United States
Department of
Agriculture

Forest
Service

North Central
Forest Experiment
Station

General Technical
Report **NC-183**

Dynamics of Forest Herbivory: Quest for Pattern and Principle

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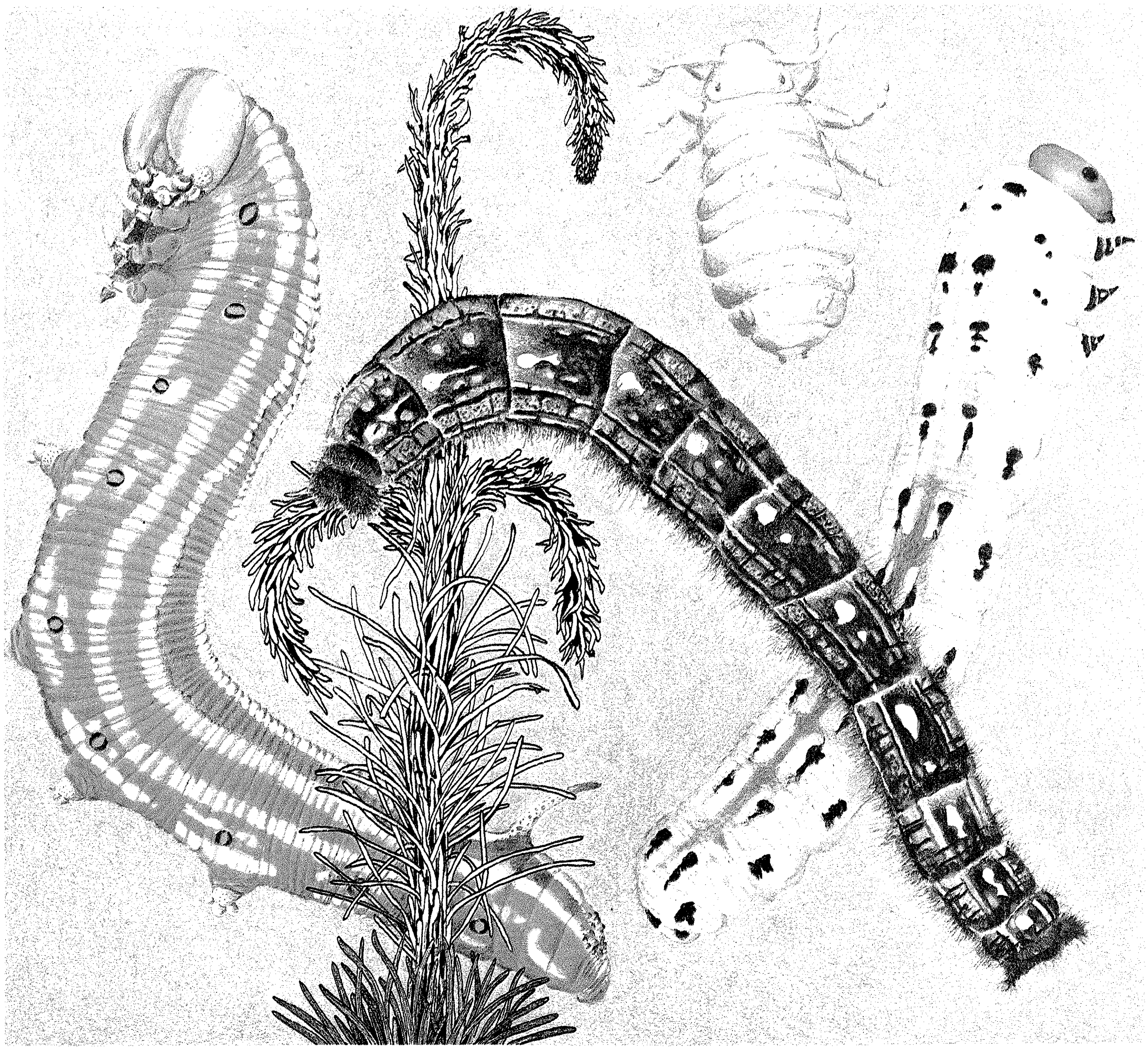
Maui, Hawaii
February 2-6 1994



ABOUT THE COVER

On the cover are artistic renditions of five forest insects and/or their damage. None are drawn to scale. The forest tent caterpillar, *Malacosoma disstria* (Lepidoptera: Lasiocampidae), (blue with white marks on its back) is a very common outbreak defoliator of several species of deciduous trees in eastern North America. The red-headed pine sawfly, *Neodiprion sertifer* (Hymenoptera: Diprionidae), is an outbreak species in young forests of various eastern American pines. The pine sphinx, *Hyloicus pinastri* (Lepidoptera: Sphingidae), is a common (green and white-striped) defoliator of pines and spruces in Europe. The balsam twig aphid, *Mindarus* sp. (Homoptera: Aphididae), feeds in the spring on balsam fir buds and shoots. The dead, wilted jack pine shoot, is typical damage caused by the white pine weevil, *Pissodes strobi* (Coleoptera: Curculiondiae), a transcontinental species attacking not just pines but also several western spruces in North America. Gyorgi Csoka provided a photograph of the pine sphinx for artistic inspiration. Kristine A. Kirkeby is the artist.

North Central Forest Experiment Station
Forest Service—U.S. Department of Agriculture
1992 Folwell Avenue
St. Paul, Minnesota 55108
Manuscript approved for publication
1996

DYNAMICS OF FOREST HERBIVORY: QUEST FOR PATTERN AND PRINCIPLE

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PREFACE

This proceedings is the result of an international symposium that was held February 2-6, 1994 in Maui, Hawaii. It was organized under the guidelines of the International Union of Forestry Research Organizations (IUFRO) by the North Central Forest Experiment Station of the U.S. Forest Service, and the Finnish Forest Research Institute. Two IUFRO Working Parties (S7.01-02, Mechanisms and genetics of plant resistance against insects, and S7.01-03, Mechanisms and genetics of plant resistance against mammals) convened for this international conference on the dynamics of herbivory and the elucidation of plant defenses against herbivores.

The proceedings is published and distributed by the USDA Forest Service, North Central Forest Experiment Station in recognition and unstinting endorsement of the IUFRO goals of promoting strong world partnerships, particularly in forest science, for the sustained management, conservation, and the betterment of the World's forest and woodland ecosystems.

ACKNOWLEDGMENTS

The editors gratefully acknowledge the generous support of the North Central Forest Experiment Station and the Finnish Forest Research Institute for underwriting the costs of organizing, assembling, editing, printing, and distributing this publication. The editors also wish to thank all of the particpants whose penetrating and spirited contributions helped make this scientific exchange highly worthwhile.

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WHY ARE TREE RESPONSES TO HERBIVORY SO VARIABLE?

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INTRODUCTION

Following defoliation the quality of tree leaves can become worse, better, or remain unchanged for herbivores. To some extent, such variation results from the different types of foliage which are studied. For example, after severe defoliation, trees may reflush in the same season, but these new leaves differ from normal, mature foliage, and leaves which grew at the time of partial defoliation may become different from leaves in an undamaged tree. Similarly, defoliated and undefoliated trees may produce qualitatively different foliage in the next growth season. The foliage produced in the year after defoliation occurred has been found to be especially different from that of undefoliated trees both in chemical traits, and herbivore bioassays, as is the case for birch (Neuvonen and Haukioja 1991), larch (Benz 1974), and oak (Rossiter *et al.* 1988). All of these trees are deciduous and there seems to be a general trend that defoliation alters the quality of deciduous foliage more noticeably than that of evergreen foliage.

We have studied defoliation-induced responses in mountain birch, *Betula pubescens* ssp *tortuosa*, and Scots pine, *Pinus sylvestris*, i.e., in a deciduous species responding strongly to herbivory (Haukioja *et al.* 1985), and in an evergreen species tending toward the other end of the continuum (Niemelä *et al.* 1984, 1991, Watt *et al.* 1991). In this paper, we briefly review the treatments which are beneficial (induced amelioration, IA hereafter) or harmful (induced resistance, IR hereafter) to herbivores. Furthermore, we discuss the validity of several hypotheses which attempt to explain how and why trees respond to herbivory as they do: the carbon-nutrient (C/N) balance hypothesis (Bryant *et al.* 1983, 1991; Tuomi *et al.* 1991), the growth-differentiation (G/D) balance hypothesis (Herms and Mattson 1992), and the sink-source (S/S) hypothesis (Haukioja 1991b, Honkanen *et al.* 1994, Honkanen and Haukioja 1994).

THE TARGET TISSUE AND TIMING OF THE DAMAGE

Whether IA or IR is elicited after damage does not depend upon the species or individual concerned. The same tree may become either better or worse depending on the type of tissue which was damaged (Haukioja *et al.* 1990), and also the timing of damage (Neuvonen *et al.* 1988).

IR can be separated into rapid (RIR) and delayed (DIR) induced resistance (Haukioja 1982). In the former, poor diet quality affects the generation of herbivores which caused the damage, while the latter influences the next generation(s) of herbivores. Note that this division is relevant as regards the consequences of the damage on herbivore populations dynamics: RIR is a stabilizing and DIR a destabilizing factor because it introduces time lags. Furthermore, the division into RIR and DIR is meaningful only in the context of a herbivore generation time; a certain response by the plant may be experienced as DIR by a short-lived herbivore species but as RIR by a long-lived one. From the plant's point of view these rapid and delayed responses do not necessarily differ. A corresponding distinction between rapid and delayed responses also applies to induced amelioration.

The mountain birch system provides an example of how herbivore-induced damage results in a multitude of changes in foliage quality. Poor quality leaves are produced after laminae of growing or newly expanded leaves are damaged by real or simulated herbivory. The RIR in birches tends to be mild when measured by its effects on herbivores (Haukioja and Neuvonen 1987), while DIR may severely curtail the potential increase of the herbivore population (Haukioja *et al.* 1985). Attempts to trigger RIR or DIR by damaging mature, late summer foliage have led to mild or no responses at all (Neuvonen *et al.* 1988, Tuomi *et al.* 1988b, Hanhimäki and Senn 1992). On the other hand, the effects of early season damage may

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Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

persist in mature leaves and affect subsequent herbivores, even from different guilds (Neuvonen *et al.* 1988, Hanhimäki 1989). The delayed effects (DIR) can remain effective on folivores consuming mature leaves produced the following summer (Hanhimäki 1989). Induced resistance in mountain birch foliage is triggered most effectively by damage at the time when there is peak feeding by the most destructive insect pest of mountain birch, the geometrid, *Epirrita autumnata* (Tenow 1972, Haukioja *et al.* 1988). Since defoliation tends to effect all members of the birch chewing guild similarly (Hanhimäki 1989), the outcomes of defoliation in birch foliage seem to represent a general response, and are not specifically targeted against *Epirrita autumnata* or other early season defoliators.

Induced amelioration in mountain birch is triggered either by removal of the apical tips of dormant twigs (Haukioja *et al.* 1990) or by damage to expanding buds or tiny leaves (Senn and Haukioja 1994). In practice, the former type of damage may result from winter browsing by mammals and the latter from insect damage very early in the season. Winter browsing makes the forthcoming foliage more preferable for a suite of different types of herbivores (Danell and Huss-Danell 1985), although the effects on insect performance tend to be mild (Neuvonen and Danell 1987, Haukioja *et al.* 1990). Larvae of *Epirrita autumnata* start feeding at, or even before, budbreak, and are known to destroy apical buds from branches (Haukioja *et al.* 1990). Early leaf damage might affect foliage quality in the same year while bud removal influences growth in the following season. In practice this means that depending on the type of target tissue and timing of the damage, larvae of *Epirrita autumnata* are potentially capable of inducing either IA, RIR or DIR in birch foliage (Haukioja 1991a).

The case with the pine is different. Most studies conducted on pines have not reported strong qualitative changes in needle chemistry after defoliation and, accordingly, the effects on insect performance also have been mild or insignificant (e.g., Niemelä *et al.* 1991, Watt *et al.* 1991, Reich *et al.* 1993). However, some studies have revealed defoliation-induced chemical changes in pines. Wagner and Evans (1985) reported that defoliation of *Pinus ponderosa* seedlings increased the production of both phenols and proteins in mature and immature foliage. Honkanen and Haukioja (1994) demonstrated increased nitrogen needle concentration after severe defoliation of Scots pine. These results indicate that the outcomes of defoliation in pines can vary substantially, and so are the consequences on herbivores.

Pruning or removal of pine buds or twigs causes more lush growth of needles and shoots (e.g., Nuorteva and Kurkela 1993, Honkanen *et al.* 1994), just like in mountain birch. Pine browsers, particularly moose, are known to cause IA (Löyttyniemi 1985), but it is not known whether insect herbivores of pine are able to induce such a response.

In summary, both in the case of mountain birch and Scots pine it is possible to induce variable effects - better or worse foliage quality, or no change at all - by selectively damaging different types of tissues and by varying the timing of the treatment.

THE RESOURCE STATUS OF BIRCH AND PINE FOR INDUCED RESPONSES: ADEQUACY OF THE C/N HYPOTHESIS

The simplest explanation for the induced resistance is that chewing or browsing of plant biomass removes nutrients and such losses lower foliage quality for later herbivores (Tuomi *et al.* 1984). If the loss of nutrients is critical for induced resistance, the tree's status can be remedied by fertilization which usually increases the nutrient concentration in foliage. The crucial question here is whether fertilization decreases the resistance of the tree and cancels any negative effects of defoliation. In the case of the mountain birch, Haukioja and Neuvonen (1985) reported that nitrogen fertilization did not alleviate the detrimental effects of defoliation (DIR) on *Epirrita* larvae. This contradicts the predictions of the carbon-nutrient balance (C/N) hypothesis, which ascribes the resistance of deciduous trees to carbon-based secondary compounds which generally decrease when trees obtain extra nutrients. However, Bryant *et al.* (1993) found that fertilized paper birches, *Betula papyrifera*, lost their ability for DIR. They criticized the mountain birch experiment by Haukioja and Neuvonen (1985) for simultaneous application of the fertilizing and defoliation treatments; the trees might not have had time to benefit from increased nutrient availability. However, another experiment with the mountain birch-*Epirrita* system (Ruohomäki *et al.* in press), accounted for the criticism by Bryant *et al.* (1993) and yet reported the same result of Haukioja and Neuvonen (1985). Furthermore, the strength of the DIR did not decline with shading, as predicted by the C/N hypothesis (Ruohomäki *et al.* in press). It is possible that mountain birch and paper birch behave differently, or the insects used in the two experiments showed differential tolerance to the altered carbon-based compounds, or that changes in carbon and nitrogen were not crucial for the herbivore. In any case, it is obvious that for birches the C/N hypothesis did not provide the general explanation for induced responses.

The C/N hypothesis does not adequately predict fertilization induced changes in pine foliage, either. Honkanen *et al.* (1994) and Honkanen and Haukioja (1994) demonstrated that branches in fertilized Scots pines produced more and bigger needles irrespective of whether the branch or the whole tree was defoliated or not. This is hard to reconcile with the prediction that carbon deficiency after defoliation restricts growth of foliage in evergreens (e.g., Bryant *et al.* 1983, Tuomi *et al.* 1988a). Furthermore, fertilization does not generally decrease, and may even increase, allocation to carbon-based secondary compounds in Scots pine (Björkman *et al.* 1991, Edenius 1993).

In summary, the C/N hypothesis does not adequately explain the effects of changes in the availability of mineral nutrients or shade for DIR in birch, or the former in pine. Since the growth-differentiation balance (G/D) hypothesis is based on the C/N hypothesis with respect to resource availability, we doubt the generality of both these to explain herbivore-induced responses of trees on the basis of resource availability (Iason and Hester 1993).

THE FUNCTIONAL ORGANIZATION OF THE TREE

Next we briefly discuss the functional organization of trees to demonstrate that some very basic physiological mechanisms behind tree structure and function actually contribute to a simple, almost mechanistic explanation for induced responses of birch. The same basic explanation may also contribute to understanding the differences between responses of the deciduous mountain birch and the evergreen Scots pine. The following reasoning forms the basis of the source-sink (S/S) hypothesis. First, trees are modular organisms. Usually this statement is interpreted as a description of the morphological design of trees: they are built of repetitive, multicellular units comprising a stem segment, leaf and meristem (White 1979). This emphasis does not help much in explaining tree functions although it vaguely makes understandable that tree parts may respond semi-independently, contrary to parts of unitary organisms like humans or fruit flies (Vuorisalo and Tuomi 1986). The term integrated physiological unit (IPU) was coined for this purpose (Watson and Casper 1984, Watson 1986). That concept emphasizes more the independence of IPUs than the integration among IPU's. However, it is the latter that makes a tree function like one individual. Haukioja (1991b) proposed that a simple set of rules can generally determine the integrity of tree functions and help not only to understand the relative independence of IPU's but also the temporal and spatial variability in their independence. Furthermore, it explicitly acknowledges other parts of the tree besides the canopy, i.e., the stem and roots.

Integration within an individual tree simply requires that IPUs at the "best" locations gain control over other, less optimally located IPUs. Within the canopy, this happens if the apical meristems, which have the best access to light, remain or become metabolically active and simultaneously suppress nearby, less favorably located, meristems (Haukioja 1991b, Sachs *et al.* 1993). For this outcome, resources should be preferentially transported to the winning meristems. This takes place by their elevated activity relative to that of nearby meristems; high meristematic activity is coupled with the production of pertinent growth hormones which, in turn, preferentially direct the flow of resources to these strong meristems. We contend that this incidentally creates the integration among above-ground parts of the plant. Integration within a tree also varies seasonally (Sprugel *et al.* 1991). We assume this results from the existence of other meristems than apical ones in the canopy. Trees have lateral meristems in branches, trunks and roots. When the sink strength of these exceeds that of apical meristems, resources are preferentially conveyed to the trunk and to the below-ground parts. When this happens, the whole tree is well integrated, and the morphologically defined IPU's have temporally lost their functional semi-independence.

The responses of IPU's ultimately are based on genetic rules which are facultative and subject to position-dependent influences. Thus, irrespective of the similar genetic basis, the behavior of an IPU depends on its position within the plant (which takes into account the possible dominance of other, especially nearby, IPUs) and on the external environment. The latter includes availability of resources. Therefore, the potential and the actual behavior of an IPU may be constrained by either internal (genetic and position-dependent) or external (resources, competition) factors, or by both. The production of a tree-like architecture requires that the genetically determined facultative and position-dependent rules include instructions for achieving, maintaining and relinquishing dominance among competing IPUs. However, we assume that, within the limits of the genetically based conditional and position-dependent rules, competition among IPUs within an individual plant is physiologically genuine yet these rules still transform the population of IPUs into an integrated "individual plant".

Variance in the strength of apical dominance (in addition to traits like dormancy/activity of meristems, internode length and branching angle, Sutherland and Stillman 1990) explains a lot of differences among different tree types. Since the relative strength of apical and other meristems has a hereditary basis (Kozlowski 1971), characteristics like the shape of the tree (resulting from the intermodular interactions) are susceptible to artificial and natural selection.

THE SINK-SOURCE HYPOTHESIS: TYPE AND TIMING OF DAMAGE ARE CRUCIAL

A crucial point underlying variable responses after damage to different types of tissues is that different meristems are provisioned seasonally at different times (Fig. 1). We claim that such a predictable temporal variation in allocation of plant resources to different sinks, as well as shifts of individual meristems from sinks to sources within the course of the season, provides an important explanation for the variability in damage-induced responses of birch and pine foliage (Table 1).

MOUNTAIN BIRCH

Early spring   sink leaves ←———— resource pool
Early summer   young source leaves ————> local sinks
Late summer   mature source leaves ————> resource pool

SCOTS PINE

Early spring   source leaves ————> resource pool
Early/late summer   apical source leaves ————> local sinks
    basal source leaves ————> resource pool

Figure 1.—The assumed seasonal variation in resource flows in the mountain birch and Scots pine.

Flushing leaves are strong sinks, and we assume that their success in drawing resources depends on their sink strength. Within few days young leaves shift from sinks to sources (Valanne and Valanne 1984) and start to preferentially provision nearby buds which will contain the leaf primordia for the next season. Simultaneously the leaves themselves mature, and when the growth of buds in short shoots, and of meristem-supporting leaves has been completed, we assume lateral meristems and meristems in the root system to be the dominant sinks for resources.

Damage to Physiological Sinks in Birch

Consequences of damage to sink organs depend on the strength and the position of the sinks. Sinks can be eliminated or weakened by clipping the tips of dormant shoots, by removing the apical dormant buds only (Haukioja *et al.* 1990), or, later in spring, by damaging flushing apical leaves which still are in the strong sink phase (Senn and Haukioja 1994). This releases extra resources for normally suppressed basal meristems (Lehtilä *et al.* ms.). The key is the apical dominance and not the amount of nutrients lost in the damage because when small but equal amounts of basal, instead of apical, meristems were removed, no changes were found in the extant foliage (Haukioja *et al.* 1990).

Damage to Physiological Sources in Birch

The consequences of damage to birch source leaves depend on the meristems which the leaves were feeding at the time of the damage (and which they would have provisioned had the damage not taken place). Damage to young source leaves reduces the sink strength of meristems in the leaf and stem primordia which the relevant leaves were feeding at the moment of the damage. For birch long-shoot leaves, those meristems are located in their axillary buds and for short-shoot leaves (which form the vast majority of all leaves in mature mountain birches) they are in short shoot buds from which the next season leaves flush. Both suffer significantly from even very limited local damage; the shoot from the axillary bud a of damaged long shoot leaf grows short the next year (Haukioja *et al.* 1990, Ruohomäki *et al.* ms). Similarly, leaves as well as

Table 1.—Typical changes in foliage growth vigor, nitrogen concentration and the amount of secondary compounds after different types of damage. + = increase, 0 = no change and - = decrease in the studied character.

Damaged tissue	Growth vigor	Nitrogen	Secondary compounds	Reference
BIRCH				
Buds/flushing leaves	+	+	-	1,2
Young source leaves	-	-	+	2,3,4,5
Mature source leaves	0	0	0	5,6
SCOTS PINE				
Buds/flushing leaves	+	+	?	7,8
Source needles feeding current year foliage	-	0,+ [a]	0	7,9,10,11
Source needles feeding general stores	0	0	0	9,10

1) Danell *et al.* (unpblished data); 2) Niemelä *et al.* 1979; 3) Haukioja *et al.* 1985; 4) Haukioja *et al.* 1990; 5) Tuomi *et al.* 1988; 6) Hartley and Lawton 1991; 7) Honkanen *et al.* 1994; 8) Långström *et al.* 1990; 9) Ericsson *et al.* 1980; 10) Honkanen and Haukioja (1994); 11) Niemelä *et al.* 1991

[a] Depends on the scale of defoliation (Honkanen and Haukioja 1994)

catkins in short shoots, whose leaves were damaged the previous year, remain smaller than in undamaged shoots (Ruohomäki *et al.* ms.). Since these outcomes follow from damage to even a single leaf, reduction of the total resource pool of the tree cannot be the relevant explanation in such a case. Instead, the meristems fed by damaged leaves presumably were unable to efficiently compete for resources in the common pool (Ruohomäki *et al.* ms).

Damage to late season foliage does not affect the sink strength of the following year's buds because they had been completed earlier. Instead, this may reduce stored resources.

The above scenario offers the simplest explanation for DIR: early summer defoliation during the previous growth season led to "weak" buds which, when still in the sink phase, were unable to get their normal load of nutrients and photosynthates. The poor quality of such foliage for herbivores may follow both from reduced amounts of nutrients and from increased secondary compounds. The same basic explanation might apply to local RIR, too: when foliage is damaged in a branch, the developing foliage loses part of its sink strength, and the whole branch might therefore become a poorer competitor of resources compared to other branches.

Damage to Scots Pine Sinks and Sources

In Scots pine, damage to apical buds has similar effects as in birch (Table 1), and we assume that these effects also can be explained in the same way (Honkanen *et al.* 1994). However, the effects of defoliation are more complex than in the case of the mountain birch. This can be explained by the simultaneous existence of several needle generations which are in different positions on a shoot, and which partially feed different meristems (Fig. 1). Defoliation of apical needles in the one-year-old needle-class that supply the developing current year foliage early in the season, decreases the growth of new shoots. However, defoliation of older needles at the same time does not affect the growth of new shoots, presumably because older needles preferentially supply lateral meristems of the tree (Ericsson 1978, Honkanen and Haukioja MS). In contrast to birch, late season defoliation decreases the growth potential of next year's pine needles more than early season defoliation (Honkanen *et al.* 1994). This presumably relates to the long developmental time of pine buds (Ågren and Axelsson 1985) and that defoliation removes the local storage in pine needles. We assume that late season defoliation of birch mainly hinders provisioning of the general resource pool (Fig. 1).

In conclusion, both the type of the damaged tissue and timing of the damage contribute to the post-defoliation responses of mountain birch and Scots pine. The important points are whether the damaged tissue was a physiological sink or a source, and which sinks the damaged foliage was provisioning at the time of the damage. Thus IA, DIR, RIR, as well as local changes in growth activity, can all be understood as outcomes of the same basic explanation.

DISCUSSION

The system of sink-source gradients is part of the mechanism regulating the allocation of resources within a plant. Therefore, it offers a basis for explanations about spatial and temporal patterns of resources, and therefore also for chemical compounds which are potentially important for herbivores and about changes in these compounds after herbivory. This is not to say that we regard resource availability unimportant; the availability of carbon and mineral nutrients obviously represents modifying and constraining factors for tree-herbivore interactions. Among other things, resources modify sink strengths of meristems and plant apical dominance (Cline 1991).

The G/D and, especially, the C/N hypotheses, emphasize plant quality and different ways that shortages of resources may cause chemical changes which are assumed to be important for herbivores. The S/S hypothesis, instead, emphasizes the ability of meristems to manufacture new biomass via spatially and temporally variable allocation of resources to specific meristems. Limitations in resource allocation may alter foliage quality either via the primary or the secondary leaf chemistry, or, most probably, both. The S/S hypothesis does not make *a priori* predictions of the importance of these chemical changes for herbivores.

We assume that the basic explanation for DIR, and perhaps for localized RIR, is the weakened state of meristems. This leads to a lower competitive ability in the plant sink-source system. Altered resistance is an automatic outcome of these changes. There are four aspects of induced tree responses which support this. First, defoliation/clipping-induced changes in plant growth activity are basically local phenomena. This also refers to the largely unspecific changes in primary and secondary chemistry which produce effects classified as RIR, DIR and IA in trees like birches. This clearly contrasts with some damage-induced systemic responses which effectively spread within and among plants (Walker-Simmons and Ryan 1977, Farmer and Ryan 1990, Takabayashi *et al.* 1991). They concern well known and obviously strictly defensive traits like production and transfer of the proteinase inhibitor inducing factor (PIIF) e.g. in many herbaceous plants (McFarland and Ryan 1974), or the resin duct system of conifers (e.g., Blanche *et al.* 1992). Second, the responses of birch foliage quality to defoliations or clippings is of a general nature and affects all species of chewing insect herbivores tested so far in a similar way (Hanhimäki 1989). Third, the existence of IA demonstrates that reasons other than defense (or recovery) must be sought when interpreting plant responses to herbivory. Such seemingly nonadaptive responses are real and presumably reflect constraints in plant design. Fourth, birch and pine are surprisingly insensitive to the amount of lost biomass: limited as well as intensive defoliations cause fairly similar degrees of responses (Haukioja and Neuvonen 1987, Honkanen and Haukioja 1994). This is consistent with sink strength regulated photosynthesis which is common in plants (e.g., Wardlaw 1990). All these aspects indicate that defoliation-induced changes in carbon-based potentially defensive chemistry, as well as in the primary chemistry, have explanations other than defense, or the relative availability of different types of resources. Note that acceptance of this explanation does not deny the importance of plant chemistry on herbivores. However, the question of whether induced tree resistance, measured as a decrease in insect performance after previous foliar damage, is a true defense or not, is outside the scope of this paper.

The S/S hypothesis has some obvious practical implications. For instance, branch or ramet specific responses of trees to defoliations (Haukioja *et al.* 1983, Tuomi *et al.* 1988b, Långström *et al.* 1990, Hanhimäki and Senn 1992, Honkanen *et al.* 1994, Honkanen and Haukioja 1994) become easily comprehensible. This fact has made it possible to use individual ramets of the same multi-stemmed tree as units on which different treatments have been applied. The question of whether such branch-specific defoliations are as effective in inducing induced resistance as more extensive tree-wide defoliations has an almost paradoxical answer: at least in pine, branches show stronger, not weaker, responses after branch-wide than after tree-wide defoliations (Honkanen and Haukioja 1994). This result agrees with the S/S hypothesis and, to our understanding, cannot be derived from any other hypothesis: within the canopy, competition of resources among meristems puts a single branch with defoliation-weakened meristems into a poorer relative position if all other branches are intact than if they also are weakened. Second, the S/S hypothesis provides a simple explanation for the multiannual "memory" of DIR (Haukioja *et al.* 1988): it might simply result from the time needed to restore the vigor of meristems. Furthermore, the implications of the S/S hypothesis also make it worthwhile to study whether plants have evolved mechanisms to prevent herbivores causing IA. For instance, it has been reported that developing current year foliage of Scots pine produces 13-keto-8(14)-podocarpen-18-oic acid which deters pine sawflies and decreases use of the young current year foliage (Niemelä *et al.* 1982). A strong deterrence of herbivores from such current year foliage may reduce the danger of induced amelioration.

Perhaps the most important empirical message of the S/S hypothesis is a call for extreme caution in damage simulations. For instance, when triggering the DIR in the foliage of the mountain birch, even small variations in timing (perhaps a day or two) of defoliations may shift the treatment from sink leaves to source leaves. That may alter the response from IA to RIR or DIR, i.e., even the direction of the response. We doubt whether any study published so far has reported both the timing of defoliations in relation to tree phenology, and the exact defoliation practice, in such detail that it would allow the treatments to be precisely repeated. Actually, we are lacking a proper classification of damages to plants. Therefore, it is not astonishing that reports of plant responses to defoliations are notoriously variable (Lawton 1991). Although insects and plants may differ with reference to species, populations and even individuals, we regard the main problem the superficial knowledge of the system under study: we have to understand and to be able to record what we are doing with our own trees when we simulate herbivore damage.

SUMMARY

The debate about damage-induced changes in food plant quality has concentrated on their active vs incidental defensive roles. We show for birch and pine that the feedback consequences for herbivores vary from favorable to harmful depending on the treated plant part and on timing of the damage. Our results cannot be directly derived from the nutrient status of the tree. Instead, damage-induced alterations in meristematic sink strength provides a general explanation. In birch, damage to dominant sinks allows other parts to obtain a greater than normal share of resources which leads to their vigorous growth and better forage quality for herbivores. However, damage to young source leaves that are supplying local meristems for next year, weakens such meristems and reduces their success in competing for resources. This leads to less vigorous growth and poorer plant quality for herbivores. Damage to mature leaves presumably disturbs provisioning of the general resource pool of the tree but such effects, especially if they are monitored locally, may be hard to reveal. An emphasis on damage-induced effects on plant sinks and sources makes induced resistance, induced amelioration, as well as localization of responses more understandable.

ACKNOWLEDGEMENTS

We thank all the persons who have helped in the practical work on which this paper is based, as well as those who have helped in formulating the ideas and who have criticized earlier versions of the present paper (Sinikka Hanhimäki, Seppo Neuvonen, Pekka Niemelä, Matti Rousi, Kai Ruohomäki, Janne Suomela, John Vranjic, and Timo Vuorisalo). John also checked our English. Financially this study has been made possible by grants from the Academy of Finland, Maj and Tor Nessling Foundation, and the Lapland Forest Damage Project.

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DAMAGE-INDUCED NUTRITIONAL CHANGES IN PINE FOLIAGE: AN OVERVIEW

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INTRODUCTION

Plant resistance can be divided into constitutive and induced defenses. Constitutive defense can deter, repel, intoxicate or interfere with insect herbivores. This can change in response to external or internal influences (e.g., weather, site factors, plant age), but is usually stable (Schult 1988). Induced responses are a form of phenotypic plasticity and can shift plant resistance as a result of herbivore attack (Haukioja and Neuvonen 1987, Haukioja 1990). Induced changes in foliage quality can take place in a density-dependent fashion and modify the stability of insect populations. Responses which are caused by one insect generation and act during subsequent generations have been called delayed induced resistance (DIR). Those responses which occur in the same insect generation have been called rapid induced resistance (RIR) (Haukioja and Neuvonen 1987, Haukioja 1990). Emphasis has usually been placed on the defensive role of chemical compounds, leading to underestimation of the role of some "positive" compounds (e.g., mineral nutrients, water, sugars). It is often questioned whether chemical changes (low quality to herbivores) represent an active defense or are only incidental by-products or reflect the abiotic environment (Tuomi *et al.* 1984, Haukioja and Neuvonen 1985).

Plant species or individuals may respond differently to insect-caused or artificial defoliation. The induced responses of deciduous trees have been studied more than those of evergreen trees. The most studied tree genera are *Betula*, *Quercus*, *Alnus* and *Salix* (Schult and Baldwin 1982, Jeker 1983, Raupp and Denno 1984, Haukioja and Neuvonen 1987, Neuvonen *et al.* 1987). Defoliation by *Zeiraphera diniana* altered the contents of nitrogen and fiber in European larch, *Larix decidua*, for a four-year period (Baltensweiler *et al.* 1977). Responses to insect defoliation have been detected in the foliage of *Pinus ponderosa* (Wagner and Evans 1985), *P. contorta* (Leather *et al.* 1987) and *P. sylvestris* (Lyytikäinen 1993). Induced reactions have also been reported in *P. radiata* after damage by spider mites (Karban 1990).

A very important question in insect ecology is whether certain tree species can exhibit induced responses and whether such responses have an impact on defoliating insect pests. The main aim of this study is to find those circumstances when induced responses have been expressed and affected insect performance or perhaps population dynamics. The discussion is focused on induced reactions in pine foliage and experiments with defoliating insects.

QUALITATIVE CHANGES IN PINES

Rather few studies have been carried out on real or artificial defoliation in conifers, especially pines. The very first investigations dealt with modified polyphenol metabolism in *P. sylvestris* after attack by *Neodiprion sertifer* (Thielges 1968), but the conclusions were erroneous due to indirect comparison between the occurrence of the compound and sawfly attack. Some of the reported studies have involved insect rearings without chemical analyses, only leading to hypotheses about the causal factors. One difficulty is the wide variability in experimental design, which makes comparisons difficult. One basic problem seems to be the analysis of needle year-classes of different age. The nutritive status, i.e., the value of foliage as photosynthetic machinery and the ability to react after damage, are not the same in different year-classes (Bernard-Dagan 1988, Haukioja 1990). The timing of treatments during the growing season can also affect the expression of the reactions (Hartley and Lawton 1991).

The nutrients most studied are nitrogen, minerals, sugars, and water content (Table 1). However, needle resin acids that are obviously harmful for diprionid sawflies (Larsson *et al.* 1986) have been analyzed only once after defoliation. Rapid

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Table 1.—Studies involving qualitative changes in foliage chemistry in different pine species. **E** = early season defoliation, **L** = late season defoliation, **M** = mature needles, **C** = current needles.

Host	Damage agent/ Induction period	Defoliation level/ Tree age	Effect on host
Pinus ponderosa[1,2]	Artificial/2 or 8 weeks (?)	0, 25, 50, 75 %/seedlings	Increased proteins, phenols, procyanidins, decreased nitrogen content (M, C)
Pinus ponderosa[2]	Artificial/11 days (?)	0, 50 % of branch/pole-size	Decreased tannin and nitrogen content (M, C)
Pinus contorta[3]	*Panolis flammea*/ 1 year (E)	0, 50 %/3 years	Changed monoterpene profiles and tannin contents (C)
Pinus contorta[4]	*P. flammea*/1 year (E)	0, 20-35, 75-90 %/22-23 years	Decreased P and β-phellandrene content, increased N:P-ratio and β-pinene, myrcene, 3-carene and terpinolene content (M, C)
Pinus contorta[5]	*Neodiprion sertifer*, *Diprion pini*/1 year (E, L)	0, 25, 50 %/5 years	Increased C, Ca, Na, Pb, Fe content (M)
Pinus sylvestris[6]	Artificial/0-2 years (E, L)	25, 50, 75, 100 %/18 years	Decreased starch content, improved water status (M)
Pinus sylvestris[7]	Artificial, *D. pini*/ 1 year (E, L)	?/10 years	Increased total lipids, abietane and pimarine diterpene acid content (C)
Pinus sylvestris[8]	Artificial/3 years (E, L)	0, 50-80 %/15-20 years	Increased nitrogen, decreased dry mass content (C, M)
Pinus sylvestris[9]	Artificial/1 year (E)	0, 5-75 %/24 years	Increased water content (M)
Pinus sylvestris[5]	*N. sertifer*, *D. pini*/ 1 year (E, L)	0, 25, 50 %/5 years	Changed C, P, K, Ca, Mg, Fe, Pb and increased water content (M)
Pinus sylvestris[10]	Artificial, *N. sertifer*, *D. pini*/0-1 year (E, L)	0, 25, 50, 75 %/14 years	Increased C, N, P, K, Ca, Cu, Na, Mn, Al, Fe, tannin and changed sugar and water content (M)

References: 1) Wagner and Evans 1985, 2) Wagner 1986, 3) Leather et al. 1987, 4) Watt et al. 1991, 5) Lyytikäinen 1993, 6) Ericsson et al. 1980a,b, 7) Buratti et al. 1988, 1990, 8) Niemelä et al. 1991, 9) Lyytikäinen 1992a, 10) Lyytikäinen 1994.

induced reactions have been studied in *P. ponderosa* and *P. sylvestris*. In *P. ponderosa* the nitrogen and tannin concentrations decreased, but those of proteins, phenols, procyanidins and certain nutrients increased in *P. sylvestris*. Needle water content tended to rise shortly after damage. After one or more years (Table 1) the nitrogen, minerals, and monoterpene concentrations in *P. contorta* increased, but only that of P decreased. A similar trend for the nutrient and water contents in *P. sylvestris* has also been reported. The most interesting aspect is the increase in diterpene acids and sugars, but there are only two reports of such findings (Buratti *et al.* 1988, Lyytikäinen 1994).

Artificial and natural defoliation induce either rapid or delayed chemical changes in pine foliage. The response to insect-caused defoliation is usually stronger than that to artificial damage (Neuvonen *et al.* 1987, Hartley and Lawton 1991), but opposing reactions have also been reported (Baldwin 1988). It would appear that pines are more insensitive to defoliation than deciduous trees. The damage level and its timing are probably more important factors for the expression of the reactions than the damage mode. Deciduous trees can respond to low foliage damage (e.g., tearing) (Haukioja and Neuvonen 1987), but conifers need at least moderate damage (Wagner and Evans 1985, Leather *et al.* 1987, Lyytikäinen 1992a). In some cases the qualitative changes in late summer were different than those in early summer (e.g., Lyytikäinen 1994). Ericson *et al.* (1980a,b) observed that Scots pines defoliated late in the season suffered from lowered starch concentrations, and the trees with the highest degree of damage lost their current year's shoots and new buds. The studies referred to in Table 1 have mostly been concerned with responses to sawfly damage. Induced reactions have unfortunately been investigated with very few other pine insect species, even though there are many other harmful pests on pines in Europe, e.g., *Panolis flammea* (Barbour 1987).

NEEDLE QUALITY AND THE PERFORMANCE OF DEFOLIATING INSECTS

Rapid Responses

Qualitative changes do occur in pines, but are these changes targeted at herbivores and are the species used sensitive enough to respond to induced reactions? Rapid reactions and insect response have been detected in two pine species: *Pinus ponderosa* and *P. sylvestris* (Table 2). Artificial damage adversely affected the growth of *Neodiprion fulviceps*, which should indicate negative reactions in *P. ponderosa*. The response of *Neodiprion sertifer* was mainly positive on *P. sylvestris* shortly after insect-caused defoliation, but after artificial damage there were no differences between control and experimental trees. Another sawfly species responded either negatively or not at all. Furthermore, the results with Scots pine were not parallel. It would appear that *Diprion pini* was the least sensitive to qualitative changes in the needles. On the other hand, the low defoliation level for the whole canopy may have an effect on the indifferent performance of different sawfly species (Niemelä *et al.* 1984). In general, slight defoliation caused no changes in insect performance. In some cases slight defoliation may even have adverse effects on insect success compared to heavy defoliation (Lyytikäinen 1992a). There were some positive responses in early summer and soon after damage, but mainly negative responses in late summer. The lack of impact on sawfly survival indicates no effect on population dynamics. On the basis of these reports (Table 2), it would appear that Scots pine possesses no effective, rapid resistance reactions against diprionid sawflies.

Delayed Responses

More studies have been carried out on delayed reactions in pines than on rapid reactions. In *P. contorta* the investigations deal only with experiments on seedlings (Table 3). The negative effect of treatment was obvious after defoliation by *Panolis flammea*, reflecting the persistence of induced reactions harmful to insects. One possible explanation for this may be that current-year needles were used in experiments and they obviously contain more secondary compounds than older needle year-classes (Buratti *et al.* 1988, 1990). There was a trend for positive or neutral responses after previous early summer defoliation. However, the responses were mostly negative following late summer damage in both pine species (Table 3). The results for *P. flammea* only are dissimilar due to the different experimental design. In general the results for *P. sylvestris* were negative or neutral. There were also experiments with *N. sertifer* and *Gilpinia pallida* in which there were no responses. As in studies on rapid responses, delayed responses did not usually affect larval survival, thus indicating negligible effects on population dynamics.

The importance of defoliation level was also obvious in the reports of delayed induced reactions in pines. The direction of the response was not similar at different levels of defoliation (Lyytikäinen 1993, 1994), or else the response in sawfly performance only occurred after moderate damage (Lyytikäinen 1992a). In some cases, the sawfly response was

Table 2.—Studies involving rapid induced resistance on pines and performance of insect defoliators. **E** = early season defoliation, **L** = late season defoliation, **0** = no statistically significant effect, **-** = statistically significant negative effect, **+** = statistically significant positive effect.

Host/ Insect	Damage agent/ Induction period	Defoliation level/ Tree age	Relative growth rate	Larval weight	Pupal weight	Larval survival
Pinus ponderosa						
Neodiprion fulviceps[1]	Artificial/11 days (?)	0, 50 % of branch/pole-size	-	?	?	?
Pinus sylvestris						
Neodiprion sertifer[2]	N. sertifer/3 weeks (E)	25 % of branch/20-30 years	+	?	0	+
N. sertifer[3]	Artificial/2 weeks (E)	0, 5-75 %/24 years	0	0	0	0
N. sertifer[4]	Artificial or N. sertifer/ 2 weeks (E)	0, 25, 50, 75 %/14 years	+	+/-	0	0
Microdiprion pallipes[2]	N. sertifer/8 weeks (E)	25 % of branch/20-30 years	0	0	0	-
M. pallipes[3]	Artificial/6 weeks (E)	0, 5-75 %/24 years	-/0	-/0	-/0	+/0
Gilpinia virens[2]	N. sertifer/9 weeks (E)	25 % of branch/20-30 years	0	0	0	0
Gilpinia pallida[3]	Artificial/6 weeks (E)	0, 5-75 %/24 years	-/0	-/0	-/0	0
G. pallida[4]	Artificial or Diprion pini/ 1 week (L)	0, 25, 50, 75 %/14 years	-/0	0	-/0	0
Diprion pini[2]	N. sertifer/8 weeks (E)	25 % of branch/20-30 years	0	0	0	0
D. pini[5]	D. pini/1-2 weeks (E)	?/?	+	0	0	0

References: 1) Wagner 1986, 2) Niemelä et al. 1984, 3) Lyytikäinen 1992a, 4) Lyytikäinen 1994, 5) Geri et al. 1990.

Table 3.—Studies involving delayed induced resistance of pines and performance of insect defoliators. **E** = early season defoliation, **L** = late season defoliation, **0** = no statistically significant effect, **-** = statistically significant negative effect, **+** = statistically significant positive effect.

Host/ Insect	Damage agent/ Induction period	Defoliation level/ Tree age	Relative growth rate	Larval weight	Pupal weight	Larval survival
Pinus contorta						
Panolis flammea[1]	*P. flammea*/1 year (E)	0, 50 %/3 years	-	-	?	-
Neodiprion sertifer[2]	*N. sertifer*/1 year (E)	0, 25, 50 %/5 years	0	+/-	0	0
Gilpinia pallida[2]	*N. sertifer*/1 year (E)	0, 25, 50 %/4 years	+	0	+	0
G. pallida[2]	*Diprion pini*/1 year (L)	0, 25, 50 %/5 years	-	-	0	0
Pinus sylvestris						
N. sertifer[3]	*N. sertifer*/1 year (E)	25 % of branch/20-30 years	0	0	0	0
N. sertifer[4]	Artificial/1-3 years (E, L)	0, 50-80 %/15-20 years	?	+/0	0	-/0
N. sertifer[5]	Artificial/1 year (E)	0, 5-75 %/24 years	0	-/0	-/0	0
N. sertifer[2]	*N. sertifer*/1 year (E)	0, 25, 50 %/5 years	+	0	0	0
N. sertifer[6]	Artificial or *N. sertifer*/ 1 year (E)	0, 25, 50, 75 %/14 years	-/0	-/0	-/0	0
Microdiprion pallipes[5]	Artificial/1 year (E)	0, 5-75 %/24 years	0	0	0	0
M. pallipes[7]	Artificial or *N. sertifer*/ 1 year (E)	25 % of branch/21 or 60 years	-/0	-/0	-/0	0
Gilpinia pallida[2]	*N. sertifer*/1 year (E)	0, 25, 50 %/5 years	0	0	0	0
G. pallida[2]	*D. pini*/1 year (L)	0, 25, 50 %/5 years	-/+	0	0	0
G. pallida[5]	Artificial/1 year (E)	0, 5-75 %/24 years	-/0	-/0	-/0	0
G. pallida[6]	Artificial or *D. pini*/ 1 year (L)	0, 25, 50, 75 %/14 years	0	0	0	0
G. pallida[7]	Artificial or *N. sertifer*/ 1 year (E)	25 % of branch/21 or 60 years	-/0	-/0	+/0	0
Diprion pini[4]	Artificial/1-2 years (L)	0, 50-80 %/15-20 years	?	0	0	-/0
D. pini[8]	*D. pini*/1 year (E, L)	?/10 or 12 years	+/0	?	-	0

References: 1) Leather et al. 1987, 2) Lyytikäinen 1993, 3) Niemelä et al. 1984, 4) Niemelä et al. 1991, 5) Lyytikäinen 1992a, 6) Lyytikäinen 1994, 7) Lyytikäinen 1992b, 8) Geri et al. 1988, 1990.

nonlinear (Lyytikäinen 1992a), indicating some kind of optimality perhaps in nutrient status due to stress (Wagner and Frant 1990). The results in these reports (Table 3) testify that lodgepole pine obviously responds negatively to defoliating insects, but in Scots pine the responses are weaker and are only expressed under certain conditions.

DISCUSSION

Foliage of different ages may respond differently to defoliation. Several reports indicate that qualitative changes induced after spring defoliation in deciduous trees are more obvious those occurring after late summer defoliation (e.g., Haukioja and Niemelä 1979). Young, expanding foliage acts as a sink for metabolites, and plant resources are converted to foliage development and expansion (Coleman 1986). Major changes in the biochemistry and physiology can occur as a result of foliage damage. On the other hand, damage to older foliage may decrease the amount of resources available to young foliage (Coleman 1986). However, no studies have been carried out on the gradual changes in needle quality after defoliation taking place during successive growing season(s). Needle quality is usually based on a single sampling. In *Pinus pinaster*, sugar, lipid, starch, and terpene concentrations have been found to vary according to season (Bernard-Dagan 1988). This phenomenon may offer one explanation for the findings concerning different responses following early or late summer defoliation.

Plant resource allocation and partitioning change in relation to age. Seedlings allocate most of their resources to growth, and mature plants allocate to reproductive organs (Kolowski 1971). Induced reactions have been found in three pine species: *P. contorta*, *P. ponderosa* and *P. radiata* (Leather *et al.* 1987, Wagner 1988, Karban 1990). The growth of *P. radiata* saplings is the fastest (Kolowski 1971). *P. ponderosa* and *P. contorta* are regarded as slow-growing species, but the growth of *P. sylvestris* is even slower than that of *P. contorta* (Lyytikäinen 1993). The lack of induced reactions is common in inherently slow-growing conifers, relying on constitutive resistance (Rhoades and Cates 1976). The induced reactions detected in these pine species were linked with age and fast growth because seedlings and saplings primarily showed the responses. In lodgepole pine, trees over 20 years old showed no induced resistance against herbivores (Leather *et al.* 1987, Watt *et al.* 1991), and only at ages ≥12 years, did saplings suffer from outbreaks of *Panolis flammea* (Barbour 1987). In Scots pine the reactions were more pronounced in seedlings than in saplings (Lyytikäinen 1992b, 1993, 1994). The defensive strategy of Scots pine is obviously also age-specific, i.e., the seedlings resemble fast-growing species in showing induced responses.

Plants usually show flexibility in their physiology within the bounds set by environmental limitations (e.g., drought, shading, nutrient deficiency). Changes in resource allocation may be directed towards equalization of all the factors limiting plant growth (e.g., Hunt and Nicholls 1986). Environmental stresses may cause shifts in the partitioning of resources to different processes (Chapin *et al.* 1987). Bryant *et al.* (1983) suggested that the phenotypic responses of secondary chemistry are influenced by plant carbon-nutrient balance. Carbon-based, secondary metabolites are supposed to accumulate in conditions where growth is limited by mineral nutrients (Tuomi *et al.* 1988, Tuomi 1992). The defense bears no cost, because secondary metabolism is supported by the resources acquired in excess of primary metabolic requirements (Tuomi *et al.* 1988). The relatively abundant nutrient status of the growing site in many studies could have reduced the probability of carbon allocation to the production of secondary metabolites (e.g., Niemelä *et al.* 1991, Lyytikäinen 1993, 1994).

The explanation for only weak rapid or delayed reactions in pines may be due to physiological differences between deciduous and evergreen trees. In deciduous trees the stems and roots provide the major carbon reserves (Bryant *et al.* 1983). In contrast, the carbon and current photosynthates in evergreen trees are stored in old foliage (Tuomi *et al.* 1984, 1988), which is mainly preferred e.g., by sawfly larvae. These physiological differences may contribute to the different pattern of long-term induced resistance (Niemelä and Tuomi 1993) because of a shortage of mineral nutrients or carbon (Niemelä *et al.* 1984).

The insect species used in the experiments covered here did not respond in a similar manner to damage. Defoliation of Scots pine seedlings and saplings (Table 3) affected the success of *G. pallida*, but not that of *N. sertifer* (Lyytikäinen 1992a, 1993). However, the effect of damage affected the performance of *P. flammea* on *P. contorta* (Leather *et al.* 1987) and *N. fulviceps* on *P. ponderosa* (Wagner 1986). One explanation for these findings could be the different sensitivity of the insects to foliage quality (Hanski 1987, Haukioja 1990, Niemelä and Tuomi 1993). Outbreak species such as *N. sertifer* may be less affected by differences in food quality than less common species, e.g., *G. pallida* on the same host species. Another explanation is the difference in larval feeding periods. The larvae of *N. sertifer* consume only mature needles in early summer, but the other sawfly species can also eat the current foliage during middle or late summer. Needle year-classes may also react differently after needle damage (Niemelä and Tuomi 1993), or interactions among needle year-classes may occur (Buratti *et al.* 1988). Thirdly, as earlier mentioned, there could also be phenological changes in the same year-classes during the summer (Bernard-Dagan 1988). The quality of mature needles is different in early season species compared to late season species.

The existence of induced responses in *P. contorta* and *P. ponderosa* may in part offer one explanation for the population dynamics of the species feeding on them (e.g., Barbour 1988). In Scots pine the weak induced reactions may be the reason for the lack of cyclicity in sawfly outbreaks (Niemelä and Tuomi 1993). The lack of damage-induced reactions in the needles of mature and pole-size Scots pine enables outbreaks of *N. sertifer* to occur for several years at the same site. The explanations for the different phases in population dynamics must be due to factors (e.g., parasitoids, diseases) other than qualitative changes in the foliage.

SUMMARY

In pine foliage, induced responses against defoliating insects have been detected in *Pinus ponderosa, P. contorta* and *P. sylvestris*. Contents of nitrogen, minerals, and water tended to increase after damage. The damage level and timing are important factors for the expression of these reactions. *P. ponderosa* showed evidence of rapid induced reactions, but not in *P. sylvestris*. Delayed induced reactions were obvious in *P. contorta*, but in *P. sylvestris* the responses were weaker and expressed only under certain conditions. Foliage of different ages may respond differently, because young foliage acts early on as a sink for metabolites. Furthermore, foliage quality can change according to season. The induced reactions detected in certain pine species are linked to their seedlings and fast growth. The defensive strategy of pines is probably highly age-specific: seedlings resemble fast-growing species. The explanation for the typically weak reactions in pines may be due to physiological differences between them and deciduous trees. The weak induced resistance in Scots pine may explain lack of cyclicity in pine sawfly outbreaks.

ACKNOWLEDGMENTS

I wish to thank Pekka Niemelä for his critical and helpful comments on the manuscript and John Derome for kindly revising the English text. The work was financially supported by The Academy of Finland (project no. 2091031).

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STAND AND LANDSCAPE DIVERSITY AS A MECHANISM OF FOREST RESISTANCE TO INSECTS

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INTRODUCTION

Plant vulnerability to herbivorous insects depends on both suitability (determined by nutritional and defensive factors) and exposure (determined by plant location relative to herbivore population sources). The importance of plant biochemical defenses against herbivores has received much attention (Rosenthal and Janzen 1979, Cates and Alexander 1982, Harborne 1982, Coley *et al.* 1985). Herbivore populations typically are aggregated on particular trees that often differ from their neighbors in chemical composition or other factors that indicate greater susceptibility to herbivores (Alstad and Edmonds 1983, Lorio 1993). Plant compounds are important as feeding deterrents or toxins for herbivores unable to adapt appropriate avoidance or detoxification mechanisms. However, herbivores have adapted various strategies for avoiding or detoxifying the chemical defenses of their hosts (Bernays and Woodhead 1982, McCullough and Wagner 1993).

Variation in suitability among plants may be important both for reducing selection for herbivore adaptations and for minimizing host exposure to adapted herbivores. Herbivores seeking new hosts must both distinguish and be able to reach suitable hosts. Therefore, plant suitability, apparency, and distance from herbivore populations function interactively to determine colonization by herbivores (Courtney 1986, Schowalter and Stein 1987).

My objective in this paper is to evaluate plant diversity as a means of reducing host exposure to herbivores. I will consider diversity at the level of genetic variability within a particular species, and at the level of stand and landscape variability in community composition. These three levels constitute a nested hierarchy of diversity that can limit population growth of herbivores within stands and across landscapes.

Genetic Diversity Within Species

Populations of plants typically vary in genotype and susceptibility to herbivores. Herbivores usually prefer, and show highest survival and reproduction on, plants or plant species whose defenses can be tolerated or detoxified, given that these plants can be discovered (Courtney 1986). Most herbivores are specialists on relatively few (usually related) plant species, because avoidance behaviors and detoxification mechanisms require an appropriate genetic template and are energetically expensive. Insects that feed on several plant species with distinct resistance mechanisms are subject to different selective forces on each host and may develop sibling species as host-specific demes diverge (Alstad and Edmonds 1983; Via 1990, 1991; Bright 1993). Conversely, stands of plants with similar resistance mechanisms permit rapid adaptation by herbivores.

Genetic diversity within a plant species confers resistance to herbivores by presenting a matrix of susceptible and non-susceptible hosts, but genetic effects are difficult to evaluate in natural forests where genotypic effects can be confounded by plant proximity to herbivore population sources or other factors varying geographically. Conifer seed orchards and nurseries provide an opportunity to examine the importance of plant genotype independent of plant location. Herbivore impact on different genotypes replicated throughout a seed orchard or nursery can be compared to assess the extent to which injury is related to genotype.

Schowalter and Haverty (1989) examined seed losses to two insect species, *Contarinia oregonensis* and *Megastigmus spermotrophus*, in a *Pseudotsuga menziesii* clonal seed orchard and in a progeny plantation in western Oregon. The various clones or families showed different degrees of resistance to the two insects (Table 1). Resistance to one insect species was

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Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

Table 1.—Percentage seed lost to two cone and seed insect species among offspring of selected parental crosses in a *Pseudotsuga menziesii* progeny plantation in western Oregon. Means above the diagonal are seed losses to a midge, *Contarinia oregonensis*; values below the diagonal are losses to a chalcid, *Megastigmus spermotrophus*.

x Parent Parent	1	2	3	4	5	6	7	8	9	10	11	12	Midge Mean[1]
1	—	51		43	55	56	54		68	39	43	67	53
2	8	—	58	55	84	75	62	85		76	51	59	67
3		10	—	54			61	79	65	60	59	58	62
4	13	17	6	—	59	63	57	52	75	55	52	62	57
5	6	7		11	—	69	62	55	63	68	40		62
6	10	10		24	6	—	59	56	60	58	61	67	66
7	6	6	8	7	16	6	—						59
8		4	18	13	7	10		—					65
9	5		14	13	8	14			—				66
10	2	11	6	11	4	20				—			59
11	9	12	15	16	10	22					—		51
12	6	5	2	15		19						—	63
Chalcid Mean[1]	7	9	12	14	8	14	8	10	11	9	14	9	

[1] 95% CI = 3.3-4.1 for the midge and 1.2-1.4 for the chalcid

not related to resistance to the second species. Of ten clones in the seed orchard that deviated significantly (based on 95% CI) from mean seed loss to either species, eight that were resistant to one species showed no resistance to the other. Four of these clones were highly susceptible to the second species. Only two clones were resistant or susceptible to both insects.

Similar results were found in the progeny plantation (Schowalter and Haverty 1989). Parental crosses that were resistant to one insect generally were susceptible to the other. In this case, resistance appeared to be heritable as a dominant trait, based on the generally low seed losses for progeny of resistant and susceptible parents (Table 1).

Schowalter and Stein (1987) compared the extent of *Lygus hesperus* feeding on different seed sources (representing different genetic backgrounds) in a conifer seedling nursery. This insect is a mobile species that feeds primarily on agricultural crops in a hit-and-run manner. Results indicated significant separate and interactive effects of conifer seed source (genotype) and of proximity to *Lygus* population sources in adjacent agricultural crops. The effect of plant proximity to herbivore populations, even at this small scale for a relatively mobile herbivore, is surprising.

Data from these studies demonstrate that, within monocultures of plants, a diversity of genotypes can limit resource availability and suitability for particular herbivore species. This diversity represents a species-level defense that limits initial herbivore population growth. Genetic diversity within a monoculture is not sufficient to prevent herbivore outbreaks over long time periods, especially when conditions that stress plants and/or inhibit production of plant defenses permit herbivore population growth (Waring and Pitman 1983). When environmental conditions increase susceptibility of a given plant species, exposure to herbivores can be reduced by surrounding non-host plants and stands.

Plant Species Diversity Within Stands

Our view of plant species interactions traditionally has focused on competitive interactions. This view has supported the tree farm (monoculture) approach to forestry. Recent studies, however, are indicating that plant species interactions are more complex. Plant species often share mycorrhizae, contribute collectively to soil fertility through differential nutrient uptake and concentration in litter and rhizosphere, and increase the chemical complexity of the forest aerosol (Visser 1986, Hunter and Arssen 1988). These mutualistic aspects of plant species interactions may reduce the likelihood of plant stress and apparency to herbivores, at least for some combinations of plant species.

If genetic diversity within monocultures can affect herbivores, then a diversity of plant species within a community matrix should limit herbivory to a greater extent. Studies with several insect species in different vegetation types have demonstrated that diverse vegetation limits overall herbivory. For example, Root (1973), Kareiva (1983) and Turchin (1988) reported that intermixed crops were subjected to lower levels of herbivory by insects than were monocultures of the same crops. Examples from forests are rare, largely because manipulating tree diversity for experimental purposes is difficult, and natural variation in diversity is confounded by geographic variation in soils, aspect, and other factors that also affect herbivory (Schowalter and Filip 1993).

Gara and Coster (1968), Johnson and Coster (1978), and Schowalter *et al.* (1981b) reported that southern pine beetle, *Dendroctonus frontalis*, populations appeared to be sensitive to *Pinus* spp. density. Populations generally grew rapidly in dense pine stands and declined in sparse pine stands. However, Johnson and Coster (1978) and Schowalter *et al.* (1981b) reported that large populations (>100,000 beetles) developing under favorable conditions also were capable of sufficient aggregation to colonize sparse hosts. Thinning experiments in western North America indicated that tree spacing also is critical to mountain pine beetle, *Dendroctonus ponderosae*, populations (Sartwell and Stevens 1975, Mitchell *et al.* 1983). The effects of tree species diversity were unclear; either hardwoods compete with pines and aggravate stress-related beetle activity (Hicks 1980) or hardwoods interfere with discovery of hosts (Belanger and Malac 1980).

Schowalter and Turchin (1993) conducted a relatively unique experiment to test effects of *Pinus* spp. density and stand diversity on *D. frontalis* populations. They manipulated pine and hardwood basal areas in a 2 x 2 factorial experiment and introduced equivalent *D. frontalis* populations into replicated plots to prevent confounding effects of plot proximity to beetle population sources. Infestations subsequently developed only in the dense pure pine stands and achieved infestation sizes (>10 dead trees) sufficient to warrant suppression in this treatment (Fig. 1). Infestations did not develop in dense pine stands where hardwoods were present or in low density stands, indicating that tree spacing and stand diversity both function to reduce insect population growth.

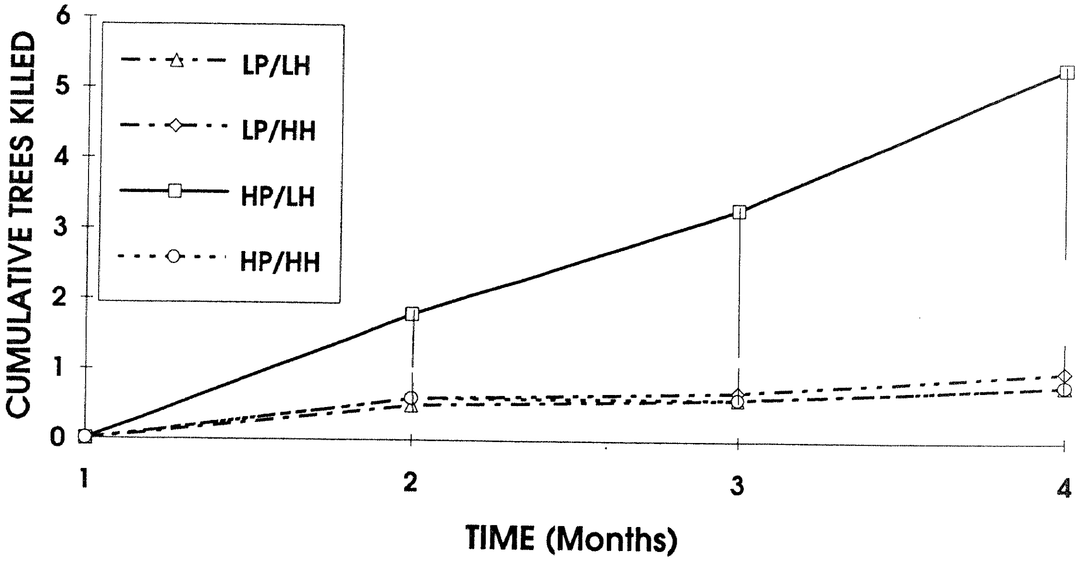

Figure 1.—Cumulative pine mortality to *Dendroctonus frontalis* by pine and hardwood basal area treatments in Mississippi and Louisiana during 1989 and 1990. Vertical lines represent 1 SEM. Low pine (LP) = 11-14 m^2/ha ba; high pine (HP) = 23-29 ba; low hardwood (LH) = 0-4 ba; high hardwood (HH) = 9-14 ba. Two infested trees were introduced into each experimental stand at the beginning of study. N=8.

Schowalter (unpubl. data) compared arthropod abundances among replicated and intermixed plots representing young Douglas-fir, *Pseudotsuga menziesii*, plantations (10-15 years old), mature natural *P. menziesii* monocultures (100-150 years old), intact old-growth *P. menziesii/Tsuga heterophylla* (450 years. old) and old-growth *P. menziesii* shelterwood (450 years old) treatments in western Oregon. Western spruce budworm, *Choristoneura occidentalis*, was significantly more abundant and caused twofold more defoliation in the mature monoculture than in other treatments. This difference also could reflect the greater predator diversity and abundance in old-growth stands (Perry 1988, Schowalter 1989, Torgersen *et al.* 1990), or unmeasured differences in mutualistic endophyte diversity or foliage quality among age classes (McCutcheon and Carroll 1993).

Stand Diversity Across Landscapes

Diversity at the landscape level augments diversity at the stand level. As the diversity of stands representing different species composition or age classes increases, the distance between stands containing suitable resources increases (Perry 1988, Schowalter 1989). Although herbivores are capable of dispersing over considerable distances, several factors reduce the likelihood of distant hosts being discovered or colonized. First, insect ability to perceive hosts over long distances is limited for most species. Plant cues or other factors conveying host location become dissipated in the forest aerosol, making distant hosts and hosts in diverse stands less apparent (Visser 1986). Second, survival decreases with distance as a result of longer exposure to mortality agents and exhaustion of energy reserves, reducing insect ability to reach distant hosts. Therefore, diverse landscapes should prevent localized outbreaks from spreading to distant hosts.

Conversely, herbivore populations are promoted in landscapes that provide greater homogeneity of resources and few barriers to population spread. Major outbreaks typically occur in relatively homogeneous landscapes.

Declining forest health in eastern Oregon and Washington is largely the result of change in landscape diversity. Forests in this region originally were a diverse matrix in which stands of shade-tolerant mixed-conifer *Pseudotsuga/Abies/Pinus* forest in moist sites at higher elevations, north aspects, and riparian areas were embedded within an arid landscape dominated by sparse fire-tolerant *Pinus/Larix* forest and savannah. Fir defoliators such as *C. occidentalis* occurred as local populations forced to search for hosts aggregated within a largely inhospitable landscape. As a result of fire suppression and selective logging of *Pinus* and *Larix* over the past century, the fir forest spread across this landscape. The landscape is now relatively homogeneous, dominated by forests of dense *P. menziesii* and *Abies* spp. Drought stress of these mesic species in addition to resource concentration has permitted *C. occidentalis* to reach epidemic population levels over most of this large area (Hadfield 1988).

In contrast to the situation in eastern Oregon and Washington, *C. occidentalis* historically has occurred at innocuous population levels in western Oregon and Washington, although localized outbreaks have occurred. Recent (and past) activity of *C. occidentalis* in western Oregon is concentrated around a major pass through the Cascade Range. This area has been affected by years of drought but also was accessible to epidemic *C. occidentalis* populations spilling over the pass from eastern Oregon (pers. obs.). Nevertheless, Schowalter (1989 and unpubl. data) found that, near this area, *C. occidentalis* was rare or absent in diverse old-growth forests but was abundant and causing measurable defoliation in mid-successional *P. menziesii* monocultures. Perry and Pitman (1983) compared suitability of *P. menziesii* foliage from eastern and western Oregon for *C. occidentalis*. They found that foliage from western Oregon was more susceptible to budworm feeding and suggested that the greater diversity of trees, predators, and parasites in western Oregon has limited budworm populations and minimized selection for resistance to this insect. If diversity has been a major factor preventing budworm defoliation in western Oregon and Washington, then widespread commercial production of mid-successional *P. menziesii* forests may result in increasing *C. occidentalis* activity in this region.

Outbreaks of *D. frontalis* in the southeastern U.S. also result from change in landscape diversity. Most of the land area in this region originally was vegetated by sparse woodlands and savannahs dominated by *Pinus palustris*, a species tolerant of the frequent fires and drought of this region and relatively resistant to bark beetles (Schowalter *et al.* 1981a). Mesic riparian and bottomland forests included a diverse assemblage of intolerant species, including *Pinus taeda* and hardwoods. Bark beetles in this landscape would have been restricted primarily to scattered injured or diseased trees. Land conversion followed by eventual abandonment and reforestation led to establishment of dense stands of rapidly growing and commercially valuable *P. taeda* over most of this region. This species is susceptible to *D. frontalis*, resulting in devastating outbreaks across the region (Schowalter *et al.* 1981a, Schowalter and Turchin 1993).

These observations indicate that diversity can be effective in limiting herbivore epidemiology. However, diversity may not provide protection when herbivores reach large population size in a sufficient proportion of the surrounding landscape.

SUMMARY

Plant vulnerability to herbivores is a function of both biochemical suitability and exposure to herbivore populations. Plant exposure to herbivores can be minimized by plant diversity. Plant diversity at genotypic, species, and landscape levels presents herbivores with a mosaic of suitable and non-suitable hosts and non-hosts. This increases the distance herbivores must travel to reach new hosts and increases the difficulty of discovering suitable hosts hidden by non-hosts in diverse stands and landscapes. By contrast, limited genetic diversity within monocultures of commercially valuable trees provides a concentrated resource for adapted herbivores. This resource inevitably will become more vulnerable to herbivores, especially during adverse conditions that stress plants and/or limit defensive capability. Localized outbreaks occur where isolated stands become vulnerable. These outbreaks can be restricted to isolated vulnerable stands in a diverse landscape that provides barriers to herbivore population spread. Regionwide outbreaks occur where a sufficient proportion of the landscape is occupied by suitable hosts. Large populations can expand into otherwise resistant stands and affect resistant or sparsely distributed hosts.

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ON NEIGHBOR EFFECTS IN PLANT-HERBIVORE INTERACTIONS

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INTRODUCTION

Coevolution involves genetic changes that occur in both plant and herbivore populations over evolutionary time. Genetic changes in turn depend on fitnesses associated with given plant and herbivore genotypes. This leads us to the ecological time scale because fitness is determined by functional interactions between individual plant units and herbivores over their life-spans. Such interactions can be formalized as a dependency of fitness on plant and herbivore phenotypes in a given context of interaction.

The basic features of co-evolutionary processes depend primarily on the direct effects that an individual's phenotype has on its fitness. However, as pointed out by Atsatt and O'Dowd (1976) and Rhoades (1979) among others, the actual interactions are often much more complicated due to various indirect effects when fitness depends on what the neighbors are doing. We label these indirect relations as neighbor effects (Eshel 1972), and argue that they may have a fundamental importance in plant-herbivore interactions (Mattson *et al.* 1991).

We discuss two aspects of indirect interactions between plants. First, in some cases palatable plants gain a benefit by growing close to unpalatable plants. This possibility was first discussed by Tahvanainen and Root (1972) and Atsatt and O'Dowd (1976), and may be called "associational refuge" effect (Pfister and Hay 1988). Several experiments have demonstrated a refuge effect (McNaughton 1978, Hay 1986), but not always (McNaughton 1978, Danell *et al.* 1991, Hjälten *et al.* 1993).

Second, it is frequently assumed that herbivores can choose their host plants depending on their nutritive value and toxicity. This presumes explicitly that herbivores can recognize the host plant and "read" cues that correlate with palatability. If the herbivore can generalize the cues (Launchbaugh and Provenza 1993), unpalatable plants that repel herbivores will naturally gain a benefit themselves. Likewise, the herbivore should avoid other plants with these very same cues. That is why we expect that plant defenses may imply "synergistic benefits" in the sense that a plant implies a benefit for other individuals sharing the same phenotype (Nee 1989, Guilford and Cuthill 1991, Rosenberg 1991, and Tuomi and Augner 1993).

We propose that these neighbor effects may well shape some aspects of plant defenses. For this purpose, we present a simplified model system where plants are interacting in pairs. Then we analyze the model system in a game theoretical context by assuming three phenotypes: non-defensive type (N), and two defensive types - one which kills the invading herbivores (D), and another that provides herbivores a signal over which to generalize (S).

MODEL SYSTEM

We assume a plant population where individual plants are interacting in pairs, i.e., in trait groups (Wilson 1975) of two plants. Each pair has a risk of herbivory, say m_p, that is a function of grazing intensity over the whole population and of the phenotypic composition of the given trait group. We call the plants the player and the neighbor (or the opponent). An invading herbivore selects the player with probability s and the neighbor with probability 1-s. We will, for simplicity, assume that the consequent risk of herbivory is equal for both the player and the neighbor so that $m_p s = m_p(1-s) = m$ in all trait groups. In other words, invading herbivores can select neither between trait-groups nor between the player and the neighbor.

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We thus neglect two potential sources of neighbor effects because experienced herbivores may well be able to evaluate the expected value of a patch or a plant from a distance, e.g., by appearance or scent. Hjälten *et al.* (1993) have discussed in a greater length how selection between and within patches can affect associational refuges.

In short, our herbivores are naive, but we assume that they can learn to evaluate the defensive status of the plants if appropriate cues are present. The consumption process is divided into two parts (bites). From our earlier assumptions, it follows that the risk of the first bite is m. Each bite by a herbivore implies a fitness cost for the plant. Plant defenses can in various ways reduce the total cost of herbivory: reducing the fitness cost caused by a herbivore per bite, increasing mortality (d) of herbivores during a bite, and inducing feeding aversion that can be either unconditional (the herbivore does not take the second bite of any food) or conditional (the herbivore does not take the second bite if a specific cue is present). In our case of two bites, unconditional feeding aversion is equivalent to increased mortality as in both cases further herbivory is stopped. We thus consider only conditional feeding aversion and the parameter a indicates the probability that feeding aversion is induced during a bite. We also assume that the cost of herbivory is h if the herbivore survives and no feeding aversion is induced, while the cost of herbivory will be h' if the herbivore happens to die or if a feeding aversion is induced during a bite ($0 < h' < h$). We assume specifically that non-defensive (N) plants cause no mortality ($d = 0$, $a = 0$), while there are two kinds of defensive plants; those (D) that will kill the herbivore during the bite ($d = 1$, $a = 0$), and those (S) that cause a conditional feeding aversion before any toxic effects appear on herbivore survival ($a = 1$, $d = 0$).

Both kinds of defenses (D and S) reduce the average costs that herbivory implies to the player. In addition to these direct fitness effects, various neighbor effects can arise if herbivores are allowed to move from the player to the neighbor and vice versa. We assume that a surviving herbivore moves after the first bite with a probability e. For simplicity, feeding aversion is not allowed to affect this probability, and the parameters h, h', d, and a assume the same values during the second bite as earlier during the first bite. Taken together, these assumptions imply that the average cost (H) of herbivory over two bites will be

$$H=[(1\text{-}d_i)(1\text{-}a_i)h + (1\text{-}d_i)ah' + d'_{ih}](1+n_{ii}+n_{ij})$$

$$n_{ii} = (1\text{-}d_i)(1\text{-}a_i)(1\text{-}e_i)$$

$$n_{ij} = \begin{Bmatrix} (1\text{-}d_i)(1\text{-}a_i)e_i & (i,j{=}S) \\ (1\text{-}d_j)e_j & (i \vee j \neq S) \end{Bmatrix}$$

where n_{ii} implies the probability of a second bite for the player by a herbivore initially invading the player (denoted by i), and n_{ij} by a herbivore moving from the neighbor (denoted by j) to the player. The latter term will be $(1\text{-}d_j)(1\text{-}a_j)e_j$ if feeding aversion induced by the neighbor protects the player (i.e., both S), and $(1\text{-}d_j)e_j$ if the neighbor does not induce a feeding aversion (i.e., the neighbor N or D) or if the player does not possess the cue required for the maintenance of feeding aversion (i.e., the neighbor S and the player either N or D). In our specific case ($e_i = e_j = e$), $H = h[1 + (1\text{-}e) + n_{ij}]$ for a player adopting N ($d = 0$, $a = 0$), and $H = h'(1 + n_{ij})$ for a player adopting D ($d = 1$, $a = 0$) or S ($d = 0$, $a = 1$). When H is multiplied by m we will get, following Augner *et al.* (1991), a measure of the herbivory load, Hm, for the payoff matrix.

This highly simplified model system elucidates clearly that the fitness of the player may well depend on the neighbor. In our case, this is so if herbivores can move between the plants ($0 < e <= 1$). If they can not ($e = 0$), the fitness of the player will be independent of the neighbor. Thus the parameter e can be interpreted both as a measure of the mobility of herbivores, and as an "interaction coefficient" that measures the extent to which the plants may potentially share the same herbivores. Note also that the model system involves only local interactions between plants within trait-groups, and that neighbor effects modify fitness payoffs during the second grazing period only. Consequently, neighbor effects in the present case are relatively weak as compared to direct fitness effects. Still, as demonstrated below, the local interactions between plants have a fundamental impact on the outcome and the dynamics of the game.

GAME THEORETICAL ANALYSIS

We analyze a game involving three strategies N, D, and S described above, and payoff matrix assuming $h = 1$ and $h' = 1/2$ (Fig. 1). We first analyze subgames with two strategies: N versus D, N versus S, and D versus S. We have formulated

		Neighbor		
		N	D	S
Player	N	$-2m$	$-(2-e)m$	$-2m$
	D	$-\frac{1}{2}(1+e)m-C$	$-\frac{1}{2}m-C$	$-\frac{1}{2}(1+e)m-C$
	S	$-\frac{1}{2}(1+e)m-C(1+k)$	$-\frac{1}{2}m-C(1+k)$	$-\frac{1}{2}m-C(1+k)$

Figure 1.—Payoff matrix for a game between two plants showing the payoffs to the player adopting either nondefensive strategy (N), lethal defense (D), or defense inducing conditional feeding aversion (S). (m) denotes the risk of herbivory, C as well as k indicate the costs of defenses, and e is the probability that a herbivore moves from one plant to another.

the model so that D and S types have similar payoffs when playing against N; the only difference is the cost of defense. Our primary interest lies in the qualitative differences between the two defensive types D (lethal defense) and S (conditional feeding aversion). We define parameter space where these phenotypes are evolutionarily stable strategies (ESS) in the three subgames (Figs. 2-4). Finally, we study the dynamics of the game when all three strategies are taken account (Figs. 5 and 6). In all cases, we have random associations and the population frequencies of N, D, and S are q, p, and r respectively.

A Disadvantage of Lethal Defense

The first subgame of N and D contrasts a nondefensive strategy (N) against a defensive strategy (D) that kills all invading herbivores and that implies a cost, C. When the trait groups are randomly derived from the population frequencies $q = 1 - p$ for N and p for D, the fitnesses will be

$$W_n = W_0 - (2-pe)\ m$$

$$W_d = W_0 - C - 1/2\ [1 + (1-p)e]m$$

where W_0 is a basic level of fitness. When $e = 0$, selection will exclusively favor either N or D depending on the balance between the cost of defense (C) and the risk of herbivory (m). When $e > 0$, some herbivores will move from N to D. This will reduce the herbivory load of N and increase that of D in mixed pairs. As a consequence, we will have three possibilities for different combinations of C/m and e (Fig. 2a):

(1) N will be an ESS for $C/m \geq (3 - e)/2$.
(2) D will be ESS for $C/m \leq (3 - 2e)/2$.
(3) Stable polymorphism ($0 < \hat{p} < 1$) is obtained when neither N nor D are ESSs.

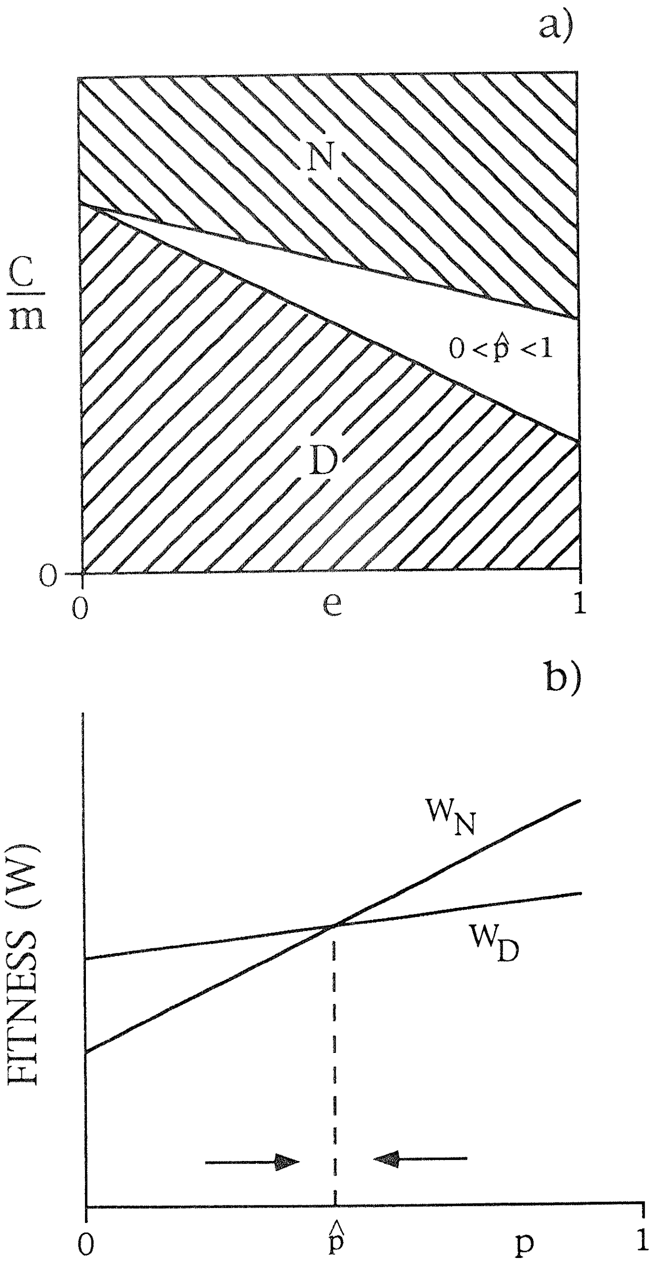

Figure 2.—ESS-conditions in N-D subgame contrasting nondefensive strategy (N) and lethal defense (D): [a] The parameter areas where N and D are pure ESSs are shaded, while in the blank area neither of them is an ESS; and [b] Fitnesses of N and D as a function of population frequency ($\hat{p}$) of D when $0 < \hat{p} < 1$. $\hat{p}$ = stable equilibrium; C, m, and e as in Fig. 1.

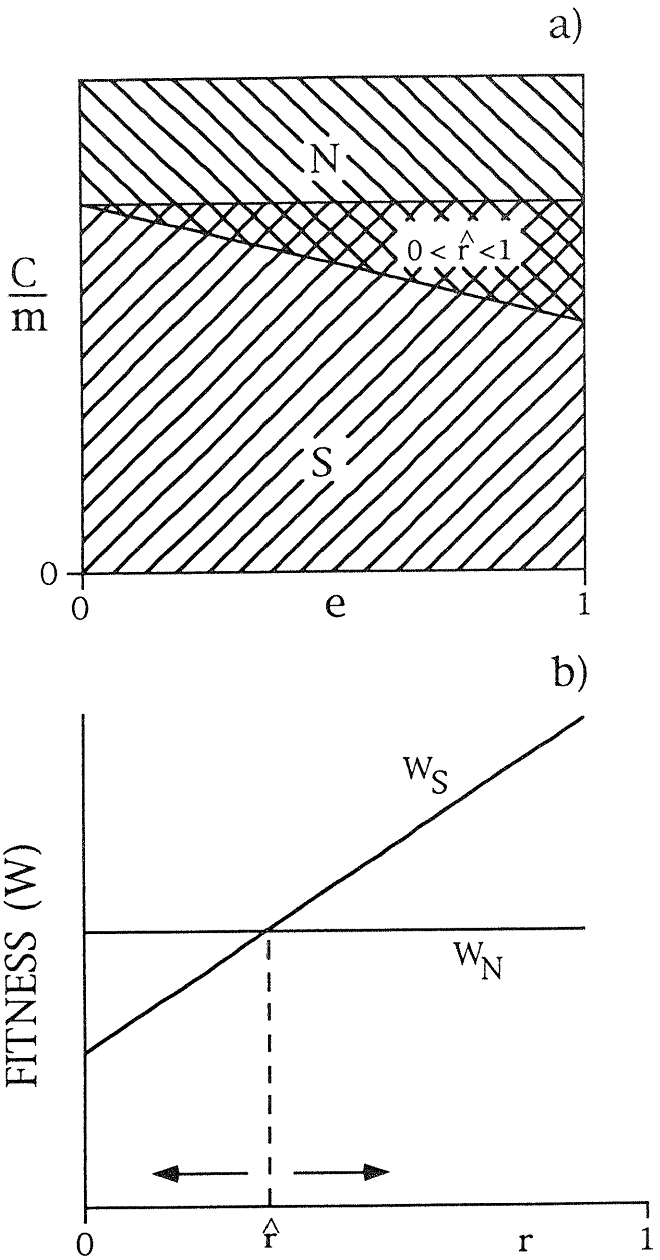

Figure 3.—ESS-conditions in N-S subgame contrasting nondefensive strategy (N) and defense (S) that induces conditional feeding aversion: [a] The parameter areas where N and S are ESSs are shaded; and [b] Fitnesses of N and S as a function of the population frequency (r) of S when $0 < \hat{r} < 1$. $\hat{r}$ = unstable equilibrium; C, m, and e as in Fig. 1.

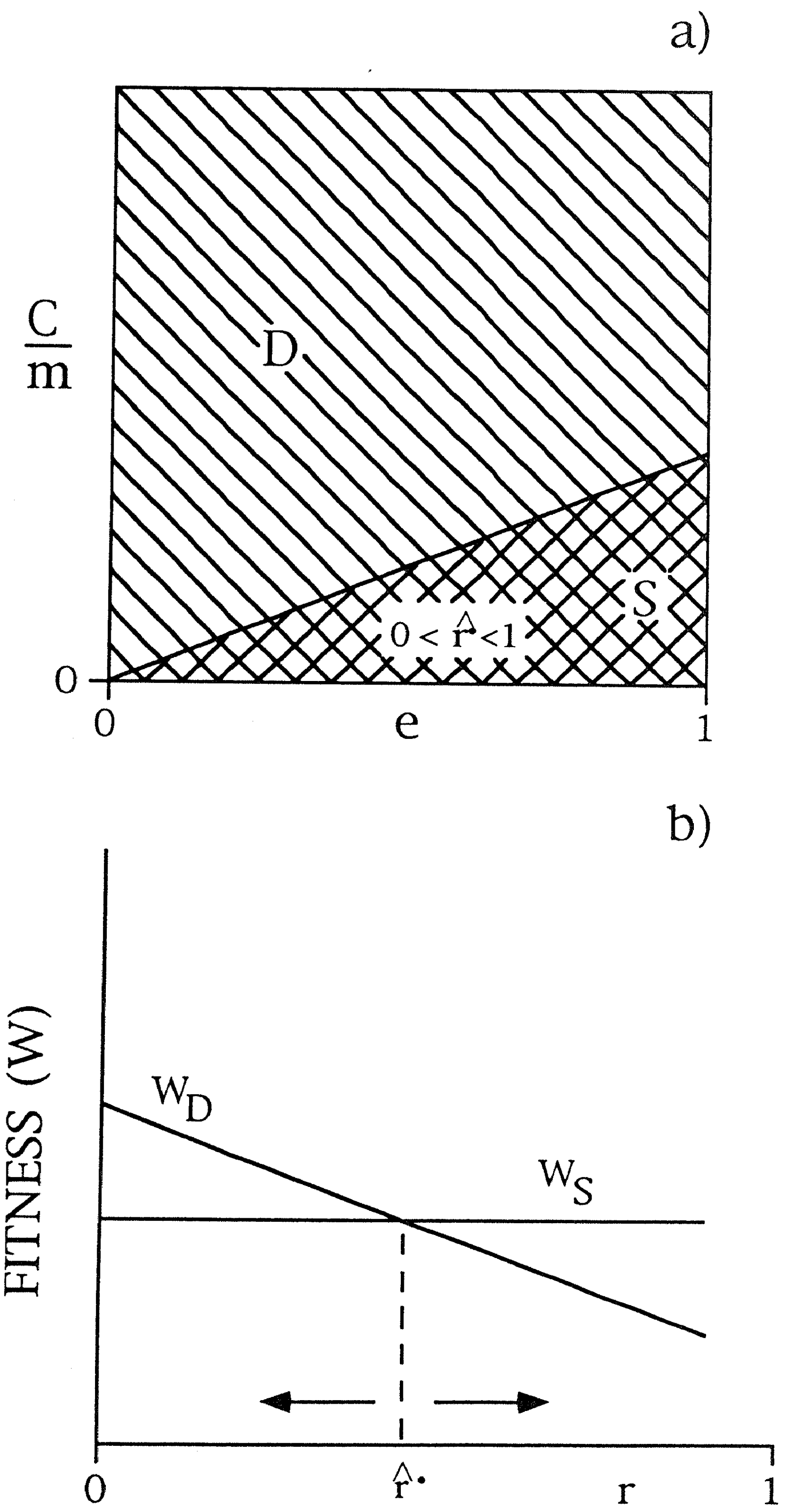

Figure 4.—ESS-conditions in D-S subgame contrasting lethal defense (D) and defense (S) that induces conditional feeding aversion: [a] The parameter areas where D and S are ESSs are shaded ($k > 0$); and [b] Fitnesses of D and S as a function of the population frequency (r) of S when $0 < \hat{r} < 1$. T $\hat{r}$ = unstable equilibrium; C, m, and e as in Fig. 1.

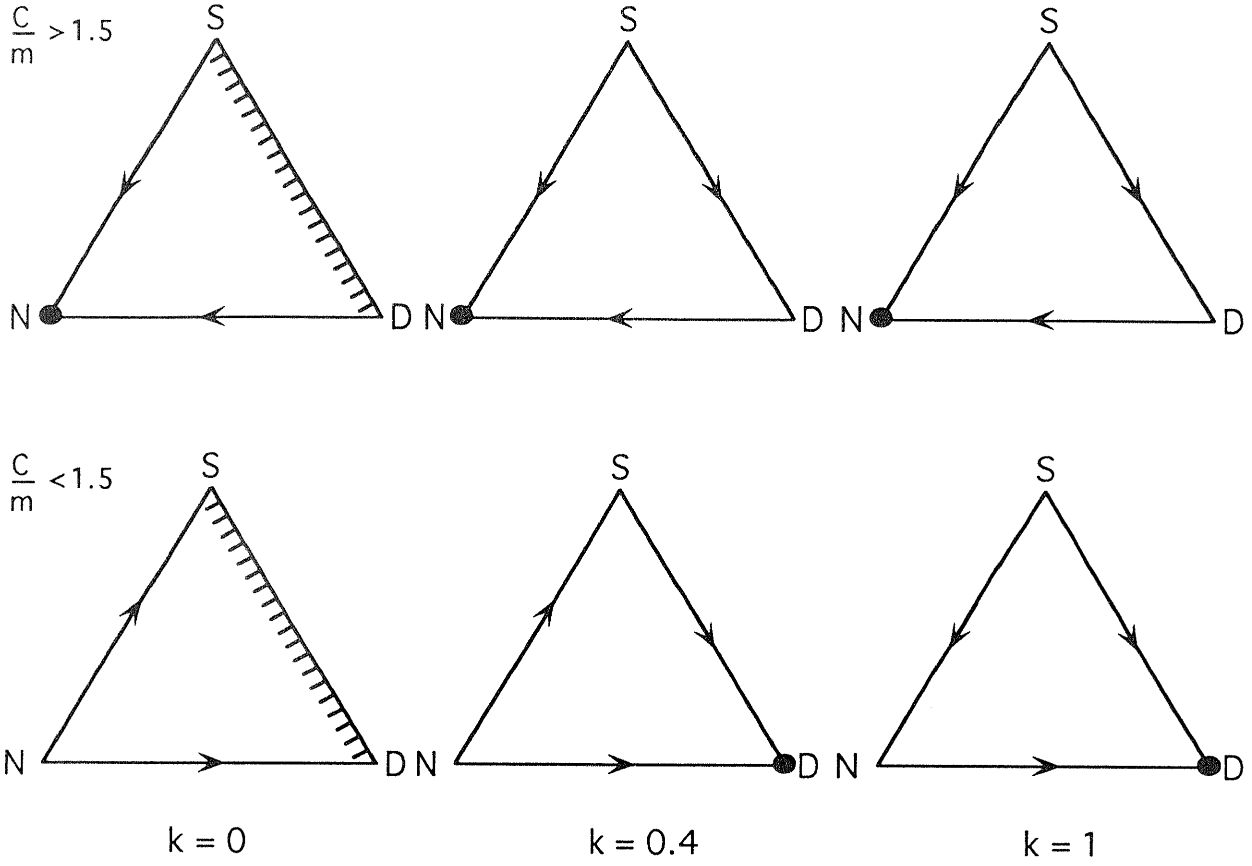

Figure 5.—Basic game dynamics when neighbor effects are excluded ($e = 0$). N, D, and S correspond to populations where $q = 1$, $p = 1$ and $r = 1$ respectively.

The third situation corresponds to "evolutionarily stable" associational refuges as both plant types coexists (Sabelis and de Jong 1988, Tuomi and Augner 1993). The coexistence is possible because both types have a selective advantage when rare, but a disadvantage when common (Fig. 2b). In our case, stable polymorphism requires that D has a higher fitness value for p close to 0. However, when p increases toward 1, W_N increases more steeply than W_D. The slope of W_N as a function p is em, while the slope of W_D is (1/2)em. The reason for this difference is that $h' = 1/2$. Consequently, the associational protection has a greater value for N that suffers $h = 1$, while D suffers only a half of that value. If both N and D would win equally from the neighbors' defenses (i.e., $h' = h$), associational protection would not maintain stable polymorphism in the present case.

The major lesson of this subgame is that the associational effects counteract the evolution of lethal defenses. We have proposed that this implies a dilemma for plant defenses: the defenses should protect the plant without benefiting nondefensive neighbors too much (Tuomi *et al.* submitted). If the defensive plants kill all herbivores, the nondefensive type can easily invade. Consequently, one could expect that "less lethal" defenses could do a better job in the long run. However, when adopting sublethal defenses the plant itself will suffer more damage by herbivores. There are at least two solutions for the dilemma. First, if a plant is allowed to adopt a lethality-level (d) between 0 and 1, associational refuge effects select for sublethal defenses with $d < 1$ (Tuomi *et al.* submitted). Second, another solution of the dilemma could be a defense strategy (S) that is potentially "lethal" to the herbivores, but that is associated with chemical or visual traits which herbivores can use as cues in order to avoid the potentially "lethal" defenses. Below, we analyze this second possibility. Since we have set $d = 0$ for S, we thus assume that feeding aversion is induced before the amount of ingested food reaches any toxic level.

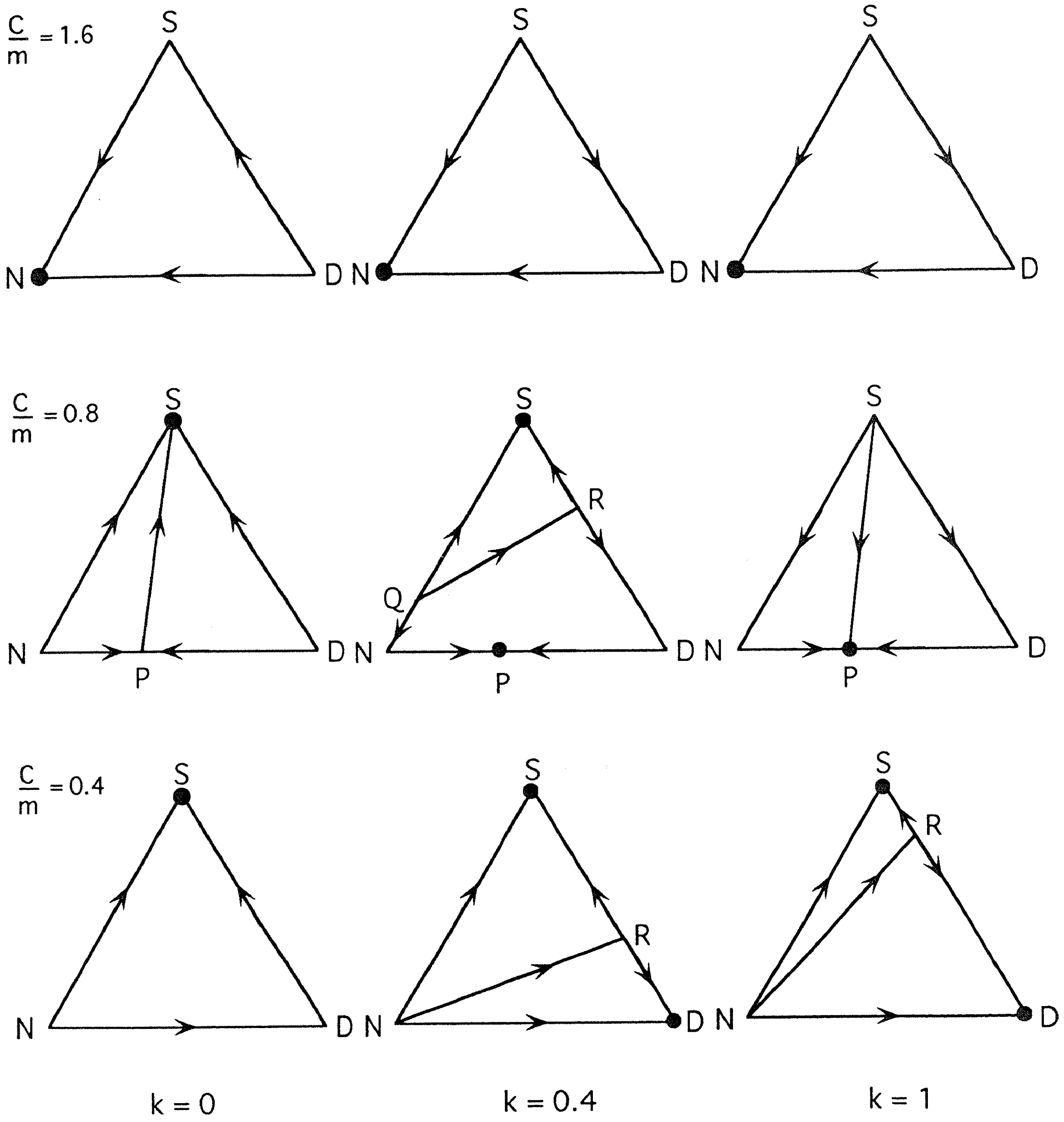

Figure 6.—Basic game dynamics when herbivores are allowed to move between the interacting plants (e = 1). N, D, and S correspond to populations where q = 1, p = 1, and r = 1, respectively. Q is an unstable equilibrium and R is a saddle point, while P can be a saddle point or a stable equilibrium. Trajectories will eventually approach the points denoted by the dots.

Conditional Feeding Aversion

We formulated the present model so that the player is subject to the exactly same herbivory load when adopting either D or S, and when the neighbor is N (Fig. 1). There are two major differences when N-S subgame is compared to N-D subgame. First, we assume that S and D may imply different costs for the plant. We have defined that the cost of S is C(1+k). To be conservative, we assume that k is non-negative indicating that it is equally or more costly to induce and maintain feeding aversion than to kill the herbivore. If both D and S require an equal investment in defensive compounds, hen $k > 0$ suggest that the cue or the signal associated with S is costly. Second, the major difference between S and D is that S does not allow N to escape from herbivory in mixed pairs. That is why the fitness of N, when playing against S, reduces to

$$W_N = W_0 - 2m$$

whereas W_S will be

$$W_S = W_0 - C(1 + k) - 1/2\ [1+(1-r)e]m$$

where r is the population frequency of S. The population frequency of N is $q = 1 - r$. Also in this case we have three possibilities (Fig. 3a):

(1) N will be an ESS when $C/m \geq (3 - e)/2(1+k)$.
(2) S will be an ESS when $C/m \leq 3/2(1+k)$.
(3) There will be a unstable equilibrium $0 < \hat{r} < 1$ when both N and S are ESSs.

In other words, no stable coexistence is possible since S will be an ESS above the equilibrium frequency $\hat{r}$, and N will be an ESS below the equilibrium (Fig. 3b).

In summary, this subgame has two interesting aspects. The first is that nondefensive plants do not benefit from S because herbivores are assumed to be able to make a distinction between N and S types. The second is that the defensive plants will benefit from each other if feeding aversion is maintained by a cue that the neighbors share. As a consequence, the fitness of defensive plants will increase as the frequency of S approaches 1, while the fitness of nondefensive plants remains constant (Fig. 3b). This situation corresponds to "synergistic selection" discussed by Guilford and Cuthill (1991) in the evolution of aposematism, and by Tuomi and Augner (1993) in relation to plant defenses.

Costs of Defenses

When allowing D and S to play against each other, a plant adopting D will obtain the same payoffs as when playing against N (Fig. 2). A plant adopting S will obtain the same payoffs when the neighbor is D or S. When r is the population frequency of S, we get the following fitnesses

$$W_D = W_0 - C - 1/2\ m(1+er)$$

$$W_S = W_0 - C(1 + k) - 1/2\ m$$

where W_D declines as a function of er, while W_S is independent of both e and r. There are four qualitatively different situations:

(1) S will be a pure ESS either if $k < 0$, or if $k = 0$ and $e > 0$.
(2) S and D are equally fit if $k = 0$ and $e = 0$.
(3) D will be a pure ESS if $k > 0$ and $C/m \geq e/2k$.
(4) There is an unstable equilibrium $0 < \hat{r} < 1$ if $k > 0$ and $C/m < e/2k$.

Consequently, the costs of defenses play a prominent role here. If S is expensive relative to D, lethal defense is most likely to be selected for. Fig. 4a indicates the last two situations obtained for $k > 0$. The unstable equilibrium

$$\hat{r} = 2Ck/em$$

represents the point where the fitness curves intersect each other (i.e., $W_S = W_D$, Fig. 4b). When $\hat{r}$ moves closer to zero, the situation becomes more favorable to S. This will happen when k becomes smaller, or when e and m become larger. The reverse changes will accordingly favor D. We interpret this so that lethal defenses may be more economical against rare herbivores (m small) and sedentary herbivores (e small), while defenses inducing conditional feeding aversion should be more effective against common herbivores that are mobile and move from a plant to another (both m and e high).

Game Dynamics

When the above subgames are combined, there arises a number of possible dynamic solutions for the game where all three strategies are taken account. We have chosen to describe some basic situations for $e = 0$ (Fig. 5) and $e = 1$ (Fig. 6) when $k \geq 0$. The population frequencies of N, D and S are q, p and r respectively ($q + p + r = 1$), and the equilibria discussed above are denoted by $P = (\hat{q}, \hat{p}, 0)$ (the stable equilibrium of the N-D subgame), $Q = (\hat{q}, 0, \hat{r})$ (the unstable equilibrium of the N-S subgame), and $R = (0, \hat{p}, \hat{r})$ (the unstable equilibrium of the D-S subgame).

When $e = 0$, there are no neighbor effects and hence no frequency dependency. Now the game dynamics can be directly derived from fitness ranks, as

$$W_N = W_0 - 2m$$

$$W_S = W_0 - C(1 + k) - 1/2m$$

$$W_S = W_0 - C(1 + k) - 1/2m$$

When C is sufficiently low relative to m, selection will favor a defensive strategy, either S or D. For $k > 0$, selection will always favor D over S. On the other hand, when C is high relative to m, N will always be an ESS (Fig. 5).

When $e = 1$, the situation is a little bit more complicated as now fitnesses depend on population composition. Using the standard methods (Taylor and Jonker 1978, Zeeman 1981, Bomze 1983, Hofbauer and Sigmund 1988), we can analyze the local stability of equilibria (Fig. 6). Also in this case, selection will favor a defensive strategy if C is sufficiently low relative to m. However, now S will have an advantage over D when $k = 0$. For a higher values of k (e.g., $k = 0.4$ or 1.0, Fig. 6), the equilibria Q and R appear with the consequence that, depending on the initial state, the population may evolve toward S, or alternatively to D (or P). Finally, if C is high relative to m, N will have an advantage over both D and S.

This analysis confirms the earlier result of the D-S subgame that high m and e favor S (Fig. 6), and low e favors D (Fig. 5). However, the suggestion that low m favors D over S, does not imply that D is an ESS. When m is low enough, N can invade and either out-compete both defensive types or establish stable polymorphism with D.

CONCLUSIONS

Plant defenses have both direct effects on plant fitness and indirect effects that arise from interactions between neighboring plants. Although direct fitness effects obviously are the primary forces of selection, neighbor effects may also shape some basic aspects of plant defenses. This is especially so if fitness differences due to the direct effects of plant phenotypes are marginal.

We analyzed two types of neighbor effects when herbivores are allowed to move from one plant to another:

(1) Palatable plants can benefit from the defenses of their unpalatable neighbors. Defenses can, for instance, increase mortality among herbivores or induce unconditional feeding aversion with the consequence that the herbivory load of palatable plants is low in mixed trait groups. Associational protection can maintain stable polymorphism if palatable plants gain more in fitness than unpalatable plants when growing close to an unpalatable neighbor. We thus expect that associational protection counteracts the evolution of plant defenses that do not provide herbivores any signal over which to generalize.

(2) Unpalatable plants can selectively benefit other such plants if they share a trait that herbivores can use as a basis of their food intake and their choice for host plants. In that case, palatable and unpalatable plants that do not share the signal do not gain associational protection. Defenses associated with a signal are most effective against abundant and mobile herbivores, and their advantage is greatest when they are common.

Consequently, we expect that there should be some correspondence between the design of plant defenses and the sensory and learning mechanisms of herbivores. Undoubtedly, thorns and spines are defensive characters that can function oth as protective weapons and as potential signals. If this also holds for chemical defenses, neighbor effects might have a undamental importance in shaping plant defenses. This may well be so as, because according to Provenza *et al.* (1992), mammalian herbivores learn to select food items through two interrelated systems. The affective system integrates the taste f food and its post-ingestive consequences, while the cognitive system integrates the odor and sight of food and its taste. Because they have to sample plants in order to adjust food intake to avoid intoxication (Provenza *et al.* 1992), the expected herbivory load of a plant is likely to depend both on its own defenses and on the defensive status of its neighbors.

SUMMARY

In order to be evolutionary stable, plant defenses should not benefit palatable neighbors too much. If they do, palatable plants can invade and eventually either out-compete unpalatable plants or establish polymorphic populations. Therefore, we expect that plant defenses that can provide signals for herbivores could be superior to defenses that have no value as signals. A game theoretical analysis is presented in order to explore the evolution of plant defenses when plant fitness depends on the defensive status of the neighbor.

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DEFENSE THEORIES AND BIRCH RESISTANCE

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INTRODUCTION

Different tree species in northern temperate forests clearly vary in their resistance to browsing mammals (Gill 1993 a,b). In addition to variation at the species level, there are also clear indications of genetic differences in herbivore resistance within species. Such variation in resistance has been explained by the duration and intensity of past exposure to herbivores and pathogens (Leppik 1970, Bryant *et al.* 1989). It is believed that the centers of genetic diversity for plant species should be the best places in which to find resistance to their common herbivores and diseases (Vavilov 1920). All of these kinds of variation have important economic and ecological implications for plantation forestry and forest industries. For example, hybridization could be used to elevate the resistance of otherwise high quality but susceptible tree species or individuals (see Rousi 1990).

It has been postulated that there is a trade-off between growth and plant defense (Rhoades 1979, Herms and Mattson 1992). That being the case, practical forestry might not be interested in slow growing genotypes, although they might be more safe to use. Trees are generally attacked by a myriad of herbivores and if there is a trade-off between resistance and growth to each of those, then generally resistant genotypes should be very slow growing. Ecological trade-offs in the form of negative correlations in resistance to different types of herbivores are also possible. Conventional wisdom suggests that trees which have to compete intensely for nutrients and light, can probably be resistant only to restricted number of herbivores.

In addition to genetic components, environment may also modify plant resistance. If carbon is the limiting factor due to shading, or to large amounts of available nutrients and water, then defensive secondary metabolite level may decline so long as the plant still has strong sinks capable of usurping the available photosynthates and nutrients (Mattson 1980, Bryant *et al.* 1983).

To test the effect of environment and birch genotype on hare resistance, we carried out several cafeteria experiments using winter dormant shoots of small birch seedlings and plantlets grown in Punkaharju, East Finland.

RESULTS AND DISCUSSION

Birch Species and Genotypes Vary In Their Hare Resistance

Birch species vary strongly in their hare resistance. According to many studies, Japanese white birches, *B. platyphylla* and *B. ermanii*, are both very resistant. Some other species, such as *B. alleghaniensis* (Fig. 1) and *B. papyrifera*, are among the most susceptible species, whereas *B. pendula* is somewhat intermediate in resistance. Interestingly, experiments made in Alaska, Finland, and Japan all give essentially the same results (Chiba and Nagata 1968, Bryant *et al.* 1989, Rousi *et al.* 1989, 1996a). This most probably indicates the wide adaptability of birch: there are no drastic changes in resistance even if birches are grown as exotics. In addition, it shows that the Japanese mountain hare, *Lepus ainu*, the Finnish mountain hare, *L. timidius*, and the snowshoe hare, *L. americanus*, make their food selection on the same bases.

Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

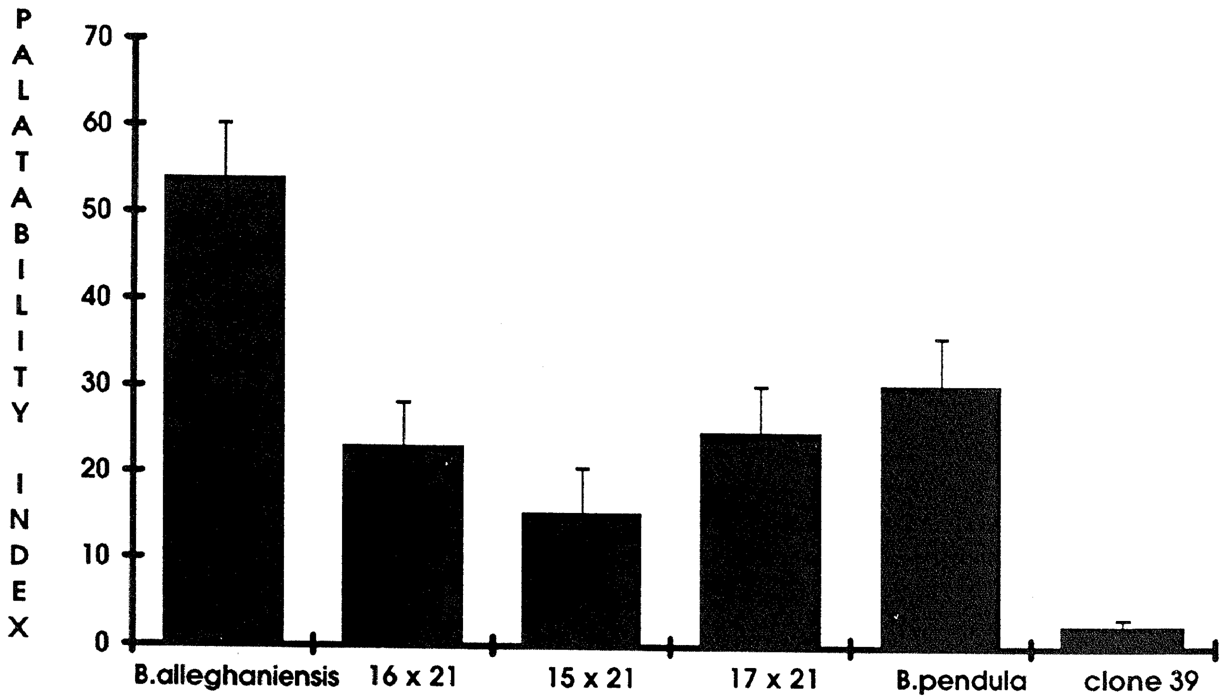


Figure 1.—Hare palatability of *B. alleghaniensis*, three backcrosses of *B. pendula* and *platyphylla*, local forest origin of *B. pendula* and one micropropagated clone of *B. pendula*. Clone 39 consists of micropropagated plantlets of *B. pendula*. Mother trees (15,16 and 17) are hybrids between the two species and full sibs. Father trees (18,19 and 21) are South Finnish plus trees. Mean ±S.E. Palatability index is the result from cafeteria experiments (two nights, six hares. For details see Rousi *et al.* 1991). Mean ±S.E.

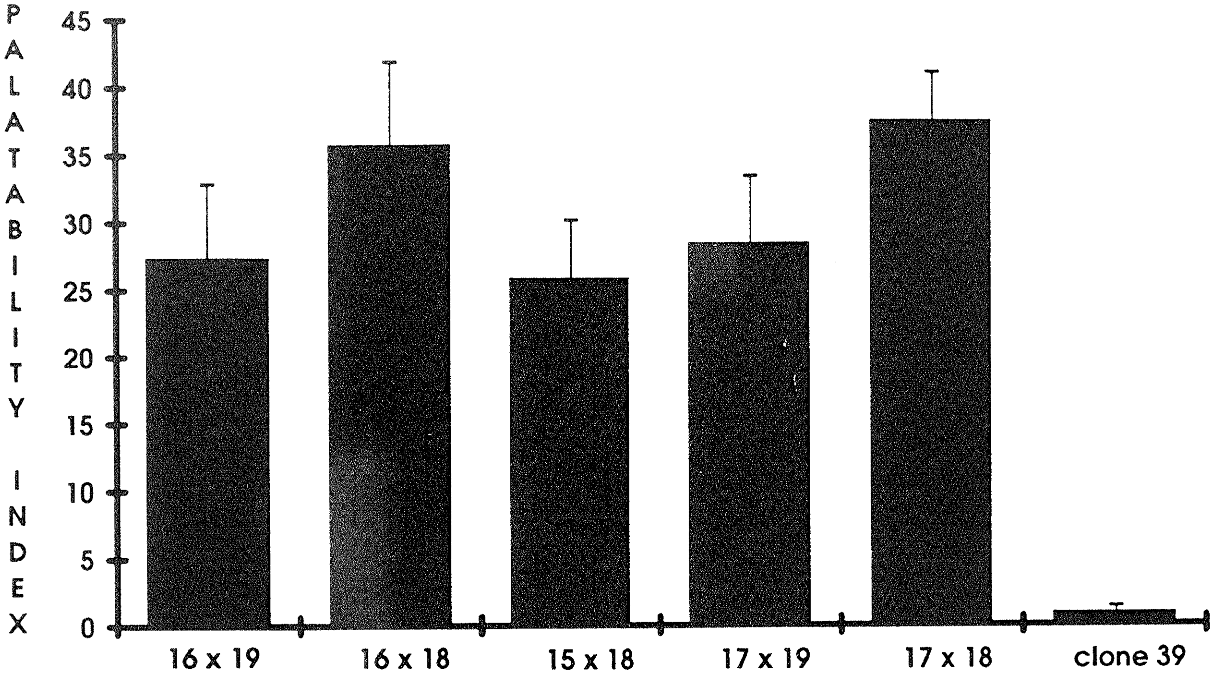
Hybridization has been shown to be a very promising method to increase e.g., vole resistance of *Larix* species (Chiba *et al.* 1982). In addition, hybrids between susceptible and resistant birch species have been intermediate in their resistance to hares (Chiba and Nagata 1969). In order to make preliminary tests of the inheritance of resistance, we tested the resistance of a backcross between Japanese and European birch [a hybrid between a Japanese white birch (very resistant) and European white birch (intermediate) was crossed with several South Finnish birch genotypes].

The Japanese white birch used as a parent tree in our crossings was always of the same genotype. Generally, resistance of hybrids was at the same level as was that of local forest origins of European white birch (Figs. 1 and 2). Hybrids between Japanese and Fennoscandian white birches have shown superior juvenile growth as reported by Johnsson (1966). But the first year growth of our backcrossed seedlings was the same as that of European white birch (see Figs. 3 and 4). Obviously the genetic base of our crossings was too limited for any far reaching conclusions.

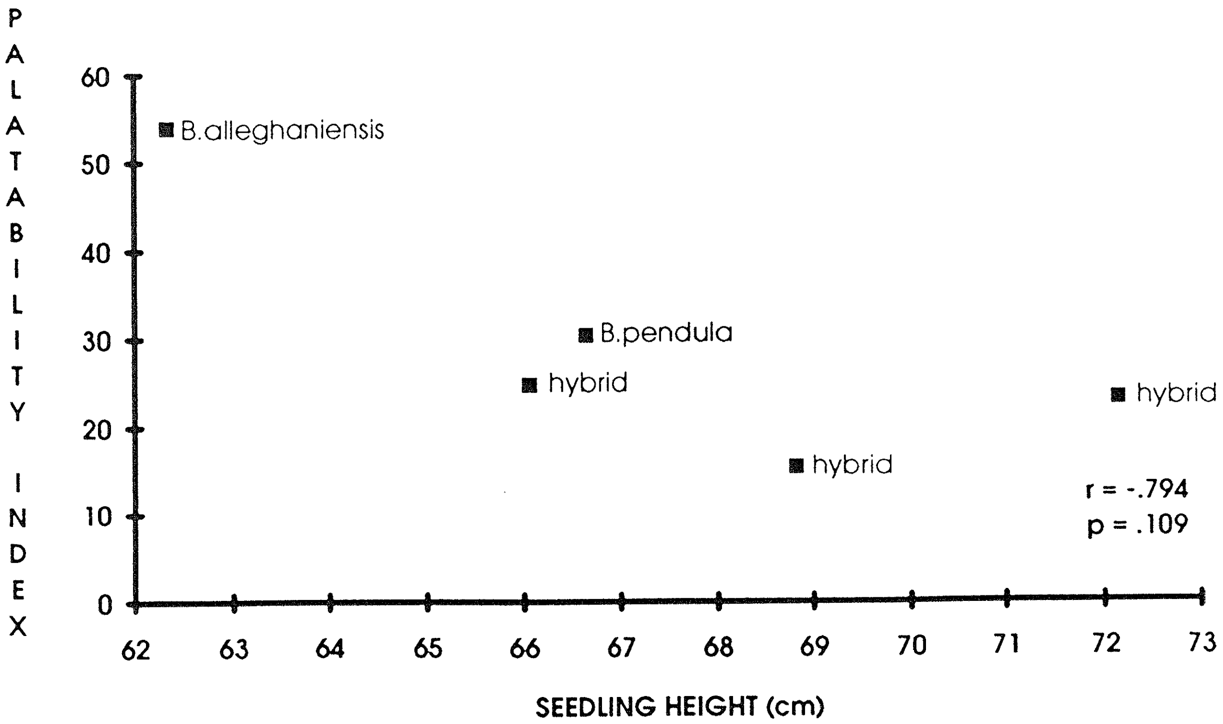
Birch species and origins derived largely from Pleistocene refugia such as Alaska, Siberia or Japan, are supposed to be more resistant than ecologically similar birches derived from nonrefugia (Bryant *et al.* 1989, Rousi 1988). It is difficult to test this hypothesis because it is difficult to verify whether particular birch populations came from authentic "refugia" or not. Moreover, no one knows the kind of selection pressure such populations have faced since release from their refugia. The most resistant and the most susceptible species in our experiments are from Japan, a country that was minimally glaciated during the Pleistocene. Moreover, *B. pendula* origins from Siberia (not glaciated) and Finland (heavily glaciated) are equally resistant to hares (Rousi *et al.* 1996a). Experiments with micropropagated plantlets have revealed very large variation in hare resistance among different *B. pendula* genotypes growing in South Finland. Clone 39 especially has proved to be highly resistant under various experimental conditions (Fig. 5, Rousi *et al.* 1996b).

Fast Growing Birches Are Also Resistant

We tested the resistance of persistence-adapted yellow birch, *B. alleghaniensis*, against fast growing, short-lived white birches in order to find out whether inherent growth strategy is related to hare resistance of the species. The first year (greenhouse) growth of yellow birch was less (ANOVA, Bonferroni t-test, $p<.05$) than that of most of the white birches.



e 2.—Hare palatability of backcrosses between *B. pendula* and *B. platyphylla*. Mother trees (15,16 and 17) are hybrids between the two species and full sibs. Father trees (18,19 and 21) are South Finnish plus trees. For more details see Figure 1. Mean ±S.E.



'e 3.—Relationship between seedling growth and hare palatability. Material is the same as in Figure 1- H is for hybrid (backcross). Clone 39 is omitted because plantlets were ca. 2-yr-old; seedlings were 1-yr-old.

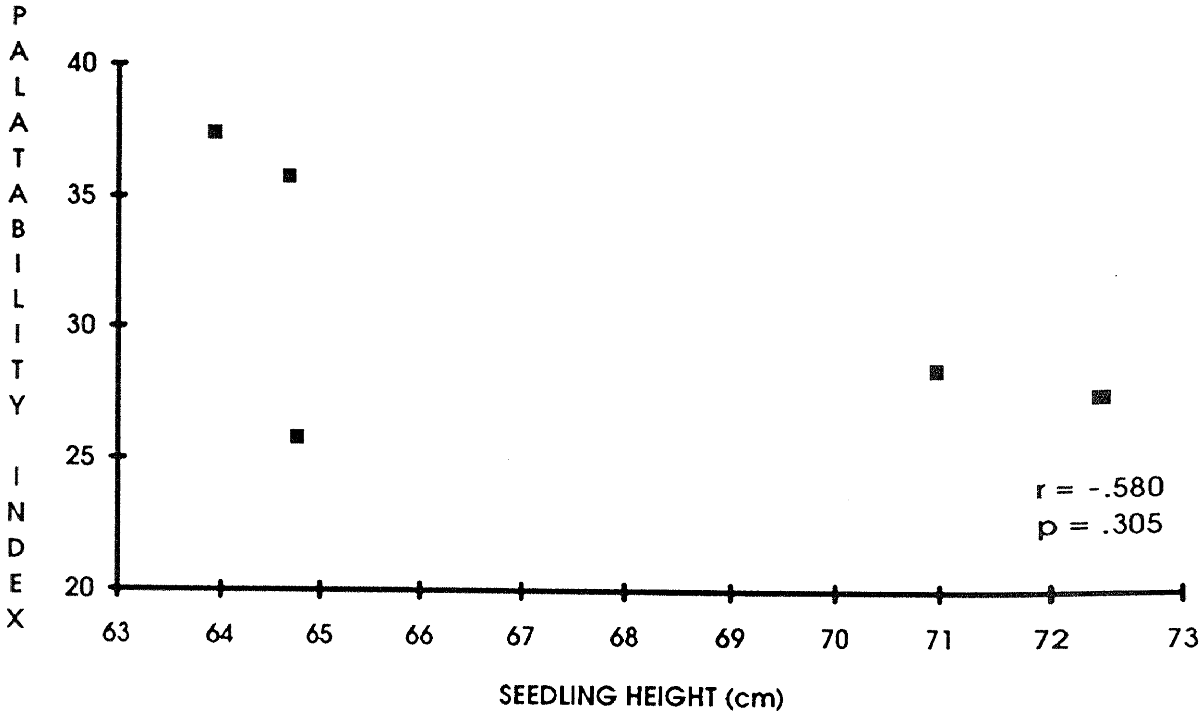


Figure 4.—Relationship between seedling growth and hare resistance. Material the same as in Figure 2. Clone 39 is omitted because plantlets were ca. 2-yr-old; seedlings were 1-yr-old.

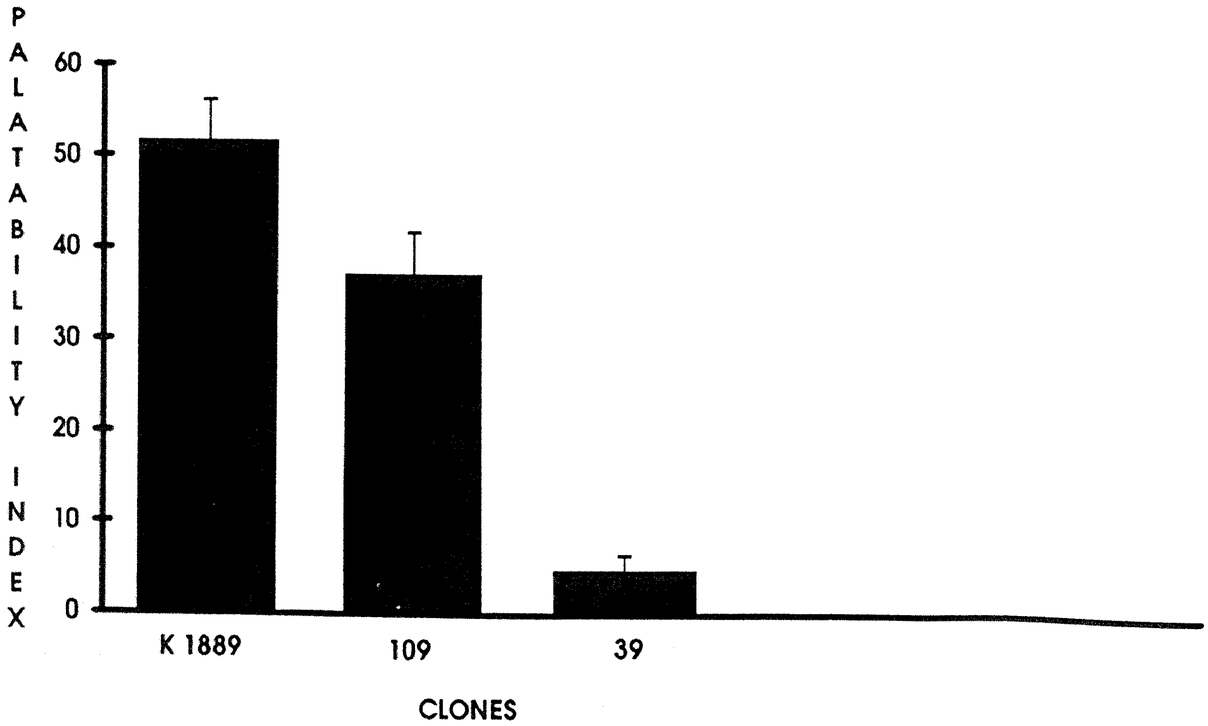


Figure 5.—Hare resistance of three micropropagated genotypes of *B. pendula*. Clone 109 stands for f. *bircalensis* (lobed leaves). Cafeteria experiments as in Figure1. Means ±S.E.

However, hares showed strong preference for it. The general trend in our experiments has been that species and genotypes resistant to hares showed better than average growth, although the relationship was not statistically significant (Figs. 3 and 4). These results support our earlier findings which indicate either a positive relationship between first year growth of birch seedlings and their hare resistance, or no relationship at all (Rousi *et al.* 1989, 1990, 1991, 1996b).

European white birch genotypes have not shown any relationship between their growth and resistance to hares, *Microtus* voles, Curculionidae weevils, or *Melampsoridium* leaf rust (Poteri and Rousi 1996, Rousi *et al.* 1996b). These experiments did not support any ecological trade-off either: resistance to any one pest was not related to resistance to others. However, vole and hare resistance in some experiments have shown a positive relationship (Chiba and Nagata 1969), and have also tended to do so in our own experiments (Rousi *et al.* 1989, 1996b). Interestingly, clone 39 has shown generally high resistance to both biotic and abiotic threats (Fig. 5, Rousi *et al.* 1996b). The same clone also seems to be exceptionally resistant to ozone injury (clone 5-M, in Pääkkönen *et al.* 1993), whereas the generally herbivore susceptible clone 36 is similarly very susceptible to ozone damage (2-M, in Pääkkönen *et al.* 1993).

Environment May Sometimes Affect Birch Resistance

Our previous experiments with various birch species (8 birch species) and origins have indicated, as expected, that growth environment (shading or fertilization) does not have an effect on low resistance species (slow or fast growing), such as *B. papyrifera, pubescens, maximowicziana, schmidtii* or *grossa*. In high resistance species, fertilization increased the palatability of two species, *B. platyphylla* and *B. ermanii*, but did not affect another, *B. resinifera*. Shading had no clear effect on any of the tested species (Rousi *et al.* 1996a).

European and Japanese white birches are closely related (Dugle 1966). However, fertilization did not diminish the hare resistance of European white birch (Rousi *et al.* 1991, 1993), but lowered that of Japanese white birch (Rousi *et al.* 1996a). On the other hand, shading tends to increase hare palatability of European white birch (Figs. 6 and 7), but its effect on Japanese white birch is negligible (Rousi *et al.* 1996a). The effect of shading on European white birch is, however, strongly dependent on genotype and site fertility. There were no statistical differences in resistance among three families that we tested in low fertility soils (Fig. 6). But, if seedlings are grown in high fertility soil, resistance was significantly different in two out of three families (ANOVA, Bonferroni t-test, $p<.05$) (Figs. 6 and 7). The importance of background genetic resistance in such comparisons is clearly seen in Fig. 6, where unshaded seedlings of the susceptible family are more palatable to hares than are shaded seedlings of the two other seed lots.

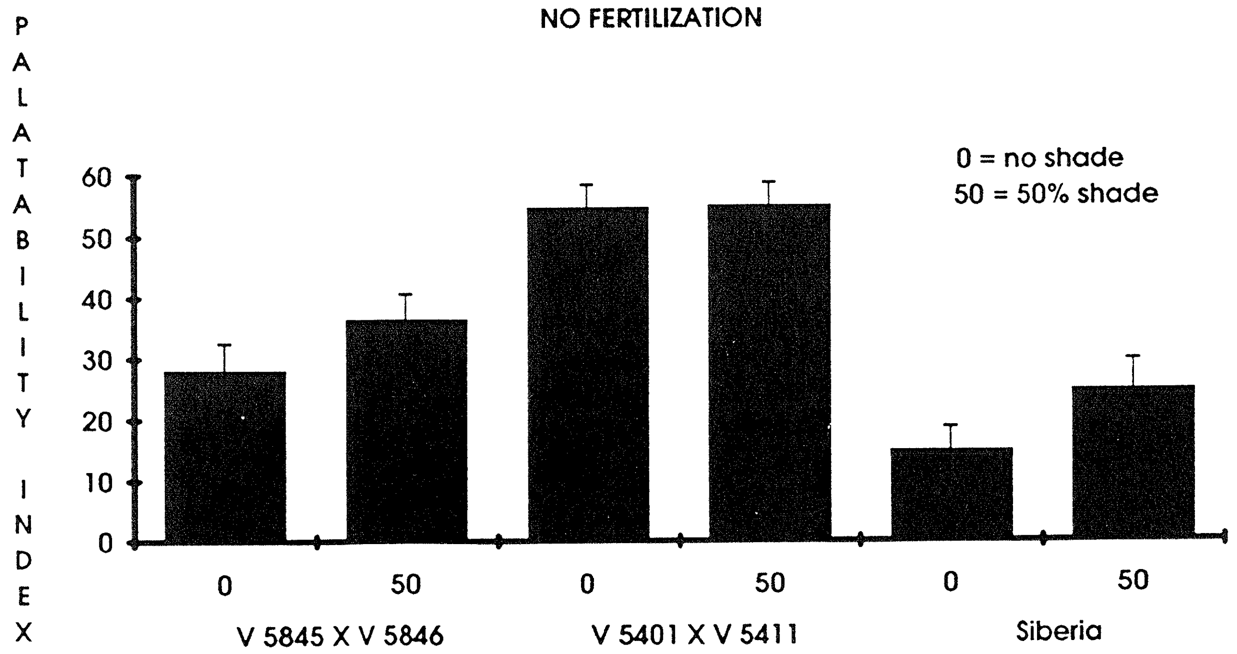


Figure 6.—Hare resistance of two *B. pendula* F_2- families and one seedlot from Siberia in two shading treatments (0% and 50% shading). Seedlings were grown in very low fertility growth medium and obtained a minimum fertilization (For details see Rousi *et al.* 1996a). Cafeteria experiments as in Figure 1. Means ±S.E.

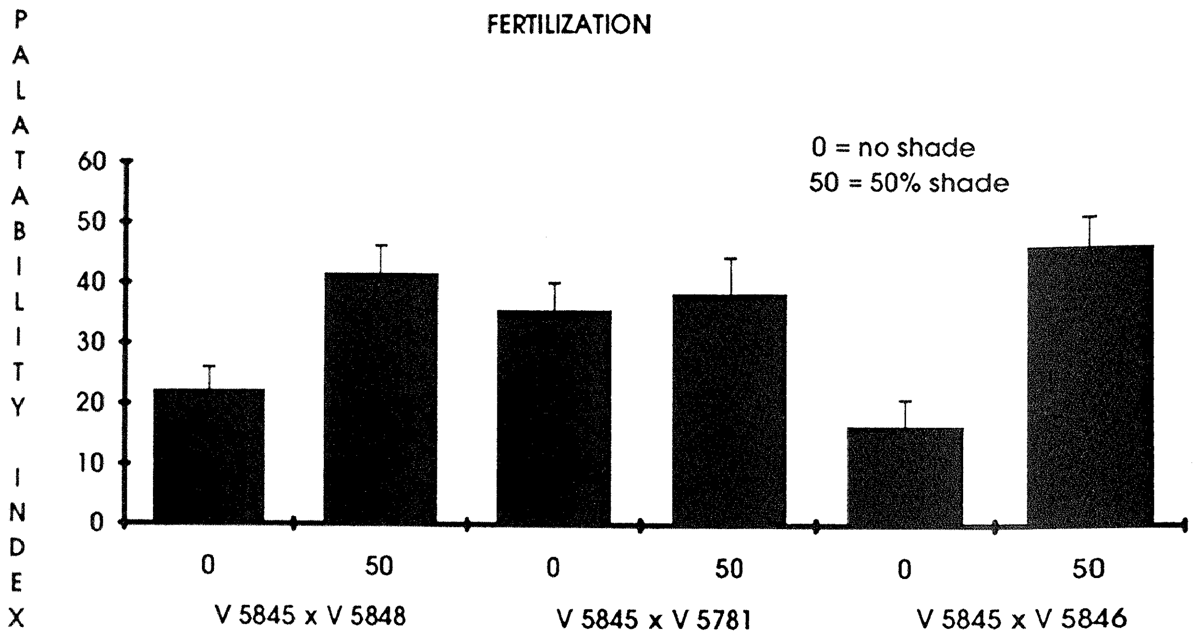


Figure 7.—Hare resistance of three *B. pendula* F_2 families in two shade treatments. Seedlings were grown in nursery peat and obtained optimum fertilization (For details see Rousi *et al.* 1996a). Cafeteria experiments as in Figure 1. Mean ± S.E.

SUMMARY

We conclude that prevailing defense theories, (e.g., Carbon-Nutrient Balance, Growth-Differentiation Balance, etc.) may not be fully applicable to birch. The reaction of birch species, origins, and genotypes to variable environments produces variable results, which depend on plant genotype, environment and the particular mechanism of resistance to each pest type. Consequently, generalized conclusions about birch resistance should be made with great caution.

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A REDOX-BASED MECHANISM BY WHICH ENVIRONMENTAL STRESSES ELICIT CHANGES IN PLANT DEFENSIVE CHEMISTRY

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INTRODUCTION

It is now clear that most, if not all, lifeforms employ chemicals in communicating with their biotic and abiotic environments. Higher plants and animals, including insects and humans, may even use many of the same compounds (Rodriquez and Levin 1976, Norris and Liu 1992, Raina *et al.* 1992). Quinol-quinone redox couples, e.g., p-hydroquinone / p-benzoquinone (Fig. 1), are prime examples of such common messengers. Hundreds of ions, free radicals (Packer and Glazer 1990) and molecules have been shown to be messengers. Alkaloids, flavonoids and terpenoids are families of compounds which are especially important as messengers (Norris 1986).

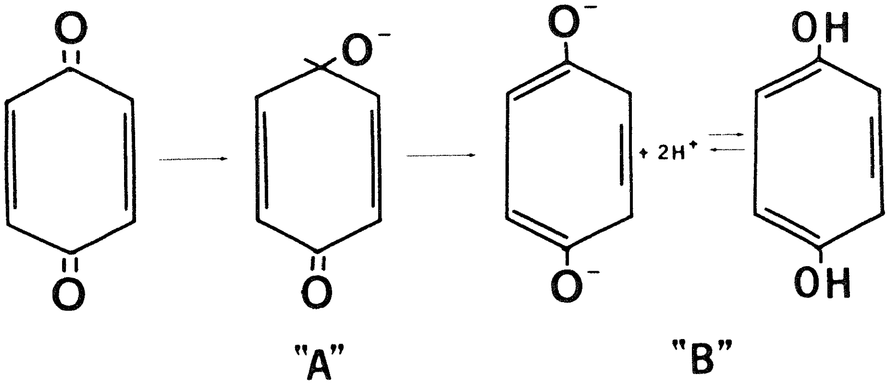

Figure 1.—The classical redox couple in physical chemistry, p-hydroquinone / p-benzoquinone.

The development of knowledge about the chemical senses has frequently been quite disconnected among many scientific disciplines. As a result, limited progress has been made in finding unifying principles. However, for several decades, I have developed hypotheses pertaining to such unifying principles and tested them experimentally (Norris 1994). The results of our and others' efforts have allowed me to propose that a common code for chemical communication exists among lifeforms, and them and their abiotic environments. The proposed name for this code is the Environmental Energy Exchange (EEE) Code (Norris 1981, 1986, 1988, 1994; Neupane and Norris 1992; Norris and Liu 1992).

In this paper, the essential characteristics of this EEE code, including its underlying sulfhydryl (thiol, -SH) / disulfide (-S-S-) -dependent oxidative-reductive, energy-transducing mechanism, are applied to the interpretation of how environmental stresses elicit changes in plant defensive chemistry.

Stress Alteration Of Phytochemical Messages

The chemistry by which plants "communicate" with their herbivores has been classified as either constitutive (i.e., pre-stress) or inducible (i.e., post-stress). Plant pathologists and plant physiologists created this classification to

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accommodate findings related to microbial interactions with plants (Bell 1981). Constitutive defensive phytochemistry is that expressed in the plant by its genome in the supposed absence of specific stress from the environment; whereas, inducible defensive phytochemistry is that expressed in response to a specific stress caused by some factor (e.g., microbial infection) in the plant's environment. Although these interpretations were developed for plant-microbe interactions, subsequent studies, especially by entomologists and chemical ecologists, have shown that much of the involved phytochemistry, and some of the developed concepts, may prove useful to understanding messenger phytochemicals in herbivore-plant communications (Kogan and Paxton 1983). Our studies of such phytochemistry indicate that the major differences between pre- and post-stress chemistry are largely quantitative (e.g., Neupane and Norris 1991a, Markovic *et al.* 1993). Thus, a plant usually responds to environmental stresses by making more, or less, of individual compounds in a given (genome-determined) array of secondary metabolites. These chemicals have sometimes been termed 'phytoalexins' by plant pathologists and physiologists.

Unifying Mechanisms Of Elicitation

A wide diversity of biotic and abiotic entities have proven effective as exo-elicitors (external to the plant cell) in the inducible alteration of plant defensive chemistry against environmental stresses (Sequeira 1983). However, plant pathologists and physiologists have especially focused on fragments of microbial-pathogen or plant-cell walls as exo-elicitors. β-*glucans* from plant-cell walls apparently play major elicitory roles (Ryan 1983, Darvill and Albersheim 1984, Templeton and Lamb 1988, Dixon and Lamb 1990). Heavy metals, including mercury, and sulfhydryl reagents are other proven exo-elicitors (Stossel 1984). Although the diversity of exo-elicitors might imply that a common mechanism of exo-elicitation is unlikely, the suggested involvements of sulfhydryls in the process led Sequeira (1983) to hypothesize their importance in such inductions. Other reports of elicitation agents reacting with sulfhydryls in phytoalexin biosynthesis (Stossel 1984) support the underlying importance of sulfhydryls in elicitation processes. More recent studies (Neupane and Norris (1990, 1991a) and Liu *et al.* (1992, 1993)) showed that several classical compounds for detecting (i.e., reacting with) sulfhydryls also alter the biosynthesis of isoflavanoid phytoalexins in soybeans, *Glycine max* (L.) Merrill. These induced phytoalexins also alter the defense of these plants to some insect herbivores (Sharma and Norris 1991).

It now seems clear that the elicitory mechanism in plants involves directly, or indirectly, the oxidative-reductive (redox) chemistry of sulfhydryl (-SH) / disulfide (-S-S-) interactions (Fig. 2). First, reagents which react specifically with sulfhydryls are effective elicitors of stress-alterable defensive phytochemistry (Stossel 1984; Sequeira 1983; Neupane and Norris 1990, 1991a; Liu *et al.* 1992, 1993, 1994; Haanstad and Norris 1992; Norris and Liu 1992; Norris 1994). Second, sulfhydryl proteins in the plasma membrane which surrounds each plant cell have been shown to react with elicitory sulfhydryl reagents (e.g., p-chloromercuriphenyl sulfonic acid, PMBS) when applied in vivo to the intact plant (Liu *et al.* 1992, 1993, 1994). Such reaction, or interaction, between a sulfhydryl reagent and a sulfhydryl protein (i.e., receptor) at the plasma membrane level may trigger significant alterations in the defensive chemistry of elicited plant cells (Liu *et al.* 1992, 1994; Norris 1994). Third, the essential mechanism by which sulfhydryl reagents and other proven elicitory entities, both biotic and abiotic, function in altering defensive phytochemistry is apparently oxidative-reductive (Neupane and Norris 1991b, 1992; Markovic *et al.* 1993; Norris and Liu 1992). The classical antioxidants, L-ascorbic acid (vitamin C) and α-tocopherol (vitamin E), have proven to be effective elicitors when applied to whole plants (Neupane and Norris 1991b, 1992). Fourth, Neupane and Norris (1992) showed that L-ascorbic acid and herbivory by *Trichoplusia ni* (Hubner) elicited analogous defensive responses in soybeans. Thus, the involvement of a common mechanism of elicitation, i.e., oxidative-reductive, is supported. Our current interpretation is that herbivory elicits inducible defensive phytochemistry through triggering oxidation / reduction-dependent reactions which are common with those caused in plant tissues by a wide variety of other environmental stresses.

-SH / -S-S-

Figure 2.—The sulfur-based redox couple, sulfhydryl (thiol, -SH) / disulfide (-S-S-), in which the addition of reducing agent favors the formation of thiols; whereas, the addition of oxidizing agent favors the reverse, i.e., formation of disulfides. This redox couple in proteins is considered the functional interface by which energy is exchanged as information between living cells and their environments.

METHODS

Antioxidant Alteration Of Ash Antixenosis To *Malacosoma disstria*

Because sulfhydryl groups may readily participate in redox reactions in living cells (Morton 1965), our studies evaluated a natural antioxidant (reducing agent), α-tocopherol (vitamin E), as an elicitor of defensive phytochemistry. Alpha-tocopherol was tested in a basal trunk band on green ash trees to alter their antixenosis to forest tent caterpillar larvae, *Malacosoma disstria* (Hubner). The trees were 4.6m tall 'Summit' green ash, *Fraxinus pennsylvanica* var. *subintegerrinia* (Vahl) Fernald, and had a mean trunk diameter of 5.1 cm at 1 m above the soil surface. The basal trunk of each tree received α-tocopherol, a proven elicitor (Neupane and Norris 1991b), as a band application at the dosages of 25.0 or 50.0 IU / ml in mineral oil. An international unit (IU) equals 1 mg of all-*rac*-α-tocopheryl acetate (U.S. Pharmacopaea 1980). Sixty ml of either concentration of α-tocopherol in mineral oil were placed on a $120cm^2$ bandage; the control trees received only 60 ml of mineral oil.

Insect Bioassays

Leaves for bioassay were removed from three distinct trees per treatment or control at each of several intervals after treatment, and two-choice feeding assays were conducted with 1.5-cm-diam disks cut with a No. 8 cork borer from such leaves and with third-instar forest tent caterpillars. The insect's feeding option thus was between a comparable leaf disk from an elicited versus a solvent-control tree. The quantitation of insect feeding was detailed by Markovic *et al.* (1993).

Chemical Analyses

Leaves for chemical analyses were collected from three distinct ash trees for each elicitation dosage and the solvent control, and for 8 and 16 days after treatment. Three compound leaves from each tree were immediately put individually into a glass jar containing 80% methanol, and then stored in darkness at -20°C until chemical analysis. Procedures used to extract chemicals from ash leaves; hydrolyze extracted chemicals; and analyze the chemicals by high performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) were as detailed by Markovic *et al.* (1993).

RESULTS

Antioxidant Altered Foliar Antixenosis In Ash

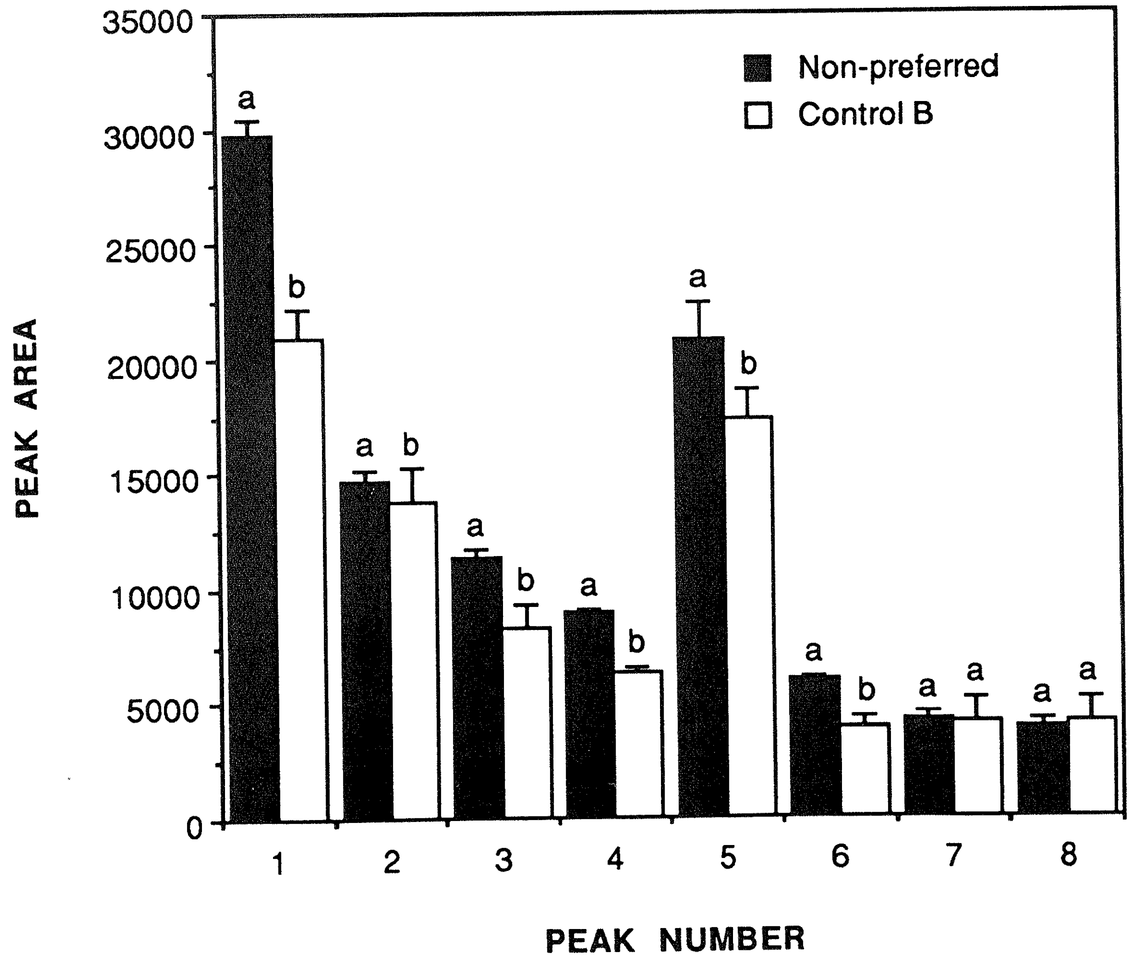
Forest tent caterpillar herbivory was altered on leaf disks from green ash trees elicited with either of the two dosages of α-tocopherol as compared to disks from solvent-treated ash trees (Table 1). High performance thin layer chromatography (HPTLC) revealed distinct differences in both the non-hydrolyzed and hydrolyzed portions of the ethyl acetate extractables from elicited versus control ash trees (Markovic *et al.* 1993). Reduced insect preference for foliage due to tocopherol elicitation was accompanied by an increased mean total HPLC-resolved peak area of ethyl acetate extractables from the non-hydrolyzed leaf sample as compared to control foliage. Whereas, increased insect preference for foliage due to elicitation was accompanied by a decreased total HPLC-resolved peak area of such extractables as compared to control. HPLC of the above non-hydrolyzed, ethyl acetate extractables showed mainly quantitative, rather than qualitative, differences between α-tocopherol-elicited versus solvent-treated controls. The differences were especially evident in five major, and three lesser, peaks; these eight peaks thus were chosen for a more detailed comparison between the non-hydrolyzed fraction of the ethyl acetate extractables from the two α-tocopherol treatments and solvent-treated controls at two times (8 and 16 days) after elicitation.

At 8 days after elicitation, the foliage from trees receiving 25 IU / ml contained significantly more of HPLC peaks 1-6, but not of peaks 7 or 8, than did leaves from solvent-control trees (Fig. 3). Foliage from these treated trees also was significantly less preferred than that from the control trees (Table 1). Conversely, at 16 days after elicitation, foliage from trees that received either 25 or 50 IU / ml was preferred over that from the solvent-control ones (Table 1). The ethyl acetate extractables at 16 days after elicitation from preferred foliage from trees that received 25 or 50 IU / ml had a smaller average total HPLC-resolved peak area than did those from the leaves of solvent-control trees (Fig. 4). The mean total HPLC-resolved peak area was also significantly different between the foliage collected from the solvent-control trees at 8 and 16 days after elicitation. Thus, time in the growing season also affected the chemical composition of the trees.

Table 1.—Mean ±S.E. area eaten by the forest tent caterpillar in leaf disks from tocopherol-elicited versus control-elicited ash trees.

Behavioral response	Area eaten (cm^2)[b]		
	Days[a]	Treatment	Control
Nonpreferred[c]	8	0.18 ± 0.04**[e]	0.41 ± 0.04
Preferred 1[c]	16	0.49 ± 0.07*	0.31 ± 0.06
Preferred 2[d]	16	0.46 ± 0.05**	0.16 ± 0.04

[a] Days after elicitation.
[b] From Markovic *et al.* (1993).
[c] Vitamin E dose was 25.0 IU / ml.
[d] Vitamin E dose was 50.0 IU / ml.
[e] Means followed by * or ** are significantly different from their control at $p < 0.05$ and $p < 0.01$, respectively.



gure 3.—Comparative mean areas of the indicated peaks in the standardized HPLC analysis of the nonhydrolyzed fraction of the ethyl acetate extractables from ash foliage. Data are for nonpreferred foliage (elicited with 25 IU / ml) and control B foliage (elicited only with the solvent). Leaves were collected at 8 days after elicitation. Peak areas not followed by the same letter are significantly different at $p < 0.05$.

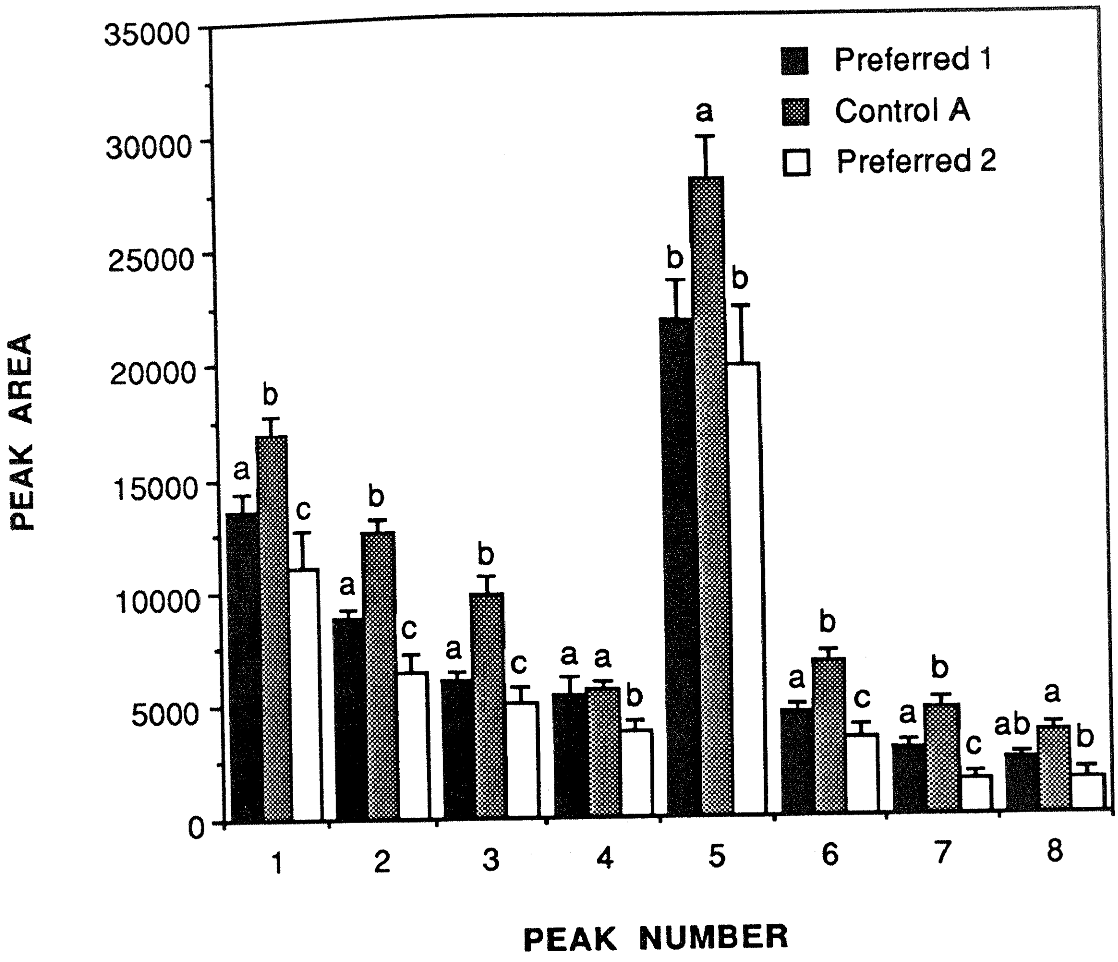


Figure 4.—Comparative mean areas of the indicated peaks in the HPLC analysis of the nonhydrolyzed fraction of ethyl acetate extractables from ash foliage. Data are for preferred 1 (elicited with 25 IU / ml), preferred 2 (elicited with 50 IU / ml and control A (elicited only with solvent) foliage. Leaves were removed from these trees at 16 days after elicitation. Peak areas not followed by the same letter are significantly different at $p < 0.05$.

DISCUSSION

Antioxidant Altered Foliar Antixenosis

The findings support the interpretation that preference / non-preference decisions by *M. disstria* larvae regarding α-tocopherol-elicited versus solvent (control)-elicited green ash foliage are probably attributable to quantitative changes in several compounds, not just in one chemical. Elicitation by α-tocopherol alters the quantitative contributions of at least several chemicals in the mixture of secondary metabolites. The effects are very dynamic, e.g., changing from increased to decreased antixenosis between 8 and 16 days after elicitation with 25 IU / ml. These findings seem compatible with the knowledge that survivor plants systematically switch their major allocation of nutrient and energy resources between primary growth and secondary growth-differentiation including defensive chemistry. Such dynamic switching seems reasonable because survivor plants must grow, but they must also defend themselves from potentially lethal environmental stresses.

Sulfhydryl-Disulfide Mechanisms In Plant And Animal Perceptions

Most, if not all, common chemical messengers between insects and plants, and between them and their environments (Rodriguez and Levin 1976, Neupane and Norris 1992, Norris and Liu 1992, Raina *et al.* 1992) are both elicited and perceived by sulfhydryl / disulfide (-SH / -S-S-) -dependent mechanisms (Neupane and Norris 1992, Liu *et al.* 1992, Norris 1994). The energy-transduction mechanism in chemical communications by both animals and plants is sulfur dependent. The initial studies in this area showed that 1,4-naphthoquinones serve as repellents and deterrents to *Periplaneta americana* L., the American cockroach, and *Scolytus multistriatus* (Marsh.), the smaller European elm bark beetle, by reacting with sulfhydryls in receptor proteins in dendritic membranes of chemosensitive neurons (Norris *et al.* 1970a, 1970b, 1971; Rozental and Norris 1973, 1975; Singer *et al.* 1975).

The redox dependency of chemoreception in insects was also shown in studies (Norris 1969, 1970) where p-hydroquinone, the reduced partner in the classical redox couple, p-hydroquinone / p-benzoquinone, excited feeding by *S. multistriatus*; whereas, the oxidized partner, p-benzoquinone, inhibited feeding. In spite of these early findings, enthusiasm for redox-based receptor and energy-transduction mechanisms in animal and human chemoreception has, until quite recently, been limited. This was especially true for insect chemoreception. Although numerous research reports have confirmed that insect chemoreception is sulfhydryl-disulfide dependent (Villet 1974; Frazier and Heitz 1975; Singer *et al.* 1975; Ma 1977, 1981; Vande Berg 1981), several workers in this entomological specialty (e.g., Kaissling 1971, 1974, 1987; Vogt and Riddiford 1986) concluded that non-covalent bonding, but not redox reactions, are involved in the transduction mechanism in insect chemoreception. Until recently, most researchers working in receptor and energy-transduction mechanisms in living cells also had concluded that redox reactions were not involved. In contrast, findings by Norris (1969, 1971, 1976, 1981, 1985, 1988, 1994) have consistently indicated that redox chemistry is fundamentally involved in receptor function and energy-transduction mechanisms. The validity of our results has been reconfirmed throughout the general field of receptors and signal transduction (e.g., Storz *et al.* 1990, Van Der Vliet and Bast 1992, Stamler *et al.* 1992, Reichard 1993, Pyle 1993, Ravichhandran *et al.* 1993). The sulfhydryl-disulfide dependent redox chemistry of receptors and energy transduction has become a major area of research throughout biology (Van Der Vliet and Bast 1992). Thus, these aspects of insect chemoreception have not only been confirmed, but also are now widely viewed as a part of a much larger chemoreception 'whole' within biology.

The Required Elemental Trait

The fundamental trait required for the evolution of a system for information generation, transfer and use in maintaining order is the transfer of chemical groups and energy. This required trait for chemical messengers singles out phosphorus and sulfur from all other atoms in the "Periodic System" (Wald 1969). The two basic atomic characteristics which qualify sulfur and phosphorus uniquely as agents of chemical group and energy transfers are (a) the possession of *d*, in addition to *s* and *p*, orbitals which increases their capacities to form linkages with a variety of energy potentials and (b) an intrinsic instability of such linkages, which facilitates the exchange of chemical groups and energy (i.e., informational units) (Wald 1969). Such roles for phosphorus are well established experimentally, but they are only just now being recognized for sulfur.

However, sulfur now seems to be the most highly qualified element regarding abilities to exchange energy as information among living forms and their abiotic environments (Wald 1969; Norris *et al.* 1970a, 1970b, 1971; Norris 1971, 1979, 1981, 1985, 1986, 1988, 1994; Neupane and Norris 1992; Norris and Liu 1992; Van Der Vliet and Bast 1992). First of all, regarding such roles, sulfur, as thiol (-SH), brings to living systems a form of organic sulfur which possesses those atomic traits which are otherwise limited to organic oxygen and are essential to organisms. However, thiol lacks most, if not all, of those traits (or degrees thereof) possessed by oxygen which make oxygen or its free-radical derivatives, especially harmful, even deadly to living organisms, unless the oxygen is handled in special ways, e.g., bound to hemoglobin in vertebrate blood (Wald 1969, Harold 1986). Second, sulfur as well as phosphorus forms relatively long (i.e., loose) bonds with other atoms; such bonds hold the attached atom less tightly and thus it may be more readily transferred, exchanged, as informational energy in living systems (Wald 1969, Harold 1986). Third, the trait which ultimately sets sulfur, as a vehicle of chemical group and energy transfers as information, apart from phosphorus is the former's ability to form a thiol (-SH). This property uniquely enables sulfur to readily accept, donate or variously share the basic energy unit in living systems, hydrogen (Wald 1969, Szent-Gyorgyi 1973). Thus, through sulfhydryl / disulfide (thiol, -SH / disulfide, -S-S-) redox chemistry, sulfur uniquely brings to living cells, and their chemical communications, a "yin and yang", a give and take, mechanism for moving hydrogens, the basic energy unit of living systems, as information between plants and animals and their abiotic environments (Norris 1986, 1988, 1994; Neupane and Norris 1992; Norris and Liu 1992).

The conversion of abiotic informational energy states, as in pheromone and phytochemical messengers, into biotic informational energy states occurs in sulfhydryl / disulfide-proteinaceous receptors in the plasma membrane which encloses each living cell (Norris 1981, 1986, 1988). Chemical messengers, by directly or indirectly oxidizing sulfhydryls or reducing disulfides in proteins of living cells, allow the folding and unfolding, respectively, of the three-dimensional structure of such macromolecules in cells (Figs. 5) (Sela *et al.* 1957, Norris 1981). Such folding and unfolding of proteins (e.g., as in muscles during their contractions versus relaxations, or in nerve membranes during impulse generation versus decay) especially bring to living systems, motion; a trait which is so commonly associated with life (Harold 1986).

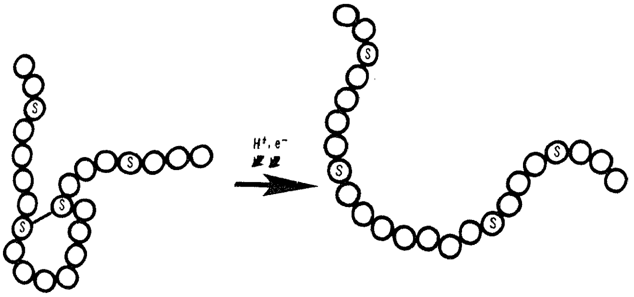


Figure 5.—A schematic illustrating how the formation of disulfide (-S-S-) bonds in proteins can stabilize the three-dimensional structure of such macromolecules in a more aggregated state (see on the right); whereas, the reduction of those -S-S- bonds by reducing agents (e.g., some chemical messengers) can yield sulfhydryls (-SHs) and a more "opened", non-aggregated, three-dimensional form to the protein in the receptor and energy-transducer macromolecule (see on the left). In this illustration, each circle in the chains of circles represents an amino acid in the chain of amino acids which make up a protein. Each circle containing a sulfur (S) represents the amino acid, cysteine, which bears one sulfhydryl (-SH).

As Norris and coworkers (Norris *et al.* 1970a, 1970b, 1971; Rozental and Norris 1973; Singer *et al.* 1975) showed about 20 years ago, the highly reversible redox system based on sulfhydryl / disulfide interactions with chemical messengers yields not only qualitative (i.e., excitatory or inhibitory), but also quantitative (i.e., quantal, unitized) exchanges of energy. An informational system requires simply an ability to map an output (i.e., efferent) message or behavior on to an input (i.e., afferent) message or stimulus (O'Connell 1981). The above described highly reversible sulfhydryl / disulfide redox system for transferring energy (hydrogens, or electrons and protons) as information is readily quantifiable in millivolts (mV) especially by polarography (Rozental and Norris 1973, 1975) or the electroantennogram (EAG) (Norris and Chu 1974; Norris 1979, 1986, 1988). Thus, this system fully satisfies the requirements of a code for the informational exchange of energy.

Theorized Environmental Energy Exchange Code

An informational code thus must be based on (a) an universal medium (i.e., messenger) and (b) a means (method) of using that universal messenger to convey both qualitative and quantitative messages. Such a system allows a special use of energy for establishing order (Harold 1986). Norris (1971, 1976, 1986, 1988, 1994) has presented theories, based on sulfur electrochemistry as the universal messenger, for chemical communications among animals, including humans, and plants; and between them and their environments. The most recent theoretical version of such a code is here termed the 'Environmental Energy Exchange Code'. In the proposed EEE code, sulfur electrochemistry is the universal medium (messenger); and the thiol (-SH), its derivatives and its redox couple, disulfide (-S-S-), are the chemical means for using sulfur electrochemistry to convey both qualitative and quantitative messages. Thus, through chemical-messenger oxidation of a sulfhydryl (thiol, -SH) in each of two molecules of the amino acid cysteine in the receptor protein (energy-transducer), a disulfide (-S-S-) may form (Fig. 2). Such a disulfide in the three-dimensional protein involves a conformational (shape) change; such energy transfer thus allows the protein to become more folded (aggregated) in shape (Fig. 2). Conversely, through chemical-messenger reduction of a disulfide, the -S-S- bridge between the two cysteines is broken and the protein may then assume a more open (i.e., unfolded) three-dimensional shape (Fig. 2). This chemical messenger-driven, highly reversible sulfhydryl / disulfide electrochemistry in the receptor protein (energy-transducer) thus brings to organisms quantitative dynamic form, which is so essential to function.

In a prior experimental analysis of such information exchange between the feeding-inhibitory messenger menadione (2-methyl-1,4-naphthoquinone) from the environment, and sulfhydryls in the dendritic membrane of chemosensitive neurons in the antenna of *Periplaneta americana*, the electrochemical transduction of informational energy into organismal response (i.e., activity) was described mathematically as a regression with **r**= 0.997 (Norris 1986, 1988). Thus, one unit of experimentally determined input (stimulus) energy from the environment can be mapped (correlated) informationally upon (related to) one unit of output (organismal-response) energy using the proposed sulfur-based encoding mechanism. Some key parameters of this experimentally elucidated sulfhydryl / disulfide-dependent electrochemical mechanism are further discussed in Table 2.

Table 2.—Some key fascets of the sulfhydryl / disulfide-dependent electrochemical mechanism involved in insect perception of phytochemical (e.g. 1,4-naphthoquinone) messengers.

1. Each of the three possible pairings of the chemoreception parameters (**a**, **b**, **c**) yields a linear regression with $r > 0.95$: **(a)** the moles of messenger required in a standardized insect behavioral assay to cause a > 99% change in that behavior; **(b)** the maximum in vitro polarographic $U_{1/2}$ shift in millivolts by the involved receptor and energy-transducer sulfhydryl / disulfide protein from the insect's antennal chemosensory neurons when saturated with the above messenger: and **(c)** the maximum percent inhibition of a standardized excitant-stimulated electroantannogram (EAG) by the above messenger (Rozental and Norris 1973, 1975; Norris and Chu 1974; Norris 1979, 1986, 1988).

2. Simultaneous solution of the three linear relationships among the parameters in (1) showed that the correlation (r^2= 0.95) between (a) the maximum $U_{1/2}$ shift elicited in the sulfhydryl / disulfide receptor and energy-transducer protein by the messenger and (b) the maximum percent inhibition of the standardized EAG by that messenger is so high that only one of these two parameters need be considered in a mathematical description of the transduction of the molar-messenger energy into insect behavioral change. $\text{Log } Y = 3.40 - 0.112 \text{ Log } X$, quantifies the energy-transduction relationship (Norris 1986, 1988).

3. Based on behavioral analyses (Rozental and Norris 1975; Norris 1986, 1988), three distinct sets of sulfhydryl (thiol)-dependent receptor sites for 2-methyl-1,4-naphthoquinone (menadione) messenger exist in the receptor and energy-transducing protein in *Periplaneta americana*. Those sulfhydryls in each of these three sets of receptor sites cause a 4-5 millivolt shift in the receptor and energy-transducing protein's $U_{1/2}$ value when they react at the mercury electrode involved in this polarographic analysis (Rozental and Norris 1973; Norris 1979, 1986, 1988).

4. Based on the EAG-inhibition assay, messenger-menadione saturation of one set of receptor sites, as described in (3), causes about an 8% inhibition (Norris and Chu 1974; Norris 1986, 1988). Thus, the theoretical maximal inhibition of the standardized EAG by saturation of the receptors in all three sites with menadione might be predicted as 3 (sites) times 8%, which equals 24%. It is interesting and significant that the experimentally determined range in maximal percent of EAG inhibition by saturation with menadione was 23-25%.

5. Data summarized in (3) and (4) above lead to the interpretation that a receptor and energy-transducer $U_{1/2}$ shift of 4-5 mV equals an 8% inhibition in the EAG. This means that each 4-5 mV shift in the $U_{1/2}$ is accompanied by an 8% inhibition in the standardized EAG. The observed linear relationship between the $U_{1/2}$ millivolt shift in the receptor and energy-transducer protein and the percent EAG inhibition shows that the primary encoding of the message dictating the whole-insect behavior occurs in the energy-transducer protein in the chemosensory sensillum (Norris 1979, 1986, 1988).

6. Our research explains for the first time, both in electrochemical and electrophysiological parameters, why the EAG is so meaningful to an understanding of the chemical senses of insects. EAG does not just measure millivolts of electrical energy, but also the energy after it has already been coded as information in the receptor and energy-transducer protein adequately to elicit a predictable behavior in (by) the insect (Norris 1979, 1981, 1986, 1988).

7. Saturation of the receptor and energy-transducer protein with p-chloromercuribenzoate (PCMB), a compound which reacts specifically and irreversibly with sulfhydryls, blocks the above characterized messenger-induced $U_{1/2}$ shift in the protein. Thus, the conversion of molar-messenger energy into electrochemically based information adequate to predict whole-insect behavior is blocked by the sulfhydryl-specific reagent, PCMB (Rozental and Norris 1973; Norris 1979, 1981, 1988).

8. Sulfur, as in sulfhydryl / disulfide redox systems in proteins, is the critical dynamic elemental interface between responsive cells and the stimulating environment, whether biotic or abiotic.

SUMMARY

Animals and plants "communicate" with each other, and with their environments, via common ion, free radical, and molecular messengers which function by electrochemical energy-transduction mechanisms. Such mechanisms depend upon the reversible sulfhydryl-disulfide redox couple in receptor and energy-transducer proteins to convert quantitatively messenger-based energy states from the environment into altered membrane potentials and/or second messengers which may serve as signals in the elicited cell, and between it and other cells in an organism. Receptor and energy-transducer proteins are associated with the plasma membrane which surrounds each living cell. Within biological constraints, the conversion of a molar-messenger energy state into a redox-based energy state in the sulfhydryl-disulfide receptor and energy-transducing protein in the plasma membrane is linear (i.e., quantal). This means that messenger-borne energy from the environment is converted to informational energy (i.e., units) by the perceiving cell.

Many entomologists and chemical ecologists have used the electroantennogram (i.e., EAG) to detect compounds from the environment which alter the behavior of insects. In using this classical technique, the experimentalist is measuring change in the energy (e.g., dendritic-membrane potential) state in the primary peripheral chemosensory neurons which are specially "housed and exposed to the external environment" within the antenna of the insect. The experimental use of the EAG and the correct prediction, thereby, of the resultant behavioral change elicited in the whole, live insect constitutes scientific proof that the information necessary for alteration of the behavior of the whole insect can be encoded in the primary peripheral chemosensitive neuron. This encoding of energy into information is dependent upon the element 'sulfur', and especially its readily reversible sulfhydryl (i.e., thiol, -SH) / disulfide (i.e., -S-S-) redox couple. This encodement of chemical-messenger energy into biologically useful information is blocked (or otherwise altered) in the intact cell or whole organism by the application of biological concentrations of reagents which react specifically with sulfhydryls and/or disulfides in proteins in plasma membrane. Recent research has proven that this transduction of environmental energy into biologically useful information also occurs in plant cells. Thus, the sulfhydryl / disulfide-dependent Environmental Energy Exchange Code is supported by extensive scientific findings from both animal and plant realms.

The unique atomic attributes of the element 'sulfur' for fulfilling this vital role in the conversion of environmental energy into biologically useful information for all cells were clearly described by Wald (1969). It is fortunate that scientists can now readily test the role of sulfur, and especially the sulfhydryl / disulfide redox couple, in the exchange of environmentally based energy into information in any living cell. Chemical ecologists seem especially fortunate in this regard through their frequent familiarity with the EAG and other electrophysiological techniques for experimentation. We have also shown the usefulness of classical electrochemical (e.g., dropping-mercury-electrode polarography) techniques for asking questions about the roles of sulfur and its derivatives in the proposed Environmental Energy Exchange Code. Further experiments on this exciting energy-exchange interface between living cells, organisms, and their vital environments should yield data which significantly improve our abilities to quantify environmental influences on the expressions of phenotypes by genomes, and on the functionalities and longevities of such phenotypes.

ACKNOWLEDGEMENTS

Research reported here from the author's laboratories was supported partially by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin, USA; in part by numerous research grants from the U.S. National Science Foundation and the U.S. National Institutes of Health; and in part by U.S. Hatch Projects 3040 and 3419, McIntire-Stennis Project 3127, and USDA Competitive Grants 84-CRCR-1-1501 and 88-37153-4043.

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FUNGAL ENDOPHYTES: CONTRASTING EFFECTS IN TREES AND GRASSES

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INTRODUCTION

Fungal endophytes, fungi that live asymptomatically and intercellularly within tissues of most plants, have recently received increasing attention from ecologists (e.g., Strong 1988). The presence of fungal endophytes in plants, especially grasses, can increase resistance to herbivores due to the production of alkaloidal mycotoxins, but also may increase resistance to drought and flooding stress, increase plant competitive abilities, and, for endophytes transmitted vertically via seeds, deter seed predators or increase seed dispersal (Clay 1990, Knoch *et al.* 1993). Endophytic fungi are typically considered plant mutualists because of these potential modes of increased plant fitness (Clay 1990). However, most research on fungal endophytes has involved introduced, agricultural forage grasses, such as tall fescue or perennial ryegrass. Little is known of the ecological role of endophytes in either native grasses or woody plants relative to plant-herbivore, plant-plant, or plant-seed predator interactions. Here, I summarize our research to date on endophytes in *Quercus emoryi* (Emory oak) and their effects on a major herbivore, the leafminer *Cameraria* sp. nov. (Lepidoptera:Gracillaridae). Leafminer larvae spend 11 months within leaf tissues and are confined to a single leaf chosen by the ovipositing female. Therefore, endophytes should alter leafminer performance more than that of mobile, exophytic insects. I contrast these results with endophytes in *Festuca arizonica* (Arizona fescue). I predict stronger effects of endophytes on herbivores and seed predators of Arizona fescue due to differences in mode of fungal transmission and specificity.

METHODS

We have monitored seasonal and spatial patterns of fungal endophyte infections and the leafminer in trees of Emory oak at Oak Flat study area in central Arizona for the past 4 and 10 years (Faeth 1991), respectively. We have isolated at least 12 species of endophytes from Emory oak, but four species, QE1 (*Asteromella* sp.), QE2 (Ascomycete:Diaporthales), QE7 (*Plecophomella* sp.), and Y1 (filamentous yeast) make up >95% of all infections. All of these endophytes are transmitted horizontally via spores, likely carried in rainsplash. In observational studies, we have correlated the presence of living and dead larval leafminers with the frequency of infection. In manipulative experiments, we have either increased (spore spraying of leaves or spore injection of individual mines) or decreased (enclosing branches with plastic or application of fungicides) to test the role of individual fungal endophytes on leafminer developmental and mortality.

We have begun to investigate the role of fungal endophytes in Arizona fescue populations and their relationship to intensity of grazing and soil nutrients. Arizona fescue harbors two endophytes, *Acremonium starrii* and a *p*-endophyte (*Phialophora*-like). Both endophytes are transmitted vertically from maternal to offspring plant via seed, but the *p*-endophyte also sporulates and can be transmitted horizontally from adult plant to adult plant. We have conducted preliminary experiments testing the role of the *Acremonium* endophyte in reducing seed predation and increasing seed dispersal by seed harvesting ants (*Pogonomymrex* species) by presenting E+ (infected) and E- (uninfected) fescue seeds to ant colonies and following proportions collected and discarded into refuse piles.

RESULTS AND DISCUSSION

Generally, endophyte infections in oaks increase seasonally, with the peak infection level coinciding with summer rains in Arizona in July-August. Overall infection levels vary with all spatial scales - between localities, between and within

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Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN

trees, and between and within individual leaves. Differences in infection levels between trees result from differences in infection by individual species, with QE7 comprising differences early in the season and QE1 later in the growing season. Leafmining is associated with increased fungal infection, and leaves with dead larvae have higher infections than those with live larvae. However, neither mass nor survival of leafminers was affected by spore-injection of the three common endophytes. One endophyte (Y1) increased developmental time. Other experiments show that oviposition is not associated with leaves that are either more or less likely to become infected with endophytes. Thus, leafmining activity appears to increase infection frequency, probably by altering the surface of the leaf, but the endophytes themselves appear to have only weak effects on leafminer development and survival.

Preliminary observational evidence indicates that Arizona fescue in areas under intensive grazing by cattle and native ungulates have higher frequency of *Acremonium starrii* but not the *p*-endophyte. These results support our prediction that endophytes that are only transmitted vertically are more likely to interact mutualistically with the plant and provide protection against herbivores. Future experiments will test the dependency of the mutualism on available resources to the grass and controlled levels of herbivory. Similarly, experiments with seed-harvesting ants show that *Acremonium*-infected seeds are less likely to be collected. Of the seeds that are harvested, E+ seeds are more likely than E- seeds to be discarded into refuse piles where germination success is higher than in surrounding areas.

SUMMARY

Endophytic fungi are diverse and ubiquitous in almost all woody and non-woody plants examined to date. The role of endophytes in plant-herbivore, plant-plant, and plant-seed predator interactions in natural systems is still largely unexplored. Testable hypotheses and predictions, however, can be made about the direction and strength of the interactions based upon mode of transmission and specificity of the fungi and plant.

ACKNOWLEDGMENTS

I thank Kyle Hammon and Dennis Wilson for preliminary data and Rindy Anderson, Carmen Febus, Dawn Hagerman, Catherine Hudson, Terri Kingrey, Myrna Miller, and Franziska Schulthess for technical assistance. This work is supported by NSF Grant BSR 91-07296 to SHF.

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PROPAGATING THE EFFECTS OF HERBIVORE ATTACK IN AN OBJECT-ORIENTED MODEL OF A TREE

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INTRODUCTION

Understanding cause and effect in forest health problems requires an understanding of the structure and functioning of trees. Before conclusions about a malfunctioning system can be made, we must first understand how a healthy one works. In industrial engineering and medicine, diagnostic reasoning about structure have long traditions (e.g., Bratko *et al.* 1989, Torasso and Console 1989), and well defined structural models exist for man-made machines and the human body. Diagnosis of disorders in trees and forests is not that advanced. One of the reasons for this is that we have not had good structural models of trees at our disposal.

By structural models, we mean models that make the parts of the system explicit. Mathematical models describing photosynthesis, respiration, water pressure, etc. are not enough alone. They have to be bound into some larger structure, like shoots, buds, needles, etc. These parts in turn must be explicitly connected with their physical immediate neighbors. Such a model of a tree would form a topology of thousands of interconnected parts. L-systems (Prunsinkiewicz and Hanan 1989) and fractal trees (Mandelbrot 1977) are probably the original approaches to this problem. However, those models are made for visualization, and do not maintain the status of the parts after they have been graphically drawn. Plant and tree growth has been studied in detail for research in plant morphology and landscape architecture (e.g., Reffye *et al.* 1989). However, structural models of tree development and survival have emerged only lately because the necessary software technologies have only been in place for a few years. Sequeira *et al.* (1991) have presented an structural model of plant. Ahonen and Saarenmaa (1991) and Saarenmaa (1991) have prototyped a topological tree. Nikinmaa (1992) has a presented the models for the functioning of a structurally explicit Scots pine tree.

Object-oriented (OO) programming is rapidly becoming the leading way of constructing software. OO software consists of objects that are more or less encapsulated entities. They combine attributes and behavior under the same encapsulation. Objects can consist of other objects and they can communicate with each other with messages. They make it possible to represent structures such as biological organisms with a great realism. Once an object-oriented tree model has been built, it can, hopefully, be reused for many different purposes. These uses may be growth prediction and physiological research. Such models should also be useful for making diagnoses and visualizing the effects of disorders. Herbivore-tree interactions are often so complicated that they can only be understood through modelling. An OO tree model should be able to propagate the effects of a herbivore attack in one part of the tree into others where the symptoms and secondary damage may be observed.

In this paper we describe briefly an OO model of Scots pine, *Pinus sylvestris* L., that we have built, and demonstrate its uses for simulating disorders caused by two herbivores.

METHODS

Object-oriented modelling allows building software that mechanistically resembles the real world. This similarity is achieved with three basic concepts: encapsulated objects, classification of objects, and message passing between objects (Rumbaugh *et al.* 1991, Saarenmaa *et al.* 1994).

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Encapsulated objects are data structures that always maintain their identity, have a clear boundary, and show to the outside world only selected facets of themselves. Objects have attributes and behavior. They can be concrete physical things, abstract ones, or events. Examples of concrete things are trees, insects, vehicles, people, etc. Examples of abstract objects are beliefs, statistical information, and time. Examples of events are decisions, insect outbreaks, treatments, etc. The attributes contain the internal state of objects in their values. Examples of the attributes of an insect object are length, wing color, etc. Examples of behaviors (also called methods) of in an insect object are development, emergence, oviposition, etc.

Objects fall into two categories: classes and instances. A class is a definition, a blueprint for actual instances. For instance, a biological taxon such as the species of "Scots pine" can be a class. Its instances, in turn, are the physical individual trees that grow outside this building. Classes often form hierarchies where subclasses inherit the attributes and methods of their superclasses. At a lower level, specialized values and new attributes and methods can be introduced in order to refine the generic superclasses.

Objects communicate by sending messages to each other. Different objects can respond to the same message in their own ways. For instance, a "time" object may send a message of "lightness change" with a value "dusk" to all the objects in a simulation. One insect object may respond to it by activating its flight behavior whereas another, that would only fly in sunlight, would deactivate its own.

Objects may consist of other objects. For instance, a tree object consists of a root system, one or several trunks, and a crown, which, in turn, consist of smaller objects. Objects may be associated and connected with other objects by having their names for some purpose as the value of an attribute. OO models should be implemented with an OO programming language. The present system, called Lignum, has been written in C++ under the Unix operating system, X-Window System, and 3-dimensional interactive graphics.

RESULTS

Description of the Tree Model

The model describes a single Scots pine tree that consists of small elementary parts. The functions of the model have been further elaborated from Nikinmaa (1992), and the model has been described in detail by Perttunen *et al.* (1994) and Salminen *et al.* (1994). The parts belong currently only into two operational classes: shoots and buds. In addition, we define classes for the stem, branch whorls, foliage, bark, phloem, sapwood, and heartwood. Each instance of a stem contains zero or more instances of shoot, branch whorl, and it ends with an instance of bud. Each pair of instances of shoot is separated by an instance of branch whorl (Fig. 1). Shoots consist of foliage, bark, phloem, sapwood, and heartwood. In this version of the model, the objects in a stem are connected to each other using a list structure.

The tree is originally started from one bud instance. Buds have methods for creating shoot instances, branch whorl instances, and new bud instances. The shoots create the parts they consist of. Shoots and branch whorls that do not have foliage any longer, are collapsed into the stems.

As we do not yet have a class for foliage, the implementation of shoot captures the productive metabolism of the tree. These include methods for photosynthesis, respiration, and growth. Foliage is implemented as an attribute for the needle mass, which must have a value greater than zero for any of the production to take place. Production is handled by a method which boasts the following function: where Wf is the foliage mass of the shoot and i the degree of interaction of shoot p which is dependent on the shadiness of the location (Sievanen 1992) with the foliage mass x above the height z. Production has to be multiplied with factor P0 which describes the photosynthesis under unshaded conditions (Nikinmaa 1992).

Respiration of buds, foliage, phloem, and sapwood is directly proportional to their mass. In the current implementation, the root system of the tree is extremely simple. It is considered to have mass that increases as the result of the production in the shoots. Part of the root mass dies annually. It also respires similarly to the foliage and the sapwood.

After production and respiration the tree can allocate its net production into the new shoots, foliage and roots. The equations for these are given by Perttunen *et al.* (1994). Nutritional status of the parts is only dealt with a single attribute in the present model.

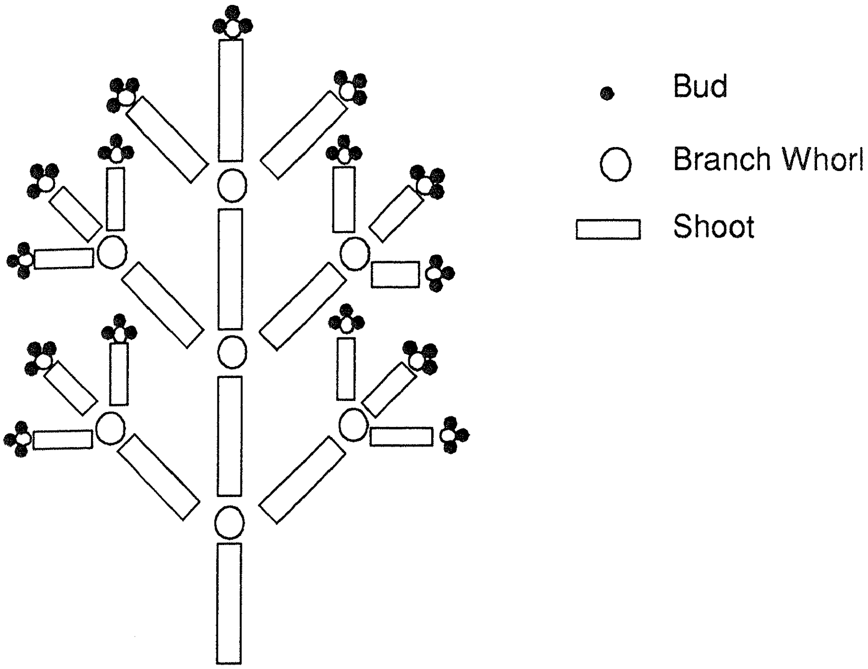
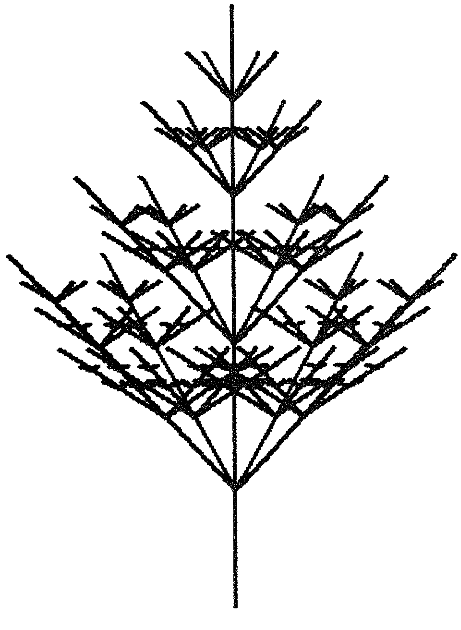


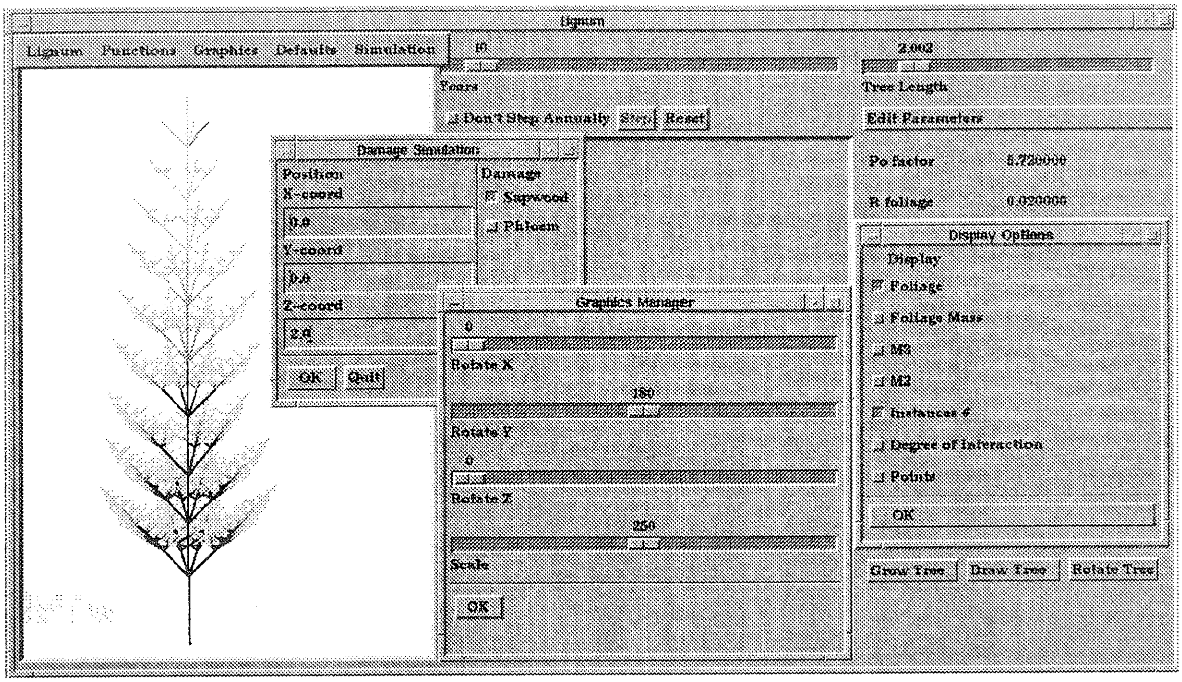
Figure 1.—Schematic representation of the object tree. (1)

When new buds are created, each bud is transferred to a new position in space. The direction is known by the position vector of the bud and the distance is determined by the adjusted length of the new shoot created by the bud. When the bud is in its new position it is able create new buds. The number of new buds is a function of the foliage mass in the new shoot. The function is user definable but default function is provided which limits the maximum number of buds to four.

The buds created are given new directions by rotating them evenly round the stem. If some of the buds is about to grow downwards (which surely will happen if the algorithm is applied strictly schematically) a simple heuristic is used to rotate these buds upwards. This makes it possible to create trees looking like in Figures 2 and 3. The elegant way of describing the topology of various tree species with the help of term rewriting systems is provided by Lindenmeyer (1968) and Kurth (1992).

Figure 2.—The model makes the 3-dimensional geometry of the tree explicit like L-systems, but also maintains the attributes of each object it consists of. In a five-year old tree (left) there are about 600 objects, and in a 20-year old about 30,000. Growing a tree into that size takes about 10 minutes for a 100 MIPS workstation with the current C++ implementation. The tree can be rotated on the screen.



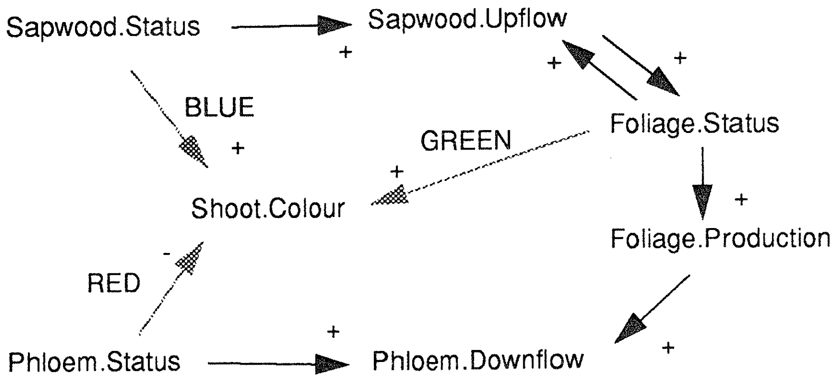


Years: 10
Tree Length: 2.002
Po factor 5.720000
R foliage 0.020000
X-coord 0.0
Y-coord 0.0
Z-coord 2.0
☒ Sapwood
☐ Phloem
Rotate X: 0
Rotate Y: 180
Rotate Z: 0
Scale: 250
☒ Foliage
☐ Foliage Mass
☐ M3
☐ M2
☒ Instances #
☐ Degree of Interaction
☐ Points

re 3.—The Lignum model can be controlled through windows and sliders and the parameters can be customized. In the graph shown, one shoot in the top whorl has turned brown due to damage in sapwood as caused by *Tomicus* spp.

Examples of Herbivore Attack on the Model Tree

Propagation of the effects of herbivore attack in the tree model can only be implemented through material flows from object to another. So far, we have only implemented an upward water flow-method for the sapwood part of shoots and rls and a downward starch flow-method for the phloem part of shoots and whorls. The status-attribute of these parts can e values between 0 and 1. The rate of the flow is directly proportional to this status. The status of phloem, sapwood, and ge is also shown graphically as the color of the part as Figure 4 shows.



re 4.—The color of the diseased shoot is shown as the combination of three main status attributes of its parts.

Boring by Pine Shoot Beetles

Examples of insects feeding on the canopies of trees are the pine shoot beetles, *Tomicus* spp. This bark beetle is the first one to invade dying or felled pines to breed in spring. The progeny emerging from breeding sites in trunks disperse to the crowns of pines during mid- and late-summer. In order to become mature, young adults feed in pine shoots burrowing into the pith. The beetles attack mainly current year shoots of upper half of the canopy. The burrows may heal over only in the thickest shoots, otherwise they turn brown, break, and fall down. Physiologically, *Tomicus* attacks in shoots have an effect like the sudden cutting of ducts (Fig. 5).

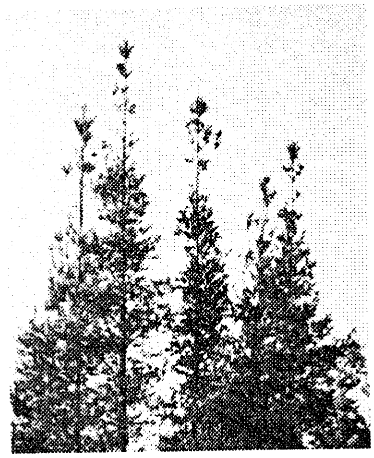


Figure 5.—After about 10 years of repeated *Tomicus piniperda* damage the tops of Scots pine have become deformed as on the right. Under a permanent beetle pressure the spike-like tops die and the pines start looking flat-topped.

The effects of removal of shoots on remaining parts of the tree can be divided into three groups: first, the environmental effects, e.g., the changes in light conditions, water availability and release of nutrients from fallen shoots; second, the structural reactions like leader shoot changes, formation of adventitious shoots, sapwood-heartwood relation, and the dying of roots as the pipe model indicates; and third, the carbohydrate dynamics of the tree, including changes in assimilation capacity, nutrient allocation, root activity and compensation processes. The last group of effects is the most difficult to include in a model. These effects are discussed thoroughly by Swedish researchers (Fagerstrom *et al.* 1977, Ericsson *et al.* 1985, Langstrom *et al.* 1990, Troeng and Langstrom 1991, Langstrom and Hellqvist 1991, 1992). In addition, the reactions seem to be different according to age, growing site, and provenance of trees.

The distribution of damage classes of crown can be used to make rough estimates of growth losses in stands repeatedly attacked by pine shoot beetles (Kukkola *et al.* 1994). With a stand of model trees, the spatial pattern of beetle dispersal and the distribution of growth losses of pines may be examined.

The present model should be able to simulate the pruning pattern of *Tomicus* attack and the growth loss. The model is currently being used this way by letting individual *Tomicus* object instances to choose a shoot for foraging. The choices of attacking beetles depend on the shoots already allocate by the previously attacked beetles, and the amount the tree stands out above or below its neighbors.

Defoliation by Insect Larvae

Scots pine is adapted to low nutrient resources and stores its major reserves in foliage. Figure 6 shows the dynamics that follow from a severe defoliation in this situation. A severe defoliation reduces the carbohydrates and nutrients overall in the tree, and also reduces the needle number. This leads to increased nutrient concentration in the remaining needles, as the fine root biomass is not immediately reduced and its nutrient uptake pumps more nutrients into the remaining needles. This increases the quality of the remaining needles for food of the defoliator. Increased nutrition in needles also leads to their increased size and increases production. The carbohydrate concentration in needles increases as well as the concentration of the carbon-based protective metabolites (for a review, see Herms and Mattson 1992).

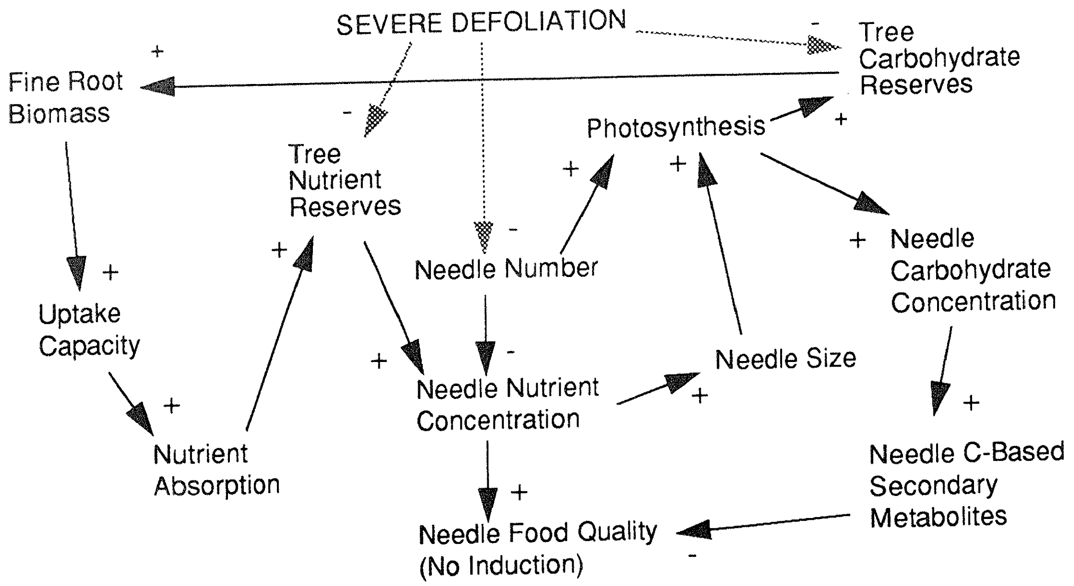


Figure 6.—Dynamics of nutrients and carbohydrates in the Lignum model, with the effects of defoliation.

The present model, as it has explicit descriptions for foliage in each shoot, is capable of duplicating this kind of dynamics. However, we are still calibrating the model, and can not yet claim error-free predictions.

DISCUSSION

The present model was created for a testbench of ideas in many different disciplines. It should be reusable for diagnostic reasoning, studying the effects of herbivore attack, visualization of symptoms, research on growth and development, and modelling tree architecture. Although we are still working on the first version of the model, the experiences have been promising. Future versions will be built to incorporate simulation on parallel computer hardware, and zooming in and out of the tree. The color schemes that we use for illustrating the status of the functions of the tree do not produce realistic visualization of the symptoms as of now. We will study the color effects that follow from various injuries, and try to animate the suffering and death of trees.

The model is a part of a larger system for integrated forest health management (Saarenmaa *et al.* 1994). It has been built compatible with object-oriented descriptions of forest insects and fungi. These have attributes that describe what parts of trees, represented as object classes, they attack and what kind of disturbances they cause in those parts. Matching OO trees with OO insects and other harmful agents will create new kind of ecological simulations and also be useful in diagnostic reasoning.

SUMMARY

A model of a tree which consists of thousands of small interconnected parts can potentially be useful for studying the effects of insect and disease attack on the whole system. We present such a model for *Pinus sylvestris*, which is based on object-oriented programming. The model consists of classes for shoots, buds, the stem, branch whorls, foliage, bark, phloem, sapwood, and heartwood. The model simulates primary production, respiration, growth, water flow, and starch flow, and has a geometry that can be monitored in 3-D color on computer screen. The effects of attacks by shoot boring bark beetles and defoliating insects are studied.

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DEFOLIATION OF FAGUS CRENATA AFFECTS THE POPULATION DYNAMICS OF THE BEECH CATERPILLAR, QUADRICALCARIFERA PUNCTATELLA

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INTRODUCTION

In spite of remarkable progress in understanding the ecology of plant-herbivore interactions during the past 20 years, there is little yet known about the interactions between beeches and their folivores (Edwards and Wratten 1983, Edwards *et al.* 1986). Two beeches, *Fagus crenata* and *F. japonica*, are dominant in the deciduous, broad-leaved forests of the cool-temperature zone in Japan. They are found from the southern part of Hokkaido, the northernmost main island, to the high mountains of Kyushu, the southernmost. The beech caterpillar, *Quadricalcarifera punctatella* (Motschulsky) (Lepidoptera: Notodontidae), is a monophagous species which feeds on beech leaves and occasionally completely defoliates the trees. We studied its population dynamics and tested the maternal effects hypothesis (Rossiter 1992) and the inducible resistance hypothesis, assuming that food deterioration occurs in beech following herbivory. A further objective was to determine the exact mechanisms by which *Q. punctatella* body size and density decrease after an outbreak has occurred.

Life History and Features of Outbreaks

Q. punctatella is a univoltine species (Igarashi, 1975). The pupa overwinters in the ground, and the adult emerges from late May to late July with a peak in mid- to late June (Kamata and Igarashi 1995a). Eggs are laid in masses on the underside of beech leaves, the total number per female being about 350. These are laid in several masses, each containing 20-100 eggs (Kamata and Igarashi 1995b). The larva feeds only on beech leaves and molts 3 or 4 times. The peak appearance of the last instar larva is in early August.

Serious outbreaks have been recorded since 1917, and outbreaks are known from central Honshu, the main island of Japan, to southern Hokkaido (Fig. 1) (Kamata and Igarashi 1995d). Most intervals between outbreaks have been 8-12 years and the average duration 1-4 years. Outbreaks in many regions have been synchronized even though the period of one or the time between two differed by time and place.

METHODS

Study Sites

Larval density was estimated in Hakkohda (Site A), Hachimantai (Site B), and Appi (Site C), located in the northern part of Honshu island (Fig. 1). Outbreaks have been recorded at Sites A and B, but no conspicuous defoliation has ever been recorded in Site C (Kamata *et al.* in preparation).

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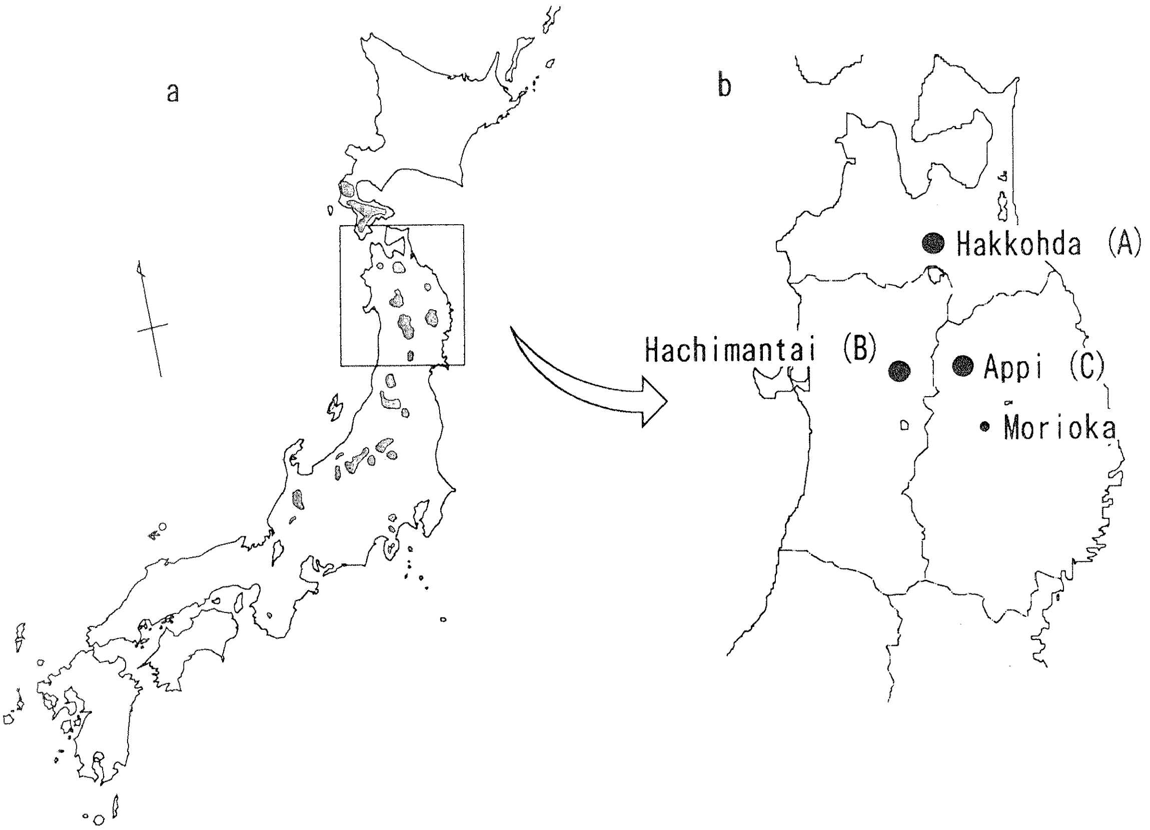

Figure 1.—Locations of beech forests which have had been conspicuously defoliated by *Q. punctatella* (dark shading), and three study sites: Hakkohda (Site A), Hachimantai (Site B), and Appi (Site C).

Estimation of Larval Density

The density of *Q. punctatella* in each plot was estimated by trapping frass pellets of the last instar larvae, because defoliation is mostly the result of its feeding (Kamata and Yanbe, 1993). The density can usually be estimated by the following equation: Density = Total number of frass pellets per square meter / 630, where 630 is mean number of frass pellets during last instar stage. Because density is underestimated when larval density becomes high and conspicuous defoliation occurs, the density must be calculated by the following equation: $\log_{10}$ (Density) = $0.988*\log_{10} X + 0.898$, where X is the maximum number of frass pellets per day per square meter (Kamata and Igarashi, 1994a).

Adult Number and Size

Adult numbers were measured at Site B. Two light traps were set in an open area in a small valley surrounded by a forest of predominantly beech. The distance between the two traps was 10m and both were 20m from the nearest edge of the beech forest where the heaviest defoliation had occurred in a 1981 outbreak. Each light trap had two blue fluorescent bulbs (20W) and a basin with water and kerosene. The relative numbers were estimated by the total catches during the 20 days from June 11th to 30th, because the peak of the adult catches was in mid- to late June (Kamata and Igarashi, 1994). Body size of the adult greatly influenced fecundity, but the fecundity of a trapped female cannot be estimated directly or indirectly from body weight, because both measurements decrease rapidly after emergence due to oviposition (Kamata and Igarashi 1995a). Rather, the area of forewing, which showed a good correlation with both fecundity and adult weight, was used as a substitute measurement of body size and fecundity (Kamata and Igarashi 1995c). The area was therefore measured by digitizer to the nearest mm^2 and its annual change was compared with population dynamics. In Site A, one light trap, which had two blue fluorescent bulbs (20W) and no basin, was set in a bare area ca. 20 m apart from the edge of beech forest completely defoliated in 1990. A sheet of white, finely-meshed cotton cloth (1.8m x 1.8m) was set behind the trap and adults were caught on it. This survey was conducted several days each year from 1988 to 1993. Area of the beech caterpillar's forewing was also measured.

Defoliation Experiments

To test the food deterioration hypothesis, the growth of larvae reared on beech saplings which had suffered previous artificial defoliation was compared with controls. It was assumed that the effects of defoliation would be apparent in mid August. Treatments were a 2 year regime where all leaves were clipped for 2 years running in 1991 and 1992. Another regime was a single year treatment during which all leaves were clipped for only the year (1992) prior to the experiment. No clipping was done in the control group. *Q. punctatella* were reared in screened enclosures on branches of treated and control beeches. Larvae were occasionally shifted from one branch to another so that food was always plentiful. Survivorship and mature body size were compared by rearing the larvae to maturity on these saplings. The *Q. punctatella* used in the experiment originated from a population on Site A three generations after the previous outbreak.

Tests for Food Deterioration and Maternal Effects Hypotheses in the Field

Three different regional *Q. punctatella* populations were reared on beech trees in the Tohoku Research Center of the Forestry and Forest Products Research Institute in Morioka to evaluate population quality (Fig. 1). Larvae were reared in screened enclosures on branches and mature body sizes were compared.

Eggs collected from these three regions were attached to beech saplings in each region. Larvae were also reared in screened enclosures on branches, and survivorship and mature body size compared among the three. From these results the influence of host and herbivore quality on population dynamics and associated changes in body size were determined.

Quantitative and Qualitative Properties of Beech Leaves

To learn about changes in leaves which had been defoliated, their number and dry weight were determined. Dry weight of 10 leaf disks (2 cm in diameter), one punched from each of 10 randomly selected leaves, was viewed as an index of leaf density. Decrease in the weight and the density of a leaf were compared to determine how leaves change due to defoliation. Leaf toughness was also measured using a "penetrometer" as described by Feeny (1970).

We used the protein-precipitation assay as a measurement of the tannins. We measured the precipitation of BSA by extracts of beech leaves according to Martin and Martin (1983) and Makkar *et al.* (1987). Leaf powder (150 mg) was extracted three times with 3 ml of 70% acetone, and the total volume of the combined solution of extracts was adjusted to 10 ml with the solvent. One ml of the above stock solution from leaf powder was added to 2 ml or 3 ml of 0.1% BSA solution (containing 2 mg or 3 mg of BSA, respectively) in acetate buffer (pH=5.0). The amount of precipitated BSA was determined by ninhydrin assay.

RESULTS

Population Dynamics and Body Size

Larval densities changed with the same pattern in all three areas (Fig. 2). Density was lowest in 1986, and increased successively until 1990 (Kamata and Igarashi 1995c). In Site A, slight defoliation was recognized in 1989 and heavy defoliation in 1990. The predator, *Calosoma maximowiczi* (Coleoptera, Carabidae), and an entomopathogenic fungus, *Cordyceps militaris* (Clavicipitales: Clavicipitaceae), greatly increased in abundance during the outbreak periods (Kamata and Igarashi 1994c, Sato *et al.* 1994). The densities in Sites B and C were not as high in 1990, and neither defoliation nor natural enemies were conspicuous. However, the density decreased in 1991 in all three areas, then increased again in 1992. The resilience of the population density in 1992 was stronger in Sites B and C, where natural enemies were not conspicuous in 1990, than in Site A.

Moth numbers in Site B fluctuated in tandem with larval density (Fig. 3). They were lowest in 1987, then increased. Interestingly, moth numbers were almost the same in 1990 and 1991, though the larval density decreased in 1991. Adult number began to decrease after 1992.

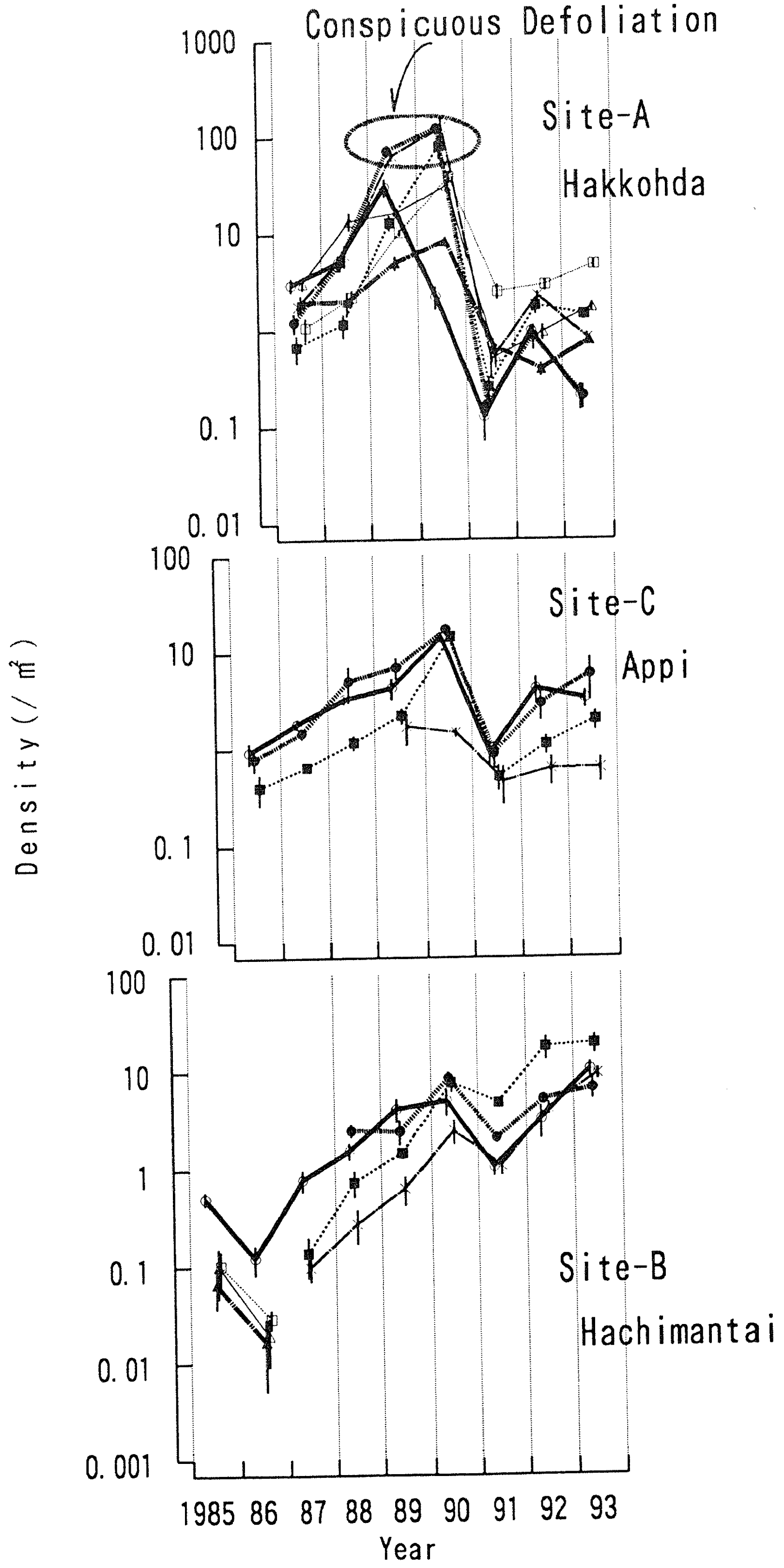

Figure 2.—Annual changes in last instar larval densities of *Q. punctatella* in the three study plots (mean ± 90% confidence intervals). Site A (Hakkohda) is where conspicuous defoliation occurred during our study, Site B (Hachimantai) is where conspicuous defoliation had been recorded but did not occur during our study, and Site C (Appi) is where no conspicuous defoliation of this species has been recorded.

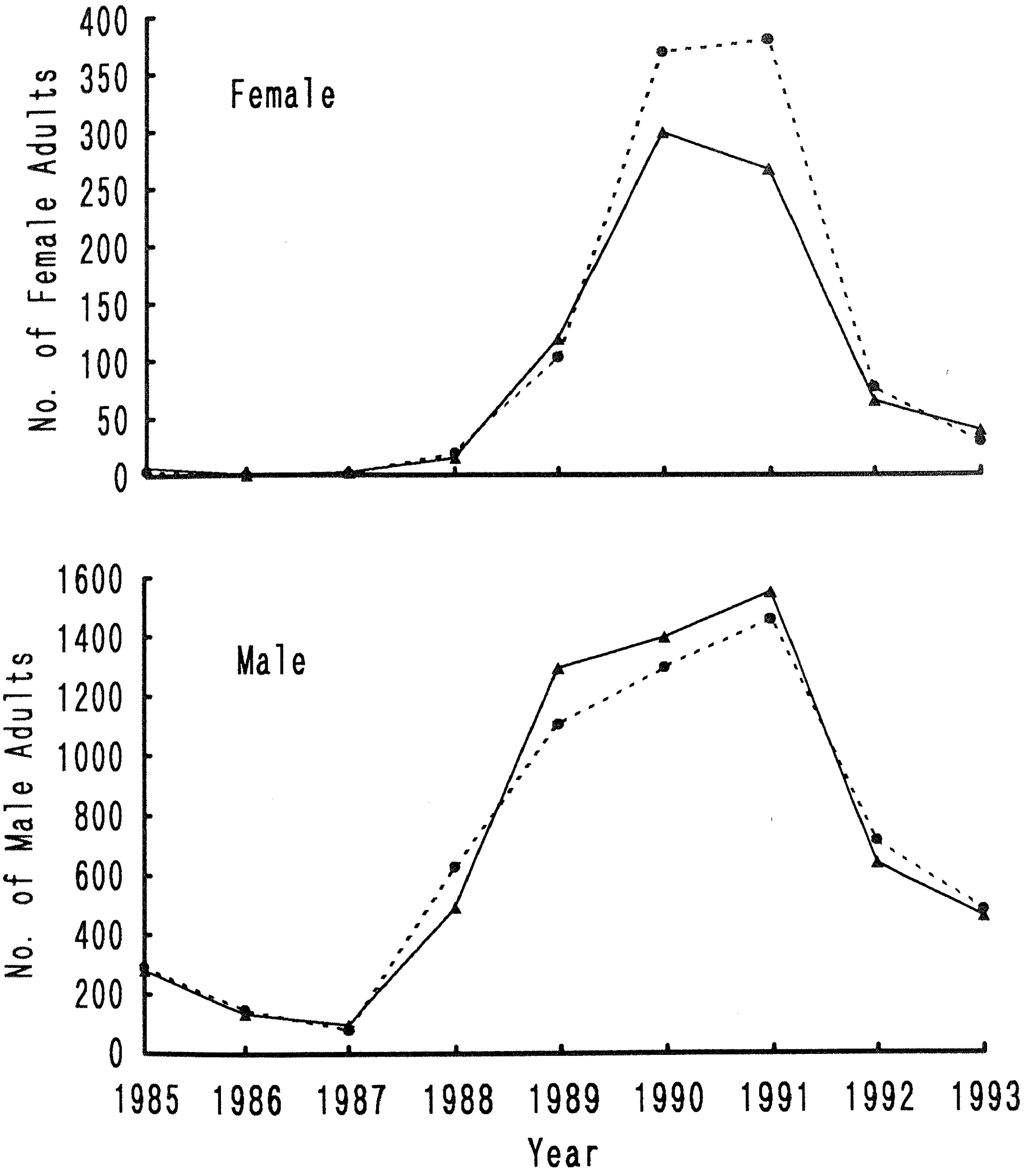

Figure 3.—Number of *Q. punctatella* adults caught in two light traps in Site B (Hachimantai) 1985-1993. The number of adults of each sex in each trap is shown. Nitrogen content of foliage was measured by a CHNS/O Analyzer (Perkin-Elmer PE2400 Series II) to the nearest 0.01%.

Body size of moths gradually increased with larval density, but decreased suddenly in 1991 in Site A where severe defoliation had occurred the previous year (Fig. 4). A quantitative food shortage in 1990 was probably the main factor causing this size reduction. Body size increased a bit but was still small in 1992 and 1993, but larval density decreased and no food shortage occurred after 1991. Thus, factors other than a shortage of food were involved in the size decrease. Because size was almost the same in the two sites before the outbreak, the great difference between the two after the outbreak was not genetically determined.

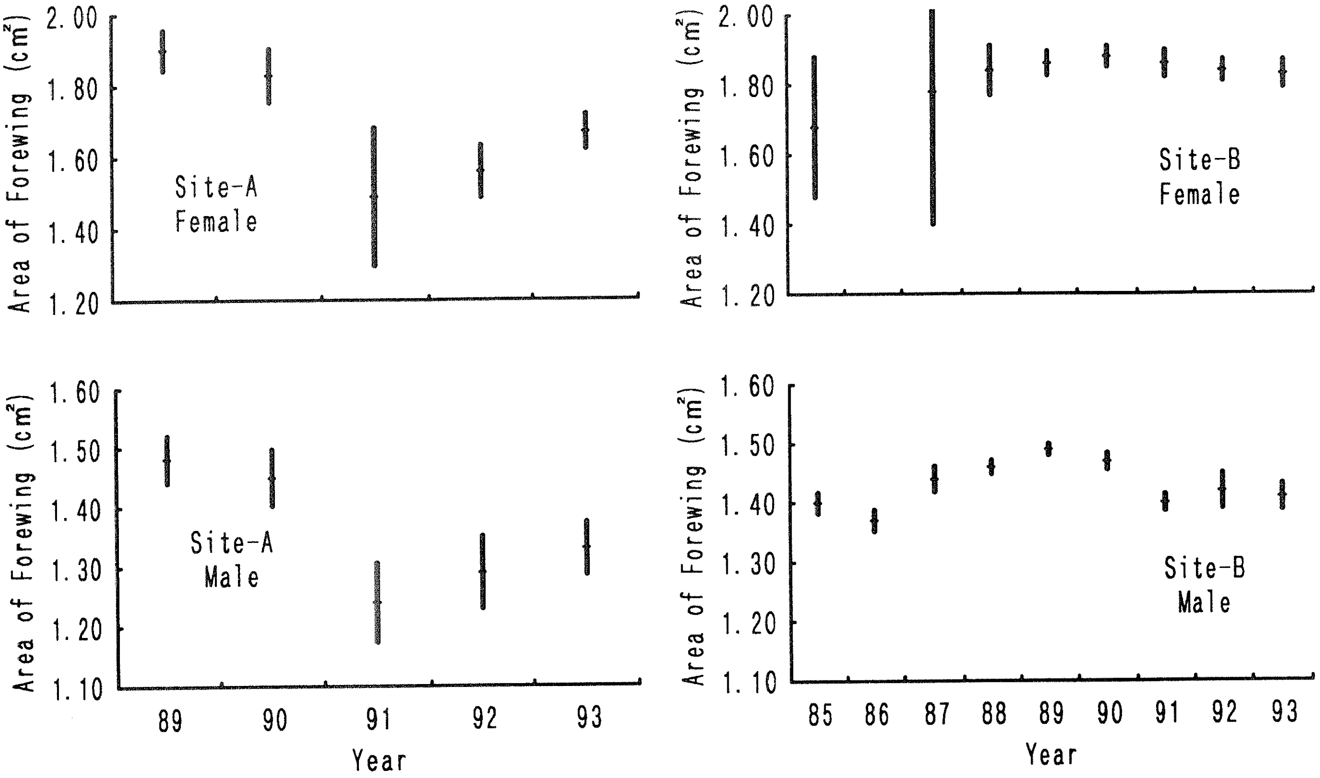

Figure 4.—Annual changes in adult size of *Q. punctatella* caught in light traps in Site A (Hakkohda) and Site B (Hachimantai) (mean ± 95% confidence intervals). Area of fore wing, which has a strong relationship to both fecundity and adult weight, is shown for each sex.

Defoliation Experiments

Quantitative Changes in Beech Leaves and Food Limitation During an Outbreak Period

The mean size and biomass of leaves declined precipitously after defoliation (Fig. 5). The 1-year treatments caused a 46.1% decline (ca. 33.4 to 18.0 mg) in mean leaf dry weight from 1991-1992, and a 32.3% decline (ca. 41.2 to 30.8 mg) from 1992-1993. Following the second year of defoliation (i.e., in 1992), biomass declined further to ca. 12 mg/leaf in 1993, a 64.9% drop from the 1991 mean weight (Fig. 5a). Leaf density or dry weight of 10 leaf disks (31.4 cm^2 total) from defoliated trees followed the same pattern: 1-year treatments caused a 14.0% decline (108 to 92.8 mg/ 10 disks) from 1991-1992, and an 8.0% decline (104 to 93.8 mg/ 10 disks) from 1992-1993 (Fig. 5b). The 2-year treatment caused a further decline in leaf density to ca. 81 mg/ 10 disks, > 25% down from the initial mass in 1991 (Fig. 5b). Besides a decline in leaf size, and leaf density, the number of leaves on each tree also decreased slightly after the 1-year treatment, though two of 12 trees exhibited a slight increase in leaves (Fig. 5c). Consequently, total leaf biomass per tree declined by about 50% due to the 1-year treatment (Fig. 5d).

In two plots at Site A, slight defoliation was observed in 1989, the amount of leaves fed on by *Q. punctatella* was estimated at ca. 60% of total canopy, and ca. 20% of the beech trees were completely defoliated. The following year, *Q. punctatella* increased in density and defoliated completely almost all beech trees. Food limitation was an important mortality factor in this generation because many dead larvae, which had not been killed by predators or pathogens and were supposed to have died of starvation, were scattered on the ground. Considering the results of the defoliation experiment, the biomass of leaves should have decreased in 1990 as a result of the severe defoliation the previous year. A negative feedback occurred: as herbivore density gradually increased the defoliation reduced the amount of available food in the following generation, thus acting as a limiting factor.

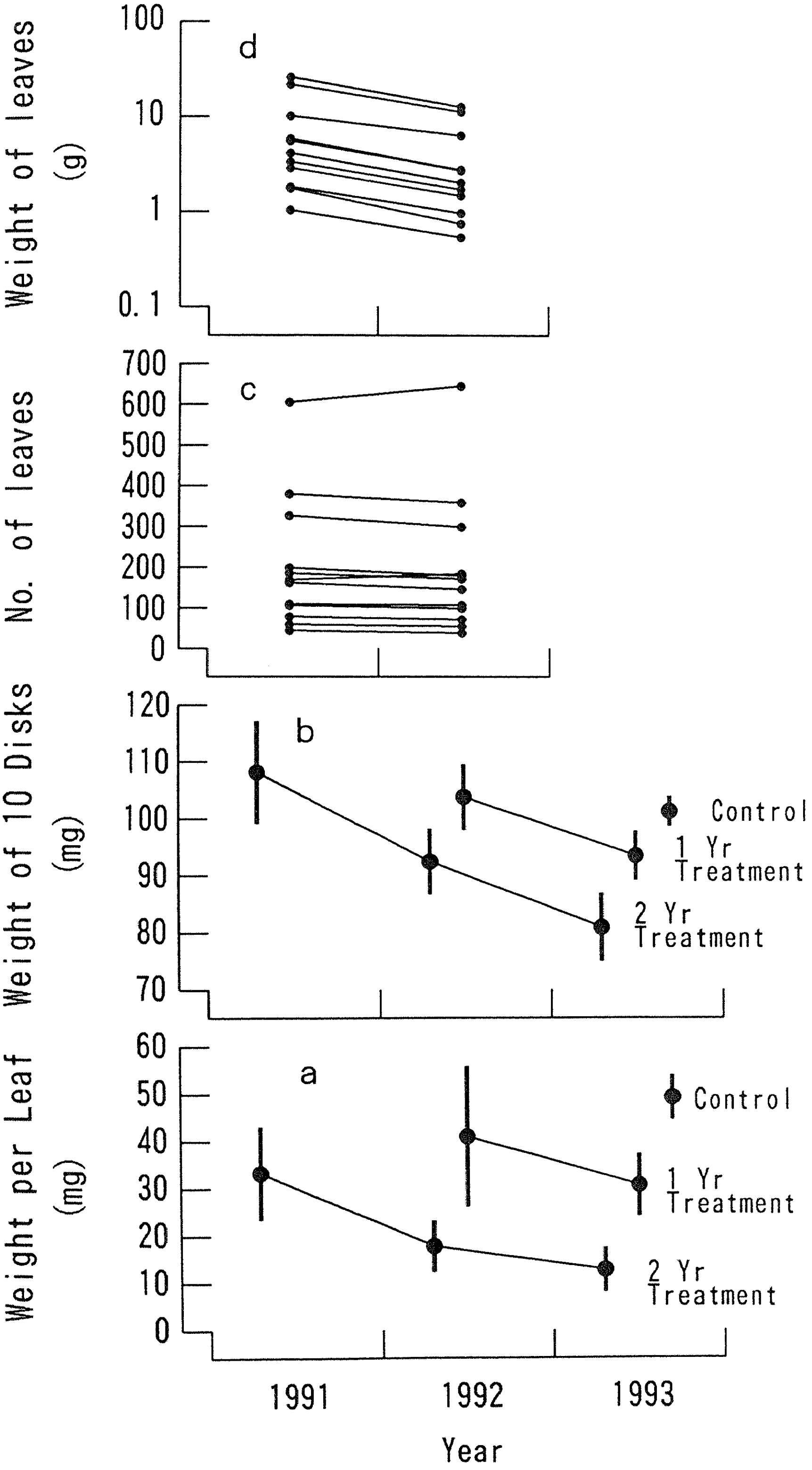

Figure 5.—Quantitative changes in beech leaves in the year following artificial defoliation. a: Dry weight per leaf, b: Index of leaf density measured by dry weight of 10 leaf disks (2 cm in diameter), c: Number of leaves in each tree, d: Dry weight of all leaves in each tree.

Qualitative Changes in Leaves and Larval Growth

Leaf toughness, like leaf density, decreased following clipping of the leaves: it was only 85.7% of the control the year following the 1 year treatment and 69.8% of the control after the 2 year treatment (Fig. 6). Nitrogen content was almost the same in untreated trees for 3 years (1991-1993) but decreased after leaves were clipped. It was 2.23-2.26% before the treatment, 2.00-2.05% the year following the 1 year treatment (ca. 10% reduction), and 1.88% the year following the 2 year treatment (16.6% reduction) (Fig. 7a). The N content following the 1 year treatment was almost the same in 1992 and 1993. These results clearly indicate that nitrogen content is reduced by defoliation and is influenced not by the year but by the degree of defoliation. Tannin content, however, increased the year following leaf clipping (Fig. 7b). When 2 mg of BSA was added, about 0.4 mg of the protein was precipitated in the regimes of 2 year treatment and 1 year treatment, although precipitation was not detected in the control regime. Furthermore, when 3 mg of BSA was added, no precipitation was recognized in the 1 year treatment or the control regimes, but 0.43 mg of the protein was precipitated following the 2 year treatment. These quantitative and qualitative changes in beech following defoliation support that the trees were less vigorous after defoliation, and also less suitable for herbivores because of the declining nitrogen and increasing tannins.

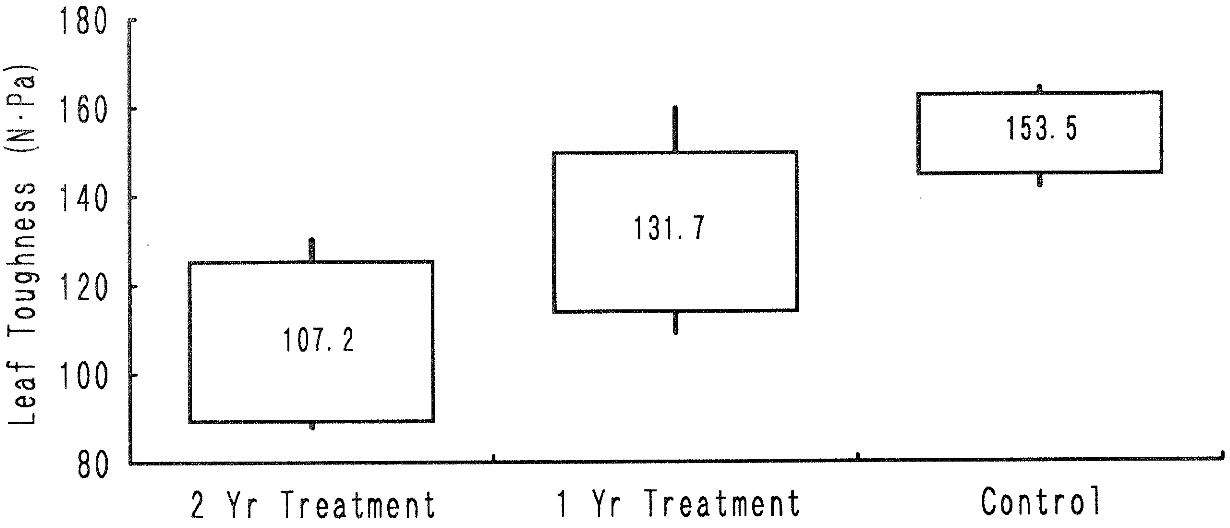

Figure 6.—Leaf toughness of beech leaves in the year following artificial defoliation. The number is a mean value, bar means maximum and minimum value, and upper and lower sides of box are one standard error.

Survivorship rates on beech saplings were determined using the number of hatched larvae as a base. The x-axis in Figure 8 is the number of days following egg attachment on June 30th. Although approximately half of the larvae reared on the control saplings survived to maturity, only about 10% of those reared on the 2 year treatment trees survived to maturity. Many of the latter died during early stages of larval development. Survivorship on the 1 year treatment was intermediate between the 2 year treatment and the control. Both female and male body size declined for larvae reared on clipped saplings, as did adult body size (Fig. 9). In the 2 year treatment regime, body size was smaller than of the 1 year treatment regime, and was just three quarters of that in the control. Defoliation lowers beech quality which, in turn, lowers larval survivorship and body size.

Test for Maternal Effects and Food Deterioration in the Field

Population Quality of Herbivore in Three Sites

The larvae from Site A had smaller body size ($p < 0.01$ t-test) than those from the other two regions (Fig. 10). This tendency was exactly the same for males and females, as well as for mature larvae and for pupae. No difference was found between the Site B and C populations. Thus it can be said that even three generations after an outbreak, the quality of the Site A population was less than that of the populations from the other two regions where density was increasing.

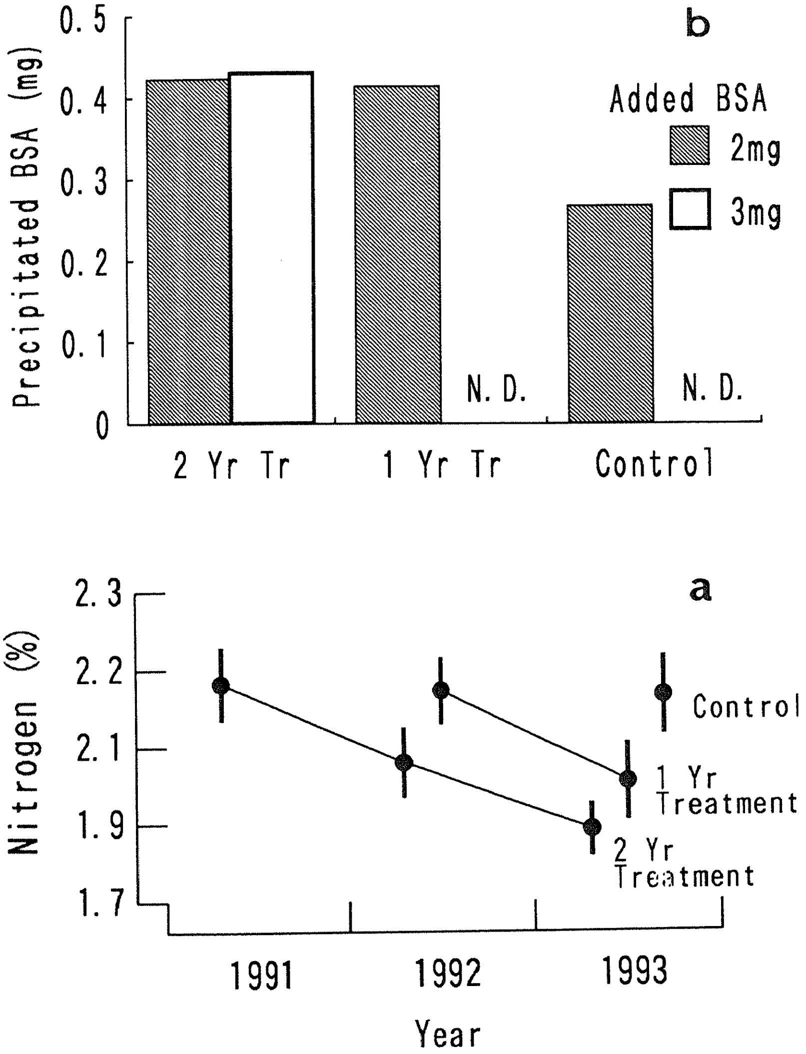

Figure 7.—Nitrogen and tannins contained in beech leaves the year following artificial defoliation. a: Total nitrogen, b: Weight of precipitated BSA by addition of 2 ml of 0.1% BSA solution (corresponding to addition of 2 mg of BSA) and 3 ml of 0.1% BSA solution (3 mg of BSA). N.D. indicates that no precipitation was detected.

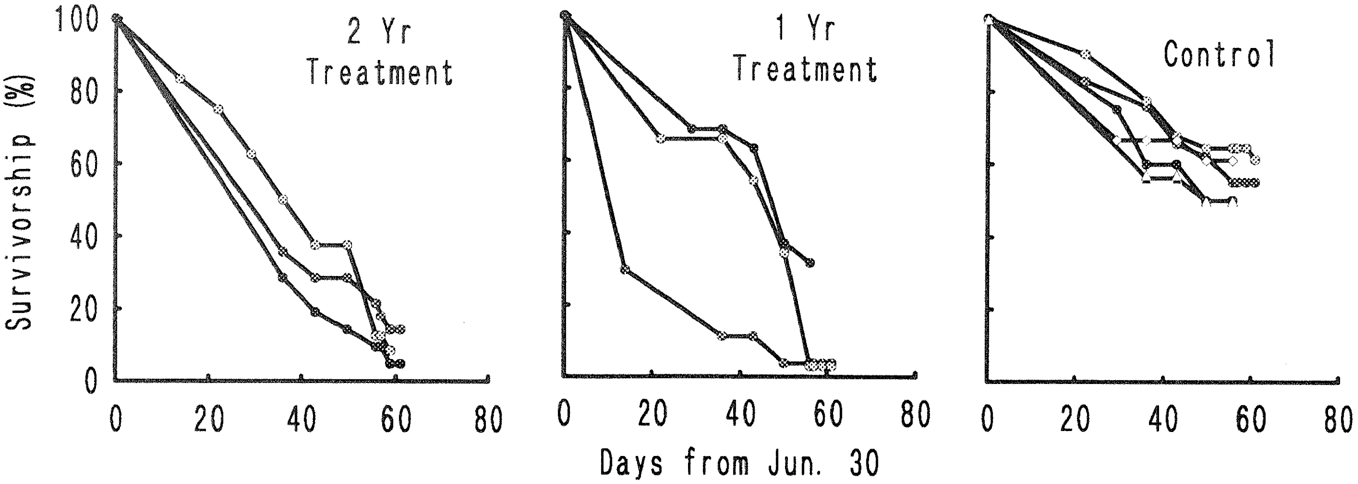

Figure 8.—Survivorship curves of *Q. punctatella* larvae reared on beech which had suffered artificial defoliation. Each line began with an egg-mass containing about 50 eggs. X-axis is the number of days following egg attachment on June 30th.

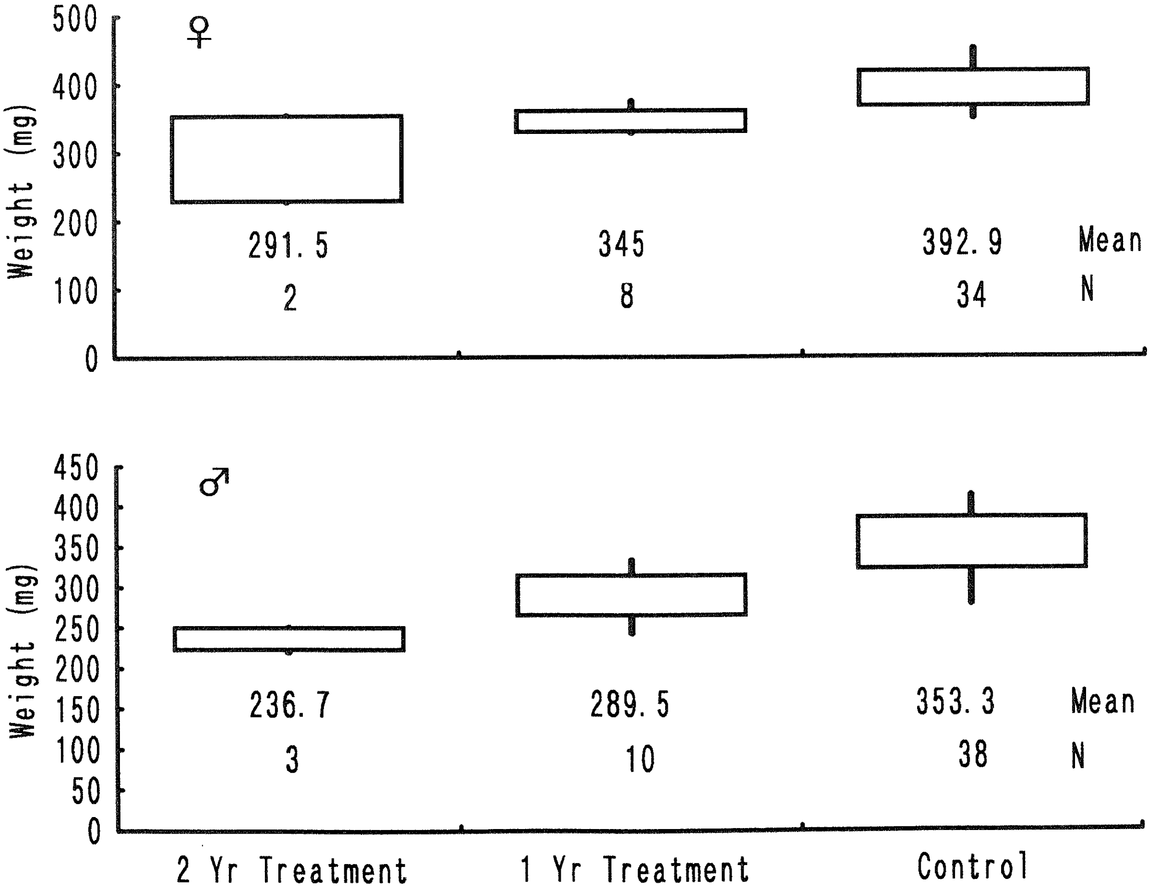

Figure 9.—Body size of *Q. punctatella* larvae reared on beech which had suffered artificial defoliation. Weight of larvae at maturation is indicated for each sex. Bar means maximum and minimum value, and upper and lower sides of box are one standard error.

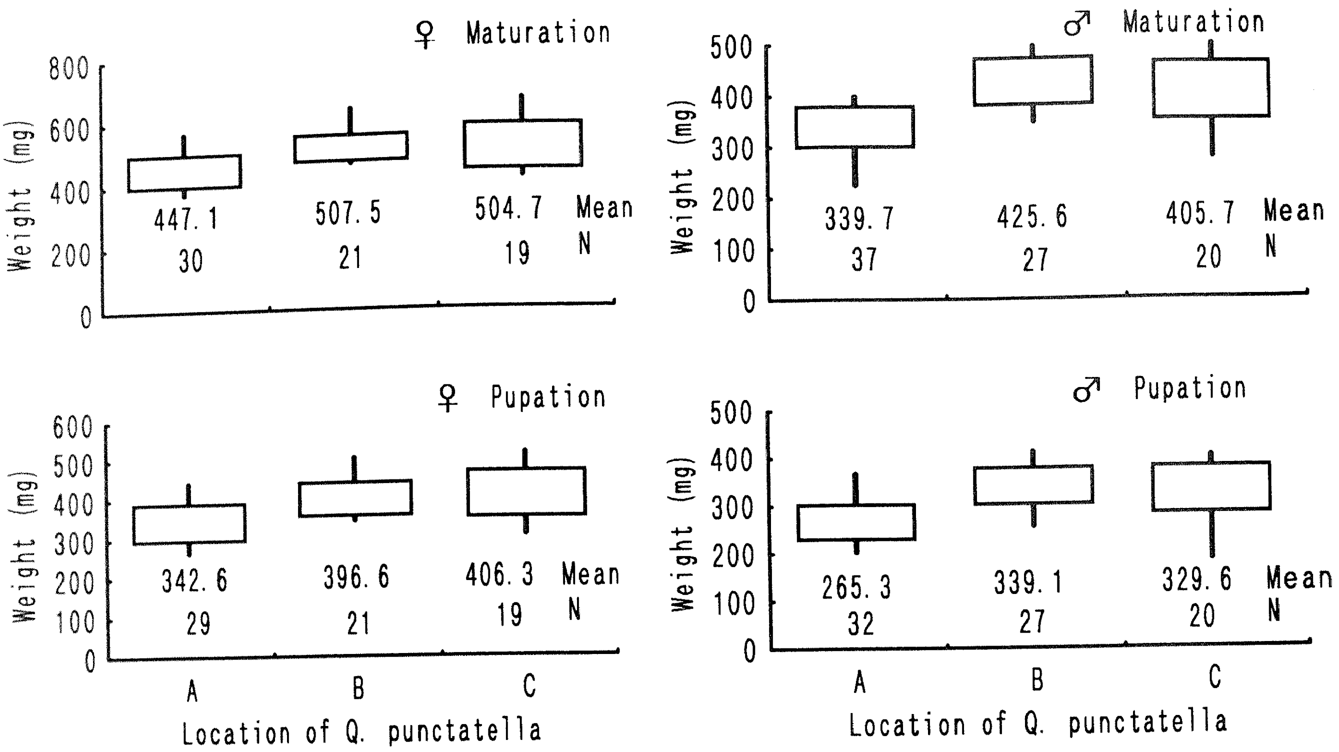

Figure 10.—Body size of three different regional *Q. punctatella* populations reared on beeches in the Tohoku Research Center of the Forestry and Forest Products Research Institute in Morioka. Weight of larvae at maturation and at pupation are indicated for each sex. Bar means maximum and minimum value, and upper and lower sides of box are one standard error.

Tests of Two Hypotheses in Field Populations

Larvae from all of the regions were reared on beech saplings in each of the respective regions. Plant quality influenced survivorship in each region. Although great differences in survivorship between regions were observed, little difference was found within a region (Table 1). Approximately half of the larvae reared on beech saplings in Sites B and C survived to maturity, whereas survivorship of all those reared on Site A beech was between 3.2% and 6.5%. The influence of *Q. punctatella* population quality in each region on body size was clearly evident. Irrespective of the location of beech saplings on which the larvae were reared, the order of average body size among the regional populations was the same: B ≥ C ≥ A (Table 2). In particular, body size of A population larvae was significantly smaller than that of larvae from the other

Table 1.—Survivorship of *Q. punctatella* larvae from each region on beech in that region.

		Q. punctatella		
		A	B	C
	A	3.6% (6/165)	3.2% (5/155)	6.5% (10/154)
F. crenata	B	48.9% (44/90)	55.0% (33/60)	53.9% (41/76)
	C	55.8% (72/129)		52.9% (36/68)

(No. of matured larvae / No. of hatched larvae)

Table 2.—Body size of *Q. punctatella* from each region on beech in that region. Weight of larvae at maturation is shown for each sex.

Female		*Q. punctatella*		
		A	B	C
F. crenata	A	376.3±68.5	442.0±15.6	414.4±48.9
		388	453	489
		369	431	360
		3	2	5
	B	398.5±75.3	484.5±108.9	451.3±58.8
		576	739	554
		309	333	340
		17	17	22
	C	392.9±26.3		455.0±68.5
		451		550
		351		342
		34		14
Male		*Q. punctatella*		
		A	B	C
F. crenata	A	288.3±60.5	369.7±15.6	336.8±41.3
		349	384	398
		228	353	298
		3	3	5
	B	294.5±63.6	384.9±36.99	335.3±45.2
		416	469	409
		204	332	254
		27	16	19
	C	353.3±31.9		396.0±39.0
		412		450
		279		299
		38		22
		Mean±SD		
		Max		
		Min		
		N		

two regional populations ($p < 0.01$ t-test). The influence of beech on body size was not as apparent as the population effect: female body size of larvae reared on Site B beech was close to that of larvae reared on Site C beech, while male body size of larvae reared on Site B beech was similar to that of those reared on Site A beech.

Leaf properties were compared among the three sites (Fig. 11), and weight per leaf among the three was almost the same. Leaves of Site A beech were significantly tougher than those of the other two sites, though they were much thinner than Site C leaves and almost the same as the Site B leaves. A remarkable point is that nitrogen content was significantly higher in leaves of Site A than in those of the other two sites (Fig. 12). Tannin content also increased greatly. Judging from the high N content of beeches in Site A, where severe defoliation had occurred three years before our experiment, trees had

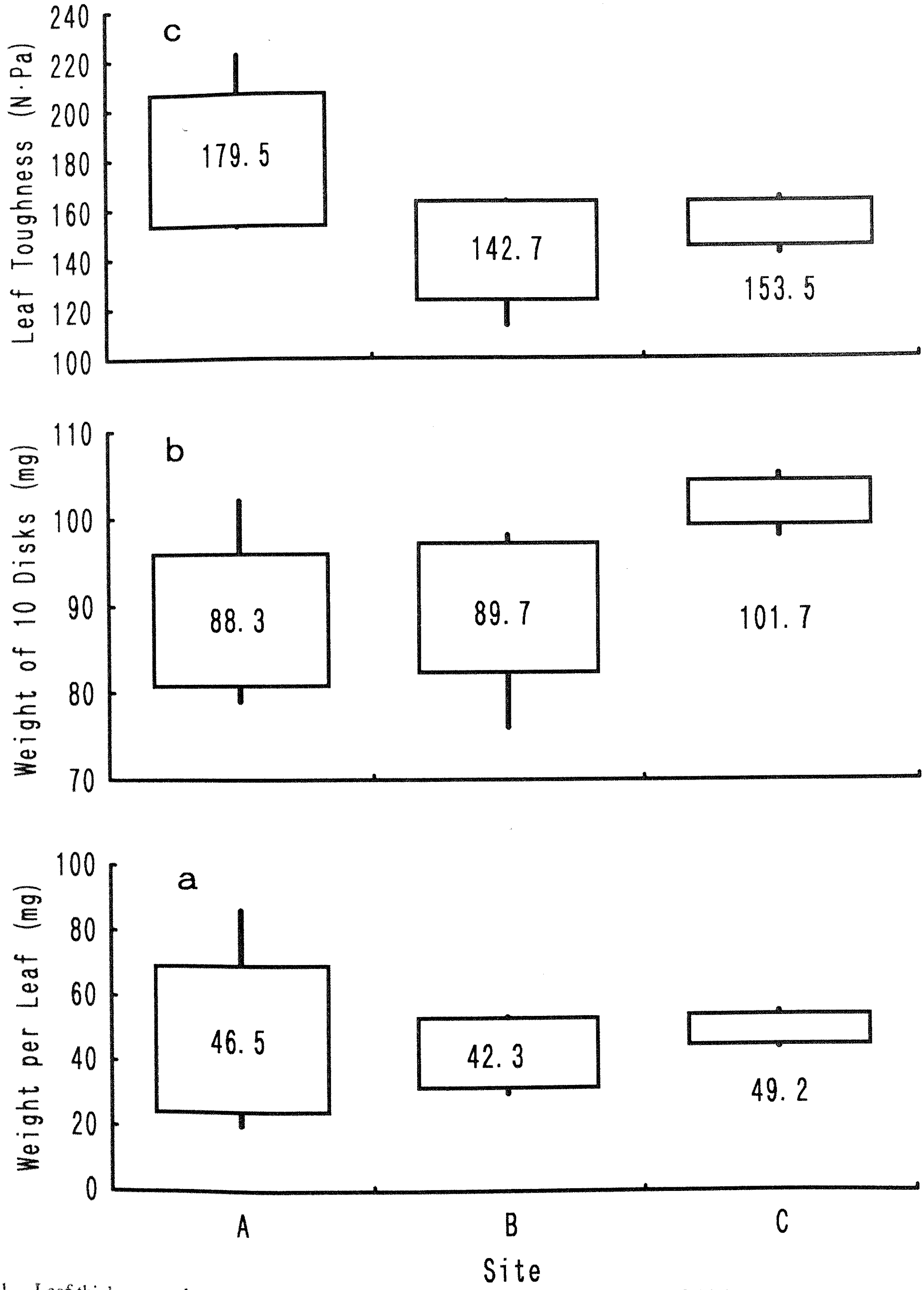

Figure 11.—Leaf thickness and toughness of beech leaves in three different regions. Leaf thickness was represented by dry weight of 10 leaf disks (2 cm in diameter). The number is a mean value, bar means maximum and minimum value, and upper and lower sides of box are one standard error.

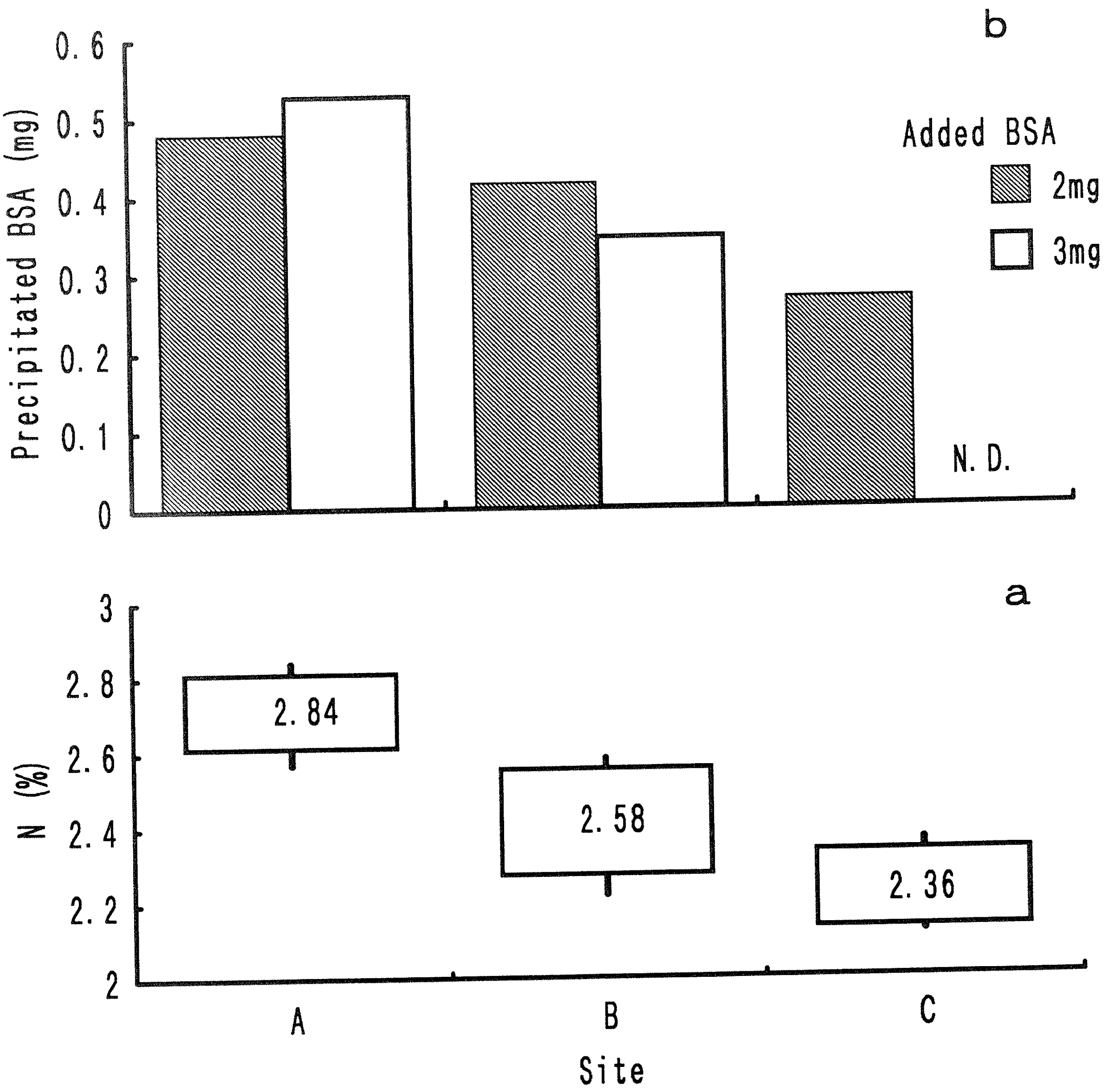

Figure 12.—Nitrogen and tannins contained in beech leaves in three different regions. a: Total nitrogen (The number is a mean value, bar means maximum and minimum value, and upper and lower sides of box are one standard error), b: Weight of precipitated BSA when added at 2 mg and 3 mg BSA. N.D. indicates that no precipitation was detected.

become vigorous. However, since the trees had also increased their defensive secondary compounds, they had also become less suitable for herbivores, and insect mortality was higher than in trees in the other two sites.

DISCUSSION

Self regulation by reducing adult body size and curtailing egg production in insects during an outbreak with resultant declines in the initial number of individuals in the next generation is thought to be one of the major factors governing the population dynamics of insects (Klomp 1966, Dempster and Pollard 1981). This has been shown to be a consequence, among other factors, of insufficient food supply due to complete defoliation of the host, as well as a density dependent outcome resulting from conditions experienced during larval development. However, this phenomenon is not restricted solely to the outbreak generation. Many insects have been shown to have body size changes associated with their population density dynamics. In other words, during a period when population density is increasing, body size also increases, and the reverse occurs when population density is declining, body size decreases also (Baltensweiler and Fischlin 1988, Myers 1990). In *Q. punctatella*, body size changed in the same way as the density. Changes in fecundity caused by these changes in body size were probably one factor causing the population fluctuation.

Because the body size of *Q. punctatella* at Site A before the outbreak was approximately the same as that for the Site B population, the current small body size of the former is thought not to be genetically determined. Body size at Site A dropped to 70% of the norm the year following the outbreak and was 80% even two generations after the outbreak. This cannot not be explained solely by a lack of food during the outbreak. The results indicate that beech which has experienced defoliation the previous year is a poor host, causing both survivorship and body size of larvae to decline. Even though 3 years had passed since the outbreak at Site A, larvae reared on Site A beech still had low survivorship. Corresponding to host quality, *Q. punctatella* quality was still bad in Site A. Even when *Q. punctatella* populations from this site were reared on beech from the other sites where density was increasing, the bodies of these larvae were still significantly smaller. High mortality, due to deterioration of food and small body size, and low egg production due to poor *Q. punctatella* quality, explains why its density continued to decline years after the outbreak. Several authors have demonstrated that poor nutritional status in one generation may deter normal development in the next. Examples include *Lymantria dispar* (Kovasevic 1956), *Malacosoma pluviale* (Wellington 1965), and *Hyphantria cunea* (Morris 1967). Such intergenerational, cumulative effects are called "maternal effects" (Rossiter 1992). Rossiter (1992) states "maternal effects are the result of resource provisioning by one generation for the next... The resource based maternal effects are the product of gene-environment interactions experienced in the parental generation." Deterioration in *Q. punctatella* quality after the outbreak was the very result caused by maternal effects.

Haukiojia and Neuvonen (1987) proved the hypothesis that poor food may select for individuals with superior ability to process low-quality diets. However, experiments conducted here did not confirm this: the survivorship of Site-A *Q. punctatella* was significantly lower than that of Site C *Q. punctatella*, and was almost the same as that of Site B *Q. punctatella* on poor-quality Site A beech.

Concerning the food deterioration hypothesis, it is necessary to distinguish between induced changes in secondary compounds such as tannin, which are an active means of defense (induced defense hypothesis) and that of passive changes in secondary chemistry following episodes of defoliation (Myers 1988). The defense strategy of beech, in relation to severe defoliation, changed as time passed after an outbreak, though in each case it resulted in high mortality and small body size of *Q. punctatella*. In the year following defoliation, beech became less vigorous and the defense response was a passive one (food deterioration): tannins increased and nitrogen content decreased. Next, trees became more vigorous and the defensive strategy changed to a more active one; both nitrogen and tannin content increased.

The results on Site B beech were unexplainable by this scenario; nitrogen content was lower than Site A beech and tannin content was almost the same, but the tree performance was comparable to Site C beech for rearing *Q. punctatella*. These results indicate that it is very dangerous to discuss the performance of plants by measuring only nitrogen or secondary compounds. Clancy (1991) also demonstrated that Douglas fir trees susceptible to the western spruce budworm had lower levels of foliar nitrogen and sugars than resistant trees, and that the susceptible trees had mineral/nitrogen ratios which were closer to optimal levels. Not only secondary compounds such as phenols and tannins but also minerals thus must be taken into consideration.

Nitrogen content in untreated trees at Site A was nearly the same in all 3 years (1991-1993) and that in beeches following 1 year of defoliation was the same for 2 years (1992-1993). It differed greatly among the three sites, but variance within a site was very minimal. Beech leaves at outbreak Sites A and B showed higher nitrogen content than at the non-outbreak Site C. There is a possibility that nitrogen level is linked to the site dependent outbreak characteristics of *Q. punctatella*.

The induced defense hypothesis advocates the following scenario. Due to the decline in food quality after an outbreak, succeeding generations decline, that is, insect growth is checked and population numbers decrease. With the recovery of plant quality, insect population density begins to increase again, thus creating the cyclical population dynamics of these insects. Two different types of beech response were recognized in the decline in food quality as time passed after an outbreak (Fig. 13). Beech becomes less vigorous soon after severe defoliation, and nitrogen content decreases but defensive

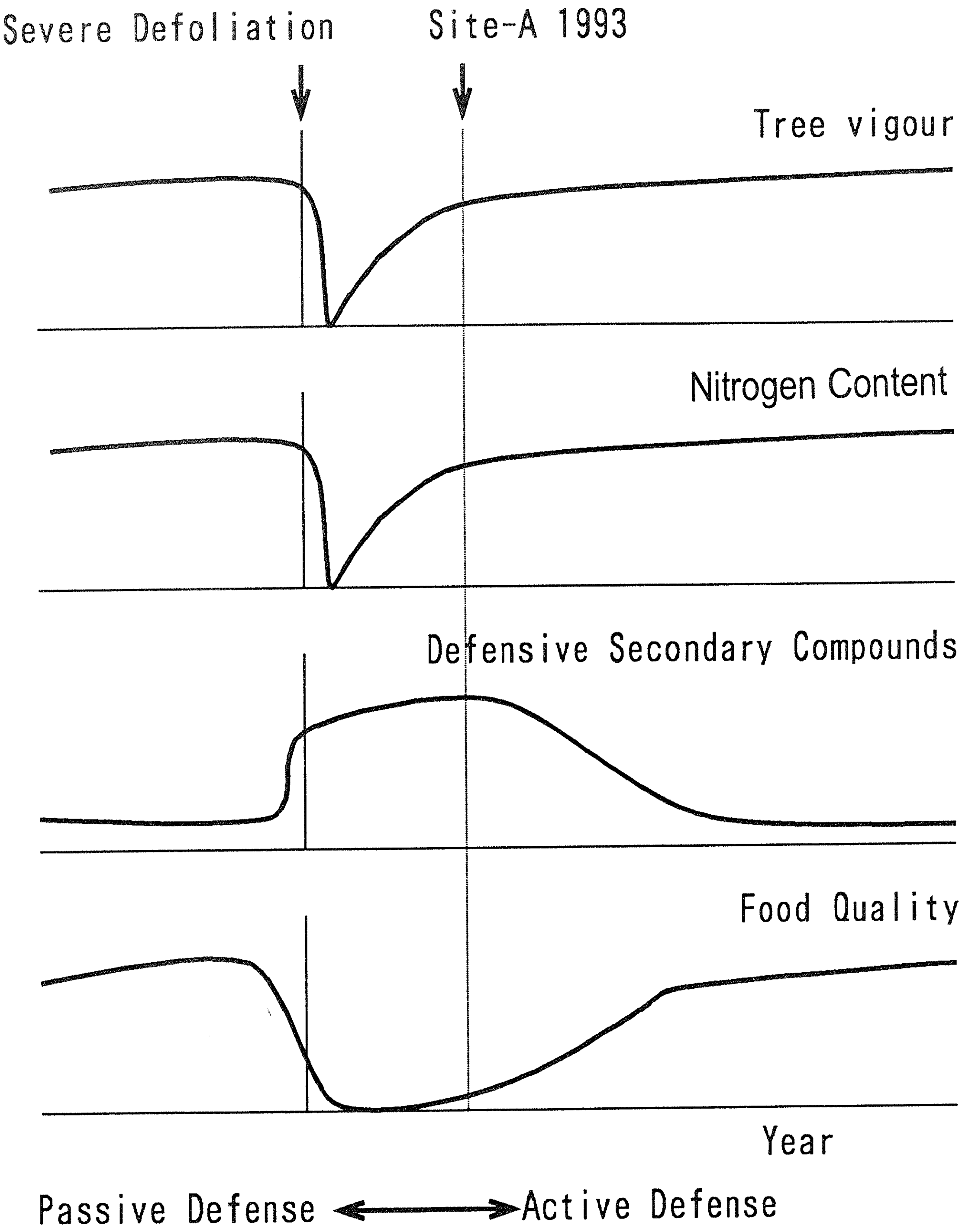

Figure 13.—Schema of defense response of beech after severe defoliation.

secondary compounds increase. This rather passive food deterioration causes high mortality and smaller body size in the *Q. punctatella* population. Thus, *Q. punctatella* density decreases and beech is released from severe herbivory pressure. Beech gradually becomes vigorous again and its defense strategy changes to a more active one; defensive compounds such as tannins increase, though nitrogen content also recovers. Such beeches are not suitable for herbivores, which results in high mortality of *Q. punctatella*.

SUMMARY

Body size of *Q. punctatella* at first increased with population density, but became very small the year following an outbreak. Its size was still small 3 years afterwards. Because adult size greatly influences fecundity, this was a factor in keeping *Q. punctatella* density low for several years after an outbreak. As for food quantity, severe defoliation caused a decrease in the amount of leaves the following year, and this limited the peak density. The defense strategy of beech in relation to severe defoliation changed as time passed. In the year following severe defoliation, nitrogen content decreased and tannins increased; this resulted in high mortality and small body size in *Q. punctatella*. Three years after an outbreak, nitrogen content recovered to the same level as prior to defoliation, but tannins were even higher. This active, induced defense kept larval survivorship low. Maternal effects were also recognized; the quality of *Q. punctatella* was poor even 3 years after an outbreak. Thus, food deterioration and the correlated results of maternal effects suppressed *Q. punctatella* density for several years after an outbreak.

ACKNOWLEDGMENTS

We would like to sincerely thank Dr. Garry J. Piller (School of Environmental Earth Sciences, Hokkaido University) for his kind advice and discussion.

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THE IMPACT OF FLOWERING ON THE SUITABILITY OF BALSAM FIR FOR SPRUCE BUDWORM VARIES WITH LARVAL FEEDING BEHAVIOR

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INTRODUCTION

The production of staminate flowers by coniferous trees is known to affect the fitness of insect defoliators. For instance, spruce budworm, *Choristoneura fumiferana* Clem., larvae on flowering balsam fir, *Abies balsamea* (L.) Miller, had 3 to 7 days shorter development times than those on nonflowering trees (Jaynes and Speers 1949, Blais 1952, Greenbank 1963). Based on this effect, Blais (1952) hypothesized that flowering of balsam fir could trigger spruce budworm outbreaks.

The impact of flowering on budworm survival and fecundity varies greatly from one study to another. Blais (1952) found no significant effect of flowering on spruce budworm survival, while Mattson *et al.* (1991) detected a 25% increase in larval survival when insects were reared on balsam fir branches bearing staminate flowers compared to those on nonflowering branches. Moreover, balsam fir flowering had no effect on the fecundity of spruce budworm (Blais 1952), whereas *Pinus banksiana* Lamb. flowering had a negative effect on the fecundity of the jack pine budworm, *Choristoneura pinus*. Although there is no agreement on whether flowering positively or negatively affects budworm mortality and fecundity, most authors believe that the impact of flowering results from either the high nutritive value of the pollen and/or the presence of staminate flower clusters that may provide a more favorable micro-habitat in term of heat and protection from natural enemies (Lejeune and Black 1950, Wellington 1950, Blais 1952, Greenbank 1963, Mattson *et al.* 1991).

In regard to budworm dynamics, the production of staminate flowers can be followed, the next year, by either an increase (Graham 1935, Blais 1952, Volney 1988) or a decrease (Batzer and Jennings 1980, Bauce 1986) in budworm population density. In order to explain such opposite trends, we hypothesize and test here that the impact of flowering on the suitability of balsam fir for budworm larvae varies according to the feeding behavior of the larvae, which is affected by the density of budworm population (Blais 1952).

METHODS

Field Rearing Experiment

The impact of flowering on spruce budworm growth, development, survival, and fecundity was determined by caging second instar larvae in the mid- and lower-crowns of flowering and nonflowering balsam fir trees. In 1992, a total of 10 flowering and 10 nonflowering dominant balsam fir trees were randomly selected in a 60 yr-old balsam fir stand located in compartment 20 of the Forêt Montmorency (47° 19' N, 79° 09' W), an experimental forest of Université Laval. The stand fits Grandtner's (1966) description of the balsam fir-white birch association and was classified as site 1 quality with good drainage (Bélanger *et al.* 1983), 15% slope, deep uncompacted glacial till, and 65% crown cover.

A severe drought in 1991 triggered an intense production of staminate flowers the following spring. Trees located near streams, or not fully exposed to sunlight did not produce staminate flowers. On each sample trees, two 90 cm long branches, facing north-northwest, were selected at the mid and lower third sections of the crown. Staminate flowers were present only in the midcrown of flowering trees. Reproductive buds burst before vegetative buds and the pollen was dispersed during a period of 3 to 4 days. Each branch was enclosed with a fine-mesh cloth sleeve cage which served as an

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Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

enclosure for 20 post-diapausing second instar larvae. Larvae were placed in the cages when 100°C-days were attained, or approximately 2 weeks prior to opening of vegetative buds.

Sample branches were cut when budworms reached pupal stage. Pupae were weighed, moths were mated, the number and the weight of eggs laid were recorded every day during the oviposition period. The progeny were placed at 2°C for a 29 week long diapause period, and the survival of the progeny was recorded at the end of diapause. On each branch, the weight of current-year foliage produced by the tree, and the weight of current-year and 1-year old foliage removed by the larvae were estimated.

Data were analyzed using the Statistical Analysis System (Sas Institute 1988). Normality and variance homogeneity tests were performed before data were subjected to a nested-factorial, multivariate analysis of variance with individual trees nested within flowering class and crown sections crossed with flowering class. Percent survival was analyzed using Chi-square analysis.

Laboratory Rearing Experiment

The impact of spruce budworm feeding behavior on the effects of flowering on the insect growth and development was determined using laboratory rearing experiments with fresh food from the field. Also, this approach allowed us to separate the food effect from the microhabitat effect provided by the staminate flower clusters.

A total of eight feeding scenarios were simulated under laboratory conditions (T = 12°C, RH = 65%, 18L:6D) using the methodology of Bauce *et al.* (1994). These conditions corresponded to the average conditions prevailing at the experimental field site during the period of spruce budworm feeding activity. The eight feeding scenarios were (1) larvae fed on staminate flowers during 4 days, and current-year foliage from the midcrown of flowering trees thereafter, (2) larvae mined 1-year old needles until vegetative budbreak, and fed on current-year foliage from the lower crown section of flowering trees thereafter, (3) larvae fed on staminate flowers during 4 days, current-year foliage from the midcrown of flowering trees until they reached their larval sixth instar, and 1-year old foliage from the midcrown of flowering trees thereafter, (4) larvae fed on staminate flowers during 4 days, current-year foliage from the midcrown of flowering trees until they reached their larval sixth instar, and current-year foliage from the lower section of flowering trees thereafter, (5) larvae mined 1-year old needles until vegetative budbreak, and fed on current-year foliage from the midcrown of nonflowering trees thereafter, (6) larvae mined 1-year old needles until vegetative budbreak and fed on current-year foliage from the lower crown section of nonflowering trees thereafter, (7) larvae mined 1-year old needles until vegetative budbreak, fed on current-year foliage from the midcrown of nonflowering trees until they reached their larval sixth instar, and 1-year old foliage from the midcrown of nonflowering trees thereafter, and (8) larvae mined 1-year-old needles until vegetative budbreak, fed on current-year foliage from the mid-crown of nonflowering trees until they reached their larval sixth instar, and current-year foliage from the lower crown section of nonflowering trees thereafter. The scenarios 3, 4, 7, and 8 simulated a lack of current-year foliage when larvae reach sixth instar. This phenomenon usually occurs when larval density is high. Scenarios 3 and 7 simulated a backfeeding (feeding on 1-year old needles) while scenarios 4 and 8 simulated dispersal of the population from the midcrown to the lower crown section. Backfeeding and dispersal to the lower crown section were observed by Blais (1952) at high budworm population density.

Two trees served as food source for two groups of 25 individually reared female larvae per feeding scenario. The food was replaced in the rearing containers at 2-d intervals and budworm growth and development were recorded for each instar separately. Insect development was monitored 24 hours a day every 6 hours and every 15 min during the moult. One hour after molting from one instar to the next, before feeding was resumed, each larvae was weighted along with he newly molted larval skin and then transferred to a new twig. Pupae were weighed 8 hours after pupation. Normality and variance homogeneity tests were performed before data were subjected to a two-stages nested multivariate analysis of variance with individual trees nested within feeding scenarios.

RESULTS

Field Rearing Experiment

The production of staminate flowers significantly affected spruce budworm larval biology (♀: Wilk's lambda F[2,17] = 18.3, p = 0.0001, ♂: Wilk's lambda F[2,17] = 13.1, p = 0.0004) (Fig. 1, 2). Larval biology also significantly varied according to crown sections (♀: Wilk's lambda F[2,17] = 458.6, p = 0.0001; ♂: Wilk's lambda F[2,17] = 331.8, p = 0.0001). However, the interaction between flowering class and crown section was significant (♀: Wilk's lambda F[2,17] = 329.3, p = 0.0001; ♂: Wilk's lambda F[2,17] = 549.9, p = 0.0001). This significant interaction results from the fact that flowering affected budworm biology only in the midcrown of flowering trees where staminate flowers were available for consumption (Figs. 1, 2).

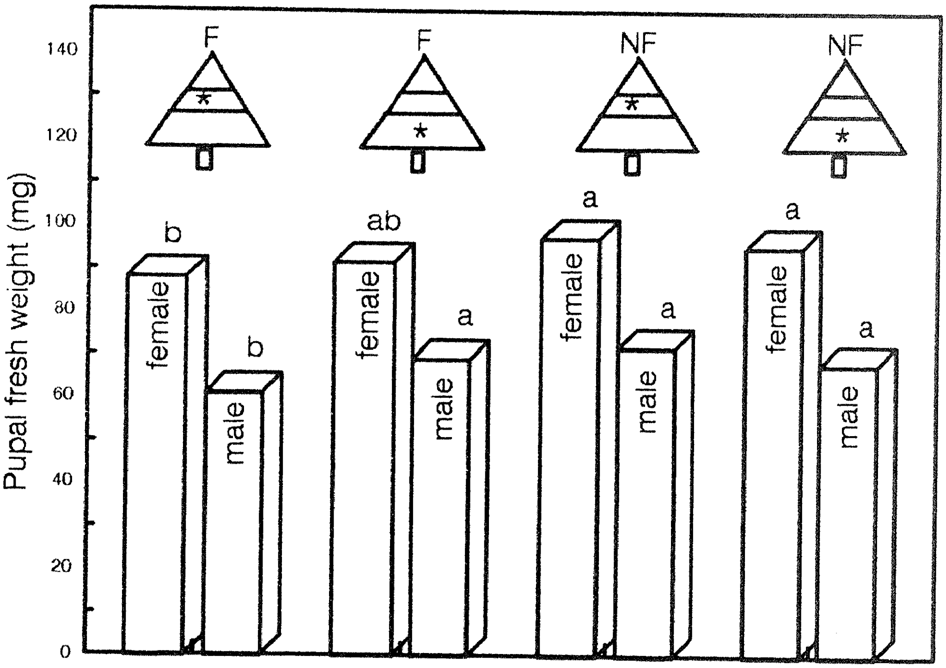

Figure 1.—Pupal weight of male and female spruce budworm reared in the midcrown and the lower sections (*) of flowering (F) and nonflowering (NF) balsam fir trees. Staminate flowers were present only in the midcrown of flowering trees (n = 10 trees). For a given sex, means followed by the same letter are not significantly different ($p < 0.05$).

Both male and female budworms that had access to staminate flowers in the midcrown of flowering trees exhibited 5 d shorter development time (♀: F[1,18] = 32.7, p = 0.0001; ♂: F[1,18] = 27.1, p = 0.0001) and 9% (♀) to 15% (♂) reduced pupal weight (♀: F[1,18] = 12.3, p = 0.0026; ♂: F[1,18] = 4.9, p = 0.0397) compared to those located where staminate flowers were not available for consumption. However, no significant impact of flowering on budworm survival was detected (Chi-square = 75, df = 69, p = 0.28, n = 40) (Fig. 3).

Insects reared on flowering branches had lower fecundity (F[1,18] = 10.8, p = 0.0041) than those reared on nonflowering branches (Fig. 4), but they produced individual eggs of similar weight (F[1,8] = 1.8, p = 0.21) (Fig. 5). At the end of the 29 week long diapause period, no significant difference was detected in terms of survival of the progeny maintained at 2°C during their diapausing stage (Chi-square = 132, df = 129, p = 0.41, n = 46) (Fig. 6).

Flowering branches produced significantly less current-year foliage than nonflowering branches (F[1,18] = 4.4, p = 0.04) (Fig. 7). Although the insects reared on flowering branches removed approximately the same amount of current-year foliage as those reared on nonflowering branches (F[1,18] = 1.95, p = 0.18), they back-fed on 1-year old foliage, while the others did not (F[1,18] = 23, p = 0.0001) (Fig. 7). This indicates a possible reduction in the nutritive quality of the current-foliage produced by flowering branches.

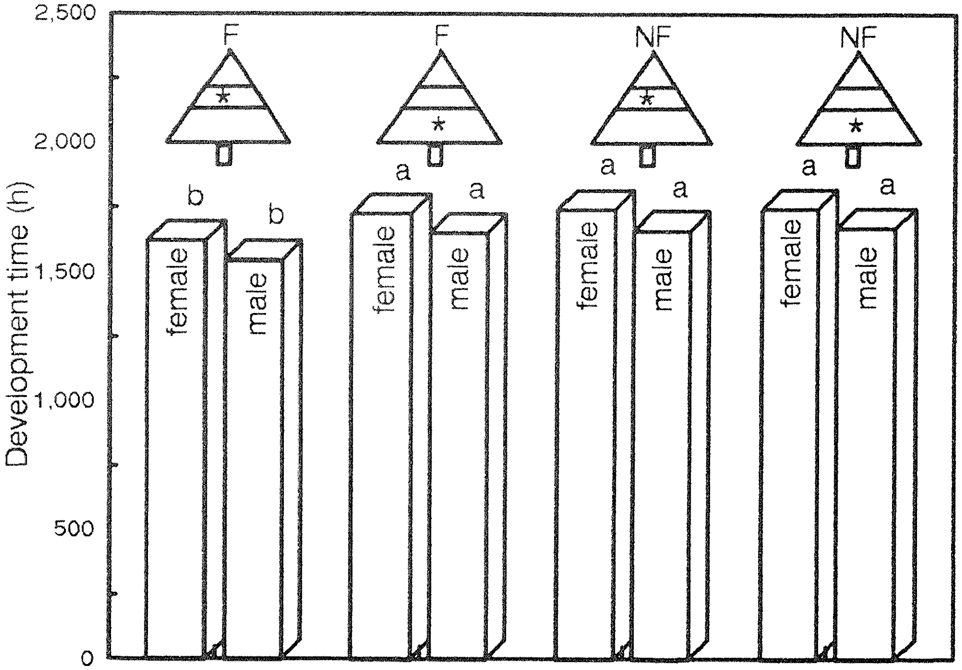

Figure 2.—Development time from post diapausing second instar to moth emergence of male and female spruce budworm reared in the midcrown and the lower sections (*) of flowering (F) and nonflowering (NF) balsam fir trees. Staminate flowers were present only in the midcrown of flowering trees (n = 10 trees). For a given sex, means followed by the same letter are not significantly different ($p < 0.05$).

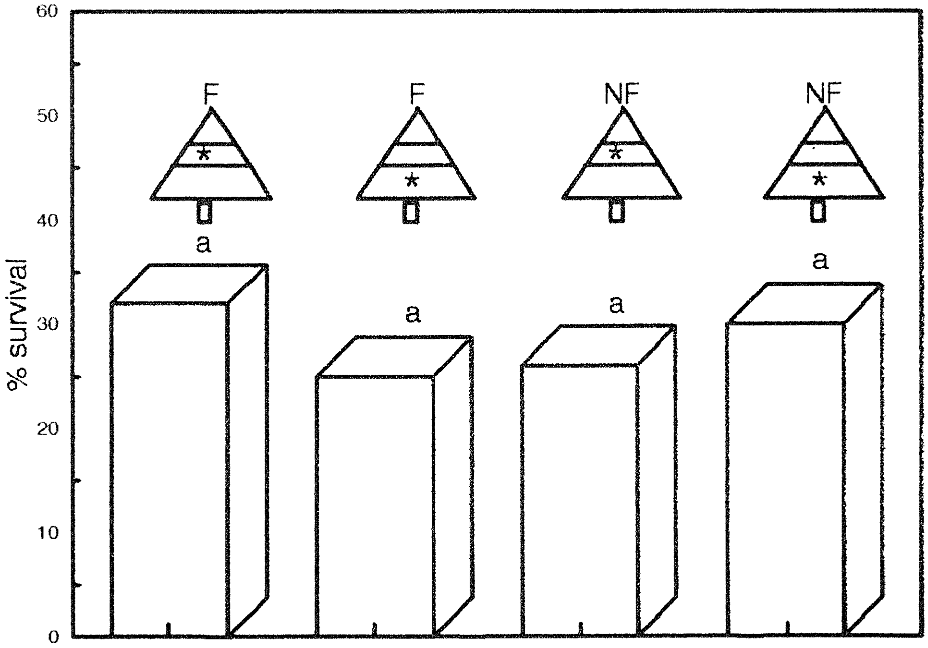

Figure 3.—Percent survival of spruce budworm reared in the midcrown and the lower sections (*) of flowering (F) and nonflowering (NF) balsam fir trees. Staminate flowers were present only in the midcrown of flowering trees (n = 10 trees). Means followed by the same letter are not significantly different ($p < 0.05$).

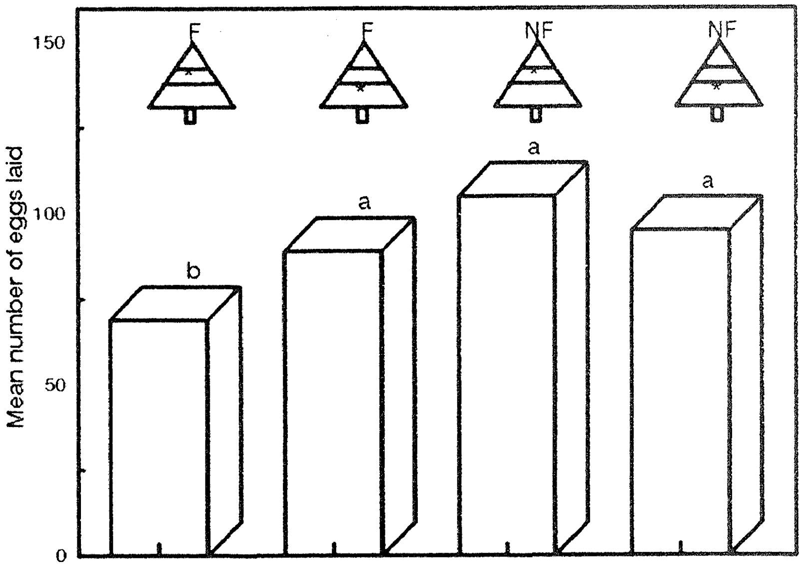

Figure 4.—Fecundity of spruce budworm reared in the midcrown and the lower sections (*) of flowering (F) and nonflowering (NF) balsam fir trees. Staminate flowers were present only in the midcrown of flowering trees (n = 10 trees). Means followed by the same letter are not significantly different ($p < 0.05$).

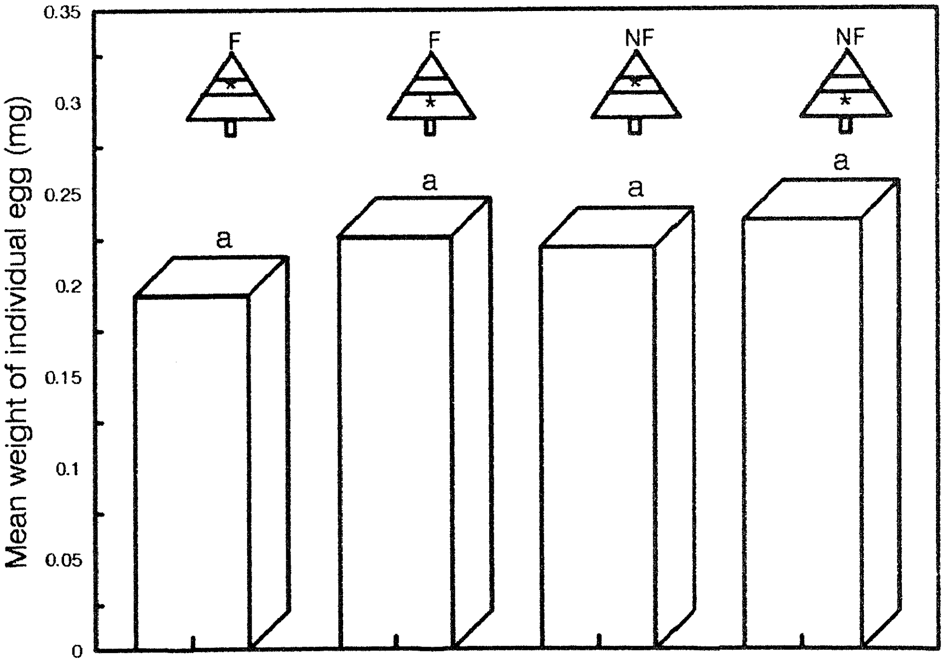

Figure 5.—Mean weight of individual eggs laid by spruce budworm reared in the midcrown and the lower sections (*) of flowering (F) and nonflowering (NF) balsam fir trees. Staminate flowers were present only in the midcrown of flowering trees (n = 10 trees). Means followed by the same letter are not significantly different ($p < 0.05$).

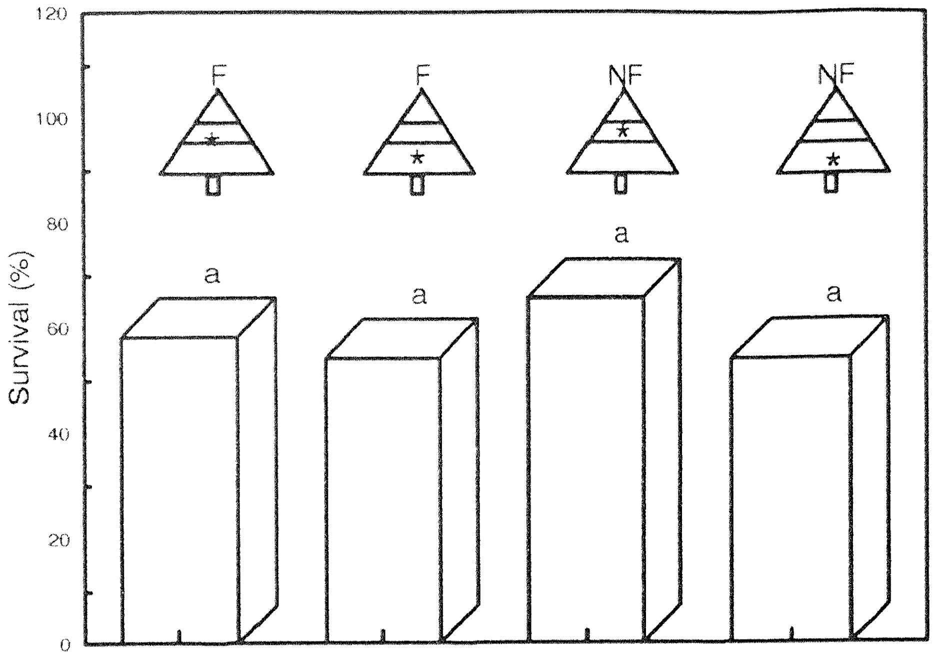

Figure 6.—Percent survival at the end of the diapause period of the progeny of spruce budworm reared in the midcrown and the lower sections (*) of flowering (F) and nonflowering (NF) balsam fir trees. Staminate flowers were present only in the midcrown of flowering trees (n = 10 trees). Means followed by the same letter are not significantly different ($p < 0.05$).

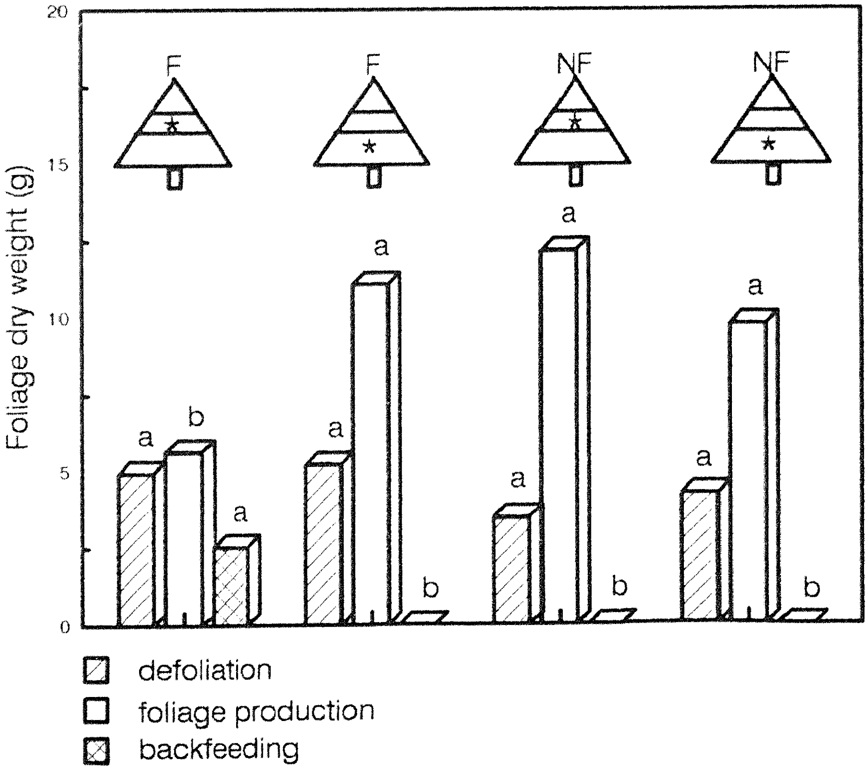

Figure 7.—Weight of current-year foliage produced by flowering (F) and nonflowering (NF) balsam fir trees at two crown levels (*), and weight of current-year and 1-year old (backfeeding) foliage consumed by spruce budworm reared on flowering and nonflowering balsam fir trees at two crown levels (*). Staminate flowers were present only in the midcrown of flowering trees (n = 10 trees). For a given parameter, means followed by the same letter are not significantly different ($p < 0.05$).

Laboratory Rearing Experiment

Results from the laboratory rearing experiment indicated that the various feeding scenarios tested in this study significantly affected female spruce budworm growth (Fig. 8) and development (Fig. 9) (Wilk's lambda F[49,15] = 4.7, p = 0.0012).

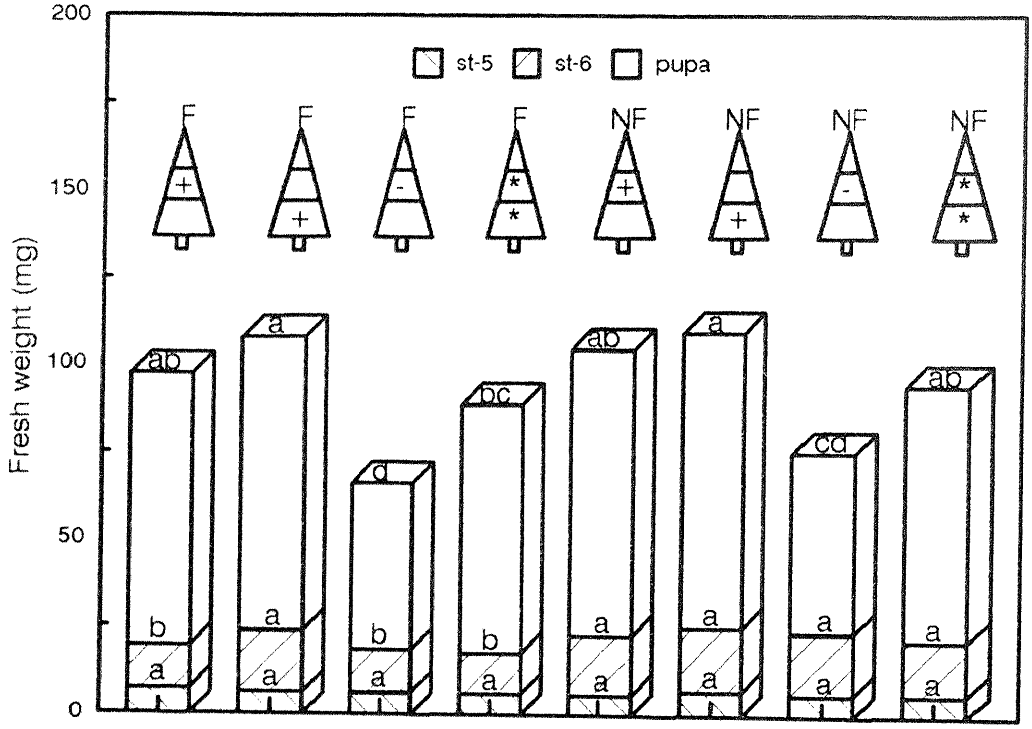

Figure 8.—Female spruce budworm weight per instar according to various feeding scenarios using fresh food from flowering (F) and from nonflowering (NF) balsam fir trees. Two trees served as food sources for two groups of 25 individually reared larvae per feeding scenario (T = 12°C, RH = 65%, 18L:6D, + larvae fed on current-year foliage from the given crown section, - larvae fed on current-year foliage prior to reaching sixth instar and 1-year old foliage thereafter, * larvae fed on current-year foliage from the midcrown prior to reaching sixth instar and current-year foliage from the lower crown section thereafter). For a given instar, means followed by the same letter are not significantly different ($p < 0.05$).

Insects fed on pollen during their early stages of development were smaller when they reached their sixth instar than those that did not have access to staminate flowers (Fig. 8). However, they had similar pupal weight as those that did not eat pollen but had access to current-year foliage during their whole development. The presence of pollen in the insect diet caused a significant 5 d reduction in development time prior to reaching sixth instar, and a significant 3 d reduction during pupal stage (Fig. 9). Although backfeeding during the insect sixth instar did not significantly affect development time, it reduced the pupal weight of larvae fed on food from flowering and nonflowering trees by 32% and 28% respectively (Fig. 8). On the other hand, dispersal from the midcrown to the lower crown section, when larvae reached sixth instar, did not significantly affect pupal weight. However, this feeding scenario resulted in a 6 d increase in the development time from sixth instar to moth emergence (Fig. 9).

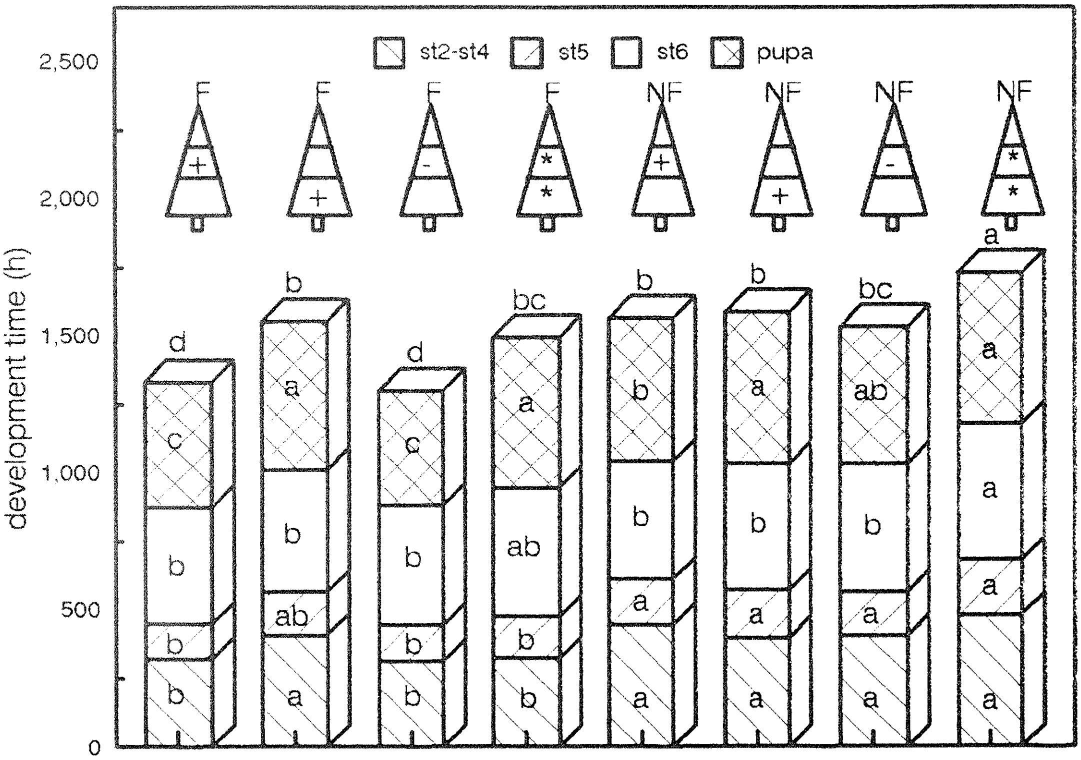

Figure 9.—Female spruce budworm development time per instar according to various feeding scenarios using fresh food from flowering (F) and from nonflowering (NF) balsam fir trees. Two trees served as food sources for two groups of 25 individually reared larvae per feeding scenario (T = 12°C, RH = 65%, 18L:6D, + larvae fed on current-year foliage from the given crown section, - larvae fed on current-year foliage prior to reaching sixth instar and 1-year old foliage thereafter, * larvae fed on current-year foliage from the midcrown prior to reaching sixth instar and current-year foliage from the lower crown section thereafter). For a given instar, means followed by the same letter are not significantly different ($p < 0.05$).

DISCUSSION

Several authors have reported that the production of staminate flowers by balsam fir trees accelerates the development of spruce budworm larvae (Jaynes and Speers 1949, Blais 1952, Greenbank 1963). However, these authors could not determine whether the effect of flowering on budworm development time was caused by the pollen, a rich source of amino acids, or by the microhabitat provided to the insect by the staminate flower clusters. Results from our laboratory rearing experiment showed that pollen is a food that can reduce development time. Moreover, because both laboratory and field rearing experiments gave similar results, the impact of flowering on development, results mainly from the presence of pollen rather than from the microhabitat of flower clusters.

It is generally believed that shortened development time of an insect reduces the probability of mortality due to natural enemies prior to reaching the adult stage (Slansky 1990). However, this hypothesis has never really been tested. Nevertheless, if this hypothesis is true, balsam fir flowering may allow spruce budworm to escape the pressure exerted by certain natural enemies, with the exception of those attacking the insect during its sixth instar for which development time was not affected by flowering. Also, the development of natural enemies and of spruce budworm could be desynchronized allowing spruce budworm population density to increase.

Results from the field rearing experiment indicated that flowering did not affect the survival of spruce budworm post diapausing larvae. Similar results were obtained by Blais (1952). However, Mattson *et al.* (1991) found a 25% increase in the survival of larvae reared on flowering branches compared to those reared on nonflowering branches.

development time that they obtained from feeding pollen during their early stages of development. On the other hand, when old larvae dispersed to the lower crown section, they avoided the negative effects of backfeeding , but they lost the advantage in development time that they obtained from feeding on pollen. Results from this study indicated that the production of staminate flowers by balsam fir trees could have opposite effects on spruce budworm population dynamics depending upon the insect population density when flowering occurs.

ACKNOWLEDGMENTS

We wish to thank M. Charest, M. Crépin, D. Verge, G. Moreau, J.F. Demers, S. Perreault, C. Paquet, I. Coté, J. Forest, L. Roy, and M. Marin for technical assistance during insect rearings. Insects were supplied by the Insect Production Unit of the Forest Pest Management Institute, Sault Ste. Marie, Ontario, Canada. Financial support came from the Natural Sciences and Engineering Research Council of Canada.

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STAMINATE FLOWERING AND TREE PHENOLOGY AFFECT THE PERFORMANCE OF THE SPRUCE BUDWORM

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INTRODUCTION

The important role of staminate flowers in the ecology and population dynamics of spruce budworm has been the subject of speculation for more than 50 years (Blais 1952, Greenbank 1963). This suspicion has been fueled by the fact that high populations of budworm have historically been associated with mature, flower-bearing trees (Mott 1963). Moreover, overwintering budworm larvae typically spin their silk shelter in the old staminate flower bracts, and spring emerging 2nd instars prefer to mine and feed in staminate flower buds rather than old needles. Budworms emerge 1 to 4 weeks before vegetative buds have begun to expand and are suitable for attack, whereas flower buds, when present, are always more advanced and thus better synchronized with the early, vernal budworms. Hence, second-stage larvae are invariably concentrated in the host's expanding flower buds. On the other hand, whenever budworm emergence is retarded relative to tree phenology, then larvae tend to go directly to expanding foliage rather than the staminate flower buds (Greenbank 1963). Obviously, then, the importance of flower buds to budworms may be linked to the phenology of the hosts' vegetative buds relative to the timing of spring emergence of the budworms.

Larvae that feed within staminate flower clusters may actually grow faster than foliage-feeding larvae because such clusters are miniature greenhouses, trapping more radiant energy than comparable pure foliage environments. However, there is little other experimental evidence to support the hypothesis that staminate flowers can enhance budworm performance and thus contribute to outbreak development. For example, Jaynes and Speers (1949) and Blais (1952) reported that there is no difference in fecundity of budworms having fed on pollen or new foliage.

Herms and Mattson (1992) hypothesized that plant reproduction can also indirectly affect herbivore performance through its strong impact on the allocation of plant nutrients and photosynthates. When plants produce large crops of pollen and/or seeds, the reproductive organs may preferentially receive scarce nutrients and energy that might otherwise have gone to plant defenses or even new growth on which phytophages depend. Hence, staminate flowering could indirectly impact budworms by reducing the concentrations of nutrients and perhaps even altering the levels of secondary metabolites in plant foliage.

This study was undertaken to simulate and explore the consequences of abundant staminate flower production on the performance of pre-outbreak spruce budworm populations. We hypothesized that budworm larval performance would depend not just on the availability of staminate flower but also on the phenology of a tree's vegetative buds. We predicted that the presence of staminate flowers would be most important on very late flushing trees, and least important on very early flushing trees.

METHODS

In 1989 and 1991 we selected 20 half-sibling balsam fir, *Abies balsamea*, that were flowering at the Kellogg Experimental Forest (Michigan State University) near Augusta, Michigan. Trees were planted in 1970 and about 6-8 m tall at the time of the experiments. Before budbreak (April 21, 1989, and April 11, 1991), we selected per tree two flowering and two nonflowering branches near midcrown, which were then enclosed with a fine-mesh, cloth sleeve cage wherein we placed about 20 ready to emerge second instar budworm larvae still in their silken hibernacula on a gauze patch. Exactly 1 week

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later, we selected one more flowering and nonflowering branch on each tree which we once again enclosed with sleeve cages and inserted about 20 second-stage budworms. These two batches came from the same population, and they were randomly allocated to treatments. Hereafter, we respectively refer to them as cohort 1 and cohort 2. Approximately 2 weeks later we removed the gauze patches and counted the number of larvae which failed to emerge from their overwintering hibernacula. This gave us the initial number of insects in each sleeve cage. When most insects had reached the pupal stage, cages were removed by cutting each branch at its base. We then searched each bag for budworm larvae and pupae. Pupae were stored immediately in plastic vials and checked daily until all adults emerged. Moths were first frozen, then oven dried to constant weight and weighed and sexed. Because male and females responded in the same way to treatments, we pooled sexes by first converting males to female equivalents. Each male observation was multiplied by a constant (k_i) that was derived from the grand means (gdm) of the particular variable in question (e.g., weight, developmental time, developmental rate, etc): k_i = gdm female/ gdm male, for each cohort and year. We calculated survival per sleeve cage by summing all larvae that achieved the final 6th larval stage (even though some were parasitized or preyed upon by stink bugs), pupae, and adults, and divided this grand total by the number of larvae that actually emerged from their hibernacula.

In 1991, we selected 32 (16 flowering, 16 nonflowering) white spruce trees, *Picea glauca*, that were growing at the edge of a provenance plantation (USDA North Central Forest Experiment Station) near Wellston, Michigan. Trees were planted in 1963, and were approximately 8-10 m tall at the time of the experiment. Just as above we selected (on May 3) two branches at midcrown level from each the flowering and the nonflowering trees for enclosure with sleeve cages and budworms. We placed only one cohort of budworm larvae on these trees, however. We collected and counted surviving budworms when most had reached the pupal stage.

All trees were scored for their phenological development according to the protocol of Nienstaedt and King (1970) at the time of placing insects on the trees.

Difference between cohorts 1 and 2 in the balsam fir study were first tested via a 2 x 2 factorial, randomized block design where trees were treated as blocks and flowers and cohort timing were treated as treatments. Next each cohort was analyzed separately to test specifically for flower by tree phenology effects on budworm performance (weight gain, growth rate/day, days to complete development, and survival to the pupal stage). We subjected the data to univariate anova after appropriate transformations of the data (i.e., arcsin (%survival)). The balsam fir study was analyzed as a completely randomized, split-plot design with phenology (3 classes) treated as the main plot and flowering (2 levels) as the subplot. The white spruce study was analyzed as a completely randomized 3 x 2 (phenology x flowering) factorial.

RESULTS

Balsam Fir: Flowering and Phenology Effects on Budworm

Larval Survival

First cohort survival was clearly affected by both flowering (F) and flowering x tree phenology (P) classes (Table 1). In 1989, the significant F x P interaction was due largely to the enhancing effect of flowering, as expected, on the late-flushing trees, where survival averaged 65.3% with, and 43.2% without flowers (Fig. 1). The smallest flowering effect occurred on early flushing tree (57.0% vs 53.6%, with and without flowers, respectively). In 1991, even though there were no significant main or interaction effects (Table 2),the largest positive impact of flowering was evident on the late flushing trees (as in 1989) where survival averaged 48.2% with, and 40.6% without flowers (Fig. 1).

Second cohort survival in 1989 was significantly ($p < 0.02$) enhanced by flowering but not affected by phenology, nor by the F x P interaction (Table 1). In 1991, there were again significant flower, and F x P effects (Table 2). This was due primarily to the huge difference in survival rates between flowering (54.1%) and nonflowering (32.0%) branches on late flushing trees (Fig. 1).

Testing the grand means for cohorts 1 and 2 in 1989 and 1991 revealed in both cases that survival was higher for the first cohorts, but only statistically significant in 1989 (55% vs 43%).

Table 1.—Mean spruce budworm performance (female equivalents) on balsam fir in 1989 on early, middle and late phenology trees (main plots) and flowering and nonflowering branches (subplots). Means varying significantly ($p < 0.05$) among main effects have different letters. For cohort one, mean survival was effected by flowering and by the flowering x phenology interaction. For cohort two, mean survival was affected by flowering.

Budworm performance variable	Trees (main plots)—Phenology class			Subplot—Flowering class	
	Early	Middle	Late	Yes	No
			Cohort one		
Survival (larvae)	0.55	0.57	0.54	0.62**a**	0.49**b**
Weight dwt (mg)	23.93	22.75	22.44	23.18	22.72
Dev. time (days)	52.94	53.37	52.93	52.91	53.34
Growth rate (mg/da)	0.45	0.43	0.43	0.44	0.43
			Cohort two		
Survival (larvae)	0.43	0.42	0.45	0.47**a**	0.39**b**
Weight dwt (mg)	26.11	23.58	24.23	24.53	24.1
Dev. time (days)	50.45	51.27	50.88	51.11	50.84
Growth rate (mg/da)	0.55	0.46	0.48	0.48	0.48

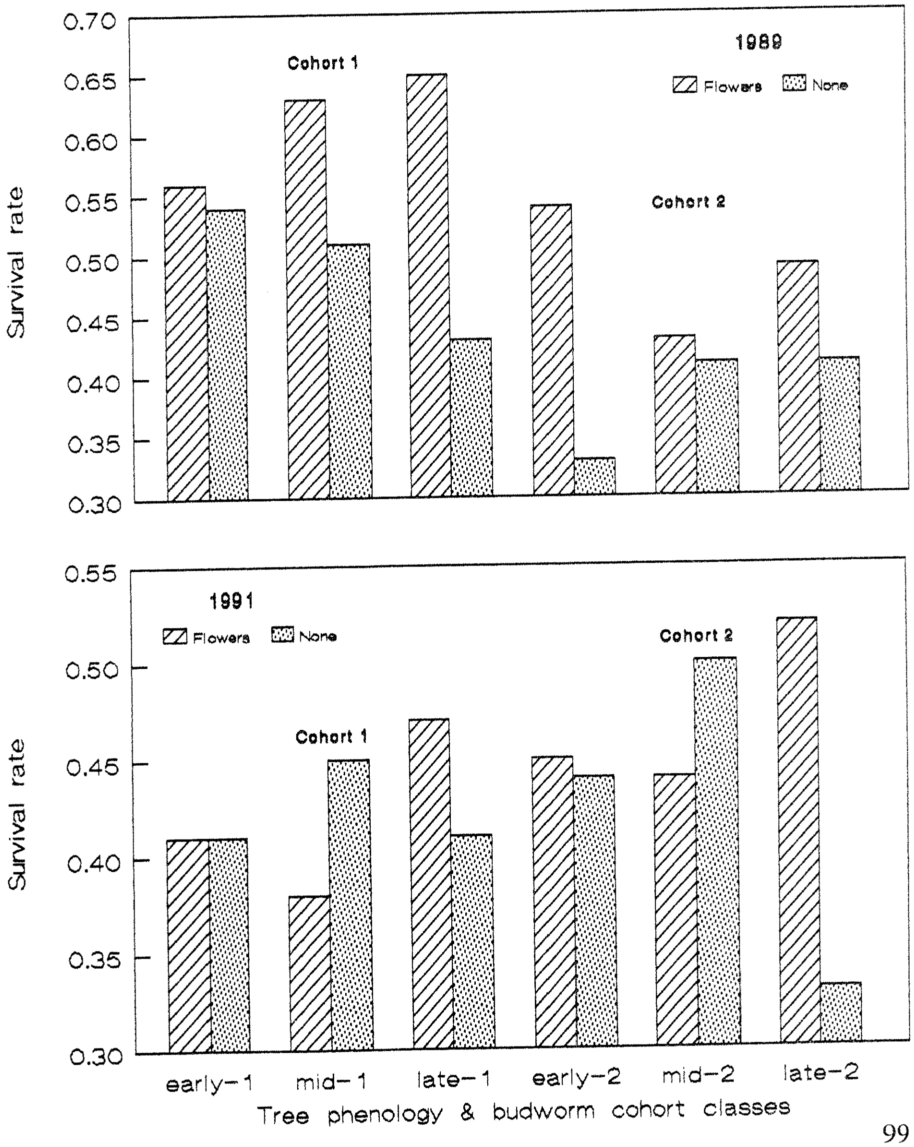

Figure 1.—Survival rates of two cohorts of spruce budworm larvae in 1989 (upper panel) and 1991 (lower panel) on flowering and nonflowering branches on 20 balsam fir trees divided into three phenology classes: early, mid and late flushing at the Kellogg Experimental Forest of Michigan State University in Augusta, Michigan. Flowering and flowering x phenology effects were statistically significant ($p < 0.05$) in one or both cohorts of both years (Tables 1 and 2).

Table 2.—Mean spruce budworm performance (female equivalents) on balsam fir in 1991 on early, middle and late phenology trees (main plots) and flowering and nonflowering branches (subplots). Means varying significantly ($p < 0.05$) among main effects have different letters. For cohort one, mean weight and growth rate were significantly affected by phenology, and mean development time by the f x p interaction. For cohort 2, only mean survival was significantly affected by flowering, and by the flowering x phenology interaction.

Budworm performance variable	Trees (main plots)—Phenology class			Subplot—Flowering class	
	Early	Middle	Late	Yes	No
			Cohort one		
Survival (larvae)	0.41	0.41	0.44	0.42	0.42
Weight dwt (mg)	23.41**b**	26.47**a**	25.72**a**	24.79	25.00
Dev. time (days)	51.29	50.97	51.05	51.05	51.23
Growth rate (mg/da)	0.46**b**	0.52**a**	0.50**a**	0.49	0.49
			Cohort two		
Survival (larvae)	0.45	0.47	0.43	0.47**a**	0.41**b**
Weight dwt (mg)	24.37	25.27	25.53	24.51	25.44
Dev. time (days)	46.02	46.16	45.65	45.92	45.95
Growth rate (mg/da)	0.51	0.55	0.56	0.53	0.55

Larval Growth

There were no significant flower or F x P effects on weight gain (mg dwt) by the first or second budworm cohort in either 1989 or 1991 (Tables 1, 2). However, there was a significant phenology effect on cohort 1 in 1991, where middle and late flushing trees yielded bigger adults than early flushers (26.5 vs 23.4 mg dwt).

Likewise, there were no significant flowering, or F x P effects on budworm growth rates (mg/da) for either cohorts in 1989, and 1991 (Table 1, 2). However, there was a significant phenology effect on the growth rate of the first 1991 cohort. Budworms on middle and late trees grew faster than those on early flushing trees (0.52 mg/da vs 0.46 mg/da). There was also a significant F x P effect on development time for cohort one (Fig. 2), but this may have been spurious.

Comparing the grand means for cohorts 1 and 2 in 1989 revealed that growth (22.95 vs 24.31 mg) was significantly higher and development shorter (53.13 vs 50.97 da) for the second than first cohort. By contrast, in 1991, there were no significant differences in overall growth between the two cohorts, but development time was, as before, much shorter for the second (51.14 vs 45.93 da), causing its growth rates to be significantly higher (0.49 vs 0.54 mg/da).

White Spruce: Flowering and Phenology Effects on Budworm

Survival

Flowering and phenology had no significant effects on survival of larvae (Table 3), but the F x P interaction was nearly significant ($p < 0.10$). There was an apparent trend for survival to increase with increasingly retarded phenology on nonflowering trees, and the opposite trend on flowering trees (Fig. 2). The fact that the two trends run counter to each explains why the main effects of flowering and phenology were insignificant. The grand means from flowering and nonflowering trees in each phenology class canceled one another out.

Larval Growth

There were strong, consistent phenology effects on both total weight gain and growth rates, the pattern being identical on both flowering and nonflowering trees (Table 3). Budworm mass and average growth per day increased with increasingly retarded tree phenology. Insects on the late flushing trees averaged 13.5% larger than those from the early flushing trees (32.5 mg vs 28.6 mg). Likewise their daily growth rates were 14.5% higher (80 mg/da vs 70 mg/da). Neither flowering, nor

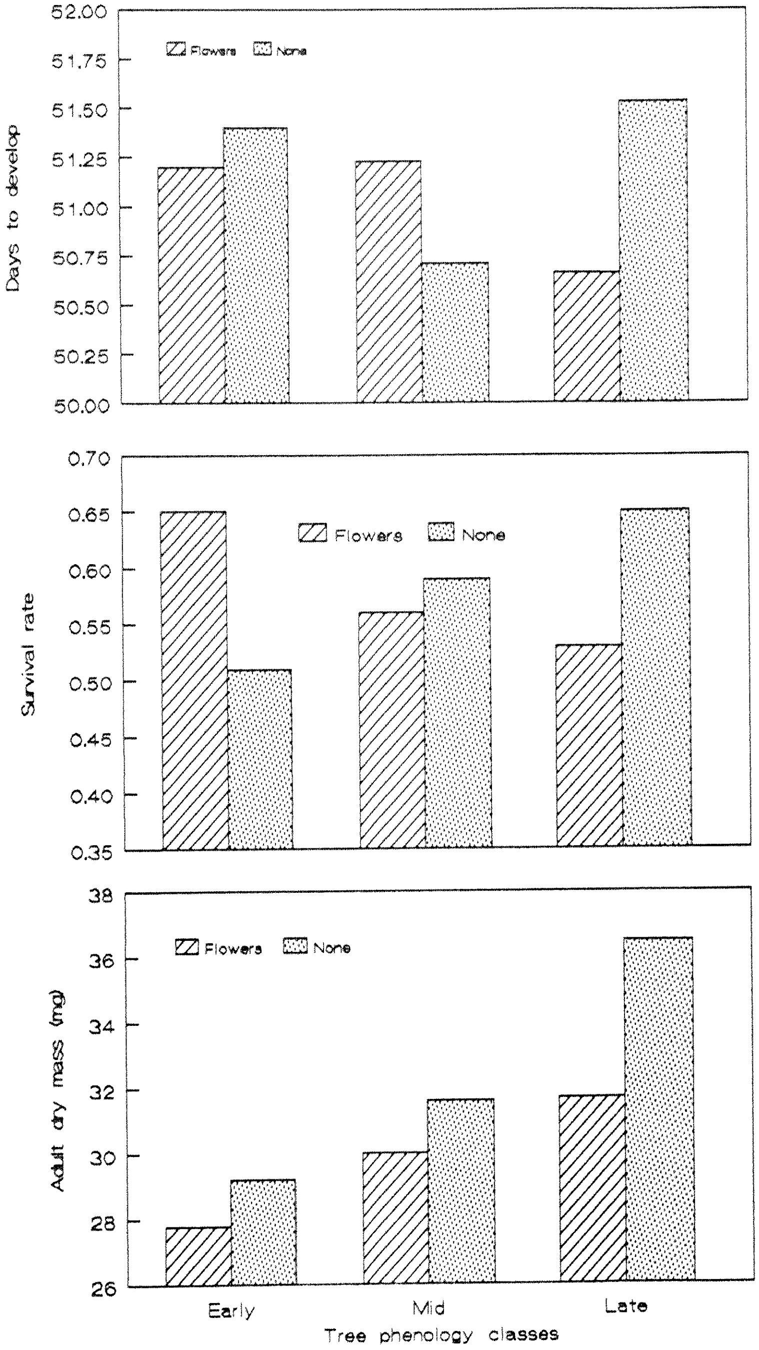

Figure 2.—Development times, survival rates, and adult dry weights, on 16 flowering and 16 nonflowering white spruces divided into three phenology classes: early, mid and late flushing at Wellston, Michigan in 1991. Flowering and flowering x phenology effects were only borderline ($p < 0.10$) statistically significant (Table 3).

Table 3.—Mean spruce budworm performance (female equivalents) on white spruce in 1991 on early, middle, and late phenology trees and flowering and nonflowering trees. Means varying significantly ($p < 0.05$) due to main effects have different letters. Only mean weight and development time were affected by phenology. There were no flowering effects.

Budworm performance variable	Tree phenology class			Tree flowering class	
	Early	Middle	Late	Yes	No
			Cohort one		
Survival (larvae)	0.57	0.57	0.55	0.57	0.57
Weight dwt (mg)	28.61**b**	30.96**a**	32.48**a**	30.13	31.33
Dev. time (days)	41.11	40.93	40.85	40.96	40.95
Growth rate (mg/da)	0.70**b**	0.76**a**	0.80**a**	0.74	0.77

F x P had any significant ($p < 0.05$) effects on growth, development time, or growth rates. Although, there was a hint ($p < 0.08$) of a tendency for nonflowering trees to be superior for larval performance than flowering trees (Fig. 2).

DISCUSSION and CONCLUSIONS

Yearly Differences in Protocol and Weather Weaken the Tests

The differences in the effects of flowering and phenology on spruce budworm performance between years is almost certainly due to the differences in host tree phenology when the studies were initiated. In 1989, we placed our first cohort of budworms on the trees at 133 degree days (dd), very close to the time native budworm populations would have been emerging (est. at 100 dd). The second cohort was placed out at 204 dd, 71 dd and 7 days later than the first. In 1991 the experiment was begun 10 calendar days earlier than in 1989 but at a phenologically later point, 210 dd, about the time of budbreak on the early flushing trees (Nienstaedt and King 1970). Hence, the first cohort in 1991 was more nearly equivalent to the second cohort in 1989. The second cohort on fir in 1991 was placed on the host plants at 262 dd thereby having no 1989 equivalents. Finally, the single cohort on spruce in 1991 was placed on the trees at 280 degree days, about the time of budbreak for the later flushing spruce trees (Nienstaedt and King 1970). In addition, in 1991, just after placing the first cohort of second instars on the trees, the weather turned cool and wet keeping the young insects in their overwintering hibernaculum until nearly 1 week later when the next cohort was being placed on the trees.

Although this study attempted to examine the combined effects of staminate flowering by host phenology on the performance of spruce budworm, we were unable to execute the experiment in perfect phenological duplication that would have allowed the most powerful tests of the hypotheses.

Flower by Phenology Effects

The data clearly suggest that the survival of budworms is dependent on the flowering x phenology interaction of its host plants. In the case of fir, abundant flowering probably enhances survival most significantly on the later flushing trees, i.e., late relative to budworm emergence (they inevitably emerge before their hosts break bud). This was apparent especially in 1989. In that year all flowering branches supported higher survival than nonflowering branches regardless of tree phenology class, and regardless of cohort timing. In 1991 there was no apparent flowering effect on survival except for the late flushing trees, especially in the case of the second cohort. Likewise, on spruce there was a strong tendency, though only nearly significant ($p < .10$), for a F x P interaction, but opposite to that on fir. If the effect is real on spruce, it may be that flowering is enhancing on early flushing trees because it allows the young larvae to somehow better cope with the small and rapidly closing phenological window of susceptibility in spruce (Lawrence *et al.* 1996). On late flushing spruce trees, the insect may be more appropriately synchronized with plant foliar development and therefore, staminate flowers do not necessarily enhance survival. Budworms are very adept at mining year-old spruce needles while waiting for buds to open for their entry. Studies in the laboratory suggest that 2nd stage budworms mine needles of spruce more readily than those of fir (Trier and Mattson 1996).

There was practically no evidence to suggest that flowering enhances the growth and development rate of budworms, not on fir, nor on spruce. Likewise, there seems to be no strong evidence that there is any kind of F x P effect on growth processes. To the contrary, the data on spruce, though not quite statistically significant ($p < 0.08$), indicate that flowering may somehow diminish growth, be it ever so small (Fig. 2). These conclusions, in fact, accord with much earlier work by Blais and Thorsteinson (1948), Jaynes and Speers (1949), and Blais (1952).

Phenology Effects

An ever increasing asynchrony of budworm with balsam fir phenology does not seem to have a particularly strong effect on growth. Cohort 2 usually grew as well or better than cohort 1, even in 1991 when the first cohort started perhaps 2 weeks later than the wild budworms.

Likewise, the ever increasing asynchrony of budworm with balsam does not severely impact its survival. In 1989, second cohort survival averaged 40.1% and first cohort averaged 51%. In 1991, the second cohort had slightly higher survival than first (45.3% vs 40.85%).

This leads us to conclude that balsam fir does not have a particularly sharp window of susceptibility to budworms. This is unlike the situation for white spruce where Lawrence *et al.* (1996) have demonstrated that a retarded budworm asynchrony causes a very sharp decline in both its survival and growth.

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FOLIVORE FEEDING ON MALE CONIFER FLOWERS: DEFENCE AVOIDANCE OR BET-HEDGING?

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INTRODUCTION

In the fifties and sixties a group of British researchers led by G.C.Varley made pioneering studies on the life history of the winter moth (Varley and Gradwell 1970). Among many other things, they concluded that the key to understanding the main mortality in this species was the timing between egg hatch and bud-burst. Larvae which hatch on trees, having buds firmly closed, fail to find food there. Instead such larvae disperse by spinning a thread of silk, and are blown away to an uncertain destiny. Larvae that hatch when buds have just burst, survive well. This first model was further developed by Feeny (1970) who in another pioneering study argued further that larvae which hatch after bud-burst face certain difficulties, this time in relation to nutritional conditions, mainly decreased nitrogen and water, and increased tannin concentrations.

In summary these factors indicate that in *Quercus robur*, there is a "phenological window" represented by recently burst, highly nutritious and poorly-defended leaves. In oak many defoliators, especially lepidopteran species, take advantage of this window and often reach high densities, even outbreak levels, at that time. Also species richness is highest in the early spring. However, this situation in *Quercus robur* does not seem to apply in a number of other tree species. The period of shoot growth in oak is rather short, and Niemelä and Haukioja (1982) demonstrated that in many other deciduous trees the period of shoot growth is much longer and seems to give defoliators a chance to find a much broader phenological window.

In this paper the nutritional status of two coniferous genera, pine, *Pinus*, and spruce, *Picea*, are considered in relation to the phenological window, including the situation when alternative food, especially flowering buds are available. The insect species studied has mainly been the nun moth, *Lymantria monacha*, a close relative of the gypsy moth. The nun moth is a polyphagous species that often defoliates large areas of conifer forest in Central Europe (Jensen 1991).

METHODS

Rearings and experiments of nun moth larvae and other insects were performed in a laboratory at constant 20°C. Eggs were collected in the forest and brought to the laboratory during winter. In growth and survival experiments nun moths were kept in batches of ten in the first three instars; fourth and fifth instars were reared singly. Larvae were fed needles and flowers attached to excised twigs put in water.

Chemical analysis were performed by means of micro-Kjeldahl analysis (nitrogen) and gas-chromatography (carbohydrates, cyclites and phenolic acids). For details see Jensen (1988).

RESULTS

Spruce

The genus *Picea* comprises about 30 species in the northern hemisphere. In parts of Europe, Norway Spruce, *Picea abies*, is the native species, but it is also widely planted, along with the introduced North American species Sitka spruce, *Picea sitchensis*. The buds burst rather early in spring along with a lot of deciduous trees, and the new shoots and needles are

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soft for quite some time. It has 2-5 year classes of older needles. Nutritional content of the new needles is high: total N reaches 4.5% dwt, carbohydrate levels 250 nanomoles/g dwt (Jensen 1991). When needles get older, nitrogen levels drop to around 1%, whereas carbohydrate levels increase somewhat (Table 1).

Table 1.—Concentrations of nutrients and secondary compounds in needles and male flowers of spruce, *Picea abies.*

Plant part	Total Nitrogen	Amino acids	Shikimic acid	Quinic acid	Pinitol	Hexoses	Inositol	Cathecin	Total Phenolics
	%	- - - - - - -	- - - - - - -	- - - - n moles/mg dwt	- - - -	- - - -	- - - -	- - - -	
Male flowers	5	~900	29	213	134	575	18	4	—
Old top needles	1.2	~450	224	52	61	390	7	12	68
New top needles	4	1,120	108	625	133	248	17	3	35

In Europe a substantial number of insect species are flush-feeders on spruce although some of them also consume older foliage, especially late in year or at outbreak densities (Table 2). This situation mainly applies to Lepidoptera although certain sawfly species also are flush-feeders. Another group feeds exclusively on older needles and is mainly comprised of Hymenoptera. In general, most spruce flush-feeders (e.g., *L. monacha*) perform substantially better when reared on the new, nutritious needles than on the old ones. Most newly hatched larvae die when put on old needles probably because their mandibles are not strong enough to cope with the hard needles (Jensen 1992). On the other hand, if third instar larvae are put on old needles, they survive and perform reasonably well. Preference indices for new needles (Table 3) decrease with instar number, indicating that larger larvae do not require young needles.

Table 2.—Needle age preferences of insect larvae feeding on Norway spruce, *Picea abies.*

Insect species	Newly flushed	Needle age class Current	Old
Lepidoptera			
Parasyndemis histrionana	*		(*) [a]
Eana argentata	*	*	(*)
Epinotia tedella		(*)	*
Epinotia nanana	*	*	(*)
Epinotia pygmaea	*		
Zeiraphera ratzeburgiana	*		
Orgya antiqua	*	*	(*)
Lymantria monacha	*	*	(*)
Hymenoptera			
Cephalcia abietis			*
Cephalcia arvensis		*	*
Gilpinia hercyniae			*
Pristiphora abietina	*		
Pristiphora ambigua	*		
Pristiphora saxeseni	*	(*)	
Pachynematus scutellatus	*	*	(*)

[a] * indicates unequivocal primary preferences, (*) indicates secondary preference, often by late instars.

Table 3.—*Lymantria monacha* preference for new needles of spruce, *Picea abies*.

Instar	Preference index	n	P
I	1.00 ± 0.00	20	< 0.001
II	1.00 ± 0.00	17	< 0.001
III	0.84 ± 0.32	9	< 0.020
IV	0.63 ± 0.15	21	< 0.020
V	0.54 ± 0.26	21	n.s.

However, as indicated in Table 2, some species actually prefer the old needles, and to such an extent that they will die if fed the tender, nutritious new needles. An example of this is the sawfly, *Gilpinia hercyniae,* known in North America as the European Spruce Sawfly. It is a former pest in Canada, but not in Europe. When offered only new foliage, this defoliator dies without eating (Jensen 1988). Experiments have shown that one possible deterring compound to *G. hercyniae* could be quinic acid, which together with shikimic acid is responsible for much of the acidity of these new needles. Quinic acid is found in huge amounts in the new needles, but in low concentrations in the old ones. When quinic acid was added to old (preferred) needles in an experiment (Jensen 1988), *G. hercyniae* refused to eat after concentrations were increased to the levels of new needles.

With respect to the phenological window, insects like *G. hercyniae*, feeding on old needles have no problems. In contrast, the flush-feeders must find the phenological window. If they hatch very early, even dispersal will not help if the food resource is not yet present. The only way to survive is to find an alternative food. In the dense monocultures of European spruce forests it is not easy to find other host species. However, occasionally, i.e., with 2-7 year intervals flowering buds might offer this alternative. The pollen buds are prime food, being soft, with high nutritional content and rather low content of secondary compounds (Table 1). Laboratory experiments on newly emerged *L. monacha* showed that these larvae are very fond of male flowers. When they hatch early and are reared on branches with only old needles, they die. If male inflorescences are present they survive and grow well (Fig. 1). Once started on flowers, they can eat new and old needles. Later in the season the larvae may struggle for some time until vegetative buds burst.

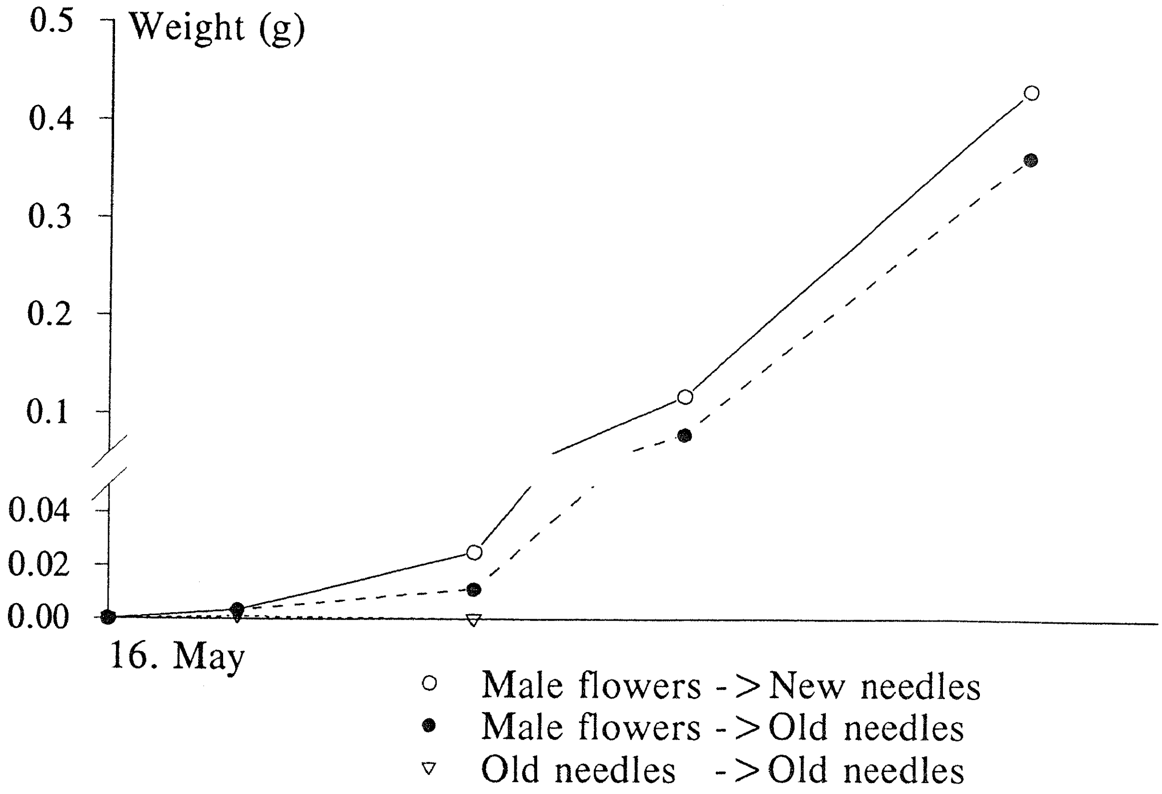

Figure 1.—*Lymantria monacha* growth on male flowers and needles of Norway spruce (*Picea abies*).

Pine

In pine species the new needles emerge rather late in spring. Old needles are tough and of low nutritive value. This means that there is a long period with few feeding opportunities for herbivores, except for the existence of male inflorescences. In most places, pines set flower buds much more consistently than do spruce.

New pine needles are less nutritive than spruce, nitrogen reaches only 2-2.5% of dwt. The toxins occurring in new needles are potent and it has been demonstrated (Ikeda *et al.* 1977) that they reduce growth rate, increase mortality, and extend larval period. Not surprisingly, most defoliator species feed on old needles or current year needles late in the year (Larsson and Tenow 1980, Table 4).

Table 4.—Needle age preferences of insect larvae feeding on Scots pine, *Pinus sylvestris*.

Insect species	Needle age class	
	Current	Old
Lepidoptera		
Bupalus piniaria	*	*
Cedestis spp		*
Cidaria firmata		*
Dendrolimus pini	*	*
Ellopia fasciaria		*
Hyloicus pinastri		*
Panolis flammea	*	*
Semiothisa liturata		*
Lymantria monacha		*
Hymenoptera		
Diprion pini	*	*
Diprion similis		*
Gilpinia frutetorum		*
Microdiprion pallipes	*	*
Neodiprion sertifer		*

Nun moth larvae normally hatch prior to bud-burst, leaving them with only old pine needles for food. Experiments in field enclosures and in the laboratory with first instar larvae on whole branches or twigs, showed almost 100% mortality. Only if the bagged branches included male flowers were larvae able to survive. In the laboratory, larvae survive and develop only if they are put on flowering branches in the first part of their life, irrespective of pine species (*P. contorta*, *P. sylvestris*, *P. mugo*) (Table 5). Surviving larvae that later were fed new or old needles, performed equally well (Fig. 2).

Table 5.—Percent survival of nun moth larvae on pines. T = 17 days, n = 85 per trial.

	P. silvestris	*P. mugo*	*P. contorta*
Flowering	71.0	50.8	14.6
Non-flowering	15.0	16.7	0.0

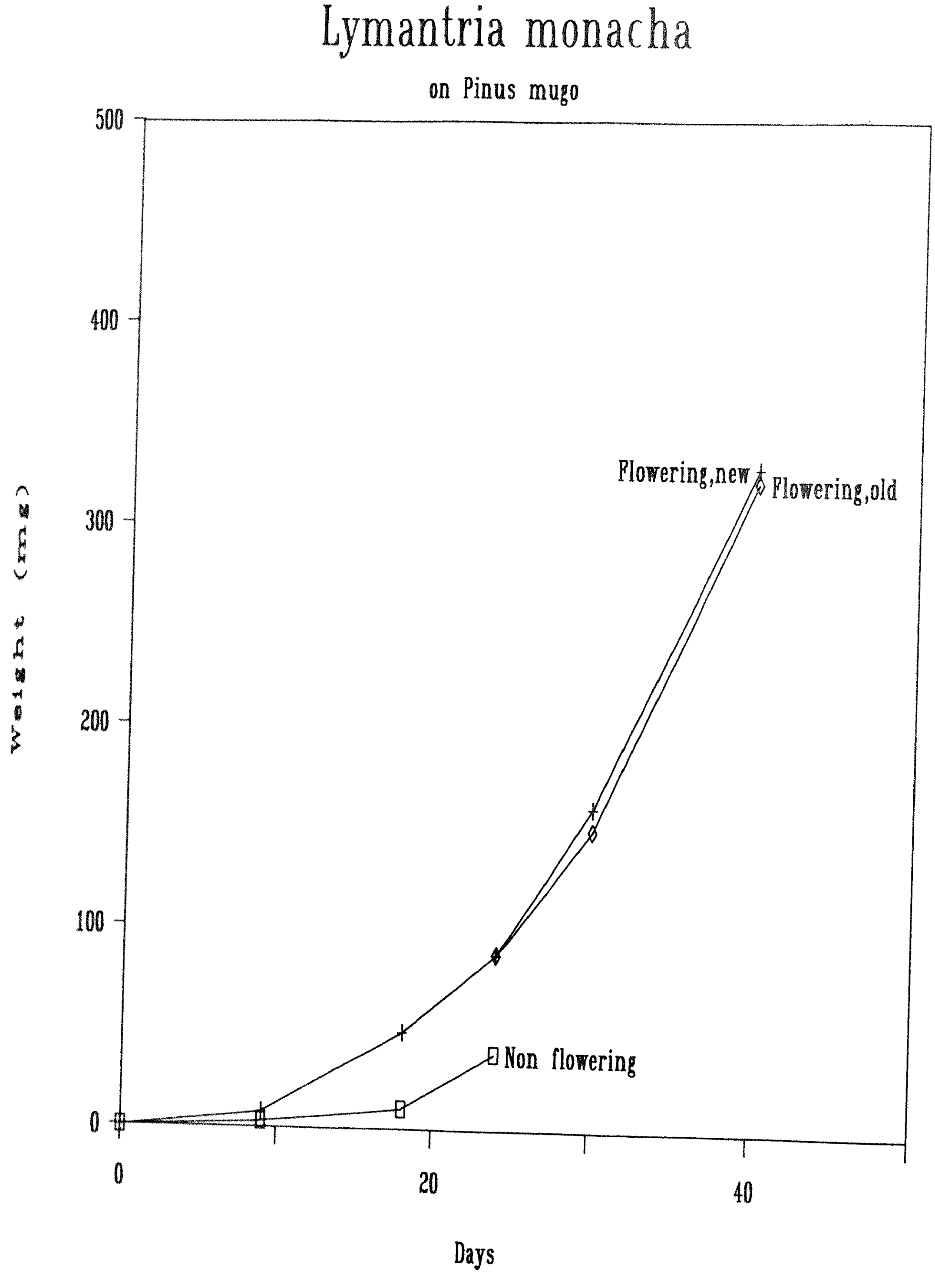

Figure 2.—*Lymantria monacha* growth on flowering and nonflowering branches of mountain pine (*Pinus mugo*). On flowering branches larvae were later separated into feeding groups on new and old needles.

DISCUSSION

Based on the above findings it seems necessary to add another set of curves to the Varley-Feeny phenological window, curves that represent the opening of male flowering buds (Fig. 3). These curves are fundamentally different in the pine and spruce. In spruce, where male flowers burst very early in spring, it can be noted that flush feeders simply get a broader phenological window in years with male flowers. This means that larvae which hatch early in such a year get a larger probability of finding highly nutritious food. However a strategy of early hatching is a risky business, because chances are high that there will be no flowers. On the other hand, if they hatch late the risk is small, but the nutritional quality is lower and hence development time and predation risk will be higher.

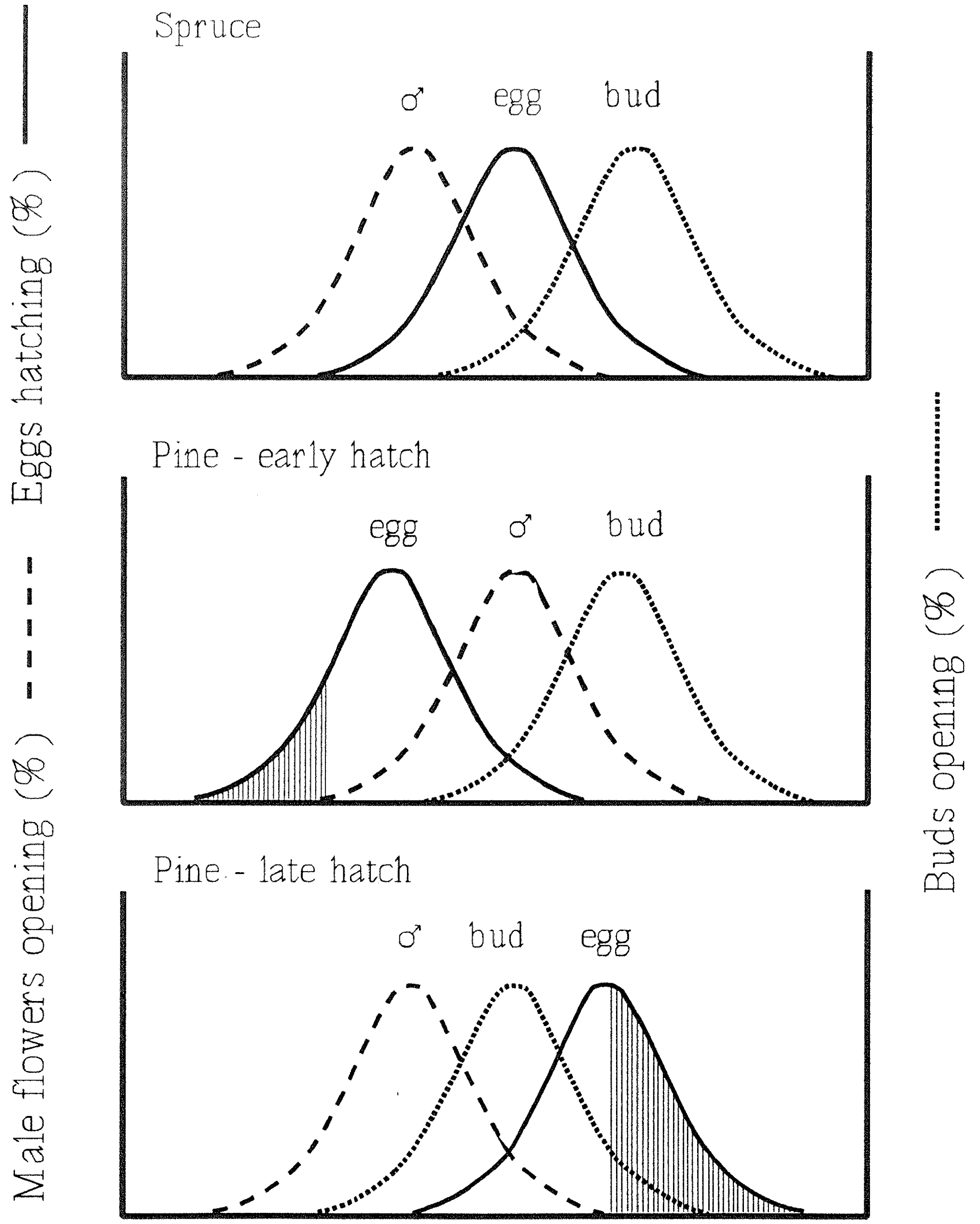

Figure 3.—Modification of the Varley-Gradwell curves for the "phenological window": insect egg hatching, male flower and bud opening on spruce and pine.

From the point of view of trees, the evolutionary response in terms of defense seems to be consistent with a concept known from deciduous species like oak or beech, as masting or mast seeding (Silvertown 1981). In other words, in some years there will be high beech-mast or acorn production, but no seeds in the years in between. In the latter years, pest populations will decrease markedly. The strategy has probably evolved against seed predators but it could also work against some defoliators.

The second line of defense is simply the variability of bud burst (Mitcherlich and Wellenstein 1942). Large variation in bud burst between provenances exist, but also large variation occurs within provenances. However, variability as a defense will mean that some larvae always will survive.

The third line of defense against flush-feeders is a chemical one. Apparently, rather few spruce herbivores are deterred from feeding on newly burst needles to such an extent that they prefer old needles (Schopf 1986).

In contrast, in pine it seems essential that flush feeders avoid plant defenses and here male flowers are of vital importance. In pine, male flowers are a more predictable resource than in spruce. Larvae that hatch after the flowering period will die and hence the risk of early hatch is less because male flowers are most often present. However, the strategy might fail; they might hatch on a tree without flowers, or worse in a year without flowers in the whole stand.

In the context of the phenological window it is actually necessary to consider the choices made by the female moth. Where does she deposit her eggs? Does she in fact spread the risk so that at least some of her larvae will survive? But at the same time, does she abandon an optimal strategy of maximizing short term fitness? Essential to all these considerations are the development time of buds and eggs, both factors being related to spring temperatures, although probably not in the same fashion. I hypothesize that the position of the eggs is a key factor in this synchronization simply because the temperature varies so much between microsites.

Lymantria monacha females deposit their egg clusters mainly on the tree trunk under bark scales. Egg numbers and egg hatch differ considerably between north- and south-facing sides of the trunk (Raae 1979), and it might be that this is the mechanism the females employ when they spread the risk. This could be the way whereby females ensure that some of their progeny in this variable environment will hatch when the phenological window is open. Another bet-hedging mechanism could be that after laying their first batch and becoming lighter, females fly to another tree and deposit the rest of their eggs. If the first tree is in the middle of a dense stand and the next is at the southern edge, the temperature regime is different and accordingly the bets change.

It is interesting to observe that many flush feeders have wingless females or have reduced flight capability due to their heavy loads of eggs. In *L. monacha*, females are winged but poor fliers and it is likely that the females lay their eggs close to if not directly on the tree where they pupated, thereby increasing the likelihood of synchronization between the egg hatch and bud burst.

In conclusion, avoiding defenses of new needles is absolutely necessary for pine defoliators but of minor importance to spruce defoliators. Bet hedging might prove to be the evolutionary response by the defoliators in order to find the host's phenological window.

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THE BLACKMARGINED APHID AS A KEYSTONE SPECIES: A PREDATOR ATTRACTOR REDRESSING NATURAL ENEMY IMBALANCES IN PECAN SYSTEMS

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INTRODUCTION

Plants defend themselves against herbivores in many ways. Conceptualization of these defenses has resulted in many terms and contexts to describe them (see Harris and Frederiksen 1984). Snelling (1941) listed 15 categories. Painter (1951, Fig. 3) depicted 11 plant factors interacting with 8 insect factors mitigated by 8 environmental factors influencing 5 insect-plant interaction factors as possible causes of resistance to insects, and then consolidated these factors into three resistance mechanisms: tolerance, antibiosis, and preference. These mechanisms were, by definition, heritable, effective in isolation, and particularly apt for usage in agriculture where producing a crop of good quality in the presence of the insect was paramount. Harris (1980) proposed a succinct and natural characterization of plant defense, listing escape in space and time, accommodation, confrontation, and biological associations as the primary mechanisms to consider.

Biological Associations

Of these, biological associations are the most complex and difficult to demonstrate as natural defense mechanisms, because both the genetics of the plant and the arthropod are involved. Perhaps the best known case of a natural biological association providing plants with a defense against herbivores is the ant-acacias. Howe and Westley (1988) review this and other lesser known ant-plant associations, as well as other biological associations including those important in dispersal, pollination, etc. Biological associations mediated by the genetics of the plant clearly play an important role in nature, yet they have seldom been deliberately exploited to a significant degree in agriculture. Exceptions could be corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphidae) on corn and sorghum, and apple rust mite *Aculus schlechtendali* (Nalepa) (Acarina: Eriophyidae) on apple, which are relatively innocuous at moderate densities and, if left alone, will often support densities of natural enemies that also suppress more pestiferous species of aphids and mites, respectively (Flint and van den Bosch 1977). The agricultural importance of corn leaf aphid and apple rust mite is clear, but the origin and role of these biological associations in natural systems are presently unknown. From an agricultural perspective, this area combines aspects of biological control and host plant resistance—two subdisciplines of entomology that lack a common paradigm.

BLACKMARGINED APHID AND PECAN SYSTEMS

This paper proposes another example of a biological association, *viz.* how pecan, *Carya illinoensis* (Wang.) K. Koch (Juglandaceae), interacts with the blackmargined aphid, *Monellia caryella* (Fitch) (Homoptera: Aphidae), to influence other biological associations that may impact plant defense within the context of the matrices of interactions noted above.

Pecan is a deciduous, monoecious, wind-pollinated, woody perennial that occupies alluvial soils from western Texas to the Mississippi Valley on the east and from southern Illinois into Mexico (Little 1971). Pecan trees can live more than 200 years, grow to heights exceeding 35 m, and can constitute as much as 50% of the tree canopy in their natural habitat (Maggio *et al.* 1991). Reproduction is by seed (≈200/kg) abundantly produced every 2-7 years (≤ 500 kg/ha) in natural populations, and each tree in nature is genetically distinct (Harris 1988). Leaves are compound and occur at a density of about 3 million leaves or roughly 50,000 m^2/ha.

Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

Pecan domestication has been a gradual process, initially consisting of thinning riverine woodlands to pure stands of pre-existing pecan trees. Wild pecan nuts are commercially attractive and usually preferred by bakers and confectioners because they are typically priced about 25% less than selected varieties; they are about 40% smaller, allowing the whole halves to be pleasingly presented on small cakes, candies, etc.; and they are comparable, if not superior, in taste to selected varieties. More than 60% of current nut production in Texas comes from such trees with the remainder produced by vegetatively propagated varieties. Thus, the pecan landscape throughout the indigenous range consists of wild trees growing in mixed species woodlands, native pecan that has been thinned from woodlands, and vegetatively propagated orchards often found adjacent to one another. Management programs like fertilization, pesticide application, irrigation, and pruning range from being most intensive in orchards to virtually nonexistent in mixed species woodlands. This variety of habitats provides unique opportunities to examine many questions including effects of plant domestication on arthropods. Most investigations of the pecan arthropod complex have been spurred by economic considerations and have concentrated on species that pose economic threats or economic relief (Harris 1983). However, given the long, close association of the arthropod complex with the pecan and the physical proximity of the wild and cultivated varieties, the opportunity to interpret results of pecan arthropod interactions from other perspectives invites attention. The arthropod complex consists of more than a 100 phytophagous, predator, and parasite species that have been associated with pecan from the earliest of times (Harris 1983).

The blackmargined aphid (BMA) is a monophagous, multivoltine, obligatorily alate, phloem feeder that overwinters as eggs placed in bark crevices and parthenogenetically infests pecan from shortly after budbreak until the fall some 7-8 months later, when males are produced, mating occurs, and the overwintering egg population is established (Harris 1983). Seasonal phenology on wild trees typically consists of BMA densities initially below 1/leaf increasing to between 1-10 aphids/leaf for a 2-3 week period during the summer, and then returning to densities below 1/leaf for the remainder of the season. Orchard trees typically experience ≥ fivefold higher densities and exhibit BMA population increases earlier in the season that last for 3-4 weeks (Liao *et al.* 1984).

BLACKMARGINED APHID AS A KEYSTONE SPECIES

Effects of BMA on nut production appear to be negligible in wild trees (Liao and Harris 1984), and with one exception (Wood *et al.* 1987), on orchard trees. However, routine use of insecticide in the early season typically results in epidemics of pecan aphids (PA) *(Monelliopsis pecanis* Bissell), mites, and leafminers (Harris 1988, 1991). The pecan aphid complex consisting of BMA and PA has particularly been considered a primary threat to nut production (Dutcher and Htay 1985, Beshears 1988). Population densities of BMA are typically an order of magnitude lower than those of PA in such epidemics and, if the epidemic proceeds to defoliation, BMA is virtually absent during the latter stages (Bumroongsook and Harris 1992).

BMA outbreaks routinely occur once per season in nature, or can also be induced once following the use of broad spectrum insecticides. Cage studies show that BMA outbreaks occur 2-3 weeks after introducing BMA into natural enemy exclusion cages throughout the season on previously unexposed foliage (Edelson 1982, Liao and Harris 1984, Liao *et al.* 1984, Liao *et al.* 1985, Bumroongsook and Harris 1992). These outbreaks naturally subside after 3-4 weeks, leaving intact photosynthetically active foliage (Bumroongsook and Harris 1992). BMA populations cannot reinfest the foliage for at least a month after the first induced outbreak (Liao *et al.* 1984). BMA outbreaks can be curtailed by opening the exclusion cages before the infestation has run its course or by introducing spiders or lady beetles into the cage at densities of about 1 per 10 leaves (Liao *et al.* 1984). Lacewing, *Chrysoperla rufilabris* (Burmeister), oviposition increases as BMA densities increase. Lacewing egg densities are also higher on foliage exposed by opening cages containing an incipient outbreak of BMA (Liao *et al.* 1984). Spider densities increase as BMA densities increase, and spiders readily accept BMA as prey (Bumroongsook *et al.* 1992). Spider densities are also higher on foliage exposed by opening cages containing an incipient outbreak of BMA (Liao *et al.* 1984). *M. pecanis* is slower to outbreak on mature bearing trees (Bumroongsook and Harris 1992), and high densities result in defoliation whether the leaves have been conditioned by BMA or not, indicating these aphids are capable of causing more loss of foliage than BMA.

These studies indicate that BMA may mitigate the impact of disruptions or natural declines of natural enemies in the pecan ecosystem by outbreaking and then attracting and reestablishing natural enemies to the system before more insidious phytophages increase in density (Bumroongsook and Harris 1992). The effects of such a role would not be limited to the pecan aphid complex, but would extend to all phytophagous species serving as prey to the polyphagous lacewings, lady beetles, spiders, etc. (Liao *et al.* 1984), that respond to *M. caryella*.

Wood *et al.* (1987) report that *M. caryella* second and third instar nymphs excrete some fortyfold more honeydew than other pecan aphids (other life stage comparisons are three- to tenfold) and noted that some 95% of the 301 joules (J) of energy removed from pecan by one *M. caryella* aphid during its lifetime was due to honeydew excretion. *M. caryella* energy consumption exceeds by 20% the largest consumption found in a review by Llewellyn (1987) for non-pecan aphids, and also has the lowest energy assimilation compared to other aphids. Honeydew is attractive to hundreds of species of insects (Klingauf 1987), including many predators and parasites. Tedders (1991) concluded pecan aphid honeydew "may be necessary for attracting and retaining large numbers of many beneficial species." Honeydew also improved the surrounding substrate for decomposers (Dixon 1985), enhancing the recycling of nutrients. These benefits must be balanced against the costs of *M. caryella* to pecan.

Initial characterization of the costs of *M. caryella* requires accounting for energy lost to the aphid in relation to the overall photosynthate production capacity of the pecan. Wood *et al.* (1987) provide the former at 301.41 J for one *M. caryella* over a 19.3 d life span and calculate 11.42 J/d are removed by the average aphid in a population. Anderson (1991) reported pecan assimilates CO_2 at a rate of 15.9 μ mol/m^2/s [Wood and Tedders (1986) report 11 μ mol CO_2/m^2/s]. Annual photosynthate productivity (PP)/ha, therefore, can be estimated by:

> Annual PP/ha = 2.65 μ mol of glucose x 50,000 m^2 pecan foliage/ha x 6.25 x 10^6 seconds/growing season x 0.5 x 0.65 [(corrected for photosynthetic efficiency due to shading (0.5) and respiration (0.65)].
>
> Where CO_2 has been converted to glucose (1 glucose = 6 CO_2), pecan foliage/ha was averaged from Wood *et al.* (1987) who reported 35,900 m^2/ha, Cutler (1976) who reported 52,580 m^2/ha and Lozano (1982) who found 62,800 m^2/ha; a growing season was defined as 217 d with 8 hr of sunlight each day; photosynthetic efficiency was estimated at 50% and respiration costs at 35%.

This results in a PP/ha of 2.69 x 10^5 mols of glucose annually or 185.98 x 10^6 kcal/ha (1 mol of glucose = 691 kcal).

Pecan foliage routinely is exposed to BMA at a level of a 100 to 500 or so aphid days per compound leaf each season (Tedders 1978, Flores 1981, Li 1990, Liao and Harris 1984, Tedders and Wood 1985, Bumroongsook 1986, Mansour and Harris 1988). Pecan leaf density estimates vary from 2.2 to 3.6 x 10^6 leaves/ha (Cutler 1976, Lozano 1982, Wood *et al.* 1987). Using an average of 3.0 x 10^6 leaves/ha and 500 *M. caryella* aphid days/leaf/season results in an energy cost of 17.12 x 10^9 J/ha annually (11.42 J/aphid day x 3.0 x 10^6 leaves/ha x 500 aphid days). Since 1 J = 0.24 cal, this equates to 4.1 x 10^6 kcal as an average cost of BMA on each ha of pecan each season.

Therefore, a 500 aphid day/leaf infestation of BMA each season would occur at a direct cost of about 2% (4.1 x 10^6 $\div$185.98 x 10^6) of the photosynthate production of the pecan. This cost would be spread across all physiological needs of the plant with only a portion appearing as yield loss. Secondary effects of factors like saprophagous sooty mold growth and additional reductions in photosynthetic efficiency are not discussed because previous studies (Tedders and Smith 1976, Wood and Tedders 1986) indicate BMA densities of 500 aphid days/leaf/season are too low to cause measurable reductions via these secondary effects in mature bearing trees.

This view of BMA is more sanguine than that presented by Wood *et al.* (1987). Resistance to pesticides in the pecan pest complex makes the prospect of maintaining BMA below its natural equilibrium level fraught with additional pesticide costs and other secondary pest problems like mites, leafminers, and other aphids (Harris 1991). Wood *et al.* (1987) report an average of 10 aphicide sprays ($500/ha at $50/spray) required to achieve this, and Dutcher (1991) reports 50 kg/ha rates of aldicarb ($330/ha at $6.60/kg) needed to achieve the same end for aphid control from mid-July onward. Although these approaches may protect the $\approx$ 2% of photosynthate production at risk from BMA, that loss represents 20 kg from an estimated average yield of 1,000 kg/ha if the entire BMA energy removed resulted in nut loss. Most insecticides currently used in pecans are highly toxic to most natural enemies of aphids (Mizell 1991) and in danger of being rendered ineffective due to aphid resistance to them (Harris 1991). We believe that the pecan aphid complex rarely poses a direct threat to pecan production unless disrupted by pesticides, especially in the early season and that the judicious use of pesticides for key pests can usually avoid these disruptions. When the problem is viewed from this perspective, pest managers may wish to re-evaluate the risks posed by BMA compared to the benefits provided by sustaining natural enemies and enhancing decomposition. Further study is needed to determine whether a natural role of BMA in the pecan ecosystem is to re-establish waning densities of natural enemies.

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PONDEROSA PINE RESPONSE TO NITROGEN FERTILIZATION AND DEFOLIATION BY THE PANDORA MOTH, COLORADIA PANDORA BLAKE

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INTRODUCTION

The pandora moth, *Coloradia pandora* Blake (Lepidoptera: Saturniidae), is a well-known outbreak species on ponderosa, *Pinus ponderosa* Laws, and lodgepole pines, *Pinus contorta* Dougl., in the western United States (Mattson *et al.* 1991). For example, it has periodically caused severe defoliation in the pineries of central Oregon. Historically, such outbreaks have been short-lived and caused little impact. But, in the 1920's, an outbreak in the Klamath Falls region did significant damage to the old-growth ponderosa pines so that they became highly susceptible to bark beetle attacks (Patterson 1929).

The insect has a 2-year life cycle (Carolin and Knopf 1968). Moths flights normally occur in June and July of even-numbered years (those near Bend, OR occur in odd-numbered years)(Patterson 1929, Carolin and Knopf 1968, Schmid *et al.* 1982). Eggs are deposited in clusters on both needles and bark. Hatch takes place in August and the young larvae feed gregariously in small colonies on current-year needles until limited by cold weather in late fall. Feeding continues on warm winter days on the south side of trees and on trees highly exposed to sunshine. Larvae eventually disperse and feed solitarily. Feeding intensity increases sharply with the onset of warm spring weather, and defoliation eventually becomes noticeable in early June as the caterpillars grow larger. By early July, fully grown larvae drop to the forest floor and pupate in the upper several centimeters of soil where they spend nearly a full year.

The current outbreak in Central Oregon (first seen in 1988) has spread south of as well as north to the city of Bend because of massive moth flights in 1991 and 1993. In May 1992, we collected larvae in the original epicenter that were infected with a polyhedrosus virus. This usually portends the collapse of an outbreak, so 1994 could have been the year of a widespread virus epizootic.

Because pandora moth outbreaks occur only every 20-30 years, the present infestation afforded an opportunity to test the effects of fertilization on the trees and the insects in the nutrient deficient soils in the central Oregon pineries. We hypothesized that increasing nutrient availability might improve canopy growth, and net photosynthesis and thereby lead to elevated carbon-based defenses against herbivory. Increased tree growth might also offset the effects of defoliation.

In another forest-insect system, we found that fertilization may temporarily reduce the effects of defoliation (Mason *et al.* 1992, Wickman *et al.* 1992). During an outbreak of the western spruce budworm, *Choristoneura occidentalis* Freeman, both trees and insects were enhanced by fertilization, but trees more than budworms because they produced more new foliage than the budworm could eat. Consequently, fertilized stands suffered less growth impact from defoliation than did untreated stands. We tested this conclusion further in the ponderosa pine-pandora moth system.

The exact test was to determine if a single treatment with nitrogen in the form of urea would significantly reduce the impact of pandora moth defoliation in a thinned second-growth ponderosa pine stand. A secondary objective was to determine the effect of the treatment on growth and feeding behavior of pandora moth larvae and evaluate the chemical composition of foliage and larval frass.

Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

METHODS

Study Site

The study took place in an active outbreak of the pandora moth in the Deschutes National Forest about 20 km south of Bend, Oregon. The stand is a ponderosa pine/bitterbrush-manzanita/fescue plant community (Volland 1976). Lodgepole pine is the only other tree species present and is scarce. The original ponderosa pine was logged in the 1930's and the second growth stand thinned in the 1970's. The average age of the trees at the time of our study was about 55 years and spacing averaged 3.6 by 3.6 m, but the latter differed across the area (the study area averaged 165 trees per acre).

Elevation of the study area is 1,250 m, slope varies from 0 to 5 percent, and aspect is slightly south. The soil mantle is 0.9-m-deep pumice, which resulted from the Mount Mazama volcanic eruption about 6,700 years ago. Bare pumice is common and there is only a thin organic layer under the trees. Soils are particularly deficient in nitrogen, phosphorus, and sulfur (Cochran 1978). Annual precipitation is approximately 51 cm and occurs mostly as snowfall from October through March. Precipitation at Bend was 23 cm in the 1988-1989 season, and first snowfall occurred within a week after we applied fertilizer.

Experimental Design and Treatments

In fall 1988, 10 pairs of circular 1/10th of an acre plots (11.35-m radius) were located in a 4 by 5 grid with plot centers approximately 40 m apart (Fig. 1). All trees on the plots were marked with metal tags. On average, treated plots

Figure 1.—Schematic layout of experiment near Sun River on the Deschutes National Forest. F = fertilized and C = control.

contained 17 trees and control plots 16 trees. The average tree diameter at breast height (dbh) was 29.5 cm. One plot in each pair was randomly selected for fertilization. Analysis of results was on a paired plot basis. In October, 27.2 kg of urea (47% N) was applied by hand to the fertilized plot in each of the 10 pairs. This simulated an application rate of 350 kg N ha^{-1}.

Insect and Tree Measurements

Effects of the treatment were evaluated by measurements of tree buds, foliage, radial increment, and insect biomass on treated and untreated plots. Samples for foliage measurements and insect weights were collected periodically from tree crowns with pole pruners according to methods recommended by Schmid *et al.* (1982). Radial increment was evaluated at the end of the study from increment cores taken from two trees per plot. All sample trees were selected from the center of plots to minimize edge effect. The schedule for measurements and collections follows:

Date	Shoot and foliage	Insect larvae	Insect pupae	Radial increment
Fall 1989	X			
May 1990		X		
Fall 1990	X		X	
Fall 1991	X			
May 1992		X		
Fall 1992	X			X

Larvae were sampled only once in 1990, using methods developed by Schmid *et al.* (1982). Sampling also was attempted in 1992, but only a few larvae were found, indicating a virtual population collapse in the study area. Larvae were collected for weighing from 2 midcrown, 45-cm branch samples on each of 3 trees per plot. Larvae were preserved in 70% ethanol and oven dried at 45 °C to a constant weight. Mean dry weight per individual larva was calculated by dividing dry mass by density. Mean fresh weights of pupae were determined from a collection made from the soil of approximately 5 pupae per plot.

Branch samples were collected each August or September from 1989 to 1992. Two 45-cm mid-crown branches per tree, from 3 trees per plot were cut with pole pruners. In the laboratory, new buds were excised and current needles were stripped from the branches. These were oven dried at 45°C to a constant weight and expressed as foliage dry weight per centimeter of twig length. Mean foliage weight was summarized by plot and year. Buds were represented as weight per bud. In August 1992, at the end of the growing season, 2 cores per tree were collected at dbh on 2 trees per plot to measure radial increment. Cores were mounted, sanded, and measured to the nearest 0.01 mm on an incremental measuring instrument interfaced with a desk top computer, as described in Wickman *et al.* (1992).

Insect frass was collected from canvas panels placed under trees on 5 treated and 5 untreated plots at the peak of larval feeding in June 1990. Frass was air dried and analyzed for total and available levels of N, P, K, and S at the University of Arizona, Tucson, in 1993. Dried foliage from 1989 and 1990 samples also was analyzed for N, P, K, and S and available nutrients at the same laboratory in 1993. Dried 1989 needles were analyzed for total reducing sugars and phenolics by Mr. B.A. Birr, North Central Forest Experiment Station, East Lansing, MI, in 1994.

Statistical Analysis

Data were analyzed using paired t-tests on the randomly selected paired plots for each year of measurement. Differences were judged to be significant when the probability was <0.05.

RESULTS

Larval and Pupal Weights

Because the population collapsed in 1992, there was only one larval collection from May, 1990. This was composed of mostly 4th and 5th instars. Head capsule measurements were made to separate the instars prior to drying and weighing of the samples. Fertilization had substantial negative effects. Mean dry weights of individual larvae from fertilized plots were 126.0 mg and 248.7 mg for 4th and 5th instars respectively, compared to 196.5 mg and 360.3 mg, respectively, for untreated plots. These were 29.6% (p = 0.036) and 32.9% (p = 0.006) reductions, respectively, due to fertilization. Mean fresh weights of treated and untreated pupae in September, 1990 were 290.1 mg and 301.4 mg, respectively, but were not significantly different (p = 0.56).

Foliage and Bud Weights

Foliage weight was consistently greater on the control plots from 1989 through 1992, but this difference was significant (p = 0.03) only in 1990 (Fig. 2). The declining weights of all foliage from 1989 through 1992 was probably due to the combined effects of chronic drought and repeated defoliation. Visual defoliation ratings made in fall, 1990, were similar for both fertilized and control trees. The trend of bud weight was similar to that found for foliage except in 1989 fertilized buds were heavier than the controls (Fig. 3). Buds from control plots were significantly heavier in 1991 and 1992 than fertilized buds (132.3 vs 120.8 mg, p = 0.053 and 69.5 vs 49.3 mg, p = 0.021, respectively). Again, the much smaller buds in 1992 probably reflect combined effects of 2 defoliation episodes and 5 consecutive years of subnormal precipitation.

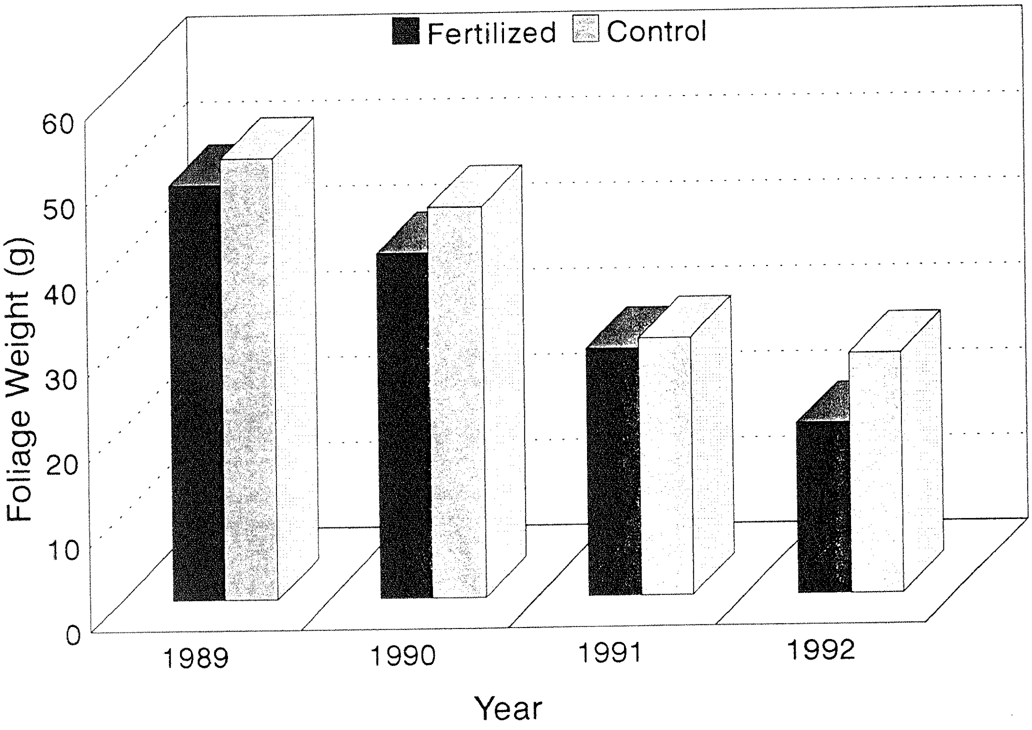

Figure 2.—Foliage per centimeter of shoot length. Each bar is an average of 10 plot means.

Foliage and Frass Chemical Analyses

Foliage from fertilized trees contained more total N in both 1989 and 1990 and more available N in 1989 (Table 1). Statistical analysis was not possible because the samples were pooled. Other nutrients (P, S, and K), apparently were similar in both fertilized and control foliage for both years (Table 1).

Larval frass collected in June 1990 was also analyzed for nutrients (Table 2). Available N was over 6 times higher in frass from larvae feeding on fertilized trees. Total P and K in frass from fertilized trees was approximately half that from unfertilized trees. Because the samples were pooled, a statistical analysis was not possible, but these differences were striking.

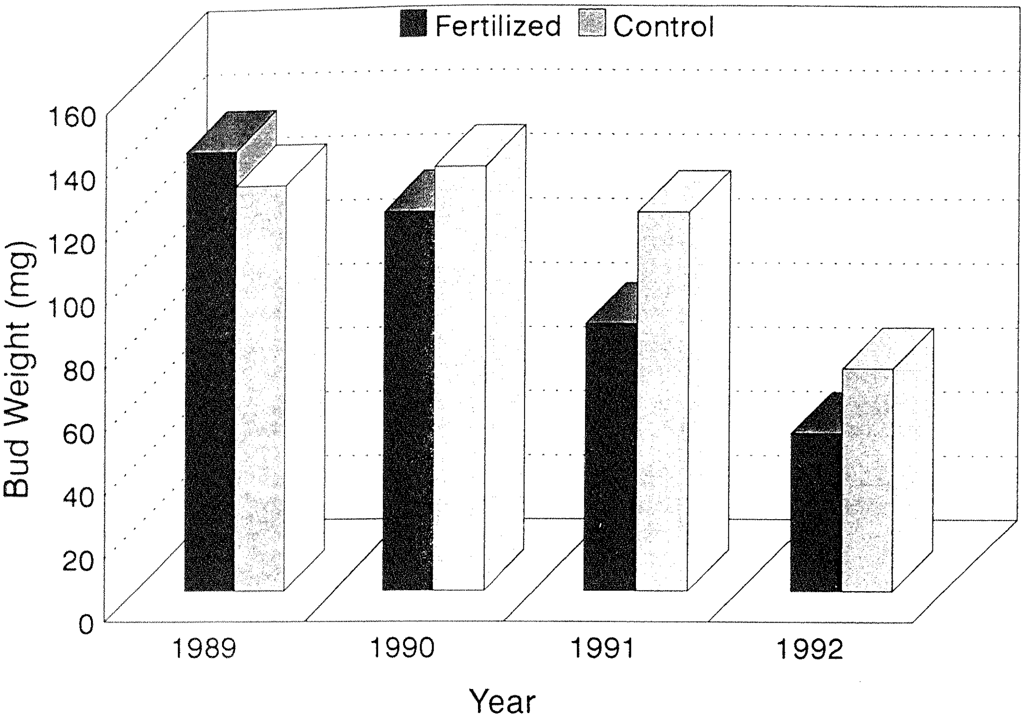

Figure 3.—Average weight per bud. Each bar is an average of 10 plot means.

Table 1.—Percent foliar nutrients in 1989 and 1990 needles (sample pooled from 10 fertilized plots and 10 control plots).

	Total			
	N	P	S	K (ppm)
1989				
Plots 1-10 (fertilized)	2.095	0.114	0.071	7,558
11-20 (control)	1.568	0.127	0.075	7,471
1990				
Plots 1-10 (fertilized)	2.012	0.167	0.082	8,641
11-20 (control)	1.750	0.185	0.088	9,995
	Available			
1989				
Plots 1-10 (fertilized)	0.0216	0.0690	0.0088	6,165
11-20 (control)	0.0134	0.0581	0.0064	5,635
1990				
Plots 1-10 (fertilized)	0.0206	0.1007	0.0039	7,082
11-20 (control)	0.0216	0.0994	0.0052	7,739

Table 2.—Percent nutrient content of larval frass in 1990 (sample pooled from 5 fertilized plots and 5 control plots).

	Total				Available
	N	P	S	ppm K	ppm N
Fertilized	0.826	0.065	0.039	1,430	625
Control	0.595	0.131	0.042	2,670	94

There was no significant difference in the total reducing sugars (p = 0.36), or phenolics (p = 0.48) from fertilized trees and controls in 1989 foliage.

Radial Growth

Radial growth showed a steadily declining trend on both fertilized and control plots from 1988 through 1992, (Fig. 4). Fertilized trees, even though they produced less foliage and smaller buds during this period, had significantly ($p \leq 0.05$) greater radial growth than did control trees. Growth in 1990, 2 years after fertilization and the year of heaviest defoliation, was particularly good compared to controls, and was highly significant (p = 0.0004). The trend continued into 1991 with fertilized trees growing more than double that of controls. Tree growth in 1992 was still 47% higher on treated plots (p = 0.036).

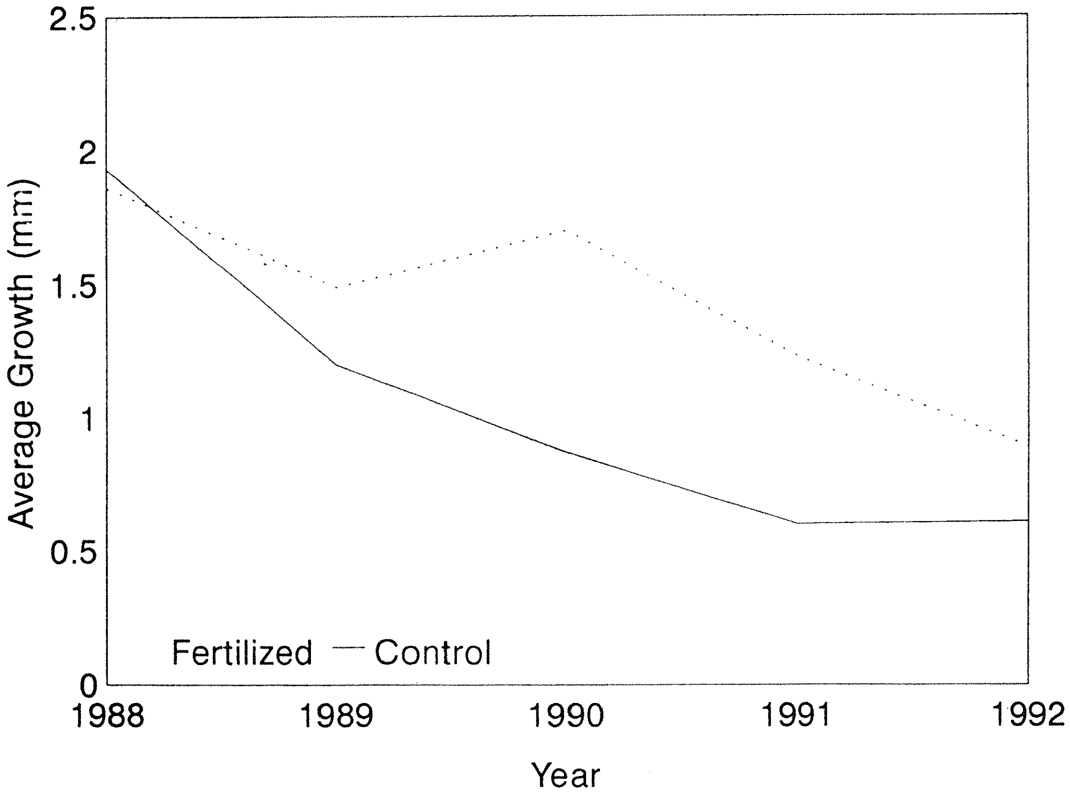

Figure 4.—Trends of radial growth of ponderosa pine at dbh in fertilized and control plots for post treatment years. Plotted points are averages of 10 plot means.

Fertilized and control trees all grew at essentially the same rates during the 16-year period prior to fertilization (Fig. 5). The downward trend starting in 1986, probably induced by precipitation deficits, was sharply reversed in 1990 for fertilized trees. Those trees eventually resumed their downward trend, but the spurt of growth in 1990 gave them a noticeable advantage over the untreated controls.

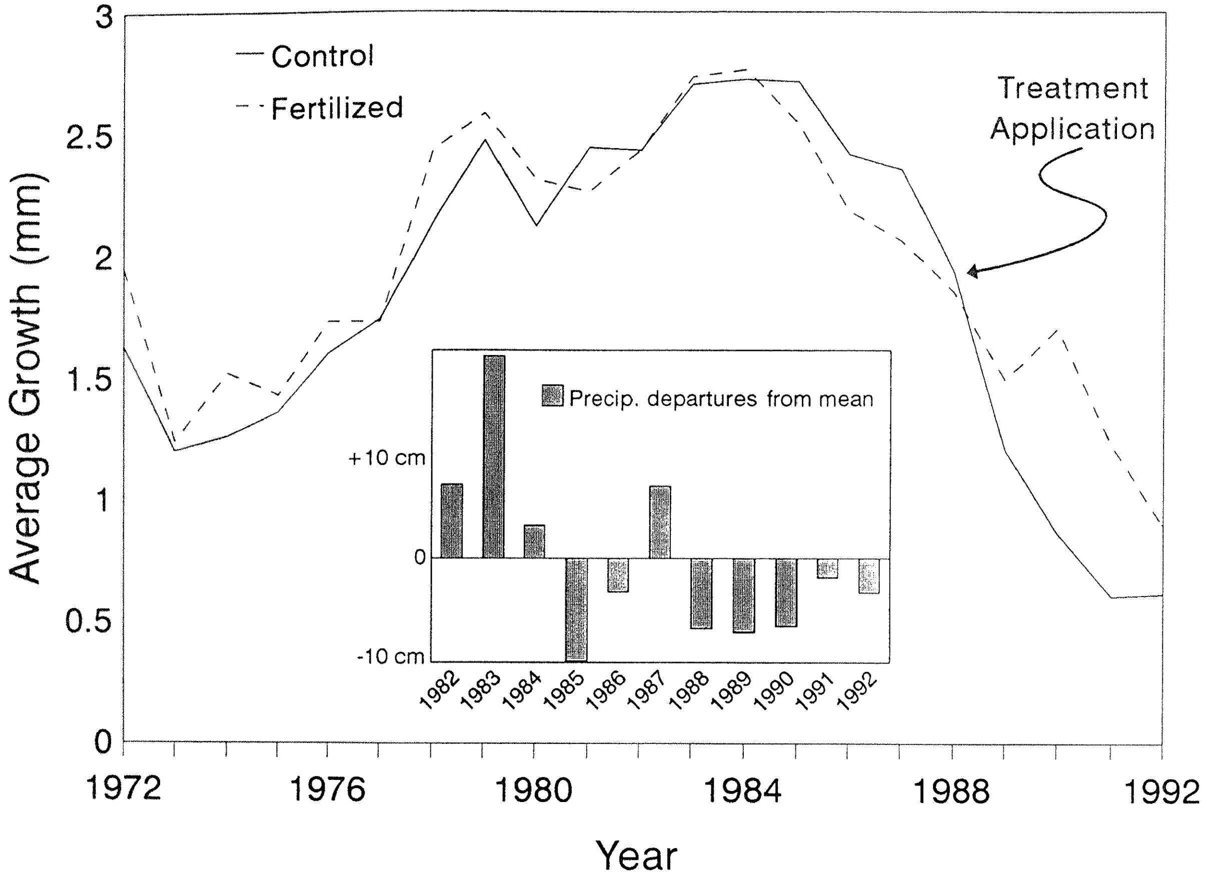

Figure 5.—Trends of precipitation and radial growth of ponderosa pine at dbh. Plotted points are averages of 10 plot means.

DISCUSSION

All stages of the pandora moth are large and easily seen, and tree defoliation is spectacular during an outbreak. Such episodes, however, occur at intervals of 20 or 30 years, and tree mortality as a result of feeding is usually negligible. Even though the pandora moth makes an interesting test insect for herbivore studies, its irregular, boom or bust dynamics limit its consistent availability. Because of life history differences between western spruce budworm and pandora moth, comparisons of fertilizer effects are tenuous. The pandora moth has a 2-year life cycle; therefore, larvae, on alternate years, feed mostly on older needles rather than emerging new foliage so that the tree has a constant supply of new foliage. Conversely, the budworm has a 1-year life cycle and feeds on new needles annually and can repeatedly remove new foliage for 7 to 10 years.

We found that larvae responded negatively in 1990, at least in terms of individual weights, to the fertilizer treatment. This occurred even though available N measured in 1989 foliage was almost 50% higher in fertilized plots. The opposite effects on larval weights were reported for a similar study of western spruce budworm in Oregon (Mason *et al.* 1992, Waring *et al.* 1992). Available N in 1990 insect frass was over 6 times higher on fertilized plots. A possible explanation is that pandora moth larvae may be differentially absorbing proteins or amino acids from fertilized compared to unfertilized foliage and concentrating N in the frass. Why the increased nutrient resource did not result in larger individuals is an interesting question. Perhaps nutrients were imbalanced.

Some tree responses to fertilization were also different than in other recent studies. Additional N provided by fertilization was expected to increase foliage production based on results of a study of fir infested with western spruce budworm (Wickman *et al.* 1992). We found fertilized trees produced less foliage per centimeter of twig length for the period 1989 through 1992 and buds also were smaller in all years except 1989.

Radial growth usually is related to foliage quantity and quality. Fertilized trees had less foliage production but more radial growth than control trees, which suggested that resources may have been allocated to the tree bole at the expense of foliage. Fertilization resulted in greater individual tree growth similar to findings of Cochran (1978). He fertilized thinned ponderosa pine stands similar and close to our study area; however, his stands were not defoliated by pandora moth at that time.

A study by Miller and Wagner (1989) of the effects of pandora moth defoliation on ponderosa pine growth in Arizona found greater radial growth in heavily defoliated trees 1 year after the last defoliation. This is not the usual growth response of trees defoliated by some other insects where growth is usually directly related to degree of defoliation and lags 1 year after defoliation (Wickman 1979, Wickman *et al.* in press)

The study raised more questions than answers. Response of insects and foliage production of host trees was contrary to findings from a recent study of western spruce budworm (Mason *et al.* 1992, Waring *et al.* 1992, Wickman *et al.* 1992). But radial growth seems to respond positively after fertilization on nutrient deficient soils irrespective of insect or host tree species, and perhaps from a site productivity perspective that is what really matters.

It may be impossible to account for the smaller larvae from fertilized trees in this small study. Perhaps the added nutrient resources (N) were only partially allocated for growth of trees and some increased defensive chemistry production in fertilized trees resulted in smaller larvae. Maybe the larval growth was related to feeding efficiency determined by effects of foliage phenolics or other foliage attributes, like needle toughness, on the previous generation of larvae that carried over to the next generation. This explanation is difficult to pursue because we were able to measure larval weights from only one generation of the three that affected the host tree through the outbreak period. Perhaps the insect frass with its high levels of available nitrogen reacted synergistically with residues of N in the soil and this resulted in increased radial growth, even while the trees were being defoliated. Miller and Wagner (1989) suggest that the heavily defoliated pines ability to compensate for foliage loss involves certain compensatory growth mechanisms, perhaps accelerated nutrient cycling of insect frass. We did find that pandora moth frass is rich in nutrients, but we had no way of accounting for interactions of frass and our N treatments.

This study, though small and short on chemical analysis, does point out the vagaries associated with interactions among fertilization, insects, defoliation, and the host tree. Our studies to date of budworm on fir and pandora moth on pine do not indicate that fertilization enhances plant defenses against herbivores through the production of secondary resistance compounds. One consistent result we have encountered is increased radial growth of fertilized trees.

SUMMARY

Responses of ponderosa pine and pandora moth to fertilization with N were studied for 4 years after treatment. Fertilization had a negative effect on larval weights. The 1990 generation of treated larvae were significantly smaller than control larvae, but there was no significant difference between pupal weights. Foliage and bud weights of fertilized trees were significantly lighter than controls. Available nitrogen in both foliage and insect frass was higher in fertilized plots. Radial growth at dbh of fertilized trees was significantly greater and almost double the growth of controls. The results indicate that effects of fertilization differ with species of host tree and insect herbivore, except for increased radial growth that has been consistently noted in all the recent fertilization studies in eastern Oregon. The highly complex interactions of increased nutrient cycling from herbivore feeding and artificial application of N may help explain variable results found in recent studies.

ACKNOWLEDGMENTS

Technical assistance was provided by Katie Bobowski, Ellen Stenard, and Wendy Sutton. We also thank Dr. Russel Mitchell for collecting insect frass and Dr. Arthur Tiedemann for arranging for chemical analysis of foliage and frass.

Dr. William Mattson arranged for analysis of sugars and phenolics in foliage. Valuable insights which helped our interpretations were provided by Drs. William Mattson and Pekka Niemelä during a visit to the study site in 1993. We appreciate helpful review comments from Dr. Arthur Tiedemann and Dr. Patrick Cochran, Pacific Northwest Research Station, and Dr. Pekka Niemelä, Finnish Forest Research Institute.

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PHYTOCHEMICAL PROTECTION AND NATURAL ENEMIES IN THE REGULATION OF A ROOT AND STEM BORING INSECT

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INTRODUCTION

Much debate has centered on why herbivores in natural systems do not more often have a large negative effect on the plants on which they feed. Except for some aquatic trophic cascades in which plants are algae, (Carpenter *et al.* 1987, Carpenter and Kitchell 1988) and a few large mammalian grazers (McNaughton *et al.* 1988), herbivores have rarely been shown to substantially depress plant populations. Two main ideas why this is are top-down suppression by predators and bottom-up plant protection (Hunter and Price 1992). The natural enemy hypothesis suggests that populations are generally controlled by their predators and/or pathogens (Hairston *et al.* 1960, Oksanen *et al.* 1981, Hairston and Hairston 1993). Thus, the plant feeders are kept too sparse to substantially depress plant populations. The endogenous plant protection hypothesis proposes that, in terrestrial and some aquatic systems, plant biomass is protected by noxious secondary chemicals (Ehrlich and Raven 1964, Hawkins 1992, Hay and Steinberg 1992). An adjunct to this is that higher plant biomass is also largely unavailable to most herbivores (Strong 1992, White 1993). This is because most herbivores cannot digest cellulose or lignin without the aid of microbial symbionts (Martin 1991). Because cellulose and lignin make up a large portion of the plant, many essential nutrients for herbivores are present only in low concentrations, which may limit the growth of herbivore populations so that they are not able to have substantial impact on plant populations.

Of course, these hypotheses are not mutually exclusive, and in the system discussed here both plant defense and herbivore natural enemies may play a role in determining the level of herbivore effect on the plant population (Price 1992). The system of the bush lupine, *Lupinus arboreus*, and its stem and root boring ghost moth or "swift," *Hepialus californicus*, is ideal for examining the roles of these different factors because the level of herbivore attack varies over space with some areas exhibiting extensive plant mortality and others with little mortality (Strong *et al.* in prep.).

Directly or indirectly, abiotic factors are probably involved in the differences between the sites in level of attack. The effect of an abiotic factor on the level of herbivory may act directly on the herbivore or be mediated through the herbivore's host plant, which would be bottom-up regulation, or through predators and pathogens, which would be top-down suppression.

Herbivory and Resistance in the Bush Lupine

The bush lupine is a dominant woody shrub of some coastal headland habitats in central California. At Point Reyes and on Bodega Head in Marin and Sonoma Counties, a major source of mortality to the bush lupine appears to be the swift, *Hepialus californicus* (Opler 1968, Davidson and Barbour 1977, Wagner 1986). The mid to late instars of the swift caterpillar bore in the roots and stem of the bush lupine. The early instars are thought to feed on litter and rootlets (Wagner 1986). The caterpillars reach 40-50 mm, and as many as 60 have been found in one bush. While those numbers are exceptional, it is difficult to find a large bush with no evidence of swift caterpillar damage (pers. obs.).

Lupines produce large quantities of quinolizidine alkaloids, which are thought to function in nutrient storage and in defense against herbivores and pathogens (Wink 1992). As little as 0.1% fresh weight of these alkaloids can be toxic to insect herbivores (Wink 1992). Bush lupine leaves contain high levels of these quinolizidine alkaloids in their foliage (Bentley and Johnson 1991). The bush lupine probably also has substantial levels of alkaloids in the stems and roots where the swift caterpillars feed (Wink and Witte 1984). The alkaloid is probably concentrated in the epidermis of the stem and root where exceptionally high levels of alkaloid have been found in other lupines (Wink 1992).

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Whether these quinolizidine alkaloids defend the plant against attack by a particular herbivore species could depend on whether the herbivore in a specialist or a generalist. A specialist could be indifferent or prefer high levels of alkaloid, and a generalist would avoid or be harmed by high levels of alkaloid. Wink (1992) reviews the evidence for both of these cases; however, conclusive evidence for effects of alkaloids in nature has not yet been shown (Hartmann 1992). For swifts, the generalist-specialist distinction is ambiguous. The insect is polyphagous, having been found to feed on a wide variety of host plants over its geographical range. In addition, it is even more indiscriminantly polyphagous and fungivorous in early instars when feeding on detritus, before entering the root. However, as with many polyphagous species, local populations may be much more restricted in their host plant use than this implies (Fox and Morrow 1981, Singer and Parmesan 1993). The swifts at Bodega Marine Reserve seem to be feeding largely on lupine (though perhaps not entirely), despite the presence of hosts they have been found on in other locations (De Benedictis *et al.* 1990), and could show characteristics of specialist rather than generalist herbivores. Hence, it is not obvious what effect, if any, quinolizidine alkaloids will have on the interaction between the swifts and the lupine. The same can be said for another class of chemicals thought to function in defense that have been found in lupine roots, the isoflavonoids (Lane *et al.* 1987).

Herbivory, and perhaps levels of endogenous resistance of bush lupine, vary over the reserve on Bodega Head. Dramatically more bushes died, and each contained many more caterpillars, in some areas than in others in 1992 and 1993 (Strong *et al.* in prep.). In addition, the areas with lower bush lupine mortality in these 2 years have had a fairly consistent cover of lupines over the past 40 years. Conversely, areas with greater recent mortality show a historical pattern of fluctuating cover (Strong *et al.* in press). The plants in areas with different levels of herbivory may differ in their resistance to swift herbivory. Thus, in terms of endogenous plant protection, lower levels of herbivory by swift caterpillars could derive from lower nutritional value of the plant tissue to the herbivore, from higher secondary chemical levels, or both since in some cases they are inexorably intertwined.

The bark and epidermis of the lupine may constitute another or different defense because the early instars may be prevented from feeding on the plant by some chemical or physical property of the outer bark. Thus, they would be more exposed to natural enemies for a longer period of time.

Natural Enemies of Swift Caterpillars

The early instars of *Hepialus californicus* are very small, with a starting length of approximately 1 mm, and could be quite vulnerable to predation because they feed externally on litter and roots. The high fecundity of the swift, followed by modest numbers of large caterpillars in lupine stems, indicates high early instar mortality. Potential predators in the soil around the roots include a geophilomorph centipede, which does eat early instars in laboratory trials (Beld unpubl. data). Wagner (1986) has reported cannibalism as a major source of mortality in laboratory colonies as well.

As the caterpillars grow larger they are found feeding externally on lupine roots, at which time swift caterpillars killed by the new nematode species *Heterorhabditis sp.* have been found. *Heterorhabditis sp.* probably kills the early instars as well, but the tiny corpses are simply harder to locate. Larger caterpillars tunnel inside the lupine root and stem where they become relatively invulnerable to predation. A fungus, *Beuvaria brogniartii*, kills the medium to large swifts inside the stem and root (Williams 1905, Wagner 1986, Strong and Kaya unpubl.).

The nematode has the potential to limit the density of swift caterpillars in the stems. The prevalence of the nematodes in the soil at the base of lupine stems, as measured by a *Galleria mellonella* bioassay (Bedding and Ackhurst 1975), is quite variable between sites (Strong unpubl. data).

Abiotic Factors

Several abiotic factors may be of importance in this system either by depressing swift populations directly or by affecting lupines or the nematode. Wind could affect swift moth populations at the different sites directly; ovipositing female swifts release eggs as they fly over suitable habitat. The prevailing winds come out of the north, correlated with low swift caterpillar density in lupine roots. However, Wagner (1986) found that the moths do not usually fly if it is too windy and one of their flight times is predawn when winds are usually light. Also, desiccation may be a major cause of mortality in the early instar caterpillars.

Indirect effects of abiotic factors on swift populations could be mediated either by their host plant or through predators and pathogens. Input of sea spray is higher at the upwind sites (Barbour *et al.* 1973). One consequence of higher salt spray loads could be higher soil phosphorous levels. A possible mechanism by which phosphorus could drastically alter allocation patterns in the plant has been demonstrated by Dinkelaker *et al.* (1989) in a congeneric species, *Lupinus albus*. Under conditions of low phosphorus, this plant secretes large quantities of citrate (up to 23% of its dry matter production) from proteoid roots. The citrate enhances the solubility of phosphorus, making it more available to the plant. Thus, plants that have more phosphorous available could have much more fixed carbon to allocate to growth rather than to acquiring nutrients since they would not need to secrete as much citrate to get their required phosphorous. If a similar mechanism is present in *L. arboreus*, this would almost certainly alter plant chemistry between sites with different levels of phosphorous in ways that could affect individual growth rates and survival of the swift caterpillars by changing the nutritional quality of their food. Differential growth and survival rates could in turn determine the density of caterpillars in the stems and roots of plants.

Abiotic factors, such as soil properties and moisture, could contribute to differences in the nematode populations between the two sites. *Heterorhabditis sp.* are probably vulnerable to desiccation (Kaya 1990), hence in the dry summer, slight differences in soil moisture between sites may be quite important to their survival.

DISCUSSION

The picture sketched by our preliminary information leads to several hypotheses concerning the differences in the impact of herbivore on its host plant among our different sites. Understanding the mechanism by which plants are "protected" from herbivory at sites with low herbivory as compared to sites with high herbivory will allow us to assess the relative importance of the top-down effects of natural enemies and the bottom-up effects of plant chemistry (including nutrition). The simplest hypotheses implicate a single abiotic factor or an abiotic factor mediated by either the nematode or the lupine. These include differences in oviposition due to wind, differences in levels of predatory nematodes due to differences in soil chemistry, and differences in the secondary chemistry of the lupine due to differences in nutrient levels. While each of these may play a role, it seems unlikely that any of them are sufficient alone.

An additional hypothesis involves the interaction of plant chemistry and predation in explaining the observed pattern. The hypothesis is that the different nutrient levels at some sites allow plants to be better defended against herbivory, or to be more vigorous and better able to withstand herbivory (Price 1991), both of which would reduce the impact of the herbivore enough that the plants would be able to persist at that site. Then, since the plants persist at that site, rather then undergoing local extinction, there is a constant supply of the swift for the nematode to prey upon. At sites that experience local extinction of lupine, the nematode could have no host for a period of time and go extinct at that site. Since it is not a very mobile predator, it may not be able to recolonize rapidly when the lupine and the swift reappear. Thus, the nematode reinforces the pattern caused by the differences in herbivore "resistance" between the sites by reducing swift populations where the lupines usually persist, but not where they experience high mortality.

Experimental manipulations in the field of natural enemies and conditions affecting plant chemistry will be needed to assess the relative roles of top-down control and bottom-up protection in controlling herbivore populations. The third alternative, as we have mentioned, is the action of abiotic factors directly on the herbivore populations; this possibility should also be examined by looking at egg deposition and the desiccation of early instars at the different sites.

SUMMARY

Mature bush lupine, *Lupinus arboreus*, die after heavy stem and root boring by caterpillars of the ghost moth or swift, *Hepialus californicus*. Mortality rates of these plants can be extremely high in some stands and quite low in other stands in close proximity. This system is ideal for testing for top-down versus bottom-up control of herbivore populations by examining the differences between sites where there is heavy herbivory and sites where there is not heavy herbivory. Nutrient levels that are likely to affect lupine plant allocation patterns probably vary over the area of interest. Population levels of an entomopathogenic nematode that prey upon the swift moth also vary over the reserve. It is hypothesized that both plant chemistry variation and the nematode natural enemy play an important role in determining the level of herbivore attack.

ACKNOWLEDGMENTS

We would like to thank John Maron for access to his data on lupine fluctuations, which is in manuscript. For financial support we would like to thank Barbara Bentley for a graduate student grant from the Bodega Field Conferences (AVW), the Sippl fund for travel support (AVW), and William Mattson and the IUFRO conference for aid with meeting fees (AVW).

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RESISTANCE OF HYBRID AND PARENTAL WILLOWS TO HERBIVORES: HYPOTHESES AND VARIABLE HERBIVORE RESPONSES OVER 3 YEARS

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INTRODUCTION

Analysis of herbivory on hybrid plants in natural populations has recently gained attention (Whitham 1989, Keim *et al.* 1989, Boecklen and Spellenberg 1990, Paige *et al.* 1991, Whitham *et al.* 1991, Aguilar and Boecklen 1992, Fritz *et al.* 1994, Whitham *et al.* 1994), and it may be important for understanding plant-herbivore interactions (Whitham 1989). To assess the significance of interspecific hybridization of plants for plant-herbivore interactions, it will be important to know how interspecific hybridization of plants influences resistance of hybrid plants in relation to resistance of pure parents. It has been hypothesized that hybrid plants may act as an "ecological sink" for herbivorous insects (Whitham 1989). In mixed stands of parental and hybrid plants, this hypothesis predicts that a large fraction of the herbivore population would reside on hybrid individuals (Drake 1981, Whitham 1989, Floate *et al.* 1993). Whitham (1989) referred to highly susceptible hybrid poplars as being an ecological sink for a galling aphid, but in terms of metapopulation dynamics, hybrid plants could function as a "source" rather than a sink (e.g., Ericson *et al.* 1993). Hybrid plants have also been hypothesized to act as "evolutionary sinks" to pests (Whitham 1989). The "evolutionary sink" idea predicts that if herbivore fitness was higher on hybrids compared to their fitness on parental plants, there would be selection for specialization on the hybrids, perhaps preventing selection for herbivore virulence on the parental species. If so, then hybrid plant-herbivore interactions may be critical to understanding why plants seem to maintain pest resistance in the face of a greater evolutionary potential for virulence in pests. Finally, Floate and Whitham (1993) suggest that hybrid plants may facilitate host shifts of herbivores onto new plants, because they could act as a selective "bridge" favoring adaptation of an herbivore to previously non-host species. Tests of these hypotheses will require knowing the general patterns of herbivore responses to hybrid versus pure parental species.

Hypotheses

Fritz *et al.* (1994) proposed several alternative hypotheses of phytophage response to hybrid plants. Those hypotheses assume that pure parent species are being compared to F1 hybrids. The reason to focus on F1 hybrids is that this comparison could suggest: (1) how resistance mechanisms are inherited from parental species, and (2) how these traits may be expressed upon backcrossing. The Additive hypothesis (Fig. 1A) predicts that hybrids do not differ from the mean of the resistances of the two parents (i.e., the midparent value). Thus, F1 hybrids are intermediate between the resistances of the parental species, which suggests that hybrid resistances are due to the additive inheritance of resistance traits from both parents.

The second hypothesis is the Dominance hypothesis (Fig. 1B). If hybrid resistance differs significantly from the mean resistance of both parents but does not differ significantly from that of one of the parents, the Dominance hypothesis would be supported. Hybrid resistance could resemble that of either the more resistant or the more susceptible parent. (Dominance in this context refers to phenotypic similarity between a parent and hybrids, not necessarily genetic dominance, though it may imply dominant inheritance of resistance traits.) Herbivore densities could be intermediate between the Additive and Dominance patterns, which would support an hypothesis of partial dominance.

The third hypothesis is the Hybrid Susceptibility hypothesis (the Hybrids-as-Sinks hypothesis of Whitham 1989; see also Keim *et al.* 1989, Boecklen and Spellenberg 1990). This hypothesis predicts higher herbivore densities and/or higher herbivore performance on hybrids compared to parental taxa (Fig. 1C). The Hybrid Susceptibility hypothesis can be distinguished from the Dominance hypothesis in that the susceptibility of the hybrid must *exceed* that of the most susceptible parent.

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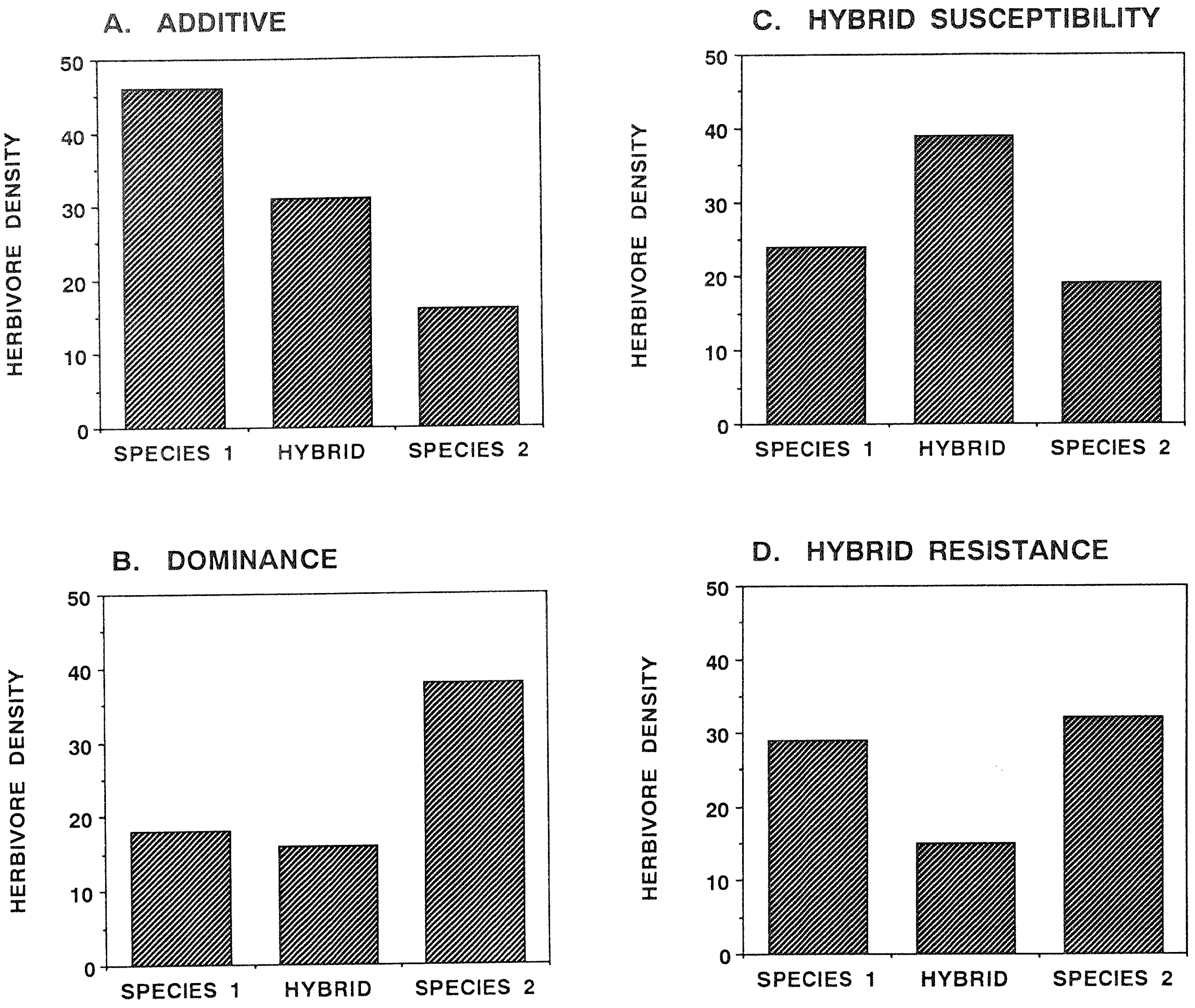

Figure 1. Hypothesized patterns that would result from the Additive hypothesis (A), the Dominance hypothesis (B), the Hybrid Susceptibility hypothesis (C), and the Hybrid Resistance hypothesis (D). Imaginary densities (Susceptibility) are plotted on the Y-axis for each parent species and their F1 interspecific hybrid.

The final hypothesis, the Hybrid Resistance hypothesis, predicts that hybrid plants will be more resistant than either parent, resulting in lower herbivore densities on hybrid plants (Fig. 1D). This hypothesis requires that hybrids be *more resistant* than the most resistant parent. A study by Boecklen and Spellenberg (1990) suggests support for this hypothesis for herbivore communities in two oak hybrid zones. Hybrids supported lower densities and diversities of herbivores than the mean of the parental species, but they did not distinguish between the fit of their data to the Hybrid Resistance hypothesis or the Dominance hypothesis. We present and discuss these hypotheses in genetic terms since support of any of the hypotheses may suggest probable underlying mechanisms of inheritance of resistance.

It is not clear a priori why one of these hypotheses should be more likely than any others. If the Hybrid Susceptibility hypothesis is prevalent and herbivory reduces plant fitness, then herbivores could be important in maintaining distinct plant species by limiting the fitness of hybrids. Boecklen and Spellenberg (1990) found evidence for the Hybrid Resistance and for our Additive hypothesis. Fritz *et al.* (1994) found support for the Additive, Dominance, and Hybrid Susceptibility hypotheses among herbivore and a pathogen species on hybrid and parental willows. The purposes of this paper are to investigate the responses of 11 herbivore species from different guilds to parental and hybrid willows over three consecutive years, and to determine if herbivore responses to hybrid plants vary over time. This paper will show that different herbivores, even those in the same guild, vary dramatically in their response to hybrid versus parental taxa, but that most species, when considered over time, fit the Additive Hypothesis.

METHODS AND MATERIALS

The System

This system has several advantages for studying herbivore-hybrid plant interactions. The two willow species that hybridize often co-occur in the same habitats with their hybrids, eliminating, to a certain extent, confounding environmental variation across naturally occurring hybrid zones with herbivore resistance variation among hybrids and parents. Willows have a number of herbivore species in different guilds, including gall-formers, leaf miners, leaf tiers, and chewers, that are well characterized and that have been studied extensively on one of the willow species in this system.

Salix sericea Marshall and *Salix. eriocephala* Michx. co-occur in swamps and along streams in central New York. *S. sericea* is a 0.5-4-m-high shrub that has lanceolate leaves with densely sericeous hairs on the lower leaf surface. Stipules are small, lanceolate, and usually absent from older leaf nodes (i.e., stipules are deciduous). This species occurs predominantly in swamps from Canada south through the northeastern U.S. and along the Appalachian range to Georgia (Argus 1986). *S. eriocephala* is a shrub that may reach 6 m in height, and its leaves are lanceolate to narrowly oblong and glabrous beneath. Stipules are large, persistent, half ovate, and half cordate at the base. It frequently occurs along streams, and its range extends from Canada south as far as Virginia and west to Missouri (Argus 1986). The ranges of the two species are broadly sympatric, and at the study site both species co-occur and intermingle. Species can be easily distinguished in the field based on leaf, stipule, and bud characteristics (Argus 1986).

These willow species hybridize to form plants that are usually distinctive from each parent. Large, persistent, half-ovate stipules (a *S. eriocephala* trait) and a sericeous (hairy) lower leaf surface (a *S. sericea* trait) serve as morphological markers to identify *S. sericea* x *S. eriocephala* hybrids in the field (Fritz *et al.* 1994). The natural hybrids at the study site do not appear to be extraordinary; *S. eriocephala* and *S. sericea* have been reported to hybridize throughout their range (Argus 1974, 1986; Mosseler and Papadopol 1989). Flowering phenology may explain the occurrence of hybridization between *S. sericea* and *S. eriocephala* (Argus 1974, Mosseler and Papadopol 1989), which differ by only a few days in the onset of flowering (foliage phenology is also similar) (Fritz, pers. obs.).

Many herbivores attack the parental and hybrid willows. Each herbivore species attacks both parental willow species and hybrids. Gall-forming sawflies (Hymenoptera: Tenthredinidae) include *Phyllocolpa nigrita*, *Phyllocolpa* sp. nov., *Phyllocolpa terminalis*, and *Pontania* sp. (leaf galls). Gall-forming flies (Diptera: Cecidomyiidae) include the stem gallers *Rabdophaga rigidae*, the beaked willow gall; *R. salicisbrassicoides*, the willow cabbage gall; and a leaf galler, *Iteomyia salicifolia*. The other common leaf galler species is the gall mite *Aculops tetanothrix* (Acarina: Eriophyidae). There are two species of leaf miners, *Phyllonorycter salicifoliella* and *Phyllocnistis* sp. (Lepidoptera: Gracillariidae), that commonly form mines on willow leaves.

There are two frequently seen leaf folder species (Lepidoptera) that have distinctive leaf folds, but have not yet been successfully reared or identified. One species (LF) folds over and sews the leaf margin to the lamina with silk. The other species (V) forms a tube of the leaf tip by sewing the edges of the leaf blade together.

Methods

These studies were performed from 1991 to 1993 at the Sosnowski site 3 km west of Milford, NY, along County Route 44. All plants are individually marked at the study site. Censuses of herbivores species on 14 *S. eriocephala*, 29 *S. sericea*, and 16 hybrids were conducted from late July to early August 1991. In 1992, we censused 20 *S. eriocephala*, 36 *S. sericea*, and 39 hybrids, and in 1993 we censused 40 *S. eriocephala*, 109 *S. sericea*, and 38 hybrids. Mostly hybrid plants known to be F1-types (Fritz *et al.* 1994) were included in the hybrid category. Plants known to be backcrosses from RAPD were not included in this analysis. Some hybrid plants added to the census group after 1991 have not been examined for their RAPD genotype, and it is therefore possible that they are something other than F1-types (Fritz *et al.* 1994).

Galls, leaf miners, or leaf folds were counted on 50 shoots per plant for hybrids and *S. eriocephala* and on 300 shoots for *S. sericea* in 1991 only. Data are expressed as number of herbivores per 300 shoots. Because leaf folds of *Phyllocolpa nigrita* and *Phyllocolpa* sp. nov. were not distinguished on *S. sericea* in 1991, we combined them for analyses in all years. All other species densities are considered separately. Species densities were considered statistically independent among plants within taxa for all years (Fritz *et al.* 1994), and species-wide significance tests rather than table-wide significance tests were applied. ANOVAs were performed for each year separately. We first performed a multivariate ANOVA, using loge transformed densities of all herbivore species, and then we performed univariate ANOVAs for each species separately.

Orthoganol contrasts were performed to test for the fit of each herbivore species to the hypotheses. We performed only two orthoganol contrasts to test the significance of herbivore densities on willow taxa (species) to avoid overparameterization of the analysis, since only three taxonomic groups were present (SAS Institute 1985). To test the Additive hypothesis, we tested the significance of the hybrid-midparent contrast (Contrast A) using sequential Bonferroni analysis with species-wide significance at $P \leq 0.05/2 = 0.025$ (Rice 1989). If this test was significant, we rejected the Additive hypothesis and then tested the contrast between herbivore density on hybrids and density on the numerically closest parent (Contrast B) ($P \leq 0.05/1 = 0.05$). This contrast tested if there was a significant departure from the Dominance hypothesis. If the Dominance hypothesis was rejected, we inspected the means to determine if the Hybrid Susceptibility hypothesis (density on hybrids exceeded that of the highest parent) or the Hybrid Resistance hypothesis (density on hybrids was lower than the lowest parent) was supported, or if the density of hybrids was intermediate between that of one parent and the midparent value. This last possibility would support a partial Dominance hypothesis.

RESULTS

Multivariate ANOVAs showed highly significant contrasts between herbivore densities on hybrids and the mean herbivore densities of parent species for all three years (Table 1). This is a rejection of the Additive hypothesis overall. Further multivariate contrasts could not be made since the test of the Dominance hypothesis requires comparison to the numerically nearest parent, which would not be the same for all herbivores . In 1991, five herbivore species had significant hybrid vs. parents contrasts (Contrast A) at $P < 0.025$, and two herbivore species had marginally significant hybrid vs. parents contrasts at $P < 0.05$ (Table 1). In 1992, four species had significant hybrid vs. parents contrasts (Table 1). Three species that had significant contrasts in 1991 did not have significant contrasts in 1992. In 1993, five species had significant hybrid vs. parent contrasts (Table 1). Overall, then, there appear to be differences among the years in the species of herbivores that had significant departures from the Additive hypothesis.

To illustrate the year-to-year differences in more detail and to draw conclusions about which hypotheses mentioned above are supported by each species, we present the separate results for each year for each species, which included all censused plants.

Both contrasts for *Phyllonorycter salicifoliella* were significant in 1991 and 1993 (Table 1), and the means support for the Hybrid Susceptibility hypothesis (Table 2). In 1992, neither contrast was significant, which supported the Additive hypothesis. The other leaf miner *Phyllocnistis* sp. also showed differences between years. In 1991 and 1993, the first contrast was not significant, supporting the Additive hypothesis (Tables 1 & 2). Fritz *et al.* (1994) concluded that this species supported the Hybrid Susceptibility hypothesis in 1991, because of the marginally significant contrast. In 1992 the Dominance hypothesis was supported with densities on *S. eriocephala* and hybrids being equal and lower than on *S. sericea* (Table 2).

Among species of the leaf galling guild, *Phyllocolpa terminalis* showed consistent results in each year (Tables 1 & 2). In all cases the Hybrid Susceptibility hypothesis was supported. *Phyllocolpa* spp. supported the Additive hypothesis in 1991 and 1993 (Tables 1 & 2). In each year, including 1992, densities on hybrids were intermediate between the two parental species. In 1992, the first contrast was significant and the second contrast was not significant, supporting the Dominance hypothesis (Tables 1 & 2). *Pontania* sp. supported the Additive hypothesis in 1991 and 1992, but the Dominance hypothesis was supported in 1993 (Tables 1 & 2). *Iteomyia salicifolia* supported the Hybrid Susceptibility hypothesis in 1991 and 1993 (Tables 1 & 2). In each year, including 1992, densities on hybrids were greater than on either parental species. In 1992, the first contrast was not significant at $P < 0.025$ (although it was marginally significant at $P < 0.05$), thereby supporting the Additive hypothesis, even though the mean density on hybrids was more than twice as high as on *S. eriocephala* (Table 2). The last leaf galling species, *A. tetanothrix*, supported the Dominance hypothesis in 1991 and 1993 (Tables 1 & 2). In both of

Table 1. —Summary of multivariate and univariate ANOVAs of herbivore densities on *S. sericea*, *S. eriocephala*, and hybrid plants for 1991, 1992, and 1993. F-values and degrees of freedom are shown for the univariate tests. Contrast A was considered significant if its P value was less than the Bonferroni criterion of $P < 0.025$. F-values for the multivariate ANOVAs are based on Wilks' lambda (other tests gave similar results). Herbivore species are grouped by feeding guild.

YEAR	1991		1992		1993	
CONTRAST	A	B	A	B	A	B
	F(1,61)	F(1,61)	F(1,92)	F(1,92)	F(1,184)	F(1,184)
Multivariate	11.22†	—	11.28†	—	7.04†	—
Leaf Miners						
Phyllonorycter salicifoliella	10.31***	5.94*	2.06	0.68	7.27**	5.82***
Phyllocnistis sp.	4.24*	4.67*	13.02†	0.22	0.45	5.35*
Leaf Gallers						
Phyllocolpa terminalis	18.35†	10.13***	16.09†	10.26***	36.91†	27.93†
Phylocolpa spp.	2.18	0.31	6.13**	0.03	1.66	11.75†
Pontania sp.	0.87	8.60***	4.28*	0.16	6.86**	1.82
Iteomyia salicifolia	13.04†	13.50†	4.32*	0.69	16.95†	11.93†
Aculops tetanothrix	13.97†	2.27	30.23†	7.62***	11.88†	2.34
Leaf Folders						
Leaf folder-LF	4.49*	1.74	0.73	0.40	0.92	0.52
Leaf folder -V	16.99†	9.30***	0.44	2.36	0.40	0.47
Stem Gallers						
Rabdophaga rigidae	1.99	0.21	0.50	0.47	2.13	0.22
salicisbrassicoides	3.24	0.06	4.77*	0.11	0.13	0.44
Total Herbivores	0.42	9.47***	1.30	0.32	11.98†	4.62*

* - $P < .05$, ** $P < .025$, *** $P < .01$, † - $P < .001$

these years, densities on *S. sericea* and hybrids were equal and lower than on *S. eriocephala*. In 1992, however, the Hybrid Resistance hypothesis was supported. Mite gall density on hybrid plants was significantly lower than on *S. sericea*. It should be noted, however, that in all three years the numbers of mite galls were lower on the hybrid plants than on the nearest parent.

For the leaf folding guild, LF supported the Additive hypothesis in all years (Tables 1 & 2), but the first contrast was marginally significant in 1991 (Table 1). Species V supported the Hybrid Susceptibility hypothesis in 1991, but the Additive hypothesis was supported in 1992 and 1993 (Tables 1 & 2).

In the stem galling guild, both *R. rigidae* and *R. salicisbrassicoides* supported the Additive hypothesis consistently in each year (Tables 1 & 2), but densities of *R. salicisbrassicoides* in 1992 were marginally significant for the first contrast, nearly supporting the Dominance hypothesis.

Seven of the 11 herbivore species showed significant changes in the hypotheses that were supported between years. In five of the seven cases, densities of the herbivores had declined between the years of the change; in two cases densities had increased or there was little change. Lower herbivore densities, therefore, might have contributed to the changes in which hypotheses were supported.

Table 2.—Summary of the hypotheses supported by each species based on the results in Table 1 and inspection of mean herbivore densities among the willow species in each year (Fritz *et al.* in prep.). When the Dominance hypothesis was supported the two equal taxa are indicated. In all cases they were more resistant than the other taxon. Herbivore species are grouped by feeding guild.

	1991	1992	1993
Leaf Miners			
Phyllonorycter salicifoliella	Susceptibility	Additive	Susceptibility
Phyllocnistis sp.	Additive	Dominance (E=ES)	Additive
Leaf Gallers			
Phyllocolpa terminalis	Susceptibility	Susceptibility	Susceptibility
Phyllocolpa spp.	Additive	Dominance (ES=S)	Additive
Pontania sp.	Additive	Additive	Dominance (E=ES)
Iteomyia salicifolia	Susceptibility	Additive	Susceptibility
Aculops tetanothrix	Dominance (ES=S)	Resistance	Dominance (ES=S)
Leaf Folders			
Leaf folder-LF	Additive	Additive	Additive
Leaf folder -V	Susceptibility	Additive	Additive
Stem Gallers			
Rabdophaga rigidae	Additive	Additive	Additive
R. salicisbrassicoides	Additive	Additive	Additive
Total Herbivores	Additive	Additive	Susceptibility

To visualize the combined response of herbivores to hybrids vs. parents, we summed densities of herbivores on each plant and reanalyzed the responses. When mites were included in the analysis, there was no significant hybrid vs. parent contrast in 1991 and 1992 (Tables 1 & 2, Fig. 2), but the Hybrid Susceptibility hypothesis was supported in 1993.

DISCUSSION

The main conclusions of this study are that: (1) there is evidence that different herbivores support different hypotheses regarding the effects of interspecific hybridization on plant resistance; and (2) there is year-to-year variation in which hypothesis is supported by a given herbivore species. These results suggest the possibility that different resistance mechanisms are inherited differently in hybrid plants, or that different herbivores have different responses to the same resistance mechanism in the hybrid compared to the parent. The results also suggest the possibility that environmental variation affects the hybrid and parental willows differently between years, which alters susceptibility to herbivores.

The hypothesized effects of interspecific hybridization on plant resistance have as an assumption that hybrids are F1's. The plants that were censused were predominantly F1's based on RAPD analysis (Fritz *et al.* 1994), but six plants were most closely identified as backcrosses to *S. sericea*. Known backcross plants were removed from this analysis, but it is possible that some backcrosses are included among the hybrid plants added to the censuses in 1992 and 1993. We have not yet analyzed these new hybrid plants using our RAPD technique to know their exact genetic status. Fritz *et al.* (1994) have shown that morphological characters are not always reliable for distinguishing *S. eriocephala* from hybrids, and F1-type hybrids are indistinguishable from backcross plants using morphological traits (Fritz, unpublished data). With these comments in mind, we will proceed to interpret the results of this study in relation to the hypotheses (Fig. 1).

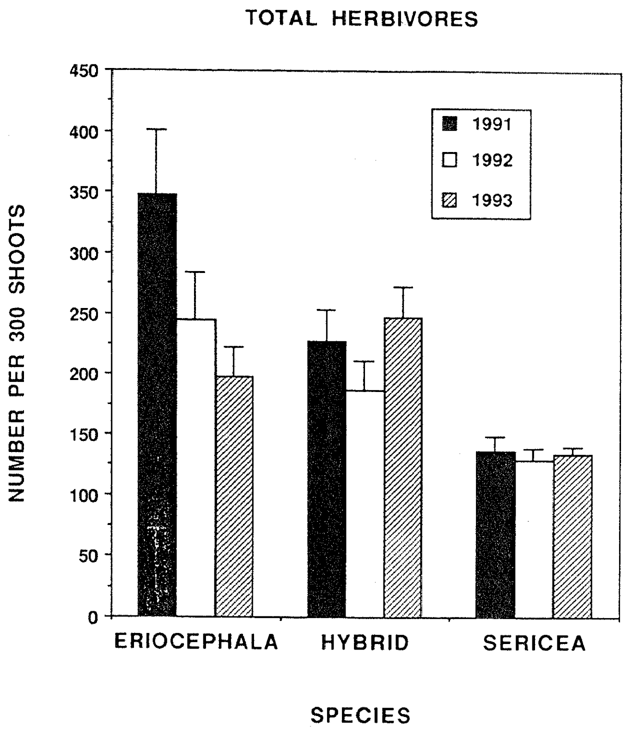

Figure 2.—Density (± 1 SE) of total herbivore numbers among *S. eriocephala, S. sericea* and their interspecific hybrids and among years. Table 2 indicates which hypotheses were supported by total herbivore numbers.

The Additive hypothesis received the greatest support in each year (7 of 11 species, Table 2). Herbivore densities on hybrids are often intermediate between the densities on the two parental species. This finding is consistent with an Additive model of inheritance of resistance traits. Other studies also provide support for the Additive hypothesis. Soetens *et al.* (1991) found for a species of *Pontania* on European willows and their hybrids that gall numbers were intermediate on hybrids (supporting the Additive hypothesis). Studies on sawflies on birches (Hanhimäki *et al.* 1994), beetles on elms (Hall and Townsend 1987), and beetles on willows (Soetens *et al.* 1991, and Fritz unpublished), and various species on oaks (Aguilar and Boecklen 1992) also indicate that hybrids are often intermediate in suitability for herbivore growth. Hanhimäki *et al.* (1994) have studied herbivore performance on hybrid and parental birches that grew in a common plantation in Finnish Lapland. Of the 14 herbivore species studied (13 sawflies and a moth), 11 species had intermediate growth rates on hybrid leaves compared to parental leaves.

Monogenic and polygenic resistance traits where allele effects on traits are additive will have half the dosage of alleles, on average, in hybrids compared to parents, unless parents have the same defense mechanisms determined by the same loci. There is evidence from willows supporting additive inheritance of phenolic glycosides, tannins, and morphological defenses (Meier *et al.* 1989, Soetens *et al.* 1991, Nichols-Orians and Fritz, unpublished data). How the altered dosage of defensive traits influences herbivores will depend on the sensitivity of the herbivores to the resistance trait and the presence of other resistance traits inherited from species that might not normally be host plants of the herbivore. A consequence of additive inheritance of resistance is that backcrossing should result in a reconstitution of defense, rather than further breakdown of resistance, as hypothesized by Whitham (1989) and Paige and Capman (1993). The resistances of backcross individuals should more closely resemble that of the recurrent parent as more parental genes are reincorporated into the genome. Whitham *et al.* (1994) found evidence supporting this hypothesis in their work on eucalypts. When F1 hybrids were compared with backcross plants and parents, often backcross plants were intermediate between F1 hybrids and parents.

The Dominance hypothesis received support from *Aculops* mites, *Phyllocnistis* sp. *Phyllocolpa* spp., and *Pontania* sp. in at least one year (Table 1). In this hypothesis, herbivore densities on hybrids resemble those of one parent but differ significantly from that on the other parental species. Hanhimäki *et al.* (1994) found for three species, a moth and two sawflies, on the Finnish birches that performance on hybrids did not differ from that of one parent but did differ from the other parent, supporting the Dominance hypothesis. The Dominance hypothesis was proposed by Fritz *et al.* (1994), and it is particularly plausible because of the inheritance of defensive chemicals in hybrids of several crop and wild plants. For *Lotus* (O'Donoughue *et al.* 1990), *Nicotiana* (Huesing *et al.* 1989), and *Papaver* (Levy and Milo 1991), chemical defense mechanisms have been shown to be inherited as dominant traits in hybrids. Zangerl and Berenbaum (pers. comm.), in a review of the inheritance patterns of secondary chemicals, found that 13 of 32 compounds had dominant inheritance. Given dominant inheritance, if the parent possessing the high levels of defensive chemical is resistant, then F1 hybrids should also be resistant. Paige and Capman (1993) showed that hybrid breakdown in resistance to *Pemphigus betae* occurs only in backcrosses of *Populus* hybrids to the susceptible parent, and F1 hybrids are as resistant as the resistant *P. fremontii*. This illustrates the Dominance hypothesis in the cottonwood-aphid system.

There was also considerable support for the Hybrid Susceptibility hypothesis. Three herbivore species consistently supported the Hybrid Susceptibility hypothesis, and over three years 9 of 33 cases supported this hypothesis. The Hybrid Susceptibility hypothesis was also supported by the analysis of total herbivore densities in 1993 (Table 1, Fig. 2). Mosseler (pers. comm.) reports that in his garden of F1 hybrids and parents of seven *Salix* species, hybrids were highly susceptible to *Melampsora* rust, but parents were immune. Fritz *et al.* (1994) has also found for *Melampsora* rust that hybrids were much more susceptible than either parental species. This observation shows that F1's appear to show breakdown of resistance.

The Hybrid Resistance hypothesis was supported only by the *Aculops* gall mite in 1992. Density of mites was lower on hybrids and on either parent. Boecklen and Spellenberg (1990) found some evidence for this hypothesis in their study of leaf mining and leaf galling herbivores on oak. A problem with the support for the Hybrid Resistance hypothesis from that study is that many herbivore species were combined into feeding guilds for analysis and they did not explicitly test for a significant departure from the Dominance hypothesis.

The results of this study suggest that interspecific hybridization of plants has a variety of effects on resistance of hybrids and it does not lead only to a pattern of breakdown of resistance (i. e., support of the Hybrid Susceptibility hypothesis). The various herbivore responses suggest that different resistance traits of plants have different mechanisms of inheritance and/or that different resistance factors could affect herbivores differently. An important question is: How can the hypothesized herbivore responses be explained by the known patterns of inheritance of resistance traits? The Additive and Dominance hypotheses both have fairly straightforward explanations. If resistance is dosage-dependent, then an Additively inherited trait should lead to support for the Additive hypothesis. A dominantly inherited trait would be fully expressed in hybrids and should result in a pattern of herbivore resistance that supported the Dominance hypothesis. Backcrossing to the susceptible parent, as seems to have occurred in cottonwoods, could lead to the loss of one or more dominant resistance genes, thereby resulting in Hybrid Susceptibility of the backcross progeny. Paige and Capman (1993) suggest that there is more than one dominant gene involved in resistance to aphids in the cottonwood system.

Additively inherited traits could also result in herbivore densities that support the Hybrid Susceptibility hypothesis. Herbivores that require a threshold amount of a chemical defense could have higher densities on hybrids (Hybrid Susceptibility) if the dosage in hybrids was less than the threshold amount. Hybrid Resistance could result from a mechanism of dominance inheritance. Hybrids with dominant inheritance of two different resistance traits from each parent would possess complete expression of two different resistance mechanisms. If particular herbivores are negatively affected by both resistance mechanisms, hybrids could be more resistant than either parent (i.e., Hybrid Resistance). It also seems possible that if two additively inherited resistance mechanisms interacted synergistically to affect herbivores Hybrid Resistance could also result. These alternative mechanisms suggest that various outcomes of interspecific hybridization on resistance are likely and that mechanisms of inheritance of resistance and their effect on herbivores need to be investigated in hybrid systems.

The year-to-year variation in support for particular hypotheses was an unexpected result of this analysis. Seven of the 11 species had at least one change in which hypothesis was supported between adjacent years. These changes could be considered either: (1) random fluctuations in herbivore numbers on hybrids and parents or accidents of sampling, (2) consequences of temporarily lower population sizes that made differences difficult to detect, or (3) real year-to-year changes in the expression of resistance of hybrids and/or parent species due to variation in environmental factors. Point 1 is always possible, but it is impossible to discount using the census data. A number of shifts in herbivore response occurred between 1991 and 1992 and then back to 1991 patterns in 1993. This may have something to do with the decreased abundance of herbivores in 1992 (unpublished results). Lower numbers could have made differences difficult to detect. While point 2 is possible, it would be wrong to attribute the shifts in support for hypotheses to this factor alone. Shifts in support for hypotheses may be real changes in the relative expression of resistance between hybrid and parental plants. Environmental variation (e. g., solar radiation, temperature, nutrients, etc.) is known to affect the expression of resistance among genotypes of many plant species. It therefore seems likely that changes in environment could affect resistance of hybrid and parent species of plants in nature. There were some substantial shifts in the weather among the years of this study. The summer of 1992 was cool and rainy compared to normal in the northeast, whereas in 1993 there were some long periods of high temperatures and drought (pers. obs.). These factors might have contributed to the variation in resistance of hybrids. However, experiments will be required to test if the relative resistance of hybrids shifts with manipulated changes in specific environmental factors.

Guild membership was not a reliable indicator of similar responses of herbivores to hybrids and parents. Among the leaf galling guild, all four hypotheses were supported in at least 1 year. Leaf miners supported two different hypotheses (Additive and Hybrid Susceptibility) considering the 3 years together (Table 2). These results indicate that combining the densities of two or more species in the same guild for hybrid-parent comparisons will obscure the underlying patterns of herbivore densities on hybrids and parents. In this analysis, numbers of *Phyllocolpa nigrita* and *Phyllocolpa* sp. nov. were combined in 1992 and 1993, because densities of these species had not been distinguished on *S. sericea* of the willow species in 1991. Therefore, the conclusions drawn from these species should be considered with caution.

SUMMARY

We studied herbivory of two species of willows (*Salix sericea* and *S. eriocephala*) and their interspecific hybrids to test four alternative hypotheses concerning the effects of hybridization on plant resistance. Individually marked plants were identified using morphological traits in the field, and RAPD band analysis was used to verify the genetic status of some parental and hybrid plants. The densities of 11 herbivore species were compared between 2 parents and their hybrids in the field. We found the most support for the Additive hypothesis and the Hybrid Susceptibility hypothesis over the three years. We found some evidence for the Dominance hypothesis, and one species in one year supported the Hybrid Resistance hypothesis. Guild membership was not a good predictor of similar responses of species to hybrid versus parental plants. This study demonstrates the diversity of responses of phytophages in response to interspecific hybridization, and indicates the presence of year-to-year variation, which might indicate the influence of environmental variation on the expression of hybrid resistance.

ACKNOWLEDGMENTS

We would like to thank Len and Ellie Sosnowski for allowing us to work on their property. We thank S. Kaufman, N. Murphy, P. Zee, M. Momot, A. Samsel, A. Dao, J. Hama, A. Samsel, and G. Sylvan for help in the field. This research was supported by the National Science Foundation grants BSR 89-17752 to R. S. Fritz and DEB 92-07363 to R. S. Fritz and C. M. Orians, and by the Vassar College General Research Fund.

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F1 HYBRID SPRUCES INHERIT THE PHYTOPHAGOUS INSECTS OF THEIR PARENTS

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INTRODUCTION

Interspecific hybridization in plants is a widespread and important natural phenomenon. In fact, Stace (1987) estimates that at least 50% of the extant angiosperm species have been derived via hybridization. Natural hybrids are also common among the gymnosperms (Wright 1955, Hanover and Wilkinson 1969, Schmidt-Vogt 1977, Zobel and Talbert 1984). In spite of the ecological and evolutionary importance of plant hybrid zones (Remington 1968, Barton and Hewitt 1985), little is yet known about how they affect the dynamics of plant/herbivore relationships. Whitham (1989) speculated that hybrids may be particularly prone to herbivory because usual resistance mechanisms derived from the parents are poorly expressed. For example, polygenic resistance traits might be intermediate in F1 hybrids, whereas monogenic and oligogenic traits might be fully expressed or not at all depending on the mode of gene action (e.g., dominant or recessive, etc.), and on whether there is genetic dysfunction preventing normal gene expression (Fritz *et al.* 1994, Whitham *et al.* 1994). Furthermore, to compound these problems, hybrids may attract specialist phytophages from both parent species, thereby being predisposed to a much larger pool of potential consumers than either of the pure parent types (Whitham *et al.* 1994).

Insect Loading in Relation to a Plant's A/R Vectors and S/D Vectors

To examine the issue of hybrid resistance to insects in a more mechanistic framework, we consider that there are at least two sets of plant traits affecting insect loading on plants: (1) the mix of properties which elicit insect attraction/arrestment/repulsion (e.g., morphology, electromagnetic and biochemical cues, abiotic requirements, etc.) and thus govern the number of insect species that attempt to colonize it, and (2) the mix of properties that are sustaining/defensive for each of the would be colonizers as they attempt to feed, oviposit, and reproduce. These two sets of properties, which together set the potential for insect loading (total insect biomass or numbers), can be represented as two vectors: the species attraction/repulsion (A/R) vector, $[a_1,a_2,a_3..a_n]$, and the suitability/defense (S/D) vector $[s_1,s_2,s_3..s_n]$. Each species of insect, Si, is represented by an element (a_i) in the species A/R vector, having a numerical value (0-1) that is proportional to the plant's average capacity to attract and arrest individuals of the particular insect species. Likewise, the S/D vector contains specific elements (s_i) that correspond to and represent the plant's average suitability, ranging from zero, where an insect colonizes a plant but cannot complete development on it (immunity), to 1 where the plant fulfills all of the insect's requirements, for each species in the species A/R vector. The product of the two vectors, $[a_1s_1+a_2s_2+..a_ns_n]$, represents the plant's overall potential "resistance expression", or potential insect loading per plant.

To give an extreme example for one insect: the white pine weevil, *Pissodes strobi*, is highly attracted to *Picea glauca* shoots as evidenced by the great number of feeding scars it produces (i.e., a = 1), but it can only rarely reproduce in these shoots (i.e., s = 0), with the consequence that *P. glauca* is considered "resistant" to the weevil (Lanier 1983). Actual insect loading depends, in addition, on the quantity and genetic diversity of insects (N_i) that continually attempt colonization. The total number of non-zero elements (all those $a_i > 0$) in an individual plant's A/R or species vector (its species richness or species loading) is positively linked to the size and density of its population, the total areal distribution of the species, and negatively to its biochemical uniqueness wherein it occurs (for a review, see Tahvanainen and Niemela 1987). For many natural hybrids, their populations and geographical distributions are usually small (compared to their parents) thereby exposing them to fewer potential colonists, but their low biochemical uniqueness or high similarity to the two nearby parent species will likely have a counteracting influence making them highly susceptible to the reservoirs of consumers from two plant species (Whitham *et al.* 1994).

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Elements of the Suitability/Defense Vector in Hybrids: Expression of S/D Traits

Most work on resistance expression (i.e., a_is_i) in natural and artificial interspecies hybrids has focused on suitability/ defense (s_i) expression for particular insects, assuming that a plant's attractiveness (a_i) is high and invariant. Fritz *et al.* (1994) hypothesized four expression scenarios: (1) the "additive" case where the hybrid's S/D ranking (s_{hi}) against an insect is the simple mean of its parents, p_1, and p_2 ($s_{hi} = (s_{1i} + s_{2i})/2$), (2) the "dominance" case where the hybrid is consistently like one of the parents, e.g., p_1 ($s_{hi} = s_{1i}$), (3) the "susceptibility" case where the hybrid has more insects than either of the parents ($s_{1i} < s_{hi} > s_{2i}$), and (4) the "resistance" case where the hybrid has fewer insects than either parent ($s_{1i} > s_{hi} < s_{2i}$).

Most studies with trees have shown that hybrid S/D expression (s_i) against an insect is not cleanly predictable but varies with the particular insect and the host plant species (Boecklen and Larson 1994, Fritz *et al.* 1994). For example, Manley and Fowler (1969) and Osawa (1989) discovered that the severity of spruce budworm, *Choristoneura fumiferana*, defoliation of spruce trees was linked to the direction of their introgression between black and red spruces, *P. mariana* and *P. rubens*. Hybrids closer to pure red were severely defoliated as were pure reds, whereas hybrids closer to pure black were only lightly defoliated as were pure blacks. Several hybrid elms, *Ulmus*, were consistently intermediate in resistance to the elm leafbeetle, *Xanthogaleruca luteola*, whenever one parent species was susceptible and the other was resistant (Hall and Townsend 1987, Hall *et al.* 1987). Hybrids of *P. sitchensis* x *P. glauca* are usually resistant to *Pissodes strobi*, and so are such backcrosses to white spruce, the resistant parent whereas sitka spruce is highly susceptible (Mitchell *et al.* 1974, Copes and Beckwith 1977). Similarly, the *Pinus coulteri* x *P. jeffreyi* hybrid carries resistance against the weevil, *Cylinrocopturus eatoni*, that the *coulteri* parent possesses, but the *jeffreyi* parent does not (Miller 1950). Hybrids of several species of Japanese pines, *Pinus*, all apparently inherited their "resistance" against the scale, *Matscoccus matsumurae*, from the more susceptible parent species (McClure 1985).

More recently, Floate *et al.* (1993) reported that *Chrysomela confluens* was invariably more abundant in a hybrid swarm of *Populus fremontii* x *P. angustifolia* than in the parental populations. Whitham *et al.* (1994) reported that of some 40 phytophagous taxa in a hybrid swarm of *Eucalyptus risdonii* x *E. amygdalina* only two were more abundant on F1 hybrids than on the parents. Specialist insects were most abundant on hybrid backcrosses to their favored parent species, and generalist insects were generally more abundant on all hybrid types. Hanhimaki *et al.* (1994) reported that in the case of 14 insects tested on hybrids of *Betula pubescens* ssp. *tortuosa* x *B. nana*, the hybrids were consistently equivalent to *B. pubescens*, but superior to *B. nana*. Fritz *et al.* (1994) tested 11 insects and one leaf rust on hybrids of *Salix sericea* x *S. eriocephala*, and concluded that 3 supported the additive, 2 the dominance, and 7 the susceptibility expression hypotheses. Boecklen and Larson (1994) measured densities of 8 species of galling wasps on hybrids of *Quercus grisea* x *Q. gambelii*, and reported that 3 supported the additive, 2 the dominance, 2 the susceptibility, and 1 the resistance expression hypotheses.

In the case of non-woody species, plant breeders have created innumerable interspecies hybrids which have become the cornerstone of breeding programs for the development of insect resistance in food and forage plants. Consequently, most resistance traits useful against native insects and pathogens have been derived from crosses to related but allopatric plant species (Harris 1975, 1980 1982; Bailey 1983). These studies reveal a surprisingly large number of cases for which insect resistance seems to be either monogenic or oligogenic, but polygenic inheritance is also important (Hanover 1980, Harris 1982, Zobel and Talbert 1984, Singh 1986, Carson and Carson 1989, Geiger and Heun 1989). Moreover, the resistance genes are usually, but not always dominant in their expression (Harris 1982, Singh 1986), and evidence suggests that gene linkages and multiple resistance alleles are uncommon. Resistance genes appear to be insect specific, not conferring cross protection against many species (Harris 1982).

Elements of the Species A/R Vector in Hybrids: Expression of A/R Traits

General plant traits (e.g., crown form, height, growth rate, phenology, etc.) in hybrids are often intermediate between those of its parents (implying that $a_{hi} = (a_{1i} + a_{2i})/2$) (Hanover and Wilkinson 1969, Bongarten and Hanover 1982, Yeh and Arnott 1986) which suggests that interspecies hybrids will probably have the unique gestalt of properties to attract the all the specialist insects from both parents (Miller and Strickler 1984). In fact, Whitham *et al.* (1994) and Morrow *et al.* (1994) have found that this is true in a eucalypt hybrid swarm. They reported that the average hybrid tree supported 53% more phytophagous insect and fungal species than equivalent trees from the pure parental stands.

Objectives

Given the importance of a plant's herbivore loading, i.e., the number of herbivore species or non-zero elements in its species vector, this study tested the hypothesis that artificial white x blue spruce F1 hybrids will inherit the phytophages from each of their parent tree species, with the consequence that the number of elements in the hybrid species vector will be the sum of the unique (specialists) plus the shared or common (generalists) elements of both parents. Because hybrid spruces do not occur naturally in Michigan, we only conducted tests in several test plantations where the trees are exposed to the natural phytophages of white spruce but not those of the other parent, blue spruce, a western North American tree species. However, one western spruce galling insect, *Adelges cooleyi*, is common in eastern North America on ornamental blue spruce. We also tested whether total insect loading on hybrids, i.e., the total number of individuals (pooled over all phytophage species) found per unit of foliage per plant, is equal to or greater than that found on each of the parent species.

METHODS

Hybrid spruces *(P. glauca* x *P. pungens)* were sampled in 1991 and 1992 at two different locations in Michigan, along with populations of the parent species in order to assess differences in species, and total insect loading. One sample site occurred in south-central Michigan at the Michigan State University Kellogg Experimental Forest. The other occurred some 450 km to the north in Michigan's Upper Peninsula at the Michigan State University Dunbar Experimental Forest. Kellogg (n = 40) and Dunbar (n = 24) sample trees were all F1 hybrids. Equivalent numbers of parent spruces were likewise sampled from adjacent or nearby (< 1 km) plantations.

At each site, trees were randomly selected, and examined twice per growing season (late May-early June, and late June-mid July). Trees were first examined visually to score for adelgid galls *(Adelges abietis, A. cooleyi,* and *Pineus similis)*. Next they were sampled using standard beating methods whereby the apical half (45 cm length) of a midcrown branch on the west side was held over a specially designed stainless steel collection pan, and the branch was gently tapped with a wooden dowel for 10 seconds to encourage free feeding species to drop off into the pan. Such insects were gathered in vials and then stored in a freezer for later identification and counting.

Data from both June and July samples (numbers of insect species, and total insect counts per tree) were analyzed separately using a completely randomized ANOVA, after log (X + 0.1) transformation. Means for each tree species were ranked and then separated using the SNK multiple range test.

RESULTS

Gall-forming Adelgid Specialists from Both Parent Spruces Successfully Attack Hybrids

At the Kellogg experimental forest in southern Michigan, there were significant populations of both *Adelges abietis*, and *A. cooleyi*, largely specialists, respectively, on *P. glauca*, and *P. pungens* and their near relatives (Furniss and Carolin 1977). We found that the hybrids contained substantial numbers of both kinds of adelgids, whereas the parent spruces had primarily their respective specialist adelgids (Table 1). One *glauca* individual had evidence of two poorly formed *A. cooleyi* galls, but in general *glauca* in eastern North America are not susceptible to the eastern *A. cooleyi* populations (personal observations) which live largely on ornamental blue and Engelmann spruces introduced from western North America. Similarly, there was only minor evidence for *A. abietis* on *pungens*: one tree, with one small, poorly formed gall. Although Table 1 shows very low populations of *A. cooleyi* on *pungens* (one infested tree in a sample of 30-42), this was because the adelgid population had drastically declined in the preceding 2 years (1989, 1990) in the sampled *pungens* plantation which was about 1 km from the hybrids. In 1988 and earlier there were much larger populations of *A.cooleyi* there: 76 of 100 sample trees were infested (Mattson, unpublished data). *Pineus similis*, another galling adelgid that is largely a specialist of *glauca* and near relatives, was not abundant enough to test hybrid susceptibility.

At the Dunbar site in northern Michigan, *A. cooleyi* and *P. similis* populations were negligible, so we could not measure their colonization of the spruce hybrids. But, we did find there that *A. abietis* readily attacked hybrids, but not so readily *pungens*, as shown in the following tabulation of the percentage of trees attacked: white (84), hybrids (83), blue (15).

Table 1.—Comparing the mean numbers of insects and adelgid galls on blue, hybrid, and white spruces, using visual examination of two random midcrown branches per sample tree at the Kellogg Experimental Forest during late May-early June, and mid-late July. N = 30 - 42 trees.

Insect Species	Time period 1: nos./ 2 branches			Time period 2: nos./ 2 branches		
	B.Spruce	Hybrids	W.Spruce	B.Spruce	Hybrids	W.Spruce
A. abietis galls	0.07b[1]	1.14a	2.25a	0.00b	2.20a	2.27a
A. cooleyi galls	0.03b	0.36a	0.00b	0.00b	0.33a	0.07b
A. cooleyi alates	0.17b	24.76a	0.00b	0.00b	4.66a	0.00b
Pineus similis galls	0.00a	0.00a	0.19a	0.00a	0.00a	0.00a

[1] Means with different letters within time periods are significantly ($p < 0.05$) different.

Species Loading, and Total Insect Loading Comparable Among Hybrids and White Spruce

At Kellogg, the hybrid and white spruce herbivore species loadings were statistically equivalent (ca. 3 - 3.3 per branch) and significantly higher than those of blue (1.9) in the first sample, but not in the second, when all three spruces were statistically equivalent. At Dunbar, the hybrids, and white spruce also had equivalent herbivore loadings (ca. 3 - 3.8 species per branch) which were significantly higher than those (ca. 1.2 - 1.5 per branch) of blue spruce in both first and second samples (Table 2).

Table 2.—Comparing mean numbers of phytophagous insect species, and total insect numbers occurring in beating samples from blue, hybrid, and white spruces at two study sites in Michigan.

Study area and sample period	Mean nos. insect species per branch			Mean nos. of insects per branch		
	B.Spruce	Hybrids	W.Spruce	B.Spruce	Hybrids	W.Spruce
Dunbar-1	1.50b[1]	2.96a	3.79a	2.42b	4.17a	6.71a
Dunbar-2	1.17b	3.21a	3.00a	1.25b	4.33a	4.71a
Kellogg-1	1.87b	3.31a	2.97a	1.13b	4.27a	4.23a
Kellogg-2	2.97a	2.17a	2.90a	2.20a	2.12a	1.97a

[1] Means having different letters within an area and sample period category are significantly ($p < 0.05$) different.

At Kellogg, there were 20 species of phytophagous insects on the hybrids, of which they shared 12 in common (60%) with white spruce (Fig. 1). At Dunbar, we found twice as many (41) species of phytophagous insects on the hybrids, of which 33 (80.5%) they shared in common with white spruce (Fig. 1). About 12-15% of the hybrid's phytophagous insects were "unique" at both sites, because they did not share these species with either white or blue spruce. About 49-60% of their total species pool, they shared with blue spruce. These data confirm the expectation that the phytophagous insects occurring on the hybrids are largely those commonly associated with white spruce. The same is true for blue spruce, which had a total of 23 species at Kellogg and 24 at Dunbar, of which 19 were shared in common with white spruce at both sites.

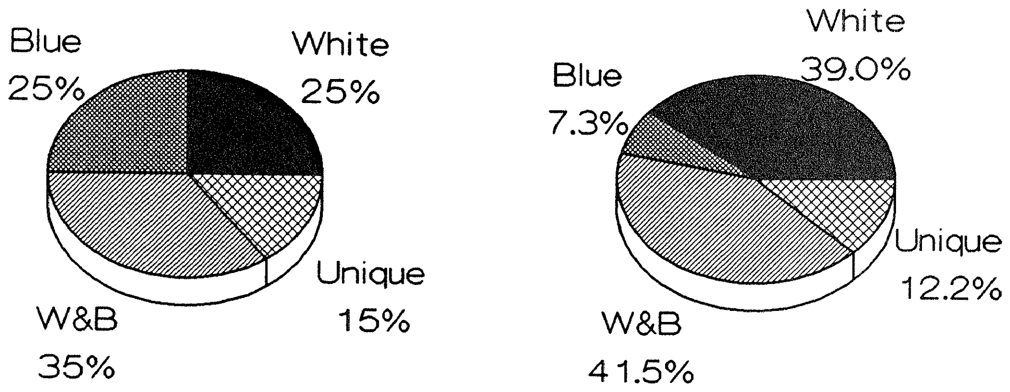

Figure 1.—The percent distribution of the phytophagous insect fauna of hybrid spruces that are shared with blue spruce only, white spruce only, both white and blue spruce, and unshared or unique at two sites: Kellogg (left side) in southern, and Dunbar (right side) in northern Michigan. At Kellogg there were 20 and at Dunbar there were 41 species of insects found in beating samples from the hybrids.

DISCUSSION

The data clearly suggest that white x blue spruce F1 hybrids inherit most of the phytophagous insect species of their white spruce parents. Although we cannot claim that they similarly inherit most of the phytophagous insect species of their blue spruce parentage because the hybrids were not exposed to these insects in Michigan, we can never-the-less say that they readily inherited at least one of them, *A. cooleyi*. Likewise, total insect loading on the hybrids was comparable to that of white spruce.

Thus, the data do not falsify the hypothesis that the number of phytophages (i.e., the number of non-zero elements) in the hybrid species vector will be the sum of the unique and shared species of its parents. We fully expect that if the hybrids were growing adjacent to large, natural populations of blue spruce, they would readily inherit its typical phytophages. Whitham *et al.* (1994) emphasized this very important point for Tasmanian eucalyptus hybrids, reporting that the average hybrid had 53% more phytophagous species than equivalent trees in pure parental stands.

Neither do our data falsify the hypothesis that the total insect loading (i.e., the total number of all individuals of all insect species per unit foliage) on hybrids will be equal to or greater than the average loading of each of its parents. This may be true because the defensive traits (s_{hi}) for any insect in the hybrids will probably never be more strongly expressed than in the less suitable or better defended of the two parent species (e.g., if $s_{1i} \geq s_{2i.}$ then $s_{2i} \geq s_{hi}$), and in many if not most cases, it will be less poorly expressed (e.g., the additive, the susceptibility, and the dominant (high defense is recessive) expression scenarios of Fritz *et al.* 1994). At the level of the whole phytophagous insect community, the hybrid studies of Boecklen and Larson (1994), Fritz *et al.* (1994), Hanhimaki *et al.* (1994), and Whitham *et al.* (1994) unequivocally support this proposition. Hybrids may therefore generally sustain more herbivores and herbivore injury than either pure parent species.

SUMMARY

F1 hybrids of the spruces *Picea glauca* x *Picea pungens* were studied at two Michigan locations to measure and compare their total phytophagous insect fauna (i.e., species and total loading) with that of the parent species in adjacent or nearby plantations. Hybrids had species compositions, and total insect loading nearly identical with that of *Picea glauca.* In addition, at least one adelgid specialist from *P. pungens* readily colonized and successfully reproduced on the hybrids. These data support the hypotheses that hybrids will (a) inherit the insect specialists from both parents, and (b) have an overall insect loading that equals or exceeds that of each parent.

ACKNOWLEDGEMENTS

The authors sincerely thank Drs. J. Kouki and J. Spence for their discussions about and their helpful critiques of earlier drafts of this manuscript. They also thank the personnel of the W.K. Kellogg Forest for their unstinting assistance.

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RECENT ADVANCES IN RESEARCH ON WHITE PINE WEEVIL ATTACKING SPRUCES IN BRITISH COLUMBIA

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INTRODUCTION

Interior spruce (a collective term for white spruce, *Picea glauca* (Moench) Voss; Engelmann spruce, *Picea engelmanni* Parry, and their varying degrees of hybrids) is a very important timber resource for the province of British Columbia. Over 100 million interior spruce seedlings are planted annually. An estimated 1.38 billion seedlings have been planted to date, and the total number of hectares planted in spruce is over 1.3 million.

White pine weevil, *Pissodes strobi* Peck, is a major cause of Sitka spruce, *Picea sitchensis* (Bong.) Carr., plantation failure in coastal British Columbia, Washington, and Oregon (McMullen 1976; Furniss and Carolin 1977; MacSiurtain 1981; Alfaro 1982, 1989). This pest is also becoming an important menace to interior spruce plantations, causing considerable damage to both yield and quality of product (Cozens 1983, Taylor *et al.* 1991).

Adult weevils overwinter in the duff layer of the forest floor and begin laying their eggs in the bark of young spruce trees (generally 3-30 years old) near the tip of the previous year's shoot in early summer. Young weevil larvae feed on the phloem of the leader, moving downwards in the process (Silver 1968). Once a feeding ring is formed, the leader of the tree dies. In their downward movement, the larvae can destroy up to 4 years of growth. Literature sources refer to the successful colonization of the leader as weevil attack; this terminology will be maintained in this report. Repeated leader destruction causes loss of height growth and stem deformities, which leads to quality reduction (Alfaro 1989 and 1992).

Selection of spruce varieties genetically resistant to weevil damage is a potential tool for the reduction of weevil damage in future plantations. Genetic resistance could manifest itself in several forms. Morphological and anatomical differences could make certain trees less susceptible to feeding, ovipositioning, and to the development of the larvae (Plank and Gerhold 1965, Stroh and Gerhold 1965). Another possible resistance mechanism consists of variations in the chemical composition of resistant trees that make them less attractive to weevils. Phagostimulants may be lacking in some trees, which can render them less attractive to weevils. Alternately, the presence of feeding repellents, deterrents, or toxic compounds may provide an effective defense mechanism (van Buijtenen and Santamour 1972; Bridgen *et al.* 1979; Alfaro 1980; Alfaro *et al.* 1980, 1984; Alfaro and Borden 1982, 1985; Wilkinson 1985; Brooks *et al.* 1987 a, b).

The impact of weevil damage could be mitigated by the ability of the trees to recover from the attack (Painter 1951). Tolerant trees are more likely to overcome the effect of attacks and will suffer less growth and quality loss. This depends largely on the ability of one of the lateral branches to gain apical dominance quickly after the leader has been killed. Trees lacking this ability will develop multiple leaders (forks) and often are stunted.

Evaluation of various trials in British Columbia provided strong evidence for genetic variation in susceptibility for weevil damage in both Sitka spruce (Ying 1991, Alfaro and Ying 1990) and interior spruce (Kiss and Yanchuk 1991).

Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

The objectives of this presentation are to *(i)* review measures taken by various agencies in British Columbia to elucidate the interrelationship between the weevil and its host, *(ii)* discuss studies in progress to take advantage of the genetic resistance of the host and *(iii)* present an overview of attempts to control one of the more destructive forest pests. The presentation will also review future plans.

WEEVIL RESEARCH

Field Studies

Prince George Progeny Trials

Due to the importance of interior spruce in British Columbia, a decision was made to embark on the genetic improvement of the species complex in the early 1960's. The senior author was hired to carry out this project in 1967.

The basic approach to improving interior spruce was to designate geographically distinct areas (selection units or SU's) and select trees based on size and form in each selection unit (Kiss and Yeh 1988, Kiss and Yanchuk 1991). The first of these selection units was in the Prince George area. A total of 180 parent trees were selected. Cones and scions were collected from most selected parents. Scions were used to establish clone banks, and seeds were used to establish genetic tests.

As part of the improvement program, genetic tests were established using open-pollinated progenies of individual parents selected in various selection units. The first set of these tests, representing the Prince George SU, was established in 1972 and 1973 (Kiss and Yeh 1988, Kiss and Yanchuk 1991) using the open-pollinated progenies of 173 parent trees.

The initial objective of the genetic tests was to identify well-performing families based on the growth performance of their progenies. This information aided in the construction of rogued first generation seed orchards and helped to identify candidates for advanced generation breeding. To this end, periodic height measurements were carried out and at age 15 diameter measurements were also made.

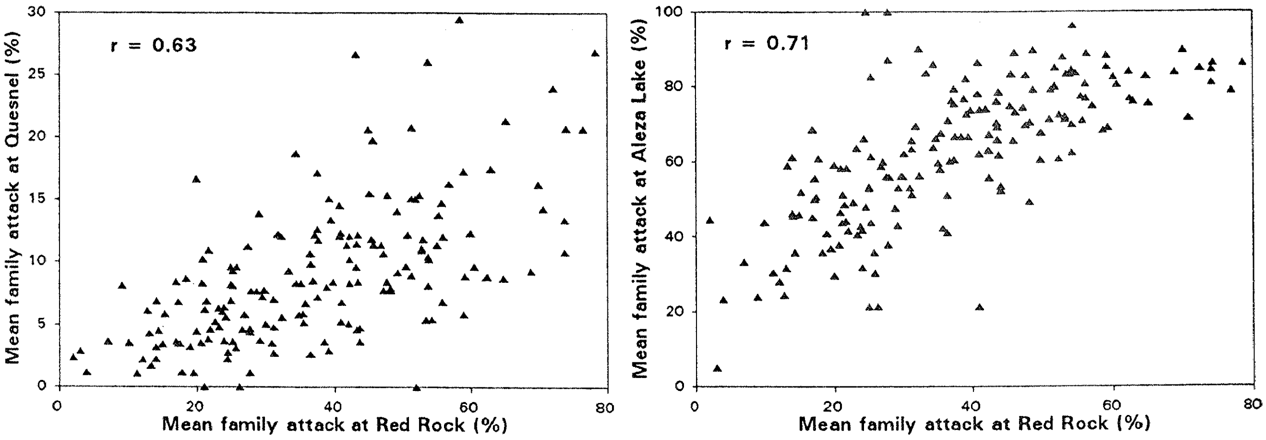
Following the 15-year height and diameter assessments, heavy infestation of white pine weevil was observed at some test sites. Cursory observation appeared to suggest a pattern to the infestation. Further studies confirmed this pattern and revealed the possibility of genetically controlled resistance mechanism(s) in a number of families. Results of this study are reported by Kiss and Yanchuk (1991).

In summary, the results showed that:

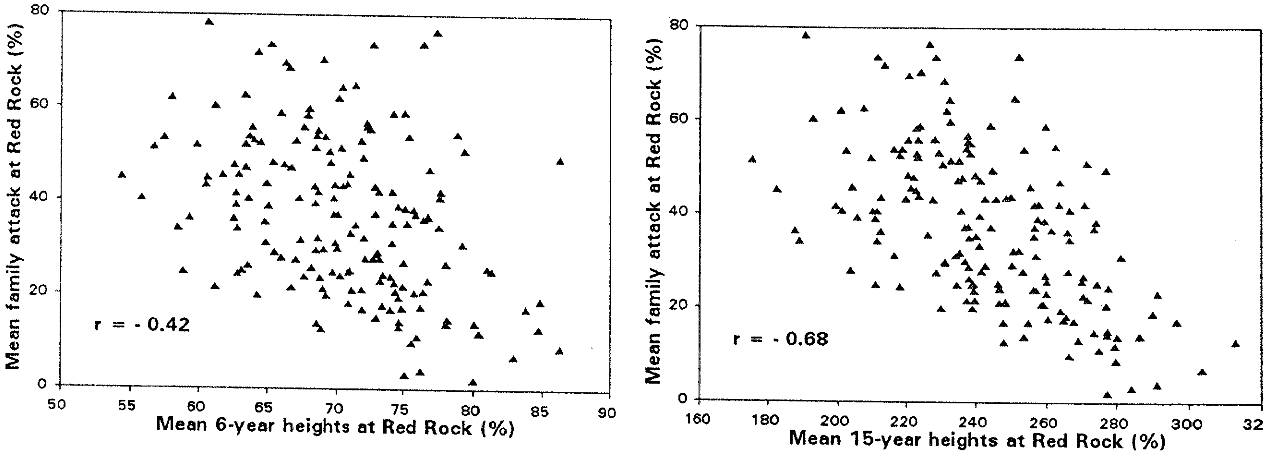
1. While there were different intensities of attack at the three individual test sites in the Prince George SU tests (i.e., 9% at Quesnel, 37% at Red Rock, and 63% at Aleza Lake), there were significant correlations between families attacked across sites (Figs. 1 and 2).

2. The estimate of family-mean heritability for weevil resistance across sites was high ($h^2_f = 0.77 \pm 0.11$).

3. Families exhibiting greater vigor at any age were generally more resistant. Correlation between 6-year family height and incidence of weevil attack was negative and significant (Fig. 3, r=-0.42), as well as 15-year height and incidence of weevil attack (Fig. 4, r=-0.68). In fact, when families were ranked based on height growth at different ages, and average attack was calculated for the top and bottom 25% of families, the top families always had significantly lower attacks than those of the bottom families (Table 1).



Figures 1 & 2.—Comparison of attack percentages at the Red Rock and Aleza Lake test sites and at the Red Rock and Quesnel test sites.



Figures 3 & 4.—Relationship between average family height measurements at 6 and 15 years of age and mean family weevil attacks at the Red Rock test site.

This latter finding was especially gratifying as a literature survey indicated that white pine weevil favored more robust, longer leaders. While this still may be true at the phenotypic level, genetically superior families are less susceptible. We would have accepted susceptibility that was equal to the mean; the fact that faster growing families are less susceptible is a bonus.

Quesnel Lakes Progeny Trials

In the fall of 1993 additional genetic test plantations were assessed for weevil attack. These plantations were established in 1983 to evaluate the genetic potential of 142 parent trees. The tests were established on three sites (Ketchum Creek, Little Benson Lake, and Clearwater) using randomized complete blocks with eight blocks per site and four seedlings per replication per family (total 32 seedlings per site per family for a grand total of 96 seedlings per family over the three sites).

Table 1.—Average weevil attack on the top and bottom 25% of families at three test sites. Families were ranked by average heights at various ages and at 15-yr dbh (diameter at breast height).

Test age	Quesnel test			Red Rock test			Aleza Lake test		
	Top	Bot.	Diff.	Top	Bot.	Diff.	Top	Bot.	Diff.
Initial ht.	6.6	10.7	4.1	31.0	42.9	11.9	56.6	68.3	11.7
3-year ht.	7.1	11.9	4.8	30.6	44.4	13.8	55.9	69.4	13.5
6-year ht.	6.6	12.1	5.5	28.8	45.1	16.3	53.6	71.3	17.7
10-year ht.	5.9	11.9	6.0	26.4	45.0	18.6	52.7	70.8	18.1
15-year ht.	5.2	13.3	8.1	23.8	49.0	25.2	50.6	73.3	22.7
DBH	6.3	11.7	5.4	28.3	44.1	15.8	55.6	70.1	14.5

Moderate to heavy weevil infestation occurred on two of these plantations (Little Benson Lake - 17%, Clearwater - 33%). As was the case with the earlier studies, the weevils again favored certain families and avoided others. Some individuals were attacked up to five times over the years, while more than 1,700 of the 2,700 intensively studied trees at Clearwater have never been attacked. There was high correlation between mean family attacks at the two sites (Fig. 5, r =0.66).

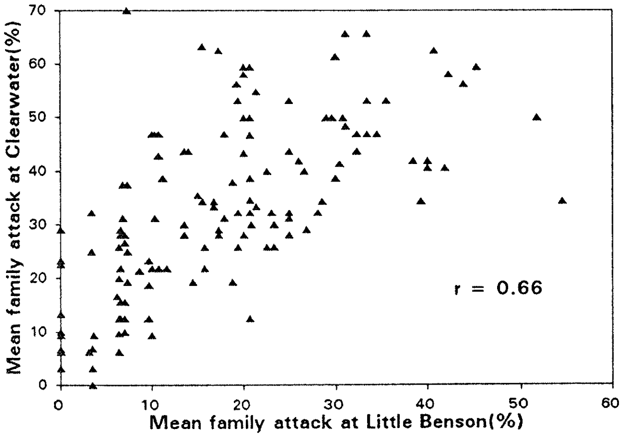


Figure 5.—Comparison of attack percentages at Little Benson and Clearwater.

Once again the more vigorous, faster growing families were less susceptible to weevil attacks. Based on the overall ranking at all sites, the top 25% of families suffered substantially less damage than the bottom 25% (Table 2).

Additional data collected on the Clearwater site in cooperation with Dr. René Alfaro relate to number of attacks per tree, recovery of attacked trees, and quality traits following recovery. These data are presently being evaluated and the results will be published elsewhere.

Table 2.—Average weevil attack on the top and bottom 25% of families at the Little Benson and Clearwater test sites. Families were ranked by average heights at various ages.

Test age	Little Benson test			Clearwater test		
	Top	Bot.	Diff.	Top	Bot.	Diff.
Initial ht.	15.5	18.2	2.7	28.7	40.8	12.1
3-year ht.	14.4	18.6	4.2	26.3	41.4	15.1
6-year ht.	10.4	19.7	9.3	22.4	43.8	21.4
10-year ht.	7.6	21.8	14.2	17.5	47.6	30.1

Comparative Trials

In 1992 another pilot project plantation was evaluated for weevil damage. This plantation was established in 1976 to compare the relative performance of native British Columbian spruces with those originating from eastern Canada. Eastern spruces up to now perform well in B.C., and we are planning to incorporate some eastern material into our breeding program.

This plantation is a replicated complete block design with 15 blocks, 10-tree row plots in each block from each of 21 families. There are 9 families from eastern Canada (ENA) and 12 families from B.C. (four each from East Kootenay (EK), Prince George (PG), and Prince Rupert (PR)).

Results indicate that eastern white spruce is very resistant to the white pine weevil. Of the 1,220 eastern trees, only 67 were damaged by weevil, whereas 379 native spruces were damaged out of a total of 1,665 (Table 3). Interestingly, 25 of the 67 individuals damaged were progenies of the same parent. These results are very significant in light of the fact that these trees also grow and survive extremely well.

Table 3.—Weevil attack differences among families of different geographic origin.

Parents		Number of trees		
Source	Number	Alive	Attacked	Percent
ENA	9	1,220	67	5.5
EK	4	545	154	28.3
PG	4	562	85	15.1
PR	4	558	140	25.1
BC Total	12	1,665	379	22.8

Further proof of the resistance is demonstrated by the eastern North American white spruce clones (about 75 clones) located in our breeding arboreta at the Kalamalka Forestry Centre. These clones are surrounded by native B.C. clones that are heavily attacked by weevil yet eastern clones are almost void of attacks. It is not possible to treat these data using statistical procedures due to the small number of ramets per clone (4), but there is no doubt about the trend.

DISCUSSION

Based on our observations, we must review weevil behavior on interior spruce. It has been postulated that in Sitka spruce, white pine weevil preferentially attacked the most vigorous, longest, and thickest leaders (VanderSar and Borden

1977, Kline and Mitchell 1979, Wood and McMullen 1983). It was suggested that the same preference holds for interior spruce as well. Our investigation confirms beyond doubt that it is not the case. Susceptibility of the individual is the overriding principle that determines whether or not it will be attacked successfully.

In field studies we observed that some seedlings of susceptible families, measuring 50 cm, were attacked by weevil. In some cases these young seedlings were attacked below the previous year's growth probably due to the inadequate thickness of the previous year's stem.

To clarify weevil behavior, a number of studies are being initiated to elucidate the biology of white pine weevil in the interior and its relationship to its host. Verification of resistance in putatively resistant families will also be carried out.

During the spring of 1993 a number of crosses were made using various putatively weevil resistant and susceptible parents. Judgement as to resistancy or susceptibility was made based on the progenies' weevil resistance performance. These crosses will form the basis of a number of experiments designed to verify resistancy and susceptibility and to study the mode and degree of inheritance of these traits.

Future plans include continuation of the inventory of resistant families, provision of material for other agencies, and incorporation of the accumulated knowledge into the breeding program.

Laboratory Studies

Terpene Studies

Our first intention following the identification of genetically controlled resistance was to try to identify the reason for resistance. To this aim, we enlisted the help of a team of biochemists lead by Dr. John Manville. They tested eight resistant and eight susceptible parental clones for terpenes that they judged to be of potential use in differentiating between them. The tests included both leaf and bark samples.

They were able to classify 15 of the 16 parental clones as to susceptibility or resistance using six bark terpenes with multivariate technique (Manville *et al.* 1994). Two of the terpenes are identified (santene and citronellyl acetate) the other four are not yet identified.

Leaf terpenes also provided similar results. Using six leaf terpenes (terpinolene and five unidentified terpenes), they were able to classify correctly 15 of 16 samples.

Future plans include validation of the results (i.e., using a random sample of attacked and unattacked trees and applying the technique to them). They are also working on the identity of the unidentified terpenes.

DNA Marker Studies

We were also interested in investigating the potential differences at the gene level. To study these differences, we enlisted the help of Dr. John E. Carlson of the University of British Columbia (Carlson *et al.* 1994).

The team investigated differences between resistant and susceptible interior spruce parents and their open-pollinated progenies. They constructed genetic linkage maps based on the recently developed Random Amplified Polymorphic DNA (RAPD) marker system, the first linkage maps available for any spruce species (Hong *et al.* 1993). Using a "Bulk Parental Analysis" (BPA) technique developed by the team, they pooled DNA from 12 resistant and 12 susceptible parents. This technique identified 20 DNA markers putatively associated with weevil resistance.

The followup investigation was carried out on half-sib progenies of the original parents. Needles were collected from 10 half-sib individuals of each parent of the previous study (a total of 240 half-sib progenies).

Results of these studies showed that three of the markers were strongly correlated with resistance. None of the markers showed 100% association with resistance or susceptibility. The senior author has two potential explanations for this:

1. The markers are only loosely linked to the gene(s) for resistance, so recombination between the marker and the gene can still take place.
2. More than one gene may be involved in weevil resistance, and/or modifier genes are also necessary for a resistance gene to be expressed.

These studies are also continuing.

Micropropagation of Resistant Genotypes

Dr. Ben Sutton and his team from the British Columbia Forest Biotechnology Centre in Vancouver are involved in utilizing elite full-sib crosses among resistant parents by multiplying them through embryogenesis (Roberts 1994). Their technique to produce large numbers of "emblings" has been developed to a point where they can mass produce these propagules.

Embryogenic tissues can be stored in liquid nitrogen indefinitely. Once clones have been tested and proven to be resistant, they can be mass produced and used for operational plantations.

Results of the studies described above will be utilized in developing an integrated weevil control system that will provide protection to newly planted spruce forests in British Columbia. There are a number of unanswered questions that will be addressed in future studies, but the results to date are encouraging. We are optimistic that our efforts will lead to success.

SUMMARY

Studies of interior spruce progeny trials revealed apparent genetic control of resistance to weevil damage in interior spruce in British Columbia. Correlation between mean family attacks across sites was high.

Contrary to previous suggestions, weevil damage was more prevalent on less vigorous families. The difference between average damage of higher and lower ranked families (based on growth performance at any age) was always significant. Families that ranked highest for growth typically suffered less damage.

Eastern North American white spruce appears significantly more resistant to weevil than are western sources. Western sources suffered more than fourfold the damage of eastern sources.

Laboratory studies appeared to confirm differences between putatively resistant and susceptible spruces:

1. Terpene analyses tentatively identified terpene content differences between the two strains. Fifteen of sixteen families could be distinguished by this technique;
2. RAPD markers could also differentiate between resistant and susceptible families. While none of the markers had 100% association with resistance, the results are clearly pointing to markers loosely associated with resistance.

Genotypes identified as resistant will be propagated using a technique referred to as somatic embryogenesis. A team at the British Columbia Research Incorporation has perfected the technique so that it can mass produce clones that are resistant to weevil.

Further research is underway to clarify the many questions that are still remaining. Nevertheless, the resistance observed in our genetic trials will allow us to select and breed for this attribute in combination with growth and other desirable traits.

ACKNOWLEDGEMENTS

The authors are indebted to many individuals who contributed to this manuscript. Technical support was provided by D. Wallden and G. Phillips over many years. The financial support of the British Columbia Forest Service was responsible for the establishment, maintenance, and evaluation of the trials that provided the database for the study.

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A SUGI CLONE HIGHLY PALATABLE TO HARES: SCREENING FOR BIOACTIVE CHEMICALS

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INTRODUCTION

Herbivores often selectively feed on some plant individuals over other conspecifics. Such differences in palatability are usually chemically determined. Although typically confounded with environmental factors (Rousi *et al.* 1989, 1991, 1993), genetic control of the differences in palatability observed among geographic origins (Matsuura and Maeda 1981; Radwan *et al.* 1982; Hansson *et al.* 1986; Rousi *et al.* 1989, 1991), among families (Rousi *et al.* 1989, 1991), and among clones (Radwan 1972, Hara 1973, Dimock *et al.* 1976, Kuwahata and Tanbara 1983) is obvious.

Although mammalian herbivores can cause severe damage to trees, it is usually difficult to control such animals, because of economical, aesthetic, and ethical reasons. Breeding for resistance is one of the possible solutions to this problem. Therefore, efforts have been made to find the chemical agents responsible for the variation in palatability among trees.

Atetsu 4 (A4), an elite sugi clone, *Cryptomeria japonica*, which originated in Atetsu, Okayama, Japan, is extremely palatable to hares. Its high palatability was first observed at a test planting site of 32 sugi clones, where A4 was highly damaged by hares just 1 year after planting. Later, an unusually high mortality rate was found in A4 at another test site of 37 clones 5 years after planting, and damage by hares were considered to be the main cause of the mortality (Kuwahata and Tanbara 1983).

This paper reconfirms the high palatability of A4 to hares, analyzes some of the chemical properties of the three sugi clones, and tests the palatability of crude chemical extracts and separated fractions using filter paper trials.

MATERIALS AND METHODS

Plant Materials and Hares

The palatable sugi clone, A4, and two other unpalatable sugi clones, Uda 36 (U36) and Niimi 4 (N4), were used. The clones were transplanted to the nursery of the Forestry and Forest Products Research Institute in Ibaraki, and leaves were collected from trees at each feeding trial and, when stocked for use in chemical analysis, frozen at -43°C. Captive hares, *Lepus brachyurus*, kept in pens at the institute, were used for palatability tests. The hares were outdoors and maintained on commercial food pellets. They were either born in captivity or captured in the wild soon after birth.

Palatability Test

Feeding trials were conducted using two hares in separate 3.6 x 3.6 m pens. Fresh leaves or extracted residues of the clones were put on shallow mesh trays (24 x 32 x 1 cm) and offered to the hares. Consumption was monitored by measuring the weight of the remnants over several days. Corrections were made for changes in leaf weight due to dehydration by measuring controls.

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Filter paper tests were used to examine the palatability of fractions. Of six pieces of filter paper (5 x 8 cm, 1 g), two were treated with the extract of A4 and two with that of N4; the remaining two were left untreated as a control. The amount of fractions applied to each piece of filter paper was a one-gram dry-leaf equivalent. These were offered to hares kept in cages (80 x 80 x 80 cm) or pens (1.8 x 3.6 m), for one night and the amount (area) of filter paper left uneaten was measured.

Commercial food pellets were always available during the trials, hence the intake of leaves or filter paper by hares was entirely voluntary.

Seasonal Stability of Palatability and Solvent Extracts

Seasonal stability of the palatability of fresh leaves of A4, U36, and N4 was investigated by feeding trials every 3 months from May 1988 to May 1989. Leaves were taken from the trees and cut into approximately 5 to 10 cm lengths. The leaves of each clone were put on two trays, which were set at opposite positions in a pen to prevent biased consumption due to tray position.

For the same materials, solvent extracts were investigated: essential oils were taken by steam distillation for 10 h; dichloromethane and methanol extracts were consecutively obtained using a Soxhlet extractor. Leaves were cut into small pieces (< 3 mm in length) with pruning shears before the analysis.

Effects of Extraction on Palatability

Two series of consecutive extractions were made: (a) steam distillation for 10 h followed by methanol extraction; and (b) extraction by n-hexane and then methanol. The latter was done in a Soxhlet extractor. Leaves were cut into small pieces (ca. 3 mm in length) with pruning shears before extraction.

Hot-water extraction was made using a common kitchen pot (capacity: 5 l) and a large steam-heated stainless pot for mass extraction (capacity: 50 l). Leaves of 10 to 20 cm in length were used. Leaves in the kitchen pot were boiled for 7 days (6 h per day) with water changed twice a day. In the steam-heated pot, leaves were boiled for 8, 16, or 24 h and once for 5 days with water changed daily.

The palatability of residual leaves were investigated by cafeteria test. Comparisons of palatability were made among the clones after each extraction.

Effects of Methanol Extracts on Fresh and Residual Leaves

The fresh leaves and residue after extraction of A4 and N4 were treated with the methanol extracts of A4 and N4 and feeding trials were made to compare the palatability before and after treatment. Methanol extracts were taken by soaking dried fresh leaves (600 g wet weight) first in 36 l methanol for 13 days and then 18 l methanol for 8 days. The extracts were combined and concentrated in a vacuum evaporator to a small volume. The concentrate was then sprayed on fresh leaves or leaf residue with the amount that was originally included in the fresh leaves.

Palatability of Extracts

Fresh leaves of 10 to 20 cm in length (260 g wet weight) were put in large flask (capacity: 10 l) with methanol and heated in a hot water bath. The 2 h extraction was repeated twice for the same material each time with 7 l methanol. The extracts were then combined (MW).

The combined extract (MW) was added to 500 ml water and then evaporated to approximately 400 ml, and the water-insoluble material was precipitated. Water-soluble (W) and -insoluble (M) fractions were separated by decanting. The water-soluble fraction was then consecutively extracted with n-hexane (Wh), ethyl acetate (We), and butanol (Wb) to leave a residual water fraction (Ww). The water-insoluble fraction was dissolved with methanol and then extracted with n-hexane (Mh) to leave a residual methanol fraction (Mm).

The palatability of the various fractions was examined by filter paper tests. Each fraction was tested in pairs of A4 and N4.

RESULTS

Seasonal Stability of Palatability and Solvent Extracts

A4 showed high palatability throughout the year and was completely consumed in the first two nights with only one exception (Fig. 1). In contrast, U36 and N4 were not eaten much even after A4 was depleted. In one instance, however, U36 was eaten after A4 was depleted although N4 did not show any such tendency. The two hares showed a notable similarity in their feeding.

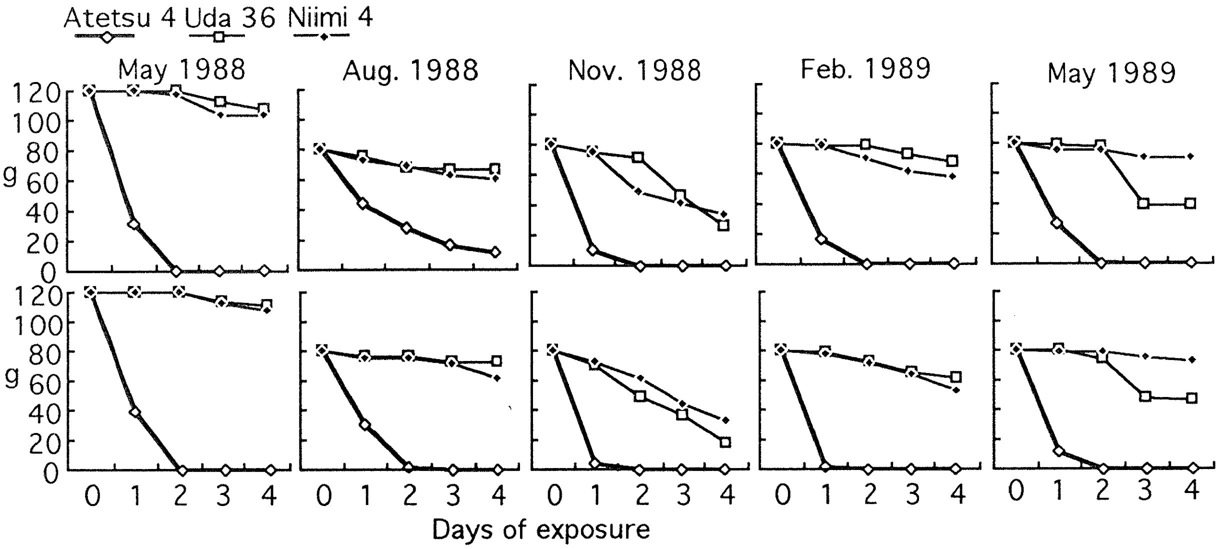

Figure 1.—Palatability of fresh leaves of three sugi clones in different seasons. Vertical axis represents the weight of uneaten leaves remaining. Upper and lower graphs each represent the results for one hare.

The mass of essential oils in A4 was much less than that in U36 or N4 throughout the year (Fig. 2). Also, the amount of dichloromethane extract from A4 was much less than that from the other two unpalatable clones in all seasons. In contrast, the amount of methanol extract was not much different among the three clones.

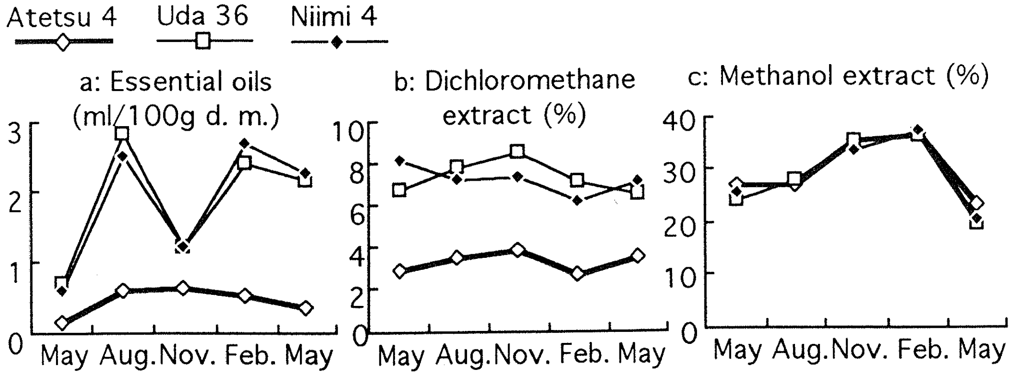

Figure 2.—Seasonal fluctuation of essential oils and solvent extracts in three sugi clones.

Effects of Extraction on Palatability

When boiled in a common kitchen pot for 7 days, the residual leaves of A4 still retained high palatability. However, boiled in the steam-heated pot for 5 days, A4 lost its palatability to hares, although it retained high palatability after boiling for up to 24 h. The percent dry matter of the fresh leaves used in these trials was 35-39%; after boiling, residual leaves were 22-25% of the original fresh weight. Thus, about one-third of the dry matter was extracted by boiling. However, when boiled in the steam-heated pot for 5 days, the residue of A4 was reduced in weight to 18% of the original, whereas N4 remained at 24%.

When essential oils were removed by steam distillation, A4 retained its palatability, but further extraction with methanol made it unpalatable (Fig. 3). Similarly, after extraction with n-hexane, A4 residue did not lose palatability but further extraction with methanol rendered it unpalatable. In contrast, the unpalatability of U36 and N4 seemed to diminish after each extraction.

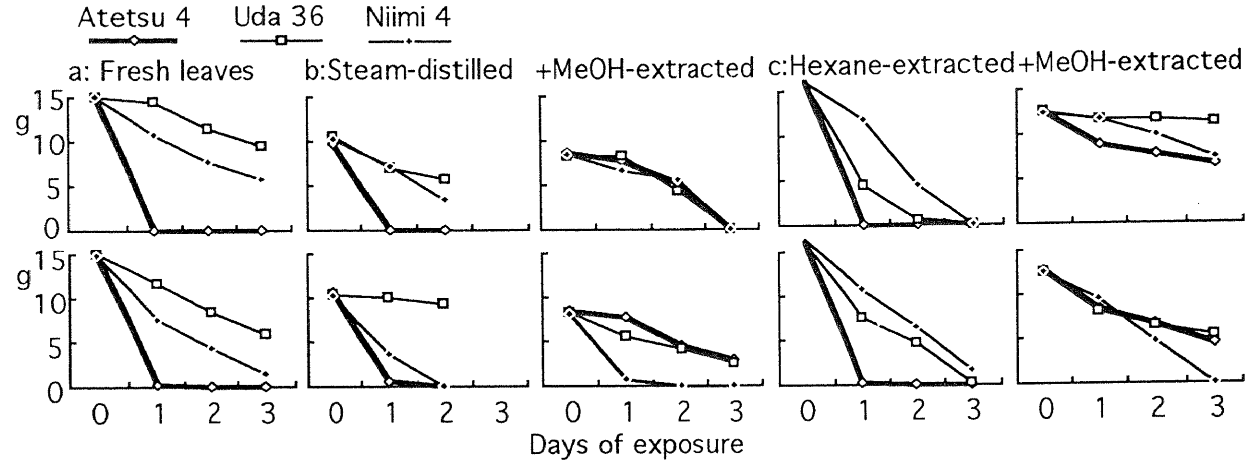

Figure 3.—Change of palatability of three clones after extraction. Vertical axis represents the weight of uneaten leaves or extracted residue leaves. Upper and lower graphs each represent the results for one hare.

Effects of Methanol Extracts on Fresh and Residual Leaves

Application of methanol extracts from dried A4 leaves did not affect the palatability of fresh leaves of A4 and N4 but made the residues after extraction palatable in both clones. In contrast, application of N4 extract made the fresh leaves of A4 unpalatable, but showed no effect on the fresh leaves of N4 nor the residual leaves after extraction in both clones.

Palatability Test of Fractions

The filter paper test was not valid for some hares, because they showed little interest in filter paper and ate it only occasionally. One hare showed constant interest and gave suggestive results. Most of the fractions of A4 extracts showed high palatability, whereas some of the fractions of N4 extracts showed only weak palatability (Fig. 4). Little of the untreated filter paper was consumed.

Of A4 extracts, both water-soluble (W) and water-insoluble (M) fractions were highly favored. The Wh fraction was less palatable than the others. The Mh fraction also showed less palatability than did the Mm. For N4, although the MW extract was moderately consumed, the separate water-soluble and water-insoluble fractions showed the least consumption. The water-soluble fractions showed light (Wh) to fairly high palatability (Wb), whereas the Mh was taken only in small amounts.

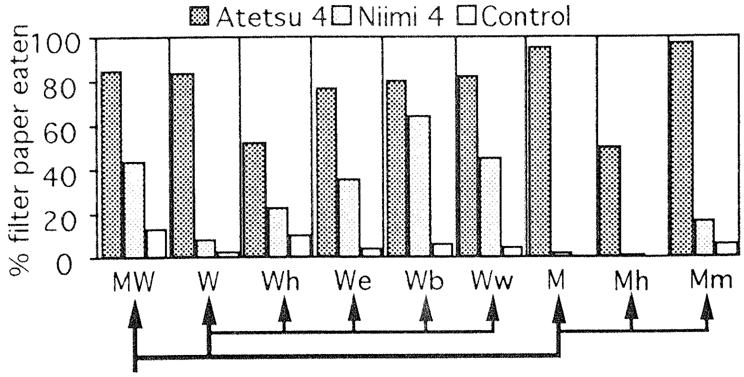

Figure 4.—Percent consumption of filter paper treated with extract fractions from A4 and N4 leaves. See text for details.

DISCUSSION

There were some seasonal changes in the palatability of A4, U36 and N4 to captive hares. A4 leaves seemed less palatable in August than in other seasons. It was not clear whether this was due to changes in chemical components of A4 because of possible effects due to the hares' own physiology.

A4 was quite different from other clones in that it yielded much less essential oils and dichloromethane extracts than did the unpalatable clones. However, the palatability tests of leaves boiled with water, or extracted with n-hexane suggested that essential oils and non-polar compounds are not the cause of the differences in palatability between A4 and N4 (Hirakawa 1989).

The relationships between gross essential-oil yields and the preferences by mammalian herbivores are not necessarily negative (Radwan *et al.* 1982; *Pinus ponderosa* - pocket gopher, Hansson *et al.* 1986; *Pinus contorta* - vole). Snyder (1992) reported that resin flow rate determined host pine tree selection by squirrels and that β-pinene and β-phellandrene were possibly the deterrent agents.

There are two possibilities for the peculiarly high palatability in A4: A4 contains certain specific feeding stimulant(s) that others do not have, or have little if any; and A4 lacks, or has only little, of the deterrent(s) that occur in most sugi trees. The cross-treatment tests showed that the methanol extract of A4 had a stimulant effect, whereas that of N4 had a deterrent effect. As a result, neither of the above possibilities was disputed.

The results of the screening using filter papers showed a tendency for n-hexane fractions (Wh and Mh) of A4 to be less palatable than other fractions. A comparison of palatability between A4 and N4 among We, Wb, Ww and Mm fractions suggested that either Mm contained the main causal compound(s) of the specific palatability of A4 or that the clue to unpalatability of N4 was in Mh.

If some specifically palatable substance or substances in A4 are involved, those substances must be insoluble in n-hexane, soluble in methanol and difficult to dissolve in water. If deterrents in N4 are assumed, the main causal component(s) must be difficult to dissolve in water and soluble in methanol and n-hexane. Multiple substances with stimulant and deterrent effects may possibly be involved.

Chiba *et al.* (1991) and Ogawa *et al.* (1992) examined the effect of extractions on the palatability of susceptible clones of the Japanese larch and its hybrid with Kurile larch to voles. Extraction with petroleum ether decreased the palatability of the residues but further extraction with ethyl ether and ethyl alcohol increased the palatability. Based on their results, Chiba *et al.* (1991) and Ogawa *et al.* (1992) suggested the possible presence of both feeding stimulants and deterrents in the susceptible larch clones.

Substances known to affect palatability of plants to mammalian herbivores are terpenes, phenols, and resins, all of which are deterrents (Radwan 1972, Bryant 1981, Farentinos *et al.* 1981, Radwan *et al.* 1982, Bryant *et al.* 1983, Reichardt *et* *al.* 1984, Tahvanainen *et al.* 1985, Clausen *et al.* 1986, Hansson *et al.* 1986, Snyder 1992). Although no specific stimulants have been reported to be involved in differences in palatability between genotypes, the possibility that A4 has some specific substance(s) that enhance browsing by hares has not been disproved. Our present results only suggest that volatile terpenes and water-soluble sugars do not have a decisive effect on the differences in the palatability between A4 and other clones.

The A4 clone is a genotype showing extremely high palatability compared to other plants of the same species. Other elite clones originating in Atetsu (Atetsu 1, 3, 5, 6) do not show higher palatability than clones from other areas, hence the palatability of A4 is difficult to attribute to its geographic origin (Kuwahata and Tanbara 1983). Although many studies describe large palatability variation within a species, A4 still seems exceptional.

SUMMARY

We examined the chemical factors determining the extremely high palatability of a sugi clone, A4, to hares. The palatability of A4 was fairly stable throughout the year. Although A4 had much less essential oil and dichloromethane extracts than the other clones, steam distillation (or boiling) and extraction by n-hexane did not reduce the palatability. Extraction by methanol made A4 unpalatable, whereas it seemed to make other unpalatable clones more palatable. Methanol extracts of A4 had a stimulating effect on feeding whereas that of N4 had a deterrent effect. A4 might contain specific feeding stimulants that are insoluble in n-hexane, soluble in methanol, and difficult to dissolve in water.

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GENETIC AND PHENOTYPIC VARIATION IN THE INDUCED REACTION OF SCOTS PINE TO LEPTOGRAPHIUM WINGFIELDII: REACTION ZONE LENGTH AND FUNGAL GROWTH

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INTRODUCTION

The induced defensive reaction of phloem generally plays a decisive role in the resistance of conifers to attack by bark beetles and their associated fungi. It is thought to be induced by fungi (Reid *et al.* 1967, Berryman 1972, Raffa and Berryman 1983, Christiansen and Horntvedt 1983, Matson and Hain 1985, Christiansen *et al.* 1987, Långström *et al.* 1992), or by the boring activity of the beetle (Lieutier *et al.* 1988, Lieutier 1993). In most cases, by stimulating that energy demanding response, the fungi can lower the threshold of attack density above which the tree is overwhelmed and beetle attacks are successful. In all situations, the individual reaction is the basic mechanism of tree resistance.

The between-tree variability in the threshold of attack density (Waring and Pitman 1980, Mulock and Christiansen 1986), as well as the between-tree variability in the induced reaction itself (Shrimpton and Reid 1973, Peterman 1977, Lieutier and Ferrell 1989, Lieutier *et al.* 1993), has been linked to tree vigor. However, the genetic contribution to this variability has never been studied.

In Scots pine, *Pinus sylvestris* L., an induced reaction is essential to resist attacks by *Ips sexdentatus* Boern. and *Tomicus piniperda* L., and their associated fungi (Lieutier *et al.* 1988, 1989a; Solheim and Långström, 1991; Långström *et al.* 1992). The blue-stain fungus, *Leptographium wingfieldii* Morelet, associated with *T. piniperda*, does not seem to play a role in the development of the induced reaction and in the success of attacks (Lieutier *et al.* 1988, 1989b). However, artificial inoculation of its sporulated cultures into Scots pine always incites strong defensive reactions (Lieutier *et al.* 1989a, Solheim and Långström 1991) and the tree's response depends on the number of spores present in the inoculum (Lieutier *et al.* 1989b). Additionally, it may play a role in tree mortality, since weakened Scots pine can be killed by a high density of artificial inoculations (Solheim *et al.* 1993).

The present study is part of a cooperative project with the following objectives: (1) to study the variation in the induced reactions (reaction zone formation, sapwood occlusion) between Scots pine provenances in two different climatic regions, (2) to study the performance of a French and a Swedish strain of *L. wingfieldii* in these provenances under different climatic conditions, and (3) to study the defensive chemistry involved in the induced reactions, particularly the roles of resin acids and phenolics, and to search for chemical markers for resistance among these groups of chemicals. This study addresses the first two issues.

Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

MATERIALS AND METHODS

Field Work

In early spring, 1991, provenances of Scots pine were selected in central France (Arboretum des Barres, Nogent-sur-Vernisson) and in southern Sweden (Remningstorp). Eleven (55 trees) and 10 (60 trees) provenances were chosen in France and Sweden respectively, so as to cover as wide a geographic distribution as possible, while taking into account the necessity of considering comparable provenances between the 2 countries (Table 1). In addition, the best lodgepole pine, *Pinus contorta*, provenance was selected at Remningstorp (6 trees) for a separate comparison with the Scots pines. French trees had been planted between 1951 and 1957, except for 2 provenances planted in 1941 (Table 1), using 3-year-old seedlings. Their DBH ranged from 14-22.5 cm. Swedish trees had been planted in 1962 using 4-year-old seedlings and their DBH ranged from 10.5-18 cm.

All trees were inoculated with a French and a Swedish strain of *L. wingfieldii*, originally isolated from *T. piniperda* in France (Lieutier *et al.* 1989a) and in Sweden (Solheim and Långström 1991) respectively. The inoculation materials were 2-week-old cultures on malt agar. Holes were made to cambium depth with a 5-mm cork borer, 5-mm pieces of agar culture were placed in the holes, and the bark plugs were put back into the tree. In this study, we inoculated five rings spaced (ca. 30 cm apart) on the lower stem (from ca. 60 to 180 cm stem height) in six points spaced evenly around the circumference. Each of two spots at opposite sides of the stem received sterile agar (control), with French and Swedish strains of *L. wingfieldii*. In order to avoid lesions growing into each other, inoculation points in subsequent rings were displaced by 30 degrees relative to the previous ring. The inoculations took place from 8 to 12 April in France and on 26 April in Sweden.

Three weeks after the inoculations, the reaction zones, i.e., the resin-soaked lenticular zones (often referred to as lesions) surrounding the points of inoculation, were sampled as follows: All 6 inoculation points of ring 2, and one of the two inoculation points of each treatment in ring 4 (i.e., 3 inoculation points per treatment in total), were carefully exposed by removing the outer bark. The reaction zones were measured on the external face of the phloem upwards and downwards from each inoculation point. For re-isolation of the inoculated fungi, the right half of two reaction zones of each treatment was cut out, put individually in plastic bags, and cold-stored immediately at 2°C. The remaining reaction zones were put in paper bags or in vials, immediately frozen on dry ice, and stored for later chemical analyses. Two unwounded phloem samples were also collected in each tree and preserved the same way as the remaining reaction zones for chemical analyses.

Six weeks after the inoculations, 3 other inoculation points per treatment, located in rings 3 and 4, were investigated in each tree and sampled as described above. Two new unwounded phloem samples were also taken. After sampling the reaction zones, 2 trees in France and 3 trees in Sweden were felled in each provenance, and discs were cut at rings 1, 3 and 5. These discs were stored in a cold room (2°C) for later examination of sapwood reaction zones and re-isolation of the fungi. The total height of the felled trees and the lengths of their current leader shoots were measured.

Twenty-one weeks (24 in Sweden) after the inoculations, the reaction zones were sampled (rings 1 and 5) from the remaining trees as described above. Trees were felled and discs were cut as described above also.

Diameter and height of the trees differed significantly between provenances in both France and Sweden. In France, the provenances "Rud" and "Die" had the highest dimensions, while "Spe", "Seg" and "Fin" had the lowest. In Sweden, the native provenances "Vit", "Väs" and "Kil" were the tallest and had the biggest diameter, while the provenances "Mal" and "Enz" were very small and thin. The provenance "Tab" was peculiar, as it had the lowest height although its diameter was comparable to other provenances. These trees were the last ones left at the plot after snow-break damage. On the contrary, lodgepole pine was taller, although its diameter did not differ from Scots pine provenances.

In France, no between-provenance differences could be noticed in the length of the current leader shoots, except in September 1991, but damage by *T. piniperda* did not permit any conclusions. In Sweden, significant provenance differences existed. Shoots of the foreign provenances were generally shorter than that of the local ones. Lodgepole pine also had long shoots. Six weeks after inoculations, the trees in France had completed ca. 2/3 of their leader growth, whereas trees in Sweden had only completed ca. 1/2 of theirs.

Table 1.—Characteristics of the Scots pine provenances and of *P. contorta* studied in Sweden and in France.

Designation	origin	latitude	longitude (m)	altitude (years)	age
Provenances studied in France (Nogent-sur-Vernisson):					
Fin	(*Finland*)				40
Kal	Kalmar (*Sweden*)	56° 30'	16° 20'	40	37
Spe	Spey valley (*U.K., Scotland*)	57° 20'	03° 30'W		41
Rud	Rudczany (*Poland*)	53° 40'	21° 29'	130	53
Klo	Klosterreichenbach (*Germany, Schwarzwald*)	48° 30'	08° 25'		43
Niz	Saint Nizier de Fornas (*France, Loire*)	45° 25'	04° 05'	900	42
Die	Saint Dié (*France, Vosges*)	48° 15'	07°	400	43
Mat	La Matte des Angles (*France, Pyrénées*)	42° 36'	02° 07'	1520	53
Ser	(*Serbie*)	43°	21°		37
Seg	Segovie (*Spain*)	40° 52'	04°W	1400	41
Can	Canakkale (*Turkey*)	40° 10'	26° 25'		37
Provenances studied in Sweden (Remningstorp):					
Mal	Malå (*Sweden*)	65° 38'	21° 07'	75	33
Sve	Sveg (*Sweden*)	62° 03'	14° 19'	385	33
Kil	Kilafors (*Sweden*)	61° 08'	16° 31'	150	33
Var	Varnhem (*Sweden*)	58° 22'	13° 11'	270	33
Väs	Västervik (*Sweden*)	57° 46'	16° 39'	20	33
Vit	Vittskövle (*Sweden*)	55° 51'	14° 01'	30	33
Olo	Olonets (*Russia, Carelia*)	61°	33°	100	33
Inv	Inverness (*U.K., Scotland*)	57° 10'	05°W	300	33
Tab	Taborz (*Poland*)	53° 45'	20° 06'	130	33
Enz	Enzklösterle (*Germany, Schwarzwald*)	48° 45'	08° 30'	700	33
Con	Stuart lake (*Canada, B.C.*) = *P. contorta*	54° 30'	124° 15'W	600	33

Laboratory Procedures

The week after collection, the samples were analyzed for fungus extension. From the reaction zones, phloem pieces were cut with a sterile razor blade at 5 mm intervals upwards and downwards from the inoculation points beyond the visible reaction zone. The sample pieces were placed on petri dishes with malt agar medium and incubated at 25°C for 1 week. Positive and negative records of *L. wingfieldii* were noted. Sapwood samples were handled the same way, except that sampling took place in the radial direction. The maximum extension of the sapwood reaction zone was also measured radially.

Data Handling and Statistics

All calculations were done with the SAS statistical package. Preliminary analyses of vertical reaction zone lengths and fungal growth revealed no differences between the vertical extension of reaction or fungal zones upwards and downwards from the inoculation points regardless of the treatment (Student's t-test). Neither were there any differences between reaction or fungal zones from different inoculation rings of the same treatment. Hence, we pooled data within the same tree, and used tree-wise treatment means (based on 3 lesions or less in some cases with missing data) as calculation units.

Treatment means were tested using one-way analysis of variance followed by Tukey's test for multiple comparisons. Two-way analysis of variance was also employed, as were pair-wise and standard t-tests, depending on the situation. Interdependency between different variables was explored by regression analyses.

RESULTS

Phloem

Reaction Zone Length

Total length of the reaction zone varied significantly between provenances and between treatments (Fig. 1). In Sweden, local provenances generally exhibited longer reaction zones than foreign provenances at 6 and 24 weeks. The opposite tendency however, was visible in France at 6 weeks, where the longest reaction zones were observed in the southern foreign provenances, particularly the Spanish and Serbian provenances, while the shortest reaction zones belonged to the local provenances. At Remningstorp, lodgepole pine exhibited shorter reaction zones than all provenances of Scots pine after 3 and 6 weeks. Despite existing differences between provenances, no systematic trends (e.g., latitudinal) in reaction zone lengths could be detected.

At Remningstorp, reactions to the two strains of *L. wingfieldii* were similar but, at Nogent-sur-Vernisson, the French strain caused a more extensive reaction zone than did the Swedish strain (Fig. 1 and 2). In both countries, reaction zones resulting from fungal inoculations were longer than reaction zones to the controls, but the latter were clearly longer in France than in Sweden. The correlation of the reaction zone length to the fungus increased more rapidly and to a higher level in Sweden than in France (Fig. 2). A plateau was generally reached after 6 weeks, except for the French fungus strain in France where the reaction zones still expanded after 6 weeks. A few provenances, however, did not fit with that description (Fig. 1).

Few of the provenances studied occurred at both study sites. The pair "Kal" (France) and "Vas" (Sweden) originates from the same region and altitude in southeastern Sweden, and should hence be comparable. We can also assume that the Finnish "Fin" (France) and Carelian "Olo" (Sweden) as well as the pairs from Scotland, Poland, and Germany are fairly similar. However, a closer examination of these pairs of provenances does not disclose any deviations from the general results given above.

Fungal Growth

Total vertical fungal expansion varied significantly between provenances only for the 6 week sampling date (Fig. 3). At Nogent-sur-Vernisson, fungal growth was the most expanded in the southern, foreign provenance "Seg", and the least expanded in the French provenances, particularly the Pyrenean one. At Remningstorp, fungal growth was the most expanded

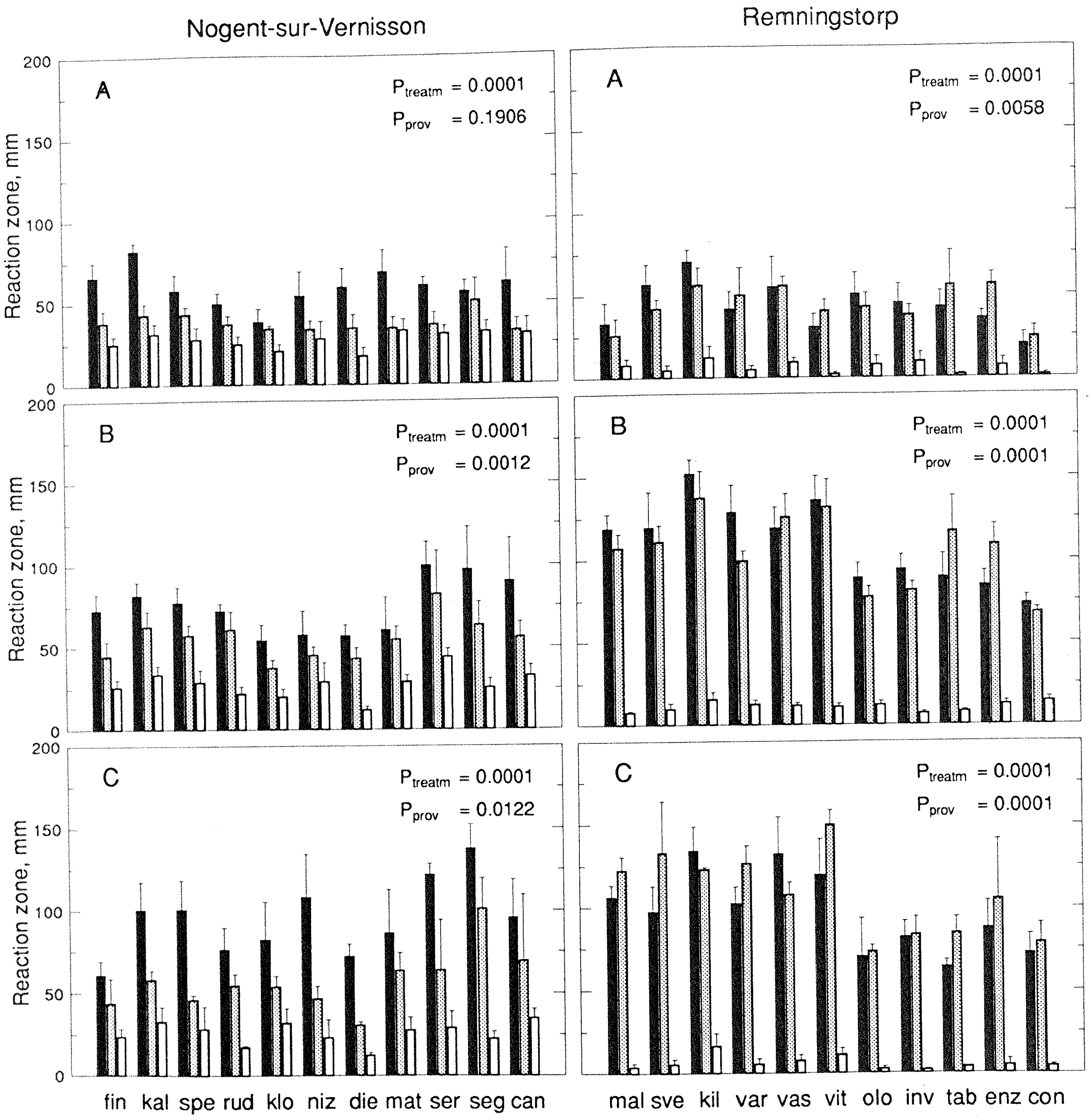

Figure 1.—Vertical extension of induced reaction zones in Scots pine phloem in response to artificial inoculations with a French (black) and Swedish (dotted) strain of *Leptographium wingfieldii* as well as a sterile control (white), in an experiment in France (left) and Sweden (right) using different provenances of Scots pine and one of lodgepole pine (Sweden only). Samples were taken after 3 (A), 6 (B) and 21 (C) (France) or 24 (C) (Sweden) weeks; provenance abbreviations are explained in Table 1. Numbers of observations (trees) were 5 and 6 (France and Sweden, respectively) for the first two sampling dates, and 3 for last sampling date at both sites. Vertical bars indicate standard error. P-values refer to two-way-anovas for differences between treatments and provenances.

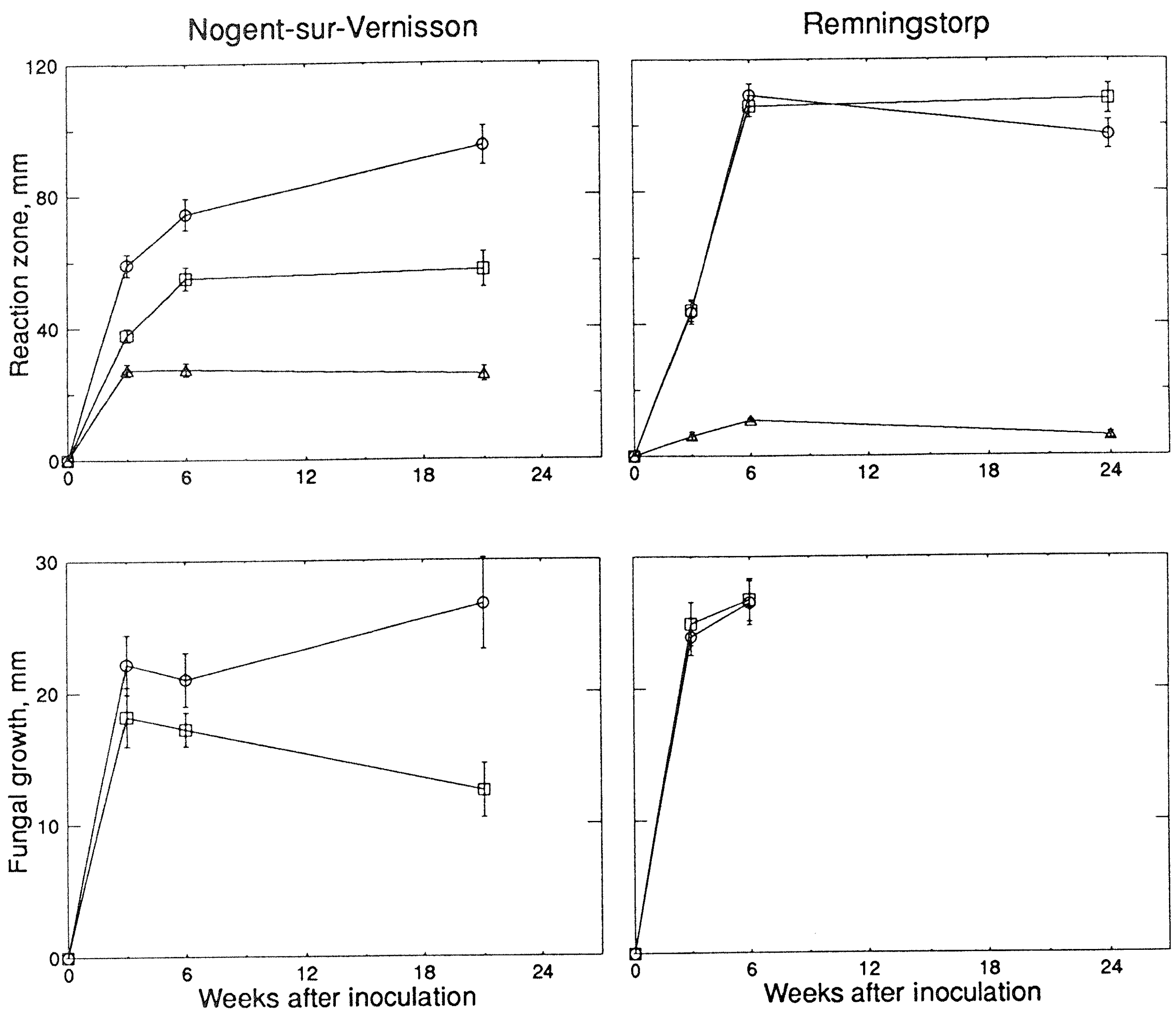

Figure 2.—Vertical progress in reaction zone size (upper row) and fungal growth (lower row) in Scots pine phloem in response to artificial inoculation with a French (circle) and Swedish (square) strain of *L. wingfieldii* as well as a sterile control (triangle), in an experiment in France (left) and Sweden (right). Tree provenances were pooled. Samples were taken after 3, 6 and 21 (France) or 24 (Sweden) weeks. Numbers of observations (trees) were 5 and 6 (France and Sweden, respectively) for the first two sampling dates, and 3 for last sampling date at both sites. Vertical bars indicate standard error.

in the native provenance "Kil". No significant differences were observed between treatments, except for the 21 week sampling date at Nogent-sur-Vernisson, where the French strain of *L. wingfieldii* grew further than the Swedish strain. However, in the Spanish provenance, that was also true for all sampling dates. In both locations, fungi expanded rapidly during the first 3 weeks, and thereafter stabilized (Fig. 2). At Nogent-sur-Vernisson, fungus expansion was higher for the French than for the Swedish strain after 21 weeks. The expansion of the Swedish strain even decreased at 21 weeks, except in the Pyrenean provenance (Fig. 3).

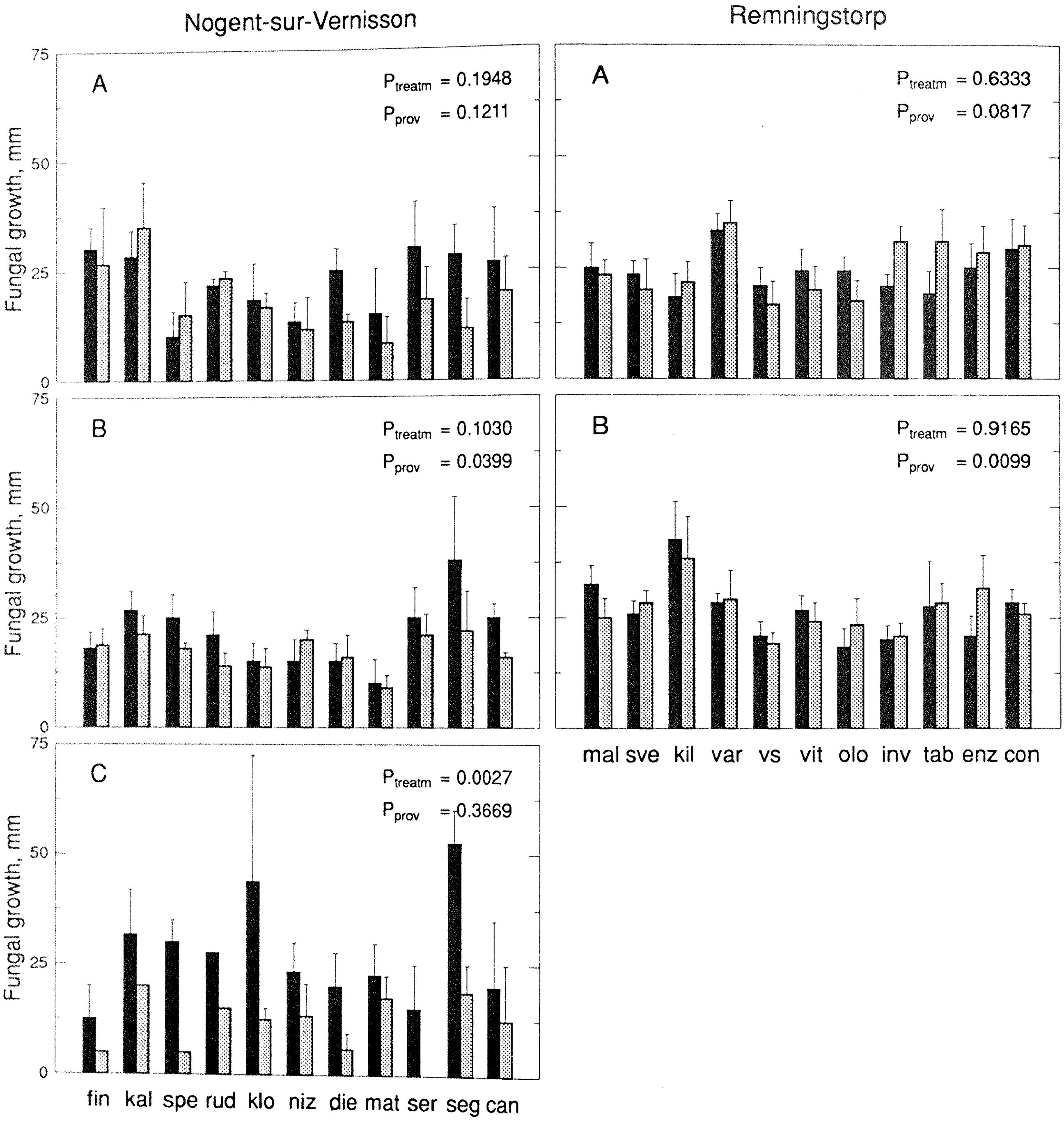

Figure 3.—Vertical expansion of fungal growth after inoculation of a French and Swedish strain of *L. wingfieldii* in the phloem of different pine provenances in an experiment in France (left) and Sweden (right). Samples were taken after 3 (A), 6 (B) and 21 (C) (France only) weeks. Provenance abbreviations are explained in Table 1. Numbers of observations (trees) were 5 and 6 (France and Sweden, respectively) for the first two sampling dates, and 3 for last sampling date (France only). Vertical bars indicate standard error. P-values refer to two-way-anovas for differences between treatments and provenances.

Interdependence Between Reaction Zone Length and Fungal Growth

In all cases, fungal growth was much less expansive than reaction zone length (cf. Fig. 1 - 3). These two parameters were positively correlated with each other after 6 weeks (r=0.73 and 0.51 in France and Sweden, respectively, and after 21 weeks (r=0.71, France only), but not after 3 weeks (r=0.38 and -0.27 in France and Sweden, respectively).

Radial Occlusion and Fungal Growth Into Sapwood

As sapwood occlusion was minimal (Fig. 4), data for the different provenances are not given in detail. After 6 weeks, the range in sapwood occlusion means was 0.4-2.1 mm and 0.2-3.1 mm in France and Sweden, respectively. After 21/24 weeks, the corresponding ranges were 1.1-5.8 mm and 0.7-4.7 mm. Sapwood occlusion was always more developed after fungus inoculation than after control inoculation, and continued to expand between 6 and 21/24 weeks (Fig. 4). This was true for both localities. At Nogent-sur-Vernisson, the French strain of *L. wingfieldii* caused a larger sapwood occlusion than did the Swedish strain, 21 weeks after inoculation.

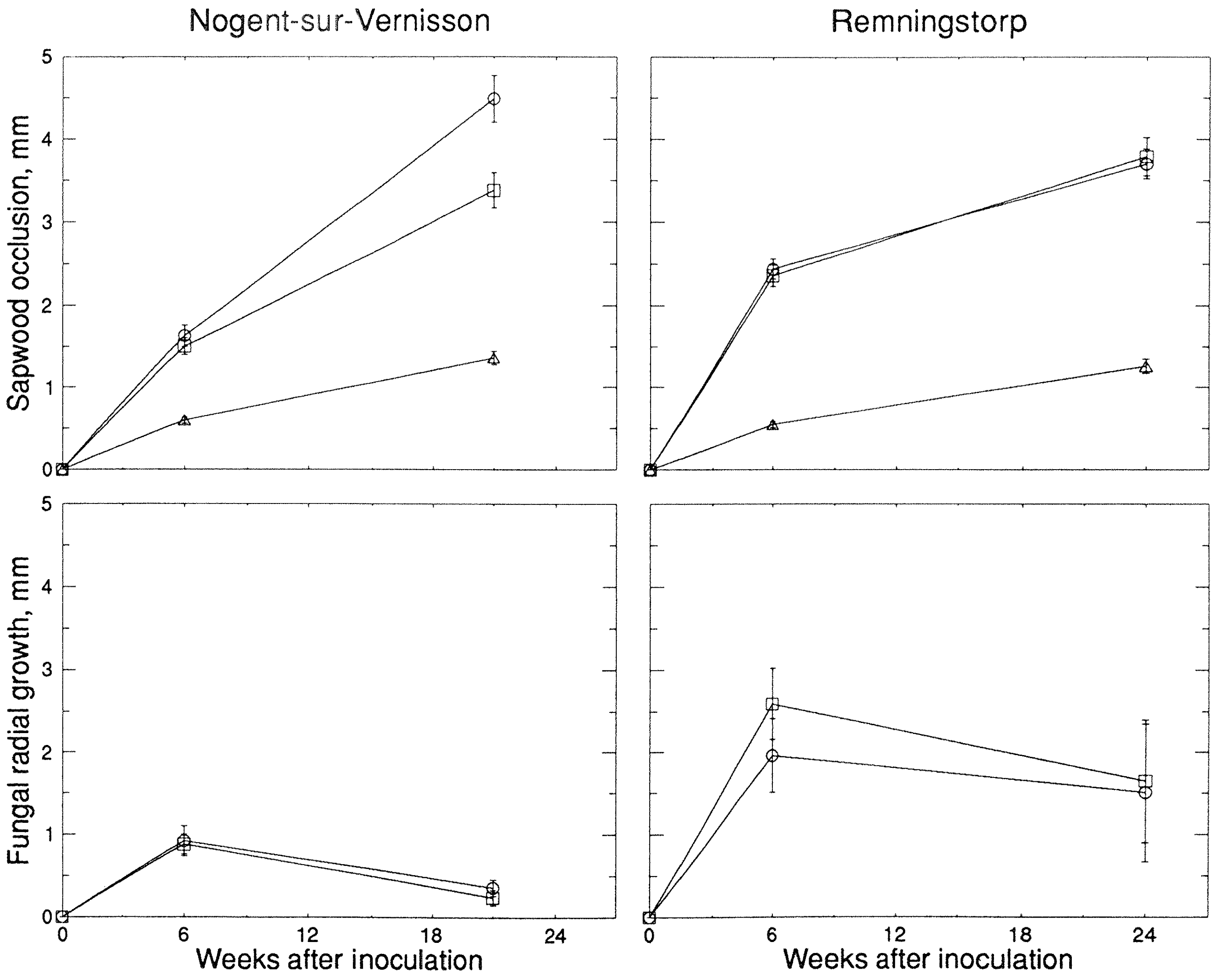

Figure 4.—Radial occlusion of sapwood (upper row) and radial expansion of fungi (lower row) following inoculation with a French (circle) and Swedish (square) strain of *L. wingfieldii* as well as a sterile control (triangle), in different Scots pine provenances, in an experiment in France (left) and Sweden (right). Samples were taken after 6 and 21 weeks (France) or 6 and 24 weeks (Sweden). Provenance abbreviations are explained in Table 1. Numbers of observations (trees) were 2 and 3 (France and Sweden, respectively) for the 6 week sample, and 3 for the last sampling date (both sites). Vertical bars indicate standard error.

As for radial occlusion, fungal growth in the sapwood was minimal (Fig. 4), and did not differ between provenances. Hence, data are not given in detail for the different provenances. After 6 weeks, the average fungal penetration into the sapwood ranged from 0.2-2.0 mm and from 0.2-6.0 mm in France and Sweden, respectively. After 21/24 weeks, the corresponding ranges were 0.0-1.6 mm and 0.0-9.0 mm. In both countries, fungi generally regressed or remained stable from 6 to 21/24 weeks (Fig. 4). There were no differences between the 2 strains of fungi, but both fungi expanded further into the sapwood in Sweden than in France.

DISCUSSION

Comparison Between Provenances

The observed differences between provenances in all variables studied (reaction zone length, sapwood occlusion, and fungal growth - vertically as well as radially) do not demonstrate a systematic pattern justifying general conclusions about provenance differences. In Sweden however, foreign provenances responded differently than native provenances to fungus infection. As this was not the case in France, no latitudinal or altitudinal trends can be detected. On the other hand, systematic differences in defense reactions may have been masked by the considerable inter-tree variation, possibly due to variations in micro-ecological conditions from one provenance plot to another, or even to genetic differences between trees of the same provenance. A larger number of replications would have been required. Even better would have been to work with clones instead of provenances. Thus in this experiment, reaction zone size alone did not provide enough information to evaluate possible genetic differences in host resistance to blue-stain fungi.

In Sweden, lodgepole pine also responded in the same manner as foreign Scots pine provenances, which suggests it may be similarly susceptible to European bark beetles.

Comparison Between Treatments

As observed earlier, fungal inoculations produced larger reaction zones than the sterile zones (Wong and Berryman 1977, Solheim 1988, Ross *et al.* 1992). For the variables measured, the two fungal strains caused larger differences in France than in Sweden, and this locality effect will be discussed below. The French strain of *L. wingfieldii* invariably caused larger reaction zones than the Swedish strain in France, and similar reaction zones in Sweden. As the pattern was similar for fungal growth, the French strain could be considered more aggressive than the Swedish one. The observed difference cannot be attributed to some environmental or host factor, as inoculations of the two strains occurred parallel in the same trees. Thus, some inherent difference between the fungal strains should cause this difference in aggressiveness. For sapwood, the French strain occluded more tissue than the Swedish strain after 21 weeks in France, hence its pathogenicity also could be higher. In all treatments however, sapwood occlusion was shallow, despite extensive reaction zone formation in the phloem (cf. Parmeter *et al.* 1992). This can be explained by the low density of inoculation, allowing the tree to contain the infection.

Comparison Between Localities

All variables measured indicated clear differences between localities. Reactions zones following fungal inoculations were larger in Sweden than in France, as was sapwood occlusion. That was also the case for fungal growth, although not so clearly for the phloem. In contrast, sterile inoculations caused larger reaction zones in France than in Sweden for the phloem, but not for the sapwood.

As these differences were also true inside comparable pairs of provenances (e.g., "Kal" vs "Vas", "Fin" vs "Olo", Spe" vs "Inv", "Rud" vs "Tab" and "Klo" vs "Enz"), a provenance effect can be discarded. However, the finding that sterile inoculations resulted in larger reaction zones in France than in Sweden whereas fungal inoculations yielded the opposite pattern, is difficult to explain. This contradiction implies a higher sensitivity to mechanical wounding in France than in Sweden, but also a more efficient containment of the fungi in the former than in the latter case. Possibly, differences in prevailing temperature conditions may have favored fungal growth in Sweden, as *L. wingfieldii* is known to have a low temperature preference (Lieutier and Yart 1989).

Although the experimental conditions were planned to be as similar as possible, trees may have differed in phenology between the localities. Judging from the relative shoot lengths 6 weeks after inoculation, Lorio's growth-differentiation balance hypothesis (Lorio 1986) cannot explain the locality effect on trees. Indeed, trees at Remningstorp were in a more intensive phase of growth than the trees at Nogent-sur-Vernisson, and should have had larger reaction zones in response to sterile inoculation. Age of the trees may be a more useful explanation, as trees were clearly older in France than in Sweden, and it has already been reported that older Scots pines have larger reaction zones than younger ones (Lieutier and Ferrell 1989, Lieutier *et al.* 1993).

General Conclusions

Although not demonstrated in our experiment, genetic variability in the tree's response to invading fungi (or beetles) probably exists in Scots pine. In addition, we have demonstrated that there is an inherent variability in strains of *L. wingfieldii*. However, phenotypic (geographic) variations concerning both tree and fungus, are superimposed on this pattern. This can be seen in the situation with larger reaction zones to fungus in Sweden than in France, whereas the opposite pattern occurred for the control. One must keep in mind all these potential variations while studying tree/bark-beetle/fungus interactions.

SUMMARY

The aggressiveness and pathogenicity of one French and one Swedish isolate of *Leptographium wingfieldii* were studied in different Scots pine provenances, both in France and Sweden. After inoculations, reaction zone length and fungal growth varied significantly between provenances, at least in the phloem, but no consistent trends could be detected. In France, fungal growth was more extended and the reaction zones were more developed for the French than for the Swedish strain, both in phloem and sapwood. In Sweden, fungal growth and reaction zone length were comparable for the two strains. Fungal growth and reaction zones induced by fungus inoculations were more extensive in Sweden than in France, but reaction zones induced by sterile wounds were larger in France. Fungus inoculations, however, always induced larger reaction zones than sterile inoculations.

ACKNOWLEDGEMENTS

This study is part of ongoing cooperative research between Institut National de la Recherche Agronomique (INRA), Swedish University of agricultural Sciences (SLU), and Norwegian Forest Research Institute (NISK), within the field of bark beetle/blue stain fungi/host tree interactions. A grant from the "Hildur and Sven Wingqvist's foundation for forest research" facilitated the Swedish part of the study, and is gratefully acknowledged. The CEMAGREF and the Arboretum des Barres at Nogent-sur-Vernisson are also acknowledged for providing experimental plots for the French part of the study. The authors express their gratitude to J. Garcia and P. Romary from INRA and to H. Knutsen, C. Nelleman and O. Olsen from NISK for their technical help.

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CAN PHLOEM PHENOLS BE USED AS MARKERS OF SCOTS PINE RESISTANCE TO BARK BEETLES?

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INTRODUCTION

Bark beetle damage is considerable in all coniferous forests in the temperate zone. In most cases, it results from beetle attacks surpassing the critical density (numbers/m^2) below which trees can successfully reject them. Above this threshold, natural mechanisms of tree resistance are overcome by the beetles and fungi which become successfully established. The level of this critical threshold of attack density is a fundamental expression of the strength or capacity of tree resistance (Berryman 1978, 1982; Christiansen *et al.* 1987).

The basic phenomenon involved in the resistance is a phloem reaction induced by the invaders and localized around them. It consists of a rapid synthesis of chemicals, including secondary metabolites such as terpenes and phenols, which impregnate tree tissues (Reid *et al.* 1967, Berryman 1972, Shrimpton 1973, Christiansen and Horntvedt 1983, Christiansen *et al.* 1987, Lieutier *et al.* 1988, Långström *et al.* 1992, Lieutier 1993). The critical threshold of attack density depends on the quantity of energy the tree is able to rapidly mobilize for various syntheses in the reaction zones, and this quantity is necessarily limited (Christiansen *et al.* 1987). Thus, the threshold can be high if the lag time for stopping an aggressor at each point of attack is very short and hence the energy mobilized for that purpose in each reaction zone is low. Under these conditions of arrested attacks, a short reaction zone has been assumed to be more effective than an extended one (Lieutier *et al.* 1993).

Markers of conifer resistance to bark beetles could be very useful; e.g., forecasting susceptibility to beetle attacks and beetle outbreaks, and aiding in selection of highly resistant trees. Because the concentrations of secondary metabolites vary considerably during tree responses to aggression, they merit a special interest. Preliminary studies on phloem phenolics have suggested dramatic changes in Scots pine, *Pinus sylvestris* L., in response to aggression (Lieutier *et al.* 1991a). This paper presents the results from two complementary approaches recently developed in France concerning the possible role of phenols in arresting bark beetle invasion and their use as biochemical markers of resistance. Detailed methods and other findings are presented elsewhere (Lieutier *et al.* 1995).

METHODS

Experimental Devices

In our first experiment, six clones located in the INRA's nursery in Orléans (France), each represented by one to three 10-year-old trees, were used to test if a relationship existed between reaction zone length and phenolic composition. Trees were each inoculated with a malt agar sporulated culture of *Ophiostoma brunneo-ciliatum* Math.-K., a fungus previously isolated from *Ips sexdentatus* Boern., and thought to play a role in the establishment of this beetle in Scots pine (Lieutier *et al.* 1988). Each tree received three inoculations. Three weeks later, the reaction zone around each inoculation point was measured and sampled. Three samples of unwounded phloem were also taken from each tree.

Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

In a second experiment, three 30-year-old Scots pines located in the Orléans forest were inoculated with *O. brunneo-ciliatum* to study the kinetics of the trees' phenolic responses. Each tree received 33 inoculations distributed in 5 rings, each separated by 35 cm. After 3, 7, 14, 30 and 60 days, the reaction zones around respectively 15, 7, 5, 3 and 3 inoculation points per tree were measured and sampled. At days 30 and 60, samples of unwounded phloem were also collected from each tree.

Inoculations were always performed with 5 mm diameter discs of culture placed in cambium-deep, equally sized holes made with a cork borer, and the bark plugs were put back into the holes. Immediately after being collected from the trees, the phloem samples were frozen in dry-ice. In the laboratory, all samples were freeze-dried.

Phenols Extraction and Analysis

Each reaction zone sample was used separately, except for the samples collected 3 and 7 days after inoculation in the second experiment, which were pooled all together (day 3) or by groups of 1 to 3 samples (day 7) to have sufficiently a high quantity of material for further chemical analyses. Extractions were performed at 4°C on ground samples. In order to remove resinous compounds (Alcubilla 1970), phloem powder was first washed with pentane before extraction of phenolics using 80% methanol. Pentane treatment was checked to have no significant effect on the compounds analyzed in our study. Gallic acid was used as internal standards for HPLC analyses.

Analyses were performed with reversed HPLC (Waters 600E, Photodiode array detector 991), in a 250 mm column and 4 mm internal diameter. Stationary phase was a C-18 grafted silica (Merck, Lichrospher^R RP18) with 0.005 mm porosity. Mobile phase was a mixture of a 1% acetic acid in ultra pure water (solvent A) with a solution of pure methanol, acetonitrile, acetic acid (49.5:49.5:1) (solvent B). The gradient is represented in Fig. 1. Readings were made at 310 nm. Results were expressed in internal standard equivalents per gram of freeze-dried powder.

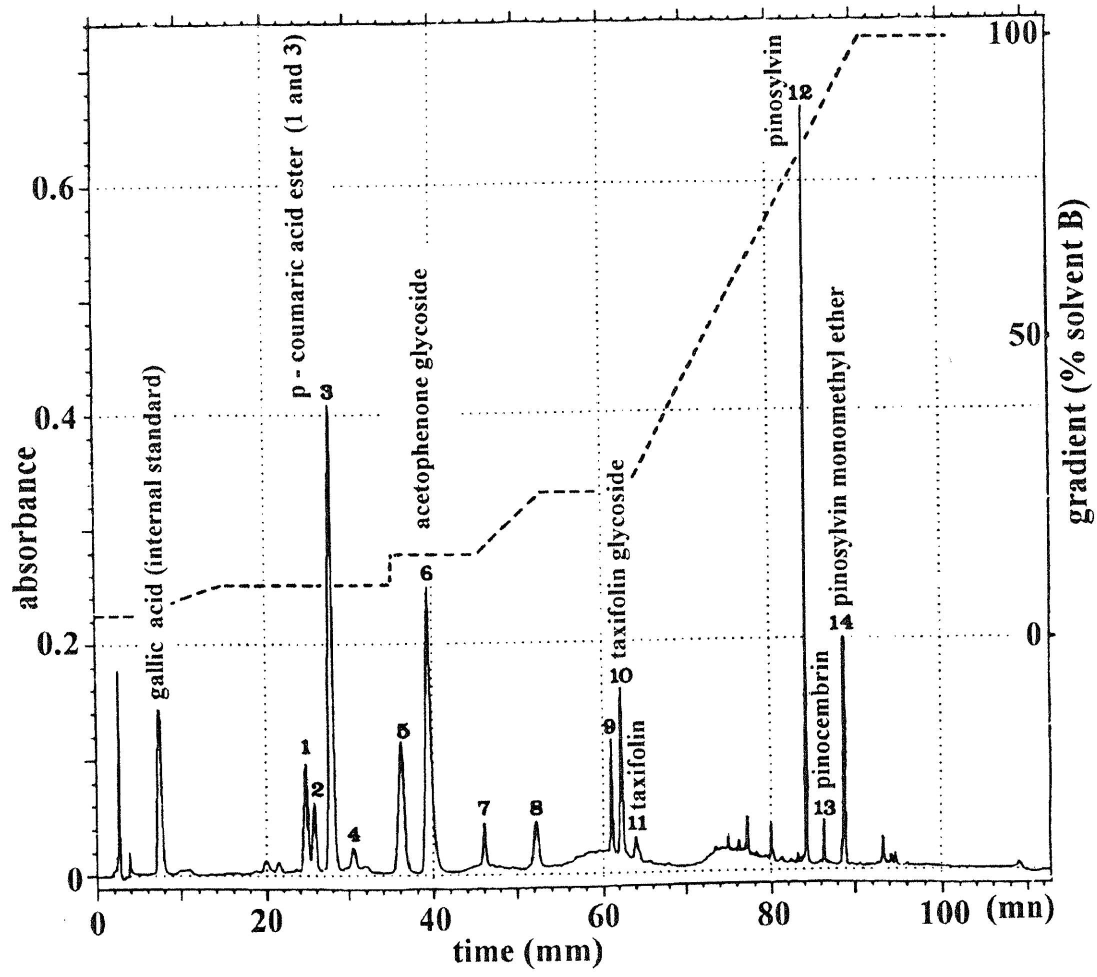

Figure 1.—Chromatogram of a reaction zone in Scots pine phloem and HPLC gradient. Solvent A = acetic acid, water (1/99); Solvent B = methyl alcohol, acetonitril, acetic acid (49.5/49.5/1).

Characterization of compounds was made by using various methods: two-dimensional thin layer chromatography (TLC) on cellulose plates to observe fluorescence and to test specific chemical reagents, observation of spectral characteristics in U.V., cochromatography in HPLC with spots obtained from TLC and with various standards. The presence of glycosidic or ester links was tested with acid or alkaline hydrolysis, respectively.

RESULTS AND DISCUSSION

Comparison Between Unwounded and Inoculated Phloem

Fourteen peaks were observed, of which eight were characterized at least at the chemical phenol family level (Fig. 1). Considerable differences existed between unwounded and inoculated phloem (Fig. 2). The most striking concerned the appearance of stilbenes, pinosylvin (Ps) and its monomethylether (Psme), and of one flavonoid, pinocembrin (Pc), in the reaction zone of all trees, while these compounds were always totally absent in the unwounded phloem. In all trees, the concentration of all compounds of the hydroxycinnamic acid group or its derivatives decreased during tree reaction. The concentration of flavonoids other than Pc increased or decreased depending on clone.

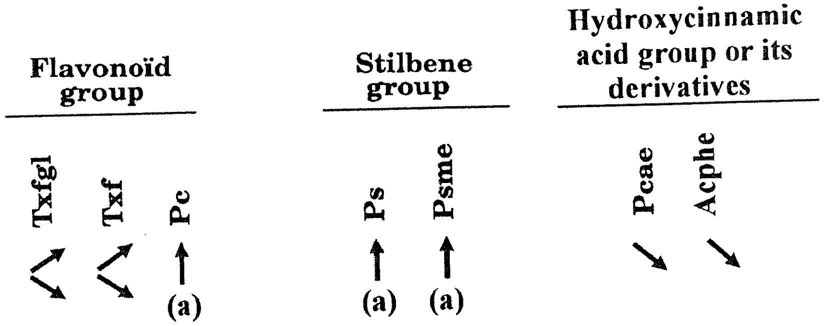

Figure 2.—Variations in the concentration of the phenolic compounds, between unwounded phloem and reactive phloem of different Scots pine clones, after inoculation with *Ophiostoma brunneo-ciliatum*. (a) = compound appearing in the reaction zone. One arrow means that the direction of variation was the same for all clones; several arrows mean that the direction of variation depended on the clone. Txf = taxifolin; Txfgl = taxifolin glycosid; Pc = pinocembrin; Ps = pinosylvin; Psme = pinosylvin monomethylether; Pcae = p-coumaric acid ester; Acphe = acetophenone glycoside.

These results confirm the preliminary study by Lieutier *et al.* (1991a) in Scots pine. Phloem phenolic response to inoculations differed greatly according to compound, which caused dramatic modifications in the relative composition of this tissue. These results differ completely from the observations on terpenes in any conifer species (Russel and Berryman 1976; Raffa and Berryman 1982a,b; Delorme and Lieutier 1990; Lieutier *et al.* 1991b; Långström *et al.* 1992), and makes certain phenols good candidates to play a role in arresting invaders and to be used as biochemical markers of tree resistance.

Stilbenes and Pc, which were characteristic of phloem induced reactions, could play a particular role in reaction efficiency, and consequently in a tree's induced resistance to bark beetle and fungi attacks. However, similar results have been reported in needles after pollution by ozone (Rosemann *et al.* 1991), as well as in the sapwood of various pine species in response to several kinds of aggression (Ref. in Kuc and Shain 1977, Hart and Shrimpton 1979, Shain 1979, Hart 1981, Kemp and Burden 1986). In addition, similar results have also been obtained with a phloem wound without fungus (Lieutier and Yart unpubl.). Lieutier *et al.* (1991a) also mentioned the non-specificity of the phloem phenolic response after various aggressions. Tree response to bark beetles and their associated fungi are thus more a response to wounding than a response to particular aggressors themselves (Lieutier 1993). Mullick (1977) has suggested previously that a tree's response to aggression is more concerned with tissue restoration than with defense.

Considering biochemical pathways, it is interesting to note that the appearance of stilbenes and Pc in Scots pine corresponded to decreases in compounds of the hydroxycinnamic acid group, since this latter group is a precursor of both stilbenes and flavonoids (Ribereau-Gayon 1968, Gorham 1989).

Between Clone Comparisons

Clonal variability was first analyzed by multivariate analysis, in order to take into account all characterized compounds together. A canonical discriminant analysis was performed by taking reaction zones as main individuals and unwounded phloem as complementary individuals. This procedure was necessary as the purpose was mainly to compare clone reaction zones with each other, and only to place unwounded phloem relatively to them.

Axes 1, 2 and 3 explained 57, 23, and 15% of the variation, respectively. In the first two axes (Fig. 3), from unwounded phloem to the reaction zone, phenolic composition seemed to evolve towards the same point of the plane, whatever the clone. That was confirmed in all significant axes (three), by calculating mean distances between points and their barycenter. Spread of the points relating to reaction zones was less than spread of the points relating to unwounded phloem, in each clone and for all clones together (data not shown). Thus, each clone would react in a different way according to its constitutive composition, but always so that the composition of the reaction zone tended to be the same.

The meaning of this phenolic modifications is not clear. It may be linked with protection against aggressors or with tissue restoration. Nevertheless, it is worthwhile to note that clone "c", whose reaction was the least efficient (reaction zone significantly longer than that of all other clones), was the only one initially separated from the others by axis 2 (Fig. 3). If phenols effectively play a role in arresting beetles and their fungi, it could thus be possible to predict reaction efficiency of a clone on the basis of the whole phenolic composition of its constitutive phloem.

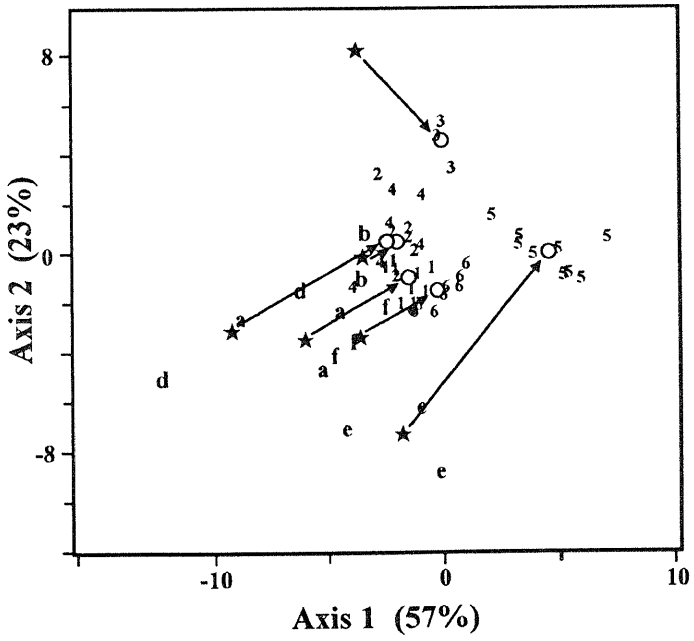

Figure 3.—Canonical discriminant analysis of phloem phenolic composition of different Scots pine clones, after inoculation with *Ophiostoma brunneo-ciliatum*. Reaction zones were considered as principal individuals and unwounded phloem as complementary individuals. a, b, c, d, e, f = unwounded phloem of the different clones (* represents the barycenters); 1, 2, 3, 4, 5, 6 = Corresponding reaction zones (o represent the barycenters). Arrows indicate the directions of variation from unwounded phloems to reactions zones. Clones a, b, c, d, e and f were respectively represented by 3, 2, 1, 2, 3 and 3 trees.

Clonal variability was also analyzed compound by compound, for the peaks characterized at least at the family level. To appreciate if certain compounds could be used as markers of efficiency of tree response, phenol concentration in each clone and for each compound, was compared to reaction zone length. The best correlation (r = 0.78) was for taxifolin glycoside in unwounded phloem (data not shown). The lowest concentrations corresponded to the least extended reaction zones, that is to the most effective response, and inversely. Concentration of taxifolin glycoside could thus be a marker of tree's response efficiency. Presence of taxifolin glycoside in the phloem prior to aggression could be favorable to the fungus, or could directly impede the development of the tree response. In the two cases, the result would be an increase in the lag time necessary to stop the fungus, thus causing a long reaction zone.

Kinetics of the Phenolic Response

A first approach to the kinetics was performed with multivariate analysis, the same way as in the previous experiment (Fig. 4). Axes 1 and 2 explained 88 and 9 percent of the variation, respectively. The composition of all unwounded samples was close to that of reaction zones at day 3, which suggest that phenolic composition of unwounded phloem did not vary during the whole experiment. Although between tree variability existed, the phenolic composition of the reaction zones at day 60 was clearly separated from that of all other samples along axis 1. Reaction zones at days 3 were separated from other samples along axis 2. Kinetics of phenolic response thus proceeded in two main phases. Concomitantly, growth of the reaction zone also proceeded in two main phases and was approximately done at day 30, indicating that the fungus had been stopped before this date (Fig. 5). One can thus hypothesize that the first phase, before day 30, is related to resistance and to interactions between tree and fungus, while the second more probably corresponds to wound healing processes. Considering that, it is interesting to note that wound periderm began to be visible at day 30.

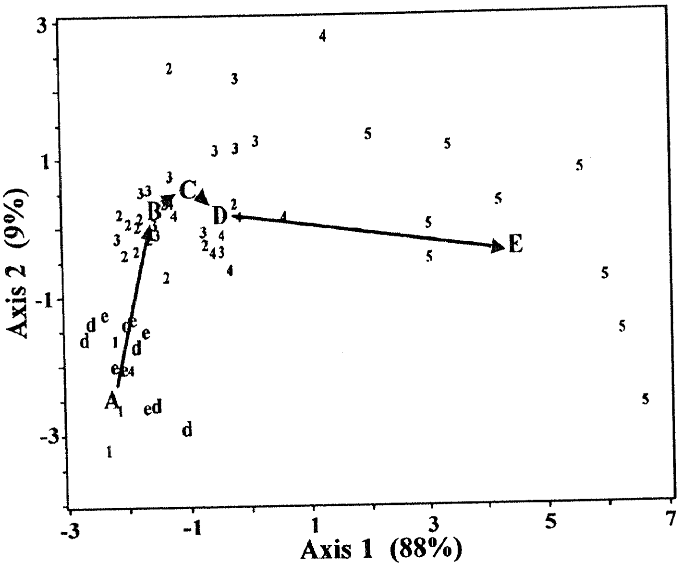

Figure 4.—Canonical discriminant analysis of the phenolic composition of Scots pine phloem collected at different dates after inoculations with *Ophiostoma brunneo-ciliatum*, in three different trees. Reaction zones were considered as principal individuals and unwounded phloem as complementary individuals. 1, 2, 3, 4, 5 = Reaction zones collected respectively 3, 7, 14, 30 and 60 days after inoculations. A, B, C, D, E = corresponding barycenters. d, e = unwounded phloem collected at days 30 and 60. Arrows indicate the directions of variation during reaction development.

Analysis, compound by compound, revealed three main kinds of kinetics. Ps, Psme and Pc increased in two phases, first early until day 7, then late after day 30 (Fig. 6a and 6b). Some other compounds, such as acetophenone glycoside, regularly decreased or remained constant (Fig. 6c), while others, such as taxifolin, increased only late, after day 30, sometimes after a decrease at the very beginning of development of the reaction (Fig. 6d).

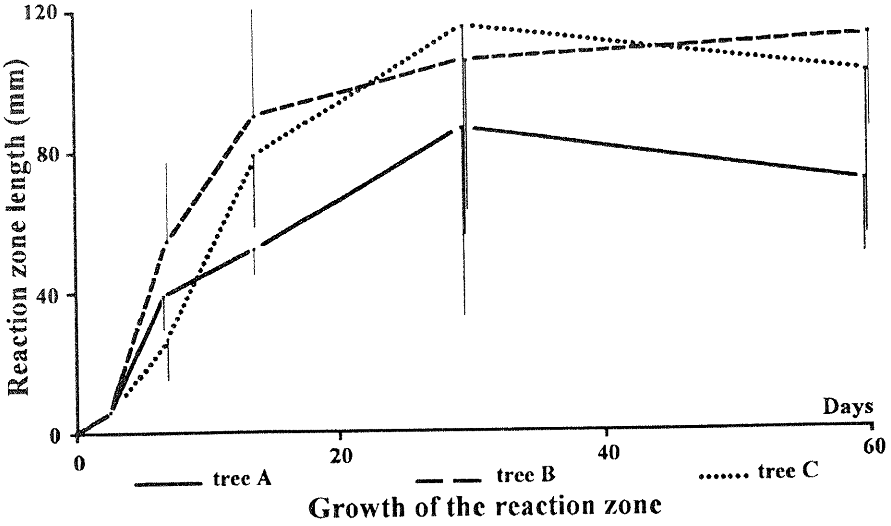

Figure 5.—Mean reaction zone length at different dates after inoculation of three trees with *Ophiostoma brunneo-ciliatum*. Vertical bars represent standard deviations.

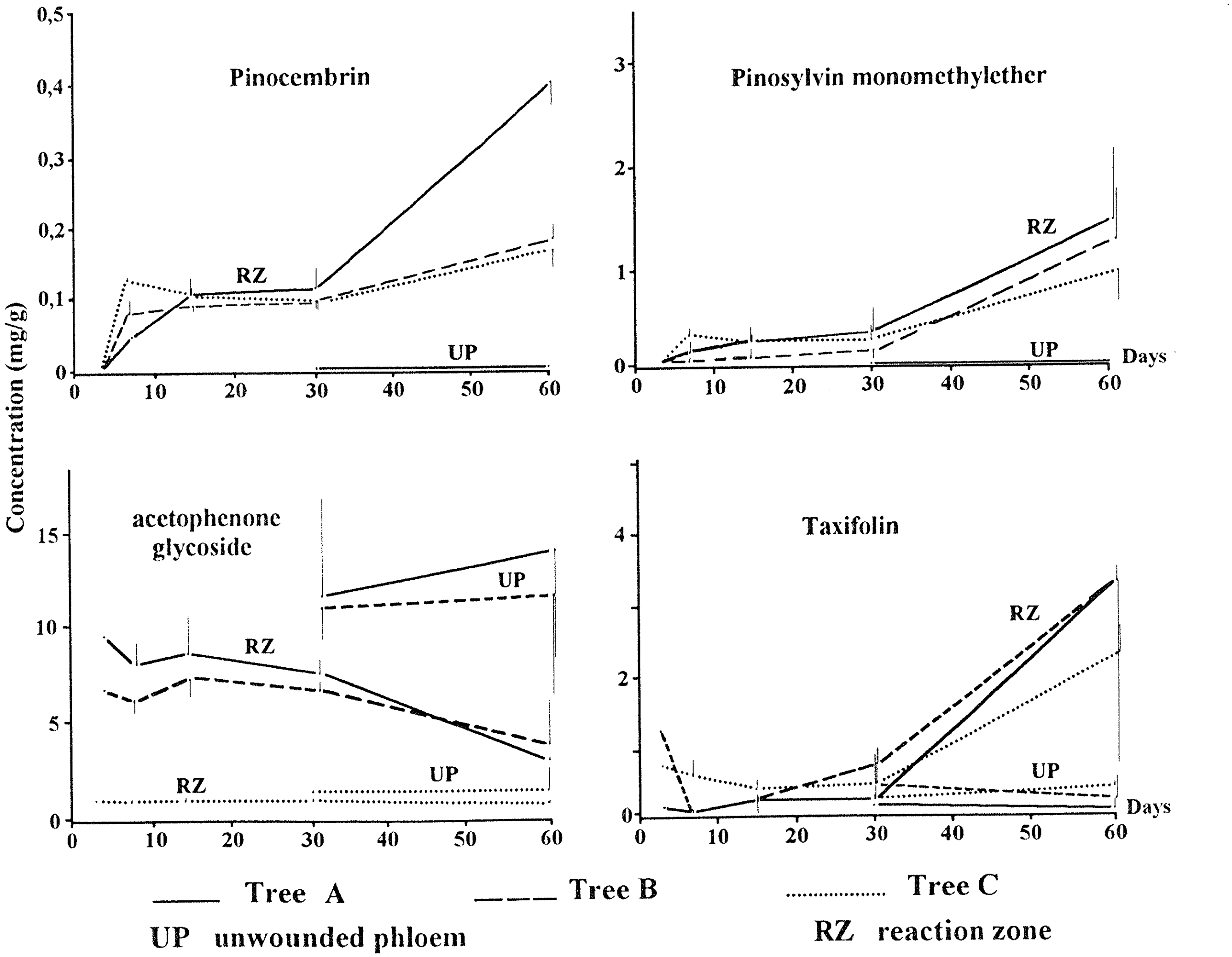

Figure 6.—Variations of the mean concentration of various phenolic compounds in reacting phloem and in unwounded phloem of three trees, after inoculation with *Ophiostoma brunneo-ciliatum*. Vertical bars represent standard deviations.

Under these conditions, according to the above hypothesis, Ps, Psme and Pc would probably be involved in tree resistance, which could be related to the tree's ability to rapidly synthesize these compounds. After an early increase, their concentration remains constant until the fungus was stopped, which could indicate that they were metabolized by the fungus because of their toxic effect, as suggested by Lyr (1962). Their increase after day 30 could thus be due to the absence of fungus activity. This increase also suggests that they are involved in wound healing processes, as could be taxifolin. In Sitka Spruce as well as in hardwood trees, accumulation of phenolic compounds takes place parallel to suberin biosynthesis, a component of the wound periderm (Biggs 1985, Woodward and Pearce 1988).

CONCLUSIONS

Tree response could be more a generalized wound response than a pure defensive response. Both stilbenes and flavonoids could be involved in tree resistance. Taxifolin glycoside is a potential marker of tree resistance, all the more interesting as it belongs to constitutive phloem, but contrary to pinocembrin and stilbenes, its high concentration would indicate a low level of resistance. Whole phenolic composition of unwounded phloem could also be a marker of resistance. However, several conditions need to be verified before definitive conclusions are possible about their use as markers. Indeed, between clone differences could depend on tree physiological status or age, environmental conditions, locality or season. In addition, if genetic markers are looked for, the corresponding compounds must be genetically dependent and the corresponding genes must always be expressed.

SUMMARY

Scots pine phloem was investigated for phenolic markers of tree resistance to bark beetles. Our approach was based on the study of relations between the reaction efficiency and phenolic composition in six different clones, and on the study of the variations in phenolic composition during tree reaction development. Considerable modifications took place after aggression. Compounds lacking in unwounded phloem appeared in the reaction zone. During the development of the reaction zone, modifications of the phenolic composition clearly proceeded in two successive phases. Stilbenes and flavonoids seemed to be involved in the induced resistance. Taxifolin glycoside in unwounded phloem looked interesting as marker, as did the whole phenol composition of this tissue.

ACKNOWLEDGMENTS

The authors thank J. Garcia and P. Romary (INRA, Orléans, France) for their technical help and Dr. D. Sauvard (INRA, Orléans) for help with the statistics. They are grateful to Prof. H. Sandermann and Dr. W. Heller (G.S.F. Neuherberg, Deutschland) and to Dr. A. Scalbert (INRA Grignon, France) for providing pure compounds. They also thank Drs B. Monties, A. Scalbert and M.T. Tollier (INRA Grignon, France) for their help in characterization. These studies were granted by the Conseil Régional de la Région Centre (France) and by the French Ministry for Research and Technology.

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DIFFERENTIAL SUSCEPTIBILITY OF WHITE FIR PROVENANCES TO THE FIR ENGRAVER AND ITS FUNGAL SYMBIONT IN NORTHERN CALIFORNIA

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INTRODUCTION

The fir engraver, *Scolytus ventralis* LeC., attacks white fir, *Abies concolor* (Gord. and Glend.) Lindl., and other true firs, *Abies* spp., in western North America. The biology, attack behavior, and ecology of this bark beetle were recently summarized by Berryman and Ferrell (1988). During the summer flight season, the attacking beetles bore into the cambial zone of fir boles, introducing a pathogenic brown-staining fungus, *Trichosporium symbioticum* Wright. Resistant firs react to the invasion by forming a resinous necrotic wound in the phloem and outer sapwood, which contains the spread of the fungus and repels or kills the beetles. In such interactions, the tree usually survives. This reaction is less intense or absent in susceptible firs, resulting in reproductive success of the beetles, and severe damage or even death of the fir. Sporadic outbreaks of the fir engraver, associated primarily with droughts, have caused widespread mortality of true firs in nearly every decade of this century in western North America.

In the drought years of 1987-88, four 26-year-old white fir provenance test plantations at Camino, California, located at 1028 m elevation in the central Sierra Nevada, sustained considerable levels of mortality. Subcortical examination of a subsample of the dead firs indicated that all had reproductively successful gallery systems of the fir engraver. Drought continued until winter 1992-1993. Surveys beginning in fall 1988 revealed pronounced differences in this mortality, among both plantations and provenances, and also within plantations.

This paper describes the observed patterns of susceptibility and resistance in relation to known patterns of geographic variation in white fir in western North America and discusses studies underway to understand the mechanisms responsible for these patterns.

METHODS

The adjacent provenance test plantations represent two studies. The "geographic range" plantations (1 and 3) consist of 39 provenances from throughout most of the western portion of white fir's natural geographic range in western North America. Each provenance was originally represented by three replications, each of three seedlings. One replication was removed by thinning in 1970. The "elevational transect" plantations (2 and 4) contain only four provenances representing a west-east elevational transect across the Sierra Nevada at the latitude of Camino. Each of these provenances is represented by 10 half-sib families, each originally with nine trees, since thinned to six. In all four plantations, provenances were planted in an interlocked randomized non-contiguous plot layout designed to minimize effects of microsite variation (Libby and Cockerham 1980).

Beginning in August 1988, trees in these plantations were surveyed annually in summer or early fall for mortality and susceptibility to fir engravers. Surveys were conducted after trees attacked and killed by fir engravers the previous year had faded crowns, but before trees killed by current attack had faded crowns. During these surveys, trees topkilled by the previous year's attack were also noted.

Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

Patterns of tree resistance were surveyed in December 1989; numbers of pitch streamers indicating unsuccessful fir engraver attacks were classified as 0, < 5, 5-10, and > 10 on stems of all live trees.

Distributions of trees classified by status (live, dead) and number of pitch streamers were compared among provenances by Chi-square Contingency Test (SAS Institute Inc. 1988). Data from the two replicates of each type of plantation (geographic range, elevational transect) were similar and were pooled for analysis. In the geographic range plantations, provenances were combined according to Hamrick and Libby (1972) as modified by Libby *et al.* (1980). The latter authors divided white fir in the western part of its range into five major geographic groups based primarily on needle morphology: (1)"Northern"— central Oregon and northwestern California; (2) "Central"— south-central Oregon, central and northeastern California; (3) "Southern California"; (4) "Interior South"— Arizona; and (5) "Interior North"— eastern Nevada and western Utah.

RESULTS

Of the 1,127 firs alive in all four plantations in 1987, 393 (35%) were killed by the fir engraver by 1993. Most (253) of the mortality resulted from the attack season of 1987, with an additional 121 resulting from the 1988 attack season. Relatively few (29) were killed by attack in 1989-1993. Only a few (10) trees were topkilled, two of which were killed in subsequent years. Firs on the outside edges of the plantations were infrequently killed (4/97 or 4%).

Through 1993, cumulative mortality in the elevational transect plantations (256 of 578 total trees, or 44%) was almost twice that in the geographic range plantations (137/549, or 25%). The associated Chi-square (adjusted for continuity) was 45.505, $p < 0.0001$ at 1 df.

In the geographic range plantations, mortality differed among major geographic groups of Hamrick and Libby (1972) and Libby *et al.* (1980). In both plantations, provenances of the Northern and Central groups had sustained the highest mortality, while very few of the firs of the Interior South and Interior North groups were killed (Table 1). Low levels (11 percent) of mortality occurred in southern California firs. The Chi-square associated with among-group variation was 40.631, $p < 0.0001$ at 4 df. Among individual provenances, the one from nearest Camino (AK; Omo Ranch; 32 km distant) had the highest mortality in both plantations.

Table 1.—Mortality of white fir provenances caused by fir engravers in the geographic range plantations[1] at Camino, CA 1987-1993

Group[2]	Plantation 1			Plantation 3		
	Total	Dead	Pct dead	Total	Dead	Pct dead
Northern	55	12	22	84	33	39
Central	106	22	21	124	55	44
Southern Calif.	40	3	8	47	7	15
Interior South	28	0	0	31	4	13
Interior North	14	0	0	20	1	5

[1] Each plantation contained 39 provenances from the western portion of white fir's geographic range.

[2] Provenances combined by major morphological groups of Hamrick and Libby (1972).

In the elevational transect plantations, mortality in the two lower- elevation westside provenances nearest Camino (AK, AL) averaged more than one-third greater than that in the two upper-elevation eastside provenances (AM, AN), which are only about 50 km more distant (Table 2). The among-provenance Chi-square was 24.757, $p < 0.0001$ at 3 df.

Analysis of the distribution of pitch streamers on boles of surviving firs revealed that in the elevational transect plantations, virtually all surviving firs had been attacked, and many heavily so (>10 streamers). In the geographic range

Table 2.—Mortality of white fir provenances caused by fir engravers in the elevational transect plantations[1] at Camino, CA 1987-1993

Provenance	Plantation 2			Plantation 4		
	Total	Dead	Pct dead	Total	Dead	Pct dead
AK	76	37	49	95	59	62
AL	72	30	42	72	42	58
AN	65	19	29	63	27	43
AM	61	9	15	74	33	45

[1] West-east transect across Sierra Nevada at latitude of Camino. AK, AL are lower elevation westside; AN, AM are upper elevation eastside.

plantations, however, more than 38% of Interior South and Interior North firs had no pitch streamers, and fewer than 11% had more than 10 pitch streamers (Table 3). In contrast, among Northern and Central firs, fewer than 6% had no pitch streamers while more than 24% had over 10. Distribution of pitch streamers on Southern California firs was intermediate between these two patterns. The among-group Chi-square was 128.424, $p < 0.0001$ at 12 df. The Central provenances from the Sierra Nevada where Camino is located had the lowest percentage of firs with no pitch streamers (ca. 2%) and the highest percentage with more than 10 streamers (ca. 41%).

Table 3.—Incidence of pitch streamers caused by fir engravers on boles of white fir provenances in the geographic range plantations,[1] Camino, 1989

Group[2]	Number of pitch streamers			
	0	<5	5-10	>10
Northern	5	34	31	23
Central	3	22	61	60
Southern Calif.	11	16	33	16
Interior South	27	14	8	6
Interior North	13	12	8	1

[1] Plantations 1 and 3, combined.

[2] Provenances combined by major morphological groups of Hamrick and Libby (1972).

DISCUSSION

Results indicated that Northern (Oregon) and Central (Northern California) provenances were susceptible to the fir engraver and its fungal symbiont in the Camino plantations, while Interior South (Arizona), and Interior North (Nevada, Utah) provenances were virtually nonsusceptible. Southern California provenances demonstrated low susceptibility. These patterns agree closely with known geographic patterns of morphological and chemical (cortical monoterpenes) variation in white fir over its natural range in western North America (Hamrick and Libby 1972, Zavarin *et al.* 1975). Susceptible provenances were those characterized as green-foliaged, with needle morphology suggesting only moderate drought resistance, and low in camphene and 3-carene, consisting of California white fir (var. *lowiana* (Gord.) Lemm.) from northern California and intermediates with grand fir, *A. grandis* (Doug.), from south-central Oregon. Provenances evidencing virtually no susceptibility were blue-green foliaged with needle morphology suggesting higher drought resistance, relatively high in camphene and 3-carene, and of the Rocky Mountain variety (var. *concolor* (Gord. & Glend.) Lindl.). Southern California provenances evidencing low susceptibility were characterized as blue-green foliaged, relatively high in 3-carene but nearly lacking in camphene and thus intermediate between the Northern and Interior South groups.

In all plantations, susceptibility was always highest in provenances nearest Camino. This was evident not only on a large scale in the geographic range plantations where Central (Northern California) provenances were the most susceptible, but also on a fine scale in the elevational transect plantations where provenances 40 km distant from Camino were more susceptible than those from 80 km distant. In loblolly pine plantations in South Carolina, Powers *et al.* (1992) also found that local provenances were more susceptible to southern pine beetle, *Dendroctonus frontalis*, than distant provenances.

Mechanisms responsible for the observed differential susceptibility among provenances are under investigation. Their basis, however, is undoubtedly primarily genetic as the plantations are all adjacent with provenances planted intermixed to minimize chances that microsite would differentially affect provenances.

The very low susceptibility of trees on the outside edges of the plantations compared with those in the interior was probably primarily determined by stand factors such as differences in soil moisture or beetle pheromone dispersal rather than tree genetics because susceptibility occurred without regard to provenance. Mechanisms underlying this difference are under investigation.

We have two studies underway testing the hypothesis that observed differential susceptibility is attributable to differential moisture stress among provenances, and between firs on plantation edge versus interior. White firs with highly negative water potentials (pre-dawn in August during summer dry, and fir engraver flight, seasons) are known to be susceptible to the fir engraver (Ferrell 1978). Results to date failed to find appreciable differences among provenances in 1989, 1990, or 1993. "Edge" firs did average higher moisture stress than "interior" firs in 1992 and 1993. Both studies will continue for one more post-drought year. Results are necessarily conditioned by the fact that moisture stress can be studied only in surviving firs. Thus, parallel studies are underway to compare radial growth patterns in surviving versus killed firs, under the hypothsis that differential moisture stress should be evident in radial growth pattterns.

Differences in fir engraver attack preference and success are being investigated by reciprocally caging "local" and "exotic" populations of beetles with bolts cut from "local" and "exotic" provenances of white fir. These tests are designed to reduce or eliminate differential resistance caused primarily by differences in environmental factors such as moisture stress and to isolate for analysis differential resistance caused by factors that are primarily genetically determined such as constitutive bark chemicals. Preliminary results indicate that beetles from the vicinity of Camino initiate far fewer attacks in Arizona bolts than in "local" bolts. No such preference was evident in tests with Nevada beetles caged with bolts of firs from Nevada and the vicinity of Camino. These tests are being repeated with beetles and bolts of other provenances. Parallel studies are also under way using various geographic isolates of the *Trichosporium* fungus inoculated into stems of surviving firs in the plantations. Among-provenance variations in inoculation wound size and monoterpene composition are being studied. Thus far consistent differences in virulence have been found between isolates regardless of provenance of the fungus or the fir. Studies continue to explore seasonal and yearly variations and to analyze monoterpene composition.

Results indicate that local populations of host conifers can be more susceptible to local populations of bark beetles and their fungal symbionts while exotic host populations can be less susceptible. If generally true, this phenomenon may have to be taken into account in tree improvement programs, most of which prefer to utilize local genetic material as planting stock because this material is preadapted to the growing site. If results at Camino are any indication, however, exotic seed sources may be less susceptible to local bark beetle populations provided that these trees are adequately adapted to the physiographic factors of the growing site. At Camino, many of the nonsusceptible exotic provenances had grown fully as tall as the best-growing local provenances, indicating that they were thus far as well adapted to the growing site.

Another important implication of the Camino results is that maintenance of genetic diversity in conifer populations may be an important safeguard in protecting conifer stands against bark beetles. The much greater mortality experienced in the elevational transect plantations containing only four provenances may be an expression of this, although it was evidently also influenced by the local origin of all four of these provenances.

Results from the geographic range plantations suggest that planting well-adapted exotic and local provenances in mixture may be a useful strategy for avoiding problems from bark beetles and their fungal symbionts. Any use of exotic provenances should probably be limited to high-value trees, particularly those grown on stressful sites, because more widespread planting could lead to loss of resistance through local pest populations becoming adapted to them.

SUMMARY

During a drought-associated fir engraver outbreak in California, local white fir provenances were more susceptible than exotic provenances, and, doubtless partly in consequence, less genetically diverse plantations were more susceptible than plantations with greater genetic diversity.

Mechanisms underlying the observed differential susceptibility remain unknown but are the subject of continuing investigations.

Results suggest that planting a mixture of well-adapted exotic, as well as local, provenances for maintenance of high genetic diversity may be an important strategy for protecting conifer hosts against bark beetles and their symbiotic fungi.

ACKNOWLEDGMENTS

We thank Dr. Tom Conkle and staff of the Pacific Southwest Research Station's Institute of Forest Genetics and Prof. Bill Libby of the cooperating University of California College of Environmental Science, Berkeley, for access to plantations and for encouragement.

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MILD DROUGHT ENHANCES THE RESISTANCE OF NORWAY SPRUCE TO A BARK BEETLE-TRANSMITTED BLUE-STAIN FUNGUS

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INTRODUCTION

Outbreaks of the spruce bark beetle, *Ips typographus* (L.), one of the most serious pests of the Eurasian spruce forests, are frequently triggered by large-scale windfelling. Although correlative evidence indicates that long-lasting drought incites and aggravates such epidemics (cf. Christiansen and Bakke 1988), very little experimental work has been carried out to test this assumption.

Ideally, the defensive capacity of the trees should be tested using prescribed numbers of bark beetle attacks. In *Pinus contorta* this has been done successfully by screening the branch-free part of the stem, and inducing a defined number of *Dendroctonus ponderosae* attacks under the screen (Raffa and Berryman 1983). Without screening, additional attacks by "wild" beetles in the neighbourhood will occur if the beetles possess aggregation pheromones.

Unlike mountain pine beetle attacks on lodgepole pine, *I. typographus* attacks often extend far into the living crown of Norway spruce, *Picea abies*, making screening impractical. For this reason we chose to assay host resistance using prescribed loads of artificial mass-inoculation with the blue-stain fungus, *Ophiostoma polonicum* Siem., a close associate of the spruce bark beetle.

Spores of *O. polonicum* are transmitted both externally and internally by *I. typographus* (Furniss *et al.* 1990), and the fungus is consistently isolated from the advancing front of blue-stain in successfully attacked trees (Solheim 1986, 1992). *O. polonicum* can kill healthy Norway spruce when artificially inoculated under the bark (Horntvedt *et al.* 1983, Christiansen 1985a, Solheim 1988). Similarly, other spruce species and even *Pseudotsuga menziesii* may succumb to mass-inoculation with this fungus (Christiansen and Solheim 1990). Parallel to the "threshold of successful attack" for bark beetles (Thalenhorst 1958), a "threshold of successful infection" exists for mass-inoculation with this fungus (Christiansen 1985b).

Drought is generally accompanied by hot weather, and the two factors are not easily separated when one attempts to find the causes for large-scale infestations. We hypothesize that (1) elevated temperatures act directly on the various life stages of the beetles to favour population build-up, and/or that (2) drought affects the physiology of the trees, making them more susceptible to attack or more suitable as food. Here, we report an experiment designed to shed light on aspects of Hypothesis 2: Norway spruce trees were artificially drought stressed, and their defensive capability was assayed and compared with unstressed, control trees.

In ordinary forest stands, trees will vary considerably in their susceptibility to beetle/fungus attack. This necessitates large numbers of experimental trees to obtain statistically significant results. In the present experiment, however, we utilized clones of spruce trees and thus permined work with relatively few trees.

METHODS

Experimental Trees and Their Treatment

The Norway spruce clones used in this study grew in a multi-clonal stand at Hogsmark Experimental Farm in Ås, Akershus, Norway, and had been produced as follows: Seeds from selected trees were sown in 1951 and cuttings from the

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Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

resulting seedlings were then rooted. In spring, 1970, rooted ramets of each clone were planted using 2x2 m spacing. The planting was done in a west-facing, gently sloping agricultural field where underground drain pipes had been installed and the clayey soil tilled before planting. The homogeneous soil and the exact planting array reduced phenotypical variation in the stand to a minimum.

From each of two clones, #194 and #582, twelve ramets were selected. Trees of Clone 194 had been used in an earlier mass-inoculation experiment with *O. polonicum*, and had proved relatively resistant to the infection (Christiansen, unpubl.). Nothing was known about the susceptibility of Clone 582 as to its susceptibility.

The continuous rows of clones followed the slope of the field. The row of trees between Clone 194 and Clone 582 had been cut in early October 1992, and so had the two rows on their other side. Thus both rows had a distance of 4 m to their nearest neighbours. Trees at both ends of the two rows were discarded as phenotypically aberrant, having much larger crowns than trees inside the stand.

In late October, 1992, all branches below ca. 2.5 m were removed from all 24 trees. These branches were all dead due to shading. The ground on both sides of the upper six trees in each row was then covered with plastic sheets to prevent autumn and winter precipitation from percolating into the ground. Instead, this water was led down to the lower six trees in the two rows. On 20 April 1993 the plastic ground cover was removed. Ropes were strung at 2 m above ground between the upper six trees of the rows, and non-transparent plastic tarpaulins were suspended from these ropes down to drains fastened to the stumps of the tree rows that had been removed. The drains collected the rain falling through the canopy, and channelled it down to the lower six trees in the rows.

This way the six upper trees in a row were deprived of most of the precipitation falling from October till the end of the experiment. The lower six trees of the two rows served as unstressed controls. Because the early summer of 1993 was very dry, these control trees were given an extra 47 mm of water, applied under the canopy by means of a garden sprinkler.

Monitoring of Drought Stress

Starting on 12 May 1993, drought stress was monitored by repeated measurements of pre-dawn xylem water potentials (hereafter termed "WP"), using a pressure chamber. Freshly excised shoots taken 4-5 m above ground were used. To detect a possible natural WP gradient along the slope, trees at the upper and lower ends of the rows were measured. By mid-June WPs of the experimental trees were clearly different from those of the controls, and after a monitoring of all 24 trees on 23 June we decided that they had reached a satisfactory level of stress for inoculation with the fungus.

Fungal Inoculation

Prior to inoculation a 80 cm long template was attached around the stem between 1.2 and 2.0 m above ground. In the template evenly spaced holes had been punched at a density of 4 per dm^2, and the points of inoculation were marked through these holes. The choice of this particular dose was based on earlier experience with the susceptibility to infection by trees of Clone 194 and of two other clones growing in the same stand. Inoculation occurred on 25 June. Bark plugs were removed with a 5 mm cork borer. The inoculum, actively growing *O. polonicum* mycelium on malt agar, was placed in the cambium-holes, whereupon the bark plug was returned to its original position (cf. Horntvedt *et al.* 1983).

Further Field and Laboratory Procedures

Around the time of inoculation, exudation of constitutive resin was estimated twice (cf. Christiansen and Horntvedt 1983): on each tree 10 thin plastic tubes were inserted into holes cut with a cork borer about 2.1 m above ground. After 24 hours the length of the resin column in the tube was measured.

On 1 August, about 5 weeks after inoculation, exudation of resin from the point of inoculation was recorded on a subjective scale from 0 to 5, and used as an estimate of induced resinosis. On 14 September 1993, 81 days after inoculation all trees were felled, and tree height and height of the lowest green whorl of branches were measured. The inoculated stem section was cut out, and brought to the laboratory.

In the laboratory two ca. 5 mm thick cross-sectional discs were cut 20 cm inside each end of the section. The heartwood-sapwood border and blue-stained areas were marked out on these two discs. Two separate measurements for fungal success were used: (1) percent of the sapwood which had become blue-stained, and (2) percent of the disc circumference where the cambial area had been killed. For both measurements, the disc having the most fungal proliferation was used to represent the tree.

Above the lower of these thin discs a thicker one was cut. This ca. 3 cm disc was used for re-isolation of fungi from the wood. Three small wood chips were taken along one radius of the disc where staining occurred, one at the front of the advancing blue-stain, one near the cambium, and one in between.

The data were analyzed using the Minitab statistical system (Ryan *et al.* 1992).

RESULTS

During the period late October 1992 to late June 1993 precipitation at Ås amounted to ca. 400 mm, and a further 200 mm fell from late June (inoculation) till mid-September (felling). Although small quantities of water must have leaked through the ground cover and later the roof, the experimental trees were still deprived of most of this precipitation. The spring of 1993 was drier than normal; only 124 mm fell during the period 1 March - 30 June, as opposed to a normal of 215 mm. Normal annual precipitation is ca. 800 mm.

Measurement of pre-dawn xylem water potentials in late June demonstrated significant differences between stressed and control trees in both clones (Fig. 1). In Clone 194 the WP values of stressed trees ranged between 0.75 and 0.95 MPa as compared to 0.35-0.50 MPa in the control trees. No stressed tree had as high a WP as any of the controls. Clone 582 exhibited less difference in WP between the two categories of trees, and one sheltered tree (#6) was no more stressed than three of the control trees. Trees of the same clones at the top and bottom of the slope showed no clear differences in WP (result not shown).

On 29 June measurements indicated that stressed trees of Clone 194 yielded less constitutive resin in 24 hours than controls ($p=0.002$), but on 6 July no difference could be detected. In Clone 582 such differences vere absent on both occasions. Subjective estimates of surface exudation of resin from the points of inoculation gave a rating that was about three time higher in stressed trees of both clones than in corresponding controls ($p=0.000$).

Upon felling on 14 September, no tree had lost its foliage. Several trees of Clone 194 had a yellow tint to their foliage, but this occurred both in stressed and control trees.

The cross-sectional discs showed sapwood blue-staining varying from 0 to 100 percent (Fig. 2). Staining was much more pronounced in controls than in drought stressed trees of Clone 194. The stressed tree that had the most fungal invasion had much less stain than the least affected control ($p=0.000$). In Clone 582, on the other hand, there was no difference between stressed and control trees. This measure of fungal success coincided with percentage of the disc periphery where the cambial area had been killed (Fig. 2). There was no overlap between stressed trees and controls in Clone 194 ($p=0.000$). In Clone 582 control trees proved to be somewhat more affected than the stressed ones ($p=0.033$).

O. polonicum was reisolated from 10 of the 12 trees of Clone 194 and 8 out of 12 in Clone 582. Because almost 12 weeks elapsed from inoculation to felling, other fungi had also invaded the wood (cf. Solheim 1992). These were mainly *Nectria* spp. (found in 14 trees), but another *Ophiostoma* species, *O. piceae* (Münch) H. and P. Syd., occurred together with *O. polonicum* in two trees of Clone 194.

DISCUSSION

In this experiment current drought stress obviously enhanced tree resistance against *O. polonicum* infection in Clone 194. Clone 582 yielded no conclusive evidence, as the fungus appeared to be almost equally successful in stressed and control trees. It should be noted that in Clone 582 there was also less difference in WP between the two groups of trees than in Clone 194.

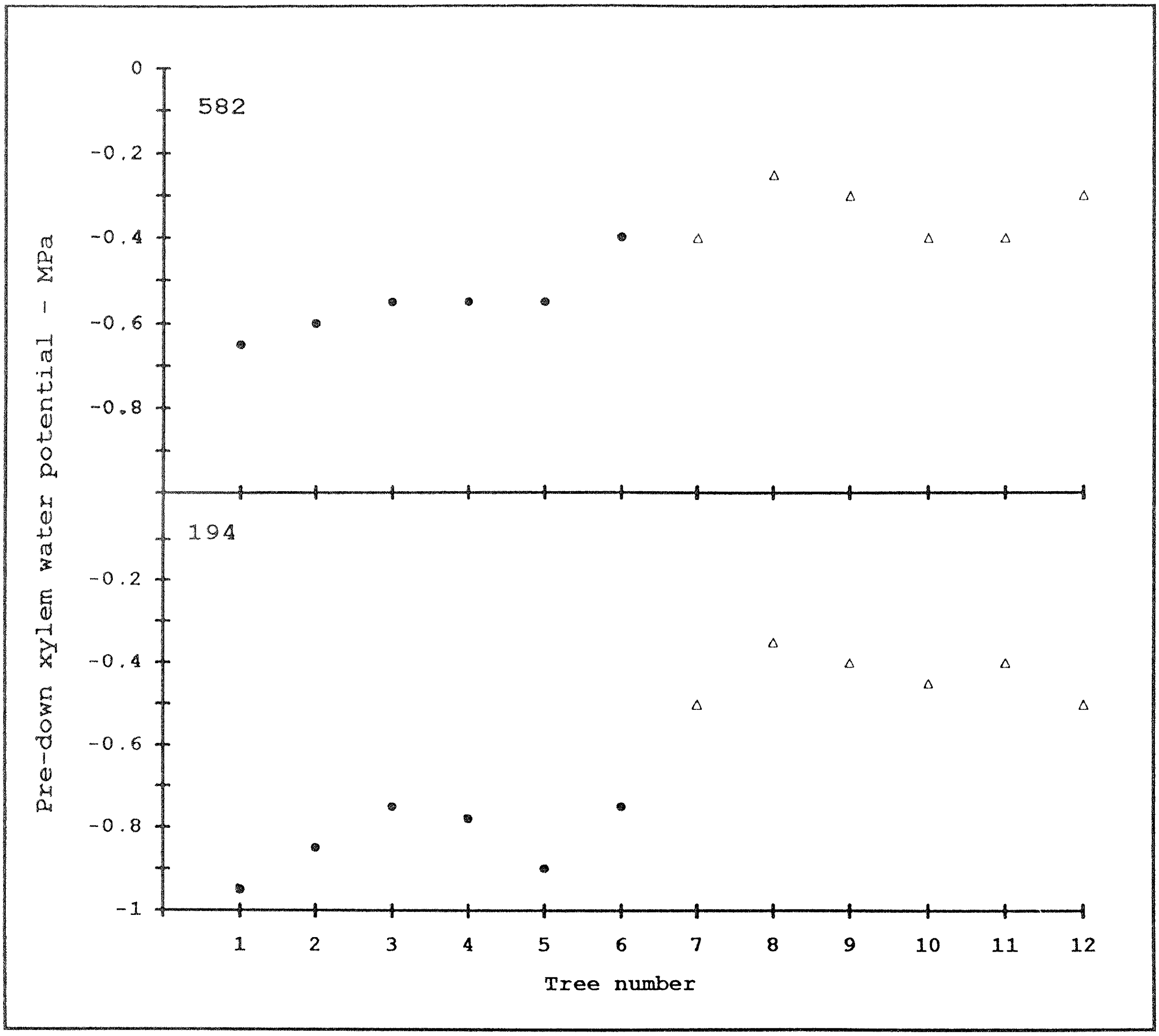

Figure 1.—Pre-dawn xylem water potential of Norway spruce trees on 23 June 1993. Trees 1-6 of both clones (194 and 582) are artificially drought stressed; trees 7-12 are controls.

An earlier experiment in Norway spruce addressed possible after-effects of drought on the resistance to *O. polonicum* infection (Christiansen 1992). In that case, three summers of severe artificial drought did not affect the resistance of the trees during a fourth season of normal precipitation. Thus neither that experiment nor the present one supports a general hypothesis that drought stress makes coniferous trees more susceptible to attack by bark beetles and their associated blue-stain fungi (cf. Christiansen *et al.* 1987).

The drought stress was relatively mild in the present study, though possibly adequate to have influenced important physiological processes in the trees (cf. Hsiao 1973), even including gas exchange (cf. Havranek and Benecke 1978). Such changes may be thought to reduce resistance, but vascular wilts are characteristically found to be more severe in wet than in dry soils (Schoeneweiss 1986).

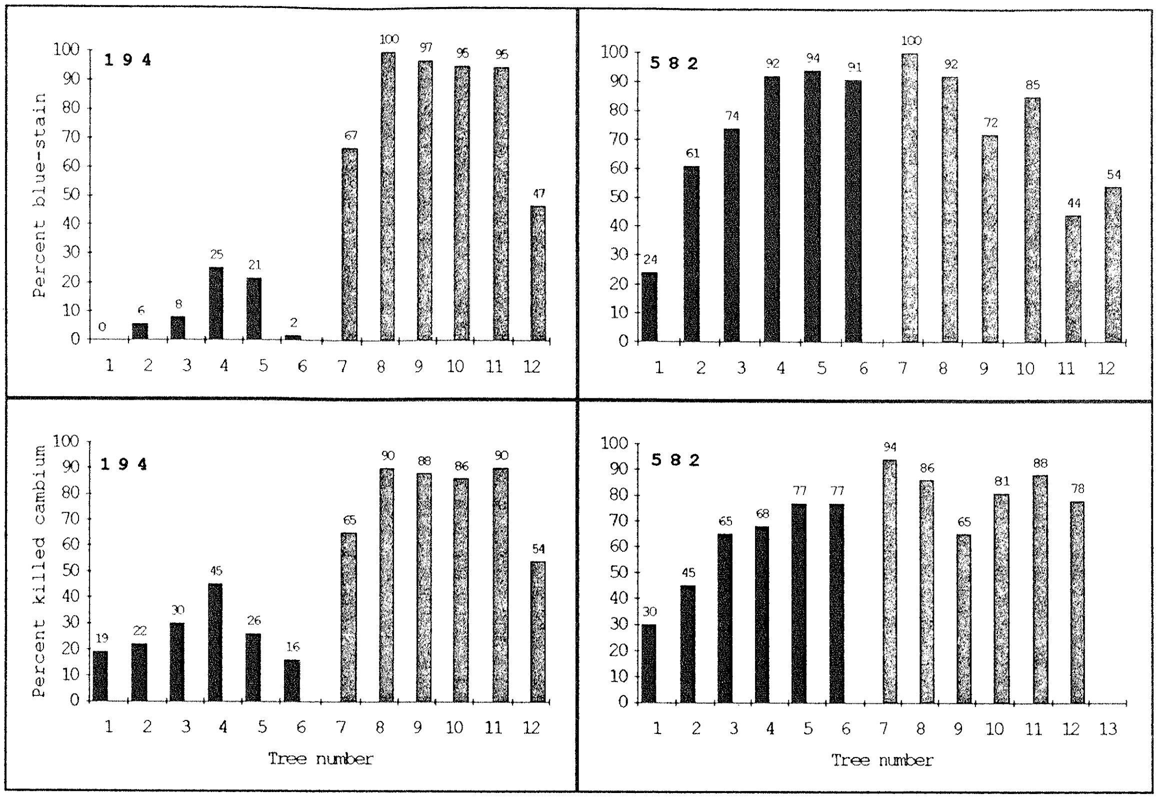

Figure 2.—Fungal proliferation in Norway spruce trees inoculated with *Ophiostoma polonicum*. Trees 1-6 of both clones (194 and 582) are artificially drought stressed; trees 7-12 are controls. Upper graphs: Percent blue staining of the sapwood cross-sectional area. Lower graphs: Percent cambial killing.

In a field experiment in young *Pinus taeda*, long-lasting water deprivation resulted in pre-dawn xylem water potentials of the same magnitude as in the present study. Although resin flow was significantly reduced in the stressed pines, they were also less attacked by *Dendroctonus frontalis* than the irrigated controls. The conclusion was that drought of this extent does not necessarily lower resistance (Dunn and Lorio 1993).

Like several other inoculation experiments in Norway spruce, the present one demonstrated that the invasive success of *O. polonicum* is negatively correlated with the quantities of resin that accumulate in "reaction zones" around the points of infection and ooze out from these sites (Christiansen and Horntvedt 1983, Christiansen 1985a, Christiansen and Ericsson 1986, Horntvedt 1988, Horntvedt and Solheim 1991). As in other conifers, this "secondary" resinosis is assumed to be due mainly to localized, induced defence reactions (Berryman 1972).

During the early stages of invasion in conifers, the bark beetles encounter a first line of defence, i.e., constitutive or primary resin oozing from severed ducts in the phloem. In our study there was no unequivocal difference between stressed and control trees in this character, although the first of two measurements gave higher resin yields in control trees of Clone 194 than in stressed ones. However, in the end, these controls turned out to be less resistant to infection than the stressed trees. In Norway spruce, the quantities of primary resin appear to be highly variable (Christiansen 1991). Despite its apparent importance for the defence of certain pines (Cates and Alexander 1982), this constitutive resin appears to be less important for others such as *Pinus sylvestris* (Schroeder 1990). It has been suggested that tree species that are repeatedly exposed to attacks by multiple generations of beetles per year may rely more on a constitutive defences than those which generally have only one critical period of attack (Matson and Hain 1983).

For some pine bark beetles (e.g., *D. frontalis* and *D. brevicomis*), studies suggest that drought has a negative effect on resistance, and that this effect is cumulative both on a shorter and a longer time scale (Craighead 1925, Miller and Keen

1960, Kalkstein 1976). Increased susceptibility may be due to a reduced yield of constitutive resins. However, this explanation may not hold when drought is only mild (cf. Dunn and Lorio 1993).

It seems likely that the degree of stress to which trees are exposed will influence their resistance. Assuming that a higher level of stress would have made the trees more susceptible, it is relevant to ask how often it occurs in the field under natural conditions. In this study the sheltered trees were deprived (depending on their root systems) of as much as half a year's precipitation (c. 400 mm) prior to inoculation, and a further 200 mm were lost thereafter. Such an abrupt loss of water does not seem likely to occur under field conditions in Norway. At the normal time of mass flight by *I. typographus* (i.e., in May), soil water reserves are not likely to be seriously depleted.

In the present case it can be hypothesized that the relativley mild drought may have triggered a mobilization of some defence mechanism in Clone 194; it may have induced the accumulation of defensive compounds or have elicited the development of defence-related cell types (V.R. Franceschi, pers. comm.). A more severe drought might have prevented the mobilization of such defences by critically reducing the availability of some important resources, but this remains to be proved.

SUMMARY

Young Norway spruce trees of two different clones were artificially deprived of precipitation water over a period of 8 months, starting in late October. In late June, pre-dawn water potentials of the experimental trees were significantly lower than those of unstressed control trees, but the stress was still fairly mild. At this time both categories of trees were inoculated with a standard dose of the blue-stain fungus *Ophiostoma polonicum*, a usual associate of the spruce bark beetle *Ips typographus*. The stressed trees of one clone proved to be significantly more resistant to infection than unstressed controls of the same clone. The other clone showed a similar but much less pronounced trend. It is suggested that the mild stress has triggered the mobilization of some defence mechanism in the former clone.

ACKNOWLEDGEMENTS

The study was supported by a grant from the Agricultural Research Council of Norway (Contract no. 205030). Thanks are due to Tore Skrøppa and colleagues for providing experimental trees; to Line Hamar, Heidi Knutsen, Olaug Olsen, and Torfinn Sæther for field assistance; to Olaug Olsen for fungal reisolation; to Olaug Olsen and Halvor Solheim for fungal identification; and to Halvor Solheim and Ye Hui for critical examination of the manuscript.

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APPROACHES TO STUDYING ENVIRONMENTAL EFFECTS ON RESISTANCE OF *PINUS TAEDA* L. TO *DENDROCTONUS FRONTALIS* ZIMMERMANN

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INTRODUCTION

Environmental conditions and the genetic potential of loblolly pines, *Pinus taeda* L., affect or determine tree resistance to attack by the southern pine beetle, *Dendroctonus frontalis* Zimmermann, by operating on physiological processes (Fig. 1). Environment and genetic potential must operate through physiological processes to determine the quantity and quality of growth, as well as to express resistance to invasion by pathogens and bark beetles (Kramer 1986). Only in this way can either environment or genetics affect growth and development at the cell or the whole tree level. Here, I would like to focus primarily on one major aspect of the environment that commonly affects the growth and development of loblolly pines and their relative resistance to attack by the southern pine beetle; that is, the water regimes under which they may grow. It is important to consider the effects of environment across a range of time frames, from very short (diurnal or even hourly) to very long (lifetime). For example, trees growing on wet sites and in humid environments may grow rapidly and reach large size over long time frames because of prolonged wet conditions; however, in short time frames of days or weeks they may be subjected to severe water deficits not evident when data are summed over long periods.

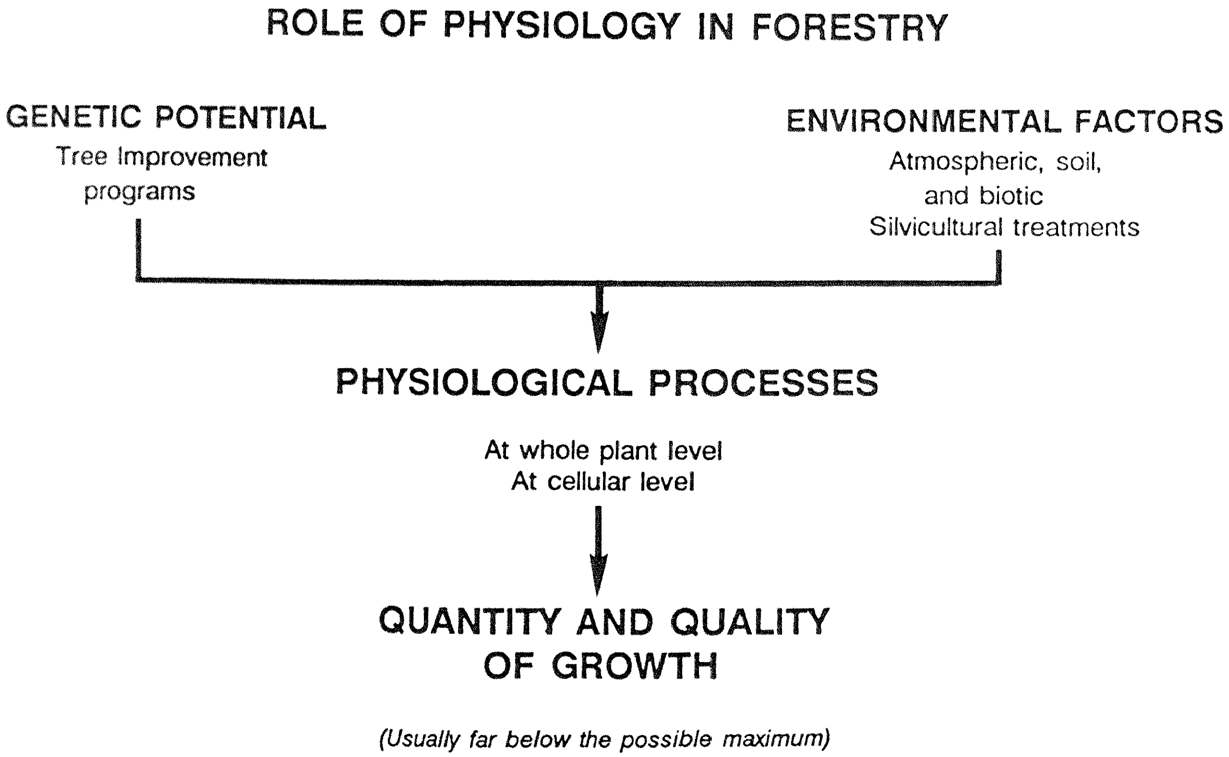

Figure 1.—Diagram illustrating the role of physiology in forestry. Genetic potential and environment operate through physiological processes in determining the quantity and quality of growth. Expression of resistance mechanisms of conifers to invasion by pathogens and bark beetles is likewise governed by these relationships (From Kramer, 1986).

Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

It is seldom feasible to control water regime in the practice of forest management as one can control the density and spacing of trees, or the sites on which trees are grown. They may be planted in various ways, on dry or wet sites, thinned to reduce competition among individuals for light, water, and nutrients, and fertilized or pruned if desired, but in the southeastern United States there is little one can do about the great variation in rainfall through growing seasons and from year to year. Varying water regime can be a major factor in tree growth and development, as well as in tree resistance to southern pine beetle attack.

One may approach the study of tree responses to water regimes in a variety of ways. Commonly, a manipulative approach is chosen to establish a range of conditions that may produce measurable responses considered important to the purpose. Although it is difficult, if not impossible, to modify one aspect of the environment without altering one or more other factors, manipulative studies can be very useful. However, it helps considerably to consider the possible effects of prevailing environmental conditions and the ontogenetic stage of trees on responses to treatment.

Observational studies can be very helpful, if not essential, to the development of the most useful manipulative studies. By conducting such studies, one can develop some understanding that can help in the design of better studies in which treatments are imposed. The two approaches can be complementary. For example, (Chou 1982) emphasized the importance of seasonal predisposition of host trees in planning inoculation trials for any purpose. He found that *Pinus radiata* susceptibility to *Diplodia pinea* varied with season of the year, being high in spring-summer but low in autumn-winter. Knowledge of structural and physiological changes associated with ontogeny can be invaluable to interpretation of results of fungal inoculation studies, as indicated in studies by (Paine 1984) and (Owen *et al.* 1987), as well as with pinewood nematode inoculations (Myers 1986). Similarly, physiological changes that occur during ontogeny of trees may alter their susceptibility to forest pests (Kozlowski 1969).

We have conducted a number of observational studies toward discovering tree and site characteristics correlated with the initiation and recurrence of southern pine beetle infestations (Lorio 1966, 1968; Lorio and Hodges 1968b, 1971; Hodges and Lorio 1971; Lorio *et al.* 1972; Lorio and Bennett 1974). These studies led to practical applications, such as the development of stand risk rating for the southern pine beetle (Lorio 1978, 1980; Lorio and Sommers 1981a, 1981b; Lorio *et al.* 1982; Zarnoch *et al.* 1984; Hedden and Lorio 1985). A compendium on the southern pine beetle was based to a large extent on observational studies (Thatcher *et al.* 1980).

Here, I would like to review some studies that we have conducted with loblolly pine and the southern pine beetle in attempts to determine the effect of water regime on tree resistance to beetle attack. They include both manipulative and observational studies. Contrary to our early assumption that stress in the form of water deficit is bad for trees and good for bark beetles, a more sound assumption is that "it depends." It depends on a number of factors, such as tree size, growth stage or age, the timing of the deficit during the growing season, how severe the deficit becomes, how long it lasts, site conditions, tree root distribution and condition, and perhaps many other factors. It is especially important to remember that any such factors affect trees by operating through physiological processes (Kramer 1986).

METHODS

Early on we conducted both manipulative and observational studies to assess the effects of environmental conditions, principally soil water supply, on loblolly pine physiology and susceptibility to southern pine beetle attack. One of our manipulations was to dig trenches around groups of trees or individuals, and line them with polyethylene sheeting to restrict lateral movement of water and prevent roots of study trees from tapping soil water beyond the trench (Lorio and Hodges 1968b, 1977; Hodges and Lorio 1969). We constructed various types of shelters that limited soil water recharge from rainfall and allowed us access to the trees for measurements. Other methods included continuously flooding tree root systems for long periods of time by maintaining ponded water on the site, and applying collars of solid CO_2 around the lower tree bole to inhibit the movement of water through sapwood to induce accute water deficit (Moeck *et al.* 1981, Stephen *et al.* 1988). In more recent times we have girdled trees (Dunn and Lorio 1992), sheltered root systems without trenching, and irrigated in attempts to provide well-watered conditions to compare with the effects of sheltering (Dunn and Lorio 1993). This and other studies included artificially inducing southern pine beetle attacks (Lorio and Hodges 1977; Dunn and Lorio 1992, 1993), but in some studies, volunteer attack was relied upon (Lorio and Hodges 1968b, Hodges and Lorio 1969).

Observational studies included examining site and stand conditions associated with southern pine beetle infested stands compared with noninfested stands (Lorio 1968), measuring soil water regimes, depth to free water in perforated tubing, and tree diameter growth (Lorio and Hodges 1971); determining tree rooting characteristics and distribution, and oleoresin exudation pressures in relation to microrelief (Lorio and Hodges 1968a, Lorio *et al.* 1972); and comparing methods of measuring field techniques for assessing the water status of trees (Hodges and Lorio 1971). One study, conducted over several years, included calculation of soil water balance (Zahner and Stage 1966), measurement of cambial growth by periodically extracting xylem plugs from tree boles and counting the number of tracheids produced in the current annual ring, counting vertical resin ducts in the current annual ring and calculating their density, and periodically sampling resin flow from wounds through the years (Lorio *et al.* 1990).

RESULTS

Manipulative studies to establish levels of water deficit that would severely weaken trees relative to nontreated controls (Lorio and Hodges 1968b, 1977; Hodges and Lorio 1969, 1975) demonstrated that severe water deficits, aside from reducing growth, caused physical and chemical changes apparently associated with reduced tree resistance to beetle attack. However, it was not demonstrated that the southern pine beetle preferentially attacked trees subjected to severe water deficit, or that they required such weakened hosts for successful attack. On the contrary, it was clear that vigorously growing hosts could be overcome readily by this bark beetle species, and that such hosts could produce abundant brood.

An observational study of relationships among xylem water potential of twigs (Scholander *et al.* 1965), relative water content of needles (Weatherley 1950), oleoresin exudation pressure in the lower main bole (Vité 1961), and short-term flow of resin (Mason 1969, 1971) showed good relationships between resin pressure and the conventional measures of water status, but no relationship between resin pressure and flow (Hodges and Lorio 1971). The question of a direct relationship, or lack thereof, between oleoresin exudation pressure and oleoresin flow from bark wounds is discussed elsewhere (Lorio *in press).*

Recent attempts to evaluate the effects of environmental conditions on loblolly pine resistance to the southern pine beetle provide some insight about the importance of environment to tree resistance. Results from over a 2-year period (1984-1985) of an observational study (Lorio *et al.* 1990) indicated substantial differences between years in calculated soil water storage, cumulative soil water deficits, cambial growth, density of resin ducts in the current year's annual ring, and the patterns of resin yields through the growing seasons. The results for 1986 and 1987 are reported here (Fig. 2). Clearly different water regimes prevailed from one year to the next, as did the apparent effects on cambial growth, resin duct densities, and resin yields. No treatments were applied and beetle attacks were not induced, and the apparent responses are correlative. However, the pattern of resin yields in 1987 closely mimics a conceptual model of seasonal changes in pine resistance to beetle attack in years having soil water balance patterns similar to the long-term average (Fig. 3). Resin flow from small bark wounds is considered indicative of the relative resistance of southern pines to southern pine beetle attack (Hodges *et al.* 1979).

A manipulative study carried out within the same stand in the spring of 1986 and the late summer of 1987 indicated a strong environmental effect preceding and during the study (Stephen *et al.* 1988). Another manuscript that focuses on important differences in environmental conditions, growth and development of the host trees, and southern pine beetle population levels is pending.

Girdling of bark to the face of the xylem prior to the development of latewood, and under well-watered conditions, produced considerably different responses to fungal inoculations or induced southern pine beetle attacks at 2 weeks compared to 8 weeks after girdling (Dunn and Lorio 1992). Two weeks after girdling, carbohydrate status, cambial growth, resin flow, phloem moisture content, lesion formation, and beetle colonization differed little above or below girdles. After 8 weeks the carbohydrate status below girdles was greatly reduced, as was cambial growth (no latewood formed). Although not statistically significant, resin flow was reduced almost 50% below compared to above girdles. Phloem moisture was significantly higher below than above girdles, and beetles oviposited three times as many eggs and constructed three times as much egg gallery below than above girdles. These results were obtained during a period of almost continually declining soil moisture and steadily accumulating calculated soil water deficits (Fig. 4).

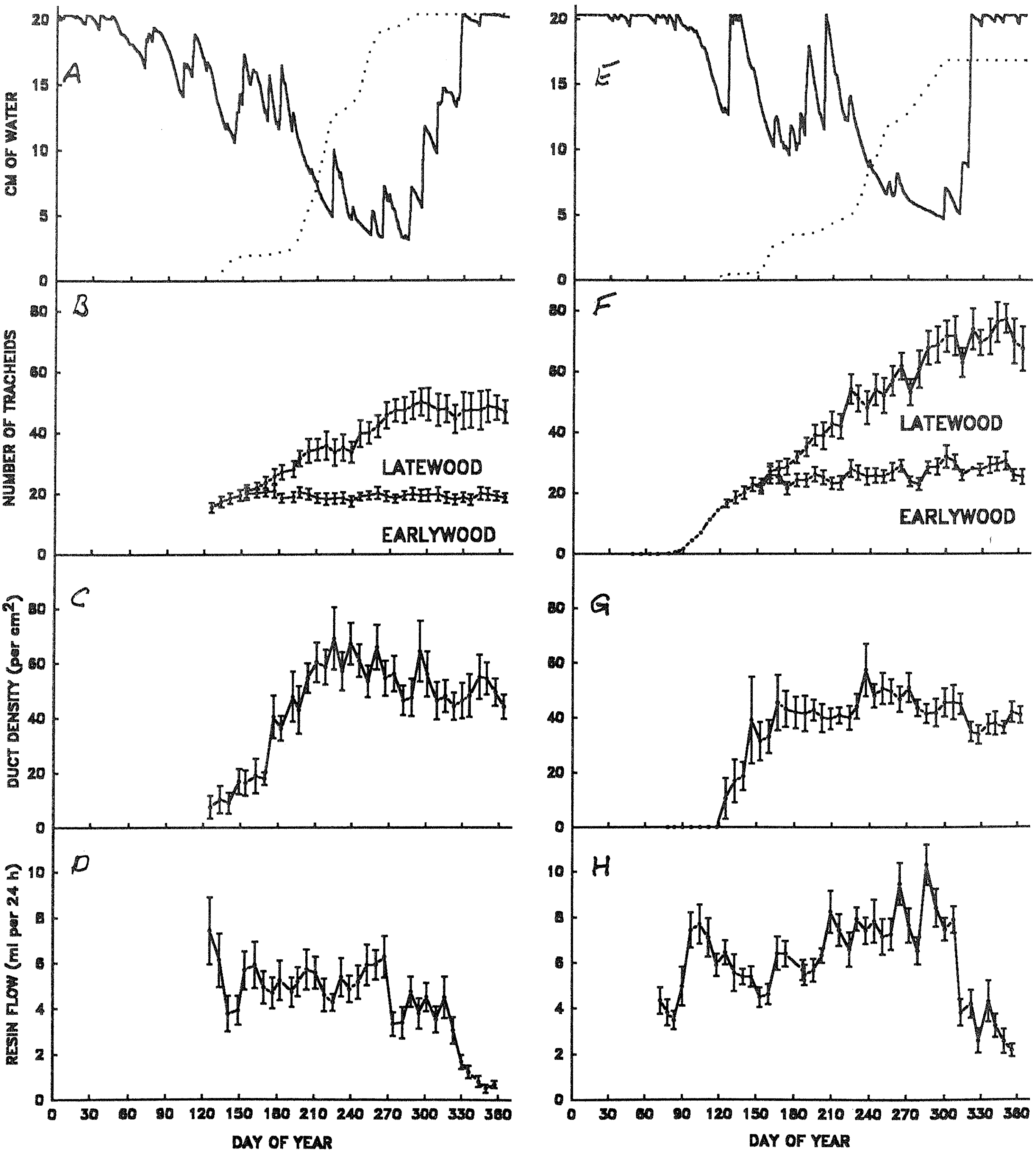

Figure 2.—Graphs of water regimes, the course of xylem growth and development, vertical resin duct density in the current annual ring, and resin yield from standard bark wounds in 1986 (A, B, C, D) and 1987 (E, F, G, H). Daily soil water storage (solid line) and cumulative daily water deficits (dotted line), (A, E). Tree bole cambial growth and development expressed as the number of tracheids formed, time of transition from earlywood to latewood, and the total amount of earlywood and latewood formed (B, F). Vertical resin duct densities in the current annual ring (C, G). Oleoresin yield over 24-hour periods (D, H). Vertical bars are standard errors, n=11 for 1986, n=13 for 1987. Water regimes, growth and development, and resin yields differ dramatically between years. Resin yields for 1987 closely approximate the seasonal changes in resistance suggested in the conceptual model shown in Figure 3 for resistance to southern pine beetle attack in years that have soil water regimes similar to that of the long-term average. Severely dry conditions in the summer of 1986 apparently not only limited carbon partitioning not only to growth, but also to secondary metabolism; e.g., oleoresin synthesis.

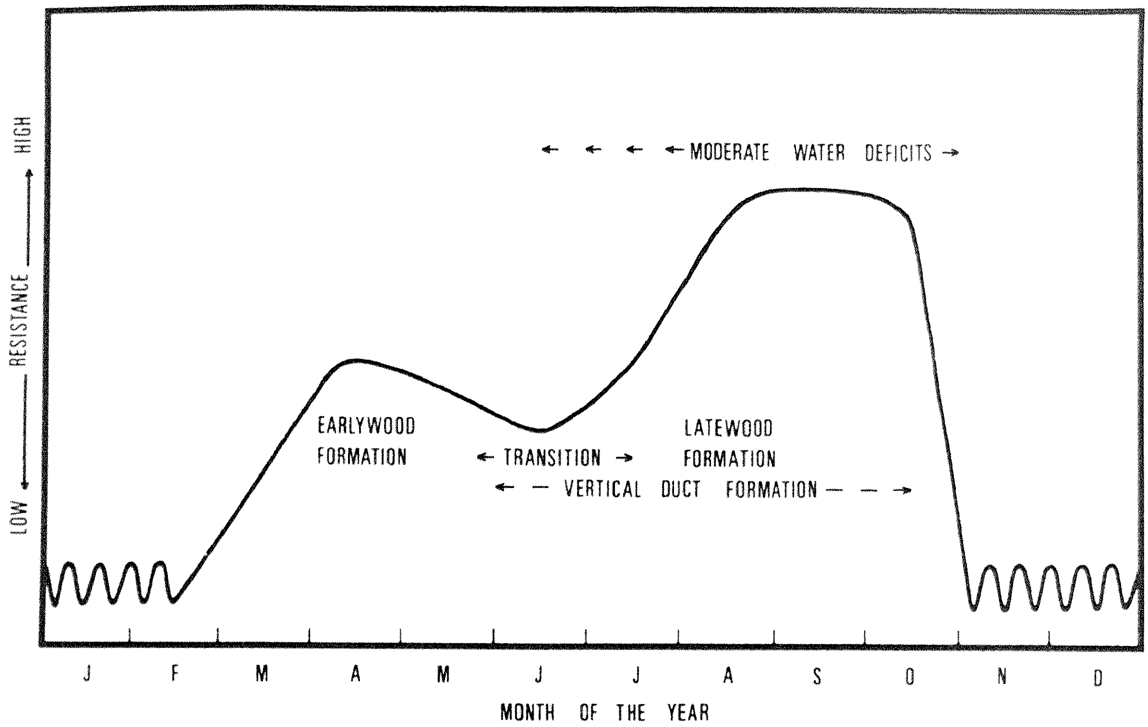

Figure 3.—A conceptual model of seasonal changes in pine resistance to southern pine beetle attack for years that have soil water balance patterns similar to that of the long-term average. Resistance to the earliest attacking beetles is considered to be highly dependent on the potential flow of oleoresin at the wound site. (From Lorio *et al.* 1990).

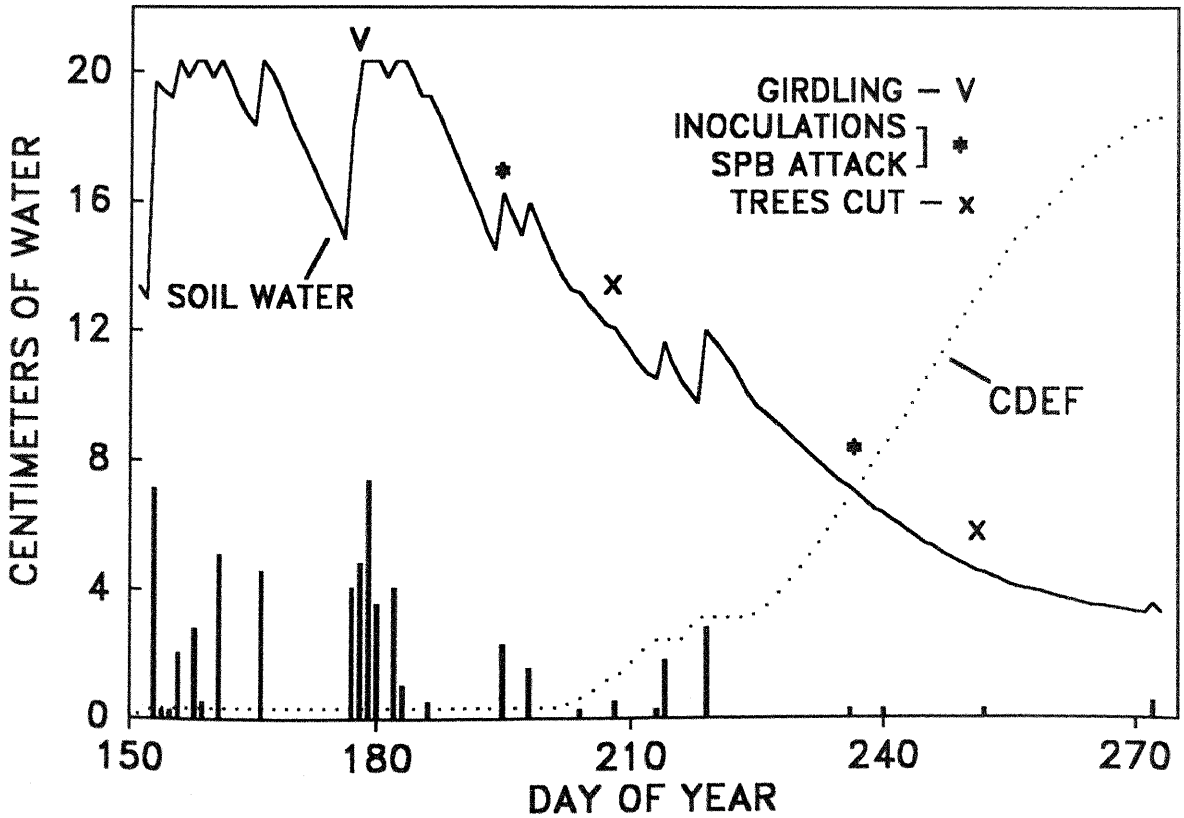

Figure 4.—Daily precipitation (vertical bars), calculated daily soil water storage and cumulative daily water deficits (CDEF) for June-September, 1989, near Alexandria, LA. The two-level soil water storage program of Zahner and Stage (1966) was used, assuming as sandy clay loam soil holding 20.32 cm of available water in the tree rooting zone. Dates of girdling, inoculations, and induced southern pine beetle attack are indicated. (Modified from Dunn and Lorio 1992).

Only recently have we conducted studies with juvenile pines (trees not yet into reproductive growth). One such study involved sheltering root systems to limit water supply and irrigating to supplement rainfall (Dunn and Lorio 1993). Environmental conditions were such that no treatment effects could be established early in the growing season because of high rainfall through the month of May. Subsequently, with very high evaporative demand and low rainfall from June through early August, it was not possible to irrigate sufficiently to recharge soils to field capacity (soil water content after excess water has drained away). Two tests were conducted in which southern pine beetle attack was induced in early August in one case and in late October in another. Compared with irrigated trees, sheltered trees exhibited lower xylem water potentials, and reduced cambial growth, photosynthesis, and resin flow. However, sheltered trees demonstrated a capacity for internal adjustments that maintained twig water potentials at relatively high levels (> - 0.8 MPa at predawn) even when photosynthesis was reduced 30-60% compared to irrigated trees. In the early test, about half as many beetles attacked sheltered trees as irrigated trees, but beetle success was low in general, with only 4.3 eggs per attacking pair of beetles for sheltered trees and 2.9 for irrigated. Over 70% of egg gallery length excavated over 13 days from initial attack was resin filled. In the late test, attacks were allowed to progress for 25 days, and egg galleries of both groups of trees were much less affected by resin soaking, but eggs per attacking beetle pair was still low for both sheltered and irrigated trees (7.9 versus 3.8). Dramatic increases occurred in resin flow during beetle attack in both tests. Although resin flow often decreases rapidly following mass attack by the southern pine beetle (Hodges *et al.* 1979), attack densities in this study were relatively low and may not have been sufficient to rapidly deplete the preformed resin supply and resin synthesized in response to invading beetles or associated microorganisms.

DISCUSSION

In our research we have taken both manipulative and observational approaches to study relationships between characteristics of the environment and loblolly pine resistance to the southern pine beetle. Although this bark beetle's behavior differs considerably from other important species within its range, such as *Ips* species and the black turpentine beetle, *Dendroctonus terebrans* (Olivier), general relationships should apply to those species as well, if modified appropriately. Typically, the *Ips* species cannot withstand resin flow as well as the southern pine beetle, but they can withstand higher temperatures and drier inner bark conditions. The black turpentine beetle apparently can withstand abundant resin flow, even when attacking in very low numbers. It appears that death of the tree is an unavoidable result only with the southern pine beetle.

Earlier studies lacked guidance of a broad conceptual framework within which to frame hypotheses and test them. Basically, we considered that stress of various kinds was *bad* for trees and *good* for bark beetles, but we had little understanding of how factors such as water deficit affected trees and reduced their resistance to beetle attack. Observational studies indicated that stand characteristics such as high density and slow growth were frequently associated with infestation incidence. However, it was not clear what mechanism of resistance was affected by the conditions. It was generally believed that the oleoresin system was an important aspect of tree resistance, and for some time it was thought that oleoresin exudation pressure could be a useful surrogate measure of resin flow and tree resistance. In time, it became evident that oleoresin exudation pressure was a reasonably good indicator of tree water status, but that it did not necessarily correlate closely with resin flow or tree resistance to beetle attack.

Work was started in 1984 to conduct research that would lead to better understanding of loblolly pine resistance to southern pine beetle attack. The concept of plant growth-differentiation balance (Loomis 1932, 1953) provided a framework for hypothesis formation and gave us a rationale for interpreting our results (Lorio 1986, Lorio and Hodges 1985). Application of Loomis's concept provided us with a sound foundation for interpreting results of previous studies, both manipulative and observational. In a sense, the concept provided a physiological basis for our research that meshed well with Kramer's (1986) thesis of how both genetics and environment influence tree growth and development by operating through physiological processes (Fig. 1).

Gordon and Smith (1987) provide a useful diagram of carbon partitioning among various meristems and aboveground and belowground tissues as it is affected by greater or lesser nutrient and water supplies (Fig. 5). It effectively incorporates concepts of both Kramer (1986) and Loomis (1932). Supplies of carbon and nitrogen assimilates are normally limited in trees, and various sinks compete for the limited supplies. If one process removes a substrate from circulation, another cannot use it. This competition results in corrdinated developmental changes (Trewavas 1985). Loomis *et al.* (1990) refer to this as a "nutritional theory" of coordination, which can be traced back at least to the work of Klebs (1903, 1910).

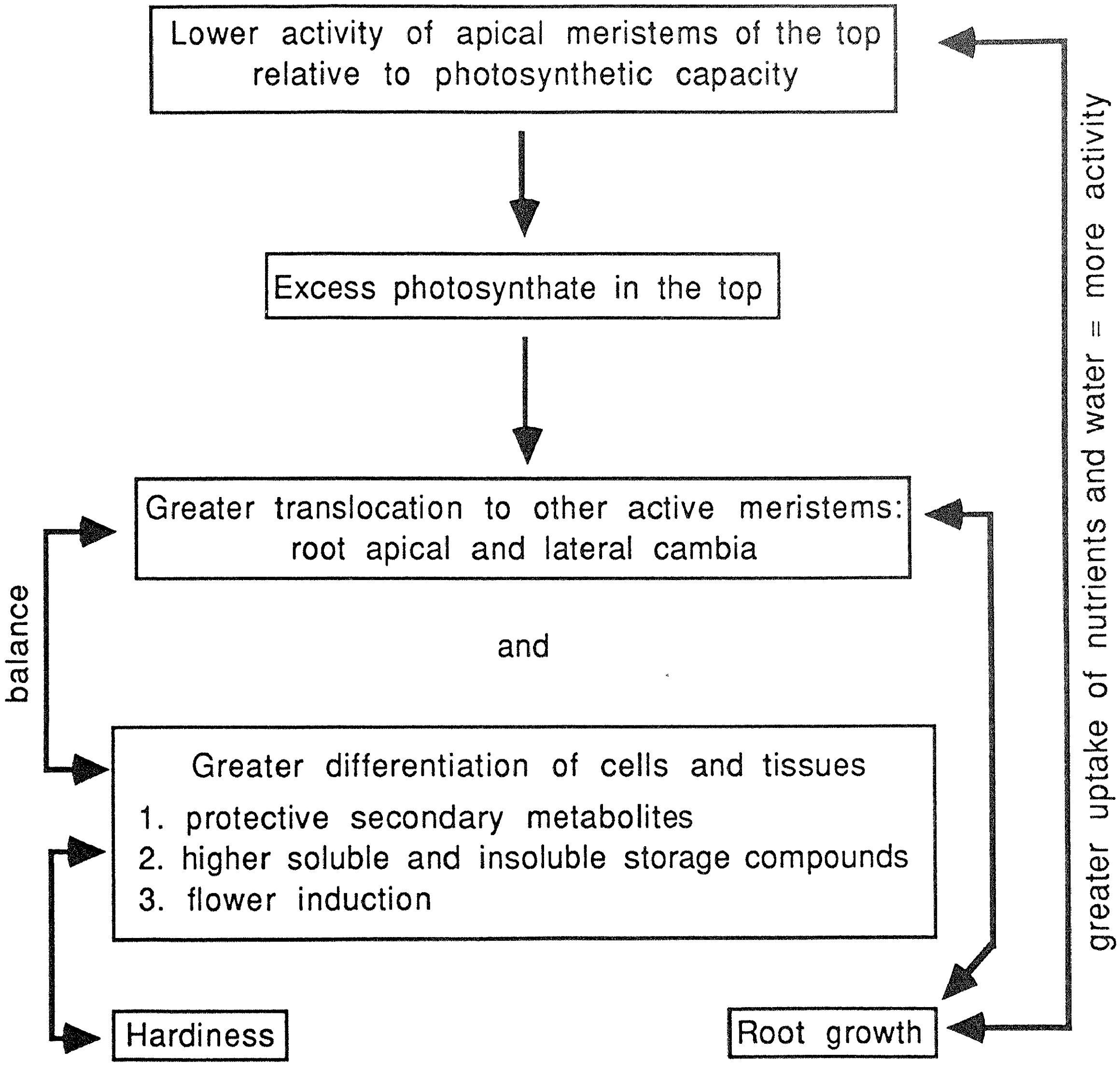

Figure 5.—Carbon partitioning among growth and developmental processes in response to changing nutrient and water supplies influence the competition and fluctuating balances between growth and differentiation processes, e.g., the various cambial meristems versus protective secondary metabolites; as well as among aboveground and belowground systems within a tree. (After Gordon and Smith 1987).

Voluminous literature in the area of physiological ecology and plant/herbivore interactions in general appears to be related closely to the growth-differentiation balance concept (Mooney and Chu 1974, Mooney *et al.* 1983, Bryant *et al.* 1983, Coley *et al.* 1985, Chapin *et al.* 1987, and others). Although the paths of research in those areas did not include consideration of Loomis's (1932) concept, I find that it provides considerable support for developments in those fields. For example, it seems to me to be essentially in agreement with the carbon-nutrient balance hypothesis (Bryant *et al.* 1983, Tuomi *et al.* 1988), but provides a broader framework, primarily by providing explicit consideration for water. Several papers include consideration of the plant growth-differentiation balance concept in forming theories of evolutionary development of plant defenses against insects (Tuomi *et al.* 1990, Herms and Mattson 1992, Tuomi 1992).

Here, I have indicated some of the research results from both early and recent studies. The more recent studies were carried out with consideration of plant growth-differentiation balance relationships in mind, and with considerable stimulation from advances in physiological ecology and studies of plant/herbivore interactions in general. We are continuing research in the same vein. The study with juvenile pines (Dunn and Lorio 1993) has been extended to an older stand, with some improvements in techniques, and with results indicating strongly that water deficits have nonlinear effects on tree resistance to beetle attack. Another study is in progress, once again with juvenile pines, in a plantation subjected to thinning and fertilization. We will be continuing work in that direction. At this time preliminary results indicate that the short-term effects of thinning and fertilization are to enhance carbon partitioning to growth and to reduce carbon committed to secondary metabolism; e.g., resin synthesis.

SUMMARY

There are a number of ways to approach the problem of assessing the effects of environmental conditions, such as water regime, on tree physiological responses and resistance to bark beetle attack. It helps to keep in mind that environmental factors operate through physiological processes (Fig. 1, and Kramer 1986), and that there are concepts, such as plant growth-differentiation balance (Loomis 1932) and carbon-nutrient balance (Bryant *et al.* 1983; Tuomi 1992; Tuomi *et al.* 1988, 1990), that can provide bases for forming testable hypotheses. It is especially important to know as much as possible about the host tree and the specific bark beetle of interest. Observational studies are particularly important because they can provide a baseline for interpreting the results of manipulative studies. Whenever feasible, it is especially helpful in manipulative studies to characterize environmental conditions before and during a study. Because physiological changes that occur during ontogeny of trees can alter their susceptibility to herbivores, knowledge of tree stage of growth and development can be especially helpful.

ACKNOWLEDGMENTS

Cooperative Agreements (19-86-036 and 19-87-20) between the Mississippi Agricultural and Forestry Experiment Station and the USDA Forest Service, Southern Forest Experiment Station, contributed in part to results reported here.

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EFFECTS OF ROOT INHABITING INSECT-FUNGAL COMPLEXES ON ASPECTS OF TREE RESISTANCE TO BARK BEETLES

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INTRODUCTION

Forest declines have proven difficult to address, because they involve complex interactions in which the key processes are only poorly understood (Houston 1981, Witcosky and Hansen 1985, Witcosky *et al.* 1986, Klein and Perkins 1988, Mueller-Dombois 1988, Huettl *et al.* 1990, Manion 1991, Saxe 1993, Woodcock *et al.* 1993). Without a basic understanding of the underlying mechanisms, it is difficult to relate field observations to various management practices, anthropogenic or environmental stresses, or natural successional events. One of our major information gaps concerns the interactions among different insect and fungal feeding guilds that both respond and contribute to plant stress physiology. We have been addressing this question in declining red pine, *Pinus resinosa*, stands in the Great Lakes region of North America. Our objective is to link field correlations with underlying physiological mechanisms.

Insects and Pathogens Associated With Declining Red Pine Stands

Red Pine Decline Disease is characterized by a progressive deterioration and death of mature plantation trees. Affected portions of stands are highly localized, and mortality progresses radially from an epicenter (Klepzig *et al.* 1991). No living trees remain in this epicenter, and the resulting gap is colonized by herbaceous and early succession vegetation. Trees killed during the current growing season ring this zone. Neighboring trees show reduced radial and crown growth, whereas trees farther into the stand show no symptoms. Each year, these zones expand. The killed trees become windblown, the low vigor trees are killed, and additional trees show signs of stress. This syndrome can be characterized at the between-stand, within-stand, and within-tree levels. In a 3-year evaluation of 19 sites at 13 locations in Wisconsin, Klepzig *et al.* (1991) found that the most consistent between-stand correlate with Red Pine Decline was the abundance of root- and lower-stem infesting beetles (Table 1). These included three curculionid and two scolytid species. Several other scolytids and curculionids were also present in high numbers, but their densities did not consistently differ between declining and asymptomatic plantations of the same age category. Likewise, there were no strong differences associated with abiotic soil factors.

The beetles associated with Red Pine Decline differ in their within-stand spatial distributions relative to the margin of tree mortality. For example, four beetles occur at higher densities in declining than asymptomatic stands. However, two of these show no significant within-stand distribution patterns, one occurs more commonly near the zone of stressed trees, and one occurs at higher densities in regions of the stand not yet showing visible symptoms (Table 1). Interestingly, the red turpentine beetle, *D. valens* Le Conte, occurs at higher densities in the asymptomatic than symptomatic portions of declining stands, yet does not show statistically significant between-stand differences.

The subcortical belowground beetle guild inhabiting Wisconsin pines shows a high degree of niche partitioning. This separation among closely related beetles is based on host tissue tree vigor, olfactory behavior, and symbiotic relationships (Raffa and Hunt 1989, Rieske and Raffa 1990, 1991, Raffa and Klepzig 1992, Hunt *et al.* 1993). For example, *H. porculus*

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Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

Table 1.—Root- and lower-stem infesting beetles associated with Red Pine Decline Disease (From Klepzig *et al.* 1991).

A. Species occurring at higher densities in declining than asymptomatic stands
    - *Hylobius radicis* (Curculionidae)
    - *Hylobius pales* (Curculionidae)
    - *Pachylobius picivorus* (Curculionidae)
    - *Hylastes porculus* (Scolytidae)

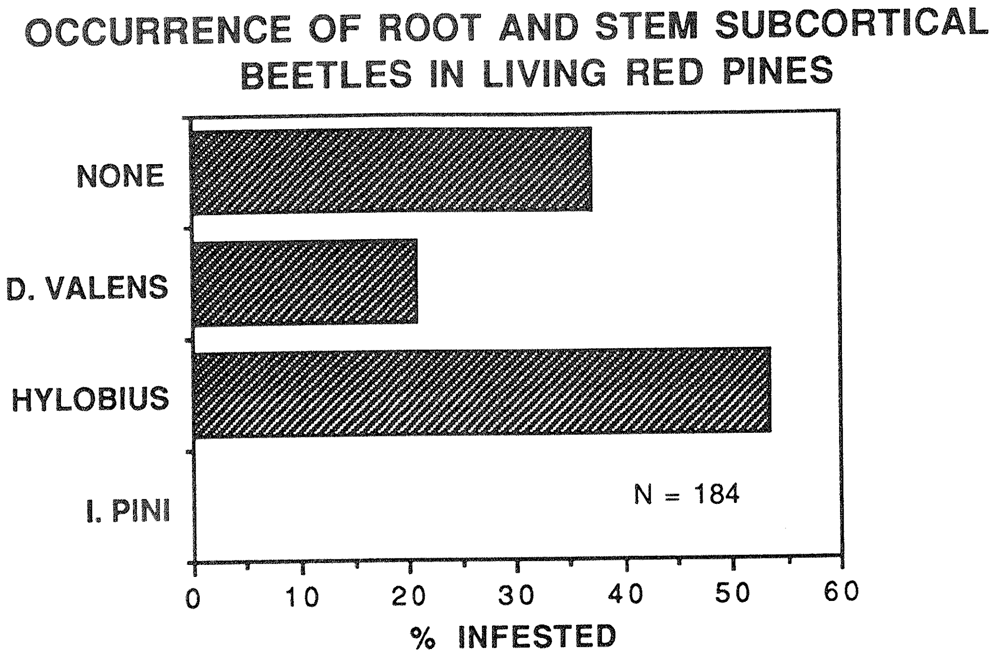
B. Species occurring at higher densities in asymptomatic portions of declining stands
    - *Dendroctonus valens* (Curculionidae)
    - *Hylastes porculus* (Scolytidae)

C. Species occurring at higher densities in symptomatic portions of declining stands
    - *Pachylobius picivorus* (Curculionidae)

and *H. rhyzophagus* colonize lateral roots; *H. pales, H. radicis,* and *P. picivorus* colonize the root collar region; and *D. valens* enter trees just above the soil line. Moreover, beetle behavioral responses to volatiles associated with stress (Kimmerer and Kozlowski 1982) reflect differences in preferred host physiology. For example, *H. pales* and *P. picivorus* differ in their relative responses to various ratios of terpene-ethanol mixtures (Rieske and Raffa 1991). Each of the beetles in Table 1 is closely associated with Ophiotomatales fungi. The scolytids are primarily associated with *Leptographium terebrantis* Barras and Perry, and the curculionids are primarily associated with *Leptographium procerum* (Kendrick) Wingfield (Klepzig *et al.* 1991). These fungi are moderately pathogenic to *P. resinosa* but cannot kill healthy trees (Raffa and Smalley 1988a, Klepzig *et al.* 1994a). The rates of association between these beetles and fungi average around 70%, and the vector efficiencies are around 50% (Klepzig *et al.* 1994b).

Colonization by the beetles shown in Table 1 and their associated fungi does not kill host trees. A survey of living red pines revealed that a high percentage of the trees peripheral to the margin of tree mortality were infested with *D. valens, H. radicis,* or both (*H. porculus* was not sampled). Such trees can remain alive for several years. In most cases, however, these trees are subsequently killed by the stem colonizing bark beetle *Ips pini* (Say), and its associated fungus *Ophiostoma ips* (Rumb.) Nannf (Fig. 1). All of the dead, but none of the living, trees were infested with *I. pini*. Thus, the *I. pini - O. ips* complex appears to be the ultimate cause of tree death, but root infection appears to be a predisposing factor. *I. pini* appears to be highly opportunistic. Despite its strong association with individual tree death, between-stand population densities were not associated with stand condition.

The above view is supported by our observations of a declining stand over a 5-year period (Fig. 2). The percentage of trees killed by *I. pini* that showed prior colonization by *H. radicis* averaged 86.8 ± 9.7(SD%). The percentage of trees killed by *I. pini* that showed prior colonization by *D. valens* averaged 19.4 ± 12.4 (SD%). Most of the trees infested by one of these root colonizing species were also infested by the other. Interestingly, the lowest proportion of trees killed by *I. pini* that had previously been infested by root insects occurred during 1988, a year of severe drought (*H. radicis*: 72.7%; *D. valens*: 8.2%). Belowground excavations of five declining and control stands revealed a similar, but even more striking, trend (Klepzig *et al.* 1991). In declining stands, fungal staining and root deterioration proceeded in advance of the insect vectors and any aboveground symptoms. Significant root infection occurs 10-15m beyond the margin of killed trees. In control stands, and in the portions of symptomatic stands far beyond the stressed trees, root infection levels are negligible. *Leptographium* fungi progress through root grafts, which are extensive throughout mature *P. resinosa* plantations. The extent of grafting is so common in these plantations that the phloem tissue of stumps can remain viable for several years (Klepzig *et al.* 1994a). The exact mechanism by which *Leptographium* fungi progress through root grafts into healthy tissue remains unknown, however.



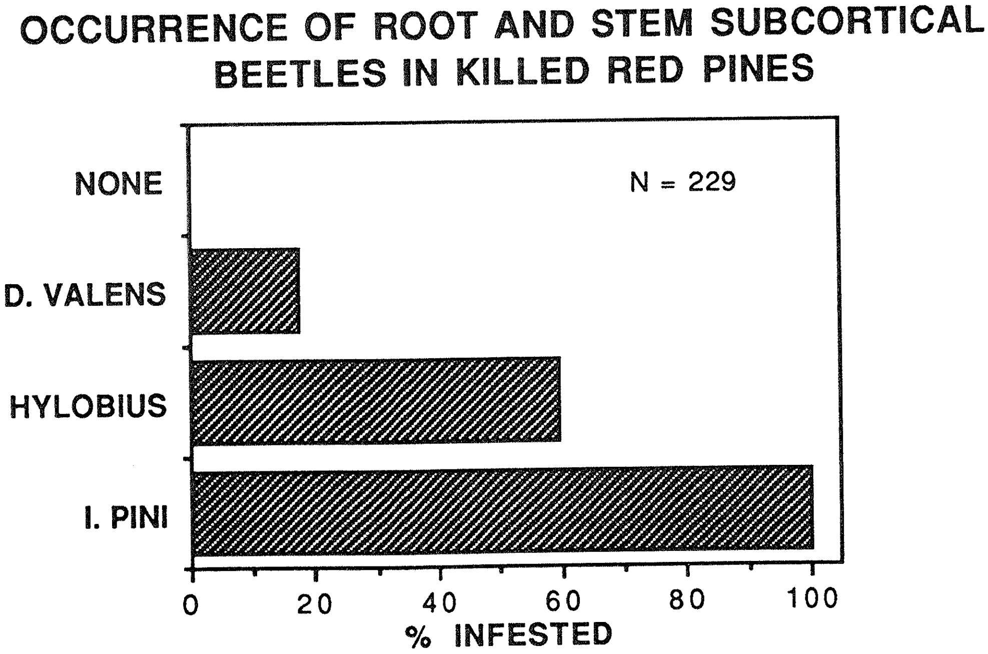


Figure 1.—Association of root- and stem-feeding subcortical beetles with living (above) and dead (below) red pines in declining plantations.

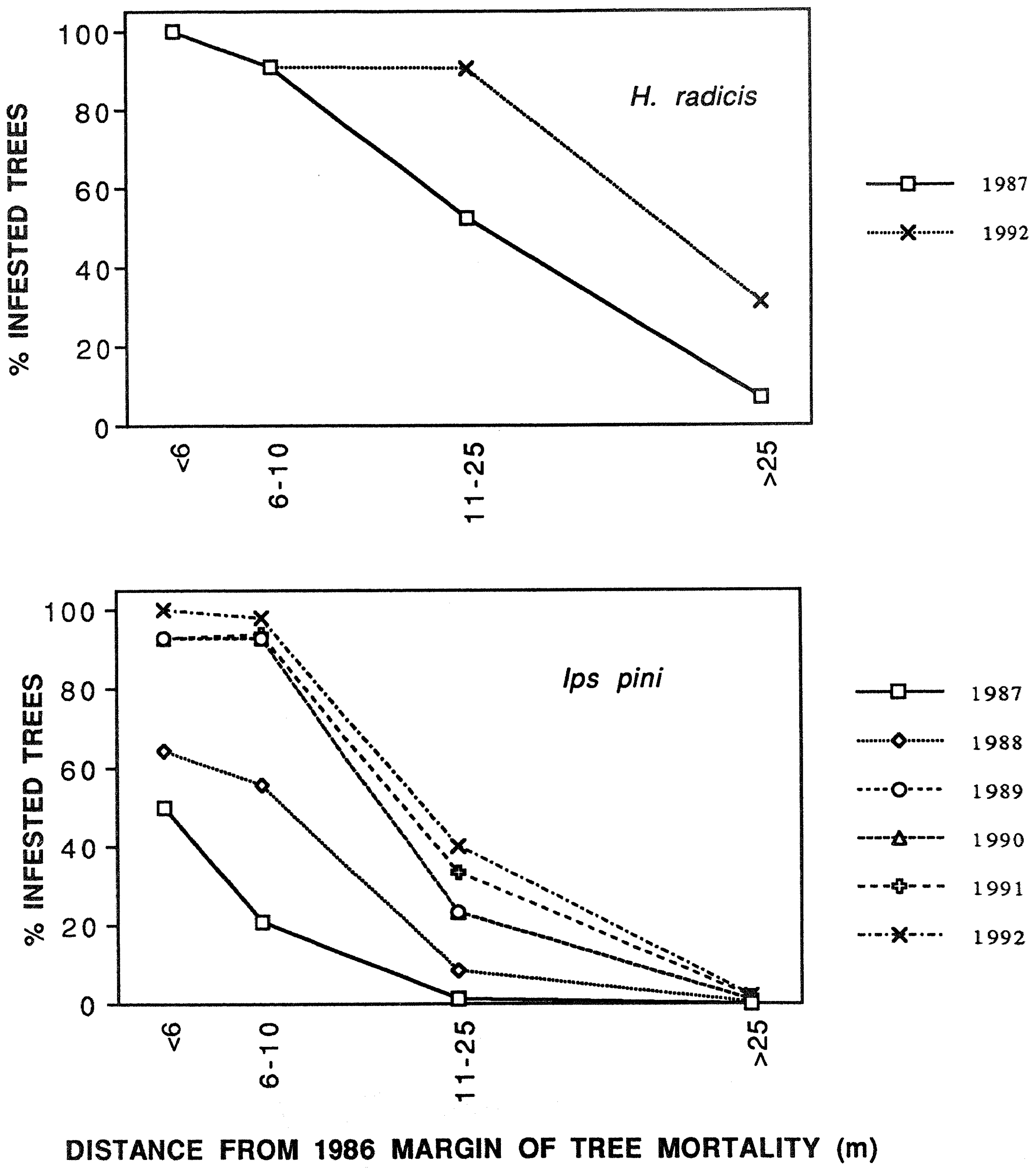


Figure 2.—Progression of infestation in a declining red pine stand. Infestation rates of the pine root collar weevil, ***Hylobius radicis*** (above) and the pine engraver, *Ipis pini* (below) relative to the originally recorded margin of killed trees are shown.

Predisposition of Root-infected Trees to Stem Colonizing Insect-fungal Complexes

Based on these observations, we hypothesized that prior infection by root- and lower-stem colonizing insects and fungi reduces *P. resinosa* resistance against *I. pini* - *O. ips* complexes. Testing this hypothesis requires both an understanding of red pine defense mechanisms against subcortical invasion, and comparisons of the key parameters between root-infected and non-root-infected trees.

The response of *P. resinosa* to invasion by biotic agents involves the secretion of preformed allelochemicals from severed resin ducts, and the rapid formation of a necrotic lesion around, and accumulation of additional allelochemicals at, the entrance site (Raffa and Smalley 1988b, 1994). These allelochemicals are comprised primarily of monoterpenes and phenolics. This is a rather generalized response that can be elicited by multiple agents and is characterized more by quantitative than qualitative allelochemical changes. It resembles those reported for other conifer-scolytid-*Ophiostoma* systems (e.g., Berryman 1972; Hodges *et al.* 1979; Cates and Alexander 1982; Raffa and Berryman 1982a,b; Christensen and Horntvedt 1983; Lorio 1986, 1993; Paine and Stephen 1987a,b; Lieutier and Berryman 1988; Nebeker *et al.* 1993; Raffa *et al.* 1993). Both constitutive and reaction allelochemicals are important to tree defense. However, allelochemical concentrations are higher in reaction tissue, and they have higher inhibitory effects on fungal germination and growth and on insect feeding and survival (Klepzig *et al.* 1994d). For example, *I. pini* adults strongly prefer media amended with extracts or simulated monoterpene doses from constitutive over reaction phloem tissue. Likewise, the monoterpene levels present in constitutive stem phloem tissue can kill 60% of adult *I. pini* within 2 days, but monoterpene concentrations present only 3 days after initiation of an induced response are sufficient to kill 90% of the beetles (Fig. 3) (Raffa and Smalley 1994). Without effective induced responses, the surviving beetles would probably attract enough additional beetles to deplete host resins and overwhelm tree resistance; without high constitutive levels, trees would be unable to delay aggregation long enough for induced reactions to be mobilized (Raffa and Berryman 1982a, 1983a,b, 1987; Raffa *et al.* 1993).

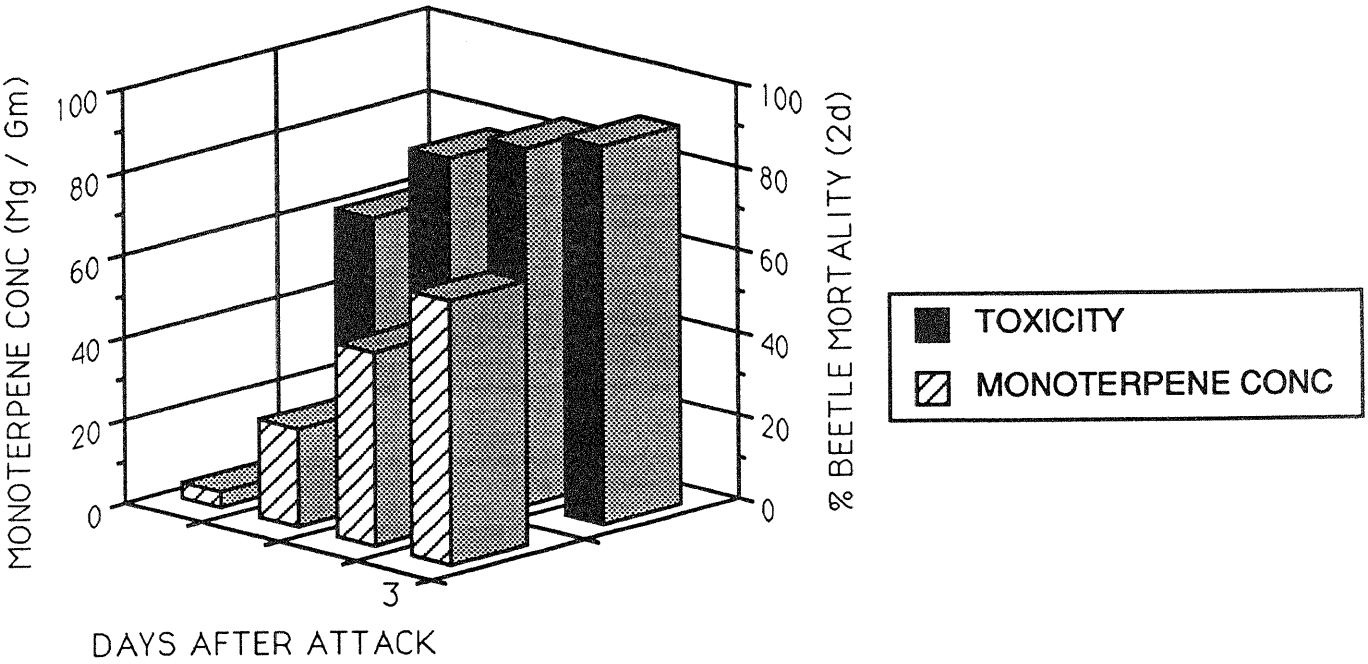


Figure 3.—Effects of constitutive and induced monoterpene concentrations on survival of *Ips pini* adults. Monoterpene concentrations in mature red pines from days 0 (pre inoculation) to 3 following inoculation with *O. ips* are shown in foreground. Mortality rate to adult *I. pini* for each concentration of synthetic monoterpene in laboratory assay is shown in background.

We conducted a series of comparisons between root-infected and asymptomatic trees for a range of parameters associated with host susceptibility to *I. pini* - *O. ips* complexes. These comparisons included field assays at the whole tree or controlled bait level, and laboratory assays involving application of controlled treatments. Both groups of assays involved behavioral tests with *I. pini*, and tree physiological responses to simulated *I. pini* - *O. ips* attack. The results are summarized in Table 2.

Behavioral assays evaluated three phases of scolytid orientation to host trees: initial landing, host entry, and gallery formation (Wood 1972, Elkinton and Wood 1980, Raffa and Berryman 1982c, Raffa 1988). Arrival rates to baited traps did not differ between stem phloem strips from root-infected or healthy trees, stems artificially infected with various fungi associated with Red Pine Decline, or volatiles associated with various intensities of stress (Table 2). When male *I. pini* were caged directly onto trees, however, the entry rates were 2.4 X higher on trees adjacent to the margin of dead trees than on trees farther into the stand. Prior excavations had demonstrated high levels of root disease in the former group (Klepzig *et al.* 1991). Three conclusions can be drawn from these results: (1) beetles can detect changes in stem phloem physiology and

Table 2.—Components of *I. pini* colonization of *P. resinosa* affected by prior infestation with root-colonizing insects and fungi

Parameter	Treatment	Comparison	Result
a. Initial landing	Host stem tissue	Rootinfested vs. healthy trees	ns
	Host stem tissue	Fungal-infected vs. non-infected stems (*Oi, On, Lt*)	ns
	Volatiles	etOH, α-pinene, EtOH & α-pinene, vs. controls	ns
b. Host entry	Caging onto whole trees	Root infected vs. healthy trees	Prefer diseased trees: 7.X
c. Gallery formation	Phloem strips	Root weevil infected vs. healthy logs	Prefer infested logs: 2.4X
	Amended phloem	Nonpolar extracts from constitutive stem phloem of root-infected vs. healthy trees	Prefer healthy trees: 10X
	Amended phloem	Nonpolar extracts from reaction stem phloem of root-infected vs. healthy trees	Prefer diseased trees: 3.0X
	Amended phloem	Monoterpene contents present in reaction stem phloem of root-infected vs. healthy trees	Prefer diseased trees: 3.5X
d. Constitutive resin	Resin flow	Stem phloem of root-infected vs. healthy trees: 1.5m height	ns
		Stem phloem of root-infected vs. healthy trees: 10m height	Higher in healthy trees: 4X
e. Reaction resin	a-pinene	Root diseased vs. healthy trees	Higher in healthy trees: 1.3X

Data from Klepzig *et al.* (in prep.), Raffa and Smalley (in prep.).

chemistry associated with root colonization; (2) there is no evidence that pre-landing behavior is influenced by factors associated with root colonization; (3) the pattern of tree mortality, in which trees near the "pocket" are more likely to be killed by *I. pini* than are trees distant from the margin of killed trees (Fig. 2), is not just a function of proximity to emerging brood. Even when the factor of beetle dispersal is removed, higher entry rates are observed on neighboring trees.

Beetle preference for root-diseased over healthy trees persists under laboratory conditions. *I. pini* excavated more extensive galleries in phloem strips of logs that had been artificially infested with *H. radicis* than in control logs. Likewise, extracts from the stem phloem tissue of root-diseased and healthy trees affect *I. pini* behavior differently (Klepzig *et al.* 1994c). When these extracts are applied to ground denatured phloem incorporated into agar, male *I. pini* show strong preferences. Both the extent of tunnelling and the location of beetles are influenced by the source of host extracts under two-way choice conditions. Interestingly, the beetles preferred extracts from the constitutive tissue of healthy over diseased trees. This suggests that low levels of monoterpenes function as feeding incitants. However, when trees are challenge inoculated with *O. ips*, the pattern is reversed. The reaction tissue of healthy trees is much more repellent than the reaction tissue of diseased trees (Table 2). The same results can be achieved by amending the media with synthetic monoterpene concentrations determined to be present in these various extracts.

Once entered by beetles, root-infected trees are physiologically less able than uninfected trees to resist attack by *I. pini* and its associated fungus *O. ips*. Differences occur in both primary and induced resin (Table 3). Interestingly, we did not observe differences in primary resin flow rates between root-infected and healthy trees when the wounds were applied at 1.5m height. However, flow rates at the base of the canopy yielded only 25% of the resin volume in root-diseased as in healthy trees. Thus, the earliest symptoms of root infection may arise relatively high up in the stem.

Based on these results, we hypothesized the following sequence: (1) root- and lower stem- infecting beetles colonize selected trees and vector *Leptographium* fungi into the root system; (2) root fungi progress through root grafts into healthy trees. These organisms do not kill mature red pines, but reduce their resistance to the stem-colonizing *I. pini* and its associated fungus *O. ips*; (3) colonization by this stem inhabiting complex is lethal to host trees; (4) killed trees provide a food base for secondary root-feeding insects, which introduce additional inoculum into the epicenter.

Table 3.—Environmental factors affecting host suitability to insects and fungi associated with Red Pine Decline Disease

Factor	Effect
Reduced lights	Increased lesion length follwoing inoculation with *L. terebrantis*: 2.5X
Defoliation	Increased lesion length following inoculation with *O. ips*: 1.2X
Defoliation	Decreased resin flow following mechanical wound: 0.45X
Defoliation	Preferential feeding by *H. pales*: 2.3X
Nitrification	Increased feeding by *H. pales*: 1.4X
Nitrification	Decreased development time by *H. pales* females: 0.89X
Nitrification	Decreased development time by *H. pales* males: 0.92X
Nitrification	Increased growth by *H. pales* females: 1.2X
Nitrification	Increased growth by *H. pales* males: 1.1X
Nitrification	incrased survival by *H. pales*: 2.1X

Data from Hunt *et al.* (1993), Klepzig *et al.* (in 1994c.), Krause and Raffa (in prep.)

Environmental Predisposition to Subcortical Insects and Fungi Associated with Declining Red Pine Stands

Although the above description may partially describe within-stand dynamics, it cannot explain the onset of decline. To address this question, we have conducted a variety of experiments on the effects of environmental stress on host suitability to insects and pathogens associated with Red Pine Decline. The results suggest that a relatively broad range of biotic and abiotic factors can improve host suitability to these organisms (Table 3). For example, defoliation decreases resin flow, reduces tree ability to confine *O. ips*, and increases feeding preference to *H. pales* (Krause and Raffa 1994). Likewise, nitrification increases feeding, development, and survival by *H. pales* (Hunt *et al.* 1993). Reduced light availability also decreases host ability to respond to *L. terebrantis* (Klepzig *et al.* 1994c). Both defoliation and reduced light availability reduce the overall pool of photosynthate for carbon-based defenses such as terpenes and phenolics (Wright *et al.* 1979, Ericsson *et al.* 1985, Lorio and Summers 1986, Miller and Berryman 1986, Peet and Christensen 1987, Waring 1987, Dunn *et al.* 1990, Reich *et al.* 1992, Krause *et al.* 1993, Krause and Raffa 1994). These stresses can themselves be initiated by biotic agents. For example, outbreaks by insect (or microbial) defoliators are often followed by mortality due to bark beetles (Wright *et al.* 1984, Paine and Baker 1993), densely crowded stands more commonly experience bark beetle outbreaks (Nebeker *et al.* 1993), and suppressed trees are less able to resist attack (Safranyik *et al.* 1975, Raffa and Berryman 1982b, Mitchell *et al.* 1983, Waring and Pitman 1985, Paine and Baker 1993, Preisler and Mitchell 1993).

Our current understanding of the biotic and abiotic interactions contributing to Red Pine Decline is depicted in Figure 4. Each of the major assumptions underlying this model has been supported, although some of the mechanisms are only poorly understood. In particular, our knowledge of the relative importance, impact, and tolerance ranges of the various inciting factors lags behind our knowledge of within-stand spread. Our current understanding is now sufficient, however, to test putative natural and anthropogenic stress agents on specific tree physiological, insect behavioral and developmental, and microbial developmental parameters, and to project these impacts to the stand level. This model can also serve as a framework for expanding our basic understanding of interactions among feeding guilds, the interaction of constitutive and inducible allelochemicals in host plant resistance, and microbial mediation of plant-insect interactions.

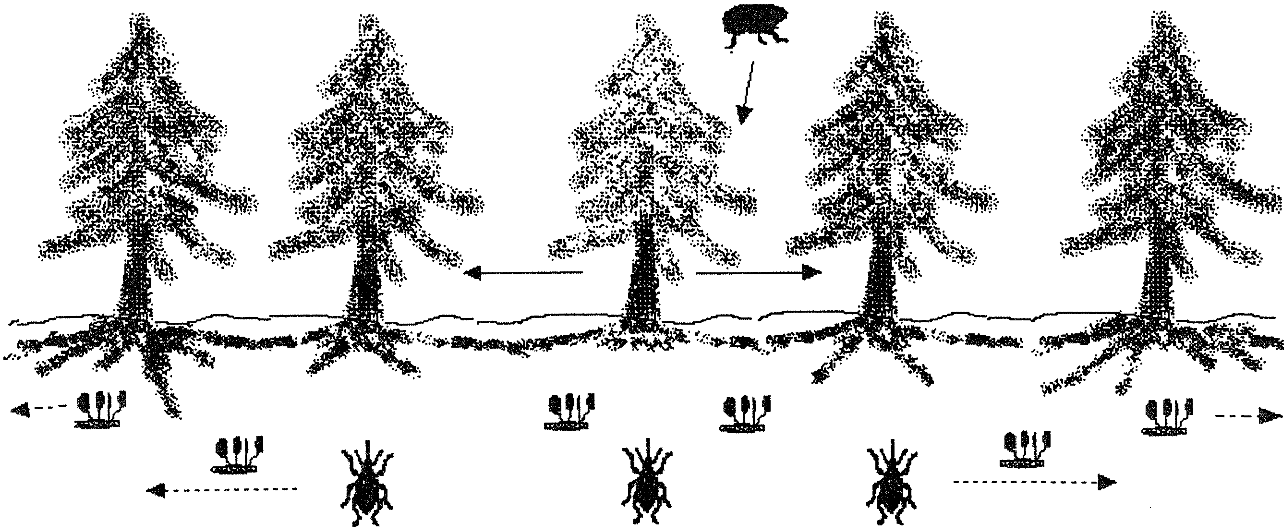


Figure 4.—Sequence of events in the infection, decline, and mortality of plantation red pines.

Implications to Natural Resource Management and Forest Ecosystem Health

The dynamics of insect and microbial colonization also suggests some specific management tactics that can prevent the onset and spread of Red Pine Decline. For example, mixed-block rather than uniform-species planting would reduce the transmission of *Leptographium* fungi through root grafts. This would contain root-diseased trees within relatively small groups rather than entire stands. Where economic incentives for red pine make large-scale planting of alternate species impractical, planting buffer strips of alternate species might serve the same purpose. *Pinus strobus* would probably be a better candidate than *Pinus banksiana*, because the former is less preferred by the root weevils in our region (Hunt *et al.* 1993). Judicious thinning would also reduce stress and thereby reduce stand susceptibility. However, the timing is quite important. Open light penetration can favor site suitability to root weevils by warming and drying the soil (Wilson and Millers 1983). Moreover, thinning should be accompanied by stump removal to avoid leaving a food base for the root- and lower-stem feeding species (Witcosky and Hansen 1985, Witcosky *et al.* 1986). Our model also suggests that severing root grafts in advance (@ 15m) of killed trees could halt the transmission of *Leptographium*. We have begun tests on this idea in Wisconsin. We further recommend sanitation removal of dead and living trees (including stumps) within the radius of root severing.

Although the insects and fungi described here are typically viewed as forest pests, these moderately pathogenic species might serve as useful bio-indicators. Reproductive success by these organisms is contingent on their ability to detect trees that are sufficiently stressed to have impaired defensive capability, but not so severely stressed as to be available to saprophytic competitors. As such, the population densities and behavior of moderately virulent endoparasites may be indicative of reduced ecosystem vigor associated with anthropogenic disturbances or injudicious forestry practices. Our monitoring data and bioassay show this idea is feasible for at least one form of forest decline. That is, populations of some root- and lower stem-feeding insects were higher in declining than in asymptomatic stands. Moreover, different species vary in how their densities relate to the progression of decline and in how they respond to tree stress physiology. We propose that the most useful bioindicator candidates might be those that show increased numbers or behavioral alteration before any symptoms become apparent, but are not by themselves major outbreak pests.

SUMMARY

Interactions between feeding guilds that affect different plant parts can strongly affect the dynamics of forest declines. Environmental stresses can mediate these outcomes, especially against highly generalized plant defense systems. Such interactions among insects and fungi are primarily responsible for Red Pine Decline, a syndrome affecting mature plantation trees in the Great Lakes region of North America. Root- and lower stem-colonizing insect-fungal complexes reduce tree resistance against stem-feeding bark beetle-fungal complexes. Between-stand transmission of moderately pathogenic Ophiostomatales fungi occurs by insect vectoring, but within-stand progression also occurs through extensive root grafts in plantations. Subterranean scolytids and curculionids show a high degree of niche partitioning based on host tissue and condition, chemical ecology, microbial associates, and spatial and temporal distributions.

Healthy trees can resist colonization by rapidly accumulating terpenes and phenolics to concentrations that repel or kill *I. pini* and inhibit *O. ips* spore germination and mycelial growth. However, tree constitutive and inducible defenses are compromised by root infection. Behavioral assays are generally the most sensitive to the early stages of root injury. Among these, post landing assays of *I. pini* acceptance behavior are most sensitive, especially those in which beetles are exposed to host reaction tissue formed in response to challenge inoculation with fungi. Host susceptibility to the root colonizing agents can be increased by environmental stresses such as reduced light, defoliation, and nitrification.

An understanding of the complex interactions underlying forest declines can improve our ability to relate various syndromes to management practices, anthropogenic stress, and natural succession. These systems can also serve as useful models to improve our overall understanding of plant-insect and insect-fungal interactions. The underlying mechanisms also suggest some specific tactics for preventing or ameliorating Red Pine Decline. Marginally pathogenic agents such as the organisms described here may also serve as useful indicators of forest ecosystem health. Because their existence is contingent on detecting trees under sufficient stress to fully resist attack but not yet available to saprophytic competitors, the population densities, behavior, and developmental success of these insects and fungi might be good indicators of incipient stress at the stand or tree level.

ACKNOWLEDGMENTS

This work was supported by USDA CSRS Competitive Grant 90-3725P-5581, the University of Wisconsin Graduate School, the University of Wisconsin College of Agricultural and Life Sciences, McIntire Stennis, and Hatch Regional Project W-187. We would also like to thank our colleagues L. Rieske, S. Krause, and K. Kleiner for input on the ideas discussed in this paper.

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DOUGLAS-FIR AND WESTERN LARCH DEFENSIVE REACTIONS TO LEPTOGRAPHIUM ABIETINUM AND OPHIOSTOMA PSEUDOTSUGAE

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INTRODUCTION

The Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, is one of the most important insects associated with Douglas-fir, *Psuedotsugae menziesii* (Mirb.) Franco, in western North America (Furniss and Carolin 1977). At high population densities, the beetle is capable of causing significant tree mortality and impacting forest resource values (Cornelius 1955, Rudinsky 1966, Johnson and Belluschi 1969, Furniss and Orr 1978, Furniss *et al.* 1979). The Douglas-fir beetle usually breeds in recently dead or live Douglas-fir trees. The only other tree species in which the beetle has been reported to successfully breed is western larch (Ross 1967). However, the beetle is apparently able to breed only in dead larch (Furniss *et al.* 1981). Live larch may be attacked, but brood production from live trees has never been observed. Reed *et al.* (1986) compared various chemical and physical properties of Douglas-fir and western larch to identify possible reasons that brood fail to survive in live larch trees. Compared with Douglas-fir, larch had a higher content of 3-carene, thinner phloem, higher phloem moisture content, larger diameter vertical resin ducts, and lower oleoresin exudation pressure. The authors speculated that the high content of 3-carene in live larch may be responsible for the failure of Douglas-fir beetle brood in those trees.

One or more species of ophiostomatoid fungi are invariably associated with each species of conifer-infesting bark beetle (Whitney 1982, Harrington 1988). Although the ecological relationships among the fungi and bark beetles are not completely understood in many cases, there is evidence that some of these fungi help the beetles to overcome the natural defenses of mass attacked trees. Conifer response to invasion by bark beetles and their associated fungi involves two different systems: (1) the flow of preformed resin from severed resin ducts and (2) the induced wound response or resistant reaction (Reid *et al.* 1967, Berryman 1972, Christiansen and Horntvedt 1983). The dimensions of the induced response in host tree tissues are generally correlated to the growth of the fungi or the extent of mechanical damage associated with beetle tunneling (Ross *et al.* 1992, Lieutier 1993, Solheim 1993). If the fungi associated with the Douglas-fir beetle are involved in overcoming tree resistance, then a possible reason for the failure of brood development in larch may be the inability of the fungi to survive and grow in larch. A significant variation in the pathogenicity of the fungi to Douglas-fir and western larch should be reflected by differences in the induced response following artificial inoculations.

Despite a considerable amount of past research related to the biology and management of the Douglas-fir beetle, little is known about its fungal associates. The two species of ophiostomatoid fungi most commonly associated with the Douglas-fir beetle are *Ophiostoma pseudotsugae* (Rumb.) von Arx and *Leptographium abietinum* (Peck) Wingf. (Rumbold 1936, Harrington 1988, Solheim unpubl.). There are no published reports on the pathogenicity of these fungi or their ecological relationships to the beetle. In 1993, we installed a study to evaluate the pathogenicity of these fungi to Douglas-fir. The final results of that study will be published elsewhere in the near future. At the same time, we installed a small test to assess the response of Douglas-fir and western larch, *Larix occidentalis* Nutt., to artificial inoculations with these fungi. The objective of our study was to measure the length of the induced response in the phloem of Douglas-fir and western larch following artificial inoculation with *O. pseudotsugae* and *L. abietinum*. This paper reports the results of this test.

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METHODS

The study was installed in a mixed conifer stand (elevation ~ 1460 m) in northeastern Oregon. On June 29, 1993, seven Douglas-fir and seven western larch were selected from an area of less than 1 ha. The trees were chosen based on similarities in size and lack of any visible injuries or disease. Three inoculation sites were established equidistant around the circumference of each tree at dbh (1.4 m height). The three treatments that were randomly assigned to the inoculation sites were: (1) sterile wound, (2) *L. abietinum*, and (3) *O. pseudotsugae*. At each inoculation site, a 1.2-cm diameter hole was drilled to the sapwood surface with a sterile bit. For the sterile wound, a cotton plug was placed in the hole that was then covered with duct tape. For the fungal inoculations, a small agar plug containing the appropriate fungus was placed in the hole that was then similarly covered. The fungi used in the study had been isolated from Douglas-fir beetles collected in British Columbia, Canada (Solheim unpubl.).

On August 25, 1993, the outer bark covering each inoculation site was removed and the length of the necrotic lesion on the phloem surface was measured.

The mean dbh for trees of each species were compared by a t-test. Lesion length was subjected to analysis of variance for a split-plot design with tree species representing whole plots and fungal inoculation representing subplots.

RESULTS

There was no significant difference ($P < 0.19$) in dbh between Douglas-fir (mean = 51.0 cm) and western larch (mean = 48.8 cm). The tree species x treatment interaction was significant ($F = 3.29$; $P < 0.05$) for lesion length. For both tree species, inoculation with either fungus resulted in a larger lesion than that produced by a sterile wound (Fig. 1). The length of the lesions induced by a sterile wound or inoculation with *O. pseudotsugae* did not differ between tree species, but the lesion induced by inoculation with *L. abietinum* was significantly shorter in western larch than in Douglas-fir (Fig. 1).

DISCUSSION

There were no obvious differences in the general appearance of lesions (i.e., intensity of resin accumulation, radial growth, etc.) between tree species, so lesion length should provide a relatively accurate indication of fungal growth within the phloem of the host trees. The smaller lesions produced by western larch in response to *L. abietinum* may reflect a more rapid and effective defensive reaction to the fungus. These results suggest that *L. abietinum* is not as well adapted to utilize western larch as a host compared with Douglas-fir. If *L. abietinum* is critical to the survival of Douglas-fir beetle brood in live host trees, then this may help to explain the complete mortality of brood in western larch. In contrast, there was no difference in the induced response to *O. pseudotsugae* between the two tree species. Our results must be interpreted cautiously since we have only measured lesion length in phloem tissue. Lesion length is not always directly correlated to pathogenicity (Solheim 1988). Fungal growth and tree response may be quite different in the xylem compared with the phloem. However, the difference in response to fungal inoculations that we observed suggests that further research on the host tree-fungus interactions in this system would be worthwhile. The significance of these results will only become apparent when we learn more about the role that these fungi play in the population dynamics of the Douglas-fir beetle.

SUMMARY

There was no difference in lesion length at the phloem surface between Douglas-fir and western larch following sterile wounds or inoculation with *O. pseudotsugae*. However, lesion length following inoculation with *L. abietinum* was significantly shorter in western larch compared with Douglas-fir. These results may help to explain the lack of Douglas-fir beetle brood survival in western larch.

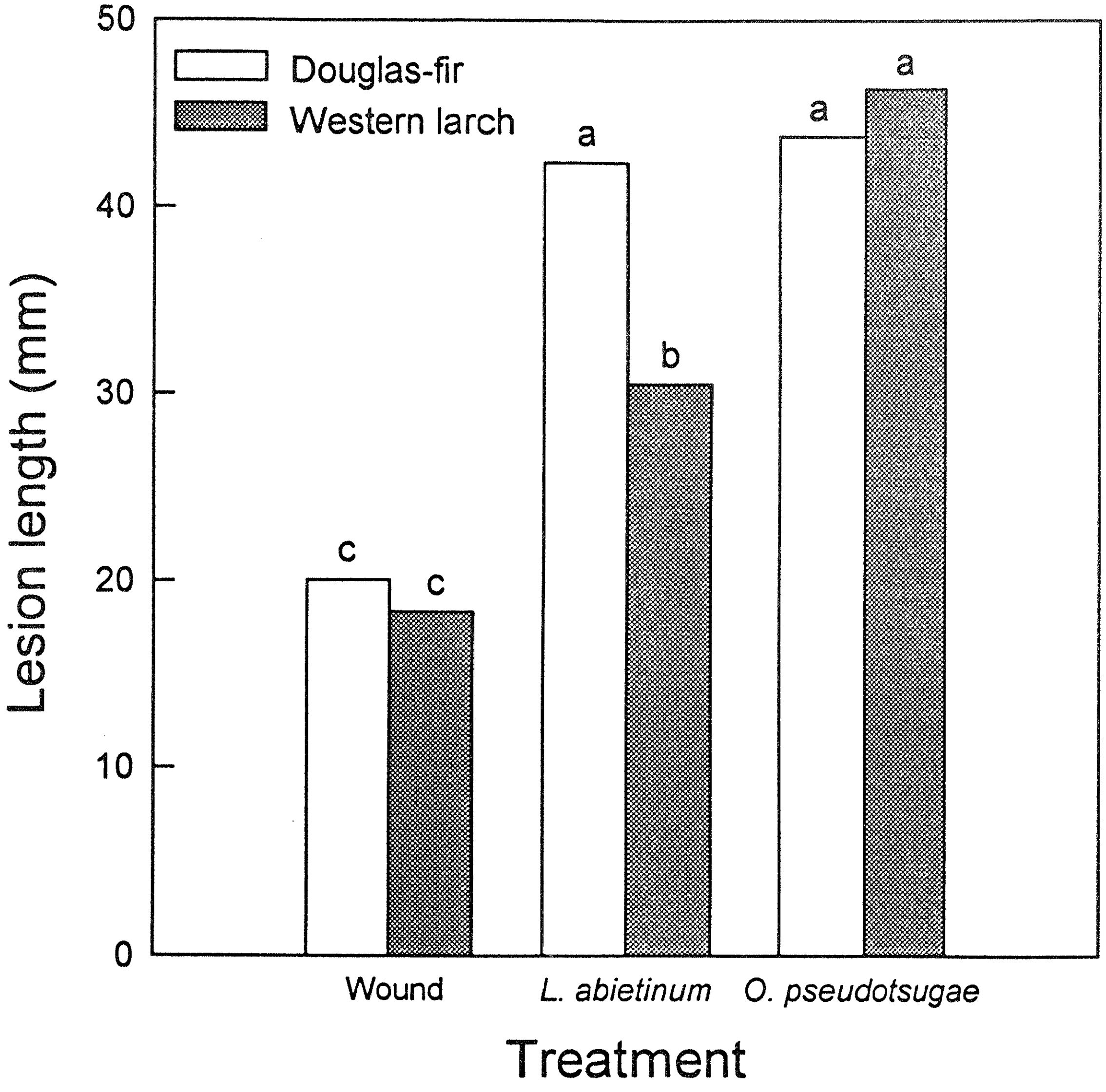

Figure 1.—Mean length of the induced lesion at the phloem surface following sterile wounding or fungal inoculation in Douglas-fir and western larch (n=7). Bars with the same letter are not significantly different (P = 0.05).

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INTERRUPTION OF BARK BEETLE AGGREGATION BY A VIGOR-DEPENDENT PINUS HOST COMPOUND

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INTRODUCTION

The attraction of bark beetles to pheromones has been abundantly demonstrated in the last quarter century (Wood 1982, Borden 1985). Much less is known about the response of bark beetles to host volatiles. This paper reports preliminary evidence that one common host compound of North American conifers is a strong repellent or interruptant of aggregation for several species of bark beetles. It also reviews work that suggests that the level of this compound may be an indicator of host tree stress typically associated with bark beetle infestation in several species of pines. This compound, methyl chavicol, fulfills the two essential requirements for a kairomonal cue that can explain the selective infestation of susceptible trees by bark beetles. It is correlated to tree susceptibility, and bark beetles respond to it.

The search for a biochemical indicator of host tree stress that might serve as an olfactory cue for bark beetles is not new (Rudinsky 1962 and references therein, Cobb *et al.* 1968). Miller *et al.* (1968) examined the resin from smog-damaged ponderosa pines, *Pinus ponderosa*, in the San Bernardino Mountains east of Los Angeles. These trees were being selectively infested by mountain pine beetle, *Dendroctonus ponderosae*, and western pine beetle, *Dendroctonus brevicomis*. Of the six monoterpenes examined, none were shown to be significantly different between damaged and healthy trees. However, Cobb *et al.* (1972) found a strong, significant difference in the level of one compound, methyl chavicol, in the foliage of smog-damaged ponderosa pines and healthy trees. Smog-damaged trees had 71% less methyl chavicol in their foliage - by far the sharpest phytochemical difference found.

More recently Nebeker *et al.* (1995) measured 18 host volatiles from healthy lodgepole, *Pinus contorta*, and those diseased by dwarf mistletoe, *Arceuthobium americanum*; armillaria root disease, *Armillaria mellea*; or comandra blister rust, *Cronartium comandrae*. The latter two diseases are reported to be the most common predisposing agents of lodgepole to mountain pine beetle attack in the Intermountain West (Tkacz and Schmitz 1986). In the case of both predisposing diseases of lodgepole, there was a large and significant difference in the level of 4-allylanisole (a synonym for methyl chavicol) between healthy and diseased trees. This was not true for dwarf mistletoe, which was not shown to be associated with bark beetle infestation (Tkacz and Schmitz 1986). For both comandra and armillaria, the difference in the level of methyl chavicol was one of the greatest phytochemical differences found between diseased and healthy trees. Trees infected with comandra had 43.6% less methyl chavicol than healthy trees; trees infected with armillaria had 63% less methyl chavicol than healthy trees. Cobb *et al.* and Nebeker *et al.* both found the strongest biochemical differences between diseased and healthy trees in one compound.

These results begged the question, "What is the response of bark beetles to methyl chavicol?" Electroantennograms (EAG) show a strong response to methyl chavicol. In 1989, Peter White found that estragole (methyl chavicol) produced the third highest response of all 11 host compounds tested on the red turpentine beetle, *Dendroctonus valens* (White and Hobson 1993). In 1993 Armand Whitehead showed a strong EAG response of mountain pine beetle to methyl chavicol (Hobson *et al.* in prep). A large EAG response does not indicate that a compound will be a strong attractant or repellent. However, ecologically relevant compounds that produce a large EAG are likely to have behavioral significance (Masson and Mustaparta 1990). These data encouraged us to conduct behavioral field studies.

Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

METHODS

In the spring and summer of 1993, we tested the response of five species of bark beetles to methyl chavicol in California and Idaho. In April and May, we tested western pine beetle, red turpentine beetle, and two species of *Ips*, *I. paraconfusus* and *I. pini*, at the University of California's Blodgett Forest Research Station in the central Sierra Nevada. Tests were conducted as described previously (Hobson 1992, Hobson *et al.* 1993). For each beetle species, attractive lures were placed on lindgren traps. In each test there were four treatments: (1) attractant, (2) attractant and methyl chavicol, (3) methyl chavicol alone, and (4) control blank.

Beetles were collected daily and treatments were randomized after each collection. All four treatments were replicated in 4-10 blocks. Methyl chavicol was released from four open 1.5-ml ependorf tubes at a rate of 0.5 ml/day. The attractant for *D. valens* was a set with four 1.5-ml ependorf tubes of S(-)β-pinene. For the western pine beetle, *I. pini* and *I. paraconfusus* commercial lures provided by Phero Tech (Delta, BC) were used. Western pine beetle lures contained *exo*-brevicomin, frontalin and myrcene. *I. pini* lures contained ipsdienol (3%+/97%-). *I. paraconfusus* lures were ipsenol (50%+/50%-), ipsdienol (97%+/3%-), and cis-verbenol.

In July and August of 1993, we tested mountain pine beetle and *I. pini* in the Sawtooth National Recreation Area in south central Idaho. The experimental design was as above. Attractant lures for mountain pine beetle (*trans*-verbenol, *exo*-brevicomin and myrcene) were obtained from Phero Tech. Attractant lures for *I. pini* were as above.

RESULTS

Methyl chavicol significantly reduced attraction of western pine beetle and mountain pine beetle to their respective aggregation pheromones (Table 1). Attraction of *D. valens* to β-pinene was not significantly interrupted by methyl chavicol. *I. pini* catch was reduced by 29%, but this was not significant. *I. paraconfusus* flew too late in California for us to collect adequate data for statistical analysis; however, in five of six blocks where *I. paraconfusus* was collected, traps baited with methyl chavicol and the bait had fewer beetles than traps with bait alone.

Table 1.—Bark beetle response to Methyl chavicol

	Pheromone bait[1]	SD	Pheromone & methyl chavicol[1]	SD	Percent reduction
Western pine beetle	53.5a[2]	69.7	21.6b	28.0	60
Mountain pine beetle	27.4c	47.5	7.9d	12.4	71
Red turpentine beetle	10.6	6.28	9.06	6.8	14
Ips pini	20.5	19.7	14.5	12.2	29

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[1] Mean catch.

[2] Numbers in a row followed by different letters are significantly different $\alpha = 0.05$ ANOVA followed by Wilcoxon test.

Where beetle response to methyl chavicol was compared by sex, mountain pine beetle males and females were both significantly less attracted to lures with methyl chavicol. The data for western pine beetle were not sufficient to statistically test gender-specific response, but methyl chavicol reduced the catch of males by 53% and females by 66%. Methyl chavicol reduced the catch of *D. valens* females by 21% and of males by 11%, but neither of these reductions was significant.

DISCUSSION

The aggregation of the two most aggressive bark beetles in this study, the mountain and western pine beetles, was strongly interrupted. The less aggressive species, *D. valens*, *I. pini*, and *I. paraconfusus* were not so strongly affected.

Subsequent testing of the *I. paraconfusus* with methyl chavicol and a larger sample size produced a consistent 40% interruption of aggregation (Storer, pers. comm.). The aggregation of two other aggressive bark beetles in Alaska, *D. rufipennis* and *D. simplex*, were interrupted by 82% and 73%, respectively (Werner 1995). Haack and Lawrence have tested methyl chavicol in traps with attractants of *Tomicus piniperda* (Haack, pers. comm.). Subsequent to the presentation of this paper, Hayes and co-workers published similar results with *Dendroctonus frontalis* (Hayes *et al.* 1994 a, b) showing 37% and 56% reduction in catch with methyl chavicol (4-allylanisole). Hayes and Strom (1994) also confirmed our results with mountain pine beetle, obtaining a 77% reduction in catch with methyl chavicol. Their work differed from this work in that they could not obtain significant interruption of attraction for western pine beetle, and they did find interruption of attraction at the 43% level for a Wisconsin population of *I. pini.*

Methyl Chavicol

Methyl chavicol, an aromatic ether or phenylpropanoid, is also commonly known as 4-allylanisole, tarragon, or estragol. (Other chemical synonyms include: isoanethole, p-allylmethoxybenzene, 4-allyl-1-methoxybenzene, chavicol methyl ether, esdragon and 1-methoxy-4-2(2-propenyl)benzene) (Aldrich Chemical - Material Data Safety Sheet). It is widespread in the oleoresin of new world pines in the subgenus *Pinus*, occurring in *P. ponderosa*, *P. taeda*, *P. palustris*, *P. elliottii*, *P. patula*, *P. jeffreyi*, *P. tenufolia*, *P. hartwegii*, *P. michoacana P. lumholtzii* (Mirov1961 and references therein), and *P. caribaea* (Smith, R.M. 1975). Among old world pines it occurs in *P. sylvestris* and *P. nigra* (Bardysev *et al.* 1970). It is abundant in the foliage of ponderosa pine (23%) and present in the foliage of lodgepole and digger pines (Mirov 1961). It is also found in 12 or more species of spruce (Zavarin, pers. comm.).

Methyl chavicol is the principal volatile ingredient or a major volatile of several strongly scented herbs: tarragon, *Artemisia dracunculus*; fennel, *Foeniculum vulgare*; star anise, *Illicium verum*; basil, *Ocium basilicum*; and cloves, *Szygium aromaticum* (Duke 1985). Methyl chavicol is also known from the leaves of three rutaceous plants, west african zigua, *Clausena anisata*, where it is the major component of the oil of leaves used to repel mosquitoes (Okunade and Olaifa 1987); *Zanthoxylum* spp., where it is toxic to *Dacus* eggs (Marr and Tang 1992); and southeast asian wood apple, *Feronia limonia* (Ahmad *et al.* 1989). In addition, it is found in oil palm, *Elaeis guineensis*, where it is attractive to the weevil *Elaeidobius kamerunicus* (Hussein *et al.* 1990). In recent plant herbivore studies, it is most well known from the work of Metcalf and his co-workers. They have found it in the flowers of *Cucurbita maxima*, where it is attractive to *Diabrotica* spp., corn root-worms. This association is the basis of a productive research effort in biorational management of *Diabrotica* (Metcalf and Lampman 1989).

Biosynthetically methyl chavicol is derived from the shikimic acid pathway (Zavarin *et al.* 1971). This pathway is disrupted by the herbicide glyphosate (Roundup, Monsanto) (Borden, pers. comm.). Interestingly, when Bergvinson and Borden (1991) applied glyphosphate to lodgepole pine, they found that treated trees were readily colonized by mountain pine beetles. They concluded that the herbicide had inhibited the treated trees' secondary defense response against the beetle's symbiotic fungi. This is consistent with the hypothesis that the success of mountain pine beetles in glyphosate-treated trees is, in part, due to a drop in methyl chavicol. Bridges (1987) found methyl chavicol was the most inhibitory host compound to the three symbiotic fungi associated with *D. frontalis*, suggesting that it may be an important defensive mechanism of loblolly pine against southern pine beetle and its vectored fungi. Hayes *et al.* (1994a) found that methyl chavicol was strongly reduced in southern pines following wounding and treatment with metham-sodium and dimethyl sulfoxide. Treated trees were attacked by *D. frontalis* after the level of natural methyl chavicol dropped. Methyl chavicol tested subsequently in field bioassays strongly reduced attraction of southern pine beetle to pheromone-baited traps.

The importance of a compound as a repellent is somewhat novel in bark beetle host selection research. Limonene and other terpenes that kill bark beetles and their vectored fungi have been thought to have a mainly short-range effect as feeding deterrents (Smith, R.H. 1975, Coyne and Lott 1976). The majority of work in this field, however, has looked for compounds that increase with stress and are attractive. Few studies have looked for compounds that decrease with stress and are repellent.

SUMMARY

The significance of these results rests in three principal areas:

1) The large difference in methyl chavicol between healthy and diseased trees provides a clear olfactory sign of stress for bark beetles, far clearer than has been demonstrated for any monoterpene to date.

2) The stresses that produce the large drop in methyl chavicol are the same stresses that are associated with bark beetle infestation, suggesting that methyl chavicol is one of the biochemical links between ecology and beetle behavior.

3) Methyl chavicol reduces the arrestment and landing of bark beetles in the field sufficiently strongly to suggest management applications for several of our most important bark beetle species with a naturally occurring, abundant host compound.

Methyl chavicol is the best candidate so far for a compound that responds to stress and to which beetles respond. It may serve as an indicator and reveal, via biochemical linkage, the elements of host defenses that most strongly affect bark beetle success. Further investigation of other host species applying stress of the sort known to favor beetle colonization may determine some new insights for our understanding of the biochemical basis of conifer defenses. Ultimately, we may understand how environmental stresses that favor beetle colonization produce the characteristics of susceptibility which beetles favor. We may be able to provide the biochemical linkage between ecological conditions and host selection.

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WILL GLOBAL WARMING ALTER PAPER BIRCH SUSCEPTIBILITY TO BRONZE BIRCH BORER ATTACK?

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INTRODUCTION

According to several general circulation models, a rapid climatic warming of 1-5°C in the annual global-mean surface air temperature is predicted by the year 2050 (Shands and Hoffman 1987, MacCracken *et al.* 1990, Karl *et al.* 1991, Schlesinger and Jiang 1991, IPCC 1992, Peters 1992, Bengtsson 1994). Some forest entomologists predict an increase in outbreak frequency of certain forest insects as a result of global warming (Kellomaki *et al.* 1988, Hedden 1989, Franklin *et al.* 1992, Fleming and Volney 1995), although the actual response may be difficult to predict due to the many interacting biotic and abiotic factors (Hedden 1989, Porter *et al.* 1991, Cammell and Knight 1992, Dewar and Watt 1992, Ayres 1993, Scriber and Gage 1995, Williams and Liebhold 1995).

Is there a way to test whether climate change will alter tree susceptibility to insect attack? One method is to look for patterns of differential insect attack within genetic test plantations where trees of known genetic background grow together at the same test site. Frequently, the primary objective of genetic test plantings is to identify either specific families or geographic seed sources (provenances) that performed well at a particular location, especially with respect to growth and form characteristics. The trees in genetic test plantings, particularly provenance tests, commonly originate from several distant locations, often representing several contrasting climates. Because tree populations become genetically adapted to their local conditions, one major factor that affects their performance when moved to a new location is the difference in climate between the original and the new location (Wright 1976, Kozlowski *et al.* 1991, Schmidtling 1994). Data from genetic test plantings have only recently been examined to elucidate how some tree species might respond to global warming (Matyas 1994, Schmidtling 1994). Similarly unexplored has been the use of provenance plantation data to predict how global warming will affect tree susceptibility to insect attack.

In this paper, I explore how global warming might alter susceptibility of paper birch, *Betula papyrifera* Marsh., to the bronze birch borer, *Agrilus anxius* Gory (Coleoptera: Buprestidae). This particular tree-insect combination was selected because (1) paper birch appears highly sensitive to environmental stress given that several large scale declines of paper birch have been reported this century in eastern North America, and (2) the bronze birch borer has usually been the ultimate mortality agent of stressed birch trees (Slingerland 1906, Swaine 1918, Spaulding and MacAloney 1931, Balch and Prebble 1940, Hawboldt 1947, Nash *et al.* 1951, Barter 1957, Redmond 1957, Clark and Barter 1958, Haack and Mattson 1989, Jones *et al.* 1993, Braathe 1995). Birch mortality as a result of bronze birch borer attack has followed stress events such as drought, extreme cold winter temperatures that follow closely behind a winter thaw, elevated growing season temperatures, high soil water tables, years of heavy seed crops, and years of severe insect defoliation (Redmond 1955, 1957; Clark and Barter 1958; Herms 1991; Herms and Mattson 1991; Auclair *et al.* 1992; Jones *et al.* 1993; Auclair *et al.* 1995; Braathe 1995).

Paper birch is a boreal species, and in Michigan it reaches its southernmost range near the center of Michigan's lower peninsula. The ecotone between boreal and temperate forests closely follows the 47°F (8.3°C) average annual mean temperature isotherm in Michigan (Fig. 1). Typically, annual mean temperature decreases steadily with increasing latitude, but in Michigan, the Great Lakes cause a semi-marine type climate, causing isotherms to closely follow the lakes' shorelines (Fig. 1). This feature of moderately vertical isotherms in portions of Michigan allows for great variation in climatic conditions within a rather narrow latitudinal band. Michigan is relatively flat, ranging in elevation from 174 to 604 m (572-1,980 ft) above sea level.

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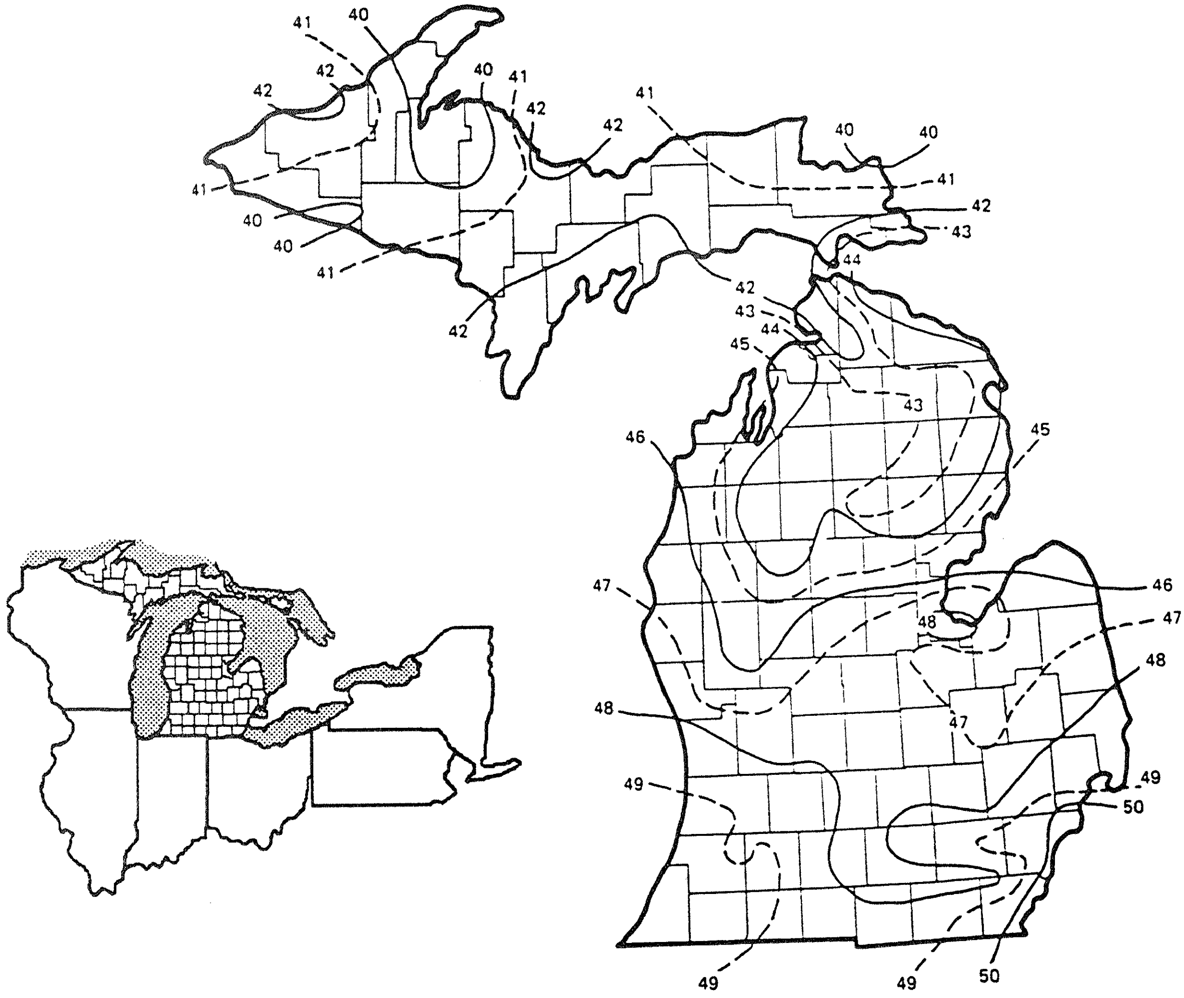

Figure 1.—Average annual mean temperature isotherms for Michigan in °F, based on the period 1940-1969 (MDA 1974). Inset shows Michigan, with county-level resolution, the surrounding Great Lakes (shaded area), and outlines of several adjacent states and Canada. Approximate °C equivalent values are: 40°F = 4.4°C, 41°F = 5.0°C, 42°F = 5.6°C, 43°F = 6.1°C, 44°F = 6.7°C, 45°F = 7.2°C, 46°F = 7.7°C, and 47°F = 8.3°C

Models of global warming in the northern hemisphere predict that a species range will change most dramatically along the southern limits of its range (Cannell *et al.* 1989, Botkin and Nisbet 1992, Woodward 1992, Matyas 1994, Sykes and Prentice 1995). Fortunately, in southern Michigan there is a paper birch progeny test (Fig. 2) that consists of over 200 half-sib (i.e., where the identity of the maternal parent is known) families that were collected from throughout the natural range of birch in Michigan. In addition, it is coincidental that the mean annual temperature varies by about 10°F (5.6°C) between the southern and northern extremes of Michigan (Fig. 1). Therefore the paper birch trees growing in the genetic test plantation in southern Michigan are theoretically experiencing an increase in mean annual temperature of 1° to 5°C. Given that the bronze birch borer preferentially attacks weakened birch trees, it seems plausible that borer attack could vary among seedlots of varying geographic origins if they were experiencing differential levels of stress.

The bronze birch borer, like many other buprestids in the genus *Agrilus*, usually attacks host trees that are stressed by drought or repeated insect defoliation (Anderson 1944; Clark and Barter 1958; Barter 1965; Carlson and Knight 1969; Haack and Benjamin 1982; Haack and Slansky 1987; Mattson and Haack 1987a, 1987b; Haack and Mattson 1989; Herms 1991; Herms and Mattson 1991; Wargo and Haack 1991; Haack 1992; Haack and Acciavatti 1992; Solomon 1995). The biology and life history of the bronze birch borer has been reported by several authors (Chittenden 1898; Balch and Prebble 1940; Anderson 1944; Barter 1957; Ball and Simmons 1980; Loerch and Cameron 1983, 1984; Akers and Nielsen 1990; Wilson and Haack 1990). Briefly, the bronze birch borer is univoltine. Adult beetles emerge in early summer from within their host

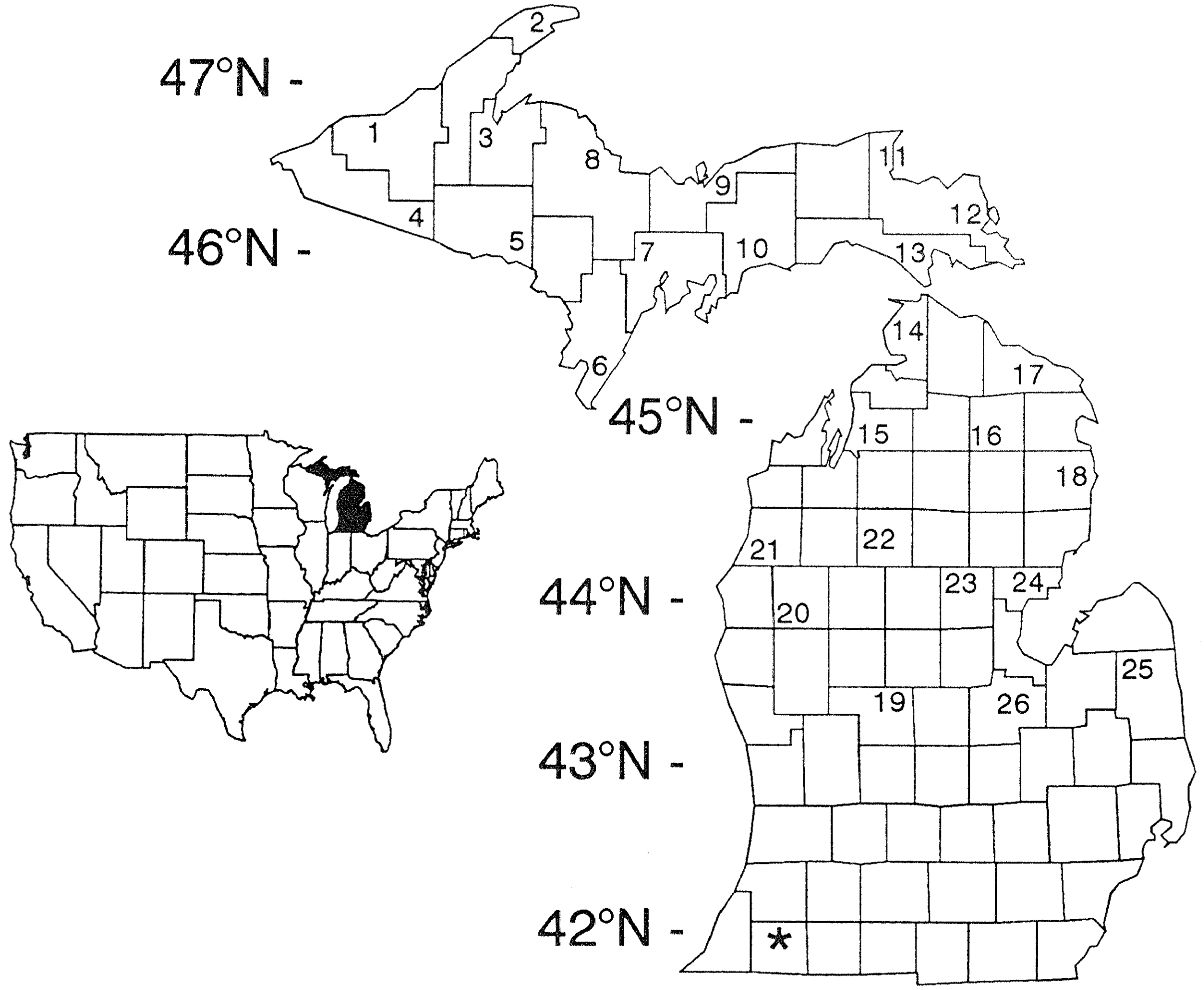

Figure 2.—State of Michigan, showing the 26 locations where paper birch seed was collected in 1975. The * in southwestern Michigan indicates the location of the plantation site where the seedlings were planted in 1976. Inset shows the conterminous 48 states of the US, with Michigan shaded.

trees, and then search for suitable trees to attack. Adults often reattack the same tree until it dies, but from year to year they attack only those portions that are still living at the time of oviposition. It often takes 2-3 years of successive attack before a given branch or trunk section dies. Adults are believed to be attracted primarily by host odors given that no pheromone has yet been discovered for a buprestid (Haack and Slansky 1987, Haack 1992). After mating, females oviposit on the bark surface of the trunk and branches. After hatching, larvae tunnel through the bark and then feed in the cambial region, scoring both the inner bark and outer sapwood. Larvae construct meandering galleries throughout the summer months, passing through four larval instars. In late summer or fall, larvae construct individual pupal chambers in the outer sapwood and there pass the winter. Pupation occurs the following spring, and soon the adults emerge to renew the cycle.

MATERIALS AND METHODS

As described in Miller *et al.* (1991), seed was collected from 218 open-pollinated paper birch seedlots (half-sib families) in 1975, representing 26 Michigan populations; 123 trees from 13 populations in Michigan's Upper Peninsula and 95 trees from 13 populations in Michigan's Lower Peninsula (Fig. 2, Table 1). All parents were growing in natural forests between latitudes 43.3°N and 47.4°N, and ranged from 5 to 22 m in height, and 9 to 46 cm in diameter (Table 1). The seed from the 218 half-sib families was planted in February 1976 and grown in the greenhouse for 5 months, held outdoors for 2

Table 1.—Summary data for the 26 populations of Michigan paper birch trees, representing 218 trees, from which seed was collected in 1975, including for each population the number of trees sampled, location data, climatic data, and mean height and diameter at breast height (DBH) of the parental trees.

Seed source[1]		Location[2]			Climatic data[3]			Height (m)[4]	DBH (cm)[4]
Pop	No	°N Lat	Area	County	Temp	DD	FFD	Mean (range)	Mean (range)
1	7	46.8	UP	Ontonagon	42	1600	110	14.0 (10-21)	25.3 (9-46)
2	10	47.4	UP	Keweenaw	40	1600	140	11.8 (5-19)	24.0 (10-45)
3	10	46.7	UP	Baraga	40	1600	130	16.2 (9-21)	21.0 (15-29)
4	7	46.3	UP	Gogebic	40	1800	60	15.0 (12-19)	22.6 (21-24)
5	10	46.1	UP	Iron	41	1800	100	15.1 (13-16)	19.6 (15-25)
6	10	45.2	UP	Menominee	42	1600	140	14.6 (12-16)	18.6 (14-23)
7	10	46.1	UP	Delta	41	1800	70	13.4 (9-16)	20.4 (13-31)
8	10	46.6	UP	Marquette	42	1600	150	14.7 (8-21)	23.5 (10-40)
9	10	46.5	UP	Alger	41	1400	140	12.0 (11-15)	16.5 (13-20)
10	11	46.0	UP	Schoolcraft	42	1600	110	16.3 (12-22)	24.6 (14-32)
11	9	46.7	UP	Chippewa	41	1400	150	11.1 (11-13)	17.6 (12-28)
12	10	46.3	UP	Chippewa	41	1400	130	13.5 (11-16)	22.6 (17-30)
13	9	46.0	UP	Mackinac	41	1600	120	13.2 (8-16)	18.3 (15-24)
14	10	45.6	LP	Emmet	42	1800	150	13.7 (7-16)	25.4 (15-32)
15	5	44.9	LP	Antrim	44	2000	140	15.5 (14-16)	24.5 (21-26)
16	10	44.9	LP	Montmorency	43	1800	70	13.7 (11-16)	19.8 (13-25)
17	10	45.5	LP	Presque Isle	44	1800	140	12.3 (9-16)	17.7 (12-28)
18	10	44.8	LP	Alcona	43	1800	130	16.3 (15-18)	25.8 (19-33)
19	4	43.4	LP	Montcalm	46	2400	130	15.2 (11-18)	21.5 (14-27)
20	5	43.9	LP	Lake, Mason	46	2200	120	14.6 (11-16)	21.7 (19-24)
21	8	44.3	LP	Manistee	46	2200	150	15.8 (11-19)	20.5 (16-27)
22	9	44.4	LP	Missaukee	43	1800	100	14.5 (11-19)	22.6 (14-38)
23	9	44.2	LP	Gladwin	44	2000	100	16.2 (11-18)	20.4 (15-28)
24	9	44.0	LP	Arenac	45	2000	130	13.8 (11-16)	17.5 (11-25)
25	5	43.6	LP	Sanilac	46	2400	130	12.4 (11-15)	15.6 (13-17)
26	1	43.3	LP	Saginaw	47	2600	150	12.1 n.a.	18.2 n.a.

[1] Seed source: Pop = seed source population number as given in Fig. 1; No = number of parental trees from which seed was collected in each particular population.

[2] Location: Area = Michigan seed source locations where UP refers to Upper Peninsula and LP refers to Lower Peninsula; County = Michigan county from which trees were sampled.

[3] Climatic data for each population of sampled trees using isopleth data from various Michigan climate maps: Temp = average annual mean temperature (°F; 1940-1969; MDA 1974); DD = seasonal (March - October) growing degree days (base 50°F; 1951-1980; Eichenlaub *et al.* 1990); FFD = average number of frost free days (i.e., number of days between last spring 32°F reading and first fall 32°F reading; 1940-1969; data drawn from MDA 1971).

[4] Height and diameter at breast height (DBH; 1.3 m) of sampled trees by population.

weeks, and then planted in a plowed and disked field in June, 1976. The plantation is located near 42°N, at the Fred Russ Experimental Forest, Michigan State University, in southwestern Michigan (Fig. 2). Trees were planted in a completely randomized design on 2.4 x 2.4 m square spacing with an average of 5 trees per seedlot. Overall, 1,088 trees were planted, with 636 trees from Upper Peninsula seed sources and 452 trees from Lower Peninsula seed sources.

Measurements of this plant material began in 1976 and continued through 1992. Height, diameter, bark color, insect damage, and tree survival were measured at varying intervals over 15 years (see also Miller *et al.* 1991). Height was measured in years 2, 6, 7, 8, and 10 post-establishment. Bronze birch borer damage was evaluated in years 10, 12, and 15 on a scale of 0 to 3, where 0 = tree apparently healthy with no evidence of borer infestation; 1 = less than half of the crown branches dead or dying with borer-related bark ridges obvious on the bark surface; 2 = more than half of the crown branches dead or dying with bark ridges obvious and borer exit holes often present; and 3 = tree dead, usually with borer-related bark ridges and exit holes present. Bark ridges (callus tissue) occur on branch and trunk sections in response to larval feeding (Anderson 1944, Barter 1957, Wilson and Haack 1990, Herms 1991). The presence of borer-related bark ridges was scored in years 12 and 15. Completion of larval development was also scored in years 12 and 15 by noting the presence of borer exit holes. Tree survival was computed for each of the 218 families in years 2, 6, 8, 10, 12, 15, and 16.

Historical temperature data were obtained from the Michigan Department of Agriculture, Climatological Division (MDA 1974). Average annual mean temperature was considered the most relevant climatic variable to use when considering global warming. The average annual mean temperature assigned to each seed source was obtained from a map showing annual mean temperatures for Michigan averaged over the 30-year period 1940-1969 (Fig. 1, Table 1, MDA 1974). This was done by locating all seed collection sites on the map and then assigning a particular annual mean temperature value to each seed source based on its location relative to the map isotherms. Although actual temperature data for each of the 26 collections sites would have been preferred, this was not possible because many collection sites had no nearby weather stations. Additional climatic data for each of the 26 collection sites were obtained in similar fashion from other maps (Table 1).

Using the map shown in Figure 1, the annual mean temperature values assigned to the 218 seed sources ranged from 40° to 47°F (4.4°-8.3°C; Table 1). Actual average annual mean temperature values from several official weather stations for this same area and time period ranged from 39.7° to 47.1°F (4.3°-8.4°C; MDA 1971). The average annual mean temperature of the plantation site was about 49.0°F (9.4°C; MDA 1971). Overall, assuming that the birch seed sources were genetically acclimated to the average annual mean temperature of their original sites, then the birch trees growing at the plantation site were theoretically experiencing levels of climatic warming equivalent to 1.9° to 9.3°F (1.1° to 5.2°C; Table 2). For ease of discussion, I will use the term climatic differential (CD) to represent the difference (in °C) between the average annual mean temperatures of the seedlot origin and the plantation site.

Table 2.—Average annual mean temperature data and percent of paper birch trees (both live and dead) that had bronze birch borer-induced ridges and/or borer exit holes along their lower trunk in years 12 (1987) and 15 (1990) post-establishment by climatic differential treatment.

CD[1]	Corresponding isotherms[2]		Mean temperature difference between origin and plantation site[3]		Percent of trees with borer-induced ridges[4]		Percent of trees with borer exit holes[4]	
	°F	°C	°F	°C	Yr 12	Yr 15	Yr 12	Yr 15
CD1°	46, 47	7.7, 8.3	2	1.1	30ab	32b	10b	13b
CD2°	44, 45	6.7, 7.2	4	2.2	24b	33b	8b	18b
CD3°	42, 43	5.6, 6.1	6	3.3	39ab	46b	12b	25b
CD4°	40, 41	4.4, 5.0	8	4.4	50a	60a	23a	40a

[1] CD = Climatic differential, which is a term that reflects the difference between the average annual mean temperature of the seed sources within a particular treatment group and the average annual mean temperature of the plantation site, which was 49°F (9.4°C). The value 1° in CD1°, for example, reflects the annual mean temperature difference between the origin and the plantation site, e.g., 1.1°C (see footnote 3 below).

[2] The isotherms of average annual mean temperature in °F (and the approximate °C equivalent), as given in Fig. 1, that correspond to each CD grouping.

[3] For each CD grouping, the mean temperature difference between the origin and the plantation site was calculated as follows. For CD1°, the corresponding isotherms were 46° and 47°F. However, these isotherms span the temperature range from 46° to 47.9°F, with a mean of about 47°F. Thus, the temperature difference between the origin and the plantation site in this case is 2°F (49°-47°) or about 1.1°C. These values represent the theoretical level of climatic warming that the corresponding trees were experiencing while growing at the plantation site in southern Michigan.

[4] Percent of trees with borer-induced ridges and/or borer exit holes along the lower trunk; percent values followed by the same letter within a column are not significantly different ($p<0.05$, Kruskal-Wallis nonparametric ANOVA on ranks followed by the Dunn's test for multiple comparisons).

One weakness of using data from genetic tests plantings to study the impact of climatic warming, however, is that other variables besides temperature change when moving seed from its site of origin to the test location. Photoperiod, for example, is one important variable for which many tree species, including paper birch, are genetically adapted (Vaartaja 1959, Wright 1976, Kozlowski *et al.* 1991, Farmer 1993). In the northern hemisphere, typically, northern sources tend to flush earlier and terminate shoot growth earlier when moved southward compared with more local sources (Wright 1976, Kozlowski *et al.* 1991). In the present study, birch seed was collected between Lat. 43.3°N and 47.4°N, and then planted at 42.0°N. On the longest day of the year there is about 15.2 hours of light at 42.0°N, 15.5 hours at 43.3°N, and 16.0 hours at 47.4°N. Similarly, on the shortest day of the year, they experience about 9.1 hours of light at 42.0°N, 9.0 hours at 43.3°N, and 8.4 hours at 47.4°N. As a means of minimizing any influence of photoperiod, linear regression analyses were also conducted on two subsets of the original 26 populations (collection sites) in which the latitudinal spread among the seed sources was further reduced. In the first case, only the 13 populations from the Lower Peninsula of Michigan were tested (populations 14-26, Fig. 2, Table 1), representing collections within a 2° span of latitude and a 5°F (2.8°C) span in annual mean temperature. In the second case, 12 birch populations were tested (populations 4-7, 10, 12-18; see Fig. 2, Table 1), representing collections within a 1.5° span in latitude and a 4°F (2.2°C) span in annual mean temperature.

Analyses were conducted using SAS and SigmaStat. Height and borer data were analyzed on an individual tree basis, whereas tree survival was calculated on a family basis (n = 218), using only those birch families that had at least one tree surviving through the second growing season (1977). Each tree was assigned to one of four "treatments" based on the average annual mean temperature of its origin. The four groupings were isotherms 40° and 41°F, 42° and 43°F, 44° and 45°F, and 46° and 47°F (Table 2). The climatic differential term assigned to each grouping was CD1° (46-47°F), CD2°(44°-45°F), CD3° (42°-43°F), and CD4° (40°-41°F; Table 2). These terms, CD1°-CD4°, reflect the average climatic differential in °C for each treatment group, e.g., for CD1° the midpoint climatic differential value is actually 1.1°C and represents the average degree of climatic warming that the trees in isotherms 46°F and 47°F were theoretically experiencing (Table 2). Analysis of variance (ANOVA) was used to test for differences among treatments; a p level of 0.05 was used for significance. Tree height and survival data were analyzed using the GLM procedure of SAS, followed by the Student-Newman-Keuls means separation test. The arcsin square root transformation was performed on the survival data prior to analysis. Borer data were analyzed using the Kruskal-Wallis nonparametric test of SigmaStat, which performs an ANOVA on ranks; Dunn's test was used to make multiple comparisons among treatment means.

RESULTS

Paper birch growth, survival, and susceptibility to bronze birch borer attack were significantly affected by the climatic differential between the origin of the seed source and the plantation site (Fig. 3). Overall, height growth decreased as the climatic differential increased (Fig. 3A). Differences in height growth were already evident by the end of the second growing season in 1977 ($p < 0.0001$; Fig. 3A). By the end of the sixth growing season (1985), CD1° trees were the tallest, with tree height becoming successively shorter for the CD2°, CD3°, and CD4° trees ($p < 0.0001$; Fig. 3A).

As the trees aged, tree survival tended to decrease as the climatic differential increased (Fig. 3B). From year 2 (1977) to year 8 (1983) post-establishment, little tree mortality occurred, but thereafter tree mortality accelerated, especially among the trees experiencing the greatest climatic differential (CD4°; Fig. 3B). Treatment survival values first became significantly different in year 12 post-establishment (1987; $p < 0.006$), when survival for the CD4° trees was lower than that for trees from the other three groups. A similar pattern of tree survival continued into years 15 (1990; $p < 0.001$) and 16 (1991; $p \leq 0.0008$) post-establishment.

As the trees became older, tree susceptibility to the bronze birch borer increased with increasing climatic differential (Fig. 3C). Of the 146 paper birch trees that died between 1985 and 1991, 143 (98%) of them had signs of borer attack along their lower trunk. A similar pattern emerged during each of the 3 years when borer damage was assessed (1985, 1987, 1990; $p < 0.001$ for each), i.e., CD4° trees experienced the highest levels of borer attack, while CD1° trees experienced the lowest levels (Fig. 3C). As would be expected from the above discussion, the occurrence of borer-related bark ridges and exit holes was more common on CD4° trees than on CD1° trees (Table 2).

Regarding the two subsets of birch populations in which the latitudinal spread was reduced, paper birch growth, survival, and susceptibility to bronze birch borer were all significantly affected by the climatic differential between the origin of the seed source and the plantation site. In the first case, linear regression analysis of the 13 populations from Michigan's

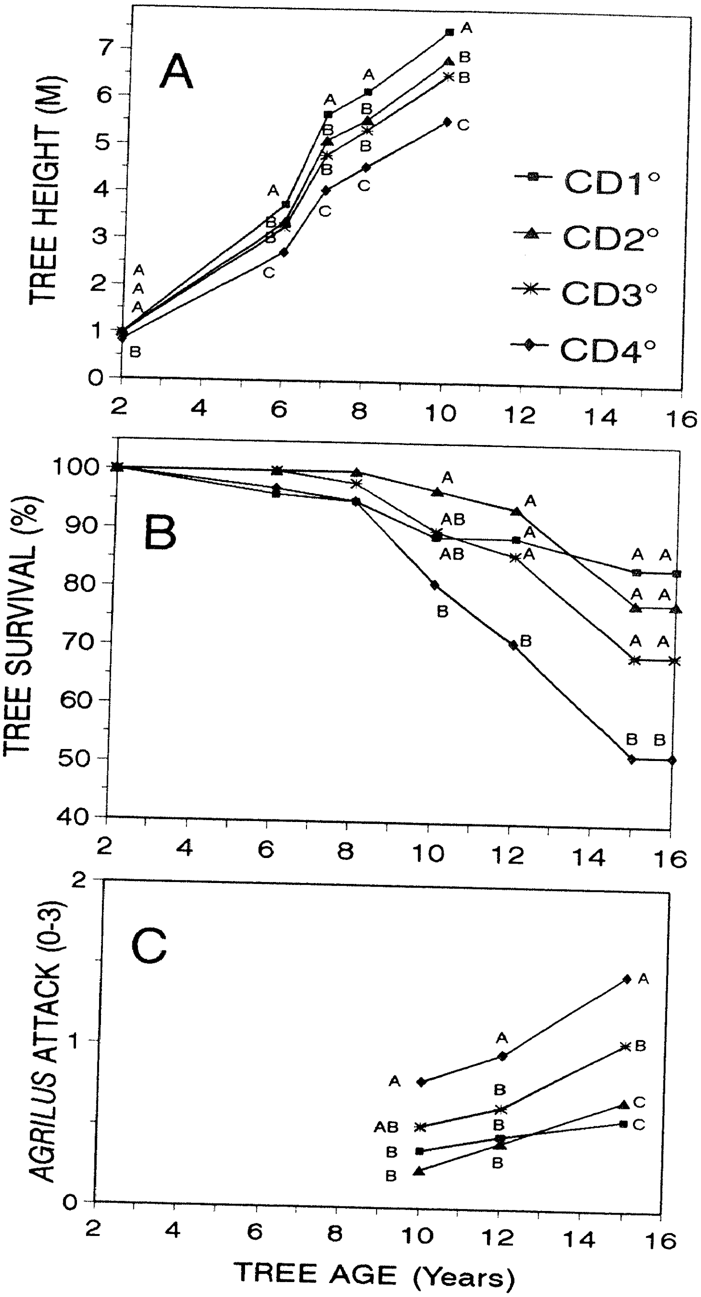

Figure 3.—Paper birch mean height growth (A), mean survival (B), and mean susceptibility to borer attack (C) over time (years post-establishment) in a provenance plantation in southern Michigan. Survival was based on only those trees that lived for the first two years. The 0-3 borer rating scale is presented in the Methods section. Trees are divided into four treatment groups based on the climatic differential (CD) between the average annual mean temperature of the origin of each seed source and the annual mean temperature of the plantation site. On average, trees assigned to treatment CD1° had a 1.1°C difference between the origin and the test site, CD2° a 2.2°C difference, CD3° a 3.3°C difference, and CD4° a 4.4°C difference (see Table 2). For each parameter, means followed by the same letter within a column (i.e., year) are not significantly different ($p < 0.05$, ANOVA and Student-Newman-Keuls test for height and survival data; Kruskal-Wallis nonparametric ANOVA and Dunn's test for borer data).

Lower Peninsula (populations 14-26) demonstrated that as the climatic differential between the origin and the test site increased, tree height decreased ($p < 0.006$, $r^2 = 0.51$, $n = 13$ birch populations), tree survival decreased ($p < 0.012$, $r^2 = 0.49$, $n = 13$), and tree susceptibility to borer attack increased ($p < 0.006$, $r^2 = 0.51$, $n = 13$). Similarly, in the second case, which included 12 birch populations (populations 4-7, 10, 12-18), linear regression analysis indicated that as the climatic differential between origin and test site increased, tree height decreased ($p < 0.02$, $r^2 = 0.43$, $n = 12$ birch populations), tree survival decreased ($p < 0.038$, $r^2 = 0.36$, $n = 12$), and tree susceptibility to borer attack increased ($p < 0.012$, $r^2 = 0.45$, $n = 12$).

DISCUSSION

Cannell *et al.* (1989) state that most tree species will benefit from an increase in annual mean temperature of 1°C, but genetic increases will often cause growth reductions or mortality. Similarly, Matyas (1994) states that an increase in temperature will positively affect tree growth only within each species' limits of physiological and ecological tolerance. Paper birch appears to have narrow limits of heat tolerance. In the present study, as the climatic differential increased for individual seed sources, birch trees grew slower, experienced higher mortality rates, and sustained higher rates of borer attack. Such results strongly suggest that paper birch will experience dramatic decline along its southern range in response to climatic warming, and that the bronze birch borer will likely be the principal mortality agent involved in future birch decline. Similar predictions for widespread decline of paper birch in response to global warming have been made by Pastor and Post (1988), Overpeck *et al.* (1991), Botkin and Nisbet (1992), Reed and Desanker (1992), Reed *et al.* (1992b), Solomon and Bartlein (1992), and Jones *et al.* (1994). Several of the general circulation models predict reduced rainfall or increased rates of evapotranspiration in association with increased air temperatures (Karl *et al.* 1991, IPCC 1992). Thus, if higher temperatures are combined with reduced rainfall, then many tree species will likely be even more susceptible to attack by trunk-boring insects (Mattson and Haack 1987a, 1987b).

As mentioned above, differences in sensitivity to photoperiod between northern and southern seed sources could partly explain the relatively poorer performance exhibited by the more northerly birch families in the present study. However, when I restricted the birch data set to seed sources that spanned as little as 1.5° and 2° latitude, the same patterns of reduced paper birch growth, survival, and resistance to bronze birch borer persisted. In a *Picea abies* provenance study, Vaartaja (1959) reported little difference in tree growth for seed sources collected between 47°N and 52°N and then planted at 47°N. Although the general rule for photoperiod is that the further north the origin, the more photoperiod sensitive the seed source, Beuker (1994) states that the exact effect of photoperiod cannot be predicted. Although changes in photoperiod are undoubtedly important, the significant relationships found here for two relatively tight latitudinal bands, strongly suggest that the differences in seed source performance in the present study were greatly influenced by temperature.

Another possible reason for the poorer growth of cold-adapted seed sources at warmer sites, is that they may have higher dark respiration rates than local sources (Kozlowski *et al.* 1991, Mebrahtu *et al.* 1991). Consequently, when growing in a warmer environment, cold-adapted sources expend more energy and have lower net assimilation rates than local sources. Reduced rates of net assimilation can help explain greater susceptibility to borer attack since compromised trees would likely produce fewer defensive compounds and have reduced rates of diameter growth (Herms and Mattson 1992). Slow stem growth has been shown to increase susceptibility of paper birch to the bronze birch borer (Herms 1991, Herms and Mattson 1991).

In Michigan, as was true for much of North America, 1988 was a year of severe heat and drought (Kerr 1989, USGS 1991). In the Great Lakes region, outbreaks of several cambial-feeding forest insects occurred in 1988, including the bronze birch borer (Haack and Mattson 1989, Jones *et al.* 1993). Part of the rapid increase in birch mortality and borer attack between years 12 (1987) and 15 (1990) in the present study (Figs. 3B, 3C) could have been triggered by the 1988 drought. In fact, it appears from the slopes of tree survival (Fig. 3B) and borer attack (Fig. 3C) for the four CD treatments, that the CD2°, CD3°, and CD4° trees were much more negatively impacted by the heat and drought than were the CD1° trees, suggesting that an increase in annual mean temperature of about 1°C will have limited detrimental effects on paper birch, but that increases of 2°C and more will be very damaging. In support of this contention are studies in the Upper Peninsula of Michigan by Reed *et al.* (1992a) and Jones *et al.* (1993) that report slower growth and higher mortality rates for paper birch, following the warm and dry years of 1987 and 1988 when mean summer temperatures were about 1°C above normal. In controlled studies on yellow birch, *Betula alleghaniensis* Britton, limited rootlet mortality was observed with a 1°C increase in soil temperature, but extensive rootlet mortality occurred with increases of 6-7°C (Redmond 1955, 1957).

As global warming occurs, I predict that those tree species with associated cambial-feeding insects that invade the trunk will be the first tree species to exhibit widespread mortality. This guild of trunk-infesting, cambial-feeding insects poses the greatest lethal threat to their host trees (Haack and Slansky 1987, Mattson *et al.* 1988, Haack and Byler 1993). Trees are typically highly resistant to cambial-feeding insects, but during periods of stress, they become more susceptible to such insects (Mattson and Haack 1987a, 1987b; Millers *et al.* 1989). Some of the boreal tree genera that occur in Michigan that can be killed by trunk-infesting, cambial-feeding insects, and thus will likely be more at risk as climatic warming occurs, include *Abies*, *Betula*, *Larix*, *Picea*, *Pinus*, *Populus*, and *Tsuga* (Table 3). Although climatic warming in the northern hemisphere is predicted to bring about tree mortality along the southern edge of a species range, the northern range of these same tree species may expand (Cannell *et al.* 1989, Woodward 1992, Sykes and Prentice 1995). In fact, paper birch has been documented to be expanding its range northward into the tundra, possibly in response to climatic warming (Woodward 1992).

Table 3.—Boreal tree genera of the Great Lakes region that will likely experience widespread insect-induced mortality as a result of climatic warming and the corresponding trunk-infesting, cambial-feeding insect that will likely be the major mortality agent.

Host tree		Insect species		
Genus	Common name	Species	Common name	Family
Abies	Fir	*Pityokteins sparsus* (LeConte)	Balsam fir bark beetle	Scolytidae
Betula	Birch	*Agrilus anxius* Gory	Bronze birch borer	Buprestidae
Larix	Larch	*Dendroctonus simplex* LeConte	Eastern larch beetle	Scolytidae
Picea	Spruce	*Dendroctonus rufipennis* (Kirby)	Spruce beetle	Scolytidae
Pinus	Pine	*Ips pini* (Say)	Pine engraver beetle	Scolytidae
Populus	Aspen	*Agrilus liragus* Barter and Brown	Bronze poplar borer	Buprestidae
Tsuga	Hemlock	*Melanophila fulvoguttata* (Harris)	Hemlock borer	Buprestidae

The present study shows that data from genetic test plantings of forest tree species can provide insight into how tree-insect interactions may change as air temperatures increase in the future. Similar studies should be conducted by others. This would be particularly useful in areas where the local geography allows seed to be collected from several temperature regimes within a narrow latitudinal band and thereby allow the effects of temperature and photoperiod to be partly disentangled.

ACKNOWLEDGMENTS

I wish to thank the staff of Michigan State University, Fred Russ Experimental Forest, for field assistance in this study; Paul Bloese, Michigan State University, Department of Forestry and Michigan Cooperative Tree Improvement Program, for making available early tree performance data from this paper birch plantation; and Paul Bloese, Daniel Herms, Dow Gardens, and Robert Lawrence, USDA Forest Service, for reviewing an earlier draft of this manuscript.

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VARIATIONS IN SPRUCE NEEDLE CHEMISTRY AND IMPLICATIONS FOR THE LITTLE SPRUCE SAWFLY, *PRISTIPHORA ABIETINA*

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INTRODUCTION

The plant-herbivore system of Norway spruce, *Picea abies* Karst., and the little spruce Sawfly, *Pristiphora abietina* Christ (Hymenoptera: Tenthredinidae), provides a good opportunity to study the synchronization between the host's bud burst and the beginning of larval feeding. Adults of the sawfly emerge in spring at about the same time as spruce bud break and oviposit in the soft, partially-expanded needles. Every single spruce bud is suitable for oviposition for only a few days. The emerging larvae almost always remain on the expanding bud where they hatch and only under starving conditions do they switch buds. After 2 or 3 weeks, depending on weather conditions, the larvae are fully grown and drop to the ground to form cocoons. They overwinter in the litter until the next spring.

The larvae of the little spruce sawfly are early-spring feeders and restricted to feeding on expanding spruce needles. Their success and survival depend on the exact phenological coincidence of bud break and oviposition, because larvae can initiate feeding only in flushing buds. Seasonal variations in the timing of bud break can therefore be of defensive value for the trees: trees that are genetically predisposed to flush needles earlier or later than the rest of the stand, thereby preceding or following the sawfly swarming, may escape larval feeding.

During the last 3 decades the sawfly has become an important spruce pest in several parts of Austria. The most severe attacks occur in lowland areas where the natural deciduous forests have been replaced by monocultures of Norway spruce. As the larvae feed exclusively on current-year needles, the affected trees do not die, but repeated infestations lead to deformed, bushy crowns and to reduced height and volume increments. In recent years, an increase in frequency and duration of epidemics has been observed, particularly in areas exposed to air pollutants like sulfur and nitrogen (Sierpinski 1985, Berger 1992). There is good reason to believe that some air pollutants may favor the sawfly's success, either by improving food quality due to changes in needle chemistry and/or by weakening the tree's defense system (Schafellner *et al.* 1993, Schafellner *et al.* 1994, Berger und Katzensteiner 1994).

This paper reports a study on the most important food quality parameters of newly emerging needles during the short period of larval feeding, and the changes that occur during the weeks of rapid growth. Additionally, the effect of excess nitrogen input (via fertilization) on needle chemistry and the nutritional value of the spruce needles for the feeding larvae is demonstrated.

METHODS

Three even-aged stands of trees 16 years old, were selected for study to see if food quality parameters show significant site-specific variation. Stand A is located at ca. 550 m elevation in Hochstrass, Lower Austria, and stands B and C are located at ca. 720 m elevation in the Hausruck, Upper Austria. Moderate sawfly attack was observed at stand A during the mid-1980's. Throughout the 3 years of attack, tree flushing and sawfly attack for every individual tree was recorded (Holzer 1988). The stand was attacked irregularly so that affected and unaffected individuals often stood close together, although the trees did not vary in the timing of bud break and should have been suitable for oviposition. From spring 1988 onwards, the sawflies vanished from the area so that during the 1989 sampling year, no frass was collected. Based on the data collected during the years of attack, 15 trees that had been affected once and 15 trees that remained unaffected, were selected for

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investigation. Sampling was done on 3 occasions at 2-week-intervals during needle expansion in spring: needles were taken from twigs of the upper crown region, where the sawflies prefer to oviposit. To record differences between the physiologically older needles at the bottom and the physiologically younger needles at the top of a given shoot, on the second and third occasion needles were separated according to their location on the expanding bud: top (t), middle (m), and bottom (b) needles.

In contrast to stand A, the trees on stand B were undergoing an actual heavy sawfly attack during the sampling period in spring 1989. Additionally, large variation in the timing of bud burst of the trees was found: the early flushing individuals flushed 4 weeks before the late flushing ones. Sawfly attack, however, occurred only on the late flushing trees. The early flushing trees (n = 10) were harvested 3 times (1,2,3), the late flushing trees (n = 10) only twice (1a, 2a). After the second sampling of the attacked, late flushing trees (2a), the larvae were fully grown and were no longer feeding. Although the sampling on early and late flushing trees differed by more than 4 weeks, needles from sampling date 1 corresponded phenologically with 1a and needles from sampling date 2 corresponded with 2a.

A fertilization experiment was done on stand C with late flushing, attacked trees. The stand was fertilized with 130 kg nitrogen per ha/year, applied on 4 occasions: in summer 1989, in spring and summer 1990, and in spring 1991. Sampling was done during needle expansion in June, 1991, from 15 fertilized and 17 unfertilized (i.e., control) trees.

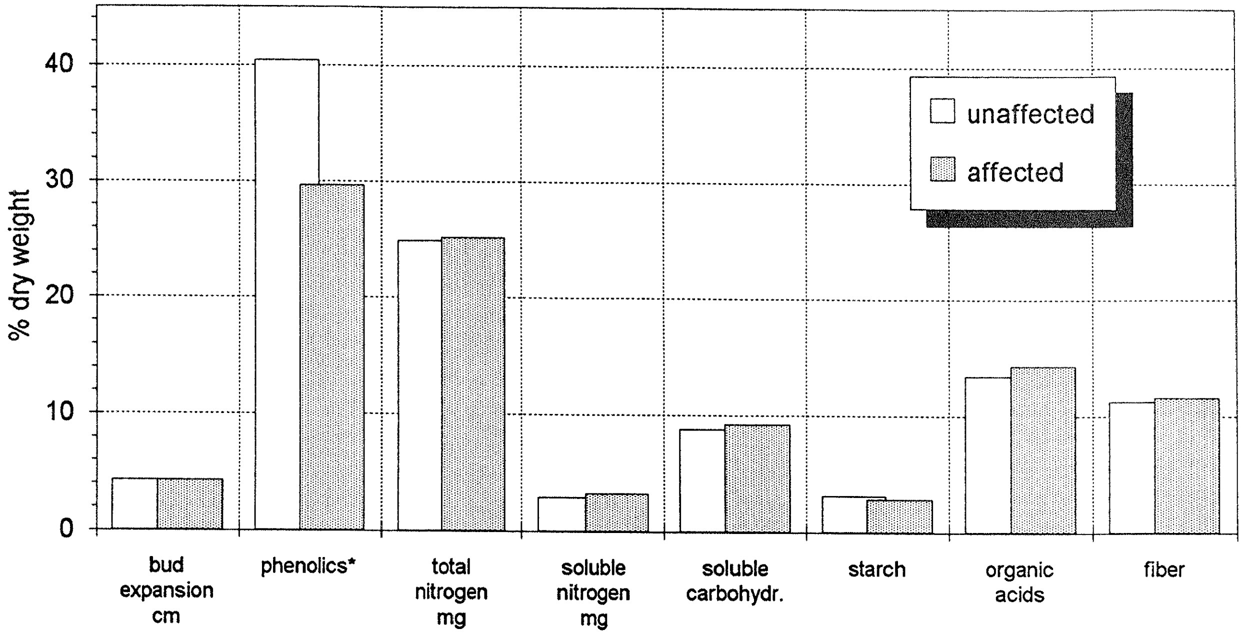
Samples for chemical analyses were put into liquid nitrogen, then freeze-dried and milled. Nitrogen (total and soluble) was determined by micro-Kjeldahl technique with selenium as catalyst. Soluble carbohydrates (sugars, cyclitols) as well as the organic acids (quinic acid, shikimic acid) were determined by GLC, starch content was estimated enzymatically as glucose-equivalents. Needle phenolics were defined and determined as the potential of the needle extract (done with boiling 50% aqueous methanol) to precipitate bovine serum albumin (BSA). Absorption was measured at 590 nm. Fiber (cellulose, hemicellulose, lignin) was estimated after a method to determine the fiber content of cattle food: after hydrolyzation with strong acids and alkalines and heating up to 550 degrees, the remaining ash residue was weighed. All values are expressed on a dry weight basis.

Larval experiments were performed at stand C with late flushing, fertilized, and unfertilized trees in spring 1991. Larvae from naturally attacked buds as well as larvae (1st to 3rd instar) placed on expanding buds and enclosed in cages were studied. After cocoon formation, larvae were collected, sexed, and weighed. Parasitized larvae were excluded from the analyses.

RESULTS

During initial feeding in mid-May (bud length 4 cm) the young sawfly larvae are confronted with both high total and soluble nitrogen (2% N tot. and 0.2% N sol.) and organic acid concentrations (12%). On the other hand, carbohydrates (sugars and cyclitols) and starch are at rather low levels (8% and 2%). Fiber was approximately 10% of the needle dry weight (dw). For all these traits, damaged and undamaged trees did not differ (Fig. 1, upper graph). The crucial difference, however, was the phenolics: concentrations were significantly lower in the attacked trees than in the unattacked trees ($p<0.05$). Four weeks later in mid-June, at the end of larval feeding, again a very much lower phenolic content was found in the attacked trees, with the differences being highly significant ($p<0.01$). Compared with the situation at the beginning of needle expansion and larval feeding, the chemical traits had changed a lot (Fig. 1, lower graph): the expanding buds were now 12 cm long, nitrogen concentrations decreased (due to dilution effects) to half of the initial value. The same was true for needle phenolics and organic acids, while sugars and starch increased. The young needles were now self-supporting with photosynthates. Fiber increased to more than 20% dw. With the exception of needle phenolics, all other traits showed comparable levels in both attacked and unattacked trees. Highest phenolic concentration always occurred in the youngest needles (Fig. 2). After one month, the concentration dropped to less than half of the initial value, in both attacked and control trees. In almost all cases a similar decline from the top (t) to the bottom (b) needles was found, as was observed during needle expansion from sampling period 1 to sampling period 3. Differences in phenolic concentrations between attacked and unattacked trees were always significant ($p<0.05$) or even highly significant ($p<0.05$-0.01).

In contrast to stand A, where the trees flushed almost simultaneously, Figure 3 presents the results of the phenolic analyses at stand B, where a 4-week-difference in the timing of bud burst occurred between early flushing, unattacked, and late flushing, attacked trees. When the larvae started feeding, the affected trees contained less than half of the phenolics of



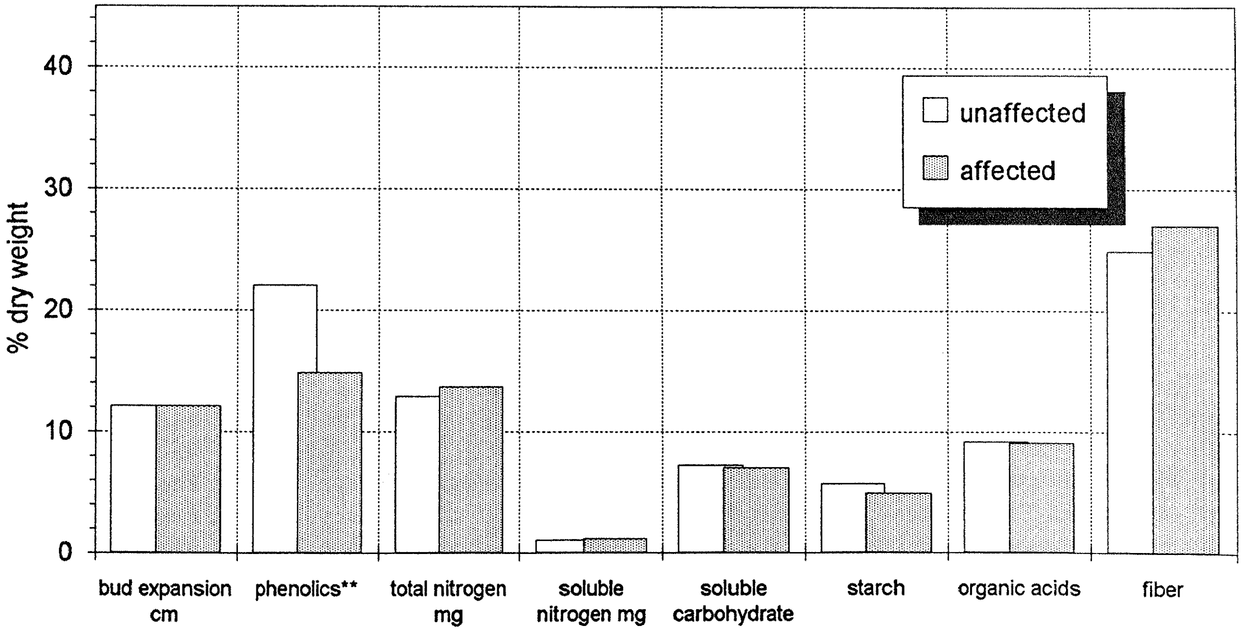


Figure 1.—Spruce needle chemistry during initial (upper graph) and at the end (lower graph) of larval feeding on stand A (identical flushing time). Asterisks indicate significant differences between once affected (n = 15) and unaffected (n = 15) trees (levels of significance: * $p < 0.05$, ** $p < 0.01$).

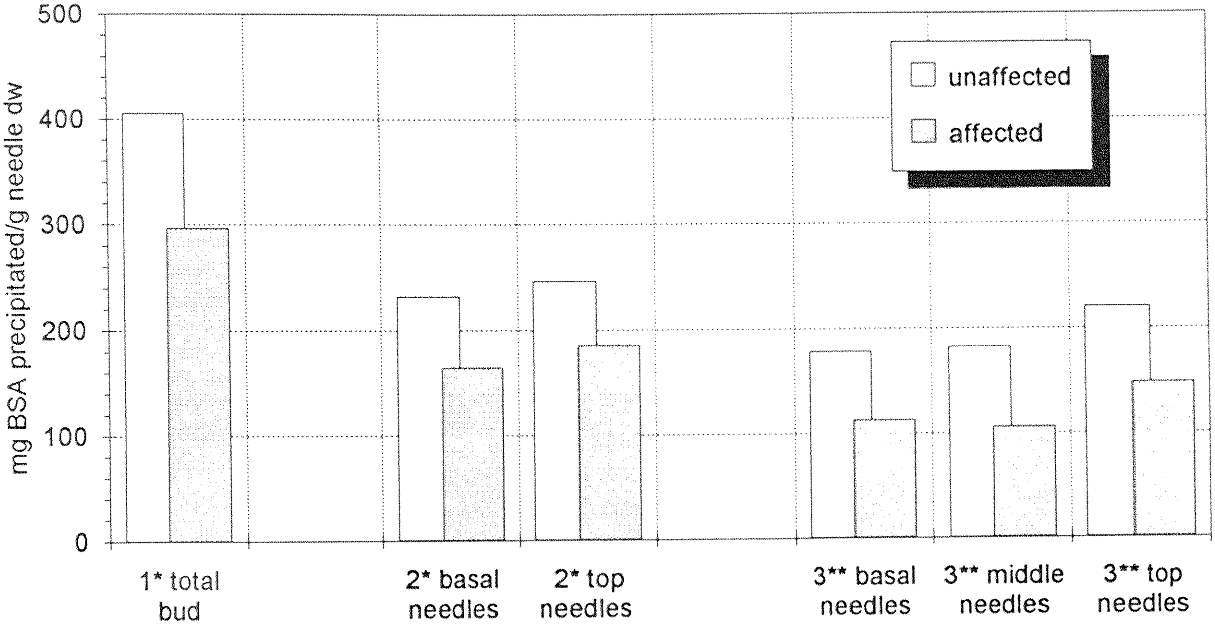


Figure 2.—Changes in protein precipitating ability ("phenolics") of spruce needle extracts during the period of larval feeding on stand A (identical flushing time). Asterisks indicate significant differences between once-affected (n = 15) and unaffected (n = 15) trees (levels of significance: * $p < 0.05$, ** $p < 0.01$). Dates of sampling: 1) 18 May 1989, 2) 1 June 1989, and 3) 14 June 1989.

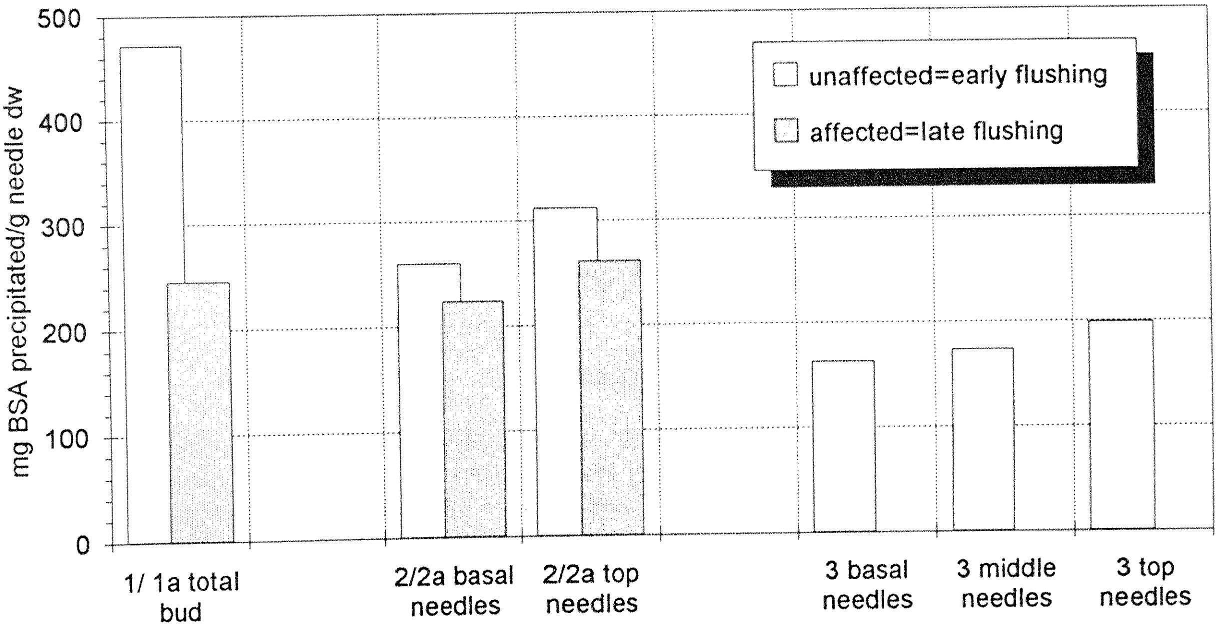


Figure 3.—Changes in protein precipitating ability ("phenolics") of spruce needle extracts of early flushers (i.e., unaffected; n = 10), and late flushers (i.e., actually affected; n = 10) during the period of needle expansion and larval feeding on stand B. Dates of sampling: early flushers: 1) 3 May 1989, 2) 17 May 1989, and 3) 1 June 1989; late flushers: 1a) 1 June 1989, and 2a) 14 June 1989.

the control trees. Within the following 4 weeks, the phenolics decreased in the controls, but did not in the attacked ones; concentrations being almost unchanged throughout larval feeding.

The effect of N-fertilization on the expanding spruce needles was clear: total and soluble nitrogen were significantly higher in the fertilized trees and so were carbohydrates ($p<0.001$). On the other hand, fiber, starch, organic acids and especially needle phenolics decreased in the fertilized trees, although differences were not significant (Fig. 4). Comparing the actual needle nitrogen content with the needle phenolics, a close relationship can be seen (Fig. 5): the higher the needle phenolics, the lower the nitrogen content and vice versa. Within the control trees, the negative correlation between nitrogen and phenolics was especially striking ($r = -0.80$). A negative correlation also occurred between larval weight and the needle phenolic concentration: female larvae feeding on trees with low needle phenolics weighed significantly more than females feeding on high-phenolic needles (Fig. 6).

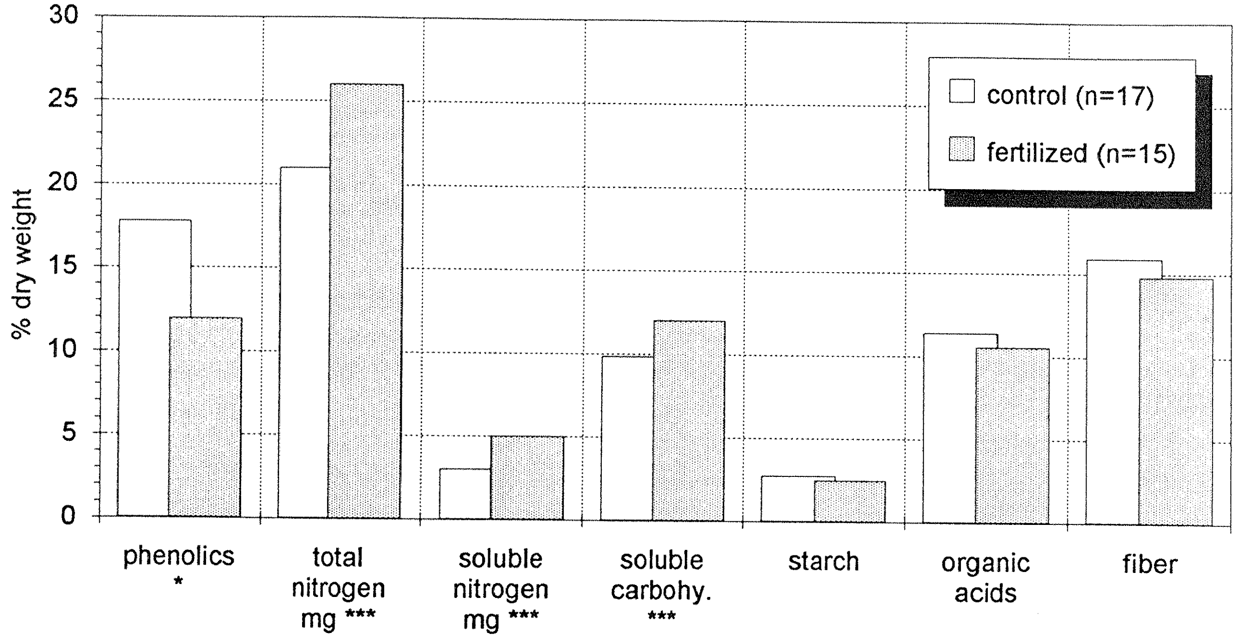


Figure 4.—Effects of N-fertilization on expanding spruce needles of actually attacked, late flushing spruce trees in stand C. Asterisks indicate significant differences between fertilized (n = 15) and control (n = 17) trees (levels of significance: * $p \leq 0.05$; *** $p \leq 0.001$). Date of sampling: 15 June 1991.

DISCUSSION

Among the potential defense systems of Norway spruce against the little spruce sawfly, variance in bud burst is of primary importance for the trees to escape from larval feeding. Phenological coincidence with bud break is a prerequisite for oviposition of the sawfly: a narrow 'phenological window' (Feeny 1976) exists between insect phenology (oviposition) and host plant phenology (bud break) and strongly affects the population dynamics of this insect.

Apart from alterations in the phenology of needle flush, variability in needle biochemicals within the host species plays a very important role in the plant-herbivore interactions. The quality of food for invertebrate herbivores in general is reduced by low water and nitrogen, and by high fiber content, toxins, and digestibility-reducing compounds (polyphenols) (Mattson 1980). Nutritional quality of foliage is considered to decline as the growing season progresses (Bryant *et al.* 1983): nitrogen and water content decrease (Mattson 1980, Scriber and Slansky 1981), while fiber (Coley 1983) and tannins (Feeny 1970) increase.

It has been suggested that tannins and phenolics are more concentrated in mature rather than immature foliage because of compartmentation problems and the potential for autotoxicity in the rapidly expanding foliage (Herms and Mattson

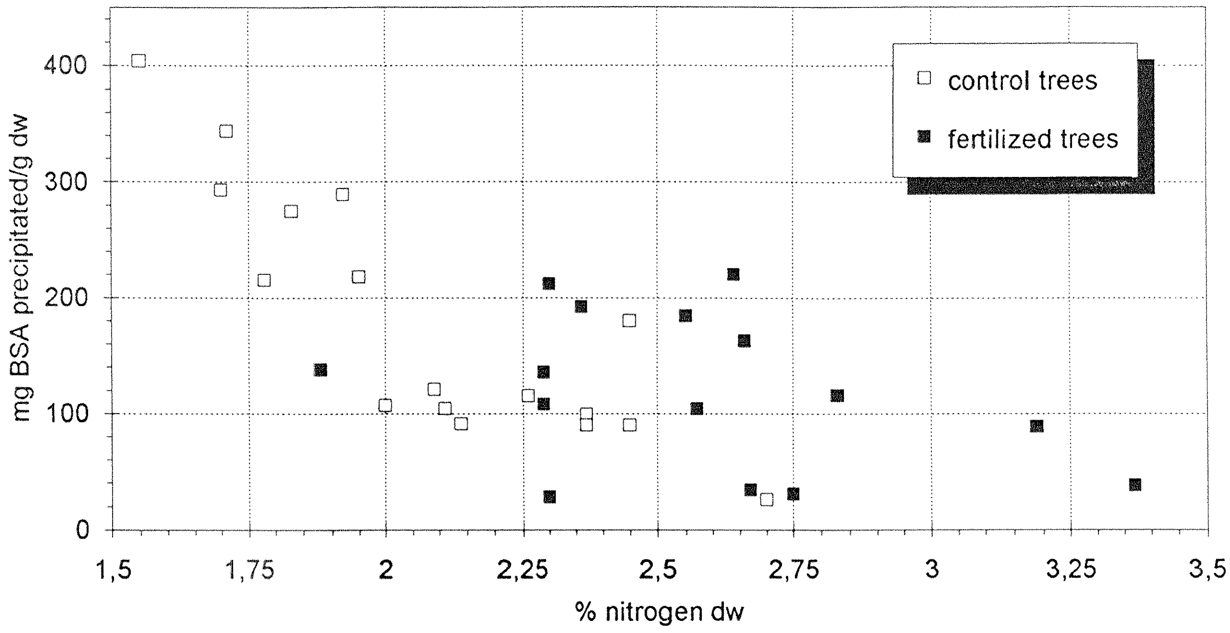


Figure 5.—Protein precipitating ability ("phenolics") and nitrogen content of expanding spruce needles from individual fertilized (n = 15) and unfertilized (n = 17) trees. Date of sampling: 15 June 1991.

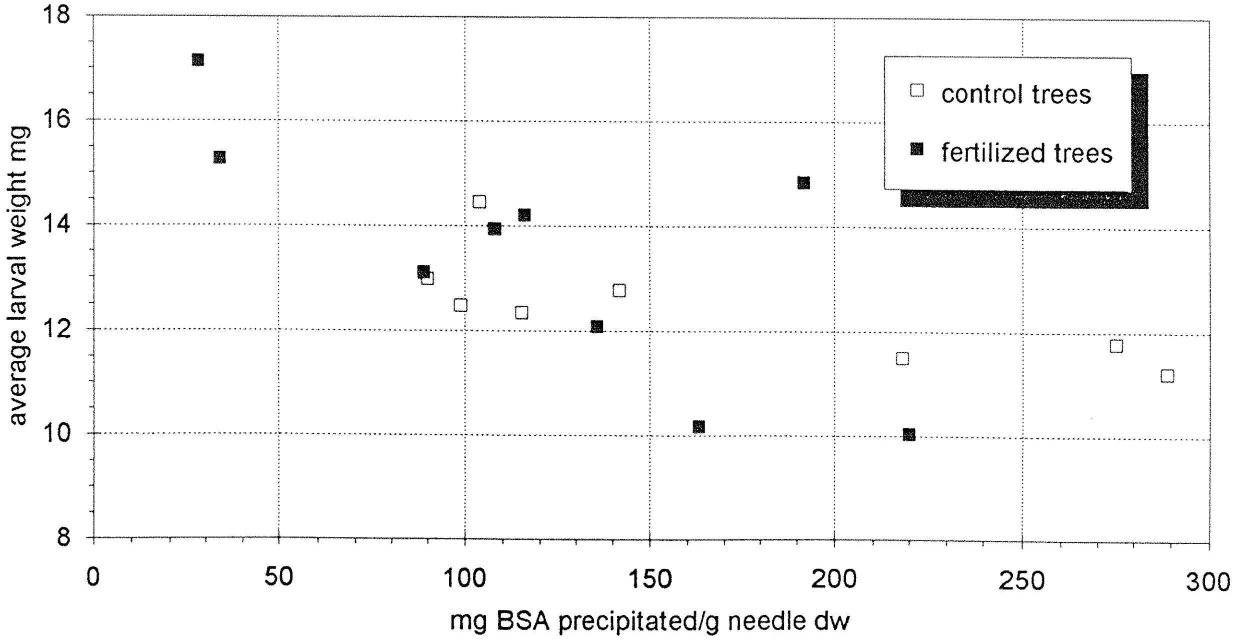


Figure 6.—The relationship between the protein precipitating ability ("phenolics") of expanding spruce needles from individual fertilized (n = 9) and unfertilized (n = 8) trees, and the average weights of female larvae (n = 30-40 per tree) feeding on these trees.

1992). However, several authors have recorded higher levels of phenolics in immature than in mature leaves (Coley 1983, Puttick 1986, Mauffette and Oechel 1989, Hatcher 1990), as did the present study, but finally a gradual increase in concentration occurred with needle maturation (Schafellner *et al.* 1993). The decline of phenolics during the first month of needle expansion may be attributed to a dilution effect resulting from the addition of other leaf constituents and/or as phenolics are important precursors to lignin, to an incorporation into the cell wall.

Flushing needles of Norway spruce have the highest concentrations of key nutrients (total nitrogen, amino acids, water), however, they also contain the highest concentrations of secondary compounds (quinic acid, phenolics). Although nitrogen is an important factor for growth and survival and positive correlations between needle carbohydrates and insect performance have been demonstrated (Schopf 1986, Jensen 1988), we propose that between-tree differences in needle phenolics caused the observed pattern of sawfly attack. The amount of phenolics in the newly emerging needles may be crucial for the feeding larvae. High phenolic levels suggest a potential defense mechanism of the host tree and seems to be determined more by intraspecific genetic differences than by site-dependent or seasonal factors (Lunderstädt 1980). Additionally, we found no hint that needle quality deteriorated for the years after sawfly attack: nitrogen and carbohydrates did not decline and phenolics did not increase.

Fertilization with N increased the nutritional value of spruce needles for *P. abietina* larvae. This increase in nutritional value is based on an increase in the concentration of needle nitrogen and carbohydrates and a reduction in needle phenolics. Our results are consistent with several reports that N fertilization increases the concentration of nitrogen and reduces the concentrations of carbon-based secondary compounds in leaves of woody plants (Waring *et al.* 1985, Haukioja *et al.* 1985, Glyphis and Puttick 1989) and that such changes in leaf chemistry are causally related to changes in leaf nutritional value for immature insects (Bryant *et al.* 1987).

An oversupply of N tends to change the metabolism of carbohydrates and nitrogen. Probably because of a higher demand for carbohydrates during N-absorption and N-assimilation, the phenolic content of spruce needles decreases (Tuomi *et al.* 1990), whereas the concentration of total nitrogen and free amino acids increase with N fertilization or N deposition (Näsholm and Ericsson 1990). The low concentrations of phenolics in the leaves of fertilized trees may explain the increased insect susceptibility of trees exposed to N deposition and excess N (Berger and Katzensteiner 1994).

In our study N fertilization also caused a significant increase in the needle concentrations of soluble carbohydrates (sugars, cyclitols), probably due to a stimulated growth of photosynthetic tissue, and a slight decrease in starch. N fertilization did not cause changes in beech foliar concentrations of total sugars, starch or soluble protein, but total phenolic compounds decreased by about a third (Balsber-Pahlsson 1992). Changes in total sugar content were found in the current-year needles of Scots pine (Ericsson 1979).

Our results indicate that a shift in the carbon/nutrient balance of spruce by N fertilization increases the nutritional value of the needles for *P. abietina* larvae in two ways: by increasing the concentration of nutritional chemicals (carbohydrates, nitrogen), and by reducing the concentrations of toxic and/or digestion-inhibiting chemicals (phenolics) in the needles. The experiment demonstrated that nitrogen fertilization enhanced larval growth. Low needle nitrogen concentrations and high concentrations of phenolics have been correlated with reduced larval weights of *P. abietina* feeding upon fertilized and unfertilized spruce trees. This supports the idea that phenolics are specific agents for protein deactivation.

It has often been shown that the effect of air pollution may be favorable to insect herbivores (Holopainen *et al.* 1991). Excess nitrogen supply (via atmospheric N-input) may promote the sawfly´s success by improving its food quality: stimulated tree growth with high carbohydrate and nitrogen levels at the cost of limited allocation of resources for the synthesis of defensive compounds (phenolics).

SUMMARY

The present study on the plant-herbivore system Norway spruce, *Picea abies*, and the little spruce sawfly, *Pristiphora abietina*, illustrates variations in spruce needle chemistry as a potential factor for successful insect attacks. While nitrogen, carbohydrates, starch, organic acids, fiber, and water content of the new emerging needles showed no significant between-tree or site-specific differences during the time of larval feeding, the levels of the needle phenolics were significantly lower in

attacked trees than in control trees. Successful larval feeding seems to be only possible on trees with low needle phenolics. High phenolic concentrations during early larval feeding suggest an effective defense mechanism of the host tree and seems to be genetically determined.

A significant negative correlation between needle nitrogen and phenolics in individual trees as well as the positive effect of nitrogen and the negative effect of phenolics on larval weight indicate a causal relationship between nitrogen and phenolics and larval growth.

We also demonstrated the effects of excess atmospheric nitrogen input via N fertilization upon different chemical properties in spruce needles and the quality of these expanding needles as food for the larvae. Nitrogen fertilization resulted in decreased phenolics, starch, organic acids, and fiber, increased nitrogen and carbohydrates, and in a better food value for the sawfly larvae. Larval weight was negatively correlated with needle phenolic concentrations. Our findings indicate that the quality of spruce needles as food for the *P. abietina* larvae are influenced in two ways: by increasing the concentrations of positive factors (e.g., nitrogen, carbohydrates) and decreasing the concentrations of negative factors (e.g., phenolics, fiber, organic acids).

ACKNOWLEDGEMENTS

This study was funded by the Austrian Fonds of Scientific Research, P 7093-CHE: Excess Nitrogen in Forest Plant-Herbivore Associations. The authors wish to thank Ing. G. Mottlik, A. Stradner, and Mag. A. Straka for technical assistance with the chemical analyses.

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ACIDIC DEPOSITION, DROUGHT, AND INSECT HERBIVORY IN AN ARID ENVIRONMENT: *ENCELIA FARINOSA* AND *TRIRHABDA GEMINATA* IN SOUTHERN CALIFORNIA

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INTRODUCTION

Except at the higher elevations, much of the arid southwestern United States and northern Mexico is not forested. The plant communities are dominated by widely spaced shrubs that are 1-3 m in height. Because these areas receive less than 20 cm of annual precipitation and mean maximum summer temperatures of 38-43 ^{o}C, plant growth is often limited by the availability of moisture. Many of the species that are components of the Creosote Bush Scrub community are drought-deciduous and remain dormant during the dry periods (Munz and Keck 1968). Even in the slightly more moderate Coastal Sage Scrub community that receives up to 40 cm of annual precipitation and less extreme mean maximum summer temperatures, the plant species are adapted to extended periods without precipitation. Growth and flowering are often limited to those periods with available moisture.

However, as the population of urban areas adjacent to these biotic communities grows, plants in those communities are subject to additional stresses arising from human activities. One of the most important anthropogenic stresses on plants is air pollution (Cowling 1982, Lee 1982, Linthurst *et al.* 1982, Treshow 1984, Shriner 1986). Although there are many different atmospheric pollutants capable of affecting plants, including ozone, NO_x, SO_x, and PAN (peroxyacetyl nitrate), the impact on plants may be enhanced when the pollutants are combined with moisture. Wet acidic deposition in the form of acidic fog is of particular concern in southern California. Acidic fog episodes in southern California last from 2 to 12 hours and often have pH's of 2.0-3.0 (Waldman *et al.* 1982). Physical changes in leaf surface structure, lesions, leaching of foliar nutrients, decreased photosynthesis, and reduced growth and yield may result from long-term exposure to acidic fogs (Granett and Taylor 1981, Granett and Musselman 1984, Musselman and Sterrett 1988, Musselman and McCool 1989, Paoletti *et al.* 1989, Takemoto *et al.* 1989, McCool and Musselman 1990, Mengel *et al.* 1990, Trumble and Walker 1991). Pollution-stressed plants also may be more susceptible to insects and diseases (Endress and Post 1985, Trumble *et al.* 1987, Trumble and Hare 1989, Jones and Coleman 1988, Paine *et al.* 1993). Consequently, if acidic deposition in the form of acidic fog occurs when the plants are actively growing and when the herbivores also are active, the pollution stress may have a significant impact on the fitness of the plant.

We have studied the influence of acidic deposition on the interactions between brittle brush, *Encelia farinosa* Gray (Asteraceae), a dominant shrub in the Creosote Bush Scrub and Coastal Sage Scrub communities of southern California, and its primary herbivore, *Trirhabda geminata* Horn (Coleoptera: Chrysomelidae), a leaf beetle that feeds in both larval and adult stages on *E. farinosa*. The system is important because it is both widespread and an indicator system for damage that may be occuring in these fragile communities occupying harsh environments. The impact of pollution stresses on the interactions between plant and herbivore has been much less studied in desert than in forest ecosystems (e.g., Johnson and Siccama 1983, Jones and Coleman 1988, Mengel *et al.* 1990); however, there may be much less environmental buffering in these desert communities for additional stress imposed by anthropogenic encroachment.

Acidic Deposition as a Single Stress

Our previous studies have demonstrated significant effects of acidic deposition on the nutritional quality and palatability of *E. farinosa* to *T. geminata* in laboratory tests. Plants fogged three times for 3 hours each time at pH 2.75 were compared to plants fogged with a control fog at pH 5.80. Acidic-fogged foliage had significantly higher concentrations of

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total nitrogen (2.33%) and higher soluble protein content (4.96 mg/g leaf tissue) when compared to leaves from control-fogged plants (1.94% total nitrogen and 3.94 mg/g leaf tissue soluble protein) (Paine *et al.* 1993). There were no differences between treatments in water content of the leaf tissues (74.8% for acidic-fogged plants and 75% for control-fogged plants). Both larvae and adult beetles preferred to feed on leaf tissue from acid-fogged plants (Paine *et al.* 1993). The studies demonstrated that the changes in *Encelia* foliage that alter insect consumption and growth indices can occur within 7 days of treatment with acid fogs.

Acidic deposition has an indirect effect through *E. farinosa* upon the growth rate and biomass of *T. geminata*. Acidic fogging (pH 2.75 vs. a control fog of pH 5.8) of *E. farinosa* resulted in a 34% increase in average larval biomass gain (Control = 2.23 mg vs. Acid= 2.98 mg) and a 31% increase in larval growth rate (Control= 0.16 $\mathrm{mg \cdot mg^{-1} \cdot d^{-1}}$ vs. Acid=0.21$\mathrm{mg \cdot mg^{-1} \cdot d^{-1}}$). The effect of fogging on growth rate and biomass gain was strongest for insects initiating feeding 14 days after treatment applications. The effect of acidic fogging and the duration of the effect (days post treatment) were independent of each other (no interaction); the effect of acidic fogging upon growth rate and biomass gain was statistically constant through time. For adult beetles, tissue consumption of acidic-fogged foliage was 65% greater than tissue consumption of control foliage (Control = 0.0964 $\mathrm{cm^2}$ vs. Acid = 0.16 $\mathrm{cm^2}$). For larval beetles, tissue consumption of acidic-fogged foliage was over 400% greater than control foliage (Control = 0.057 $\mathrm{cm^2}$ vs. Acid = 0.259 $\mathrm{cm^2}$) (Redak *et al.* In Prep.).

Simultaneous Impact of Acidic Deposition and Drought as Multiple Stresses

Drought stress has been shown to mitigate the impact of gaseous air pollutants and, to a certain extent, acidic wet deposition (acid rain and fog) on plant physiological and growth processes (Tingey and Hogsett 1985, Meier *et al.* 1990, Bender *et al.* 1991, Showman 1991, Beyers *et al.* 1992, Temple *et al.* 1992). Our field data investigating the impact of acidic fog upon the *E. farinosa-T. geminata* system are consistent with the theory that drought stress may ameliorate the impact of acidic fog upon the susceptibility of plants to insect herbivory (Warrington and Whittaker 1990, Von Sury and Fluckiger 1991). Drought stress may make the plant less susceptible to the effects of the air pollution, or alternatively, the two stresses may act independently but antagonistically on the plant so that the net effect on herbivore success is neutral. Using an undeveloped area (remnant coastal sage-scrub habitat) on the UCR campus, we applied acidic (pH = 2.0) or control (pH = 5.8) fog treatments (3, 3-hour exposures, every other day for 5 days) to 40 mature *E. farinosa* plants (20 plants per treatment application). Following fog exposure, we caged 50 second instar *T. geminata* larvae in nylon screen bags on each treatment plant. Larvae were allowed to feed and grow on the plants for 16 days at which time they were removed and their fresh and dry masses were determined. Additionally, 7 days after fog treatments, we conducted a 72-hour laboratory bioassay determining the consumption and growth rates of third instar *T. geminata* fed foliage taken from the field treated plants. After removal of experimental insects, foliage was collected from each plant and analyzed for total N and percent water.

Acidic fogging showed no effect on larval growth or consumption values or any plant foliage quality values (Table 1). For the field experiment described here, we feel the drought status of the experimental plants may account for the lack of treatment effects (acidic fog effects) upon insect performance. At the time of the experiment, southern California was in the sixth to seventh year of a sustained drought. For the year of this study, 1990, the UCR area had received less than 15% (approx. 40-50 mm) of normal precipitation by the end of the growing season (CIMIS weather data). Indeed approximately 1 week after treatment applications, several experimental plants (of both treatments) began to show characteristic signs of drought-induced leaf senescence (leaf curling, browning, dropping older more mature leaves), and by the second week following removal of experimental insects, all of the plants were undergoing severe leaf-loss. *Encelia farinosa* normally is a drought deciduous plant (Ehleringer and Clark 1988); however, in this case, the period of leaf-drop began approximately 6 weeks to 2 months earlier than normal. Given the sustained 6 years of drought and the very early period of leaf abscission, we are confident that all experimental plants were experiencing relatively severe drought stress.

The response of *E. farinosa* to drought stress includes (among other processes) an increase in leaf pubescence (Ehleringer and Bjorkman 1978, Ehleringer 1982). We suspect that (1) the experimental plants were so severely drought stressed that additional stress, in the form of acidic fog deposition, may have no further influence on the plants' suitability as a host for *T. geminata* and (2) the morphological changes that occur in *E. farinosa* with drought stress are such that the plant may be physically protected to some extent from the impact of acidic fog (i.e., drought is functioning as a "filter" to the impact of acidic fog). The dense covering of pubescence (as a result of drought stress) could have physically shielded and/or buffered the plant from acid fog deposition (Caporn and Hutchinson 1987, Musselman 1988).

Table 1.—Impact of acidic fog upon the foliage quality of *Encelia farinosa* and performance of *Trirhabda geminata*[1,2]

A. Field Bioassay.

	Final Insect Biomass (mg)	Total Nitrogen Content (%)	Total Water Content (%)[3]
Fog pH 2.00	0.0049±0.0001	2.83±0.06	60.82±0.54
Fog pH 5.80	0.0049±0.0001	2.80±0.07	60.08±0.54

B. Laboratory Bioassay.

	Insect Biomass Gain (mg)	Insect Growth Rate ($mg\bullet mg^{-1}\bullet d^{-1}$)	Insect Consumption Rate ($mg\bullet mg^{-1}\bullet d^{-1}$)
Fog pH 2.00	0.0027±0.0002	0.9008±0.0816	27.729±4.171
Fog pH 5.80	0.0026±0.0003	0.8252±0.0826	26.179±4.259

[1] There were no significant differences in any of the measured parameters between acid- and control-fogged treatments, ANOVA.

[2] Values shown are means ± 1 standard error.

[3] Analyses carried out on arcsine transformed data. Values shown are back transformed.

CONCLUSIONS

The implications of altered host plant quality for insect herbivore development are considerable (Rhoades 1983, Strong *et al.* 1984, White 1984). Researchers have clearly demonstrated that nitrogen availability and plant secondary chemistry affect such basic insect life processes as growth rates, survival, and reproductive capacity (Onuf 1978, Rosenthal and Janzen 1979, Prestidge 1982, Prestidge and McNeill 1983). Since acidic fog incidents typically occur over broad geographical areas (Perkins 1974, Hoffman *et al.* 1985), the cumulative effects on insect herbivores at the population level may be quite significant. Thus, potential changes in plant physiology due to stress or direct injury resulting from acidic fog may well have more serious consequences than previously believed. The dynamics of herbivorous insect populations associated with areas subjected to acidic fogs may be dramatically affected depending on the type and magnitude of the chemical changes that can occur in plants subjected to this type of pollution.

By using *E. farinosa* and its most common natural herbivore, *T. geminata*, we are able to draw from and build upon a relatively thorough understanding of the relationships between the effects of acidic fog on *E. farinosa* foliage quality and *T. geminata* success. Previous studies have shown that the major defensive compounds in *E. farinosa* are the sesquiterpene farinosin and the two chromenes euparin and encecalin (Wisdom and Rodriguez 1982, 1983; Wisdom *et al.* 1983; Wisdom 1985, 1988). These compounds reduce growth rates, survivorship, and quantity of food consumed for early instar larvae of *T. geminata*. Additionally the foliar concentrations of these compounds are highest at that time of year when early instars are present and actively feeding. We are currently investigating whether or not the concentrations of these compounds are affected by exposure to acidic fogs. Changes in the concentrations of the secondary chemistry of the plants may partially explain the results presented here. However, Wisdom (1985) showed that *T. geminata* larvae apparently feed on tissues high in total nitrogen content. We have shown that acidic fog exposure may change primary plant chemistry, resulting in increased plant total nitrogen and soluble protein. These increases are associated with increased larval preference (Paine *et al.* 1993) and may also be associated with better larval performance. If the herbivore consumes more plant tissue as a direct consequence of an anthropogenic stress and decreases plant fitness, there may be a significant impact on the plant community if the stress continues for a significant period of time. Although drought may ameliorate the impact of the anthropogenic stress, the long-term consequences of the pollution stress on the ecosystem are not clear.

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THE RESISTANCE OF SCOTCH PINE TO DEFOLIATORS

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SUMMARY

Scotch pine, *Pinus sylvestris*, is the most widely distributed tree species in Eurasia. It is often damaged by several defoliating insects: *Dendrolimus pini*, *Panolis flammea*, *Bupalus piniarius*, and *Diprion pini*. The foci of mass outbreaks of these insects typically occur in pure pine stands on poor, dry sandy soils, and on richer soils (loamy sands and sandy loams) depleted by long agricultural use.

Chemical analyses of needles showed that N contents are always higher in pines growing outside (i.e., on richer, moister soils) than inside the foci of mass outbreaks. With respect to sugars, no differences were found. Larvae on trees enclosed in gauze bags, showed a high mortality of early instars (I-III) in stands on rich, moist soils. Young larvae cause oleoresin droplets to form where they chew into needles, the mechanism of pine resistance to them. Such exudations are weak from trees on poor, dry soils. Therefore, larvae can feed on them without impediment, and their mortality is low.

To monitor this resistance, the ends of living needles (about 1/3 - 1/4 their length) on live trees, were cut. After 5 minutes the clipped needles were scored as follows: 0 - oleoresin does not exudate from the needle; 1 - a thin film of oleoresin appears on the cut, or separate tiny droplets which do not converge to a single lens; 2 - a small oleoresin lens appears (no more than 0.5-1.0 mm thick); 3 - a larger oleoresin lens appears. The following indices of July oleoresin exudation intensity (I) and efficiency (E) were calculated:

$$I = (n1+2*n2+3*n3) / N \qquad E = (n2+n3) * 100 / N$$

where I is a mean index of oleoresin exudation; n1, n2, n3 are number of needles with scores of 1, 2, or 3; N = total number of investigated needles (including those scored as 0); and E = oleoresin exudation efficiency.

The relationship between mortality (M) of larvae (I-III instars) and (I) was tested using regression: $M = a*I - b$, where a and b are parameters specific for each insect species. Thus, for *Dendrolimus pini*, $M = 65.0\,I - 34.4$, for *Acantholyda stellata*, $M = 37.3\,I - 8.3$, for *Diprion pini*, $M = 66.9\,I - 51.2$, and for *Neodiprion sertifer*, $M = 42.4\,I - 29.9$. The r^2 values for these regressions vary from 0.53 - 0.79.

One can calculate that Scotch pine is resistant to young larvae of *Neodiprion sertifer* beginning at $I \geq 1.7$ and $E \geq 70$. For other defoliators, Scotch pine is resistant at $I \geq 1.4$ and $E \geq 40$. It should be noted that July oleoresin exudation intensity must be determined no later than 3-4 days after heavy rains when the humus layer moisture is ≥ 6-8%, and the moisture of the lower layers (down to 50 cm depth) is ≥ 3-4%, and air temperature, ≥ 10 °C.

Oleoresin exudation intensity from the needles of several pine species show that they can be divided into three groups: (1) low oleoresin exudation intensity (*Pinus banksiana*, *P. mugo*, *P. maritima*), (2) intermediate intensity (*P. sylvestris*, *P. pallasiana*, *P. nigra*, *P. strobus*), or (3) high intensity (*P. sibirica*, *P. cembra*, *P. pithyusa*).

The connection between the oleoresin exudation intensity of various pine species and their resistance to various defoliators is not perfect. The resistance of pine species depends also upon terpene composition of oleoresin.

Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

COMPANION PLANTING OF THE NITROGEN-FIXING *GLIRICIDIA SEPIUM* WITH THE TROPICAL TIMBER SPECIES *MILICIA EXCELSA* AND ITS IMPACT ON THE GALL FORMING INSECT *PHYTOLYMA LATA*

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INTRODUCTION

In Ghana, West Africa, there is increasing concern over the loss of valuable commercial timber species that contribute to the economic development of this country (Wagner and Cobbinah 1993). Part of the diminishing supply of timber relates to the high rates of deforestation (Zerbe *et al.* 1980, Mergen 1983, Repetto 1988). But an equally important factor that is often overlooked is the failure of native species to regenerate following disturbance in the natural forest or when planted in plantations (Fontaine 1985). Forest insects can significantly contribute to the poor regeneration rates of tropical timber species. An example of this is the complete failure to artificially regenerate *Milicia excelsa*, an extremely valuable tropical timber species in Africa, because of heavy damage by the gall-forming psyllid, *Phytolyma lata* (White 1968, Wagner *et al.* 1991).

Milicia excelsa and *M. regia* are important timber species in Africa, known in the timber trade as Iroko, Mvule, and locally in Ghana as odum. The wood is durable, uniformly grained, and extremely resistant to termites. While the species is a common component of the natural overstory in much of the remaining tropical forests of West Africa, it is not currently regenerating and has a predicted resource life of less than 10 years in Ghana (Alder 1989). Reforestation efforts have focused on establishing plantations of Iroko which have failed in part because of massive damage by *Phytolyma lata*, the odum gallfly.

The odum gallfly oviposits on new foliage of *Milicia* spp., and the first instar nymphs damage leaf tissue and induce galls (White 1968; Orr and Osei-Nkrumah 1978; Cobbinah 1986, 1988). The nymphs feed within the gall tissue. Numerous attack sites result in large gall masses. After about 2-3 weeks, the galls burst open releasing the adult psyllid. The gall tissue that has burst open exudes gall and leaf fluids that are heavily colonized by saprophytic fungi. These saprophytic fungi result in decay of leaf and stem tissue and dieback of young shoots (Wagner *et al.* 1991). Multiple attacks within the same season result in heavy damage and frequent death to young seedlings.

Considering that conventional plantations had largely failed, we decided to examine the influence of companion planting of *Gliricidia sepium*, a well-known nitrogen-fixing agroforestry species, and *Milicia excelsa* on odum gallfly attack. We hypothesized that *Gliricidia sepium* would provide nitrogen and overstory shade more similar to conditions that occur in the natural tropical forests. In addition, we examined the influence of shading and nitrogen fertilization independently in an effort to determine which factor contributed more to the observed effects. In this manuscript we report that companion planting, shading, and fertilization all reduce attack and damage by the odum gallfly on Iroko.

Mattson, W.J., Niemelä, P., and Rousi, M., eds. 1996. Dynamics of forest herbivory: quest for pattern and principle. USDA For. Serv. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108.

EXPERIMENTAL METHODS

Experiments were carried out at the Forestry Research Institute's experimental nursery about 5 km south of the University of Science and Technology, Kumasi, Ghana. The area is within the moist semi-deciduous forest zone (Hall and Swaine 1983), a common tropical forest type in West Africa that generally supports populations of *Milicia excelsa*. Seedlings used in the companion planting and shading experiments were collected from a single parent tree (AA 17) from the village of Abofour in the Ashanti Region of Ghana. Other studies have indicated that this particular provenance is of intermediate susceptibility to *Phytolyma lata* (Cobbinah and Wagner 1994). Seedlings used in the fertilizer trial were of mixed origin. Seeds were collected in 1991, sown on raised beds, and transplanted into polyethylene bags. Trees were kept under shade and watered regularly until used in the experiment. The spacing on all experiments was 1m x 1m.

Companion Planting Trial

The companion planting trial was established in September 1991. The trial consisted of four rows of 10 (40 total) *Milicia excelsa* planted in alternating rows with *Gliricidia sepium* cuttings and four rows of 10 *Milicia excelsa* planted without *G. sepium*. At periodic intervals the following data were collected: seedling height, number of leaves, number of galls/plant. Regression analysis was used to estimate the number of galls in large gall masses as described by Cobbinah and Wagner (1994). For this experiment, the data were averaged for each row of 10 trees to reduce variability, consequently $n = 4$ for both treatments.

Shading Trial

The shading experiment was established in October 1992. The shade was created by supporting commercial shade cloth (Hummerts International) over wooden structures. Four levels of shade were established: full sun = no shade, light shade = 33% shade cloth, medium shade = 57% shade cloth, and deep shade = 82% shade cloth. Under each shade level, 50 seedlings were planted in five rows of 10 seedlings each. Data collection was as indicated above. For this experiment, $n = 50$ per treatment.

Fertilizer Trial

The fertilizer experiment was established in December 1992 and ran for 6 months. The effect of fertilizer was tested using a randomized complete block design with four replicates (25 seedlings/plot and 100 seedlings/block) and four treatments: control (no fertilizer), NPK® (15-15-15), Phostrogen® (10-4-22), Sampi® (8-3-3). Fertilizer was applied in the irrigation water at the rate recommended by the manufacturer. Seedlings were kept under shade. Data were collected as above and the average number of diebacks was determined.

Statistical Analysis

Data for the companion planting trial were analyzed using a paired t-test on STATGRAPHICS software. Each row of 10 trees was treated as the sample unit. Data for the shading trial were transformed [$\text{Log}_{10}(x+1)$] to meet assumptions of homogeneity of variance and were analyzed using an ANOVA on STATGRAPHICS software. Data for fertilizer trials were analyzed using ANOVA and LSD multiple range test using STATGRAPHICS software. The P value to reject the null hypothesis was set at 0.05.

RESULTS

Companion Trial

Milicia excelsa seedlings growing together with the nitrogen fixing *Gliricidia sepium* were not significantly taller (Fig. 1) and they did not produce more leaves (Fig. 2) than *Milicia excelsa* seedlings growing alone during any sample period. However, there were significantly fewer galls during two sample periods on *Milicia* grown with *Gliricidia* (Fig. 3). There was also a tendency for fewer galls in the presence of *Gliricidia* for most of the 1992 sample periods (Fig. 3).

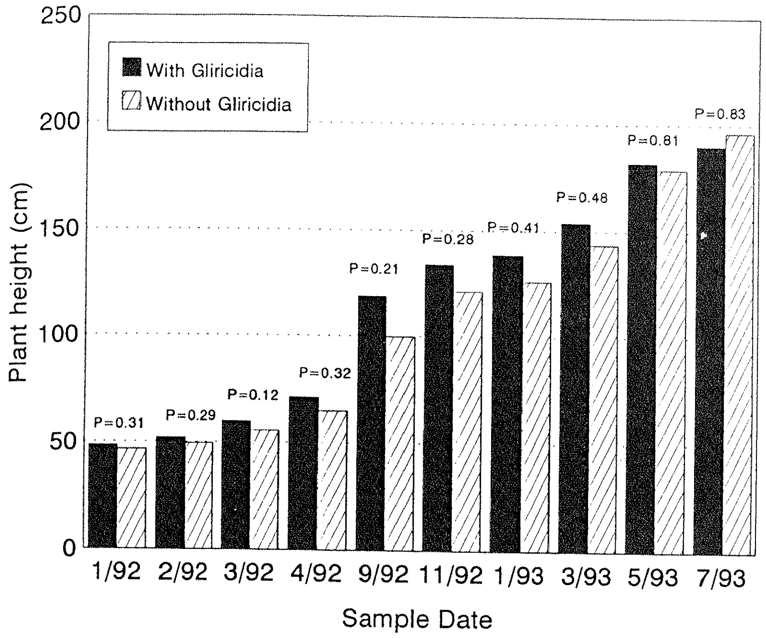

Figure 1.—Influence of companion planting *Gliricidia sepium* with *Milicia excelsa* on *M. excelsa* growth at nine sample dates over 14 months. P-value based on paired t-test.

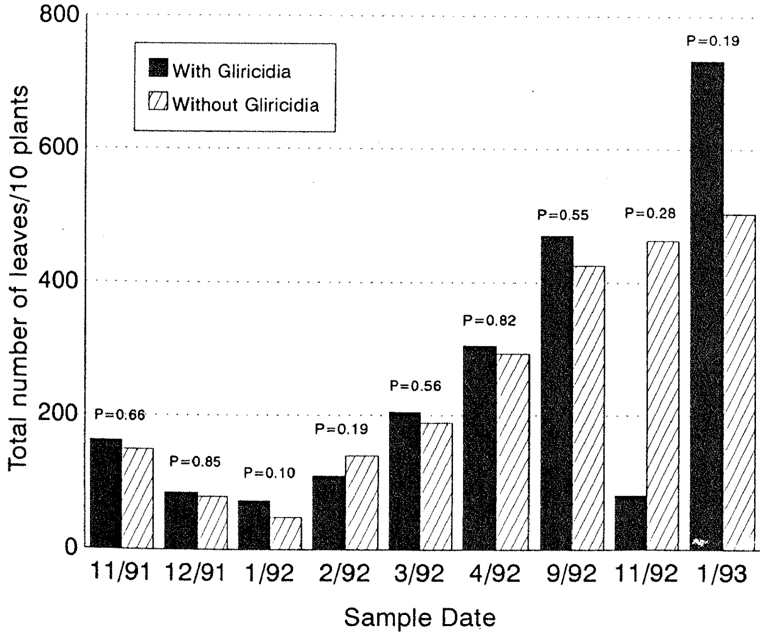

Figure 2.—Influence of companion planting *Gliricidia sepium* with *Milicia excelsa* on the number of leaves of *M. excelsa*. P-value based on paired t-test.

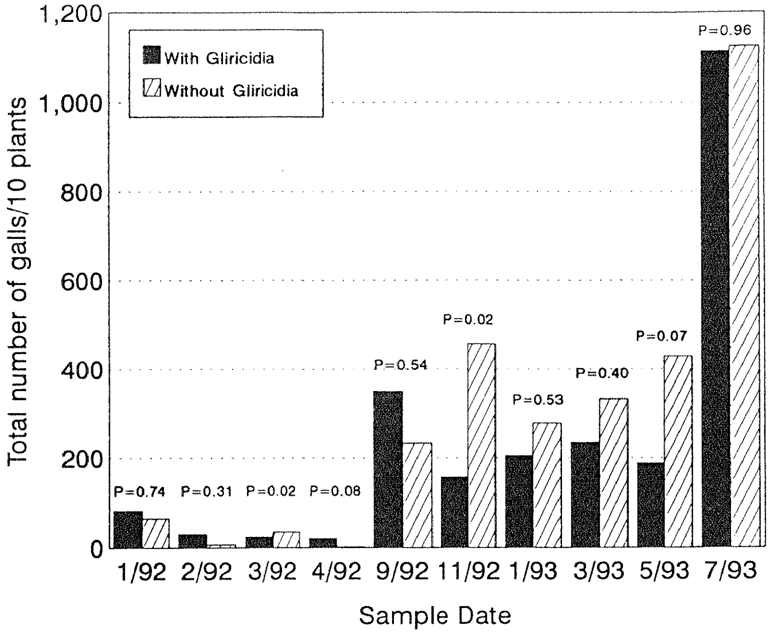

Figure 3.—Influence of companion planting *Gliricidia sepium* with *Milicia excelsa* on the total number of *Phytolyma lata* galls on *M. excelsa*. P-value based on paired t-test.

Shading Trial

Shading had a significant positive impact on height (Fig. 4). After 1 year, trees grown at the highest level of shade (82%) were nearly 50% taller than trees at 57% shade and 100% taller than trees grown in full sun. In general, there were more leaves produced under shade than in full sunlight, but this pattern was not consistent across sample dates (Fig. 5). Despite the fact that more leaves were available to *Phytolyma lata* under shade, there were fewer galls per plant under shade (Fig. 6). While this pattern is statistically significant at only two of the sample dates, the pattern is very consistent across all sample dates.

Fertilizer Trial

Two of the fertilizer treatments, 15-15-15 and 10-4-22, significantly affected *Milicia* height when compared to the control (Fig. 7). In general the higher the nitrogen the greater the impact. The highest nitrogen content fertilizer also resulted in significantly fewer branch diebacks. Diebacks are positively related to the number of galls (Cobbinah and Wagner 1994).

DISCUSSION

We are unaware of other studies that have clearly linked companion planting or agroforestry techniques and modifications in the incidence and impact of damaging insects in a tropical ecosystem. While the patterns we observed are not absolutely consistent for all the sample periods, there is sufficient evidence to warrant further study.

There is, however, a considerable body of evidence on the relationship between fertilization and insect attack (see Stark 1965, and Schowalter *et al.* 1986 for reviews). Strauss (1987) observed that fertilization tended to have a positive impact on sap feeding insects and no effect on chewing insects. Several studies have examined the relationship between fertilization and population parameters of the gall-forming adelgid *Adelges coolyei* (Mitchell and Paul 1974, Johnson *et al.* 1977, Mitchell and Miller 1976). In each case they found a general trend of fertilization increasing population parameters of *Adelges coolyei.* While these results are opposite to our own findings, the environmental conditions and plant species are

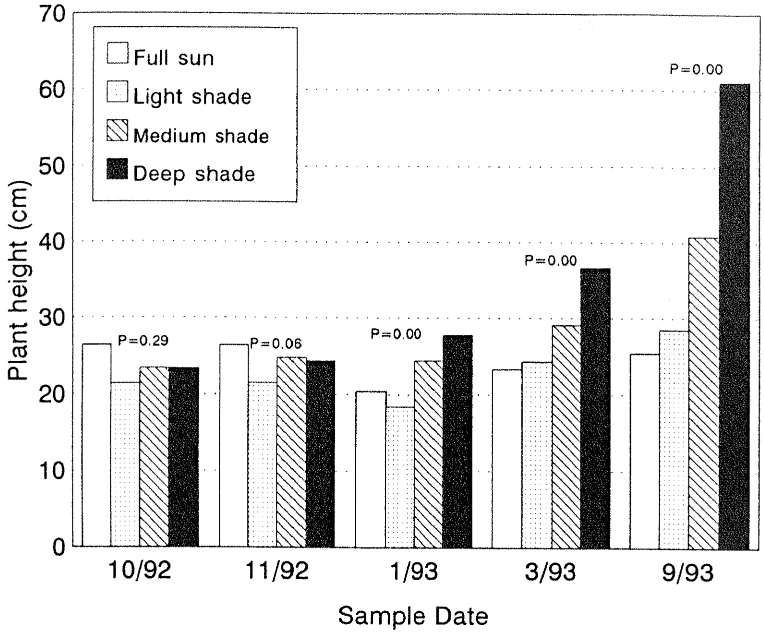

Figure 4.—Height (cm) of *Milicia excelsa* grown under four levels of shade. Light shade = 33%, Medium shade = 57%, Deep shade = 82%.

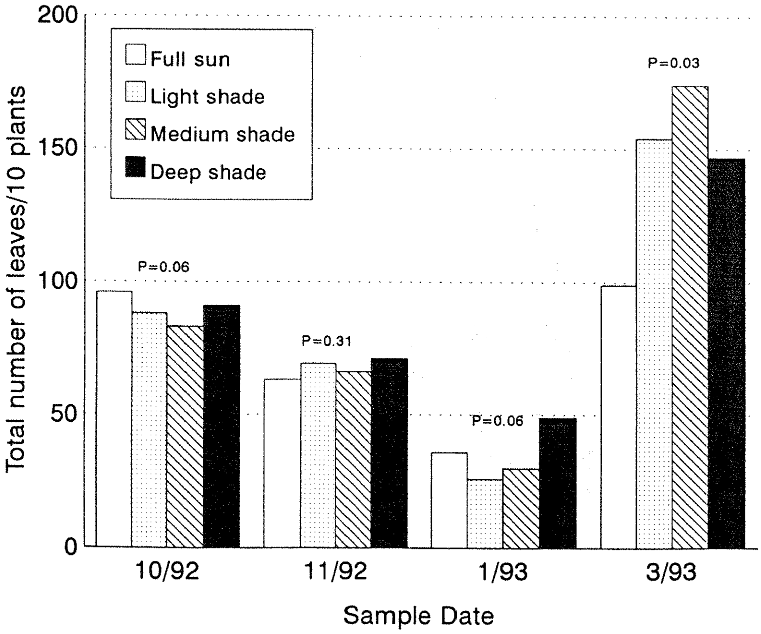

Figure 5.—Number of *Milicia excelsa* leaves produced under four levels of shade. Light shade = 33%, Medium shade = 57%, Deep shade = 82%.

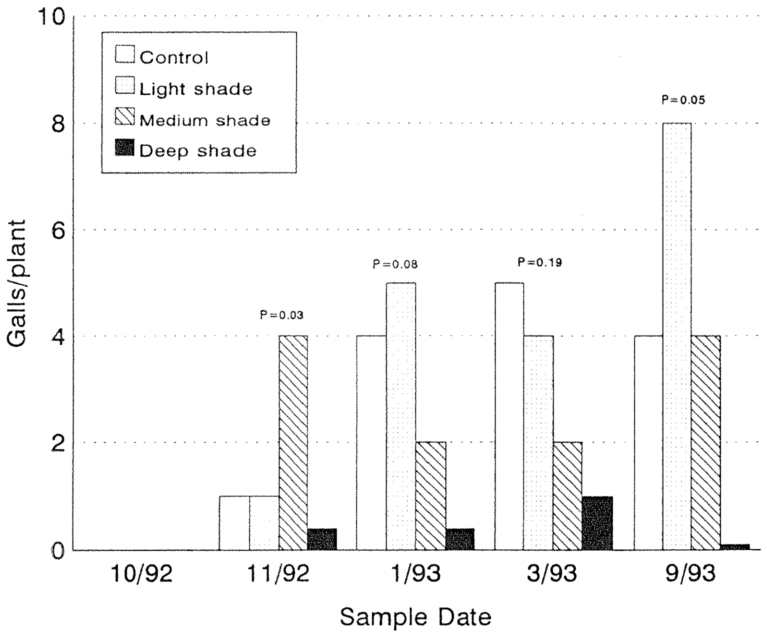

Figure 6.—Number of *Phytolyma lata* galls per *Milicia excelsa* plant grown under four levels of shade. Light shade = 33%, Medium shade = 57%, Deep shade = 82%.

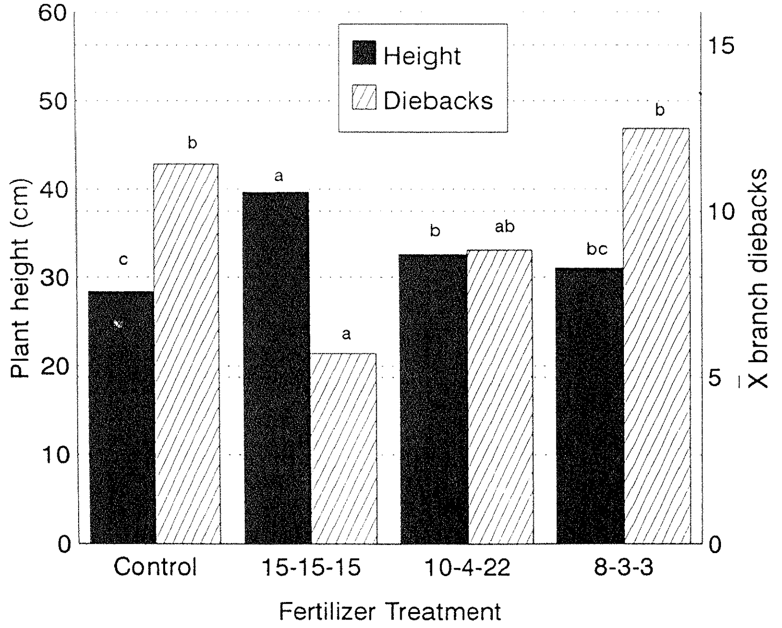

Figure 7.—*Milicia excelsa* height (cm) and average number of *Phytolyma lata* induced diebacks as the result of four fertilizer treatments.

very different from the *Milicia/Phytolyma* system we examined. There is very little consistency in the response of herbivores to fertilization of their hosts (Stark 1965, Schowalter *et al.* 1986). Insufficient evidence exists to postulate any general pattern in this regard.

Hall and Swaine (1983) reported that Iroko is not abundant in the evergreen tropical forest because it requires high light for germination and establishment. However, as previously noted, plantations of this species planted in full sun have been heavily attacked by the odum gallfly and have largely failed. In contrast to the report of Hall and Swaine (1983), we have been told by a tropical tree physiologist that the optimal photosynthesis for Iroko seedlings occurs at 8-10% of full sunlight, suggesting this is a shade tolerant species (E.M. Veenendaal, pers. comm.). The results we report in this paper indicate that the abundance and subsequent impact of this insect is greater in full sun than under shade.

We recognize that there would be considerable utility in examining the relationship between Iroko and the odum gallfly under natural field conditions. However, naturally regenerating seedlings of this species are difficult to find. In a recent field excursion to the Tain II Forest Reserve, which is noted for its high density of Iroko in the overstory, we were able to locate only a single seedling in 12 person/hours of searching. The low incidence of Iroko seedlings has been confirmed anecdotally by several botanists and forestry field surveys. Growing stock as of 1987 was immature stock (1-7ft girth) 834,000m^3 and mature stock (> 7ft girth) 5,269,000m^3 (Alder 1989).

The results we report suggest that standard agroforestry practices such as mixed planting with nitrogen-fixing trees may have utility as part of a pest management program to control the odum gallfly. Other approaches including selection for genetic resistances have resulted in considerable success (Cobbinah and Wagner 1994). Cooperative effort between silviculturists, physiologists, and genetists is required to fully understand how to reduce the impact of this insect and provide for a continuing supply of one of Africa's most valuable timber species.

SUMMARY

Planting the nitrogen-fixing *Gliricidia sepium* in conjunction with the tropical timber species *Milicia excelsa* reduces the abundance and damage caused by the gall-forming pysllid, *Phytolyma lata*. This effect appears to be related to both the increased shading and enhanced nitrogen environment. Shading independently has a strong impact on gall abundance with the lowest gall abundance occurring under the highest level of shade (82%). Gall abundance is reduced under shade even though more leaves are available to the insect. Fertilization at the highest levels of nitrogen also reduces the abundance of galls.

ACKNOWLEDGEMENT

These studies were supported by the International Tropical Timber Organization through research grant No. PD75/90 to J.R. Cobbinah and M.R. Wagner.

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FUNCTIONAL HETEROGENEITY OF FOREST LANDSCAPES: HOW HOST DEFENSES INFLUENCE EPIDEMIOLOGY OF THE SOUTHERN PINE BEETLE

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INTRODUCTION

Insect outbreaks are autogenic disturbances that are normally observed at the landscape scale as levels of herbivory above an average or expected amount. When favorable environmental conditions coincide with optimal resource availability, populations increase in size and outbreaks occur (Rykiel *et al.* 1988). Epidemiology is the study of causes for change in the distribution and abundance of an insect species at the landscape level of ecological integration. Typically, studies of epidemiology have considered herbivore populations in the context of their impact on food and habitat resources. In this paper we focus on the association of forest landscape structure and the process of herbivory, i.e., the effect of landscape pattern on the process of herbivory (Turner 1989). Our goal is to examine epidemiology of the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), in the context of functional heterogeneity of forest landscapes. Our objectives are: (i) to review briefly the current state of knowledge on epidemiology of *D. frontalis*, (ii) to consider the concept of functional heterogeneity of forest landscapes and how it relates to epidemiology of *D. frontalis*, (iii) to consider ways and means of measuring functional heterogeneity of forest landscapes, and (iv) to illustrate the relation of functional heterogeneity and herbivory by the insect.

Although emphasis is directed to *D. frontalis*, it is noteworthy that this insect is a member of a guild consisting of four other bark beetle species: *D. terebrans* (Olivier), *Ips calligraphus* (Germar), *I. grandicollis* (Eichhoff), and *I. avulsus* (Eichhoff) (Coleoptera: Scolytidae). The population systems of these insects are interrelated. How the members contribute to epidemiology of the guild is unknown.

Epidemiology of the Southern Pine Beetle

Background

Flamm *et al.* (1988) provide a review of literature on the natural history of *D. frontalis*. A general conceptual model of epidemiology of the insect was proposed by Coulson *et al.* (1983, 1985a, b, 1986a) and Rykiel *et al.* (1988). This model considers the process of herbivory in the context of variables thought to influence epidemiology, i.e., meteorological conditions, the lightning disturbance regime, forest stand conditions, background levels of the insect, and landscape structure. Studies by Flamm and Coulson (1988), Flamm *et al.* (1992), Lovelady *et al.* (1991), and Lovelady (1994) have employed empirical methods to address specific aspects of the conceptual model.

Outbreaks of *D. frontalis* are the result of population fluctuations observed at the landscape scale. There are three separate processes associated with *D. frontalis* epidemiology. The first, which operates at the population and community levels of integration, involves factors associated with initiation of infestations. The second, which operates at the ecosystem level of integration, deals with factors that influence growth of established infestations. The third, which operates at the landscape level of integration, deals with the network of habitat units and population centers occurring in a heterogeneous land area consisting of multiple interacting ecosystems. An insect outbreak is an epidemiological condition that represents a composite of the three processes operating across different scales of time and space. The actual process of herbivory by *D. frontalis* occurs at the population and community levels of ecological integration and results in mortality to host pines. However, the effects are propagated through component ecosystems to the landscape level (Coulson *et al.* 1986b, Rykiel *et al.* 1988, Lovelady *et al.* 1991).

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Host Defenses

An important component of epidemiology of bark beetles centers on the interaction of the insect with its host. Considerable effort has been directed to research on host defense mechanisms, because of their critical role in regulating colonization by bark beetles. In pines (*Pinus* spp.) both preformed and induced defense systems have been identified. During colonization, bark beetles directly confront the defense mechanisms of the host. The colonization process is greatly influenced by variation in defense capacity associated with different pines, (e.g., longleaf pine is more resistant to *D. frontalis* colonization than loblolly or shortleaf), and with different individuals of the same host species. Within a forest landscape there is also a seasonal variation in host defense capability. Lorio (1988) suggested that normal physiological changes associated with tree phenological development and response to variable environmental conditions cause regular and predictable fluctuations in resistance/susceptibility to *D. frontalis*. Lovelady (1994) provides a comprehensive review and interpretation of seasonal variation in host defense in relation to population dynamics of *D. frontalis*.

Lightning-struck hosts, which have greatly reduced capacity for defense against colonization, serve as refuges for bark beetles. For many years foresters have recognized that infestations of *D. frontalis* are often associated with a lightning-struck host. The initial conceptual model of epidemiology of *D. frontalis* included lightning-struck hosts as a prominent component, although only circumstantial evidence supported the contention (Coulson *et al.* 1983). Subsequent research has suggested that lightning-struck hosts are an essential feature of the natural history of the insect. This research considered the interaction of *D. frontalis* with disturbed hosts (Coulson *et al.* 1986a, Flamm and Coulson 1988, Flamm *et al.* 1992) as well as an analysis of lightning as a disturbance regime (Lovelady *et al.* 1991, Lovelady 1994).

Of particular concern in the study of epidemiology is how host defenses are distributed at the landscape scale. Herbivory by *D. frontalis* produces disturbance patches (infestations) in the forest matrix that can be observed relative to other features of the landscape, i.e., the crowns of infested trees become discolored and are visually detectable. The disturbance patches are typically associated with old-growth forest stands occurring on poor sites. Hazard rating systems have been developed to grade forest stands with respect to vulnerability to *D. frontalis* infestation (Lorio 1980, Branham and Thatcher 1985, Mason *et al.* 1985). Several different methods have been devised. Each rating system has novel features, but all involve integration of a subset of variables: tree species, radial growth, height, and DBH; stand basal area (pine, hardwood, total), species composition, site index, and degree of crown closure; and landform classification. When applied to a forest landscape, the hazard rating systems provide a general view of the distribution and abundance of host defenses against the insect. It is noteworthy that the hazard rating systems integrate a substantial knowledge base on the interaction of *D. frontalis*, host plants, and site conditions.

To summarize, we have described the nature of the interaction of *D. frontalis* with host defenses and have identified several sources of variation important to population dynamics of the insect. Although the hazard rating systems have proven useful in defining vulnerability of forest landscapes to herbivory by *D. frontalis*, they ignore the seasonal dynamics of host defenses associated with different tree species, with individuals of the species, and with lightning-struck hosts. Below we will consider how the spatial and temporal sources of variation in host defense can be integrated in the context of the natural history of the insect. In effect, we view host defense capacity as a variable in landscape structure that can be characterized and defined. The distribution of this variable across the forest landscape influences epidemiology of *D. frontalis*.

Outbreaks and Forest Landscapes

Herbivory at the landscape scale is significant because it is at this level of integration where the ecological and forest management consequences of insect outbreaks are interpreted (Pickett and White 1985, Forman and Godron 1986, Barbosa and Schultz 1987, Turner 1987, Platt and Strong 1989, Turner and Gardner 1991, Holling 1992a, Wiens 1992, Coulson *et al.* 1993). Advances in the geographic information system (GIS) technologies and the development of statistics for analysis and description of spatially referenced data have greatly expedited studies of insect outbreaks in forest landscapes (Liebold and Barrett 1993).

Historically, interest in epidemiology of *D. frontalis* has been primarily associated with the economic impact of the insect on forest resources. Emphasis has focused on investigations of herbivory in the context of impact on forest stand structure, i.e., the research has considered the effect of process (herbivory) on pattern (the configuration of elements that constitute the landscape mosaic). For example, Fitzgerald *et al.* (1994) examined the relation of various suppression tactics, employed to modify *D. frontalis* populations, on the subsequent development of new infestations within the surrounding forest landscape.

However, in pre-colonization forests of the southern US, herbivory by *D. frontalis* (along with fire) was likely involved in structuring the landscape (Schowalter *et al.* 1981, Rykiel *et al.* 1988). As *D. frontalis* infestations are normally suppressed in an effort to reduce economic loss by the insect, it has not been possible to evaluate this hypothesis until recently. Outbreaks of *D. frontalis* in Rare II Wilderness Areas on National Forests in Texas have been allowed to follow a natural course, with only modest intervention to protect endangered species. The outbreaks, which occurred over a period of 1983 to 1994, have radically changed the pattern of the forest landscape mosaic of these wilderness areas (Fig. 1). Under this epizootic condition, individual infestations coalesced and the pattern and extent of tree mortality substantially modified the structure of the forest landscape.

Figure 1.—The impact of herbivory by *D. frontalis* in pine forest landscapes in east Texas. Under this epizootic condition, individual infestations coalesced and the pattern and extent of tree mortality substantially modified the structure of the forest landscape.

The alternate way of viewing insect outbreaks is to consider how landscape pattern affects the process of herbivory. Again, because *D. frontalis* infestations are normally suppressed, it was not possible in the past to examine this relation. Figure 1 clearly illustrates how the process of herbivory is influenced by the pattern of patches that were created by previous infestations of *D. frontalis*. There are few examples of empirical studies of the effects of landscape pattern on the process of herbivory (Turner 1989).

By definition, epidemiological studies are dynamic and therefore the process of herbivory and the pattern of the forest landscape mosaic change through mutual interaction, i.e., the pattern influences the process and the process influences the pattern. Although not investigated in a rigorous manner, the scenario observed on the National Forest wilderness areas of Texas suggests that bark beetle herbivory drives the process of ecosystem succession (from the release to reorganization phase in Holling's scheme 1992b) (see also Brown 1991).

Functional Heterogeneity of Forest Landscapes: How Bark Beetles Perceive and Respond to Their Environment

Studies of epidemiology begin with a consideration of issues associated with landscape structure. The term *landscape structure* refers to the spatial relationships between distinctive ecosystems (ecotopes, see Coulson *et al.* 1993), i.e., the distribution of energy, materials, and species in relation to the sizes, shapes, numbers, kinds, and configurations of components (Turner 1989). Characteristics of landscape structure (porosity of the forest matrix, boundary configurations, patch size and shape, etc.) influence epidemiology of bark beetles. Structural characteristics of landscapes are often summarized and represented as heterogeneity. Although defined in several ways, the term *heterogeneity* is generally taken to mean composition of parts of different kinds. In considering the interaction of *D. frontalis* with the forest landscape it is necessary to distinguish between measured and functional heterogeneity. *Measured heterogeneity* deals with physical features or components of a landscape. *Functional heterogeneity* deals with how an organism perceives and responds to its environment (Kolasa and Rollo 1991). There is obviously a direct link between patches, boundaries, and heterogeneity as boundaries define patches, and patchiness is what produces heterogeneity (Wiens 1992, Hansen and di Castri 1992).

Tied to concepts associated with landscape heterogeneity is the subject of habitat structure. In general, habitat structure refers to the physical arrangement of objects in space (Bell *et al.* 1991). In studies of epidemiology, an essential task is to define the distribution, abundance, and location of habitat units. In effect, habitat structure is an element of measured heterogeneity, i.e., habitat can be viewed as a mappable element of a landscape mosaic. Southwood (1977) suggested that habitat serves as the template for ecological strategies.

Examination of how bark beetles perceive and respond to their environment at the landscape scale, i.e., evaluating functional heterogeneity, was difficult before the availability of (i) GIS technologies, (ii) spatially referenced databases, and (iii) spatial statistics. Also, until the text by Forman and Godron (1986), there was no standardized nomenclature for describing landscapes from an ecological perspective (McIntosh 1991). With these problems partially solved, it is now possible to consider the substantial knowledge base on natural history of bark beetles (and other organisms) in the context of the forest landscapes where they occur (Dunning *et al.* 1992).

In reference to *D. frontalis*, we have indicated that a great deal is known about host selection relative to the initiation and subsequent growth of infestations. In the case of initiation of infestations, lightning-struck hosts play a prominent role. For infestation growth, variables associated with forest stand hazard become significant. With knowledge of how these variables are spatially and temporally distributed, we can consider scenarios, based on the natural history of the insect, that explain causes for changes in the distribution and abundance of *D. frontalis* at the landscape scale.

Ways and Means of Measuring Functional Heterogeneity

Ways and means for measuring landscape heterogeneity have been reviewed by Kolasa and Pickett (1991), Turner (1987, 1989), S. Turner *et al.* (1991), and M. Turner *et al.* (1991). Although there are several methodologies available, no single index is suitable for characterization of functional heterogeneity for a given organism. Each organism perceives and responds to its environment in unique ways. The elements of landscape structure that are important in defining herbivory by bark beetles, for example, may be substantially different from those needed to characterize the process for deer populations. Figure 2 illustrates the relation of functional and measured heterogeneity. In Figure 2 functional heterogeneity is represented as a gradient ranging from homogeneity on one end to heterogeneity on the other. Interpretation of the significance of index values in the mid-range of the gradient is difficult. We emphasize, also, that intermediate heterogeneity conditions are most difficult to interpret from the behavioral perspective of the organism, i.e., it is difficult to predict how an organism will react to conditions of intermediate heterogeneity. For this reason measurement of functional heterogeneity for a specific organism will likely require use of several indices, each of which is sensitive to different aspects of landscape structure, e.g., connectivity, porosity, fragmentation, boundary configuration, etc. To address this issue, we developed (or adapted) three different procedures to characterize functional heterogeneity of forest landscapes relative to herbivory by *D. frontalis*. These indices are described briefly below.

Indices of Heterogeneity

Our initial goal in defining functional heterogeneity for *D. frontalis* was to combine knowledge of natural history of the insect with information on landscape structure. We developed (or modified) three indices to characterize functional heterogeneity. All use a weighted pattern-recognition computational approach, and each is sensitive to a different aspect of

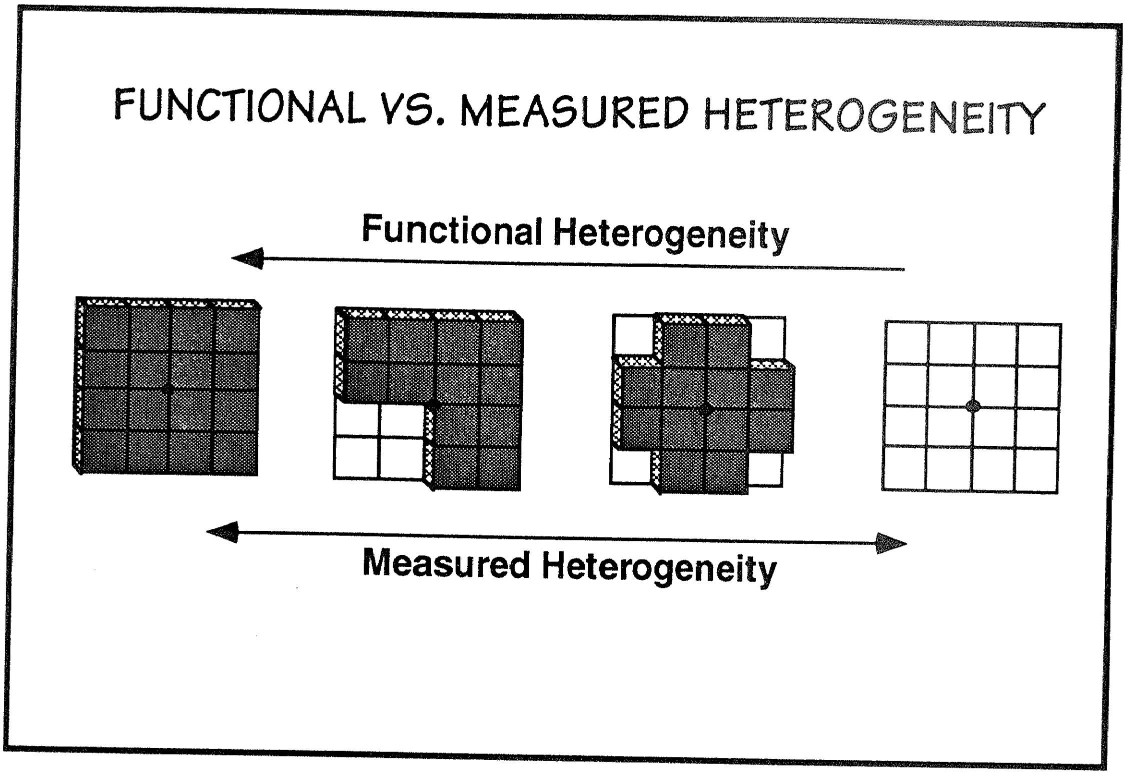

Figure 2.—Relationship of measured heterogeneity (composition of parts of different kinds) to functional heterogeneity (how an organism perceives and reacts to its environment). Note that measured heterogeneity for the left and right matrices is the same, but the functional heterogeneity is different.

Interpretation of the significance of index values in the mid-range of the gradient is difficult. The intermediate heterogeneity conditions are also the most difficult to interpret from the behavioral perspective of the organism, i.e., it is difficult to predict how an organism will react to conditions of intermediate heterogeneity.

landscape pattern. Application of the procedures involves (i) classification of land elements based on ecological function, (ii) tiling of the landscape data (usually maps or images), and (iii) quantification of spatial pattern. Following is a brief description of each index along with an explanation of the calculation procedure. These methods apply for any NxN array of weighted landscape elements. We are currently investigating the sensitivity of these indices.

The Angular Moment of Inertia Index (AMI). The AMI is an index that is sensitive to variation in the dispersion of landscape elements. It is the normalized product of a second moment calculation. The index is a measure of the dispersion of weighted matrix elements about the centroid of the landscape area. The AMI is calculated using the following procedures. First, the centroid of the weighted surface (matrix) is calculated. Because each element, a_{ij}, within the matrix has a value and locational indices attached, the horizontal (X_o) and the vertical (Y_o) centroids can be calculated by

$$X_o = (\Sigma_j \Sigma_i (a_{ij}) * j) / \Sigma_i \Sigma_j a_{ij}$$
$$Y_o = (\Sigma_i \Sigma_j (a_{ij}) * i) / \Sigma_i \Sigma_j a_{ij}.$$

Second, X_o and Y_o are used to derive the second moment (M) about the centroid

$$M = \Sigma_{ij}((j - X_o)^2 + (i - Y_o)^2) * a_{ij}).$$

However, because each element in the matrix represents a cell that has a real extent, the second moment for each cell must be calculated. The equation becomes

$$M = \Sigma_{ij}((j - X_o)^2 + (i - Y_o)^2) * a_{ij}) + \Sigma_{ij} a_{ij} / 12.$$

The Connectivity Index (CI). The CI index is based on a run-length encoding strategy that is commonly used to encode raster (matrix) data. Run-length encoding is a good compression technique for rasters that have a high degree of spatial autocorrelation. Run lengths are measured geometrically rather than arithmetically, giving more than linear value to longer runs. The computational procedures for the CI index are given below.

First, count the runs of matrix elements with the same value (i). For example, 1111 would be a run of length $L(1) = 4$. A row, column, or diagonal may have several runs. For example, vector 111222233333 has three runs. There can be multiple runs for a given i.

For each row, column, or diagonal, we sum the runs for each distinct element value. For example, vector 1112222211111 has three runs and two values. Converted to run values, the vector is 123, 12345, 12345. The sum of runs, by element value, for this vector is $\Sigma S(1) = 21$ and $\Sigma S(2) = 15$. For a given run i of length L, the run value is $S(i) = L * (L + 1) / 2$. For a given element value i, all possible S(i) are calculated by row, column, and diagonal, with the diagonal S(i) divided by 2^{-2} to compensate for separation distance. The total run value for a matrix and a given i is simply the $\Sigma S(i)$ for that matrix.

For the general NXN matrix, the smallest possible $\Sigma S(i)$, $i = 1$ to n where n is the number of different values, is

$$\Sigma Smin(i) = 2 (N^2 + N^2 / 2^{-2}).$$

$\Sigma Smin(i)$ exists where all run lengths are 1. The largest possible value, $\Sigma Smax(i)$, must be calculated using NXN matrix of constant i.

$$\Sigma Smax(i) = N^2(N+1) + 2(\Sigma K(K+1))/2^{-2} - N(N+1)/2^{-2}$$

Thus, the range is

$$R = \Sigma Smax(i) - \Sigma Smin(i).$$

The unweighted CI is calculated as

$$H = (\Sigma S(i) - \Sigma Smin(i)) / R.$$

The CI for a matrix where i values are relative to a function (i.e., hazard rating or probable use) can also be computed. The run values are calculated as weighted sums (WCI).

$$WCI = \Sigma\, i(\Sigma S(i)),\ i = 1, 2, \ldots .$$

The Spatial Interaction Index. The third index of heterogeneity is based upon the gravity model and is sensitive to fragmentation of the highest valued landscape elements. When applied to geographic analysis, the gravity model is called spatial interaction. Therefore, we have called this index spatial interaction (SI).

SI is based on Newton's law of attraction between masses. It states that attraction is proportional to the product of two masses (assigned weight) and inversely proportional to the distance between them. For a matrix of landscape elements represented in matrix form:

, $k = 1$, where

m_k and m_l are distinct elements of the matrix representing the attraction measure, r is the distance separating m_k and m_l, and t is the maximum value in the matrix.

Functional Heterogeneity of Forest Landscapes and Host Defenses

In this section we examine functional heterogeneity of forest landscapes in relation to epidemiology of *D. frontalis*. Our approach is to provide (i) a general overview of the procedure used to characterize functional heterogeneity, (ii) illustrate how the methodology facilitates integration of information on landscape structure and natural history of *D. frontalis*, and (iii) conclude with an interpretation of how host defenses influence epidemiology of the insect.

Approach for Application of the Indices to Define Functional Heterogeneity of Forest Landscapes

Use of the indices of heterogeneity is predicated on having a spatially referenced database that embraces the features of landscape structure essential to *D. frontalis.* Typically, development of GIS databases requires a substantial commitment to map and image processing in order to identify and reference pertinent landscape variables. In the case of *D. frontalis*, we are particularly interested in the habitat of the insect, i.e., patches of pines (graded by vulnerability) and lightning-struck hosts.

In addition to the spatially referenced database, it is also necessary to define how the organism interacts with its environment, i.e., to relate the natural history of the insect to habitat structure. We indicated earlier that a great deal is known about the interaction of *D. frontalis* and its hosts. Literature on this subject was reviewed in our treatment of host defenses. An essential feature of *D. frontalis*/host interaction centers on the process of dispersal. The parameters for this process determine how the network of population centers (infestations) can be connected to habitat patches and lightning-struck hosts. Although the dispersal process as a whole is poorly understood, certain elements of it have been studied in great detail, e.g., response of the insect to semiochemicals (Smith *et al.* 1993). We define boundaries for the dispersal process, based on estimates available from the published literature (Coulson *et al.* 1979, Pope *et al.* 1980, Wagner *et al.* 1984).

With the GIS database and information on how the insect interacts with its environment, we can use the indices to examine functional heterogeneity of the forest landscape. The general approach involves development of three types of maps: hazard, modified hazard, and heterogeneity. The hazard map is an abstract of the variables associated with different degrees of vulnerability of forest stands to infestation by *D. frontalis.* The modified hazard map includes additional information on location of lightning-struck hosts, infestation centers, and insect behavior. The heterogeneity map integrates these variables, i.e., information on habitat units, population centers, and insect behavior. This map represents a view of how *D. frontalis* might interact with its environment. The map is produced by applying the indices of heterogeneity to the modified hazard map. A moving-window calculation (Fig. 3) is used. The moving-window approach is a common technique in landscape ecological studies (Turner *et al.* 1991). It is particularly useful in defining functional heterogeneity, as window size and shape can be changed to accommodate different spatial scales (Weins 1989, Levin 1992) (Fig. 4). In the particular application, we are interested in the spatial scale used by *D. frontalis*, which is largely set by the distribution and abundance of habitat units and population centers and the dispersal capability of the insect.

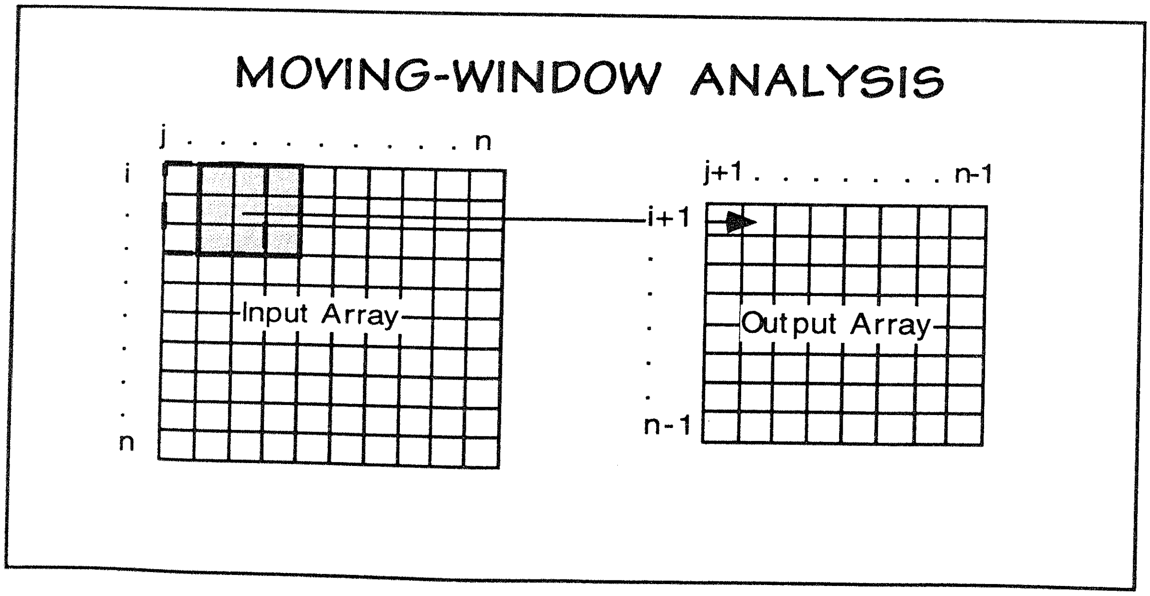

Figure 3.—The moving-window analysis applied to an array. The arrays can be raster renditions of landscape maps. Each cell (i,j) represents a 2-d sample of the landscape. In this example the window is 3X3 cells. In our methodology functional heterogeneity is calculated for land attributes in the windows of the input array and placed in the output array.

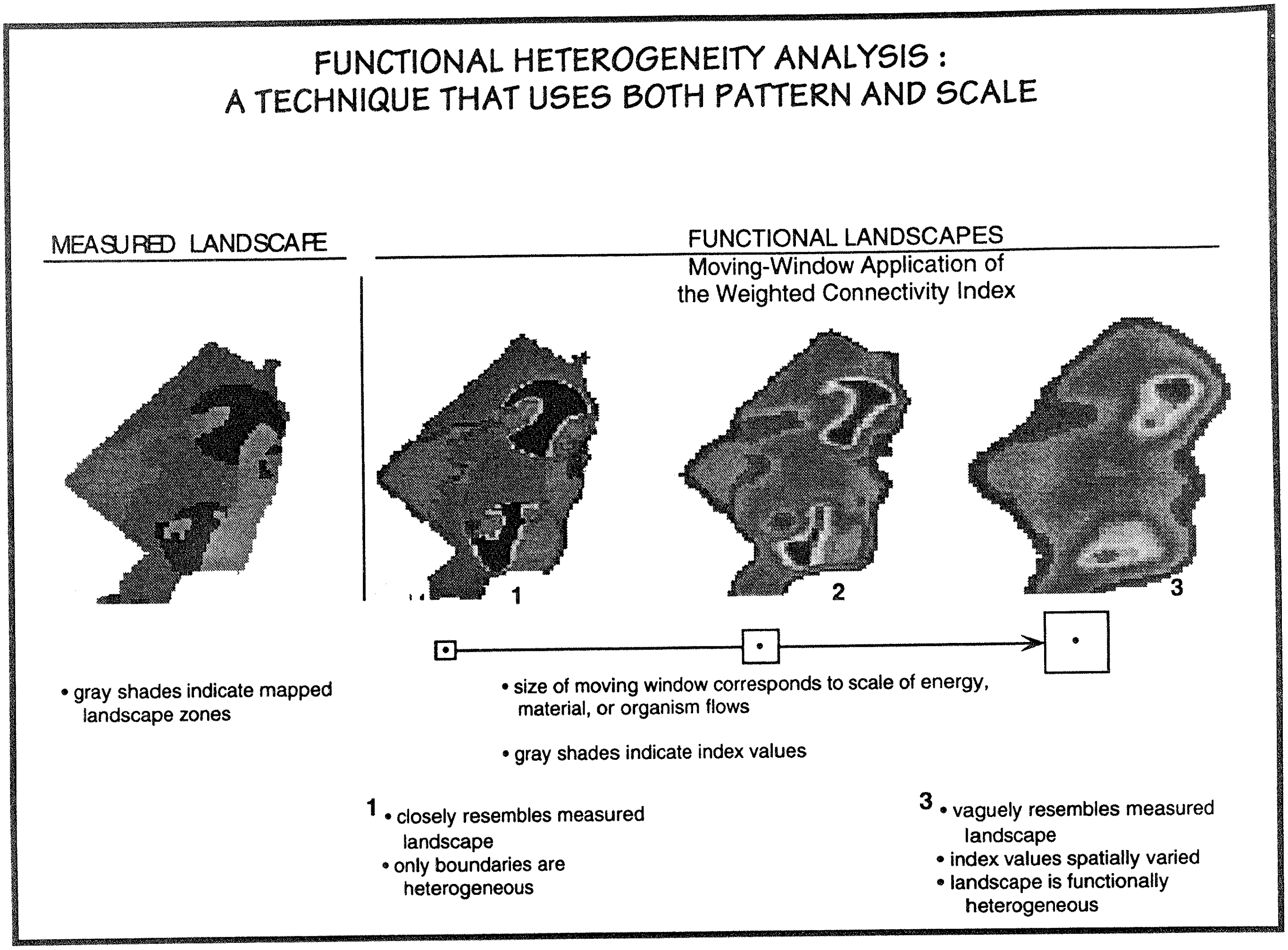

Figure 4.—Functional heterogeneity analysis using pattern recognition algorithms and moving-window calculations.

Relating Functional Heterogeneity to Epidemiology of *D. frontalis*

Although *D. frontalis* is among the most thoroughly studied pest species, there is no mechanistic explanation for how the natural history of the insect operates at the landscape scale to produce observed patterns of epidemiology (Dunning *et al.* 1992). We suggest that bark beetle epidemiology in a pine forest landscape involves a network of [i] lightning-struck hosts (Lovelady 1994), which serve as sinks during dispersal (Pullian 1988, Pullian and Danielson 1991); [ii] previous existing infestations, which serve both as sources and sinks; and [iii] high hazard stands, which also serve as sinks and are needed for development of infestations. With only modest requirements for dispersal distance (1 km), the bark beetles can reduce high measured heterogeneity in pine forest landscapes into high functional homogeneity, i.e., the insects can link dispersed food and habitat resources. Specific attributes of landscape structure will influence how this scenario is played out in different forest environments.

In the following example, we examine how *D. frontalis* interacts within a meso-scale (100 to 1,000,000 ha) forest landscape. The GIS-based study (McFadden 1994) was conducted on the Sam Houston National Forest in southeast Texas. This landscape is vegetated primarily with loblolly, *Pinus taeda* L., and shortleaf, *P. echinata* Mill, pines and mixed hardwood species. Populations of *D. frontalis* in the forest have cycled from enzootic to epizootic levels for more than two decades.

Data for the study were acquired from thematic maps, aerial photographs, lightning-strike records, and forest management records. The thematic map information included: ownership boundaries, roads, utility lines, hydrology, and forest compartment boundaries. Locations of *D. frontalis* infestations were obtained by georeferencing and interpreting aerial photographs, using a computer-assisted mapping system called MIPS (Map and Image Processing System). Aerial photographs were provided by the USDA Forest Service. Once located on aerial photographs, the UTM coordinates for infestation centers were collected and entered into the GIS (ARC/INFO) as point locations. Lightning strike data for the region were purchased from R-Scan Corporation. Again, UTM coordinates for lightning strikes were entered as point locations. Information on forest stands was taken from a database (CISC-Continuous Inventory of Stand Conditions), which is maintained by the Forest Service. These data sources were used to develop a sequence of three maps: the hazard, the modified hazard, and the heterogeneity. Each map is described below.

The Hazard Map. First in the sequence is a general *D. frontalis* hazard map (Fig. 5). This map was developed by hazard rating forest stands in the study area. In this specific application, we used tree species and age as the principal variables of hazard. We mentioned previously that several hazard rating systems have been developed (Lorio 1980). The utility of this map is that it defines patches in the forest matrix that are suitable for the development of infestations, given the presence of the insect (and acceptable weather conditions). The map also reflects habitat patches graded by host defense capability, i.e., the high hazard stands have the lowest capacity for host defense. This rendition of the landscape will have seasonal trend reflecting variation in host defenses associated with the normal physiological changes associated with tree phenological development described by Lorio (1988). The general hazard map will change slowly, i.e., it will require periodic updating, perhaps every 2 or 3 years, to remain an accurate portrayal of hazard.

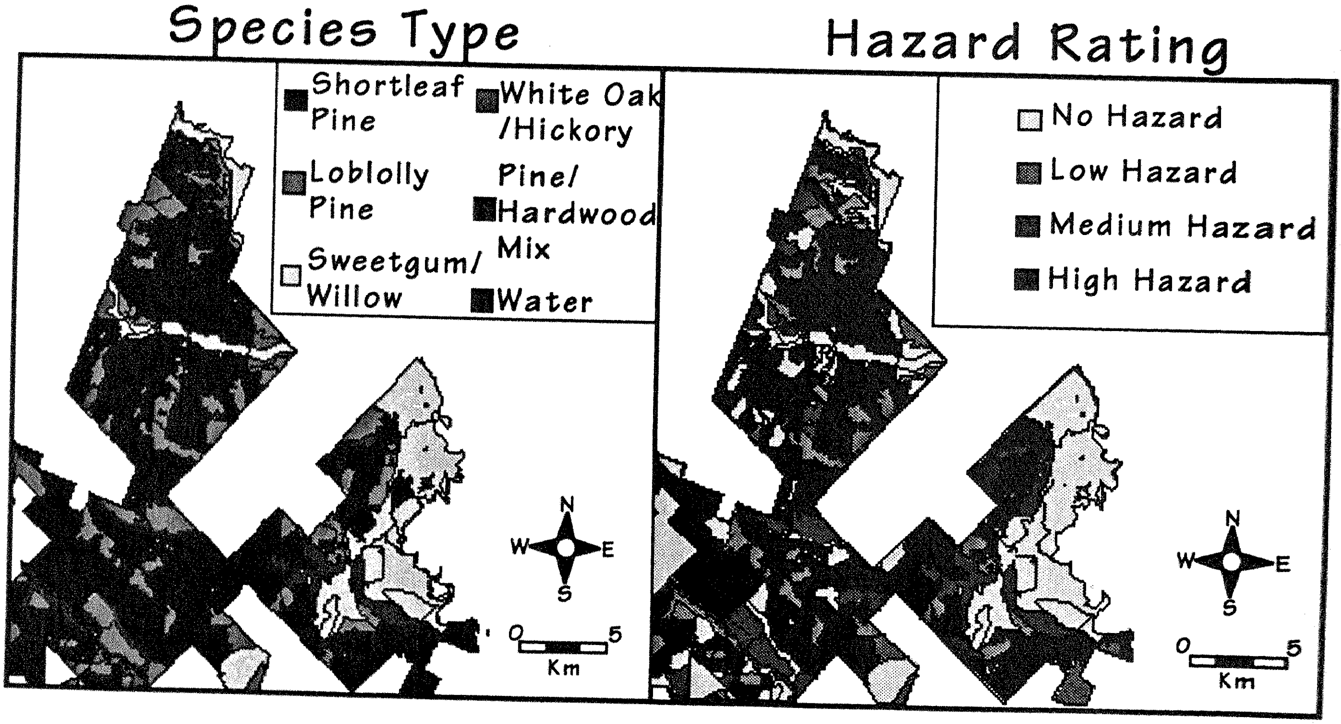

Figure 5.—Maps of a forest landscape classified by species types and hazard to herbivory by *D. frontalis*. Several systems for hazard rating have been developed. All involve integration of a subset of variables: tree species, radial growth, height, and DBH; stand basal area (pine, hardwood, total), species composition, site index, and degree of crown closure; and landform classification. When applied to a forest landscape, the hazard rating systems provide a general view of the distribution and abundance of host defenses against the insect.

The Modified Hazard Map. The next level of complexity in the map sequence involves adding information on the location of lightning-struck hosts and existing infestations (Fig. 6). The lightning-struck hosts represent sinks for dispersing beetles. We indicated above that these hosts have greatly reduced defense capacity, and they can be located, presumably through olfactory cues, by *D. frontalis*. The role of lightning-struck hosts has been examined in detail in Coulson *et al.* (1986a), Flamm *et al.* (1992), Lovelady *et al.* (1991), and Lovelady (1994). The existing infestations can serve both as sinks and sources for dispersing beetles. The product of adding the lightning-struck hosts and infestation centers to the hazard is illustrated in Figure 7 (left panel). The modified hazard map also incorporates information on dispersal distance of the insect. We assumed that the insect could disperse a distance of ca 1 km. This conservative estimate was based on reports from the literature cited above. On the modified hazard map (Fig. 7 - left panel), the dispersal distance was represented as an area (a circle with a 1 km radius) around the lightning-struck hosts and infestation centers. The lightning-struck hosts are point locations in the forest matrix. However, these hosts have a substantial impact on epidemiology when their locations are considered within the context of the dispersal range of *D. frontalis*. The modified hazard map contains the elements of landscape structure involved in epidemiology, i.e., hazard rated forest stands, lightning centers, and population centers. The addition of dispersal distance provides the means for connecting the elements. This rendition of the landscape has a significant weather-related annual pattern. Thunderstorm (and thus lightning) activity follows seasonal cycles (Lovelady 1994). Insect development and dispersal behavior also have seasonal trends. The information content of the modified hazard map will change during the course of a year, i.e., it will require frequent updating, perhaps weekly or monthly, to remain an accurate rendition of hazard.

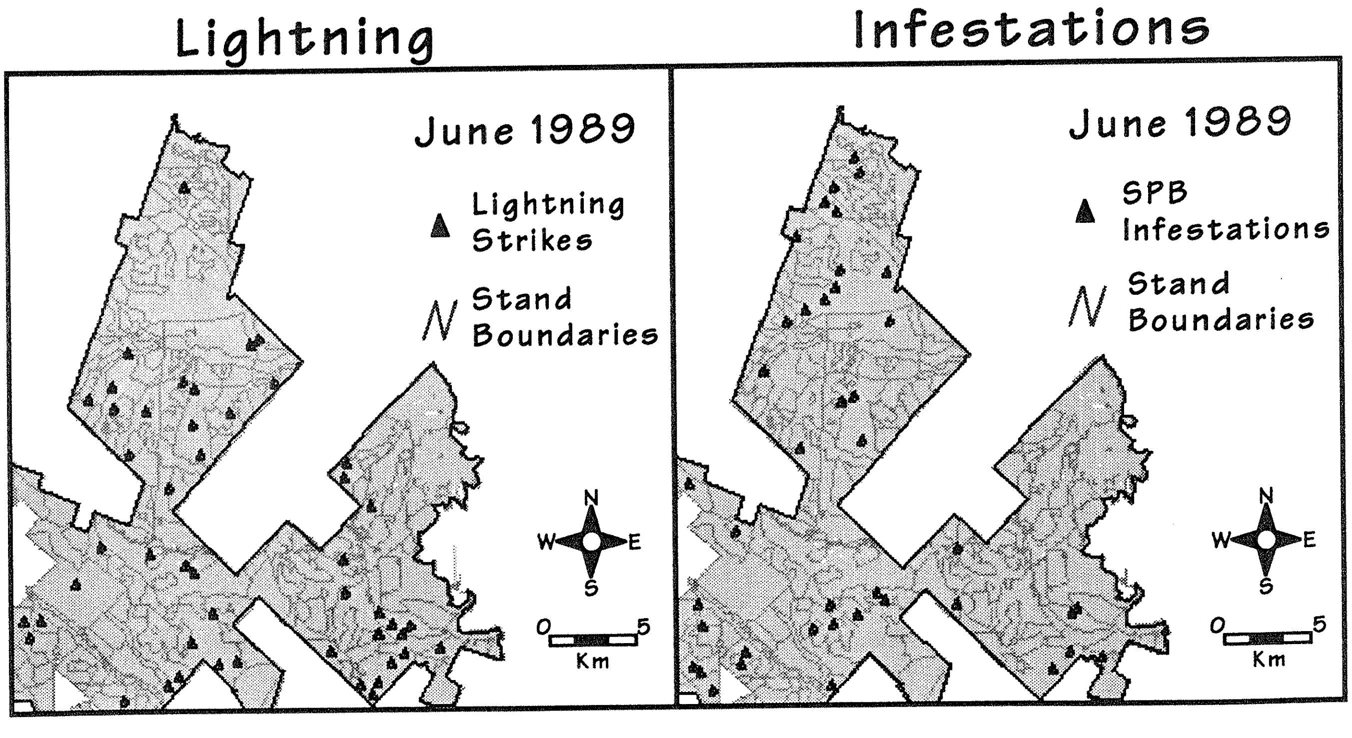

Figure 6. Maps of a forest landscape with point locations for lightning-struck hosts and existing infestation centers. Lightning-struck hosts have greatly reduced defense capacity and are used as refuges by *D. frontalis*, i.e., they are sinks for dispersing populations of the insect. The infestation centers serve as both sinks and sources for dispersing populations of the insect.

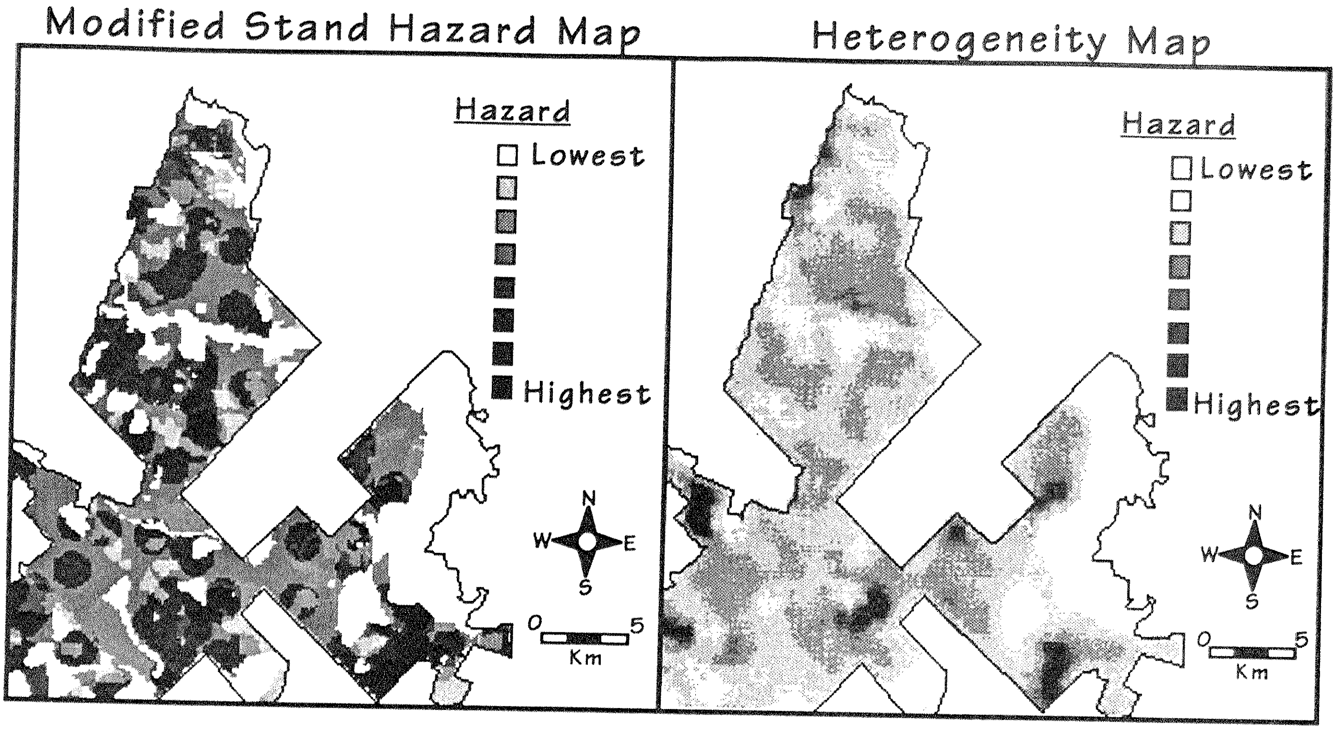

Figure 7. Modified hazard map (left panel) incorporates information on lightning-struck hosts and existing infestation centers. This map also contains information on the dispersal behavior of *D. frontalis*. The dispersal distance was represented as an area (a circle with a 1 km radius) around the lightning-struck hosts and infestation centers. Dispersal and host selection behavior of the insect link the network of high hazard stands, infestation centers, and lightning-struck host. The heterogeneity map (right panel) was developed using the AMI index applied to the modified hazard map, using the moving-window calculation. This map illustrates how the network of high hazard stands, lightning-struck hosts, and existing population centers is connected through dispersal behavior of the insect.

The Heterogeneity Map. Third in the sequence is the functional heterogeneity map (Fig. 7 - right panel). In this particular example, the AMI index was applied to the modified hazard map, using the moving-window calculation described above. Recall that the AMI index is sensitive to dispersion of landscape elements. The functional heterogeneity index allows for integration of the information on landscape structure and insect behavior known to influence epidemiology of *D. frontalis*. The heterogeneity map (Fig. 7-right panel) is a visualization of the results. This map illustrates how the network of high hazard stands, lightning-struck hosts, and existing population centers is connected through dispersal behavior of the insect. Knowledge of this interaction will certainly increase with new research on epidemiology, and this added complexity can be accommodated by the approach. Figure 7 (right panel) is a characterization of only one point in time. During the course of the year, functional heterogeneity of the landscape will change for the reasons outlined above. Functional heterogeneity will also change during successive years of an insect outbreak, as herbivory creates additional fragmentation of the forest matrix. Furthermore, there will be a flux in habitat available to the insect in successive years, as high hazard stands are depleted through herbivory and new stands grow into the vulnerable age classes. The index values are a quantitative measure of functional heterogeneity and therefore can be used for the analysis and description of sequential map data. Recall, also, that the three indices are sensitive to different aspects of landscape pattern. Therefore, each index will provide alternative insights into how *D. frontalis* perceives and reacts to its environment.

Host Defenses and Epidemiology of *Dendroctonus frontalis*

To summarize, at the onset of this study, we indicated that epidemiology of *D. frontalis* was related to the distribution of host defenses in forest landscapes. Previous research had clearly established that infestations typically occur in high hazard stands consisting of hosts with reduced capacity for defense against colonization by bark beetles. Hazard rating systems facilitate mapping of forests according to their vulnerability to bark beetle infestation. These hazard maps define a landscape mosaic comprised of forest stands graded by defense capacity (Fig. 5). Typically, the high hazard stands are also the preferred habitat of the insect, i.e., populations of bark beetles are clustered in high hazard stands. As long as population density remains sufficient to overcome host defenses, infestations grow in size through colonization of neighboring hosts. When population density falls below the level necessary for successful host colonization, the insects must disperse to find suitable refuges. Two strategies are likely. The insects can disperse to supplement populations in existing infestations at other locations or they can colonize lightning-struck hosts, which have greatly reduced defense capacity. These two elements, existing infestations and lightning-struck hosts, add to the picture of host defense for the forest landscape (Fig. 7-left panel). However, forest landscapes in the southeastern US are often highly fragmented, and the vulnerable stands, existing infestations, and lightning-struck hosts are dispersed. Behavioral strategies associated with dispersal and host selection link populations of the insect to suitable habitat centers (Fig. 7-left panel) (Southwood 1977). Finally, the indices of functional heterogeneity serve to integrate the information on the distribution of host defenses with knowledge on how the insect perceives and responds to its environment (Fig. 7-right panel). Further insight into mechanisms of epidemiology of *D. frontalis* can be gained through analysis of changes in functional heterogeneity of sequential landscape scenes.

SUMMARY

This paper focused on functional heterogeneity of forest landscapes and epidemiology of *D. frontalis*. Specific conclusions from the study include the following:

1. The general premise of this study was that epidemiology of *D. frontalis* involves a network of high hazard stands, lightning-struck hosts, and existing population centers. This network is connected at the landscape scale through dispersal behavior of the insect.

2. The concept of functional heterogeneity (how an organism perceives and responds to its environment) provides a useful way to organize knowledge about landscape structure and insect behavior. Although we have focused on functional heterogeneity of forest landscapes and epidemiology of *D. frontalis*, the approach can be used for other insects and taxa.

3. The indices of functional heterogeneity were found to be useful in integrating the landscape variables known to influence epidemiology of *D. frontalis*.

4. Measurements of functional heterogeneity of forest landscapes incorporate two types of knowledge about host defenses. The first deals with knowledge embedded in the various types of hazard rating systems used to grade forest stands by vulnerability to *D. frontalis*. The second deals with knowledge on the interaction of lighting-struck hosts and *D. frontalis*. The functional heterogeneity map provides a visualization of the distribution of host defenses within a meso-scale forest landscape.

5. The functional heterogeneity indices are a tool that can be used to investigate several fundamental issues in epidemiology. For example we hypothesize that (i) functional heterogeneity of forest landscapes influences the rate and extent of herbivory by bark beetles and that (ii) initiation, growth, and decline phases of forest insect outbreaks can be predicted by definition of the change points in functional heterogeneity of landscapes.

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ACKNOWLEDGMENTS

We acknowledge and thank Audrey M. Bunting for assistance in the preparation of the figures and text of this paper. We are grateful to the USDA Forest Service, National Forests in Texas for providing access to GIS databases and forest management records; and to the Texas Forest Service for providing access to their operational records on *D. frontalis*. The work reported herein was supported by the USDA Competitive Grants Program [TEX06961 (Landscape Study of Bark Beetle Herbivory and the Lightning Disturbance Regime) and TEX08185 (Predicting Forest Insect Outbreaks)], and by the Texas Agricultural Experiment Station. This paper is TA No. 31482 of the Texas Agricultural Experiment Station.

☆U.S. GOVERNMENT PRINTING OFFICE: 1996-757-939

U.S. Department of Agriculture, Forest Service.
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Herbivory on woody plants is highly variable in both space and time. This proceedings addresses one of its root causes, the highly intricate and dynamic relationships that exist between most herbivores and their hosts plants. It emphasizes that the consequences of herbivory both to the consumer and to the producer plant often balance on a razor's edge—depending on the exact timing of herbivore attacks, and the specific plant tissues being injured. Herbivory also varies substantially among individual plants in relationship to the inherent resistance/susceptibility of individual plants—which itself heavily depends on the particular physical and biotic (community, ecosystem, landscape) environmental matrix in which the plant grows. No two plant-herbivore systems are exactly alike, and hence generalities are difficult to establish.

KEY WORDS: Plant resistance, plant-herbivore interactions, inducible defenses, hybrid sinks, phenological windows, bark beetles, folivores, population dynamics, plant stress.