QUANTITATIVE CHEMICAL ECOLOGY OF THE LINGONBERRY FRUITWORM, GRAPHOLITA LIBERTINA HEINR.

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by

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A thesis submitted to the
School of Graduate Studies
in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy

Department of Biology
Memorial University of Newfoundland

November 2001

St. John's Newfoundland

ABSTRACT

The lingonberry or partridgeberry, *Vaccinium vitis-idaea* L. var. *minus* Lodd, is a low-growing ericaceous shrub, which produces edible berries that ripen in Newfoundland in mid-September. *Grapholita libertina* Heinrich, the lingonberry fruitworm, is a tortricid moth whose larvae feed within the lingonberry fruit.

The objectives of this study were to identify sex attractants for *G. libertina* and to evaluate its use in monitoring and controlling populations in wild Newfoundland lingonberry fields. A synthetic sex attractant was developed from among chemicals attractive to other *Grapholita* species and tested in 1997 to monitor *G. libertina* populations. Identification of this attractant led to a series of questions. Could this attractant prove useful in estimating future larval infestations? What would be the most effective delivery system for this attractant? Could field trapping accurately predict flight? How similar is the synthetic male attractant to the naturally occurring female produced pheromone?

Field trials were conducted with the sex attractant in 1998, 1999 and 2000 to correlate the adult trapping rate with the subsequent densities of larvae and damaged berries to examine the effects of berry distribution and heterogenous vegetation coverage in the wild. Trials in 1999 were conducted to determine the most effective trap design for monitoring *G. libertina*. The efficacy of mass trapping using sex attractants of *G. libertina* was tested in 2000 as a potential control measure. In addition, information on

population trends and phenology of *G. libertina* were examined through recording of the flight season, degree day accumulations and population size. Field-collected *G. libertina* were reared in order to identify the naturally occurring female sex pheromone. Solid phase microextraction was used to collect insect effluvia and gas chromatography-mass spectrometry attempted to identify pheromone components and relative amounts in the pheromone blend.

The results of this study indicated that a blend of: 85% E-8-dodecen-1-ol acetate, 10% Z-8-dodecen-1-ol acetate, and 5% Z-8-dodecen-1-ol was a suitable synthetic sex attractant for male *G. libertina*. The adult capture rate in Pherocon 1C® wing traps was correlated with subsequent larval and damaged berry density in wild fields. Berry densities were important in determining the distributions of larvae and damaged berries when berry levels were low (1999), perhaps indicating that a limited or patchy host berry distribution affected female oviposition. Heterogenous vegetation present at study sites showed no significant effects.

The Pherocon 1C® wing trap was the most effective for use with the 85:10:5 blend. Mass trapping indicated a possible disruption of mate location by *G. libertina*, however no significant decrease in larval populations was noted. As a result of trapping, it was established that the adult male flight season extends over 6 weeks from late June to early August. The number of degree days above base 5° C required for 10% emergence was recorded as 270±20.5 by rearing, and 334±8.1 by field trapping. Identification of the female sex pheromone by gas chromatography- mass spectroscopy was not successful.

ACKNOWLEDGMENTS

I would like to thank my supervisors, Dr. Peggy Dixon and Dr. Dave Larson for their endless patience, support and inspiration. You are both fantastic scientists and very dear friends. I consider it a privilege to have worked with you. I am thankful to Dr. Jean Finney-Crawley for acting on my supervisory committee, reviewing this draft, and for being a source of motivation and advice. I'd also like to thank Dr. Bill Seabrook from the University of New Brunswick for his initial project design and mentoring during the early years of this project. I am grateful for all of the help given to me by Barbara George, Janet Coombes, and the other fine folks at the Atlantic Cool Climate Crop Research Centre. I am also appreciative of my fellow graduate students in the Biology Department, you have kept my spirits high and my 'bitterness' low, best of luck to you all.

I would like to extend my thanks and appreciation for the never-ending support and assistance provided by my family, particularly my parents - Neil and Marty. Finally, I'd like to thank my wife, Wendy, for her encouragement, understanding and patience in this project. Your help has ranged from raising my spirits and preventing me from burning out, to pushing me to work harder and assisting in the joyous task of picking endless bags of berries in less than desirable weather conditions.

I appreciate and am grateful for the financial support provided by the Green Plan and the Safety Nets Council, the Biology Departmental Graduate Frellowship and the Special Scholarship for Natural Resource Development.

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ABBREVIATIONS

Site abbreviations:

P:	Pouch Cove, NF.
L:	Little Catalina, NF.
F:	Freshwater, NF.
C:	Chance Cove, NF.
B:	Bryant's Cove, NF.

Chemical abbreviations:

E,E8,10-12:OAc	E,E-8,10- dodecadien-1-ol acetate
E8-12:OAc	E-8-dodecen-1-ol acetate
Z8-12:OAc	Z-8-dodecen-1-ol acetate
E7-12:OAc	E-7-dodecen-1-ol acetate
Z8-12:OH:	Z-8-dodecen-1-ol
85:10:5:	85% E-8-dodecen-1-ol acetate 10% Z-8-dodecen-1-ol acetate 5% Z-8-dodecen-1-ol
90:7:3	90% E-8-dodecen-1-ol acetate 7% Z-8-dodecen-1-ol acetate 3% Z-8-dodecen-1-ol
94:4:2	94% E-8-dodecen-1-ol acetate 4% Z-8-dodecen-1-ol acetate 2% Z-8-dodecen-1-ol

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1.0 INTRODUCTION

1.1 Insecticides

Insect control has been an important issue for humanity throughout history. While the class Insecta and their arthropod kin represent an essential (and large) part of the earth's ecosystems, many species are defined as pests. Flint and van den Bosch (1982) described pests as "organisms which compete with people for food, fiber and shelter; transmit pathogens; feed on people; or otherwise threaten human health, comfort, or welfare". Coinciding with humanity's disdain for such pests has been the need to control, eradicate and exterminate harmful species.

The twentieth century has been dominated by the advent of synthetic (organic) insecticides. Synthetic chemicals belonging to the organochlorines (e.g. DDT, toxaphene, endrin, aldrin, dieldrin) and organophosphates (e.g. parathion, diazinon, malathion) were first found to be insecticidal during World War II (WWII)(Mellanby, 1970). The infamous DDT (dichloro-diphenyl-trichloro-ethane) became very widely used during WWII to control the lice vectors of typhus, trench and relapsing fever, and malarial mosquitoes in southern Europe and Africa (Mellanby, 1970). DDT was heralded as a "wonder chemical" with a significant broad spectrum range, low acute mammalian toxicity compared with other synthetic pesticides, and persistent activity in the environment (Edwards, 1993). Following the war, DDT was used on a large scale in the forestry, medical and agricultural industries to control pests worldwide.

It soon became evident that the extensive use of DDT and other insecticides was

accompanied by serious health and environmental problems (Hall, 1995). Control of pests was becoming more difficult due to the development of insecticide resistance in pests, for example by 1948 the common housefly was resistant to DDT throughout the United States. In addition, the destruction of predatory and parasitic arthropods which had served as natural controls prior to broad spectrum pesticide application in turn released other innocuous insects previously controlled naturally (Perkins, 1982).

Organochlorines such as DDT were found to persist in the environment and also in the tissues of living organisms. In the case of DDT, this resulted in biomagnification through the food chain. Originally applied at acceptably safe levels to crops and pests, it reached dangerous levels in top level carnivores such as raptorial birds and humans (Edwards, 1993). Other synthetic chemicals, such as the organophosphorous compounds, which were originally derived from nerve gas, may be highly toxic to vertebrates and caused problems when contaminated seed, food or insects are eaten (Carson, 1962; Edwards, 1993).

The environmental consequences of large-scale pesticide application were massive fish kills in contaminated waterways, destruction of delicate wildlife and ecosystems, and risks to human health (Edwards, 1993). Pesticides such as DDT and other organochlorines were found to have high chronic toxicity and contaminated areas and organisms far beyond the scope of their application, passing into human food and milk (Pimentel *et al.*, 1993a).

While the use of organochlorines has been significantly reduced in the first world, 1600 pesticides are available worldwide, with 2.5 - 4.4 million tons (or 21 billion dollars

worth) used annually, with nearly 30% being insecticides (Matthews, 1992). Pimentel *et al.* (1993b) have estimated that worldwide, 850,000 to 1.5 million people are poisoned from pesticides annually, resulting in 3,000 to 20,000 deaths each year, largely due to accidental exposure and poisoning. In the United States, despite a tenfold increase in insecticide use, crop losses due to insects grew from 7 % to 13% between 1945 and 1989 (Pimentel *et al.*, 1993a). It should be noted that these increases were partially due to evolved insecticide resistance by pests, and also due to changes in cropping practices (reduced crop rotation, large monocultures, reduced genetic diversity in seed) (Pimentel *et al.*, 1993a; Flint & van den Bosch, 1982). Awareness of the hazards of synthetic insecticide use has resulted in a demand for alternatives to and ways of reducing pesticide use, both publicly (through greater awareness), and in industry (through stricter regulation) (Carson, 1962; Sachs, 1993).

Alternatives to insecticides have largely been generated through a better understanding of pest biology. Biological control tactics use natural enemies (microbial pathogens, predatory and parasitic insects, and vertebrate predators) to control pest species through the importation of exotic enemies versus imported pests, or by conservation of enemies already present (Ehler, 1998). A pest species may also be used to disseminate its own doom, through sterile insect release programs directed specifically at pest eradication. GMO's, or genetically modified organisms may be used in pest management either through producing pest-resistant plants, superior biological control insects and pathogens or by acting directly on a pest species. Alternatives include the use of naturally produced chemicals to regulate and control insect populations. These may be

insecticidal in nature, such as botanical pesticides, or have an effect on the insect's growth and behaviour, such as semiochemicals (insect pheromones, repellents, insect growth regulators).

1.2 Semiochemical diversity

Alternatives to insecticides include the use of semiochemicals. Semiochemicals are chemicals that mediate interactions between organisms: those which act on different species are allelochemicals, those used for intraspecific communication are pheromones (Law & Regnier, 1971; Nordlund *et al.*, 1981). Some semiochemicals, such as defensive secretions by plants, act as repellents, antifeedants or insect growth regulators to pest insects, and others, such as insect sex pheromones, may function as attractants. The various effects of semiochemicals on insects make them useful in pest management for monitoring insects by trapping, or as area-wide control measures via spray formulations.

1.2.1 Allelochemicals

Allelochemicals, chemicals which mediate interspecific communication, are divided into four groups: allomones, which benefit the producer and deter the receiver; kairomones, which are detrimental to the producer and of benefit to the receiver; synomones, which benefit both (e.g. floral scents and pollinators); and apneumones, chemicals produced by non-living things (e.g. carrion) (Howse, 1998a). Allomones and kairomones are the most important for insect-plant interactions. Allomones are generally defensive substances which have toxic or antifeedant properties, whereas

kairomones are attractants and phagostimulants (Jutsum & Gordon, 1989). Some semiochemicals may be both allomonal and kairomonal in action, for example cruciferous plants produce isothiocyanate, which is toxic to most herbivorous pests, but is highly attractive to specialized pests such as the cabbage root maggot, *Delia radicum* L. (Diptera: Anthomyiidae), and the cabbage butterfly, *Pieris rapae* L. (Lepidoptera: Pieridae) (Metcalf & Metcalf, 1992). Hypericin, found in St. John's wort *Hypericum perforatum* L., is a pigment which induces photosensitivity when the plant is consumed, and is normally a general insect antifeedant. However, hypericin is phagostimulatory to *Chrysolina hyperici* Forster (Coleoptera: Chrysomelidae) beetles and in this case is a kairomone (Metcalf & Metcalf, 1992). Further, *C. hyperici* sequesters the hypericin into the cuticle, where it acts as an antifeedant to predators.

Allelochemicals have great potential for use in pest management, with strategies based primarily on their ability to attract or repel pest species. Azadirachtin, produced by the Neem tree, *Azadirachta indica* L., may act as an antifeedant as it has been shown that extracts artificially applied to a plant's surface act systemically to prevent insect feeding (Warner *et al.*, 1997). Other strategies use the attractive odour of hosts to trap and kill insects, for example the tsetse fly *Glossina pallidipes* Austen (Diptera: Glossinidae), can be attracted to and killed in insecticidal cloth target traps baited with host-produced phenolic compounds (Jones, 1998).

Allelochemicals are naturally occurring products, typically having low toxicity (Howse, 1998a; Jutsum & Gordon, 1989). Those which are toxic, such as antifeedants, are targeted at pest species feeding directly on the host plants, and have little direct effect

on non-target organisms (Warner *et al.*, 1997). The number of insect species affected by allelochemicals is variable, and dependant on both the host's (plant or animal) natural resistance and a pest species' ability to overpower it.

1.2.2 Pheromones

The first use of the term "pheromone" (Greek: phereum, to carry; horman, to excite) was by Karlson and Luscher (1959) who defined them as 'substances which are secreted to the outside by an individual and received by a second individual of the same species in which they release a specific reaction, for example a definite behavior or developmental process' (Hall, 1998).

The existence of insect pheromones was known long before their first description or identification. In 1609, Charles Butler described attraction and mass stinging by bees drawn to a substance found in a single bee sting (Nordlund *et al.*, 1981). Research has since shown that alarm pheromones, which attract and recruit other members of the same species to attack, are ubiquitous in the social Hymenoptera (Howse, 1998a).

Fabré (1879) provided the first scientifically documented evidence of pheromone communication in insects by recording the number of marked male emperor moths *Saturnia pavonia* L. (Lepidoptera: Saturniidae) attracted to a single caged female several kilometres away. Pheromones have now been isolated from over 477 species of Lepidoptera alone, with field attraction demonstrated in 1151 additional species (Hall, 1998; Arn, 1999).

Production and function of pheromones is variable throughout the class Insecta.

The honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), has a complex system of pheromone communication with at least ten different glands which produce pheromones for attraction, identification, alarm and territorial cues (Howse, 1998a). Further, queen honey bees dictate growth and behaviour of workers and drones within the hive through queen pheromone (Winston & Slessor, 1992).

Within the Coleoptera, research has focused on economically important cryptic species, such as the Scarabaeidae, whose larvae damage vegetation from beneath the soil, or the Scolytidae, which excavate galleries under the bark of host trees. Scolytid beetles use pheromones in conjunction with defensive allomones, such as myrcene or terpenes, to aggregate on stressed host plants (Byers, 1989). These aggregations serve in colonization of host trees and in courtship between the sexes.

"The most widespread and widely documented types of pheromones are those which are used to increase the probability of successful mating, sex pheromones" (Jutsum and Gordon, 1989). Butenandt *et al.*(1959) were the first to isolate an insect sex pheromone, bombykol, from the silkworm moth, *Bombyx mori* L. (Lepidoptera: Bombycidae). Sex pheromones are usually, but not exclusively, aerosol chemicals released by females which induce flight and mate-location and/or mating behavior in the male (Stoffolano & Romoser, 1994).

In a generalized sex pheromone communication system, a virgin female will produce a volatile pheromone from glands located on her abdominal tip. The pheromone is carried by diffusion and air currents from the emitting female. A male insect of the same species will perceive the pheromone with specialized antennal receptors, and begin

windward anemotactic flight. In many species, this involves upwind locomotion using a series of counter-turns which allow the male to maintain a heading in the direction of the female. This system allows the female to attract males from long distances with minimal energetic investment (Howse, 1998a).

The previously described system may be applied to many insect species, however several specialized mating strategies have evolved. As mentioned, *Ips* De Geer and *Dendroctonus* Erichson spp. (Coleoptera: Scolytidae) use aggregation pheromones, rather than individual female-male attractants to attract members of both sexes (Byers, 1989). In some insect species, males have been shown to produce pheromones through abdominal hairpencil glands, which are arrestant and aphrodisiac pheromones to make the female more receptive to copulation. Hairpencilling compounds in *Grapholita molesta* Busck (Lepidoptera: Tortricidae) have been identified, acting both to attract females and repel other males in close proximity (Nishida *et al.*, 1985). In the saltmarsh caterpillar, *Estigmene acrea* (Lepidoptera: Arctiidae) Drury, males emit aggregation pheromones, producing leks, and cumulatively attract large numbers of females (Willis & Birch, 1982). Receptive structures may also vary. Langley *et al.* (1987) demonstrated that in *Glossina morsitans* Westwood (Diptera: Glossinidae) pheromone-receptive sensillae are located on the tibia and tarsus, rather than antennal segments.

1.2.3 Pheromone identification

Elucidation of the chemical structure of bombykol took over twenty years, and required the processing of several million silkworm moths (Butenandt *et al.*, 1959; Hall,

1998). Early procedures for pheromone identification involved bioassays of insect behavior based on exposure to extracts. This was followed by separation and functional group determination by paper chromatography, infra-red and ultra-violet spectroscopy, and derivatization, and followed by repeated bioassay of separated components (Hall, 1998). In recent years, pheromone research has benefitted from technological advances in chromatography, spectroscopy and collection techniques.

The development of gas chromatography (GC) has provided a sensitive means for separation of volatile compounds on a stationary absorbent. The gas chromatogram may be coupled with either an electroantennogram to simultaneously measure depolarization across antennae and GC recordings, or a mass spectrometer which provides masses and intensities of target molecules from the GC (Howse, 1998b).

Pheromone collection has improved through the use of brief gland washing, which reduces contamination of extracts used in chromatographic separation (Howse, 1998b). Solid absorbents, such as activated charcoal or Porapak Q®, filter insect-produced volatiles from aerated chambers for analysis (Hall, 1998). More recently, the advent of solid phase microextraction (SPME), in which analytes are absorbed directly on to a solid absorbent and injected into a gas chromatogram, has been used in pheromone analysis (Frerot *et al.*, 1997). This reduces contamination from solvents and allows for collection of pheromones in headspace (vapor surrounding sample) non-destructively, so insects may be bioassayed more than once.

Laboratory identification and bioassay are important for insect pheromone development, however, evaluation of pheromone blends through field trials is also

important in order to determine the efficacy of pheromones as management tools. Knowledge of appropriate trap design, trap placement and pheromone concentration must be tested for each species, along with the effects of field tested pheromones on non-target species. Further, the ability of pheromone trapping to accurately predict pest emergence and levels in monitoring programs, or reduce pest infestation in lure and kill or mating disruption, should also be evaluated before incorporation into a management strategy.

The identification of the chemical structure of an insect pheromone can be a difficult task (George, 1965). However, identification of pheromone analogues (chemicals which elicit a similar behavioral response to that of pheromone) may produce suitable sex attractants for monitoring insects, with much less cost and effort (Ando *et al.*, 1977). Within certain subfamilies of the Lepidoptera many species use similar attractant structures (Roelofs & Comeau, 1970). Mozüraitus *et al.* (1998) used field screening of C_{12} and C_{14} alcohols and acetates in Delta© traps to isolate male sex attractants for 17 species of moths from the families Gracillariidae, Tortricidae, Yponomeutidae, Oecophoridae, Pyralidae and Gelechiidae. Therefore, screening of prospective compounds based on similarity among taxa is a potential method for rapid identification of field attractants.

1.2.4 Pheromones in pest management

Insect pheromones, especially sex pheromones, have been used widely for pest management. Booth (1988) recorded 250 pheromone products in use to control 436 pest species in the United States. In 1998, the pheromones, primarily sex pheromones (97%),

were applied worldwide over 1.3 million hectares of crops and forest, targeting primarily lepidopterous (95%) insects (Shami, 1998). Pheromones are currently used in pest management for population monitoring, mass trapping and attracticide control, and mating disruption (Jones, 1998). The benefits of pheromone products are that they are moderately species-specific, non-toxic and rapidly biodegradable (Jutsum & Gordon, 1989).

Pheromone trapping is a key element in eradication programs, both to monitor population size during pest suppression, and to monitor for pest reinvasions following eradication (Myers *et al.*, 1998). In the case of the Mediterranean fruit fly, *Ceratitis capitata* Weidemann (Diptera: Tephritidae), countries must maintain 'fly-free zones', a requirement for export to the United States, in which attractant traps are used to verify pest absence (Jones, 1998). The most extensive pheromone monitoring network is maintained by the U.S. Department of Agriculture (USDA) to identify areas infested by the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) (Leonhardt *et al.*, 1993). Through the use of 'thresholds' (predetermined pest population levels which warrant control measures in agricultural systems) growers can use capture rate in pheromone traps to determine the need for insecticidal application and to permit precise timing of control measures, thus preventing unnecessary insecticide application when populations are low (Pitcairn *et al.*, 1992; Pruess, 1983). This maximizes the effect of pesticide application by targeting vulnerable (those most susceptible to insecticides) life stages (such as neonate larvae) and may reduce the total number of applications required.

Pheromones and other attractants may be incorporated directly into a control

strategy by combining a pheromone lure with either an insect trap or other agent to remove insects from the population (Trimble & Hagley, 1988). Other lure and kill strategies attract insects to insecticide-treated areas, sterilants or electric grids. These strategies eliminate problems encountered by trap saturation (decreased trapping ability due to congestion of traps with dead insects). In the case of the oriental fruit fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae), the use of attracticide-baited traps led to its eradication from the Okinawa Islands in 1982 (Koyoma *et al.*, 1984).

More recently, insect pheromones have been investigated as a control measure through mating disruption. Many groups of insects (especially the Lepidoptera) rely primarily on olfactory stimuli to locate a mate, typically by males locating 'calling' (pheromone-emitting) females (Mayer & McLaughlin, 1991). Mating disruption relies on upsetting communication between male and female insects by saturating an area with the appropriate pheromone (Baker, 1985). This prevents mate location by: a) saturating the male insect's antennal receptors, resulting in confusion or habituation to the signal; or b) masking scent trails, the 'pheromone plume' produced by the calling females which stimulate males to engage in upwind flight to the female (Campion *et al.*, 1989). One of the most successful mating disruption programs was undertaken in Australia for the oriental fruit moth, *Grapholita molesta*, an important pest of fruit crops. In 1984, commercial use of oriental fruit moth mating disruption pheromone was initiated in an attempt to replace the heavily organophosphate-insecticide dominated control program (Jones, 1998). After one year, the mating disruption system gave equivalent control to the insecticides, with a one third reduction in cost.

1.3 Lingonberries

The lingonberry or partridgeberry (Figure 1.1), *Vaccinium vitis-idaea* L. var. *minus* Lodd (Ericaceae) is a low-growing, evergreen shrub, which produces edible berries that ripen in mid-September. It occurs in Newfoundland and Labrador on rocky and dry peaty soils, wet heaths, barrens and coastal headlands (Ryan, 1978).

The lingonberry has long been an important wild berry, traditionally used by boreal native people and Europeans as a food source and medicinal agent (Johnson *et al.*, 1995). Historically, its berries were the third most important fruit in the boreal forest, exceeded only by blueberries *Vaccinium angustifolium* Aiton, and cloudberries *Rubus chamaemorus* L.

1.3.1 Commercial potential of the lingonberry

Lingonberries are currently commercially harvested from the wild and are economically important in Newfoundland (Morris *et al.*, 1988). Berries are popular locally for use in jams, jellies and wine making, and there is a good export market of berries to Scandinavia. From 1987 to 1997, the wild lingonberry harvest has averaged at 308,000 lbs/year with a market value of $269,000/year (Anon., 1997). In 1994, domestic berry crops in Scandinavia failed due to frost damage, and export prices for lingonberries increased dramatically to $1.16/lb from $0.64/lb in 1993 (P. Hendrickson, p.c., 2001). Since that time, imports of wild berries such as lingonberries have taken over the domestic share of the market in Finland (Kangas, 1999).

The lingonberry holds great potential, not only as a food crop, but also in the

Figure 1.1: Lingonberries, *Vaccinium vitis-idaea* L. var. *minus* Lodd.

nutraceutical industry. The fruits have high levels of flavonoids, which are phytochemicals important in human health (Hertog *et al.*, 1995; Knekt *et al.*, 1996). In a study by Häkkinen *et al.* (1999), levels of the flavonol quercetin were shown to be very high in lingonberries (146 mg/kg, fresh weight), second only to bog whortleberry, *Vaccinium uliginosum* L., out of 25 species of edible berries tested. These flavonoids have antioxidant and anticarcinogenic properties, decreasing the risk of heart disease, lung cancer and stroke (Hertog *et al.*, 1995; Keli *et al.*, 1996).

Increasing industrial utilization of the lingonberry, in the form of food harvest, wines, juices and nutraceuticals will produce increased market demand and a need for more berries harvested from the wild. The potential market and value of wild and cultivated berries in Newfoundland may increase with new lingonberry products, such as nutraceutical tablets. The Newfoundland Department of Forest Resources and Agrifoods, and Agriculture and Agri-Food Canada have been evaluating a number of domesticated European varieties since 1991, for potential development of a U-Pick market. These varieties, differing from the local wild *minus* variety, offer several benefits, including higher growth habit which results in larger plants, an increased area for greater berry yield and easier harvesting (Penney *et al.*, 1996). Further, many European varieties bloom twice annually, permitting two harvests per season.

1.4 The lingonberry fruitworm

The first record of insects and disease on wild Newfoundland lingonberries was in 1914. Torrey (1914) describes a club-shoot disease or witch's broom, which may be

caused by a number of plant-pathogenic viruses, mycoplasmas or fungi, and a high prevalence of an unidentified leaf spot disease. Two insect pests were also recorded, a "flower worm" which attacked the bud and ate the pistil and stamens, and a "fruit worm" which devoured the pulp of several berries within a cluster. Although incidences of all these pests were generally low, Torrey (1914) stated "plants near the lighthouse at Western Bay Head are so infested (with fruit worm) as not to be worth picking. There is no profitable means of combating these insects upon plants in a wild state". Though the "fruitworm" in Torrey's study remains unidentified, it was quite likely *Grapholita libertina* Heinr. (Lepidoptera: Tortricidae), the primary pest of wild Newfoundland lingonberries. *Grapholita libertina,* the lingonberry fruitworm, is a tortricid moth whose larvae feed within the lingonberry fruit (Morris *et al.*, 1988) (Figure 1.2). It has been reported in Canada from Newfoundland, Nova Scotia and British Columbia (Morris *et al.*, 1988). In the United States, it has been recorded from California, New Jersey and Maine (J.A. Powell, p.c., 2000; J. Brown, p.c., 2000). Therefore, it is potentially distributed at points between these records and throughout the range of it's host plant. The life history of *G. libertina* has been described by Morris *et al.*(1988). Adults emerge in June, July and occasionally August and lay eggs on developing berries from July to August. Larvae hatch and bore into the developing berries, where they feed for about four weeks. During this time, larvae move and feed within clusters (corms) of berries, each damaging up to ten fruit. After reaching maturity, larvae exit berries and overwinter in the prepupal stage in a hibernaculum in debris on the ground (Morris *et al.*, 1988). Research on this pest has been limited, as it has not been recorded in European

Figure 1.2: *Grapholita libertina* Top: Adult moth, scale: 1cm = 1.18 mm. Bottom: Late instar larva, showing damage and frass within lingonberry fruit, scale: 1cm = 0.85 mm.

lingonberry production areas, and there are few areas in North America in which lingonberries are commercially harvested. Further, previous research has shown that *G. libertina* is difficult to rear, making laboratory analyses a challenge (P. L. Dixon, p.c., 1996).

Morris *et al.* (1988) reported infestations as high as 276 larvae per kilogram of unripe berries in a survey of sites in eastern Newfoundland. The presence of larvae is a significant concern whether for domestic or export markets. Current control of pest infestation is through delay of the wild harvest until late September, after larvae have exited the fruit. Since this pest causes not only a decrease in berry quantity, but a diminished quality of product and an obvious export concern, it is important to develop a means of monitoring and controlling pest populations, particularly if commercial cultivation of a marketable product is to be achieved.

1.4.1 Taxonomy of the lingonberry fruitworm

Grapholita libertina belongs to the family Tortricidae, subfamily Olethreutinae, tribe Eucosmini, subtribe Grapholitina (Heinrich, 1926). The family Tortricidae contains many pest species of economic importance. Within the genus *Grapholita*, many species are important pests on fruit crops, e.g. the lesser appleworm, *Grapholita prunivora* Walsh (Lepidoptera: Tortricidae), is a pest of apple, cherry and plum throughout North America (Mantey *et al.*, 2000). *Grapholita molesta*, the oriental fruit moth, a primary pest of stone fruit in North America, required three to four insecticide applications per season, prior to development of mating disruption techniques (Jones, 1998).

1.4.2 Pheromone structure and attractants of the genus *Grapholita*

Insect pheromones are organic compounds, which may be of low or high volatility (based on function). Pheromone structure is similar within groups, with closely related taxa having more structural similarity than distantly related taxa. Cross attraction may occur between closely related species, although allopatric populations of the same species may exhibit differences in pheromone blend ratios and attraction (Lewis & Cane, 1990; Gemeno *et al.*, 2000). Tephritid flies use host plant odors or their derivatives in mate attraction (Corneilius *et al.*, 1999; Shelley, 2000). Lepidopteran pheromones are typically derived from fatty acids, and are usually 10-18 carbon aliphatic chains, which vary by species in their double bonds and terminal or internal functional groups such as alcohols, aldehydes, esters or ketones (Hall, 1998; Jurenka & Roelofs, 1993).

The only published study of *G. libertina* (Morris *et al.*, 1988) was restricted to pest biology and distribution, and did not include any information on pheromones. Other species of *Grapholita*, however, have been extensively studied. Sex pheromones of *G. prunivora* and *G. molesta* have been isolated, and as many as 15 other species in this genus have shown attraction to certain compounds (Arn *et al.*, 1992). Most of these compounds and pheromone components are unsaturated 12-carbon chain alcohols with or without an acetate. The similarity in the structure of these attractant compounds within the genus suggests that *G. libertina* may have an attractant of similar composition.

1.5. Objectives of study

The objectives of this study were to identify sex attractants for *G. libertina* from among chemicals attractive to other *Grapholita* species, and evaluate their uses in monitoring and controlling moth populations. The initial question was to determine which *Grapholita* spp. attractants were most attractive to *G. libertina*, according to the high degree of similarity in attractant compound structure in the genus. Identification of an effective sex attractant for this species then gave rise to a series of other questions. How similar is the synthetic attractant to the naturally occurring female sex pheromone? Could this attractant prove useful both in estimating larval infestations, and in direct population control? What would be the most effective delivery system for this attractant? Could field trapping using this attractant accurately predict adult flight?

The rate of male moth trap capture at various sites was tested as a means of estimating larval populations, and berry damage within the same year. The efficacy of attractants within various trap types was tested by trap design trials, and the potential of mass trapping using high density pheromone lures as a control measure evaluated. Trap capture and rearing data were used to predict adult flight periods by degree-day accumulations. In addition, information on population trends and life history of *G. libertina* was obtained through records of the flight season and population size. Attempts were also made to isolate the female-produced sex pheromone.

This research will help to produce a monitoring tool for use in commercial or wild settings, and explore the potential uses of sex attractants in controlling *G. libertina* with minimal pesticide application.

2.0 MATERIALS AND METHODS

2.1 Study sites:

Attractant trapping was carried out in five wild lingonberry fields in eastern Newfoundland (Figure 2.1, Table 2.1) during 1996-2000. All sites were headlands, adjacent to the ocean, and well exposed to coastal weather conditions. The area is within the Boreal Shield ecozone and the Maritime Barrens ecoregion (Ecological Stratification Working Group, 1995), which is characterized by cool, foggy summers and short moderate winters, with a mean annual temperature of 5.5°C. Vegetation of study sites was heathland dominated by low growing shrubs, moss and lichens, specifically a mixture of *Empetrum* and *Kalmia* heaths, with a carpet of low growing vegetation (*Empetrum nigrum* L., *Vaccinium vitis-idaea, Potentilla tridentata* Aiton, and *Cladina* lichens), punctuated by thickets of *Kalmia angustifolium* L., *V. angustifolium* and *Ledum groenlandicum* Oeder in more sheltered areas (Meades, 1983) (Figure 2.2).

Details of study sites including location, duration and types of sampling are given in Table 2.1.

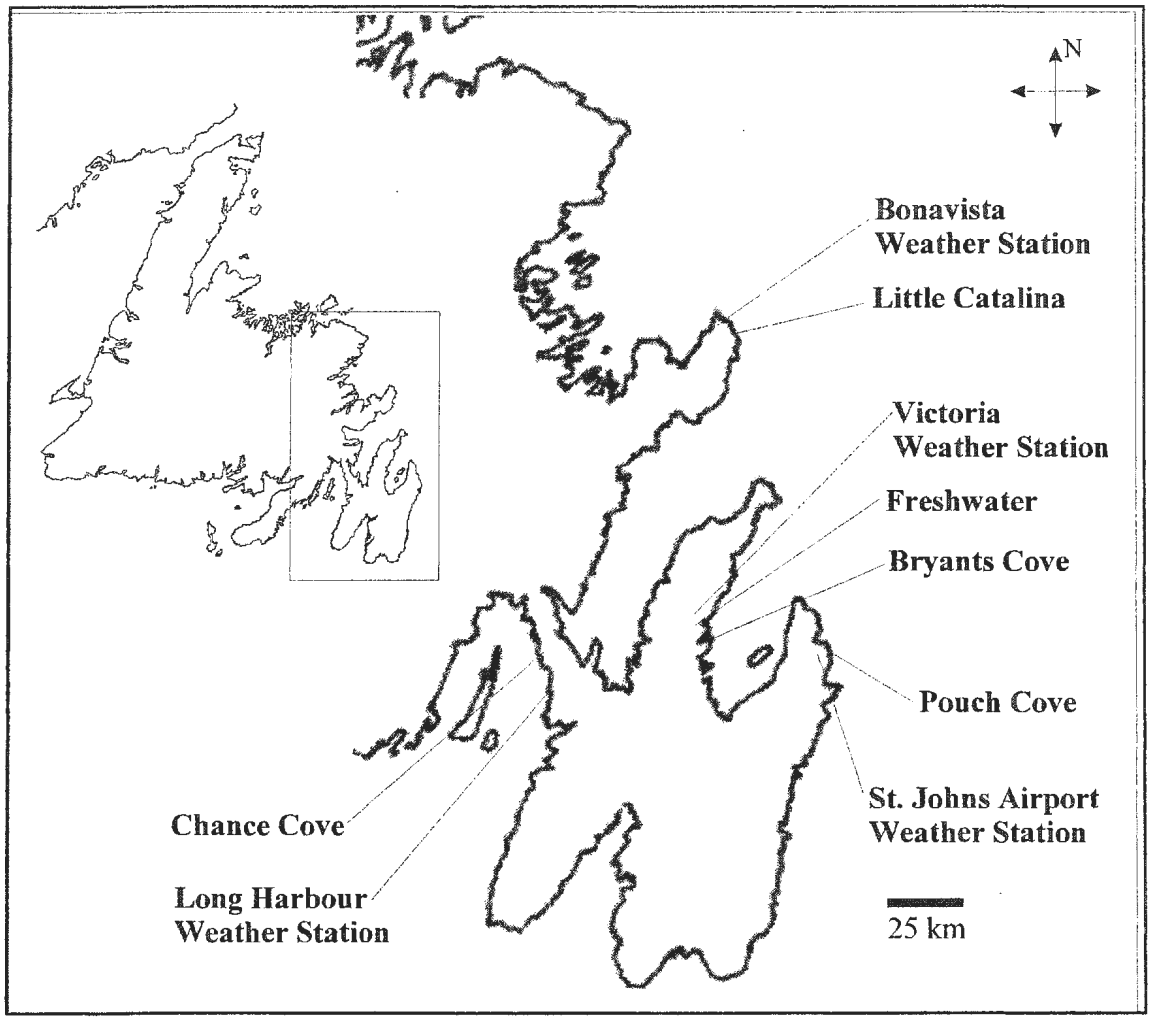

Figure 2.1: Map of eastern Newfoundland indicating sites for *G. libertina* trapping and weather stations used during 1996 to 2000. 1 cm = 25 km.

Table 2.1: Study sites used for trapping *Grapholita libertina* with sex attractants from 1996 to 2000. *Attractant identification, larval correlation, trap design trials and mass trapping are discussed in sections 2.2, 2.3, 2.5 and 2.6, respectively.

Sites	Relative location	Position	Elevation (above sea level)	Time period sampled	*Types of sampling
Little Catalina (L)	2 km west of Little Catalina	48°34' N, 53°02' W	100m	1996:June 24-Aug 19	Blend identification
				1997:June 30-Aug 25	Blend identification
				1998:June 25-July 29	Larval correlation
				1999:June 22-July 26	Larval correlation, Trap design trials
				2000:June 22-July 31	Larval correlation, Mass trapping
Pouch Cove (P)	Meetinghouse Rd., 1 km west from Pouch Cove	47°46' N, 52°47' W	300m	1996:June 24-Aug 12	Blend identification
				1998:June 25-July 29	Larval correlation
				1999:June 17-July 20	Larval correlation, Trap design trials
				2000:June 25-July 31	Larval correlation, Mass trapping
Freshwater (F)	4 km north of Carbonear	47°45' N, 53°14' W	300m	1996:June 24-Aug 19	Blend identification
				1998:June 25-July 29	Larval correlation
				1999:June 18-July 20	Larval correlation, Trap design trials
				2000:June 25-Aug 3	Larval correlation, Mass trapping
Bryant's Cove (B)	4 km east of Harbour Grace	47°41' N, 53°11' W	300m	1997:June 30-Aug 18	Blend identification
				1998:June 25-July 29	Larval correlation
				1999:June 18-July 20	Larval correlation, Trap design trials
				2000:June 25-Aug 3	Larval correlation, Mass trapping
Chance Cove (C)	On the Trans-Canada Highway at the Chance Cove exit	47°38' N, 54°50' W	300m	1997:June 30-Aug 18	Blend identification

Figure 2.2: Photograph of typical study site - Pouch Cove, NF.

2.2 Attractant identification:

2.2.1 Chemicals tested:

Compounds tested for attraction of *G. libertina* males to synthetic lures were selected based on their attractiveness to males of other *Grapholita* species (Table 2.2). Five compounds, which constitute the major attractant compounds of *Grapholita* listed in Table 2.2, were selected for study in 1996 (superscripts 1-4 correspond with compounds in Table 2.2) : EE-8,10-dodecadien-1-ol acetate (E,E8,10-12:OAc)[1], E-7-dodecen-1-ol acetate (E7-12:OAc)[2], Z-8-dodecen-1-ol acetate (Z8-12:OAc)[3], E-8-dodecen-1-ol acetate (E8-12:OAc)[4], and Z-8-dodecen-1-ol (Z8-12:OH)[5]. Acetone was used as a solvent for all compounds and acetone blanks (i.e. no attractive compound added) were used as controls. Lures were made by RPC Laboratories, Fredericton, NB, Canada, by pipetting 1 ml of attractant solutions at various concentrations onto rubber septum dispensers (Figure 2.3), which degraded and released attractant compounds at a constant rate.

In 1996, each of the five compounds was tested at four different concentrations: 0.01 mg, 0.1 mg, 1mg, and 10 mg (Table 2.3). However, septa which were to contain the E-7-dodecen-1-ol acetate were fabricated incorrectly, and their exact composition was unknown. The E7-12:OAc catch was low, and the resulting data from these traps were not considered in this study.

During the 1997 season, blends of the three most attractive compounds, and the top three compounds themselves, from the 1996 season were field tested. Blend ratios were selected based on typical ratios of other lepidopteran and tortricid pests (Mayer & McLaughlin,1991). Blends were tested in ratios of 85:10:5, 90:7:3, and 94:4:2

Table 2.2: Compounds shown to be sex attractants of *Grapholita* spp. (Arn,1999).

***Grapholita* species:**	**Attractant compounds: source (Arn, 1999)**
G. caerulana (Walsingham)	EE-8,10-dodecadien-1-ol acetate[1]
G. compositella (Fabricus)	EE-8,10-dodecadien-1-ol acetate[1]
G. conversana (Walsingham)	EE-8,10-dodecadien-1-ol acetate[1]
G. endrosias (Meyrick)	E-7-dodecen-1-ol acetate[2]
G. funebrana (Treitschke)	Z-8-dodecen-1-ol acetate[3], E-8-dodecen-1-ol acetate[4], Z-8-dodecen-1-ol[5]
G. gemmiferana (Treitschke)	EE-8,10-dodecadien-1-ol acetate[1]
G. inopinata (Heinrich)	Z-8-dodecen-1-ol acetate[3]
G. janthinana (Duponchel)	Z-8-dodecen-1-ol acetate[3], E-8-dodecen-1-ol acetate[4]
G. lobarzewskii (Ragonot)	Z-8-dodecen-1-ol acetate[3], E-8-dodecen-1-ol acetate[4]
G. lunatana (Walsingham)	EE-8,10-dodecadien-1-ol acetate[1]
G. molesta (Busck)	Z-8-dodecen-1-ol acetate[3], E-8-dodecen-1-ol acetate[4], Z-8-dodecen-1-ol[5]
G. packardii (Zeller)	E-8-dodecen-1-ol acetate[4]
G. prunivora (Walsh)	Z-8-dodecen-1-ol acetate[3]
G. succedana (Denis and Schiffermüller)	Z-8-dodecen-1-ol acetate[3], E-8-dodecen-1-ol acetate[4]
G. tenebrosana (Duponchel)	Z-8-dodecen-1-ol acetate[3], E-8-dodecen-1-ol acetate[4]

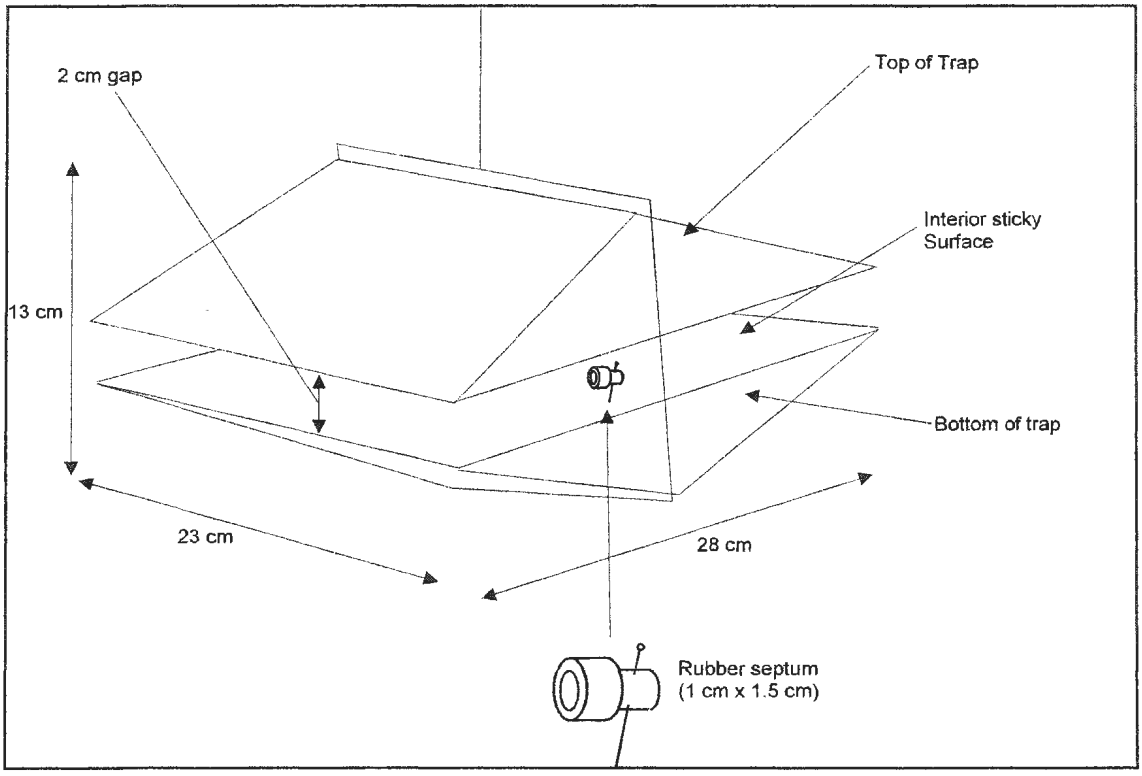

Figure 2.3: Schematic diagram of a Pherocon® 1C wing trap showing rubber septum.

Table 2.3: Compounds and concentrations tested for attraction to *G. libertina* during the 1996 and 1997 field trials.

Year	Compound tested	Concentration (mg/ ml of acetone)			
1996	E-8-dodecen-1-ol acetate (E8-12:OAc	0.01	0.1	1	10
	Z-8-dodecen-1-ol acetate (Z8-12:OAc)	0.01	0.1	1	10
	Z-8-dodecen-1-ol (Z8-12:OH)	0.01	0.1	1	10
	E,E-8,10-dodecen-1-ol acetate (E,E8,10-12:OAc)	0.01	0.1	1	10
	E-7-dodecen-1-ol acetate (E7-12:OAc)	0.01	0.1	1	10
1997	E-8-dodecen-1-ol acetate (E8-12:OAc)	-	-	1	-
	Z-8-dodecen-1-ol acetate (Z8-12:OAc)	-	-	1	-
	Z-8-dodecen-1-ol (Z8-12:OH)	-	-	1	-
	85:10:5 blend (85 E8-12:OAc: 10 Z8-12:OAc: 5 Z8-12:OH)	-	-	1	-
	90:7:3 blend (90 E8-12:OAc: 7 Z8-12:OAc: 3 Z8-12:OH)	-	-	1	-
	94:4:2 blend (94 E8-12:OAc: 4 Z8-12:OAc: 2 Z8-12:OH)	-	-	1	-

(E-8-dodecen-1-ol acetate: Z-8-dodecen-1-ol acetate: Z-8-dodecen-1-ol), all at a concentration of 1 mg/ml (Table 2.3).

2.2.2 Trapping design:

At all sites in 1996 and 1997, Pherocon® 1C wing traps (Figures 2.3, 2.4) were used. The Pherocon® 1C consisted of a cardboard bottom covered inside with adhesive, and a cardboard cover placed above the bottom tray such that there is a 2 cm gap around the perimeter to permit entry of moths. Each trap was baited with a single rubber septum, centrally positioned and held in place with an insect pin. Rubber septa were not replaced during the field season unless they were lost or damaged. Traps were suspended by wire from wooden stakes at 5 to 10 cm above ground (Figure 2.4). At all study sites, traps baited with different lures were set up in a randomized grid (Figure 2.5), within which traps were spaced at least 20 metres from each other.

In 1996, each of the five compounds was present in six traps at each site. Of these six traps, three were at a concentration of 10 mg, whereas the other three contained a lure at 0.01 mg, 0.1 mg or 1 mg concentrations (Table 2.3). Two traps baited with acetone blanks, which acted as controls, were also present at each site. This resulted in 32 traps at each site.

In 1997, traps were again set up in a randomized grid. Traps were baited with the three most attractive compounds from 1996 (E8-12:OAc, Z8-12:OAc, and Z8-12:OH), the three types of blended lures (85:10:5, 90:7:3 and 94:4:2) and acetone blanks, all at a concentration of 1mg/ml. Each test lure and acetone control was replicated three times at

Figure 2.4: Pherocon® 1C wing trap mounted on a wooden stake at Pouch Cove, NF.

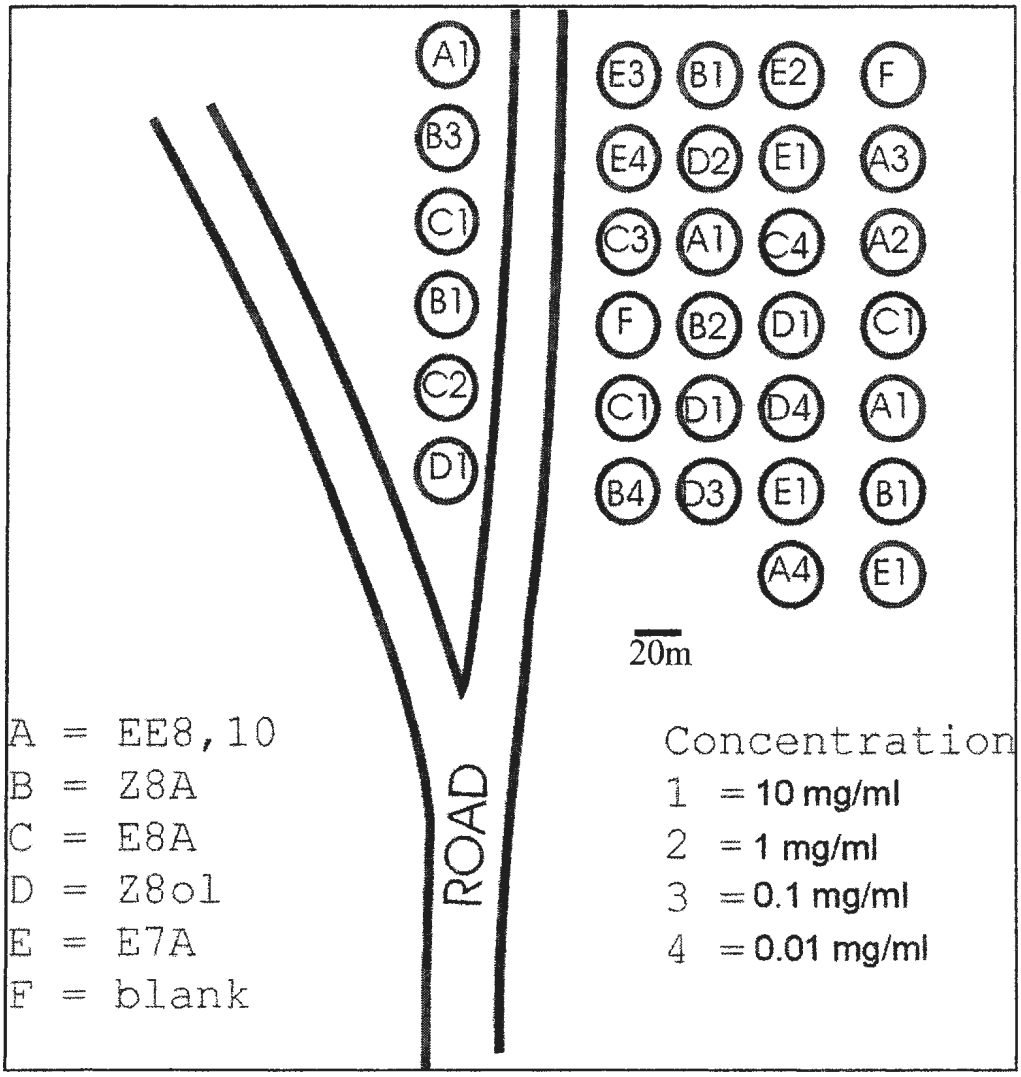

Figure 2.5: Example of a randomized trap grid used in 1996 at Little Catalina. Letters refer to compounds and numbers to concentration.

each site, for a total of 21 traps at each site, testing pure compounds, blends and controls.

2.2.3 Sampling regime:

Traps were checked twice weekly. At each check, the number of *G. libertina* in each trap was recorded, and traps were advanced by one position through the grid to minimize any position or edge effects. When a trap catch exceeded 50 moths, or when the sticky surface of the trap was congested with debris, the trap bottom was changed to provide a clean surface. All *G. libertina* moths captured were counted, and a total of 100 insects were selected at random from traps to determine the sex of trapped moths.

In 1996, trapping began on 24 June and continued until the end of the flight season, 12 August in Pouch Cove and 19 August in both Freshwater and Little Catalina. Trapping in 1997 began on 30 June and continued until 18 August in Bryant's Cove and Chance Cove, and 25 August in Little Catalina.

2.2.4 Data analysis:

The basic data unit was the mean daily trap catch per compound. In 1996, the daily trap catch of the three replicates of the 10mg concentrations were averaged for comparison with other concentrations. Due to the high number of zero values obtained, all catches were transformed by square-root transformation ($\sqrt{0.5+X}$), before being subjected to an analysis of variance (Sokal & Rohlf, 1995). Effects of attractant compound, concentration and site during the 1996 season were analyzed by Three Way ANOVA. Fisher's Least Significant Difference (LSD) (Sokal & Rohlf, 1995) was then

used to locate groups of variables which were found to have significant differences ($p<0.05$). Data from the 1997 season, having one less variable, concentration, were evaluated by Two-Way ANOVA and means were compared using Fisher's LSD (Sokal & Rohlf, 1995). SPSS® (Morgan *et al.*, 2001) was used for all ANOVA calculations. Graphs were produced using Sigmaplot®(Kuo & Fox, 1992).

2.3 Larval correlation:

During the 1998, 1999 and 2000 field seasons, the number of adult moths trapped using the previously identified attractant was correlated with fruit damage and larval density in the field.

2.3.1 Trapping design and sampling regime:

At each of four sites (Little Catalina, Pouch Cove, Bryant's Cove, and Freshwater), a 2 x 4 grid of traps was set up, including blank traps (Figure 2.6). Pherocon® 1C wing traps were separated by 20 metres, and suspended by wire from wooden stakes 5 to 10 cm above ground. Each trap in the grid was baited with a rubber septum lure of the 85:10:5 blend (the most attractive blend from 1997) at a concentration of 1mg/ml of solvent, along with two control traps baited with acetone blanks.

Traps at each site were checked and bottoms changed weekly during the six week period of adult flight. Traps were not circulated through the grid. During 1998, trapping took place from 25 June to 29 July in Freshwater, Bryant's Cove and Pouch Cove and from 26 June to 30 July in Little Catalina. During 1999, trapping took place from 17

1A	1B	2A	2B
1C	1D	2C	2D
3A	3B	4C	4B
3C	3D	4C	4D
5A	5B	6A	6B
5C	5D	6C	6D
7A	7B	8A	8B
7C	7D	8C	8D

Figure 2.6 Trapping grid used for 1998, 1999 and 2000 field trials. Each plot (shown numerically,1-6) was divided into four equal subplots (shown alphabetically, A-D). Traps were located at the centre of each larger plot (i.e. the intersection of the dashed lines). Each subplot was 10 metres x 10 metres, and total plot size was 80 x 40 metres. Two control traps were also at each site (7-8).

June to 20 July in Pouch Cove, 18 June to 21 July in Freshwater and Bryant's Cove, and 22 June to 26 July in Little Catalina. During 2000, trapping was from 25 June to 31 July in Pouch Cove, 25 June to 3 August in Freshwater and Bryant's Cove, and 22 June to 31 July in Little Catalina.

2.3.2 Berry and larval collection (Quadrat sampling):

Collections of berries and larvae were made at each site. Dates of berry sampling at each site during each year are listed in Table 2.4. Samples of berries were taken randomly within the 20 m square surrounding each trap within the grid. This was accomplished by dividing the area surrounding each trap into four 10 m x 10 m quadrats (Figure 2.6). Within each quadrat, 2 samples were taken by throwing a 1 m^2 square (Figure 2.7) and collecting all fruit within that square. This resulted in eight 1 m^2 samples per trap and 64 1 m^2 samples per site (including blank traps).

Berries collected were individually examined for larvae and larval damage (frass, burrowing, bore holes) (Figure 1.2). Total numbers of larvae and damaged berries (bore holes, frass present) were recorded per quadrat and per trap.

2.3.3 Vegetation analysis:

Percent cover of all vegetation within each quadrat (including lingonberry plants) was recorded to determine whether landscape or vegetation might significantly affect adult trapping or presence of larvae. This included estimates of plant, lichen and bare ground coverage per plot. All plants were identified to species.

Table 2.4: Berry collection dates during larval correlation study at four sites, 1998 to 2000.

Year	Site	Collection date
1998	Pouch Cove	August 20
	Bryant's Cove	August 22
	Freshwater	August 23
	Little Catalina	August 26
1999	Pouch Cove	August 17
	Bryant's Cove	August 18
	Freshwater	August 26
	Little Catalina	August 23
2000	Pouch Cove	August 16
	Bryant's Cove	August 17
	Freshwater	August 23
	Little Catalina	August 30

Figure 2.7: Sampling grid for lingonberries (1 metre square).

2.3.4 Data analysis:

Data were subjected to logarithmic transformation to resolve any positive correlations between means and the variance (Sokal & Rohlf, 1995). Total numbers of larvae/trap and total numbers of damaged berries/trap (within four quadrats surrounding each trap) were recorded and regressed against the adult capture rate/trap in the same year. Larval counts in 1998 and 1999 were also regressed against subsequent adult counts in 1999 and 2000, respectively.

Percent cover for vegetation within each quadrat was averaged for each site and principal component analysis was conducted to determine any relationships between heterogenous vegetation and insect densities at each site. Principal component analysis was restricted to species which were present in at least 50% of all plots. Eigenvector scores for the first three principal components were used to determine major factors (vegetation types) which contributed to variance along each axis. The number of adults, larvae, and damaged berries in each plot for all study sites (six plots at each of four sites) were graphed as bubble plots (Sigmaplot©, Kuo & Fox, 1992) along the first two principal component axes. Any significant relationship between insect populations and habitat (or vegetation type) were evident by the position of large bubbles along each axis.

Dominant vegetation at all sites, primarily *Vaccinium angustifolium, V. vitis-idaea*, and lichen species were run as covariates with adult and berry densities in a generalized multiway analysis of variance (MANOVA) (Sokal & Rohlf, 1995). Larvae and damage were selected as response variables with site and year as explanatory variables. *Vaccinium angustifolium* and lichen species were found to be not significant in

relation to larval and damage densities (p=0.05). Therefore, in subsequent analysis *V. angustifolium* and lichen were removed. Regressions, principal component analyses and MANOVAs were conducted using SPSS 9.0® (Morgan *et al.*, 2001) and graphed with Sigmaplot® (Kuo & Fox, 1992).

2.4 Sexing of trapped moths:

Specimens trapped during the 1996, 1997, and 1998 field trials were examined in order to determine their sex, and thus the nature of attraction (aggregation or sexual attraction). One hundred specimens were extracted from traps collected each year by soaking with ethyl acetate. Abdominal tips were cleared with 10% potassium hydroxide and examined to determine the sex of the specimens.

2.5 Trap design trials:

During the 1999 season, five trap types were tested for their relative ability to capture *G. libertina* using the 85:10:5 lure at a 1 mg concentration.

2.5.1 Trap types:

Five common trap designs were selected. Four impaction (sticky) traps were used, namely: Pherocon® 1C Wing trap (Trécé, Salinas, California), Wing trap II® (Pherotech, British Columbia), Delta® trap (Scentry, Buckeye, Arizona), and the Diamond® trap (Pherotech, British Columbia). A single example of a non-saturating, or high capacity trap was also tested, the Unitrap® (Pherotech, British Columbia).

The sticky traps all had a sticky surface on which insects are trapped, and become saturated once this surface is full. The non-saturating trap captured insects within an enclosed bucket, killing them with a Vapona® insecticidal strip (18.6% dichlorvos, Zoecon Industries Ltd., ON, Canada). The characteristics of each trap are shown in Figure 2.8 and listed in Table 2.5.

2.5.2 Trapping design:

At each of the four sites (Little Catalina, Pouch Cove, Bryant's Cove and Freshwater), a randomized grid of 15 traps was used (Figure 2.9). Traps were spaced 20 m apart within the grid and each suspended 5-10 cm above ground from a wooden stake. Each trap type was replicated three times at each site - two with 85:10:5 lures at a concentration of 1mg/ml, the other a control trap baited with an acetone blank. Traps were checked and advanced by one position within the grid weekly.

On the perimeter of each grid, a series of 10 Pherocon® 1C wing traps were placed (40 m from one another and at least 20 m from traps in grid). All were baited with the 85:10:5 lure, and placed as guard traps in an effort to reduce edge effects in the trapping data. Guard traps were checked weekly but not circulated. Bottoms of guards were changed if traps were saturated.

Figure 2.8: Trap designs tested with the 85:10:5 lure during the 1999 field season. Four impaction-style (sticky traps):A-Pherocon® 1C wing trap, B-Diamond® trap, C-Delta® trap and D-Wing Trap II® (bottom, right); and one non-saturating trap: E-Unitrap®.

Table 2.5: Characteristics of various trap designs tested using the 85:10:5 lure for *G. libertina* *Bulk is typically more than 50 traps (Prices quoted in 1997 Canadian dollars).

	Trap Characters			
Trap Design	**Material**	**Shape**	**Trapping Surface Area**	**Cost per trap (Cdn) (Bulk)***
Pherocon® 1C Wing Trap	White, waterproof cellulose (cardboard).	Wing-style	637 cm^2 on inner lower surface.	$ 3.89
Wing Trap II®	White, waterproof cellulose (cardboard).	Wing-style	342 cm^2 on removable lower surface inserts.	$ 4.00
Delta® Trap	White, corrugated plastic.	Triangular	288 cm^2 on removable lower surface inserts	$ 2.98
Diamond® Trap	White, waterproof cellulose (cardboard).	Diamond	527 cm^2 on top and bottom inner surfaces.	$ 2.20
Unitrap®	Green, heavyweight plastic (reuseable).	Bucket, with a funnel and cover on top.	2827 cm^3 (Volume) in a high capacity bucket.	$ 10-12

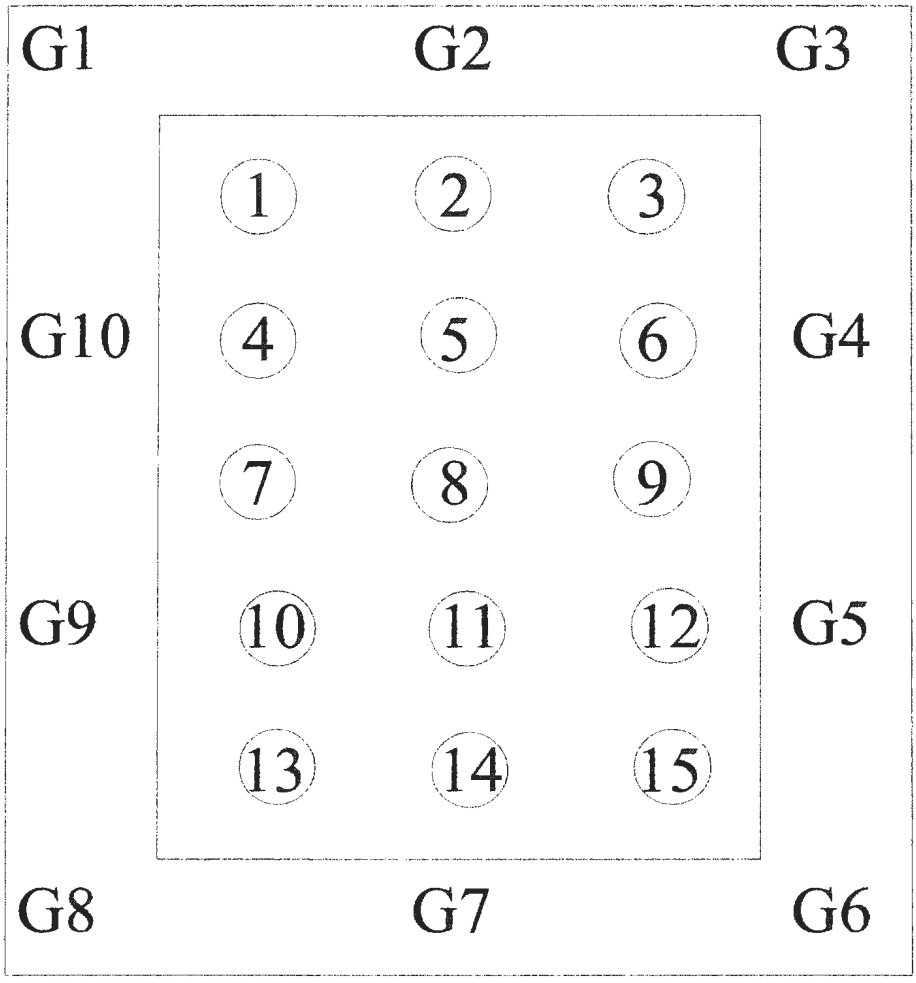

Figure 2.9: Grid used for trap trials in 1999. Different trap designs were randomly placed throughout the grid (1-15) and advanced by one position weekly. On the perimeter, 10 guard traps were placed to reduce any edge effects (G1-10). Traps within the grid were separated by 20 metres, guard traps were 40 metres from one another, and a minimum of 20 metres from traps in the grid.

2.5.3 Data analysis:

Mean number of adults captured per trap per season was recorded for each trap type at each site. Data were subjected to square-root transformation ($\sqrt{0.5+X}$), and the effects of trap type and site were analyzed using a two way ANOVA.

SPSS® was used for all ANOVA calculations, Sigmaplot® for graphs (Morgan *et al.*, 2001; Kuo & Fox, 1992). Fisher's LSD (Sokal & Rohlf, 1995) was then used to locate differences in the means ($p<0.05$).

2.6 Mass trapping trial:

Trials were conducted during the 2000 field season to evaluate mass trapping as a potential control tactic for *G. libertina*. By using a high density of traps with high concentration lures to trap adult males, it was hoped that subsequent larval infestations would be reduced.

2.6.1 Trapping design and sampling regime:

During the 2000 field season, Pherocon® 1C wing traps were placed in a 2 x 4 grid at Pouch Cove, Freshwater, Bryant's Cove and Little Catalina, in a similar configuration as in larval correlation studies (see 2.3). Six test traps baited with the 85:10:5 blend at a 1mg/ml concentration and two controls with acetone blanks were used in each plot, positioned parallel to and separated from the larval correlation grids by at least 40 metres

to reduce any interactions between traps in each plot. Each 2 x 4 trapping grid in the mass trapping trial was surrounded by a 3 x 5 grid of Pherocon® 1C wing traps, which were baited with a high-dose lure of the 85:10:5 blend (10mg/ml, Figure 2.10). Traps at each site were checked and bottoms changed weekly during the six week period of adult flight, at the same time as correlation grids. Traps were not circulated through the grid. Trapping was from 25 June to 31 July in Pouch Cove, 25 June to 3 August in Freshwater and Bryant's Cove, and 22 June to 31 July in Little Catalina.

2.6.2 Larval sampling and vegetation analysis:

Berry samples were collected and vegetation recorded at Pouch Cove on 16 August, Freshwater on 23 August, Bryant's Cove on 17 August, and Little Catalina on 30 August. Samples of berries were taken randomly and vegetation recorded within the 20 m square surrounding each trap within the grid, as in the larval correlation grids (2.3.2, 2.3.3). Berries were again analyzed for presence of larvae and damage.

2.6.3 Data analysis:

Data were subjected to logarithmic transformation to stabilize variance (Sokal & Rohlf, 1995). Total numbers of larvae/trap and numbers of damaged berries/trap (within four quadrats surrounding each trap) were recorded and regressed against the adult capture rate/trap in the same year. Percent vegetation cover within each quadrat was averaged for each site and principal component analysis was used to determine the

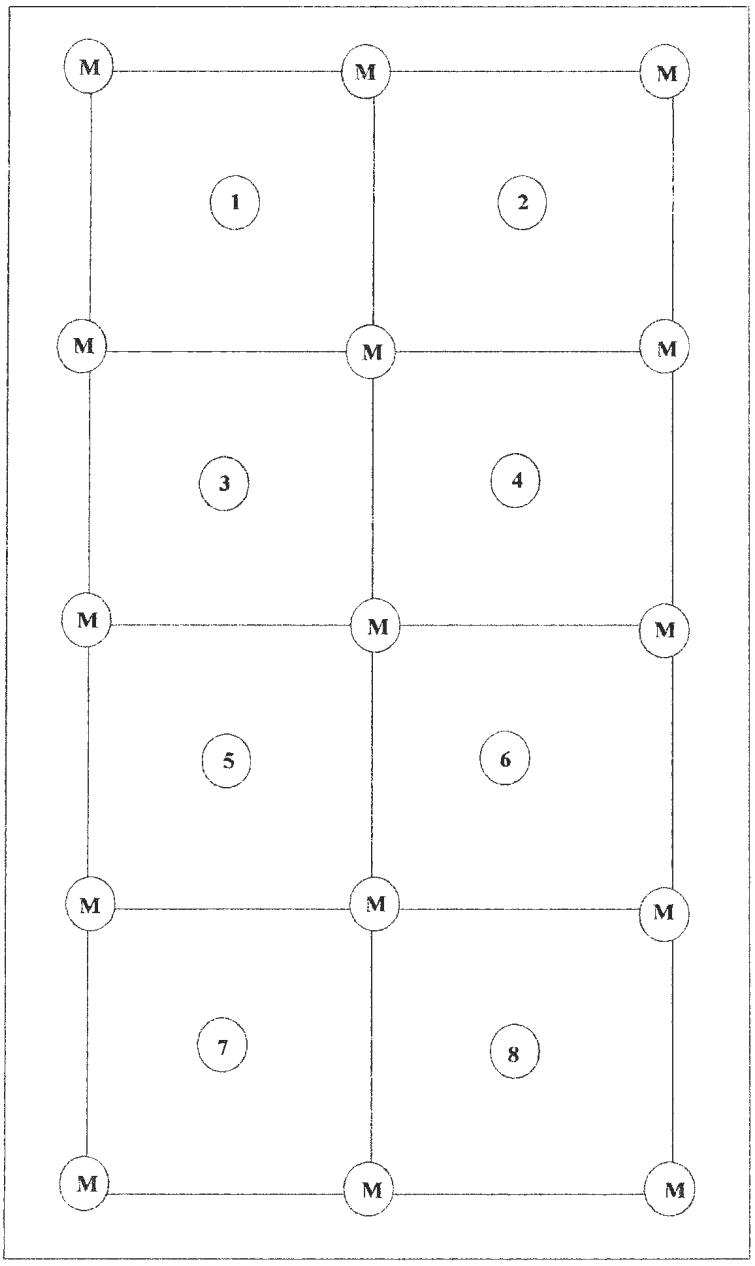

Figure 2.10: Mass trapping grid used in lingonberry sites during the 2000 field season. Numbers 1-6 indicate traps baited with 85:10:5 lure at a 1 mg/ml concentration (as in section 3.2), 7 and 8 were blank traps, M's indicate 'mass traps' baited with 85:10:5 lure at a concentration of 10 mg/ml.

abundance of adults, larvae, and damaged berries in each plot, relative to significantly correlated vegetation (as in section 2.3). MANOVA was conducted using vegetation types as covariates, site and year as explanatory parameters and lingonberry data as response variables to identify any relationships between vegetation types, the distribution of lingonberry plants or total berries at each site, and densities of lingonberry fruitworm adults, larvae and damaged berries. Mass trapping grids were analyzed independently by MANOVA with lingonberry coverage, and adult and berry density as covariates and larval and damaged berry densities as response variables.

To determine significant relationships between variables, mass trapping grids were compared with normal correlation grids through Three Way ANOVA using site, lingonberry variables (adult, larval and damaged berry densities) and grid type (standard - as in correlation grids, versus mass trapping - surrounded with traps baited with high density lures) to determine any significant differences in adult trapping, larval infestation or berry damage between plots. Means were then compared using Fisher's Least Significant Difference (LSD) (Sokal & Rohlf, 1995). Regressions, ANOVAs and principal component analyses were conducted with SPSS 9.0®, and graphing with Sigmaplot® (Morgan *et al.*, 2001; Kuo & Fox, 1992).

2.7 Rearing and chemical analysis:

2.7.1 Rearing:

Rearing of *G. libertina* was attempted in order to obtain samples for laboratory

identification of pheromones. Also noted were records of emergence and degree-day calculations for post-diapause adult emergence. All rearing chambers were maintained on a 14:10 (L:D) photoperiod, and were located at the Atlantic Cool Climate Crop Research Centre, of Agriculture and Agri-Food Canada at St. John's, NF, Canada. Substrates, sources, numbers of larvae, and dates associated with rearing are shown in Table 2.6. Detailed descriptions of rearing procedures are in appendix A.

Parasitoids which emerged from overwintering *G. libertina* were sent to the Eastern Cereal and Oilseed Research Centre (ECORC), of Agriculture and Agri-Food Canada, ON, Canada, for identification. Voucher specimens of *G. libertina* were deposited in the Canadian National Collection at ECORC.

2.7.2 Chemical analysis:

Female moths from the 2000-2001 rearing study (section 2.7.1) were used in chemical analysis to isolate the naturally produced female sex pheromone. Newly emerged females were collected from rearing chambers (tupperware containers) each day, and kept isolated in petri dishes at 8-10°C until analysis 2 to 6 days later. All samples were analyzed using a Hewlett-Packard 5890 Series II (Hewlett-Packard®, Palo Alto, CA) gas chromatogram (GC) with a 30 m DB-5 (Durabond®) column, linked to a Hewlett-Packard® 5971 series mass selective detector with electron impact ionization and electron multiplier detection. Synthetic standards of known male *G. libertina* attractants (Z-8-dodecen-1-ol, Z-8-dodecen-1-ol acetate and E-8-dodecen-1-ol acetate)

Table 2.6: Sources and numbers of larvae, types of rearing containers, substrates tested and dates associated with each year of rearing study, 1995 to 2001 (No rearing occurred during 1996 to 1998).

Year	Source	Number of Larvae	Rearing container	Volume	Substrate	Date placed at 3.5°C	Date removed to 25°C
1995 -1996	Various locations	Unknown	Bin	10 L	Berries	-	-
1998-1999	Little Catalina, Pouch Cove, Freshwater, Bryant's Cove	86	Plastic pill bottle	100 ml	Vermiculite	October 20	16 March put into 12°C, then 1 May into 20°C
1999-2000	Little Catalina, Pouch Cove, Freshwater, Bryant's Cove	504	Clear plastic margarine container	500 ml	Vermiculite, with 5-10 berries	October 14	May 5
2000-2001	Little Catalina	97	Tupperware®	4.2 L	Sand	November 21	March 23
	Pouch Cove	138	Tupperware®	4.2 L	Sand+leaf litter	November 21	March 30
	Bryant's Cove	92	Tupperware®	4.2 L	Sand+paper towel	November 21	April 4
		105	Tupperware®	4.2 L	Sand+paper towel+leaf litter	November 21	April 18
	Freshwater	270	Tupperware®	4.2 L	Sand+paper towel+leaf litter	November 21	April 14

were tested to obtain retention times for comparison with test extracts from females. Pheromone samples were obtained through collection of headspace volatiles, direct collection from emitting females, vial washing and excised ovipositor washing. Details of collection methods are described in appendix B.

2.8 Seasonal history:

Trap capture data from each year of study were recorded for each site, and used to determine the flight season of *G. libertina* and the effects of weather on trap captures.

2.8.1 Weather data collection:

Weather variables during the 1996-2000 field seasons were collected in order to calculate degree-day predictions and determine any effects of weather conditions on adult trapping. Weather data were obtained from Environment Canada climate stations within 25 kilometres of each study site (Figure 2.1). Details of each climate station are shown in Table 2.7. Maximum, minimum and mean daily air temperature (°C), total daily precipitation (mm) and wind speed (kph - only for St. John's Airport weather station) were collected to determine if temperature, precipitation or wind speed affected trapping rate.

2.8.2 Data analysis:

Temperature was measured every minute, and averaged daily. Rainfall amounts

Table 2.7: Environment Canada weather stations used in weather data collection, showing position, relative position to study site and equipment used in collection at each site.

Weather Station	Latitude and Longitude	Elevation	Distances from study sites	Temperature readings*	Precipitation readings	Windspeed readings
Bonavista	48° 40' 53° 07'	26 m	22 km from Little Catalina	Yellow Springs International Model 44212 thermistor	Modified Fischer and Porter potentiometric rain gauge	None
Victoria	47° 46' 53° 13'	43 m	2 km from Freshwater, 15 km from Bryant's Cove	Clear Spirit Celsius thermometer	Meteorological Service of Canada Type B plastic rain gauge	None
Long Harbour	47° 37' 52° 45'	141m	25 km from Chance Cove	Clear Spirit Celsius thermometer	Meteorological Service of Canada Type B plastic rain gauge	None
St. John's Airport	47° 25' 53° 49'	8 m	20 km from Pouch Cove	Campbell-Stokes MarkIII C recorder, with unspecified temperature sensor	Modified Fischer and Porter potentiometric rain gauge	Meteorological Service of Canada U2A speed detector

* All temperature sensors were housed within Type B Stevenson screens.

were summed as a daily total. Wind speed data from St. John's Airport were obtained in an hourly format, which was converted to an average daily wind speed. To test the effects of each weather variable on adult catch, daily weather values were averaged during the period prior to and between trap sampling. Weather variables were collected during the entire trapping periods by automated weather stations. During 1996 and 1997, trap sampling was biweekly, therefore weather data were averaged for 3-4 days up to and including the sample date (and record of adult catch). During 1998-2000, trap sampling was weekly, therefore weather variables were averaged during the week prior to trap examination. Average weekly/biweekly weather was tested for a relationship with numbers of adults per trap/night by Pearson correlations (Sokal & Rohlf, 1995).

Degree- day accumulations for 10% and 50% capture at each site and during each year were calculated using Arnold's Formula [mean daily temperature - base temperature] (Arnold, 1960). Since the base developmental temperature for *G. libertina* is not known, a base of five degrees Celsius was used, as recommended by Pruess (1983), and a variable readily available from Environment Canada weather summaries. The relationship between the dates of first emergence/capture of *G. libertina*, and the calculated degree days was examined to determine any consistent pattern between years and sites. Degree day accumulations determined by field trapping for each site and year were compared to emergence data from mass rearing in 2000 by Three Way ANOVA, and means compared by Fisher's Least Significant Difference (LSD)(Sokal & Rohlf, 1995).

3.0 RESULTS

3.1 Evaluation of *Grapholita* species attractants:

3.1.1 1996 Field trials:

The E8-12:OAc attracted the most moths ($p<0.05$, Table 3.1), followed by Z8-12:OAc, Z8-12:OH and the E,E8,10-12:OAc Numbers were not significantly different from each other but were significantly greater compared to the controls.

There were significant differences in moth catches (Table 3.1) between sites. Total trap captures were greater in Little Catalina than in Pouch Cove or Freshwater. However, within each site the ranking of compounds was similar (except the Z8-12:OAc catch in Freshwater was relatively lower than was found at the other sites (Table 3.2)). Since compound rankings based on moth catches were similar at each site, the intersite variation was dismissed.

The effects of different concentrations of the compounds were not significant (Tables 3.1 & 3.3). However, catch rates were generally greater at the 1 mg and 10 mg concentrations, with the exception of 0.1 mg in the E8-12:OAc (Figure 3.1). There was no significant interaction between treatment, concentration, or site.

Table 3.1: Mean (standard error) of daily catches per trap x 10^3 of *G.libertina* moths by 4 different compounds at 4 concentrations (standardized by subtraction of the mean daily blank catch), in each of 3 trapping areas from 24 June to 19 August, 1996. Total trap-nights per compound was 52 to 59. Note that 10mg lures are replicated 3 time in each grid.

Compound	Concentration	Site (Mean # of Moths(SEM))			
		Pouch Cove	Freshwater	Little Catalina	All Sites
EE-8,10-dodecadien-1-ol acetate (E,E8,10-12:OAc)	10mg*	3 (1.1)	4 (1.4)	42 (7.6)	17 (3.1)
	1mg	1 (1.4)	2 (1.4)	108 (19.0)	39 (10.1)
	0.1mg	6 (4.4)	7 (4.5)	29 (9.3)	14 (4.1)
	0.01mg	9 (4.0)	0 (0)	30 (7.1)	12 (3.3)
Z-8-dodecen-1-ol acetate (Z8-12:OAc)	10mg*	22 (5.6)	12 (2.5)	212 (37.0)	85 (15.0)
	1mg	22 (9.2)	1 (1.1)	205 (119.0)	79 (43.2)
	0.1mg	14 (8.8)	10 (3.9)	67 (45.1)	31 (16.2)
	0.01mg	9 (4.0)	21 (11.0)	79 (28.4)	37 (11.0)
E-8-dodecen-1-ol acetate (E8-12:OAc)	10mg*	24 (8.8)	21 (6.1)	305 (63.1)	119 (24.1)
	1mg	26 (11.0)	3 (1.6)	216 (74.0)	93 (29.3)
	0.1mg	46 (16.0)	4 (3.4)	295 (139.6)	153 (55.6)
	0.01mg	1 (1.4)	2 (2.2)	143 (51.1)	51 (20.2)
Z-8-dodecen-1-ol (Z8-12:OH	10mg*	7 (2.1)	4 (2.0)	125 (22.0)	47 (8.9)
	1mg	1 (1.4)	4 (2.5)	179 (44.2)	65 (19.1)
	0.1mg	1 (1.4)	6 (3.3)	34 (10.9)	15 (4.7)
	0.01mg	13 (6.2)	1 (1.1)	57 (12.1)	24 (5.7)
	Total Catch	239	203	3228	3770

Table 3.2: Ranking of 3 different attractant compounds by mean daily catches of *G. libertina*, at 1mg/ml, from 24 June to 19 August, 1996 and from 30 June to 25 August, 1997, at 5 different trapping sites. Rank values followed by different letters were significantly different ($p < 0.05$) from other compounds at the same site and year (Fisher's LSD).

Year	Site	Compounds tested			
		E-8-A	Z-8-A	Z-8-ol	Control
1996	Little Catalina (L)	1^{a}	2^{ab}	3^{b}	4^{c}
	Pouch Cove (P)	1^{a}	2^{a}	3^{b}	4^{c}
	Freshwater (F)	1^{a}	3^{b}	2^{c}	4^{d}
	Total	1^{a}	2^{ab}	3^{b}	4^{c}
1997	Little Catalina (L)	2^{a}	1^{b}	3^{a}	4^{c}
	Chance Cove (C)	2^{a}	1^{b}	3^{c}	4^{d}
	Bryant's Cove (B)	2^{a}	1^{b}	3^{c}	4^{d}
	Total	2^{a}	1^{b}	3^{c}	4^{d}

Table 3.3: Standardized total catches of *G. libertina* moths by 4 different compounds, at 4 different concentrations, (3 traps per site/standardized by subtraction of 2 blank trap captures) from 24 June to 19 August, 1996, at 3 different study sites. Catches at 10mg concentration were the mean of 3 traps.

Compound	Concentration	Site			Total catches per Compound & Concentration
		Pouch Cove	Freshwater	Little Catalina	
EE-8,10-dodecadien-1-ol acetate	10mg	1.3	2.2	26.7	30.2
	1mg	0.0	0.5	85.0	85.5
	0.1mg	3.0	4.5	13.0	20.5
(E,E8,10-12:OAc)	0.01mg	5.0	0	14.0	19.0
	Total	9.3	7.2	138.7	155.2
Z-8-dodecen-1-ol acetate	10mg	14.7	9.8	188.3	212.8
	1mg	16.0	0.0	174.0	190.0
	0.1mg	9.0	7.5	48.0	64.5
(Z8-12:OAc)	0.01mg	5.0	17.5	59.0	82.5
	Total	44.7	34.8	469.3	548.8
E-8-dodecen-1-ol acetate	10mg	16.3	18.5	271.3	306.1
	1mg	17.0	22.5	184.0	223.5
	0.1mg	31.0	2.5	347.0	380.5
(E8-12:OAc)	0.01mg	0.0	0.5	117.0	117.5
	Total	64.3	44.0	919.3	1027.6
Z-8-dodecen-1-ol	10mg	4.0	2.2	105.7	111.9
	1mg	0.0	2.5	151.0	153.5
	0.1mg	0.0	3.5	18.0	21.5
(Z8-12:OH)	0.01mg	8.0	0.0	39.0	47.0
	Total	12.0	8.2	313.7	333.9
Site Totals		130.3	94.2	1841.0	2065.5

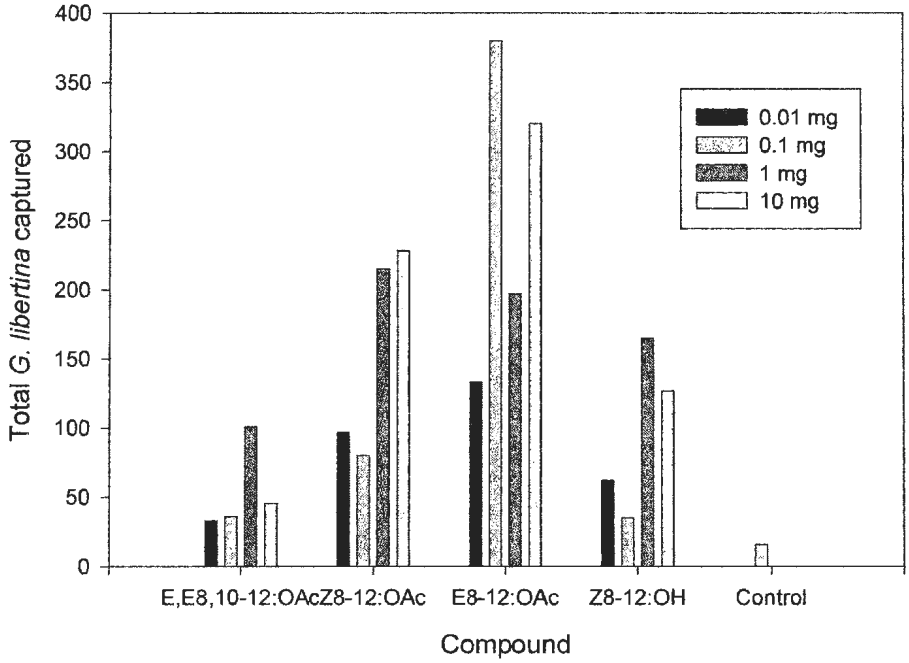

Figure 3.1: Total catches (not standardized by controls) of *G. libertina* in traps baited with four different compounds, at four different concentrations, from 24 July -19 August, 1996.

3.1.2 1997 Field trials:

There were significant differences in moth catches between sites (Table 3.2, Table 3.4), and some treatment-site interactions. Total trap captures were lower at Bryant's Cove than in Little Catalina or Chance Cove. The ranking of compounds was similar at all sites with the exception of the 90:7:3 blend in Bryant's Cove and the 94:4:2 at Chance Cove, which ranked higher than the 85:10:5, but were not significantly different ($p>0.05$) from the other sites. Inter-site variation was therefore dismissed.

Within the unblended compounds, the Z8-12:OAc was the most attractive ($p<0.05$, Tables 3.4 & 3.5). The E8-12:OAc was more attractive than the Z8-12:OH , which was not significantly different from the control traps (Figure 3.2).

The 85:10:5 was the most attractive of the blended compounds, being significantly different from the control, Z8-12:OH and E8-12:OAc lures (Figure 3.2). It was followed in attractiveness by the Z8-12:OAc, 90:7:3 and 94:4:2 blends. All three blends and the Z8-12:OAc captured significantly ($p<0.05$) more than the control and Z8-12:OH lures. The Z8-12:OH, 90:07:03 and 94:04:02 were not significantly different from the E8-12:OAc. The blends were not significantly different from one another, or the Z8-12:OAc, which was slightly more attractive than the 90:7:3 and 94:4:2 blends.

3.1.3 1996-1997 comparison:

Trap captures by each compound varied between years (Table 3.5). E8-12:OAc caught relatively more moths than the Z8-12:OAc in 1996, although this

Table 3.4: Mean adult catch per trap per night x 10^3 and relative rank of *G. libertina* by 6 attractant compounds and blends (1mg/ml) and control traps, from 30 June to 25 August 1997, at 3 different study sites.

Lures	Site					
	Bryant's Cove		Little Catalina		Chance Cove	
	Mean (SEM)	Rank	Mean (SEM)	Rank	Mean (SEM)	Rank
85:10:5	350 (75)	2	820 (230)	1	330 (77)	3
90:7:3	440 (120)	1	630 (140)	2	290 (39)	4
94:4:2	200 (43)	4	530 (190)	3	420 (90)	2
E8-12:OAc	54 (36)	5	230 (27)	5	220 (60)	5
Z8-12:OAc	340 (110)	3	360 (21)	4	720 (99)	1
Z8-12:OH	7 (7)	7	140 (26)	6	67 (49)	6
Control	34 (14)	6	60 (16)	7	54 (25)	7

Table 3.5: Mean (SEM) daily catches of *G. libertina* moths per trap x 10^3 by 3 different unblended compounds (1mg/ml), from 24 June to19 August, 1996 and from 30 June to 25 August, 1997, at 5 different study sites. Values for compounds which are followed by different letters were significantly different ($p<0.05$) from other compounds at the same site and year (Fisher's LSD).

		Compounds		
Year	Site	E8-12:OAc	Z8-12:OAc	Z8-12:OH
1996	Little Catalina (L)	220 (74.0)^{a}	210 (120.0)^{ab}	179 (44.0)^{b}
	Pouch Cove (P)	26 (11.0)^{a}	24 (9.2)^{a}	1 (1.4)^{b}
	Freshwater (F)	30 (17.0)^{a}	1 (1.1)^{b}	4 (2.5)^{c}
	Total (All sites)	93 (29.0)^{a}	79 (43.0)^{ab}	65 (19)^{b}
1997	Little Catalina (L)	11 (2.9)^{a}	19 (5.6)^{b}	7 (2.2)^{a}
	Chance Cove (C)	15 (4.0)^{a}	45 (16)^{b}	4 (2.8)^{c}
	Bryant's Cove (B)	3 (1.4)^{a}	22 (8.5)^{b}	0 (0.3)^{c}
	Total (All sites)	10 (1.7)^{a}	28 (6.1)^{b}	4 (1.2)^{c}

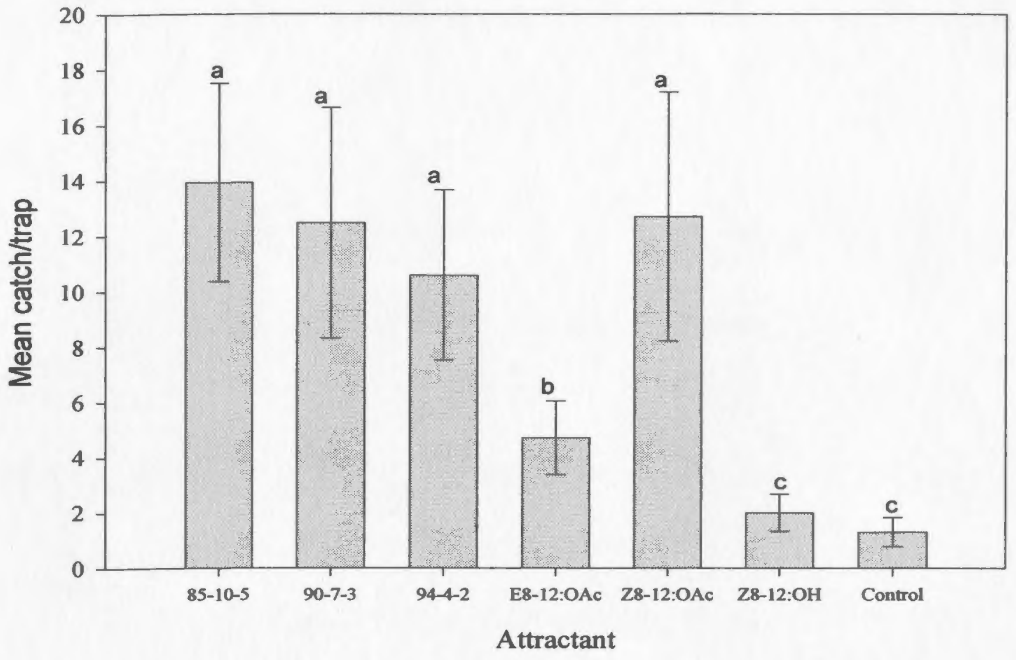

Figure 3.2: Mean number of *G. libertina* captured per trap over flight season by 6 types of attractants at 1mg/ml concentrations, and controls, from 30 June - 25 August, 1997. Means represented by the same letter did not differ ($p<0.05$) by Fisher's LSD.

was not significant, while the Z8-12:OAc caught relatively more moths than the E-8-A in 1997. Z8-12:OH caught fewer moths than the Z8-12:OH or E8-12:OAc in both years. Total trap catches for all compounds tested declined by more than 90% in 1997. No significant position effects were observed during the 1996 or 1997 flight seasons.

3.2 Correlation of larvae and damage density with adult trap capture:

3.2.1 1998 Field trials:

Total and mean numbers of berries, damaged berries, larvae and adults per site during each year are shown in Tables 3.6 and 3.7, respectively. Total adults captured ranged from 46 in Pouch Cove to 386 in Freshwater (Table 3.6). Berry densities were highest at Pouch Cove, which was followed by Little Catalina, Freshwater and Bryant's Cove, respectively. Damage and larval population densities were greatest at Freshwater, followed by Little Catalina, Bryant's Cove and Pouch Cove.

MANOVA of lingonberry variables showed a significant relationship between adult trap capture and both numbers of larvae and damage (Table 3.8) . Linear regression analysis indicated significant positive correlations between larval populations, damaged berries and adult capture for each trapping area (Table 3.9). Larval populations correlated with damage ($R^2 = 0.66$, Figure 3.3), and adult trap rate correlated with larval populations ($R^2 = 0.35$, Figure 3.4) and damage density ($R^2 = 0.56$, Figure 3.5). No significant relationship was found between berry density and adult, larval or damage densities.

Table 3.6: Total numbers of berries, damaged berries and larvae collected from 48 $1m^2$ quadrats at each site, and adults trapped, 1998 to 2000. Total number of *Phanerotoma* spp. parasitoids collected from pooled collections.

Site	Variable	Total per site/year		
		1998	1999	2000
Pouch Cove	Berries	9337	1347	2399
	Damaged berries	70	167	278
	Larvae	9	109	107
	Adults trapped	46	106	14
Freshwater	Berries	7616	1157	7723
	Damaged berries	186	288	663
	Larvae	38	130	128
	Adults trapped	386	220	3
Bryant's Cove	Berries	5873	751	8694
	Damaged berries	97	154	400
	Larvae	14	83	92
	Adults trapped	191	122	1
Little Catalina	Berries	8778	2285	4910
	Damaged berries	116	264	141
	Larvae	25	182	39
	Adults trapped	273	200	5
All Sites	*Phanerotoma* spp (Wesmael).	10	24	5

Table 3.7: Mean number of adults captured per trap during the 1998 (25 June to 29 July) 1999 (18 June to 26 July) and 2000 (June 22 to Aug 3) *G. libertina* flight seasons, and mean number of berries, damaged berries and larvae per trap (eight 1 m^2 quadrats corresponding to each trap) in attractant trapping grids at four wild lingonberry fields.

		Year (Mean/Trap (SEM)) N=6		
Site	Variable	1998	1999	2000
Pouch Cove	Berries	1556 (255.0)	224 (88.8)	281 (87.3)
	Damaged Berries	12 (2.3)	28 (10.5)	40 (12.2)
	Larvae	2 (0.6)	18 (6.7)	17 (5.1)
	Adults	8 (2.2)	20 (2.6)	2 (0.4)
Freshwater	Berries	1269 (238.2)	193 (104.1)	936 (299.3)
	Damaged Berries	31 (1.9)	48 (19.9)	82 (25.1)
	Larvae	6 (1.0)	22 (8.7)	15 (3.5)
	Adults	64 (9.9)	38 (4.3)	0.5 (0.3)
Bryant's Cove	Berries	979 (140.9)	125 (59.1)	1127 (174.7)
	Damaged Berries	169 (2.0)	26 (14.2)	55 (12.4)
	Larvae	2 (0.4)	14 (8.4)	13 (3.3)
	Adults	32 (11.0)	20 (9.4)	0.2 (0.16)
Little Catalina	Berries	1463 (164.7)	381 (71.1)	1109 (469.3)
	Damaged Berries	19 (1.8)	44 (5.0)	37 (15.8)
	Larvae	4 (0.9)	30.3 (3.9)	9 (4.1)
	Adults	46 (6.8)	33.3 (10.9)	1 (0.6)

Table 3.8: Multi-way analysis of variance results with percent coverage of lingonberry foliage, berry density and adult capture as covariates, year as an explanatory variable and larvae and damage as response variables. Asterisks represent significant correlations, $p < 0.05$.

Year	Covariate/Factor	Response variable	F	p
1998	Lingonberry foliage	Larvae	0.69	0.42
		Damage	0.39	0.54
	Berry density	Larvae	0.10	0.75
		Damage	0.18	0.67
	Adults	Larvae	10.9	0.00*
		Damage	13.3	0.00*
1999	Lingonberry foliage	Larvae	0.29	0.59
		Damage	0.01	0.92
	Berry density	Larvae	24.44	0.00*
		Damage	16.68	0.00*
	Adults	Larvae	2.51	0.12
		Damage	0.96	0.34
2000	Lingonberry foliage	Larvae	9.85	0.00*
		Damage	71.93	0.00*
	Berry density	Larvae	27.04	0.00*
		Damage	21.23	0.00*
	Adults	Larvae	17.58	0.00*
		Damage	25.75	0.00*
2000 Mass Trapping Grids	Lingonberry foliage	Larvae	135.68	0.00*
		Damage	30.58	0.00*
	Berry density	Larvae	0.10	0.75
		Damage	0.12	0.73
	Adults	Larvae	41.83	0.00*
		Damage	15.68	0.00*

Table 3.9: R^2 values for regressions based on total adult capture, larval population, damaged berries and berries, during the 1998, 1999 and 2000 field seasons. All variables were log-transformed prior to analysis. Asterisks represent significant correlations, $p < 0.05$.

Year	Regression	R^2 value
1998		
	Berries vs. larvae	0.00
	Berries vs. damaged berries	0.01
	Berries vs. adults	0.03
	Larvae vs. damaged berries	0.66*
	Adults vs. larvae	0.35*
	Adults vs. Damaged berries	0.56*
1999		
	Berries vs. larvae	0.90*
	Berries vs. damaged berries	0.85*
	Berries vs. adults	0.01
	Larvae vs. damaged berries	0.89*
	Adults vs. larvae	0.00
	Adults vs. damaged berries	0.00
2000		
	Berries vs. larvae	0.33*
	Berries vs. damaged berries	0.56*
	Berries vs. adults	0.13
	Larvae vs. damaged berries	0.89*
	Adults vs. larvae	0.36*
	Adults vs. damaged berries	0.22
2000 Mass trapping grids		
	Berries vs. larvae	0.75*
	Berries vs. damaged berries	0.89*
	Berries vs. adults	0.02
	Larvae vs. damaged berries	0.89*
	Adults vs. larvae	0.03
	Adults vs. damaged berries	0.01
1998-1999	1998 Larvae vs. 1999 adults	0.020
1999-2000	1999 Larvae vs. 2000 adults	0.038

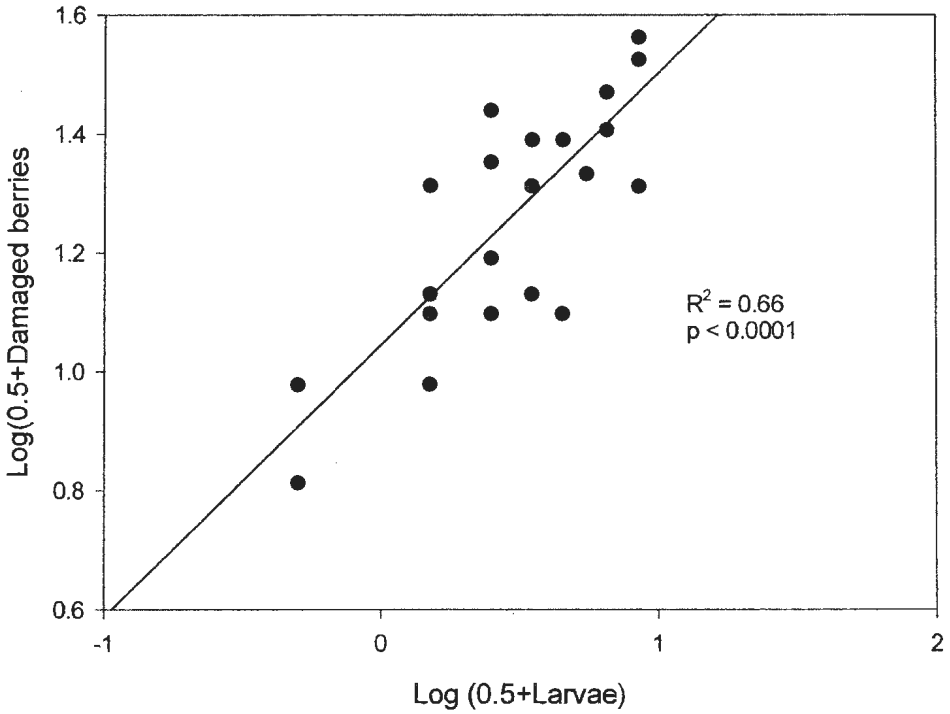

Figure 3.3: Regression of larvae vs. damaged berries within 6 plots at each of 4 sites during the 1998 field season. Data log transformed (Log (X+0.5)).

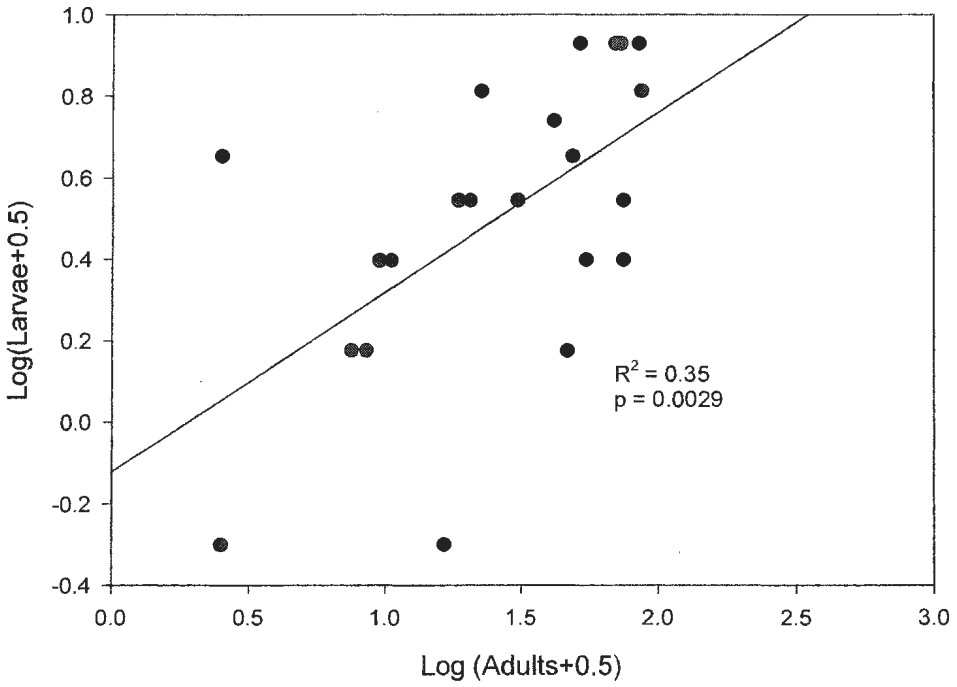

Figure 3.4: Regression of adults vs. larvae within 6 plots at each of 4 sites during the 1998 field season. Data have been log transformed (Log (X+0.5)).

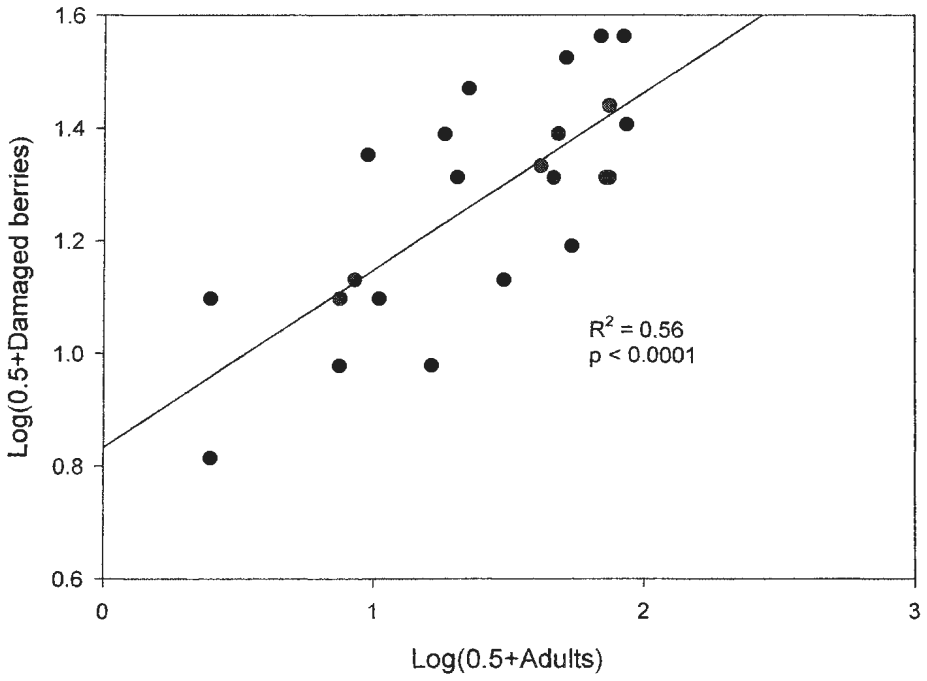

Figure 3.5: Regression of adults vs damaged berries within 6 plots at each of 4 sites during the 1998 field season. Data have been log transformed (Log (X+0.5)).

3.2.2 1999 Field trials:

The total number of adults trapped per site during the 1999 field season ranged from 106 in Pouch Cove to 220 in Freshwater (Table 3.6). Mean berry, larval and damaged berry density are shown in Table 3.7. Berry densities were highest at Little Catalina, followed by Pouch Cove, Freshwater and Bryant's Cove. Larval populations and damaged berry density were highest in Freshwater and Little Catalina, followed by Pouch Cove and Bryant's Cove (Table 3.7).

MANOVA analysis in 1999 showed no relationship between the adult capture rate and either larval, damage, or berry densities ($p<0.05$). A significant positive relationship was found between larval populations and damaged berries, with an R^2 of 0.89 (Table 3.9, Figure 3.6). Berry density was also significantly correlated with both larval populations and berry damage, with R^2 values of 0.90 and 0.85, respectively (Table 3.9, Figures 3.7 and 3.8).

3.2.3 2000 Field trials:

Total number of adults trapped per site during the 2000 field season ranged from 1 in Bryant's Cove to 14 in Pouch Cove (Table 3.6). Mean berry, larval and damaged berry density are shown in Table 3.7. Berry densities were highest in Bryant's Cove, followed by Little Catalina, Freshwater and Pouch Cove. Larval populations were greatest at Pouch Cove, followed by Freshwater, Bryant's Cove and Little Catalina.

MANOVA in 2000 indicated significant relationships between adults, berries and lingonberry coverage as explanatory variables with larvae and damage

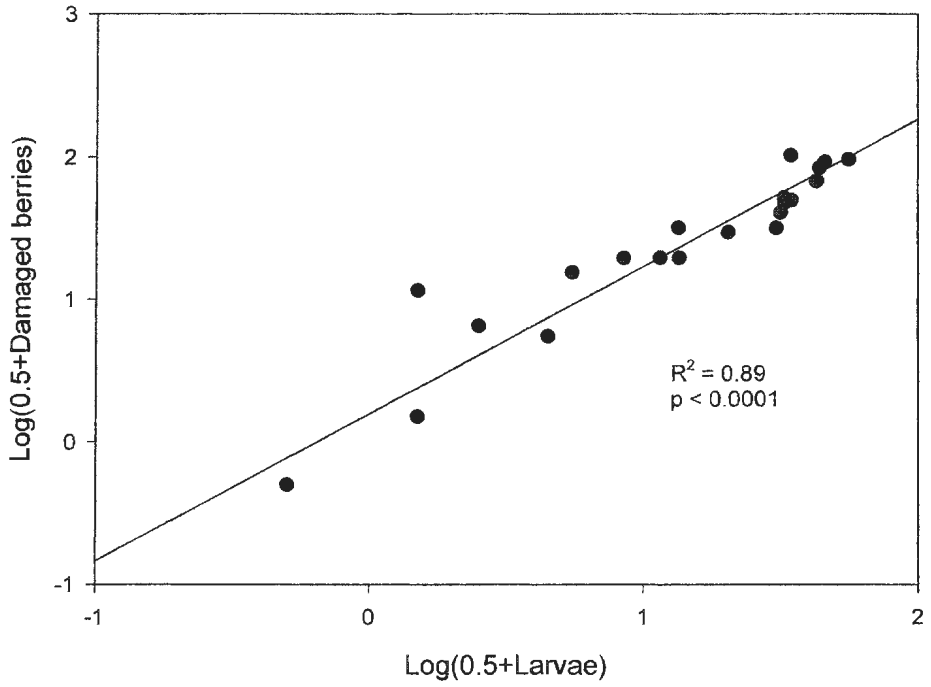

Figure 3.6: Regression of larvae vs. damaged berries within 6 plots at each of 4 sites during the 1999 field season. Data have been log transformed (Log (X+0.5)).

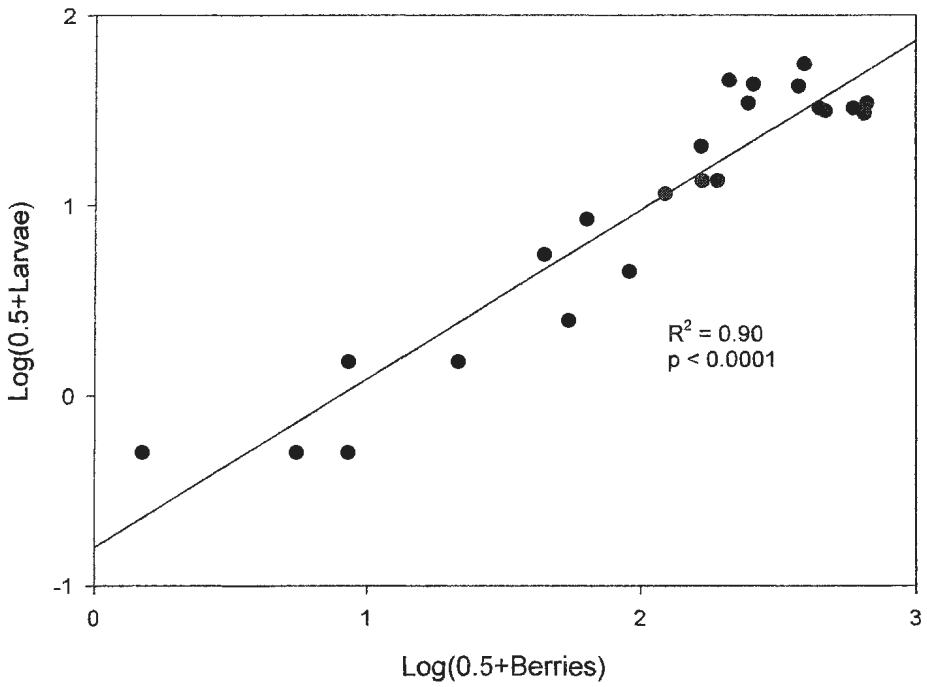

Figure 3.7: Regression of total berries vs. larvae within 6 plots at each of 4 sites during the 1999 field season. Data have been log transformed (Log (X+0.5)).

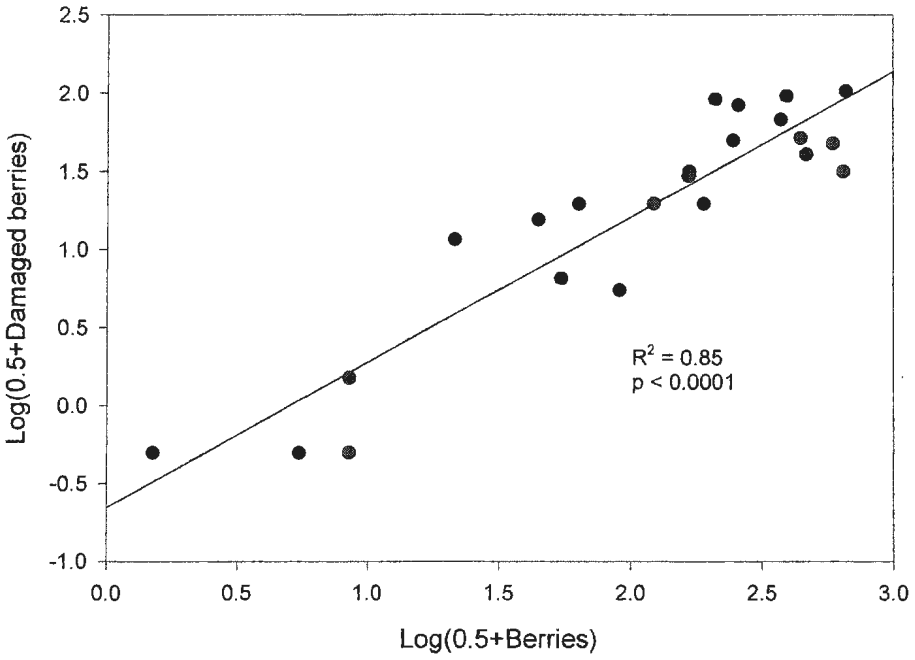

Figure 3.8: Regression of total berries vs. damaged berries within 6 plots at each of 4 sites during the 1999 field season. Data have been log transformed (Log (X+0.5)).

as response variables ($p < 0.05$, Table 3.8). Since adult densities were low, plots from which no adults were captured were not included in regression analysis for 2000. Linear regression showed significant positive correlations between larval populations and damaged berry densities and between adult and larval populations. Larval populations correlated with damage ($R^2 = 0.89$, Figure 3.9), and adult trap rate correlated with larval populations ($R^2 = 0.36$, Figure 3.10). Berry densities correlated with larvae and berry damage ($R^2 = 0.33$, Figures 3.11 and $R^2 = 0.56$ respectively, Figure 3.12). Correlation was not significant between adults and damage or berry densities ($R^2 = 0.22$ and 0.13, respectively ($p<0.05$)).

3.2.4 Comparison between years:

Total berries, percent larval infestation and damaged berries were significantly different between years, whereas differences between sites were not ($p<0.05$). Larvae and damaged berry densities were not significantly different between control and pheromone-trapped quadrats in any year. A significantly lower berry density was evident at all sites in 1999 compared with 1998 and 2000, whereas percent larval infestation and berry damage were significantly higher (Figures 3.13, 3.14, 3.15). Total adult capture at each site was less in 2000 (1 in Bryant's Cove to 14 in Pouch Cove) compared to 1999 (106 in Pouch Cove to 220 in Freshwater) or 1998 (46 in Pouch Cove to 386 in Freshwater). Site rankings based on adult capture rates were variable between years. Rankings of berry densities, larval counts and damaged berries at each site also differed between the 1998, 1999 and 2000 field seasons (Table 3.7). Linear regression

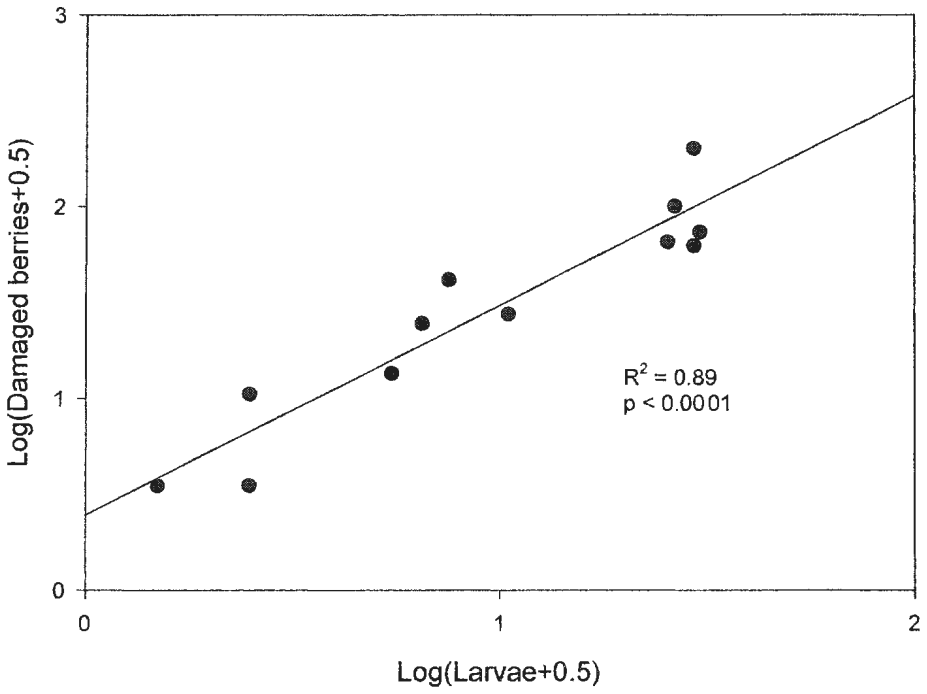

Figure 3.9: Regression of larvae vs. damaged berries within 6 plots at each of 4 sites during the 2000 field season. Data have been log transformed (Log (X+0.5)).

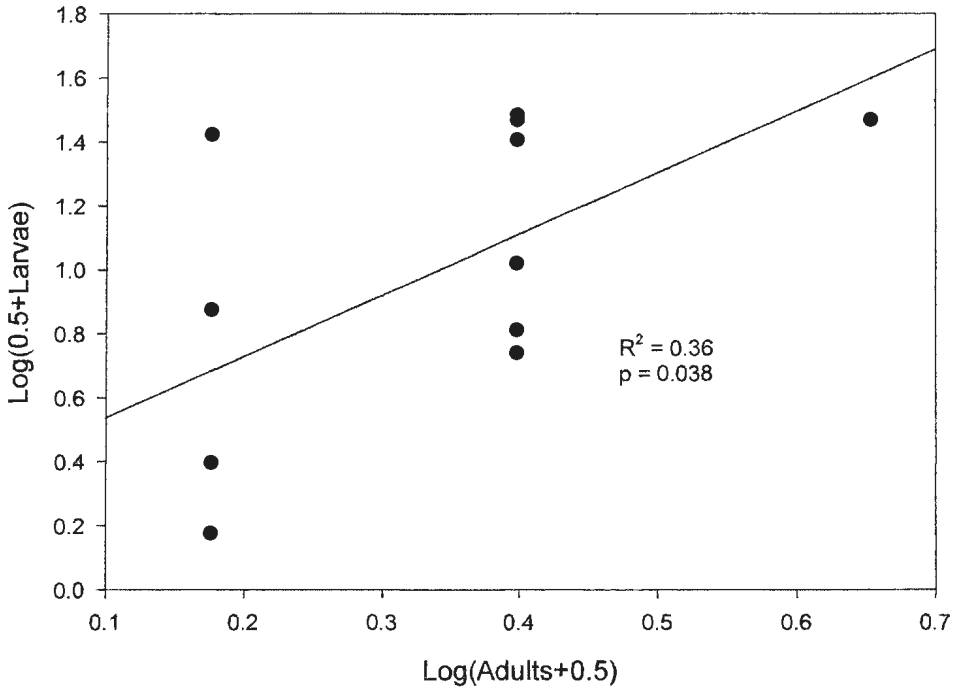

Figure 3.10: Regression of adults vs. larvae within 6 plots at each of 4 sites during the 2000 field season. Data have been log transformed (Log (X+0.5)).

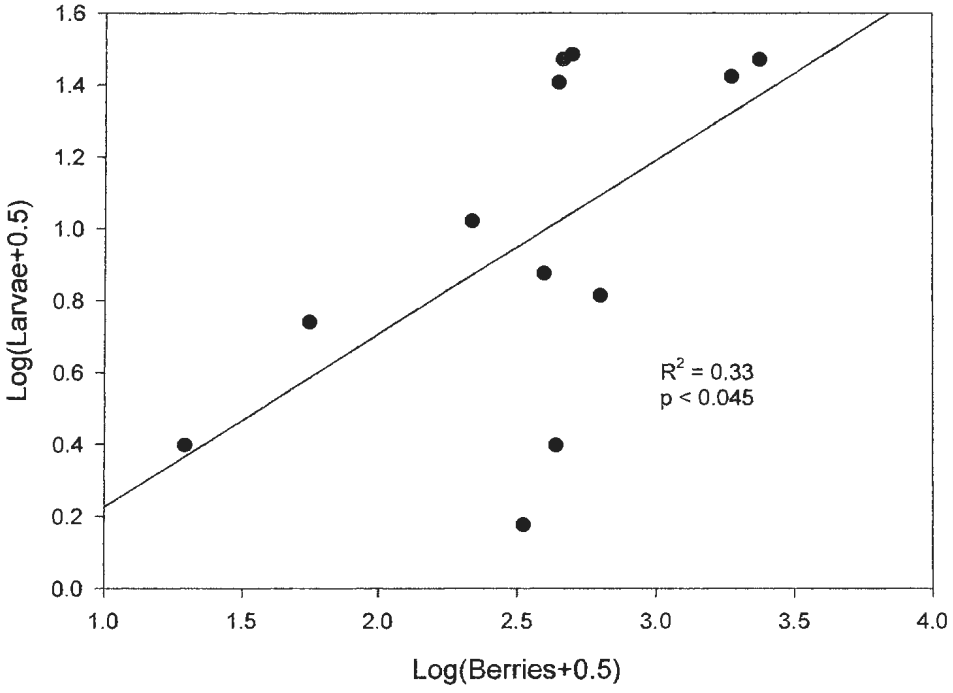

Figure 3.11: Regression of total berries vs. larvae within 6 plots at each of 4 sites during the 2000 field season. Data have been log transformed (Log (X+0.5)).

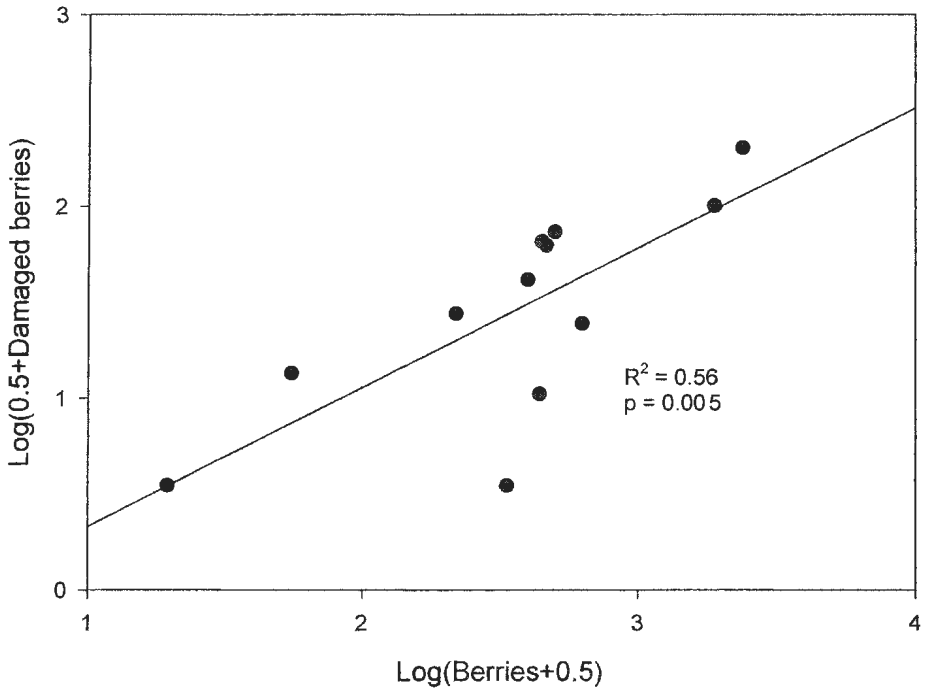

Figure 3.12: Regression of total berries vs. damaged berries within 6 plots at each of 4 sites during the 2000 field season. Data have been log transformed (Log (X+0.5)).

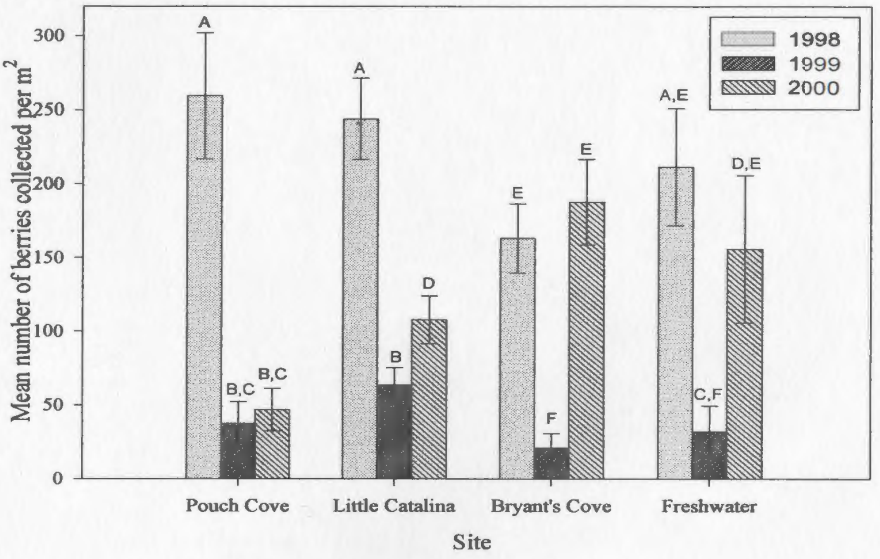

Figure 3.13: Mean number of lingonberries collected in each plot within four sites during 1998, 1999 and 2000 (Means represented by the same letter did not differ ($p<0.05$) by Fisher's LSD).

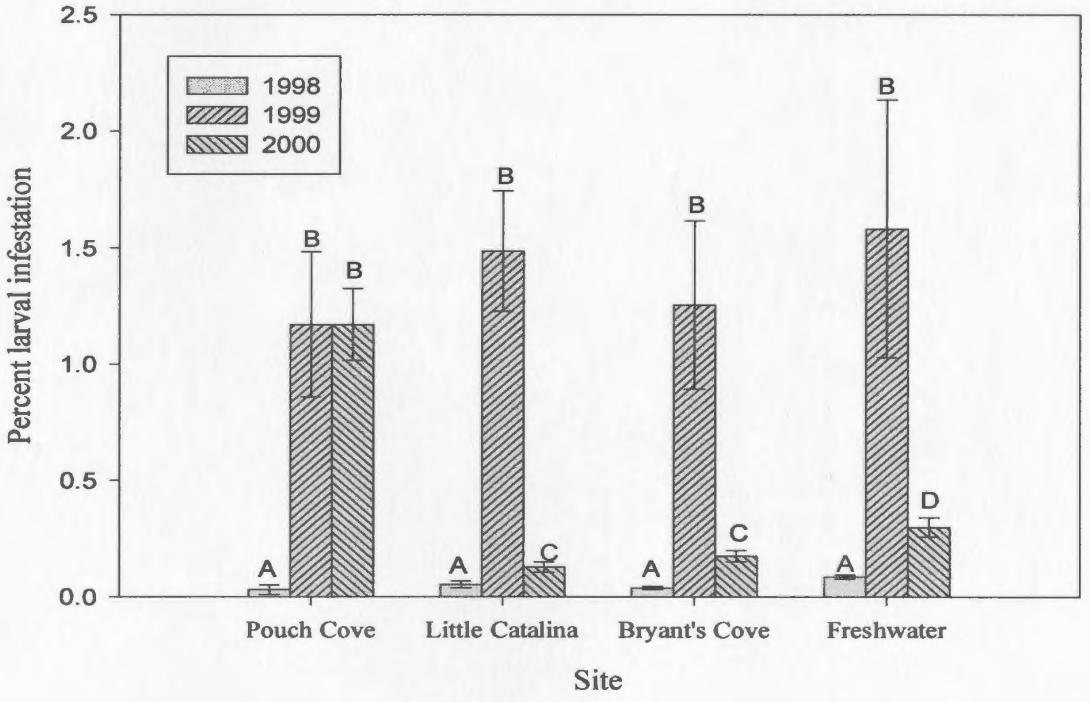

Figure 3.14: Percent of lingonberries infested with *G. libertina* larvae at four sites during 1998, 1999 and 2000. Percentages are means across all plots within each site during each year (Means represented by the same letter did not differ ($p<0.05$) by Fisher's LSD).

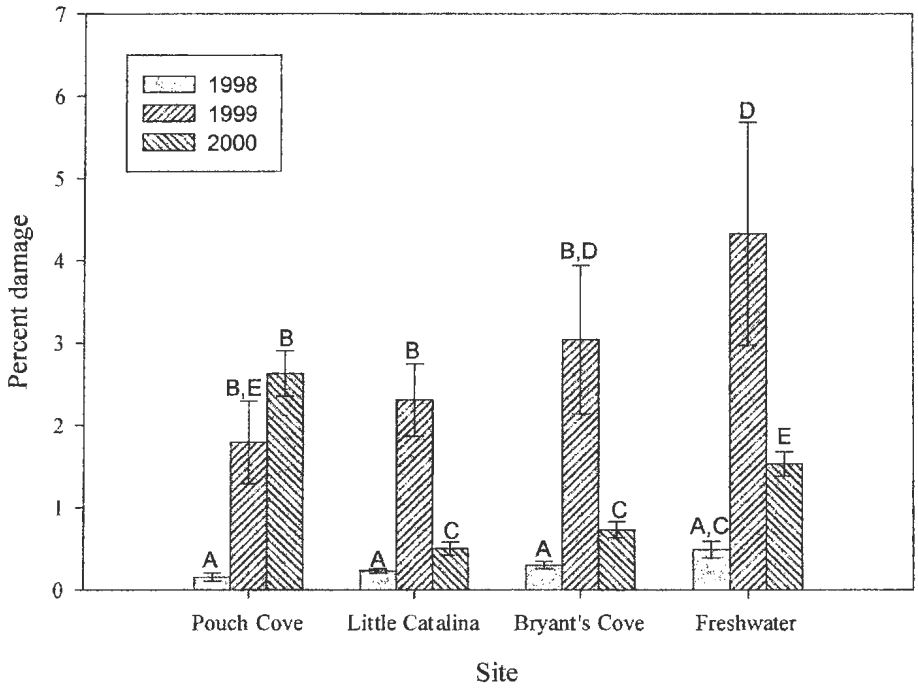

Figure 3.15: Percent of lingonberries damaged by *G. libertina* at four sites during 1998, 1999 and 2000. Percentages are means across all plots within each site during each year (Means represented by the same letter did not differ ($p<0.05$) by Fisher's LSD).

analysis also indicated that there were differences in the relationships between variables in each year, for example larval and berry damage counts were positively correlated with the adult capture rate in 1998, but were correlated with berry density in 1999 (Table 3.9).

Regression of larval counts in 1998 and 1999 with subsequent adult counts in 1999 and 2000 respectively indicated no significant relationship between larval populations and adult population size in following years (Table 3.9). Ratios of damage : larvae and adults : larvae were variable between sites and years (Table 3.10). Adult : larvae ratios in 2000 were very high in Freshwater and Bryant's Cove, and may be attributed to low adult trapping. Reported *Phanerotoma* spp. parasitism decreased from 11.6% in 1998 to 1.5% in 2000.

All of the one hundred (total over all years) randomly selected *G. libertina* moths from the traps in 1996, 1997 and 1998 were found to be males, by genitalia examination (Figure 3.16) and comparison with dissected genitalia of reared females (Figure 3.17).

3.3 Vegetation analysis:

A complete listing of plant species, densities and ranges at each site during each year is listed in appendix C. Results of principal component analysis of vegetation types from 1998-2000 are summarized in Table 3.11 and Figure 3.18.

Table 3.10: Damage : larvae and larvae : adult ratios based on 1998, 1999 and 2000 trap catch at each site.

		Year		
	Site	**1998**	**1999**	**2000**
Damage : larvae	Pouch Cove	7.8	1.5	2.6
	Freshwater	5.0	2.2	5.2
	Bryant's Cove	6.9	1.9	4.3
	Little Catalina	4.6	1.5	3.6
Larvae : adults	Pouch Cove	0.2	1.0	7.6
	Freshwater	0.1	0.6	42.7
	Bryant's Cove	0.1	1.9	92.0
	Little Catalina	0.1	0.9	7.8
Percent *Phanerotoma* spp. parasitism	Overall	11.6%	4.8%	1.5%

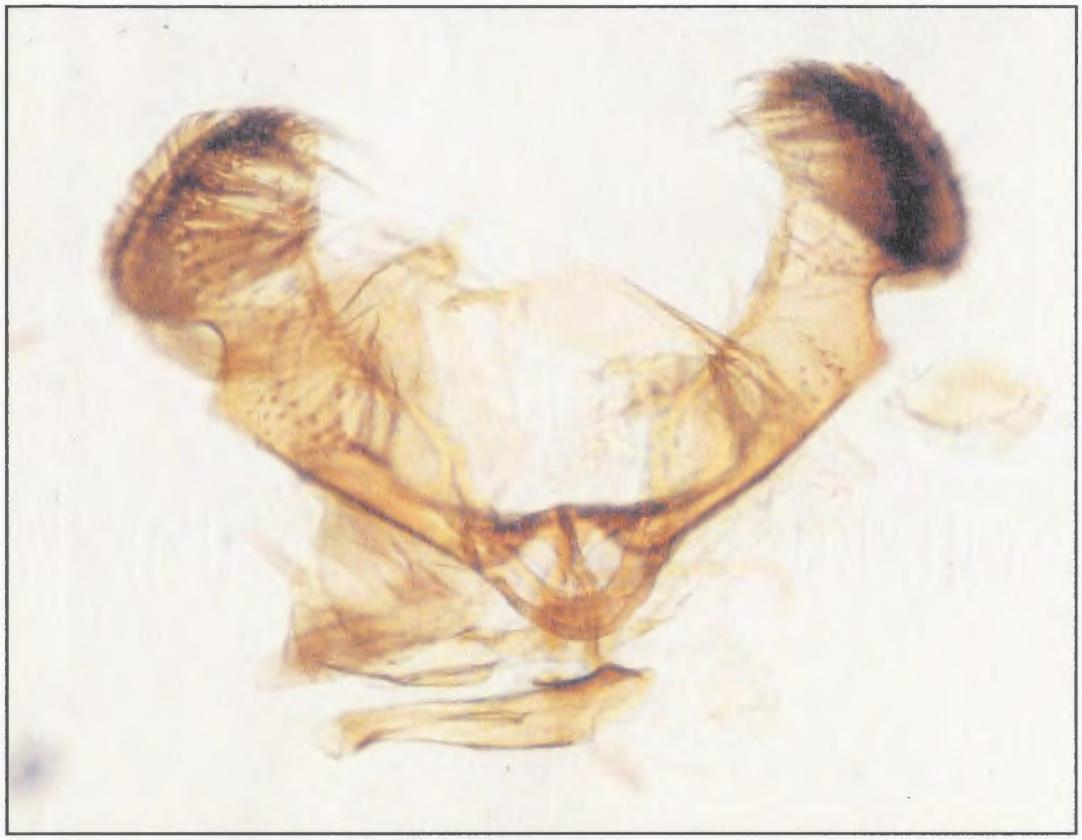

Figure 3.16: Light micrograph of male *G. libertina* genitalia, following extraction from pheromone traps with ethyl acetate, clearing with potassium hydroxide and mounting in Rubin's medium. Scale: 1 cm = 0.1 mm.

Figure 3.17: Light micrograph of female *G. libertina* genitalia, dissected from reared insects, cleared with potassium hydroxide and mounted in Rubin's medium. Scale: 1 cm = 0.1 mm.

Table 3.11: Principal component analysis of vegetation coverage data for four study sites in Little Catalina, Pouch Cove, Bryant's Cove and Freshwater, NF., during the 1998, 1999 and 2000 field seasons. For principal component 3, only those species which were most positive or negative on are shown.

Principal Component Axis	Vegetation	Minimum	Maximum	Eigenvector scores	Percent variance explained
1	*Vaccinium vitis-idaea*	0	85	0.866	**30 %**
	Vaccinium angustifolium	0	90	0.769	
	Juniperus communis	0	80	0.727	
	Sphagnum spp.	0	80	0.597	
	Potentilla tridentata	0	50	0.542	
	Maianthemum canadensis	0	40	0.521	
	Cornus canadensis	0	40	0.299	
	lichen spp.	0	90	0.225	
	Kalmia angustifolium	0	80	0.173	
	Empetrum nigrum	0	90	0.155	
2	lichen spp.	0	90	0.728	**22 %**
	Kalmia angustifolium	0	80	0.650	
	Empetrum nigrum	0	90	0.623	
	Cornus canadensis	0	40	0.337	
	Potentilla tridentata	0	50	0.292	
	Vaccinium vitis-idaea	0	85	0.156	
	Vaccinium angustifolium	0	90	0.004	
	Sphagnum spp.	0	80	-0.609	
	Juniperus communis	0	80	-0.470	
	Maianthemum canadensis	0	40	-0.174	
3	*Cornus canadensis*	0	40	0.486	**14 %**
	Maianthemum canadensis	0	40	-0.635	
	lichen spp.	0	90	-0.558	

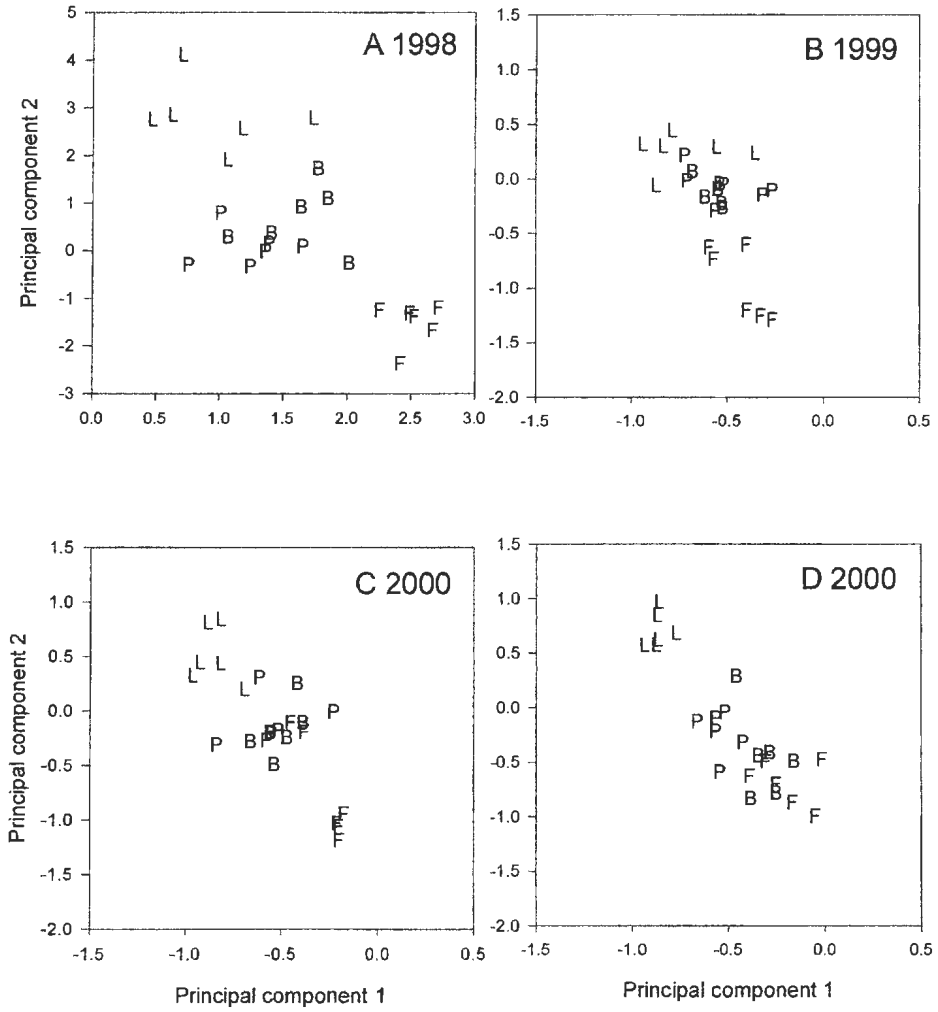

Figure 3.18: Scatterplot of plots within trapping grids at four study sites in A-1998, B-1999, C-2000 and D-2000 (mass trapping grids), along axes 1 and 2 of a principal component analysis of vegetation using data from 1998-2000. P = Pouch Cove, F = Freshwater, B = Bryant's Cove, L = Little Catalina. Yearly data were grouped for analysis and plotted on separate graphs, B, C and D were plotted on a larger scale than A.

Vegetation with high and low eigenvector scores for each principal component were assumed to contribute the most variability along each axis. Principal components 1, 2 and 3 explained 30%, 22% and 14% of the variance, respectively. Principal component 1 was represented by high positive scores for *Vaccinium vitis-idaea* (lingonberry), *Vaccinium angustifolium*, *Juniperus communis*, *Sphagnum* spp. and *Potentilla tridentata*. Vegetation on principal component 1 had no negative values, indicating positive autocorrelation between all vegetation types on this axis. Principal component 2 maintained negative scores for *Sphagnum* spp., *Juniperus communis* and *Maianthemum canadensis*, and high positive scores for *Kalmia angustifolium*, Lichen spp. and *Empetrum nigrum*. Principal component 3 had *Cornus canadensis* at the negative end, and *Maianthemum canadensis* and Lichen spp. at the positive end of the axis.

Figure 3.18 shows a number of trends evident from the vegetation data. Plots from various sites were closely associated along each principal component . Along principal component 2, the Little Catalina plots fell into the positive region, whereas Freshwater plots clustered at the negative end. Pouch Cove and Bryant's Cove clustered in the middle region of principal component 2. Site differences were not negative on principal component 1. Fisher's LSD comparisons of sites along each axis showed significant differences between Freshwater and Little Catalina along principal component 1, and between all sites except Pouch Cove and Bryant's Cove on principal component 2 (Table 3.12). Differences between plots on principal component 1 were based on differences between years (Figure 3.18). In 1999, 2000 and 2000B, plots clustered in the

Table 3.12: Least significant difference comparisons between sites on axes 1 and 2 of a principal component analysis of vegetation types at four study sites, Little Catalina, Pouch Cove, Bryant's Cove and Freshwater, during 1998-2000 field seasons. (2000B denotes mass trapping grid, variables followed by different letters were significantly different at $p<0.05$).

Axis	Site	Mean	Standard Deviation	Difference
Principal Component 1	Pouch Cove	-9.48×10^{-3}	0.811	A, B
	Freshwater	0.4	1.253	B
	Little Catalina	-0.37	0.826	A
	Bryant's Cove	5.82×10^{-3}	0.946	A, B
Principal Component 2	Pouch Cove	-8.47×10^{-3}	0.272	A
	Freshwater	-0.98	0.489	B
	Little Catalina	1.08	1.114	C
	Bryant's Cove	-3.35×10^{-3}	0.589	A

slightly negative region of principal component 1. Plots in 1998 were more variable on principal components 1 and 2, being in the more positive region of principal component 1 and variable across principal component 2. Fisher's LSD comparisons indicated that plots in 1998 were significantly different from those in 1999, 2000 and 2000B (which showed no differences between them) (Table 3.13). Mean coefficients of variation for each year also indicated more variability was present in 1998 samples (R=81% in 1998 versus R=96% in 1999 and R=97% in 2000).

Principal component 1 varied between 1998 data and other years, and variation within the 1998 data itself (relative to 1999 and 2000 data). Principal component analysis was therefore conducted again, without the 1998 data, to remove autocorrelation in the vegetation data, and variation due to the 1998 data (as evident on principal component 1, Figure 3.18).

Results of principal component analysis without 1998 vegetation data are shown in Tables 3.14 and 3.15. Principal components 1, 2 and 3 explained 30%, 20% and 11% of the variance, respectively. Principal component 1 was represented by negative scores for lichen spp., *Kalmia angustifolium*, *Empetrum nigrum*, *Cornus canadensis* and *Potentilla tridentata*, and positive scores for *Sphagnum* spp., *Vaccinium vitis-idaea*, *Juniperus communis*, *Vaccinium angustifolium* and *Maianthemum canadensis*. Principal component 2 had negative scores for *Potentilla tridentata*, *Empetrum nigrum*, *Sphagnum* spp., *Juniperus communis*, *Kalmia angustifolium* and *Vaccinium vitis-idaea*, and positive scores for *Cornus canadensis*, *Maianthemum canadensis*, *Vaccinium angustifolium*, and lichen spp. Table 3.15 describes vegetation abundance at

Table 3.13: Least significant difference (LSD) comparisons between years on axes 1 and 2 of a principal component analysis of vegetation types at 4 study sites, Little Catalina, Pouch Cove, Bryant's Cove and Freshwater, during 1998-2000 field seasons. (2000B denotes mass trapping grid, variables followed by different letters were significantly different at $p<0.05$).

Axis	Year	Mean	Standard Deviation	Difference
Principal Component 1	1998	1.6	0.675	A
	1999	-0.56	0.189	B
	2000	-0.54	0.244	B
	2000B	-0.48	0.276	B
Principal Component 2	1998	0.52	1.69	A
	1999	-0.22	0.497	B
	2000	-0.13	0.545	B
	2000B	-0.16	0.595	B

Table 3.14: Principal component analysis of vegetation coverage data for four study sites in Little Catalina, Pouch Cove, Bryant's Cove and Freshwater, NF., during the 1999 and 2000 field seasons. For principal component 3, only those species which were most positive or negative on are shown.

Principal component axis	Vegetation	Minimum	Maximum	Eigenvector scores	Percent variance explained
1	*Sphagnum* spp.	0	80	0.747	**30 %**
	Vaccinium vitis-idaea	0	85	0.637	
	Juniperus communis	0	80	0.603	
	Vaccinium angustifolium	0	90	0.572	
	Maianthemum canadensis	0	40	0.277	
	lichen spp.	0	90	-0.859	
	Kalmia angustifolium	0	80	-0.473	
	Empetrum nigrum	0	90	-0.448	
	Cornus canadensis	0	20	-0.215	
	Potentilla tridentata	0	30	-0.214	
2	*Cornus canadensis*	0	20	0.759	**20 %**
	Maianthemum canadensis	0	40	0.593	
	Vaccinium angustifolium	0	90	0.542	
	lichen spp.	0	90	0.208	
	Potentilla tridentata	0	30	-0.532	
	Empetrum nigrum	0	90	-0.457	
	Sphagnum spp.	0	80	-0.376	
	Juniperus communis	0	80	-0.338	
	Kalmia angustifolium	0	80	-0.135	
	Vaccinium vitis-idaea	0	85	-0.0047	
3	*Kalmia angustifolium*	0	80	0.691	**11 %**
	Empetrum nigrum	0	90	-0.614	

Table 3.15: Descriptive comparison of the principal component analysis of vegetation coverage data for four study sites in Little Catalina, Pouch Cove , Bryant's Cove and Freshwater, NF., during the 1999 and 2000 field seasons.

Site	Relative Position on each axis		Principal Component I	Principal Component II
	I	II		
Little Catalina	Negative	Negative	Low relative abundances of *Sphagnum* spp., *Vaccinium vitis-idaea*, *Juniperus communis*, and *Vaccinium angustifolium*. High relative abundances of lichen spp., *Kalmia angustifolium*, and *Empetrum nigrum*.	Low relative abundances of *Cornus canadensis*, *Maianthemum canadensis*, *Vaccinium angustifolium*. High relative abundances of *Potentilla tridentata*, *Empetrum nigrum*, *Sphagnum* spp., and *Juniperus communis*..
Pouch Cove	Neutral	Positive	No evidence for strong correlation with vegetation abundance relative to other sites.	Low relative abundances of *Potentilla tridentata*, *Empetrum nigrum*, *Sphagnum* spp., and *Juniperus communis*.. High relative abundances of *Cornus canadensis*, *Maianthemum canadensis*, *Vaccinium angustifolium*.
Bryant's Cove	Neutral	Neutral	No evidence for strong correlation with vegetation abundance relative to other sites.	No evidence for strong correlation with vegetation abundance relative to other sites.
Freshwater	Positive	Negative	Low relative abundances of lichen spp., *Kalmia angustifolium*, and *Empetrum nigrum*. High relative abundances of *Sphagnum* spp., *Vaccinium vitis-idaea*, *Juniperus communis*, and *Vaccinium angustifolium*.	Low relative abundances of *Cornus canadensis*, *Maianthemum canadensis*, *Vaccinium angustifolium* High relative abundances of *Potentilla tridentata*, *Empetrum nigrum*, *Sphagnum* spp., and *Juniperus communis*.

each site, based on plot positions along principal components one and two. Clustering of plots within each site, during each year is shown on Figure 3.19. Pairwise comparisons (Fisher's LSD) indicated that Little Catalina scores were significantly lower and Freshwater was significantly higher than other sites on principal component 1 (Table 3.16). All sites were significantly different on principal component 2 except Freshwater and Little Catalina, which were not different from one another.

By overlaying data on the insect's density and damage, few trends were noticed between the plots. Variation was evident between adult, larval and damage densities at plots within each site. Figure 3.20 shows that adult density was variable between all plots in 1999, but was lower in 2000 and C 2000. Larval and damaged berry densities in 1999 and 2000 were variable (Figures 3.21 and 3.22), but showed no trend along either axis. Berry density differences were not apparent between sites, but densities were lower in 1999 relative to 2000 (as in section 3.2) (Figure 3.23). As previously discussed, Freshwater and Little Catalina maintained higher adult, larval and damage densities than the other sites (see section 3.2).

Multi-way analysis of variance using vegetation coverage and berry density as covariates, and sites and years as explanatory variables found that both lingonberry coverage (*Vaccinium vitis-idaea*) ($F=6.7$, $p<0.05$), and berry densities ($F=10.4$, $p<0.05$) were significantly related to larval density, and that berry

densities (F=114.8, p<0.05) were related to damage (Table 3.17). No other vegetation showed a significant relationship to larvae or damage. Interaction

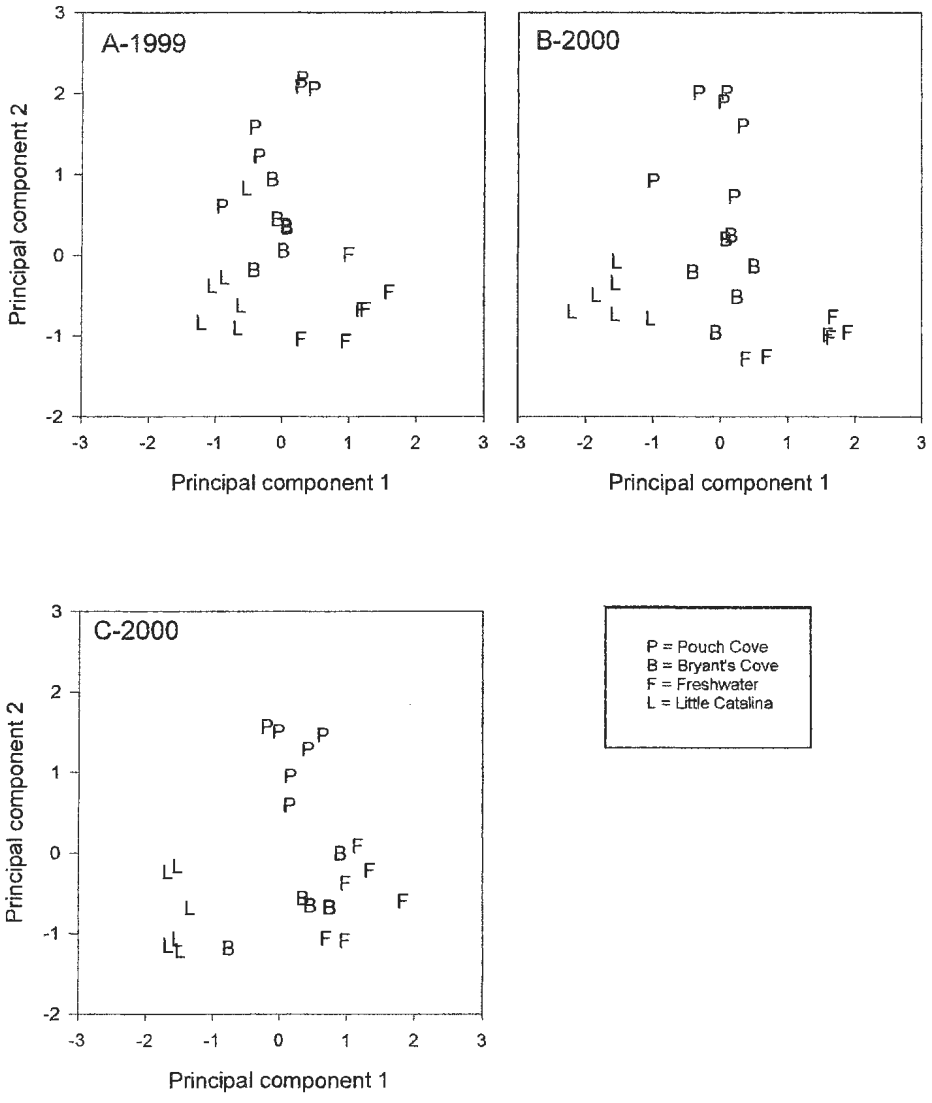

Figure 3.19: Scatterplot of plots within trapping grids at four study sites in: A-1999, B-2000 and C-2000 (mass trapping grids), along axes 1 and 2 of a principal component analysis of vegetation using data from 1999-2000.

Table 3.16: Least significant difference comparisons between sites on axes 1 and 2 of a principal component analysis of vegetation types at four study sites, Little Catalina, Pouch Cove, Bryant's Cove and Freshwater, during 1999-2000 field seasons. (2000B denotes mass trapping grid, variables followed by different letters were significantly different at $p<0.05$).

Axis	Site	Mean	Standard Deviation	Difference
Principal Component 1	Pouch Cove	1.60 x 10-3	0.449	A
	Freshwater	1.18	0.477	B
	Little Catalina	-1.32	0.454	C
	Bryant's Cove	0.14	0.436	A
Principal Component 2	Pouch Cove	1.46	0.53	A
	Freshwater	-0.74	0.413	B
	Little Catalina	-0.54	0.48	B
	Bryant's Cove	-0.17	0.553	C

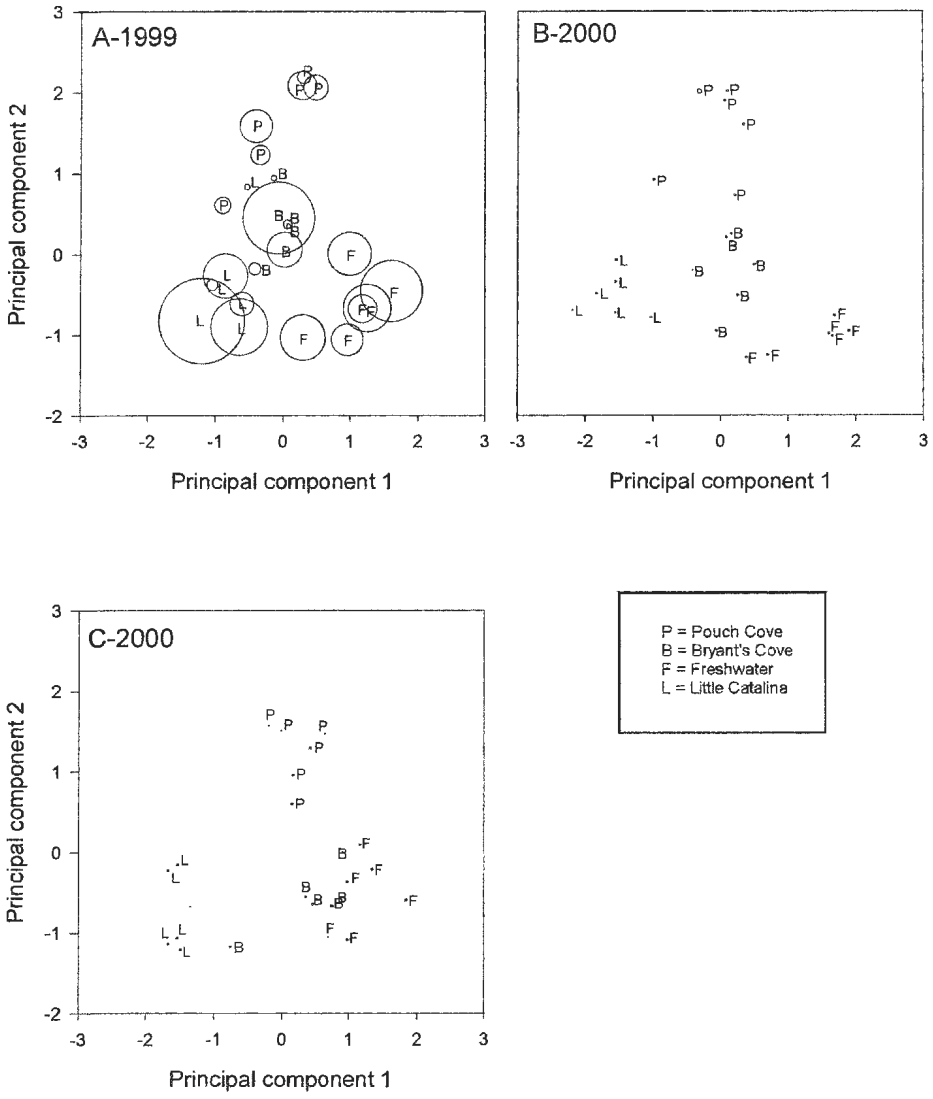

Figure 3.20: Bubbleplot of adult densities (total moth catch/trap/season) in: A-1999, B-2000 and C-2000 (mass trapping grids), along axes 1 and 2 of a principal component analysis of vegetation using data from 1999-2000.

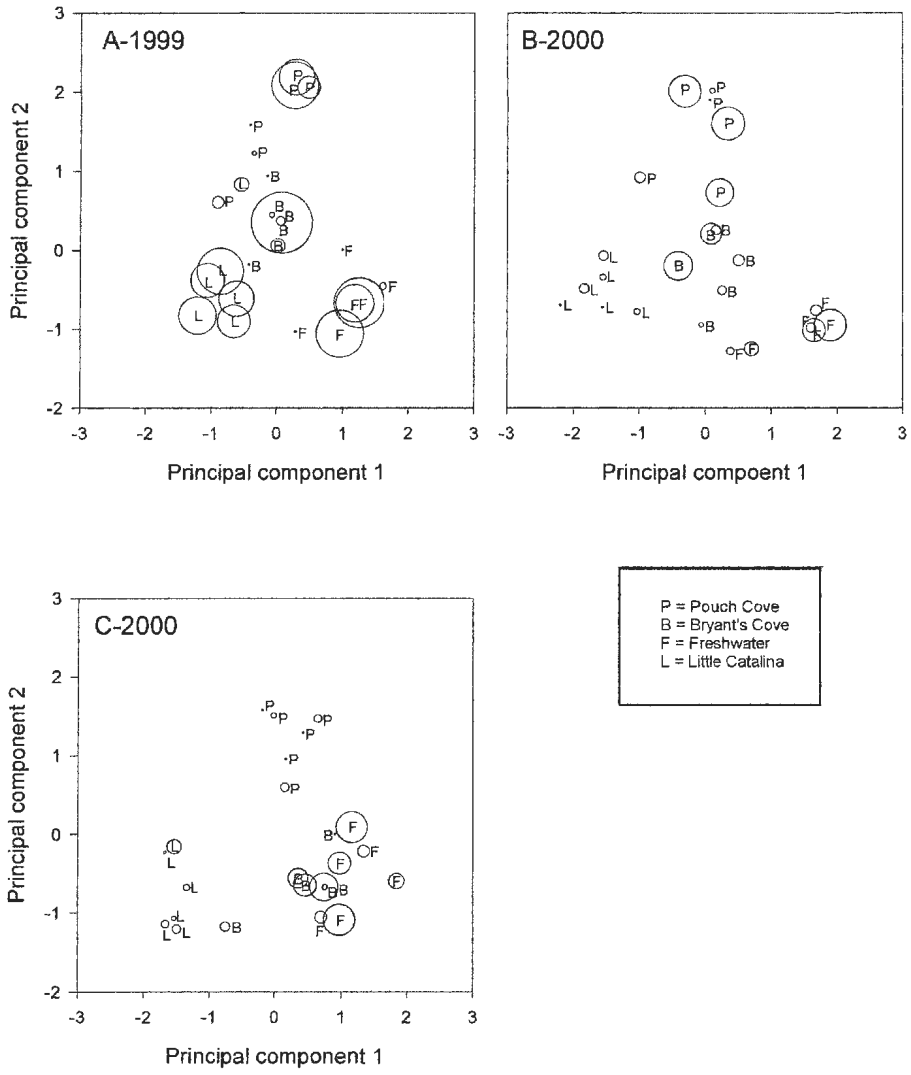

Figure 3.21: Bubbleplot of larval densities (total larvae/quadrat/season) in: A-1999, B-2000 and C-2000 (mass trapping grids), along axes 1 and 2 of a principal component analysis of vegetation using data from 1999-2000.

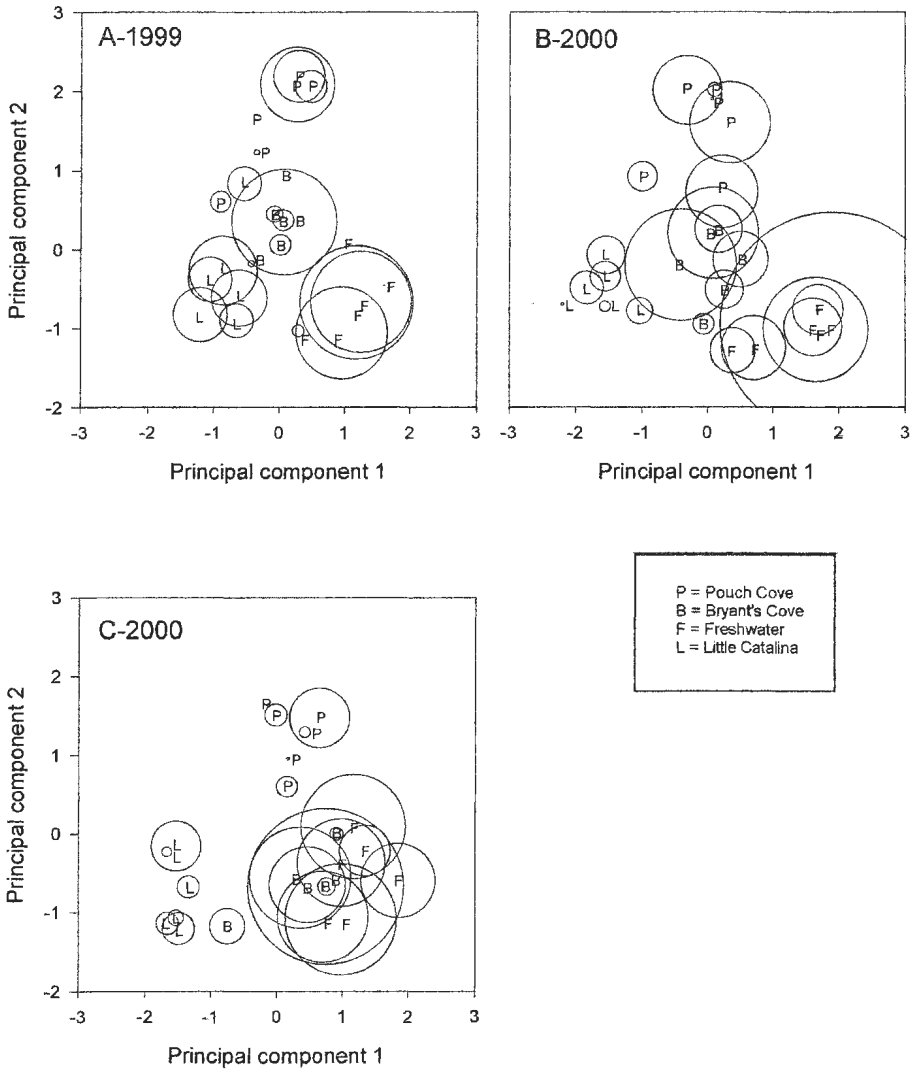

Figure 3.22: Bubbleplot of damaged lingonberry densities (total damaged berries/quadrat/season) in: A-1999, B-2000 and C-2000 (mass trapping grids), along axes 1 and 2 of a principal component analysis of vegetation using data from 1999-2000.

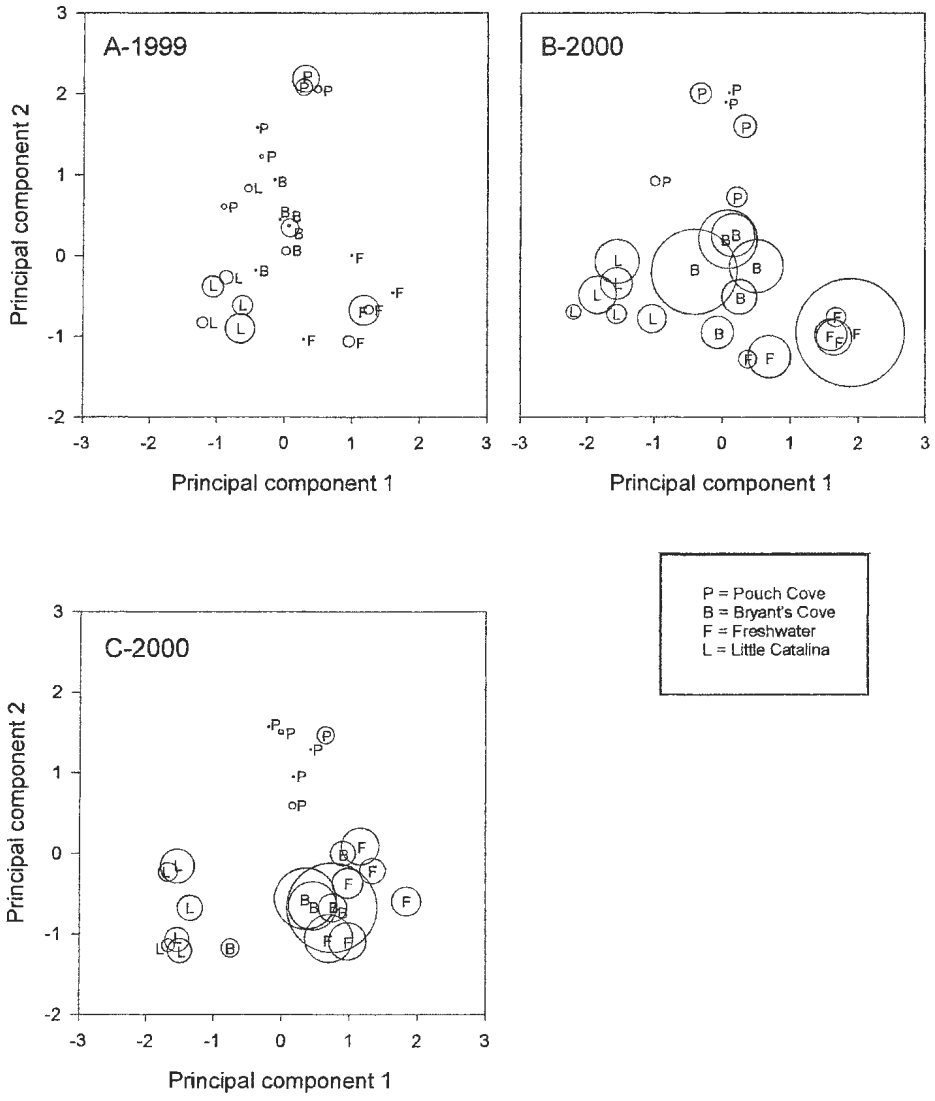

Figure 3.23: Bubbleplot of berry densities (total berries collected/quadrat/season) in: A-1999, B-2000 and C-2000 (mass trapping grids), along axes 1 and 2 of a principal component analysis of vegetation using data from 1999-2000.

Table 3.17: Multiway analysis of variance with vegetation and berry densities as covariates, site and year as factors, and larval and damaged berry densities as dependant variables. Significant relationships ($p<0.05$) are indicated by asterisks.

Variable	Dependant	F	p
Berry density	Larvae	10.4	0.00*
	Damage	114.8	0.00*
Vaccinium vitis-idaea	Larvae	6.7	0.01*
	Damage	2.1	0.16
Vaccinium angustifolium	Larvae	3.6	0.06
	Damage	2.8	0.1
Juniperus communis	Larvae	0.5	0.5
	Damage	0.3	0.6
lichen spp.	Larvae	0.2	0.67
	Damage	0.1	0.75
Potentilla tridentata	Larvae	0.6	0.44
	Damage	1.3	0.25
Empetrum nigrum	Larvae	0.4	0.51
	Damage	0.2	0.68
Maianthemum canadensis	Larvae	0	0.87
	Damage	1	0.33
Sphagnum spp.	Larvae	3	0.09
	Damage	1	0.32
Kalmia angustifolium	Larvae	1.5	0.23
	Damage	0.9	0.36
Cornus canadensis	Larvae	0.5	0.47
	Damage	1.5	0.23
Year	Larvae	3.1	0.05*
	Damage	2.6	0.08
Site	Larvae	0.6	0.62
	Damage	2.4	0.07
Year*Site interaction	Larvae	2.5	0.04*
	Damage	3.5	0.00*

between year and site was also noted for both larvae ($F=2.5$, $p<0.05$) and damage ($F=3.5$, $p<0.05$).

3.4 Trap design trials:

The Pherocon® 1C traps captured significantly more moths ($p<0.05$) than any other trap types (Table 3.18, Figure 3.24). The Diamond® trap was ranked second, followed by the Wing Trap® II and Delta® traps (Table 3.19). The Diamond® and Wing Trap® II were not significantly different from each other, and the Wing Trap® II and Delta® traps were not different from one another ($p>0.05$) (Figure 3.24). The Unitrap® captured significantly fewer moths ($p<0.05$) than all other trap types, except the Delta® trap. All trap types, except the Unitrap® captured significantly more moths than the control traps. Standardizing of overall mean catch by trap surface area ranked wing traps first, followed by Diamond and Delta traps. Some variation ($p<0.05$) was noted in the ranking of traps between sites (Table 3.19).

3.5 Mass trapping trials

During mass trapping trials in 2000, total adult capture per trap ranged from 2 in Little Catalina to 5 in Pouch Cove (Table 3.20). In addition, data on total and mean berries, damaged berries and larvae per 1 m^2 quadrat were also collected in mass trapping grids and standard correlation grids (Table 3.20). In mass trapping grids berry densities were greatest at Bryant's Cove, followed by

Table 3.18: Mean (SEM) number of moths captured per trap per season for five trap designs baited with 85:10:5 lure at four wild lingonberry fields in 1999.

Site	Trap Design (Mean(SEM))				
	Pherocon® 1C Wing trap	Diamond® trap	Wing II® trap	Delta® trap	Unitrap®
Pouch Cove	4 (3.0)	3 (0.5)	8 (6.5)	1 (0.5)	1 (0.0)
Bryant's Cove	32 (2.5)	19 (6.0)	3 (1.0)	6 (0.5)	2 (0.0)
Freshwater	56 (11.5)	25 (10.0)	33 (1.0)	17 (8.0)	2 (1.0)
Little Catalina	30 (7.0)	19 (10.0)	14 (2.5)	8 (5.5)	3 (2.5)
Overall Mean	30 (7.4)	16 (4.0)	14 (4.5)	8(2.9)	2 (0.6)
$\left(\frac{Mean}{Surface\ Area}\right) x100$	4.7	3	4.1	2.7	N/A

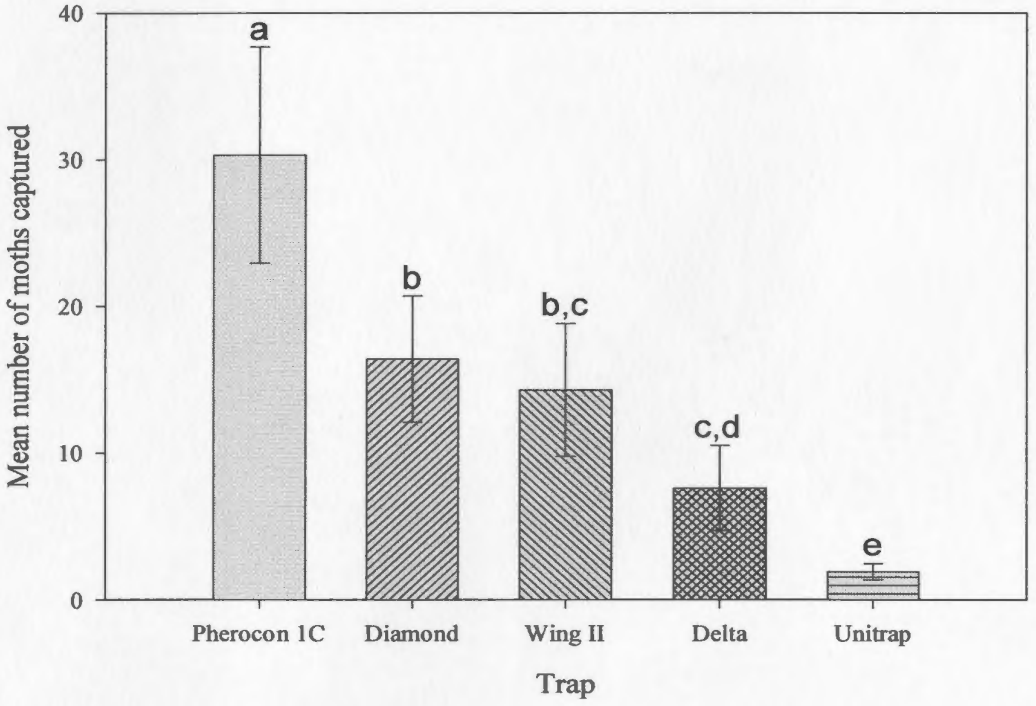

Figure 3.24: Mean trap catch of *G. libertina* for five trap designs baited with 85:10:5 lure at four sites in 1999 (All sites combined, means represented by the same letter did not differ ($p<0.05$) by Fisher's LSD).

Table 3.19: Ranking of five trap designs according to mean trap catch at each of four sites. 1 = most *G. libertina* captured, 5 = least *G. libertina* captured.

Site	Trap Design				
	Pherocon 1C Wing trap	Diamond trap	Wing II trap	Delta trap	Unitrap
Pouch Cove	2	3	1	5	4
Bryant's Cove	1	2	4	3	5
Freshwater	1	3	2	4	5
Little Catalina	1	2	3	4	5
Overall	1	2	3	4	5

Table 3.20: Total and mean berries, damaged berries, larvae and adults per site and per square metre in 2000 in standard and mass trapping grids. Berries, larvae and berry damage recorded from 48 1 m^2 quadrats at each site.

		Standard grid		Mass trapping grid	
Site	Variable	Total per site	Mean/trap (SEM)	Total per site	Mean/trap (SEM)
Pouch Cove	Berries	2399	281 (87.3)	1623	122 (59.6)
	Damaged berries	278	40 (12.2)	142	18 (8.0)
	Larvae	107	17 (5.1)	31	4 (1.4)
	Adults trapped	14	2 (0.4)	5	1 (0.3)
Freshwater	Berries	7723	936 (299.3)	6102	761 (72.3)
	Damaged berries	663	82 (25.1)	620	78 (8.0)
	Larvae	128	15 (3.5)	142	19 (3.2)
	Adults trapped	3	0.5 (0.3)	4	0.5 (0.34)
Bryant's Cove	Berries	8694	1127 (174.7)	7823	990 (241.8)
	Damaged berries	400	55 (12.4)	481	60 (20.3)
	Larvae	92	13 (3.3)	105	13 (3.7)
	Adults trapped	1	0.2 (0.16)	4	0.5 (0.34)
Little Catalina	Berries	4910	1109 (469.3)	4875	516 (62.7)
	Damaged berries	141	37 (15.8)	178	23 (5.2)
	Larvae	39	9 (4.1)	58	6.8 (1.4)
	Adults trapped	5	1 (0.6)	2	0.2 (0.2)

Freshwater, Little Catalina and Pouch Cove, respectively. Damage and larval population densities were greatest at Freshwater, followed by Bryant's Cove, Little Catalina and Pouch Cove. In the standard trapping grids, Freshwater had the highest larval densities, followed by Pouch Cove, Bryant's Cove and Little Catalina. Damaged berry densities in standard grids were greatest in Freshwater, followed by Bryant's Cove, Pouch Cove and Little Catalina.

Vegetation analysis in mass trapping grids via Principal Component Analysis was discussed in section 3.4. A generalized MANOVA with sites as explanatory factors indicated no significant relationship between dominant vegetation (*V. angustifolium*, *V. vitis-idaea* and lichen spp.) and larval or damaged berry densities in mass trapping grids. When mass trap data were analysed independent of dominant vegetation by Multi-way ANOVA, a significant relationship was found between berry density and lingonberry coverage with larval and damage densities (Table 3.8). Adults were not significantly related to numbers of larvae or damage within the mass trapping grids ($p<0.05$). Three Way ANOVA indicated that mean numbers of berries, damaged berries, adults and larvae were similar between standard and mass trapping grids at each site ($p < 0.05$). Differences between sites were significant, however there was no significant interaction between sites, trapping grids or lingonberry variables, indicating relatively similar numbers of berries, damage, adults and larvae between grids at the same site ($p < 0.05$).

3.6 Rearing and chemical analysis:

3.6.1 Rearing:

Rearing of larvae from 1996 to 2000 was unsuccessful. No surviving adults were obtained from the 1995-1996 trial. Only one adult moth, and ten *Phanerotoma* spp. (Hymenoptera: Braconidae) parasitoids emerged from rearing during 1998-1999. Rearing during 1999-2000 produced four adult *G. libertina*, and 13 *Phanerotoma* spp. Inspection of containers from 1999-2000 revealed that few larvae had pupated, and some corpses were infected with an unidentified fungus (unknown if infection was pre- or post-mortem).

In 2000-2001, survivorship ranged from 5.1% in the Pouch Cove container to 17.1 % in Bryant's Cove 2 (Table 3.21). Highest survivorship was in containers which had large amounts of paper towelling (Bryant's Cove and Freshwater). Parasitism by *Phanerotoma* spp., as determined by emerged parasitoids, ranged from 1.1% in Bryant's Cove 1 to 15.2% in Bryant's Cove 2. The rate of parasitism determined by emerged adult *Phanerotoma* spp. was higher than that determined by pupal collections (1.4%) during fall 2000 (Table 3.6). Parasitism was also noted by two (same species) unidentified chalcid wasps.

Total survivorship across all rearing containers was 9.5%, with 5.3% mortality being attributed to emerged parasitoids, and 85.2% mortality to unknown causes. Emergence of mass reared moths showed a 1 male:1.9 female sex ratio (Table 25). Fecundity was determined by ovarian egg count and was 61 ± 9.1 eggs/female (N= 10) in 3-5 day old virgins.

Table 3.21: Survivorship, parasitism (by *Phanerotoma* spp.) and mortality of reared *G. libertina* during 2000-2001.

Rearing Chamber	Survivors			♂:♀ Sex ratio	Total at start	% Survival			Mortality		
	Males	Females	Total			% Total	% Males	% Females	Parasites	% Parasitism (by emergence)	% Loss (excluding parasitism)
Freshwater	13	17	30	0.76	270	11.0 %	43 %	57%	11	4.1 %	84.9 %
Bryant's Cove 1	1	4	5	0.25	92	5.4 %	20%	80%	1	1.1 %	93.3 %
Bryant's Cove 2	3	16	19	0.16	105	17.1 %	16%	84%	16	15.2 %	67.7 %
Little Catalina	3	3	6	1	97	6.2 %	50%	50%	2	2.1 %	91.7 %
Pouch Cove	3	4	7	0.75	138	5.1 %	43%	57%	7	5.1 %	89.8 %
Total	23	44	67	0.52	702	9.5 %	34%	66%	37	5.3 %	85.2 %

3.6.2 Chemical analysis:

Calling behaviour of female moths was observed from 8 pm until 10 pm during 2 separate days when behavioural observations were made. Analysis of female *G. libertina* effluvia and pheromone gland extracts did not reveal any evidence of a compound resembling suspected pheromone components when compared with retention times for standards of synthetic pheromone (Figure 3.25). No other detectable peaks were found which could be related to any other potentially attractive compounds.

3.7 Seasonal history, degree day accumulations and weather analysis:

3.7.1 Seasonal history:

The 1996 flight season of *G. libertina* at all sites began on 2 July and continued until 5 August for Pouch Cove and Freshwater, and 19 August in Little Catalina (Figure 3.26-A). The population peaked in mid-July, best shown in Little Catalina, and declined afterwards.

The 1997 flight season began 4 July and ended by 18 August at Bryant's Cove and Chance Cove, but persisted until 25 August in Little Catalina (Figure 3.26-B). Bryant's Cove catch numbers peaked on 11 July at a lower density relative to the other sites, and declined thereafter. Little Catalina peaked in mid-July, but dropped sharply along with trap captures at the other two sites on 18 July. Both Little Catalina and Chance Cove trap capture recovered and peaked again in the last week of July.

During 1998, adults were recorded from 24 June to 14 July at Freshwater

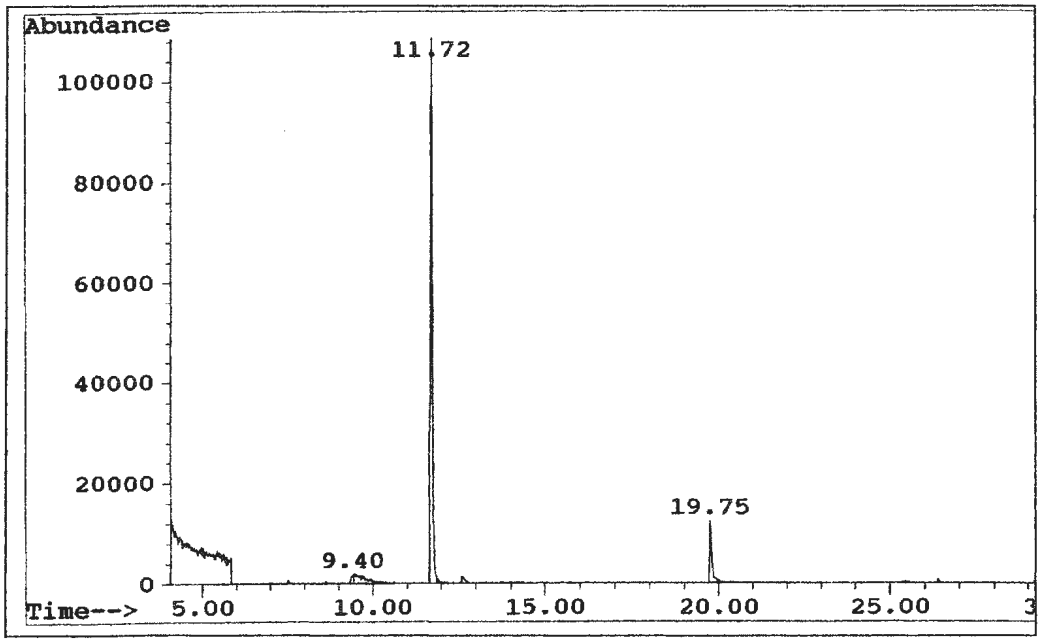

Figure 3.25: Gas chromatograph showing peaks for retention time of a 85:10:5 blend of E-8-dodecen-1-ol acetate, Z-8-dodecen-1-ol acetate and Z-8-dodecen-1-ol, using a HP 5890 Series II gas chromatograph, with a 30 metre DB-5 (Durabond) column. Z-8-dodecen-1-ol peaked at 9.40 minute retention time, Z-8-dodecen-1-ol acetate and E-8-doecen-1-ol co-eluted at 11.72 retention time.

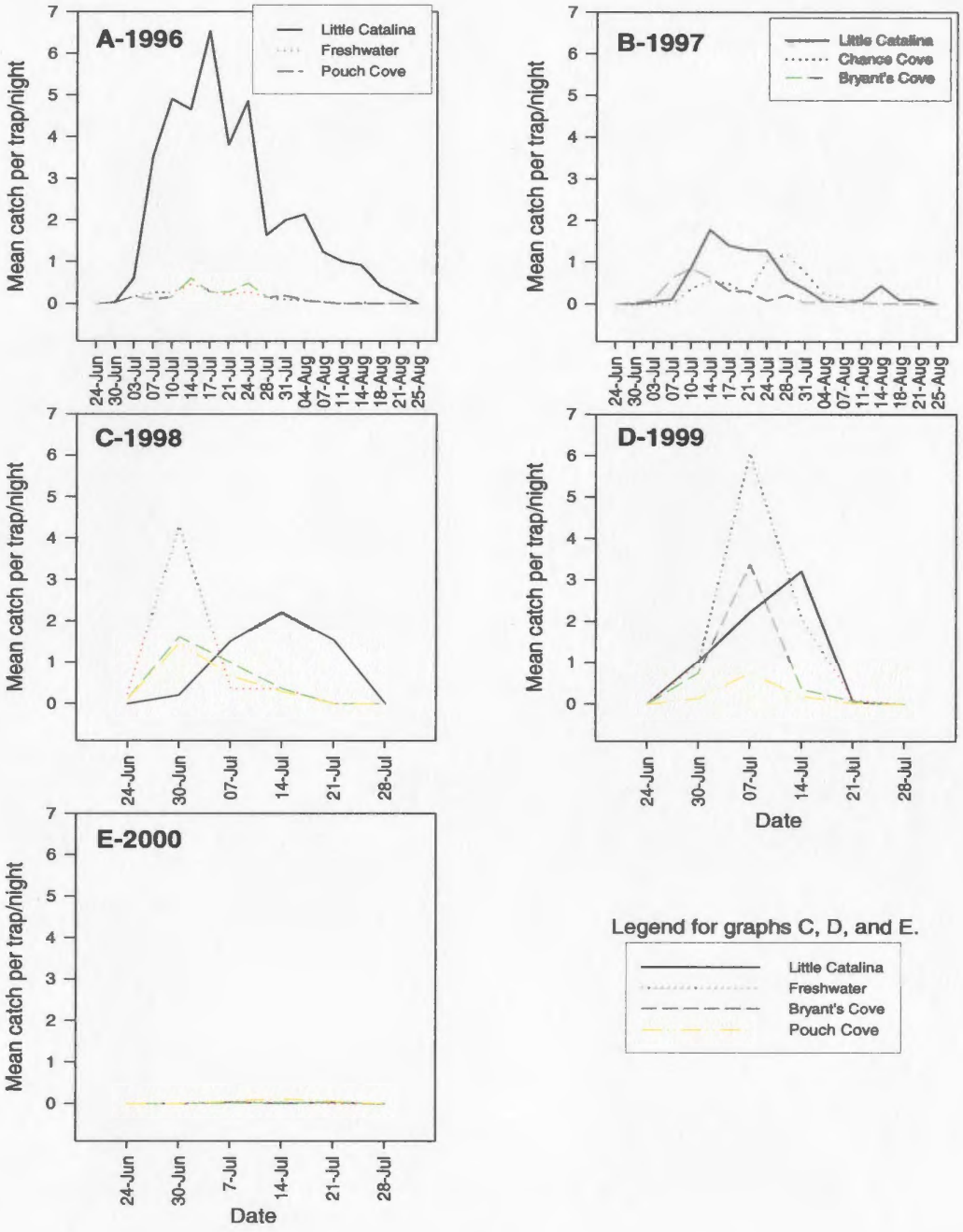

Figure 3.26: Mean catch per trap/night of *G. libertina* at various sites during: A-1996, B-1997, C-1998, 1999-D, 2000-E.

Little Catalina (Figure 3.26-C). Numbers trapped peaked in early to mid July and declined rapidly afterwards.

During the 1999 season, adults were recorded from 2 July to 21 July at Little Catalina, and from 1 July to 20 July at Freshwater, Bryant's Cove, and Pouch Cove (Figure 3.26-D). As in 1998, numbers captured peaked in early to mid July, and declined afterwards.

During the 2000 season numbers again peaked in mid-July, and adults were recorded from 1 July to 26 July at Little Catalina, Pouch Cove and Bryant's Cove, and from10 July to 17 July at Freshwater (Figure 3.26-E).

Peak trapping in 1996 and 1998 reached 6 moths per trap/night in July, while 2000 trap catches were low relative to other years.

3.8.2 Degree day accumulations:

Degree days accumulated for 10% *G. libertina* capture ranged from a low of 310±22.2 in Little Catalina, to 354±26.3 in Bryant's Cove. Across all sites 10% of captures occurred at 334±8.1 degree days, and 75% at 467±12.7 degree days (Table 3.22).

Three-way ANOVA comparing sites/bins, percent emergence and type (field trapped or laboratory reared) found significant differences within sites/bins ($F=4.9$, $p<0.05$), percent emergence ($F=8.4$, $p<0.05$) and field vs. laboratory rearing ($F=31.3$, $p<0.05$)(Table 3.23). A significant interaction was also noted between Type (emergence indicated by either reared larvae or trapped adults) and Site ($F=5.7$, $p<0.05$). A significant difference in emergence in the field was

Table 3.22: Mean (SEM) degree-day calculations for 10%, 25%, 50% and 75% capture in the field across all study sites for each year from 1996-2000. * 2000B denotes mass trapping grids.

Percent Emergence	Year (Mean (±SEM))						
	1996	1997	1998	1999	2000	2000B*	**Overall Mean**
10%	253.3 (8.8)	310.0 (40.0)	310.0 (23.3)	362.8 (6.1)	354.5 (29.6)	339.8 (12.2)	**321.7 (16.3)**
25%	300.7 (7.9)	357.5 (47.5)	334.3 (19.4)	382.5 (5.2)	422.8 (35.4)	377 (14.8)	**362.5 (17.2)**
50%	358.7 (10.7)	412.5 (62.5)	362.5 (20.7)	415.0 (10.2)	465.0 (32.5)	447.5 (23.3)	**410.2 (173.7)**
75%	444.0 (14.2)	465.0 (45.0)	388.2 (15.4)	463.5 (16.0)	501.3 (28.3)	493.8 (22.2)	**459.3 (16.6)**

Table 3.23: Comparison of mean (SEM) degree-day accumulations between laboratory reared and field-trapped *G. libertina* for 10%, 25%, 50% and 75% emergence of population. *Number of field trapped moths are cumulative means across all years, number of laboratory reared moths are cumulative counts.

Type	Site	Percent Emergence			
		10%	# Moths*	50%	# Moths
Field trapped	Freshwater	325 (20.3)	0.4 (0.13)	393 (13.6)	2.7 (0.70)
	Bryant's Cove	354 (26.3)	0.3 (0.02)	428 (27.9)	1.8 (0.21)
	Pouch Cove	311 (23.3)	0.6 (0.19)	412 (42.3)	1.3 (0.43)
	Chance Cove	350 (0.0)	0.4 (0.00)	475 (0.0)	5.8 (0.00)
	Little Catalina	310 (22.2)	1.4 (0.70)	404 (17.6)	8.2 (2.40)
	All sites(Mean)	334 (8.1)	0.6 (0.35)	422 (14.3)	3.5 (0.91)
Laboratory reared	Freshwater	136 (4.6)	5	208 (39.2)	11
	Bryant's Cove 1	356 (4.0)	2	364 (8.0)	4
	Bryant's Cove 2	196 (4.0)	2	248 (16.7)	10
	Little Catalina	352 (9.2)	1	388 (7.7)	5
	Pouch Cove	308 (12.0)	1	344 (10.3)	4
	All sites(Mean)	270 (20.5)	2	310 (16.8)	6
Laboratory (All sites combined)	Males	262 (44.9)	2	291 (38.3)	12
	Females	278 (46.0)	4	323 (31.8)	18
	Phanerotoma spp.	278 (43.7)	4	310 (41.0)	10

found only between Freshwater and Chance Cove trap data, with capture at 50% degree day accumulations. Ten percent of laboratory reared *G. libertina* from Freshwater and Bryant's Cove '2' (Table 3.23) emerged at lower degree day accumulations (136 and 196, respectively) than field records for those sites. Laboratory reared and field trapped insects from Bryant's Cove '1', Little Catalina and Pouch Cove were similar in degree day requirements for 10% emergence. The period of emergence in all mass reared containers, based on degree-day accumulations, was much shorter than in the field. The 25%, 50% and 75% emergence levels were reached rapidly at all sites (Table 3.21).

Laboratory-reared males generally emerged earlier than females, however this was not significant ($F=0.05$, $p=0.98$). Cumulative degree-days for parasitoid emergence were not significantly different from male or female moth emergence ($p>0.05$).

3.8.3 Weather factors:

Figures 3.27 and 3.28 show mean daily maximum, minimum, and mean temperatures and average total daily precipitation during adult flight seasons at each site and during each year. Mean maximum daily temperature in 1997 and 1999 was significantly lower than in 1996 (Figure 3.27). Mean daily temperatures in 1997 were also significantly lower than in 1996. Freshwater had significantly higher mean maximum daily temperatures than Little Catalina and Chance Cove sites, while Little Catalina had lower precipitation than Freshwater or Pouch Cove sites (Figure 3.28). Pearson correlations of weather factors

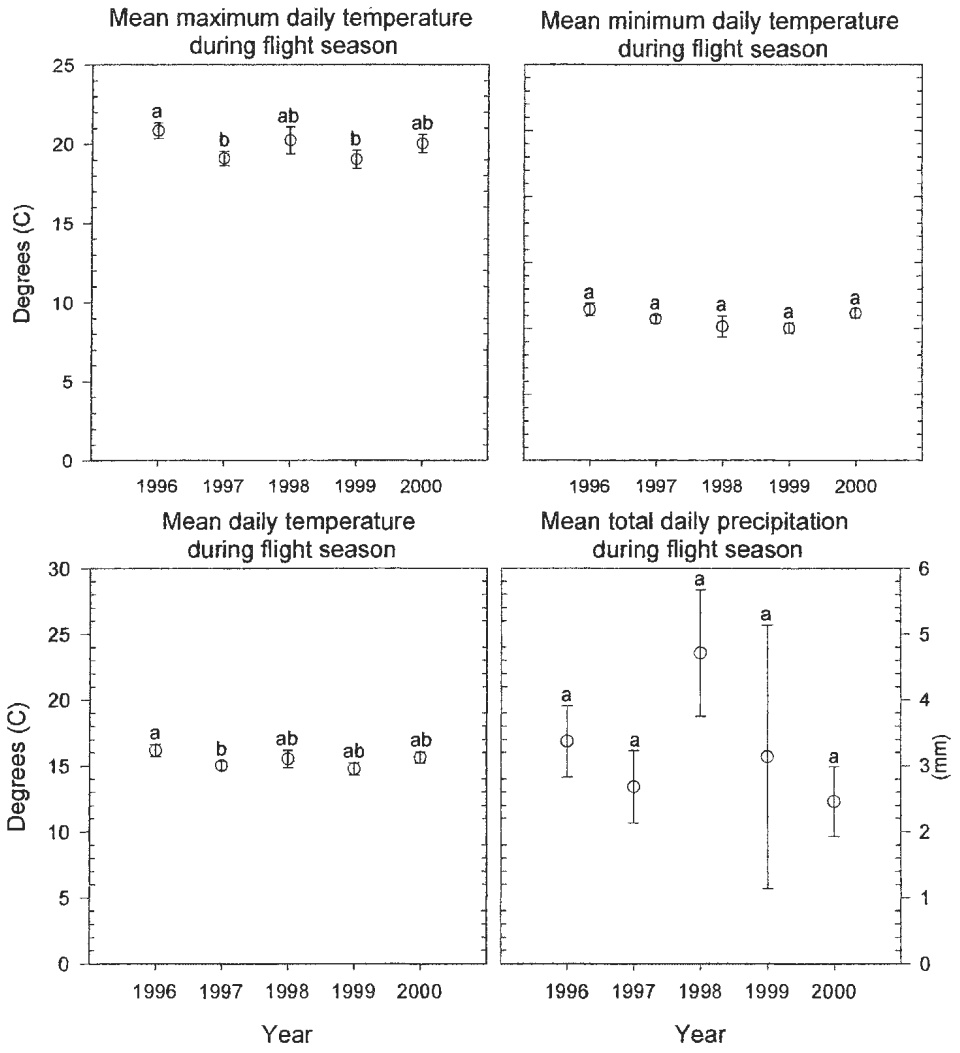

Figure 3.27: Weather variables recorded during adult flight seasons, 1996-2000, across all study sites: A-Mean maximum daily temperature, B-Mean minimum daily temperature, C-Mean daily temperature, and D-Daily total precipitation. (Means represented by the same letter did not differ ($p<0.05$) by Fisher's LSD).

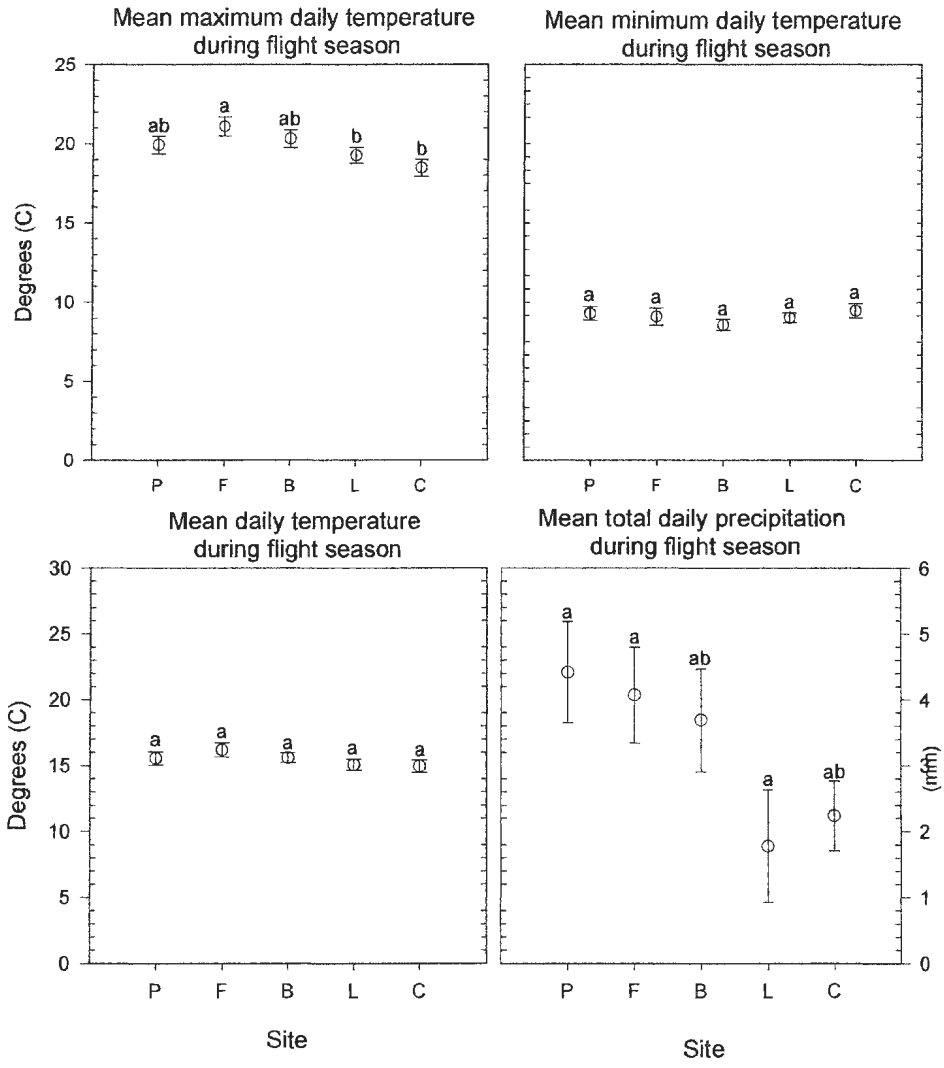

Figure 3.28: Weather variables recorded during adult flight seasons, corresponding to each study site, across all years (1996-2000): A-Mean maximum daily temperature, B-Mean minimum daily temperature, C-Mean daily temperature, and D-Daily total precipitation. Sites: P = Pouch Cove, F = Freshwater, B = Bryant's Cove, L = Little Catalina, C = Chance Cove. (Means represented by the same letter did not differ ($p<0.05$) by Fisher's LSD).

(maximum, minimum, mean temperature, total daily precipitation and wind speed) did not show any significant relationship to adult trapping (Table 3.24).

Figure 3.29 shows mean minimum daily temperatures during June 1999, at each weather station. Temperatures between June 5-10 decreased to, or below 0°C. This corresponded to mid-bloom (approximately 50% of plants had bloomed) for lingonberry plants at the study sites during 1999 (personal observation).

Table 3.24: Correlations between mean weekly weather and daily adult trap catch for all localities pooled, during 1996-2000. *Windspeed data were only available for Pouch Cove.

	Pearson Correlation with adults per trap/night						Overall (All sites)		
Variable (Daily readings averaged biweekly or weekly)	Pouch Cove	Freshwater	Bryant's Cove	Little Catalina	Chance Cove	Overall (All sites)	($p<0.05$)	Mean	Standard deviation
Maximum daily temperature (C°)	-0.05	-0.126	-0.174	0.018	-0.124	-0.041	0.605	19.9	3.32
Minimum daily temperature (C°)	-0.104	-0.032	0.019	0.009	0.033	-0.056	0.477	10.9	2.8
Mean daily temperature (C°)	-0.08	-0.118	-0.137	0.014	-0.06	-0.037	0.639	15.5	2.75
Total daily precipitation (mm)	0.025	0.041	0.061	0.085	0.045	-0.025	0.775	3.2	4.82
Average daily windspeed (kph)*	-0.2	-	-	-	-	-0.2	0.256	20	5.98
Adults per trap/night	-	-	-	-	-	-	-	1.15	1

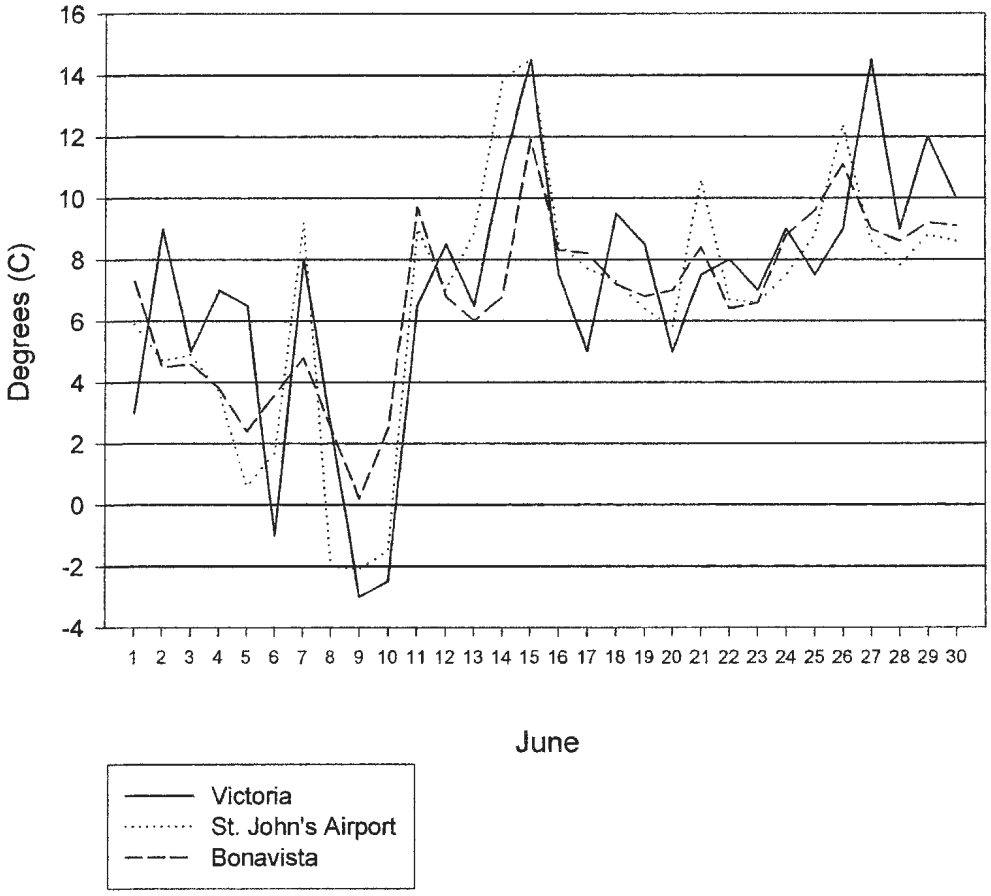

Figure 3.29: Mean daily minimum temperatures during June 1999 at three weather stations: St. John's Airport, Victoria and Bonavista.

4.0 DISCUSSION

The study of target insects in the laboratory and field, the effects of weather and landscape, distribution and condition of hosts, along with refinement of chemical blends and investigation of trapping efficacy, are all required for the development of a precise predictive trapping model, and may take many years to produce. Such studies must address variation associated with both population dynamics (population estimation) and behavioural research (pheromone trapping).

4.1 Field evaluation of attractant compounds:

Closely related insect taxa often share similarity in pheromone structure, and this is particularly notable in the Lepidoptera (Ando *et al.*, 1977). Within the genus *Grapholita*, it has been shown that attractants for different species may vary by a single carbon, or by cis-trans (E/Z) isomerism (Mayer & McLaughlin, 1991; Arn *et al.*, 1992, Arn, 1999). This similarity in structure has provided a basis for inquiry into the structure of the female-produced *Grapholita libertina* sex pheromone.

The differences in attractiveness of compounds between years (E8-12:OAc in 1996, Z8-12:OAcin 1997), cannot be readily explained. Similar effects have been shown in the spruce seed moth, *Cydia strobilella* L. (Lepidoptera: Tortricidae), in which the pheromone blends most attractive to moths differed from year to year (Grant *et al.*, 1989). It is possible that by using the 1mg/ml concentration for the lures in the 1997 season, moth attraction to the Z8-12:OAc was enhanced, while the performance of the other

compounds was diminished. In *G. molesta*, it has been found that abnormally high concentrations of pheromone (1 mg and higher) decrease the efficiency of trapping due to moths terminating their upwind flight prior to reaching the source (Baker *et al.*, 1981). In other words, unusually high concentrations make the insect behave as though it is closer to the source than it actually is, inhibiting upwind flight behavior. This may explain the decreased efficiency of the E8-12:OAc, particularly if the optimal concentration was closer to 0.1 mg than 1 mg. Z8-12:OAc was as attractive as the E8-12:OAc at 1 mg/ml in 1996, but not significantly more attractive than the E8-12:OAc as found in 1997. Assuming decreased attractiveness, due to concentration, fails to explain the low catch rate of Z8-12:OH in 1997, since 1 mg/ml was the most attractive concentration in 1996.

The total trap catches for all compounds diminished in 1997, possibly indicating a population decline, or climatic variation affecting catch rates. In Little Catalina it was possible that trapping in 1996 caused a reduction in the population, but this was unlikely as the study site was surrounded by large lingonberry-rich barrens from which *G. libertina* could re-invade. Variability in abundance noted between sites may also be due to differences in the abundance of lingonberries. Lingonberries were more abundant at Little Catalina than at the other sites and should have been able to support a larger population of *G. libertina*.

Concentration is important for attraction. Attraction to a pheromone source is dictated by a lower concentration below which there is no activation of flight, and by an upper concentration, above which insects will become disoriented (Roelofs, 1978; Baker *et al.*, 1981). Compound concentration was expected to have an important effect on the

trap catches, but there were no significant differences in catch rates between various concentrations.

Many studies have shown that a dosage-dependant relationship exists between concentrations of attractant substances and their trapping ability. Turgeon and McNeil (1983) found trapping of male *Pseudaletia unipuncta* Haworth (Lepidoptera: Noctuidae) increased with a corresponding increase in concentrations of Z-11-hexadecenyl acetate, up to a maximum of 1mg/ml (lures at a 3 mg/ml concentration were less attractive than 1 mg/ml). Polavarapu and Seabrook (1992) showed a dosage-dependant relationship between attractant concentration and trap capture in *Croesia curvulana* L. (Lepidoptera: Tortricidae). In male *G. molesta*, Roelofs and Cardé (1974) showed that synthetic pheromone blends were less attractive at concentrations of 200 ug/ml and greater. Stockel and Sureau (1980) found a logarithmic relationship between trapped males of the angoumis grain moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae), and pheromone concentration. However, differences in attraction were minimal between 0.1 and 10 mg/ml concentrations and there was no inhibition in trapping at high dosages, due to a theorized increase in the drawing range which may have attracted males from adjacent fields. The lack of a dosage-dependant relationship in *G. libertina* may have been due to an insufficient range (or replication) of concentrations tested. Also, population levels may have been too low at some sites (such as Pouch Cove or Freshwater in 1996) to indicate concentration effects.

All three blends tested in 1997 were attractive to *G. libertina*. Differences between the attractiveness of the blends were not significant, and attractiveness was

slightly variable between sites.

In the genus *Grapholita*, naturally occurring sex pheromones appear to be blends of acetates and alcohols (Arn, 1999). Therefore, high trap captures were expected for all blends tested, relative to single compounds. The 85 % E-8-dodecen-1-ol acetate:10 % Z-8-dodecen-1-ol acetate: 5% Z-8-dodecen-1-ol blend ranked the highest among the blends, and was selected as an appropriate synthetic attractant for *G. libertina* based on the first two field seasons. The lack of significant differences between the blends suggests that the ideal ratio may not be 85:10:5.

Many closely related insect species are believed to have evolved reproductive isolation through distinct differences in pheromone blend production by females, and a corresponding difference in attraction by males. Löfestedt *et al.* (1990) showed that cross-attraction was prevented in closely related *Yponomeuta* moths through specialized male antennal sensillae which perceive similar blends and cause repulsion.

Several authors have shown, however, that cross-attraction occurs between related species and between allopatrically distinct populations, which produce different pheromone blends from one another (Lewis & Cane, 1990; Mozüraitus *et al.*, 1998; Gemeno *et el.*, 2000). Gronning *et al.* (2000) have shown that 'generic' sex attractant blends, containing pheromone elements of four species within a pest complex, may be used as mating disruptants for leafroller pests on apple. Gemeno *et al.* (2000) found that females of allopatric Nearctic and Palearctic black cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae), produced distinct pheromone blends, however males showed no behavioural differentiation between local or isolated populations. Lewis and Cane

(1990) stated that the degree of cross-attraction found in *Ips* beetles was consistent with phylogenetic relatedness. Therefore, males of *G. libertina* may have been responsive to a range of blend ratios, resembling that which was produced by females of local populations.

The high capture rate of the Z-8-A relative to the blends in 1997 suggests that it may be more appropriately listed as a major component in a synthetic attractant for *G. libertina*. As well, adult capture in 1997 increased as the proportion of E-8-A decreased, and that of Z-8-A increased in the lures tested. The natural pheromone is possibly an attractant similar to the *G. molesta* pheromone / attractant, which is an optimized blend of 74% Z8-12:OAc, 4% E8-12:OAc, and 22% Z8-12:OH (Arn *et al.*, 1992).

The specific composition of the *G. libertina* sex pheromone is unknown, and further refinement and ratio testing might produce a more efficacious synthetic attractant. It should be noted that further refinements of these blends were to be carried out by gas chromatography-mass spectroscopy bioassay, however, rearing in 1995-1997 was unsuccessful, preventing such analyses.

For the purposes of this study, however, the 85:10:5 blend proved to be an effective field attractant, capturing 237 *G. libertina* and 25% of the total catch in 1997. It was the most attractive of the top four lures, making it the best choice of the synthetic lures tested.

All dissected moths from the 1996-1998 seasons were male, verifying that the 85:10:5 blend is clearly a male sex attractant for *G. libertina*, and does not demonstrate female or bisexual attraction (aggregation) when used in the field. This is important, as it

showed that the 85:10:5 blend indicated only male populations. In addition, knowing that the 85:10:5 blend was a male sex attractant may provide clues to composition of the naturally occurring female sex pheromone of *G. libertina*.

4.2 Correlation of larval and damage densities with adult trapping rate:

The general predictive value of pheromone trapping has been proven many times, however the ability to calibrate trap counts to specifically estimate later stages in an insect's life history can be difficult (Hall, 1998). Adult capture rates in pheromone traps provide useful information on relative population levels between different areas and years. For precise estimates of larval infestation, or the expected crop damage in a year, correlations must be made to calibrate adult trapping rate with larval and damage levels.

4.2.1 Correlation of larval and damaged berry densities:

There was a good correlation between numbers of larvae and numbers of damaged berries in all years. This result was expected as larvae were found within the infested berries. In addition, an individual larva appeared to feed within a single cluster (corm), rather than migrating between clusters of berries. For this reason, levels of damage may have been dictated by plant phenology and numbers of fruit at each site. Latheef *et al.* (1991), stated that variation in crop phenology due to variable planting dates and cultivars contributes to variation in larval correlations. Fruit set (and subsequent corm size) was variable between sites and three to eight fruit were set per corm (Ryan, 1978; personal observation). It may be the case, therefore, that damage

levels by larvae may have been determined by the number of berries in a corm, and larvae feeding within a single cluster during development (no migration between clusters). Damage dictated by corm size was supported by the damage : larvae ratios which ranged between 1.5-7.8. This agrees with Willson and Trammel (1975) who found that the abundance of three different tortricid species was related to the foliar density (and ratio of insects to fruit) in apple orchards. Assuming the degree of larval damage is limited by corm size, this may explain the decrease in damage : larvae ratios at each site in 1999. Other factors due to variable vegetation (plant architecture, kairomonal cues) which may have affected adult oviposition activity, and directly influenced larval distributions are discussed in the following section.

4.2.2 Correlation of larval density with adult male trap capture:

In 1998 and 2000, the mean adult capture/trap correlated well with larval and damaged berry density within grids at each site. Adult capture by attractant traps was not a good predictor of larval infestation or damage in 1999. The lack of correlation between larval populations and adult capture rate in 1999 may have been due to a number of factors.

Indirect weather effects:

Weather may directly or indirectly have an influence on insect activity and distribution. A hard frost from June 8-10, 1999, caused tremendous bloom loss, and resulted in a much lower berry set in 1999. Since *G. libertina* rely on fruit for oviposition

and development, this loss of berries created a much more patchy environment within already heterogenous sites. Sweeney *et al.* (1990) showed that correlations between adults and larvae of *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae) are dependant on host plant density.

The total number of larvae at each site increased between 1998 and 1999, and remained relatively constant between 1999 and 2000, but the percent infestation was largely affected by berry abundance. This was noted in Pouch Cove in 2000, where larval and damage levels remained high with a low berry abundance. Percent larval infestation and damage at other sites (Little Catalina, Bryant's Cove and Freshwater) decreased from 1999 to 2000 for although berry abundance increased, relatively constant numbers of larvae occurred in each site each year.

Availability of suitable oviposition sites (lingonberries), will affect the distribution of subsequent larvae. It is possible that smaller and more separated host (berry) distributions during 1999 affected female ovipositional behaviour, such as attraction to distinct host patches or using alternative hosts, like low sweet blueberry (*V. angustifolium*). The occurrence of higher percent larval infestation and damaged berries in 1999 demonstrated that larval concentrations were much higher than the sampled berries from 1998, perhaps indicating selection for specific host patches due to food resources being reduced relative to ovipositing moth populations. Whereas random sampling was expected to eliminate any such bias, intrasite variability in the number of larvae was notably higher in 1999, than 1998, as noted by the standard error for mean larvae sampled. This may be an indication of microhabitat differences in frost

susceptibility by berries, however, since the standard errors of berry counts were relatively low in 1999, it is likely that berry loss was moderately uniform within sites.

Vegetation analysis/Foliar effects:

Adult-larval correlation studies often are conducted in agricultural settings, with homogeneous host plant distributions (Latheef *et al.*, 1993). Availability of host plants, and kairomonal (repellent or attractant) properties of other plant species and alternative host plants may influence insect behaviour and distribution in the field (Metcalf & Metcalf, 1992).

Differences in vegetation composition between years were expected to be minimal, as the vegetation types sampled were largely perennial shrubs. However, sites in 1998 clustered separately from other years on the positive end of principal component one. The discrepancy between 1998 and other years may have been due to the sampling technique (random grid tossing) being insufficient to accurately show vegetation presence, and a greater sample size might have been required. An increased consistency (more experience sampling) in estimating vegetation coverage in 1999 and 2000 may have also contributed to variability from 1998 and other years. This is further evidenced by plots being significantly more variable in 1998 with a coefficient of variation of 81% compared with 96% and 97% for 1999 and 2000, respectively.

Removal of 1998 data resulted in a similar clustering pattern for sites between 1999, 2000 and 2000 mass trapping grids, which showed that each site had distinct vegetation types and densities. Adult, larval, damaged berry and berry densities were all

variable between sites and years, but no trends were noted in the principal component analysis of vegetation composition.

Since values for infestation (adults, larvae, damage) were somewhat uniform within sites along principal component two, little can be said about the influence of specific vegetation types on the insect's distribution within plots at each site. Any differences in infestation may have been therefore be due to variable insect population levels between sites, rather than variable population levels between plots (and variable vegetation compositions) within sites.

MANOVA analysis indicated no relationship between vegetation and the distribution of larvae or damaged berries. Infestation was dependant on lingonberry plant and berry density, and was influenced by both site and year of study. *Vaccinium angustifolium*, an rare alternative host for *G. libertina*, may have had an effect on larvae ($F=3.6$, $p=0.06$) and damage ($F=2.8$, $p=0.10$), however it was not significant at $p<0.05$. With few lingonberry fruit present in 1999, ovipositing females may have selected *V. angustifolium* fruit for oviposition. *Vaccinium angustifolium* fruit were abundant in plots during all years (personal observation), and experienced less berry loss due to frost in 1999. It is not clear if *V. angustifolium* had a direct effect on adult distributions (and subsequent distributions of larvae and damaged berries), or if this was a coincidental effect based on lingonberry distributions. No *V. angustifolium* fruit were sampled, and infestation levels of this alternative host in plots were unknown. However, this is an uncommon host for *G. libertina*, and infestations were expected to be minimal (Churchill, pers. com., 2001). The position of larval and damaged berry densities in principal

component analysis of vegetation did not support a trend based on *V. angustifolium*.

Kairomonal host plant cues may also affect adult insect distribution. Darnell *et al.* (2000) suggested that the semiochemical cues produced by pollinating early or late corn attracted adult *Diabrotica virgifera* LeConte *virgifera*, affecting insect distributions within fields. Insects such as *Mamestra brassicae* L. (Lepidoptera: Noctuidae) use host plant kairomones to orient, being most attracted to damaged plants (Rojas, 1999). Volatiles in corn which attract *D. virgifera virgifera* have also been shown to repel western corn rootworm, *D. barberi* Smith and Lawrence (Coleoptera: Chrysomelidae)(Hammack *et al.*, 1999). The heterogenous vegetation present in this study may have therefore acted as potential attractants or repellants which would have influenced *G. libertina* adult distribution. Although generalized odors would unlikely affect pheromone perception in males (pheromone perception is narrowly tuned), alternative host plant (i.e. *V. angustifolium*) odors may have affected female ovipositional behaviour. Complex neural coding of food resource olfactory stimuli has been found in some insects, such as honey bees and cockroaches, however, assuming that *G. libertina* is a specialist herbivore, female receptors will likely be specialized to perceive host plant (*Vaccinium*) odors as oviposition sites, minimizing "noise" from coincident vegetation (Lemon & Getz, 1999).

Other studies have shown that the density of foliage affects adult distributions, oviposition activity and larval phenology (Carter *et al.*, 1992; Willson & Trammel, 1975; Summy *et al.*, 1986). As mentioned, Sweeney *et al.* (1990) found that correlations between trapped adults and larval counts of the western spruce budworm, *Choristoneura*

occidentalis, were not significant unless standardized by basal area or foliar biomass sampled per hectare, therefore producing problems in estimation of *C. occidentalis* due to variation between stands. Rowe and Potter (2000) showed that shading reduced infestation of rose plants, *Rosa floribunda*, by Japanese beetles, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), and suggested that shaded plants were less apparent to or accessible by beetles. Foliar density has also been studied relative to parasitoid and predator populations. Plant architectural characteristics of coleus, *Solenostemon scutellarioides* L. Codd. (height, leaf number, leaf surface area and branch number) have all been negatively correlated with attack rate by *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae), a parasitoid of the citrus mealybug, *Planococcus citri* Risso (Homoptera: Pseudococcidae)(Cloyd & Sadof, 2000). In the seed bug, *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae), light reflectance of preferred cones of Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, was a selective character in locating hosts, with preferred clones reflecting more light, at broader wavelengths (Blatt & Borden, 1999).

Host plant phenology is also important in determining insect distributions in agricultural systems. Densities of adult western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), were suppressed in late-planted corn, *Zea mays* L., relative to adjacent early planted corn (Darnell *et al.*, 2000). Willson and Trammel (1975), however, found in a study of 3 orchard-inhabiting tortricids, that there was a positive relationship between foliar density and pheromone trap catch. Therefore, foliar densities and heterogenous plant distributions may affect adult insect distributions, and

subsequent larval infestation.

Direct weather effects:

Weather conditions between sites and years may have also acted directly on insect activity. Variable weather conditions may reduce moth activity by both limiting mating and trap capture in males, and flight (reducing mating, ovipostion) in females.

Weather variables were significantly different between sites and years. Pearson correlations (Sokal & Rohlf, 1995) between weather variables and adult trap capture showed no relationship. Insects require a minimal temperature to engage in flight, while higher wind speed can limit pheromone trap capture by preventing upwind flight (especially if the insects are not strong fliers), and by rapidly dissipating attractant plumes necessary for trap location (Howse, 1998a). The release of pheromone from lures and its revaporation from foliage will also be dependant on temperature (Isaacs *et al.*, 1999). Variability between sites may have been due to factors such as precipitation or light cycles, which may have influenced emergence and activity, which in turn, influenced trapping patterns (Schouest & Miller, 1994). Increased wind speeds and rainfall directly affect insect flight, decreasing their ability to reach attractant sources (Sappington & Spurgeon, 2000).

Precipitation, temperature and wind variables are all factors which affect insect activity and pheromone trapping. In the case of the spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae), male capture in pheromone traps was positively correlated with temperature (up to 25°C) and negatively correlated with

humidity (Sanders, 1981). Schoest and Miller (1994) demonstrated that rainfall, temperatures below 20°C and wind speeds of 2.5 m/s or greater all suppressed male pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae), capture in pheromone traps. Pitcairn *et al.* (1990) showed that pheromone trap capture of male *Cydia pomonella* L. (Lepidoptera: Tortricidae) was dependent on a minimum 12°C air temperature, and low wind speed, vapour pressure and rainfall. Rainfall may act to limit insect activity, however flights of some species, such as *Leucania commoides* Guenée (Lepidoptera: Noctuidae), were activated following heavy rainfall (Ayre *et al.*, 1983). Baker *et al.* (1980) stated that maximum daily temperature must exceed 15°C for *G. molesta* flight to occur. The specific weather conditions required for *G. libertina* flight are not known, however temperatures during scotophase at each site likely remained below 15°C at all study sites (personal observation).

Discrete changes and daily weather events were lost in this study due to weather variables being averaged during the term between trap counts. More frequent sampling to prevent averaging over time and weather conditions would have reduced this effect.

Much of the variance between sites may be attributed to the weather data not precisely describing the conditions to which the pupae and adults were exposed. Air temperature is not always a good predictor of the soil temperature, where the prepupae overwinter (Elliott *et al.*, 1990; Gupta *et al.*, 1984). As well, the distance from the weather stations, and microclimatic differences within the rolling landscape of the study sites would have reduced the ability of a weather station to predict individual site conditions. Unfortunately, since study sites were wild areas, weather stations were not

able to be placed on site due to the risk of vandalism.

Predation/parasitism:

Larval populations relative to adult capture rates were much lower in 1998 and 1999 than in 2000. Larval mortality may have occurred, affecting any relationship between the number of trapped adults and later larval counts. The rates of predation and predators present which might prey upon eggs and early instar larvae are unknown. One hymenopteran, *Phanerotoma* spp. Wesmael (Hymenoptera: Braconidae), is a known larval parasitoid of *G. libertina* (Morris *et al.*, 1988). Ten parasitoid pupae were found during larval collections in 1998, 24 in 1999 and 5 in 2000. The direct effects of this parasitoid on larval counts were minimal since they killed the host larvae inside the berries. Thus, the dead larvae and parasitoid puparium were intact for larval counts, and counted as larvae. In 2000, however, it was found that parasitism determined by number of emerging parasitoids was three times higher than that detected by larval collection in the fall. Many parasitoids were not detected when larvae were extracted from berries and were found later emerging from larvae selected for laboratory rearing, thus direct counts of individual *Phanerotoma* may be higher than those recorded from larval collections. This indicated that many parasitized larvae may have not have been detected by emergence counts in 1998 and 1999, and that parasitism may have been much higher than reported during those years.

Phanerotoma spp. parasitoids present a potential method of augmentative biological control, through their rearing and release. Whereas this method could facilitate

a chemical-free method of population control of *G. libertina*, and avoid the risks associated with importation of biological control agents, it should be noted that *Phanerotoma* spp. will not reduce berry damage within a single year. Since berry damage will not be prevented within a single year, control with *Phanerotoma* spp. may not be desirable from a commercial perspective. This species could be useful, however, to reduce large populations over several years, and conservation should be considered if chemical control measures are used on *G. libertina*.

Female competition:

Female *G. libertina* densities in the field may also directly affect the male trapping rate. While trap counts increased with population levels, a critical level may be reached at which calling females strongly competed with attractant traps, as found in studies of the codling moth, *C. pomonella* (Howell, 1974; Reidl *et al.*, 1976). Witz *et al.* (1992), have shown that efficiency of pheromone traps for male *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae) decreased with increasing female moth densities. If such competition (between females and traps attracting males) occurred in *G. libertina*, it would have suppressed a linear increase in trap capture which would be expected to correspond with an increase in larval population density in the same year.

Drawing range:

Shepherd *et al.* (1985) have shown that dispersal, flight ability and drawing range for traps caused variability in pheromone trap capture of male Douglas-fir tussock moth,

Orgyia pseudotsugata McDunnough (Lepidoptera: Lymantriidae). Variability in drawing range was also discussed by Stockel and Sureau (1980), wherein distant populations of *S. cerealella* may have influenced captures in traps with high concentration lures and an increased drawing range. In such cases, the area of larval sampling may have been unrelated to male trap counts, if mating or oviposition occurred at a long distance from the trap. Walker and Welter (1999) found, however, that a reduction of lure dosage from 1 mg ranging to 0.001 mg in *A. citrana* did not improve correlations between larval densities and male moth counts in apple orchards, and suggested a limited change in the drawing range due to changes in lure dosage. While the dispersal ability of *G. libertina* was not known, good correlations between trapped adults and larval populations collected within blocks in 1998 appeared to support a relationship.

4.3 Trap design trials:

The Pherocon® 1C wing trap captured significantly more moths than the other traps ($p<0.05$). The Unitrap® design trapped few *G. libertina* relative to the other trap types. Non saturating traps provide benefits over sticky traps in terms of higher capture capacities (and thus less servicing), better specimen condition, and recycling ability (Knodel & Agnello, 1990; Sanders, 1986a). Sanders (1986b), however, has shown that in the case of the spruce budworm, *C. fumiferana*, half of the moths entering non-saturating funnel traps escaped, and that the presence of insecticides acted as repellents to moths. Sticky traps do not require insecticides, and therefore do not have the repellent qualities which might reduce the potential number of insects trapped.

Differences in the trapping ability of the sticky traps may have been due to several factors. Brown (1984) showed that in trapping trials with the apple budmoth, *Platynota idaeusalis* Walker (Lepidoptera: Tortricidae), trap saturation with dead moths was directly related to sticky surface area. Consequently, trap efficiency at different moth densities may have been significantly affected by capture surface area. The accumulation of dead insects not only causes trap saturation by reduction of sticky surface area, it may also act as a semiochemical repellent to incoming target pests (Sanders, 1986b). Sanders (1986a, 1986b) has suggested that male moths may produce alarm pheromones which repel other males, however no evidence has been provided to support this. In this study, the overall ranking of the sticky traps based on their trapping efficiency of *G. libertina* followed a scale of increasing surface area. Data also suggested that the wing-style traps were slightly more attractive once standardized by surface area.

Trapping efficiency is also affected by the pheromone plume. Trap design and interaction with variable wind conditions at each site will have significantly influenced the shape and dispersal distance of pheromone plumes in the field. Whereas no records were made of plume shape or dispersal effects on *G. libertina*, Lewis and Macaulay (1976) found that trap design significantly affect plume structure and subsequent catch of *Cydia nigricana* Steph. (Lepidoptera: Tortricidae) male moths. Other factors such as trap age, lure concentration, intertrap distance and trap height have also been shown to significantly affect efficiency of different trap designs (Housewart *et al.*, 1981). However, all of these factors were constant across the trap designs tested, so their effects should have been minimal. Since traps were randomized through the grid, and

surrounded by guard traps, any position or edge effects should have been negligible.

4.4 Mass trapping:

Comparisons between standard (control) and mass trapping grids indicated no significant differences between mean numbers of adults, larvae or damage at each site ($p<0.05$). In 2000, adult moths may have been too few to be accurately detected.

Mass trapping is an effective means of controlling many pest populations. Faccioli *et al.* (1993) suggested that a trap density of ten traps per hectare was sufficient to control *Cossus cossus* L. (Lepidoptera: Cossidae) in European orchards. Trimble and Hagley (1988) also found that mass trapping of male *Phyllonorycter blancardella* Fabricus (Lepidoptera: Gracillariidae) could reduce infestations by as much as 50% in selected cases. In the current study, adult trap rate was not correlated with larval densities in mass trapping grids. Since there were low adult captures in peripheral mass traps, and no reduction in larval infestation, the disruption of adult-larval correlations did not appear to be due to increased trapping effort. Total berry densities between standard and mass trapping grids were similar, removing any effect that reduced host berry densities might have had on adult-larval correlations in mass trapping grids.

Attractant release by high density lures in mass trapping grids may have caused saturation of the immediate aerial environment of the trapping grid with attractant, resulting in disruption of trapping by monitoring traps in the grid. In their study on *P. blancardella*, Trimble and Hagley (1988) suggested "pre-adult leafminer densities were lower in trapped plots because of mating disruption due to male confusion, and not

because males were trapped before they could mate". High attractant concentrations have been shown to disrupt location of source by *G. molesta* (Baker *et al.*, 1981), and the reduced trapping ability of *G. libertina* attractants at 1 and 10 mg/ml concentrations which occurred in 1996 may support a premise of decreased trapping at higher concentrations (due to disruption or confusion of male moths). Therefore, the high density of lures in this study may have disrupted the location of monitoring traps by male moths.

Studies by Vickers and Rothschild (1985) in Australia validated mating disruption as an effective alternative for *G. molesta* control at a district level. Pree *et al.* (1994) also found mating disruption of *G. molesta* provided commercially acceptable control when pest populations were relatively low. A low moth capture by mass traps within grids, and failure to reduce larval infestations does not support mass trapping or mating disruption as control techniques for *G. libertina* at the population levels observed in 2000. Saturation of the environment with attractant from high dose lures would theoretically have prevented males from reaching attractant sources (in monitoring traps or virgin females), however, populations of adult male moths were not sufficient in 2000 to prove this occurred.

4.5 Seasonal history, Degree-days and Rearing:

The seasonal occurrence of adult male *G. libertina* ranged from late June to early or mid August, peaking in mid-July. This agrees with the flight history described by Morris *et al.* (1988).

Male *G. libertina* showed slight protandry (earlier emergence) than females, but this was not significant ($p<0.05$). Emergence of *Phanerotoma* spp. adults corresponded with *G. libertina* adult emergence ($p<0.05$). Since differences in emergence indicated by trapping and rearing were not due to differences in emergence between males and females, there may have been a delay between insect emergence and the onset of male receptiveness to pheromone (and attraction to pheromone traps). In the Caribbean fruit fly, *Anastrepha suspensa* Loew (Diptera: Tephritidae), there may be as much as a ten day delay before sexual activity is initiated (Nation, 1990). In *G. libertina*, it was possible that such a delay would be due to delayed sexual maturity, or possibly a necessity to feed before mating and oviposition.

The synchrony observed between male and female emergence times is important, as it allows for timing of control measures to target both sexes of adult, and any neonate larvae at the same time (neonate larvae are expected to be most susceptible to contact by insecticidal sprays, before they have entered fruit). As females and males emerged at similar times, female emergence can be predicted accurately by male pheromone trapping. This synchrony was also consistent with work by Baker *et al.* (1980) on *G. molesta*, in which male pheromone trap capture was highly correlated with female emergence.

Elevation and exposure of each site to wind and sunlight may have caused variation in heat sums and insect activity at each site. Variable plant composition such as canopy height, thickness and shading may have therefore created unpredictable microclimates in small areas. Increased shading could have caused cooler conditions, and

subsequent delays in emergence. Study sites were barren areas, with low growing vegetation, however vegetation analysis indicated significant differences in vegetation composition between sites.

Degree day accumulations at selected weather stations enabled prediction of *G. libertina*'s flight period with some variability between years and sites. Variation may have been due to climatic differences between sites and years, along with exposure and microhabitat conditions within plots at each site. In the endangered Californian butterfly, *Euphydras editha quino* Beher (Lepidoptera: Nymphalidae), overstory and shading of wild shrubs have been shown to produce microhabitat cooling which delays diapause break in larvae, affecting size and survivorship (Osbourne & Redak, 2000).

Differences were noted in total degree-day accumulations between laboratory-reared and field collected *G. libertina*, however the relative rates of accumulation were consistent between both. The laboratory study should be more accurate than the field data due to more controlled conditions during rearing (and removal of the effects of microhabitat, weather station distance, weather factors).

Many insects have a discrete developmental threshold temperature used as a base for degree-day calculations (Reidl *et al.*, 1976). Rice *et al.* (1984) used degree-day accumulations for *G. molesta* to optimize timing of insecticide applications. In *Cydia pomonella*, a combination of pheromone trapping, as a reference point, and subsequent degree-day calculations, has been developed to determine egg hatch and insecticide spray timing (Pitcairn *et al.*, 1992; Reidl *et al.*, 1976). Whereas 5°C was selected as the base temperature for degree day calculations, this may not have been accurate. Production of a

precise developmental threshold typically involves large amounts of rearing (coinciding with degree-day calculations), which were not possible with *G. libertina*. However, knowing the approximate degree - day accumulations for *G. libertina* emergence in eastern Newfoundland will permit a more accurate means for timing both trap placement prior to adult flight, and the application of control measures in a commercial setting.

Rearing of *G. libertina* showed low survivorship during all years. In 2000, percent survival was as low as 5.4%. The prepupae may have been vulnerable to dessication and humidity changes, making rearing difficult. Pupal survivorship was higher in Freshwater and Bryant's Cove 2 containers, both of which had large amounts of paper towelling. This may have maintained humidity at a more constant level than did other rearing media (sand or vermiculite). It was also noticed that larvae tended to form prepupae in debris such as *Cladina* lichens, which provided a concave surface and possibly maintained consistent moisture levels.

A 1 male : 1.9 female sex ratio resulted from the rearing in 2000-2001. This differs from Powell (1964), who stated that in most Tortricidae, there is generally a one-to-one male-female sex ratio. Survivorship was very low, and the sex ratio shown by rearing may have been indicative of differential survival (i.e. females may be more tolerant to rearing conditions than males), rather than a naturally occurring sex ratio. Fecundity of reared females was 61±9.0 (N=10). It should be noted, however, that rearing conditions may affect fecundity. For example, Milonas and Savopoulou-Soultani (2000) determined that temperature during larval rearing would affect fecundity of *Adoxophyes orana* Fischer von Röslerstamm (Lepidoptera: Tortricidae) females, with a

significant decrease in egg production at a temperature of 14°C or lower. Naturally occurring egg counts might have deviated in *G. libertina* (61±9.0) as well, depending on temperatures during female development. Both sex ratio and fecundity may produce deviations in female numbers and egg production relative to male populations determined by pheromone trapping, particularly if one male inseminates many females. Polygamy is variable in the Tortricidae, with males of some species, such as *Argyrotaenia citrana* Fernald (Lepidoptera: Tortricidae) typically inseminating multiple females, while *Choristoneura fumiferana* rarely mates more than once (Powell, 1964; Stehr, 1954). As well, if fecundity was variable between years, this might affect larval infestations independently from male capture rates.

4.6 Chemical Analysis

Chemical analyses of insect pheromone glands and effluvia by gas chromatogram-mass spectroscopy (GCMS) were not successful. Problems may have occurred in either sample collection, contamination by solvents, or insufficient amounts of material to be detected by the GCMS. Borg-Karlson and Mözuraitis (1996) found solid phase micro extraction (SPME) to be effective for pheromone collection in the tentiform leafminer, *Phyllonorycter sylvella* Haworth (Lepidoptera: Gracillariidae), wherein the amount of volatiles collected from a single calling female were as much as those collected from the excised glands of twenty females. Since this method required few specimens, and is non-destructive, it was thought to be ideal for pheromone collection in *G. libertina* (due to previous rearing problems).

Pheromone collection in this study was conducted through analysis of gland extracts, and collection by SPME, being a combination of a standard method (gland analysis), and a relatively new, non-destructive technique (SPME). Because gland extraction may produce contamination and dilution of samples by solvents, other authors have explored techniques to improve pheromone collection. Morgan (1990) suggested whole sample injection into a gas chromatogram (GC), by sealing a specimen (tissue or gland) into a capillary tube which is crushed within the GC. While this technique avoids dilution and contamination by solvents, it requires a solid sampler (Keele injector) for crushing samples. The SPME method of sampling was used for *G. libertina* to eliminate solvent dilution and contamination.

Solid phase micro extraction does have limitations, due to differential adsorption of test materials on the sampling fibres (Anon, 1998). Testing and selection of the 100 um polydimethylsiloxane (PDMS) fibre (Supelco®) through sampling of diluted pheromone standards, however, was expected to optimize extraction. Maille *et al.* (1998) have shown that heating of samples during headspace extraction of fatty acids shows better extraction than those sampled at room temperature. This method was not used for *G. libertina*, as it was believed heating would potentially injure specimens or alter insect behaviour (pheromone emission).

Minimum detection levels of Z-8-dodecen-1-ol acetate, E-8-dodecen-1-ol acetate and Z-8-dodecen-1-ol by the gas chromatogram was 10 nanograms. Cardé (2001, pers. com., unpublished) recently reported that female *G. molesta* produced 0.7 nanograms of pheromone/female/hour. If *G. libertina* female pheromone production was as low,

detection by the GCMS used in this study would have been unlikely, as pheromone compounds would be obscured by background peaks. In addition, since rearing produced few moths for chemical analysis, concentrating a large number of insects for effluvia collection, or extracting a large number of pheromone glands was not possible.

5.0 CONCLUSIONS

This study provides information on the attractant trapping, population dynamics and natural occurrence of *G. libertina* in eastern Newfoundland.

The results of this study have shown that a monitoring system and predictive model can be produced through a quick method of isolating a semiochemical sex attractant with relatively little laboratory investigation or chemical analysis. E-8-dodecen-1-ol acetate, Z-8-dodecen-1-ol acetate and Z-8-dodecen-1-ol acetate are all attractive to adult male *G. libertina*, both as single compounds or blends. A ratio of 85% E-8-dodecen-1-ol acetate, 10% Z-8-dodecen-1-ol acetate and 5% Z-8-dodecen-1-ol is, of the blends tested, the most attractive synthetic blend for *G. libertina*. While this blend may not represent the naturally occurring sex pheromone, it is effective for use in monitoring populations of *G. libertina* in the field.

The adult trapping rate with the 85% E-8-dodecen-1-ol acetate: 10% Z-8-dodecen-1-ol acetate: 5% Z-8-dodecen-1-ol blend, when used in Pherocon® 1C wing traps, appeared to be correlated with larval and damage densities in the area surrounding the trap. This relationship, however, was subject to a number of variables, which may affect adult moth behaviour. While the coverage and type of vegetation in the plots had little effect on adult or larval distribution, host berry distribution appeared important, particularly when berry levels were reduced. When the density of host berries in the field was reduced, they became strongly related to larval density, regardless of adult populations . This, combined with the fact that the quantity and distribution of host

berries in wild fields was not uniform, produced significant variability in the predictive model for *G. libertina*. Detection of adult moths by pheromone trapping was limited when populations were low, however, and damage to berries occurred at virtually undetectable levels of the male populations.

Trap trials validated that the Pherocon 1C® style trap was the most suitable for monitoring *G. libertina* at low population levels. The Diamond®, Wing Trap® II and Delta® traps were also effective but captured significantly fewer moths. The non-saturating Unitrap® was not effective.

Mass trapping, using a high density of Pherocon 1C® traps with high concentration attractants was not effective for management of *G. libertina* at the population levels tested in 2000. Use of high density traps and lures disrupted adult-larval correlations observed by pheromone monitoring traps.

Rearing of *G. libertina* was unsuccessful. However, survivorship improved during 2000-2001, with rearing media containing paper towelling. Parasitism by *Phanerotoma* spp. was as high as 15%. Parasitism by an unidentified chalcid wasp was also noted.

Adult flight of *G. libertina* began in late June to early July, and continued until late July to early August. This agrees with a previous study by Morris *et al.* (1988). Mean degree days above base 5° C for 10% emergence was recorded as 270±20.5 by mass rearing, and 334±8.1 by field trapping. Weather factors did not show a significant influence on insect trapping.

The naturally occurring sex pheromone of *G. libertina* still remains a mystery.

5.1 FUTURE DIRECTIONS

The current 85:10:5 field attractant will be useful for population monitoring of *G. libertina* in wild lingonberry fields of Newfoundland. Further research on the naturally occurring female sex pheromone through mass rearing and chemical analyses would permit further enhancement of this blend by identifying any minor components which might act synergistically, increasing attraction. Mantey *et al.* (2000) have produced an effective method for rearing the lesser appleworm, *G. prunivora*. Mass rearing using appropriate rearing media might permit establishment of a laboratory colony, and larger numbers of insects for chemical analysis. In addition, more sensitive techniques for detecting pheromones at low levels (which were not currently available), such as electroantennography or gas chromatography-flame ionization detection would lower detection thresholds for pheromone components.

Studies of *G. libertina* at high population levels or in other areas would validate the predictive value of adult-larval correlations. Sampling of other *Vaccinium* species to determine the degree of alternative host use would also be valuable in future correlation studies.

Mass rearing would also permit investigation of degree-day accumulations and identification of a base developmental temperature.

Two species of parasitoids were identified from reared *G. libertina*. Augmentation of naturally occurring *Phanerotoma* spp. populations by rearing of parasitoids could be implemented as a control tactic for *G. libertina*. This would provide

pesticide-free biological control of *G. libertina* populations, and minimize the risks involved with importation of exotic natural enemies to control pest species.

Future studies on weather factors applied to *G. libertina* trapping should investigate trapping during a short time span, with readings of trap capture either daily or hourly to prevent averaging of weather data. Such a study would require the availability of a large number of adult moths, to detect changes during a short period of time. As well, weather readings would be improved by using weather stations placed on study sites.

REFERENCES

Ando, T., S. Yoshida, S. Tatsuki, & N. Takahashi. 1977. Sex Attractants for Male Lepidoptera. Agricultural & Biological Chemistry. 41: 1485-1492.

Anon. 1997. Handbook of Selected Agricultural Statistics, 1997. Government of Newfoundland and Labrador, Department of Forest Resources and Agri-Foods, St. John's, NF. Pp.25.

Anon. 1998. Solid Phase Microextraction of Volatile Compounds, Supelco® Application Note 11. Sigma Aldrich Co., Bellefonte Park, Pennsylvania.

Arn, H. 1999. The Pherolist: List of Pheromones of Lepidoptera. [www document] http://www-pherolist.slu.se.

Arn, H., M. Toth, & E. Priesner. 1992. List of Sex Pheromones of Lepidoptera and Related Attractants. International Organization for Biological Control, Paris.

Arnold, C.Y. 1960. Maximum - minimum temperatures as a basis for computing heat units. American Society of Horticultural Science. 76: 682-692.

Ayre, G.L., W.J. Turnock & D.L. Struble. 1983. Spatial and temporal variability in sex attractant trap catches of *Leucania commoides* and *Peridroma saucia* (Lepidoptera: Noctuidae) in relation to their biology in Manitoba. Canadian Entomologist. 115: 1573-1582.

Baker, T.C. 1985. *Chemical Control of Behavior*. Pp. 621-672. In: Kerkut, G.A. and Gilbert, L.I. (Eds). Comparative Insect Physiology, Biochemistry and Pharmacology, Vol. 9. Pergamon Press, Oxford. Pp. 735.

Baker, T.C., R.T. Cardé & B.A. Croft. 1980. Relationship between pheromone trap capture and emergence of adult oriental fruit moths, *Grapholita molesta* (Lepidoptera: Tortricidae). Canadian Entomologist. 112: 11-15.

Baker, T.C., W. Meyer, & W.L. Roelofs. 1981. Sex pheromone dosage and blend specificity of response by Oriental Fruit moth males. Entomologica experientia & applicata. 30: 269-279.

Blatt, S.E. & J.H. Borden. 1999. Physical characteristics as potential host selection cues for *Leptoglossus occidentalis* (Hemiptera: Coreidae). Environmental Entomology. 28(2): 246-254.

Booth, W. 1988. Revenge of the 'nozzleheads'. Science 239:135.

Borg-Karlson, A-K. & R. Mözuraitis. 1996. Solid phase micro extraction technique used for collecting semiochemicals: Identification of volatiles released by individual signaling *Phyllonorycter sylvella* moths. Zeitschrift Für Naturforschung. 51c: 599-602.

Brown, M.W. 1984. Saturation of pheromone sticky traps by *Platynota idaeusalis* (Walker) (Lepidoptera: Tortricidae). Journal of Economic Entomology. 77: 915-918.

Butenandt, A., R. Beckmann, D. Stamm, & E. Hecker.1959. Uber den Sexual-Lockstoff des Seidenspinners *Bombyx mori*. Reindarstellung und Konstitution. Zietschrift für Naturforschung 14:2 83-284.

Byers, J.A. 1989. Chemical ecology of bark beetles. Experientia. 45:271-283.

Campion, D.G., B.R Critchley & L.J McVeigh. 1989. *Mating Disruption.* In: Jutsum, A.R. & R.F.S Gordon. (Eds). Insect Pheromones in Plant Protection. John Wiley and Sons, Chichester. Pp. 89-122.

Carson, R. 1962. Silent Spring. Houghton-Mifflin Company, Boston. Pp 368.

Carter, M.R., F.W. Ravlin & M.L. McManus. 1992. Effect of defoliation on larval phenology and trap capture of Gypsy moth. Environmental Entomology. 21(6): 1308-1318.

Cloyd, R.A. & C.S. Sadof. 2000. Effects of plant architecture on the attack rate of *Leptomastix dactylopii* (Hymenoptera: Encyrtidae), a parasitoid of the citrus mealybug (Homoptera: Pseudococcidae). Environmental Entomology. 29(3): 535-541.

Corneilius, M.L., L. Nergel, J. D. Duan & R.H. Messing. 1999. Responses of female oriental fruit flies (Diptera: Tephritidae) to protein and host fruit odors in field cage and open field tests. Environmental Entomology. 29 (1): 14-19.

Darnell, S.J., L.J. Lance & L.J. Young. 2000. Influence of corn phenology on adult western corn rootworm (Coleoptera: Chrysomelidae) distribution. Environmental Entomology. 29(3): 587-595.

Ecological Stratification Working Group. 1995. A National Ecological Framework for Canada. Agriculture and Agri-Food Canada, Research Branch, Centre for Land and Biological Resources Research and Environment Canada, State of Environmental Directorate, Ecozone Analysis Branch, Ottawa/Hull. Report and national map at 1:7500000 scale. Pp. 125.

Edwards, C.A. 1993. *The Impact of Pesticides on the Environment.* In: Pimentel, D. and H. Lehman. (Eds). The Pesticide Question. Chapman and Hall, New York and London. Pp. 13-46.

Ehler, L.E. 1998. *Conservation Biological Control: Past, Present and Future.* In: Barbosa, P. (Ed). Conservation Biological Control. Academic Press, San Deigo. Pp 1-6.

Elliott, N.C., J.J. Jackson & R.D. Gustin. 1990. Predicting western corn rootworm beetle (Coleoptera: Chrysomelidae) emergence from the soil using soil or air temperature. Canadian Entomologist. 122: 1079-1091.

Fabré, J.H. 1879. Souvenirs Entomologiques. Paris: Delagrave. Pp. 287.

Faccioli, G., E. Pasqualini & P. Baronio.1993. Optimal trap density in *Cossus cossus* (Lepidoptera: Cossidae) mass-trapping. Journal of Economic Entomology. 86(3): 850-853.

Flint, M.L. & R. van den Bosch. 1982. Introduction to Integrated Pest Management. Plenum Press, New York. 240 pp.

Frerot, B., C. Malosse & A.H. Cain. 1997. Solid-Phase Microextraction (SPME) - A new tool in pheromone identification in Lepidoptera. Journal of High Resolution Chromatography. 20(6): 340-342.

Gemeno, C., A.F. Lutfallah & K.F. Haynes. 2000. Pheromone blend variation and cross-attraction among populations of the black cutworm moth (Lepidoptera: Noctuidae). Annals of the Entomological Society of America. 93 (6): 1322-1328.

George, J.A. 1965. Sex Pheromone of the Oriental Fruit moth *Grapholita molesta.* Canadian Entomologist. 97:1002-1007.

Grant, G.G., W.H. Fogal, R.J. West, K.N. Slessor & G.E. Miller. 1989. A sex attractant for the spruce seed moth, *Cydia strobilella,* and the effect of lure dosage and trap height on capture of male moths. Canadian Entomologist.121: 691-697.

Gronning, E.K., D.M. Borchert, D.G. Pfeiffer, C.M. Felland, J.F. Walgenbach, L.A. Hull & J.C. Killian. 2000. Effect of specific and generic sex attractant blends on pheromone trap captures of four leafroller species in mid-Atlantic apple orchards. Journal of Economic Entomology. 93(1): 157-164.

Gupta, S.W., W.E. Larson & R.R. Allmaras. 1984. Predicting soil temperature and soil heat flux under different tillage-surface residue conditions. Soil Science Society of America Journal. 48: 223-232.

Häkkinen, S., S. Kärenlampi, M. Heinonen, H. Mykkänen, J. Ruuskanen & R. Törrönen. 1999. Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. Journal of Agricultural and Food Chemistry. 47: 2274-2279.

Hall, D.R. 1998. A rose by any other name? Inaugural lecture, University of Greenwich. Pp. 41.

Hall, R. 1995. *Challenges and Prospects of Integrated Pest Management.* In: Reuveni, R. (Ed). Novel Approaches to Integrated Pest Management. CRC Press, Boca Raton. Pp. 1-20.

Hammack, L., B.E. Hibbard, C.W. Holyoke, M. Kline & D.M. Leva. 1999. Behavioral response of corn rootworm adults to host plant volatiles perceived by western corn rootworm (Coleoptera: Chrysomelidae). Environmental Entomology. 28(6): 961-967.

Heinrich, C. 1926. Revision of the North American moths of the subfamily Eucosminae of the family Olethreutidae. U.S. Natural Museum Bulletin 123. Pp. 298.

Hertog, M.G.L., D. Jromhout, C. Aravanis, H. Blackburn, R. Buzina, F. Fidanza, S. Giampaoli, A. Jansen, A. Menotti, S. Nedeljkovic, M. Pekkarinen, B.S. Simic, H. Toshima, E.J.M. Feskens, P.C.H. Hollman & M.B. Katan. 1995. Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. Archives of Internal Medicine. 155: 381-386.

Housewart, M.W., D. T. Jennings & C.J. Sanders. 1981. Variables associated with pheromone traps for monitoring spruce budworm populations (Lepidoptera: Tortricidae). Canadian Entomologist. 113: 527-537.

Howell, F.J. 1974. The competitive effect of field populations of codling moth on sex attractant trap efficiency. Environmental Entomology. 3(5): 803-807.

Howse, P.E. 1998a. *Insect Semiochemicals and Communication.* In: Howse, P.E., I. Stevens & O. Jones (Eds.). Insect Pheromones and their Use in Pest Management. Chapman and Hall, London. Pp. 1-37.

Howse, P.E. 1998b. *Chemical structures and diversity.* In: Howse, P.E., I. Stevens & O. Jones (Eds.). Insect Pheromones and their Use in Pest Management. Chapman and Hall, London. Pp. 105-130.

Isaacs, R., M. Ulczynski, B. Wright, L.J. Gut & J.R. Miller. 1999. Performance of the microsprayer, with application for pheromone-mediated control of insect pests. Journal of Economic Entomology. 92(5): 1157-1164.

Johnson, D., L. Kershaw, A. MacKinnon & J. Pojar. 1995. Plants of the Western Boreal Forest and Aspen Parkland. Lone Pine Publishing, Edmonton, Alberta. Pp. 392.

Jones, O. 1998. *Practical Applications of Pheromones and other Semiochemicals*. In: Howse, P.E., I. Stevens & O. Jones (Eds.). Insect Pheromones and their Use in Pest Management. Chapman and Hall, London. Pp. 263-356.

Jones, G.R. & N.J. Oldham. 1999. Pheromone analysis using capillary gas chromatography techniques. Journal of Chromatography A. 843:199-236.

Jurenka, R.A. & W.L. Roelofs. 1993. Biosynthesis and endocrine regulation of fatty acid derived sex pheromones in moths. In: D.W. Stanley-Samuelson & D.R. Nelson (Eds.). Insect Lipids: Chemistry, Biochemistry and Biology. Lincoln: University of Nebraska Press. Pp. 353-388.

Jutsum, A.R., & R.F.S. Gordon. 1989. Insect Pheromones in Plant Protection. John Wiley and Sons Ltd., Chichester. Pp. 369.

Kangas, K. 1999. Trade of main wild berries in Finland. Silva Fennica. 33:159-168.

Karlson, E., & M. Luscher. 1959. *Ectohormones in Insects*. In: Stoffalano,W.S. & J.G. Romoser. (1994). The Science of Entomology. Pp. 231-234.

Keli, S.O., M.G.L. Hertog, E.J.M. Feskens & D. Kromhout. 1996. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. Archives of Internal Medicine. 156: 637-642.

Knekt, P., R. Järvinen, A. Reunanen & J. Maatela. 1996. Flavonoid intake and coronary mortality in Finland: a cohort study. British Medical Journal. 312: 478-481.

Knodel, J.J. & A.M. Agnello. 1990. Field comparison of sticky and nonsticky pheromone traps for monitoring fruit pests in Western New York. Journal of Economic Entomology. 83(1): 197-204.

Koyoma, J., T. Teruya, & K. Tanaka. 1984. Eradication of the Oriental fruit fly (Diptera: Tephritidae) from the Okinawa Islands by a male annihilation method. Journal of Economic Entomology 77: 468-472.

Kuo, J & E. Fox. 1992. Sigmaplot Graphing Software- Getting Started. Jandel Scientific. 68 Pp.

Langley, P., P.M. Huyton & D.A. Carlson. 1987. Sex pheromone production by males of the tsetse fly *Glossina morsitans morsitans*. Physiological Entomology. 42: 425-433.

Latheef, M.A., J.D. Lopez Jr. & J.A. Witz. 1991. Relationships among pheromone trap catches of male corn earworm moths (Lepidoptera: Noctuidae), egg numbers, and phenology in corn. Canadian Entomologist. 123: 271-281.

Latheef, M.A., J.D. Lopez Jr. & J.A. Witz. 1993. Capture of Corn Earworm (Lepidoptera: Noctuidae) in pheromone traps and hand nets : relationship to egg and adult densities in field corn, Texas Brazos River Valley. Journal of Economic Entomology. 86(2): 407-415.

Law, J.H. & F.E. Regnier. 1971. Pheromones. Annual Review of Biochemistry. 40: 533-548.

Lemon, W.C. & W.M. Getz. 1999. Neural coding of general odors in insects. Annals of the Entomological Society of America. 92(6): 861-872.

Leonhardt, B.A., V.C. Mastro & E.D. Devilbiss. 1993. New dispenser for the pheromone of the Gypsy Moth (Lepidoptera: Lymantriidae). Journal of Economic Entomology 86:821-827.

Lewis, E.E. & J.H. Cane. 1990. Pheromonal specificity of the southeastern *Ips* pine bark beetles reflects phylogenetic divergence (Coleoptera: Scolytidae). Canadian Entomologist. 122: 1235-1238.

Lewis, T. & E.D. Macaulay. 1976. Design and elevation of sex-attractant traps for pea moth, *Cydia nigircana* (Steph.), and the effect of plume shape on catches. Ecological Entomology. 1: 175-187.

Löfestedt , C., B.B. Hansson, H.J. Dijkerman & W.M. Herrabout. 1990. Behavioral and electrophysiological activity of unsaturated analogues of the pheromone tetradecenyl acetate in the small ermine moth *Yponomeuta rorellus*. Physiological Entomology. 15: 47-54.

Maille, R., F.R. Dani, G.R. Jones, E.D. Morgan & D. Ortius. 1998. Sampling techniques for gas chromatographic-mass spectrometric analysis of long-chain free fatty acids from insect exocrine glands. Journal of Chromatography A. 816: 169-175.

Mantey, K.D., H.R. Moffitt, L.G. Neven. 2000. Laboratory rearing of lesser appleworm (Lepidoptera: Tortricidae). Journal of Economic Entomology. 93(3):1021-1024.

Matthews, G.A. 1992. Pesticide Application Methods, 2nd Edition. Longman, Harlow,

UK. Pp. 403.

Mayer, M.S. & J.R. McLaughlin. 1991. Handbook of Insect Pheromones and Sex Attractants. CRC Press. Pp.457-462.

Meades, W.J. 1983. *Heathlands*. Pp.267-318. In: G.R. South (Ed.). Biogeography and ecology of the Island of Newfoundland. Dr. W. Junk Publishers, The Hague, Netherlands. Pp. 723.
Mellanby, K. 1970. Pesticides and Pollution. Collins Press, London. Pp. 221.

Metcalf, R.L. & E.R Metcalf. 1992. Contemporary Topics in Entomology: Plant Kairomones in Insect Ecology and Control. Chapman and Hall, New York.. Pp. 168.

Milonas, P.G. & M. Savopoulou-Soultani. 2000. Development, survivorship, and reproduction of *Adoxophyes orana* (Lepidoptera: Tortricidae) at constant temperatures. Annals of the Entomological Society of America. 93(1): 96-102.

Morgan, E.D. 1990. Preparation of small-scale samples from insects for chromatography. Analytica Chimica Acta. 246: 227-235.

Morgan, G.A., O.V. Greigo & G.W. Gloeckner. 2001. SPSS for Windows: an introduction to use and interpretation in research. L. Erlbaum Associates, New Jersey. Pp 214.

Morris, R.F., B.G. Penney, G. Greenslade, P.A. Hendrickson & K.B. McRae. 1988. Notes on the occurrence, distribution, population levels and control of *Grapholita libertina* Heinr. (Lepidoptera: Tortricidae), a pest of lingonberries in Nfld. Canadian Entomologist. 120: 867-872.

Mozüraitus, R., V. Buda, A.K. Borg-Karlson, P. Ivinskis, V. Karalius, M. Laanmaa & D. Plepys. 1998. New sex attractants and inhibitors for 17 moth species from the families Gracillariidae, Tortricidae, Yponomeutidae, Oecophoridae, Pyralidae and Gelechiidae. Journal of Applied Entomology. 122: 441-452.

Myers, J.H., A. Savoie & E. van Randen. 1998. Eradication and Pest Management. Annual Review of Entomology 43: 471-91.

Nation, J.L. 1990. Biology of pheromone release by male Caribbean fruit flies, *Anastrepha suspensa* (Diptera:Tephritidae). Journal of Chemical Ecology. 16: 553-572.

Nishida, R., H. Fukami, T.C. Baker, W.L. Roelofs & T.B. Acree. 1985. *Oriental fruit moth pheromone: attraction of females by an herbal essence.* In: Acree, T.E. & D.M. Soderlund (Eds.). Semiochemistry, Flavors and Pheromones. Walter de Gruyter & Co.

Berlin. Pp. 47.

Nordlund, D.A., R.L. Jones, & W.J. Lewis.1981. Semiochemicals: their role in pest control. John Wiley and Sons Ltd., Chichester. Pp. 1-18.

Osbourne, K.H. & R.A. Redak. 2000. Microhabitat conditions associated with the distribution of postdiapause larvae of *Euphydras editha quino* (Lepidoptera: Nymphalidae). Annals of the Entomological Society of America. 93(1): 110-114.

Penney, B.G., Hendrickson, P.A., Gallagher, C.A., Churchill, R.A. & Butt, E. 1996. The wild partridgeberry (*Vaccinium vitis-idaea* L. var. *minus* Lodd) industry in Newfoundland and Labrador and the potential for expansion utilizing European cultivars. Acta Horticulturae 446:139-142.

Perkins, A.D. 1982. Insects, Experts, and the Insecticide Crisis. Plenum Press, New York and London. Pp. 304.

Pimentel, D., H. Acquay, M. Biltonen, P. Rice, M. Silva, J. Nelson, V. Lipner, S. Giordano, A. Horowitz & M. D'Amore. 1993a. *Assessment of Environmental and Economic Impacts of Pesticide Use*. In: Pimentel, D. & H. Lehman. (Eds). The Pesticide Question. Chapman and Hall, New York and London. Pp. 47-87.

Pimentel, D., C. Kirby & A. Shroff. 1993b. *The Relationship Between "Cosmetic Standards" for Foods and Pesticide Use*. In: Pimentel, D. & H. Lehman (Eds). The Pesticide Question. Chapman and Hall, New York and London. Pp. 85-105.

Pitcairn, M.J., F.G. Zalom & R.E Rice. 1990. Weather factors influencing capture of *Cydia pomonella* (Lepidoptera: Tortricidae) in pheromone traps during overwintering flight in California. Environmental Entomology. 19(5): 1253-1258.

Pitcairn, M.J., F.G. Zalom & R.E Rice. 1992. Degree-day forecasting of generation time of *Cydia pomonella* (Lepidoptera: Tortricidae) populations in California. Environmental Entomology. 21: 441-446.

Polavarapu, S. & W.D. Seabrook. 1992. Evaluation of pheromone-baited traps and pheromone concentrations for monitoring blueberry leaftier (Lepidoptera: Tortricidae) populations. Canadian Entomologist. 124: 815-825.

Powell, J.A.1964. Biological and Taxonomic Studies on Tortricine Moths, with Reference to the Species in California.. R.F. Smith, R.M. Bohart, R.L. Doutt, E.A. Steinhaus & R.L. Usinger (Eds). University of California Publications in Entomology, Volume 32. University of California Press, Berkeley and Los Angeles. Pp. 317.

Pree, D.J., R.M. Trimble, K.J. Whitty & P.M. Vickers. 1994. Control of oriental fruit moth by mating disruption using sex pheromone in the Niagara peninsula. Canadian Entomologist. 126: 1287-1299.

Pruess, K.P. 1983. Day-Degree Methods for Pest Management. Environmental Entomology. 12: 613-619.

Reidl, H., B.A.Croft & A.J. Howitt. 1976. Forecasting codling moth phenology based on pheromone trap catches and physiological time models. Canadian Entomologist. 108: 449-460.

Rice, R.E., C.V. Weakley & R.A. Jones. 1984. Using degree-days to determine optimum spray timing for the oriental fruit moth (Lepidoptera: Tortricidae). Journal of Economic Entomology. 77: 698-700.

Roelofs, W.L. 1978. Threshold hypothesis for pheromone perception. Journal of Chemical Ecology. 4: 685-699.

Roelofs, W.L. & R.T. Cardé. 1974. Oriental Fruit moth and Lesser Appleworm attractant mixtures refined. Environmental Entomology. 3: 586-588.

Roelofs, W.L. & A. Comeau. 1970. Lepidopterous sex attractants discovered by field screening tests. Journal of Economic Entomology. 63(3): 969-974.

Rojas, J.C. 1999. Influence of host plant damage on the host-finding behavior of *Mamestra brassicae* (Lepidoptera: Noctuidae). Environmental Entomology. 28(4): 589-593.

Rowe, W.J. & D.A. Potter. 2000. Shading effects susceptibility of *Rosa* spp. to defoliation by *Popillia japonica* (Coleoptera: Scarabaeidae). Environmental Entomology. 29(3): 502-508.

Ryan, G.A. 1978. Native Trees and Shrubs of Newfoundland and Labrador. Nfld. Forest Service. Pp. 116.

Sachs, C.E. 1993. *Growing Concern Over Pesticides in Food and Water.* Pp. 380-389. In: D. Pimentel & H. Lehman (Eds). The Pesticide Question. Chapman and Hall, New York and London. Pp. 441.

Sanders, C.J. 1981. Sex attractant traps: their role in the management of spruce budworm. Pp. 75-91. In: E.R. Mitchell (Ed.).Management of Insect Pests with Semiochemicals. USDA, Gainesville, Florida. Pp. 514.

Sanders, C.J. 1986a. Evaluation of high-capacity, nonsaturating sex pheromone traps for monitoring population densities of spruce budworm (Lepidoptera: Tortricidae). Canadian Entomologist. 118: 611-619.

Sanders, C.J. 1986b. Accumulated dead insects and killing agents reduce catches of spruce budworm (Lepidoptera: Tortricidae) male moths in sex pheromone traps. Journal of Economic Entomology. 79: 1351-1353.

Sappington, T.W. & D.W. Spurgeon. 2000. Variation in boll weevil (Coleoptera: Curculionidae) captures in pheromone traps arising from wind speed moderation by brush lines. Environmental Entomology. 29(4): 807-814.

Schouest, L.P. Jr. & T.A. Miller. 1994. Automated pheromone traps show male pink bollworm (Lepidoptera: Gelechiidae) mating response is dependent on weather conditions. Journal of Economic Entomology. 87(4): 965-974.

Shami, A. 1998. Integrated pest management using pheromones. Chemtech. 28(3): 30-35.

Shelley, T.E. 2000. Fecundity female oriental fruit flies (Diptera: Tephritidae): Effects of methyl eugenol-fed and multiple males. Annals of the Entomological Society of America. 93(3): 559-564.

Shepherd, R.F., T.G. Gray, R.J. Chorney & G.E. Daterman. 1985. Pest management of douglas-fir tussock moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae): monitoring endemic outbreak populations with pheromone traps to detect incipient outbreaks. Canadian Entomologist. 117: 839-848.

Sokal, R.R. & F.J. Rohlf. 1995. Biometry, 3rd Ed. W.H. Freeman & Company. New York Pp. 887.

Stehr, G. 1954. A laboratory method for rearing the spruce budworm, *Choristoneura fumiferana* (Clem.), (Lepidoptera: Tortricidae). Canadian Entomologist. 86(9):423-428.

Stockel, J. & F. Sureau.1980. *Monitoring for Angoumis grain moth in corn.* Pp.63-74. In: E.R. Mitchell (ed.).Management of Insect Pests with Semiochemicals. USDA, Gainesville, FL . Pp. 514.

Stoffolano, W.S., & J.G. Romoser.1994. The Science of Entomology. 3rd ed. Wm. C. Brown Publishers Inc. Dubuque, Iowa. Pp. 605.

Summy, K.R., F.E. Gilstrap & W. G. Hart. 1986. Correlation between trap response and foliar densities of citrus blackfly, *Aleurocanthus woglumi* (Homoptera: Aleyrodidae). Canadian Entomologist. 118: 81-83.

Sweeney, J.D., J.A. McLean & R.F. Shepherd. 1990. Factors affecting catch in pheromone traps for montioring the spruce budworm, *Choristoneura fumiferana* Freeman. Canadian Entomologist. 122: 1119-1130.

Torrey, G.S. 1914. The partridgeberry: A report. Department of Agriculture and Mines, St. John's, NF. Pp. 12.

Trimble, R.M. and E.A.C. Hagley. 1988. Evaluation of mass traping for controlling the spotted tentiform leafminer, *Phyllonorycter blancardella* (Fabr.) (Lepidoptera: Gracillariidae). Canadian Entomologist 120: 101-107.

Turgeon, J. & J.N. McNeil. 1983. Pheromone testing for Armyworm monitoring. Environmental Entomology 12:891-894.

Vickers, R.A. & G.H.L. Rothschild. 1985. Control of the oriental fruit moth, *Cydia molesta* (Busck) (Lepidoptera: Tortricidae), at a district level by mating disruption with synthetic female pheromone. Bulletin of Entomological Research. 75: 625-634.

Walker, K.R. & S.C. Welter. 1999. Effects of lure dose on efficacy of pheromone traps for monitoring *Argyrotaenia citrana* (Lepidoptera: Tortricidae) in California apple orchards. Journal of Economic Entomology. 92(3): 574-582.

Warner, K.W., B.V. Helson & B.C. Kostyk. 1997. Foliar and systemic applications of neem seed extract for control of spruce budworm, *Choristoneura fumiferana* (Clem.)(Lepidoptera: Tortricidae), infesting black and white spruce seed orchards. Canadian Entomologist. 129: 645-655.

Willis, M.A. & M.C. Birch. 1982. Male lek formation and female calling in a population of the arctiid moth *Estigmene acrea*. Science. 218: 168-170.

Willson, H. R. & K. Trammel. 1975. Relationships between the sex pheromone trapping of six tortricids and a foliage index of apple orchard canopies. Environmental Entomology. 4(3): 361-364.

Winston, M. L. & K. N. Slessor. 1992. The essence of royalty: honey bee queen pheromone. American Scientist. 80: 374-385.

Witz, J.A., J.D. Lopez & M.A. Latheef. 1992. Field density estimates of *Heliothis virescens* (Lepidoptera: Noctuidae) from catches in sex pheromone-baited traps. Bulletin of Entomological Research. 82: 281-286.

Appendix A: Rearing procedures during each year of study:

1) 1995-1996:
In September 1995, bins of lingonberries were collected from random sites across Newfoundland, and placed in an outdoor insectary from October 1995 - May 1996. Bins were moved into a 20°C rearing chamber (80% humidity) in May. Bins were misted and checked daily from May - August for any emerging insects.

2) 1996-1998:
No rearing took place during 1996-1997 or 1997-1998.

3) 1998-1999:
In September 1998, larvae collected from correlation studies (Section 3.2) in Pouch Cove, Little Catalina, Freshwater and Bryant's Cove were used in rearing. A total of 86 larvae were individually placed in pill bottles with a vermiculite substrate (to help maintain humidity), which were covered with a fine mesh. Bottles were moved to rearing chambers (3.5°C, 80% humidity) on 20 October 1998. Bottles were periodically misted to maintain humidity. On 16 March bottles were moved into a 12°C, 80% humidity rearing chamber. On 1 May, bottles were again moved to a 20°C chamber. Bottles were checked every second day for emerging insects.

4) 1999-2000:
Larvae (504) collected in 1999 (correlation studies) were individually placed in plastic containers, containing vermiculite (N=350) or sand (N=154) as a substrate. A few berries (5-10) were added to each container to permit any further feeding required by late instar larva. Containers were moved to a 3.5°C rearing chamber on 14 October. Humidity was maintained by misting. Containers were moved to a 20°C, 85% humidity chamber on 5 May.

5) 2000-2001:
Larvae collected from individual sites were placed together in Tupperware® containers. Holes were cut in covers, and replaced with fine mesh. Berries were suspended inside on a wire frame and within each container, and various substrates were used (Table 6). Containers were placed in a 3.5°C rearing chamber on November 21 and removed to warmer chambers at variable times.

Appendix B: Methods used to collect pheromone components from female *G. libertina* (N refers to the number of trials attempted:

1) Headspace analysis (N=10): (Jones & Oldham, 1999) Samples of 10-20 calling virgin females were placed in a 2 ml glass vial, sealed with a teflon septum. A 100 um polydimethylsiloxane (PDMS) fibre (Supelco®) was lowered into the center of the vial, and volatiles were collected for one hour. The fibre was then injected directly into the GC for analysis. Other fibres (7 um PDMS and 85 um ployacrylate) were also tested for their ability to extract test standards of E-8-A, Z-8-A and Z-8-ol. The 100 um PDMS had the best extraction of test standards, showing higher and more distinct peaks at low concentrations, than the other fibres. Where possible, used virgin females were 'recycled' and used in subsequent headspace analyses.

2) Direct collection (N=5): This technique is described in Frerot *et al.* (1997). A single calling female was placed in a 2 ml glass vial, and a 100 um PDMS fibre was placed on the extruded pheromone glands, to obtain a direct sample of volatiles being released. The fibre was then injected directly into the GC.

3) Vial washing (N=5): Vials which had contained calling females were rinsed with 2 20 ul washes of hexane, to extract any volatiles adhered to the glass vial. A 2 ul sample of this extract was then injected for analysis.

4) Ovipositor washing (N=3): Extruded ovipositors were excised from samples of 8-10 females and extracted for 5 minutes in 20 ul of hexane. A 2 ul sample of this extract was then injected for analysis.

Appendix C1: Mean and range of percent coverage for vegetation recorded during the 1998, 1999 and 2000 field seasons at the Pouch Cove site. 2000A denotes a normal larval correlation grid, 2000B denotes a mass trapping grid.

	Mean % coverage (+/- SEM) (Range) Minimum-Maximum							
Plant Species	1998		1999		2000A		2000B	
Vaccinium vitis-idaea	28.5	2.77	21.1	2.03	18	2.15	24.3	2.7
	0	60	0	50	0	60	0	70
Vaccinium angustifolium	31.1	2.9	35.5	2.72	39.2	2.89	36.9	1.86
	0	85	0	90	10	80	15	80
Juniperus communis	5.3	1.98	1.8	0.64	1.4	0.59	2.9	0.84
	0	50	0	20	0	20	0	20
Juniperus horizontalis	0	0	2.3	1.05	0.2	0.15	0.1	0.1
	0	0	0	35	0	5	0	5
Lichen spp.	10.3	1.65	42.6	3.07	31.6	2.51	29.2	1.95
	0	50	5	90	5	75	5	60
Potentilla tridentata	0	0	0.3	0.31	0.6	0.32	0	0
	0	0	0	15	0	10	0	0
Empetrum nigrum	0	0	0.5	0.43	1.4	0.8	0.6	0.35
	0	0	0	20	0	30	0	10
Maianthemum canadensis	0.1	0.1	2.1	0.42	1.9	0.39	1.9	0.35
	0	5	0	10	0	10	0	5
Festuca ovina	3.6	1.21	2.9	0.65	4.5	1.27	1.6	0.54
	0	40	0	15	0	40	0	20
Sphagnum spp.	4.6	1.52	1.7	0.69	2.6	1.02	3.1	1.17
	0	50	0	20	0	35	0	40
Kalmia angustifolium	2.7	1.85	2.9	1.13	4.3	1.8	3.9	1.25
	0	80	0	30	0	60	0	35
Ledum groenlandicum	0.2	0.21	0.5	0.37	0	0	1.4	0.69
	0	10	0	15	0	0	0	25
Cornus canadensis	3.6	1.21	6	0.73	6.4	0.51	4.3	0.26
	0	40	0	15	0	15	0	5
Sorbus canadensis	0.8	0.5	0.1	0.1	1	0.15	0.6	0.28
	0	20	0	5	0	5	0	10
Bare ground	5.7	2.18	0.3	0.23	0.2	0.15	1.3	0.46
	0	70	0	10	0	5	0	15
Epilobium angustifolium	1.4	0.83	0.5	0.27	0.1	0.1	0	0
	0	30	0	10	0	5	0	0
Gaultheria hispidula	0.8	0.37	2.5	0.65	1.4	0.49	1.7	0.58
	0	10	0	20	0	20	0	20

Appendix C2: Mean and range of percent coverage for vegetation recorded during the 1998, 1999 and 2000 field seasons at the Bryant's Cove site. 2000A denotes a normal larval correlation grid, 2000B denotes a mass trapping grid.

	Mean % coverage (+/- SEM) (Range) Minimum-Maximum							
Plant Species	1998		1999		2000A		2000B	
Vaccinium vitis-idaea	19.2	2.07	21.1	2.03	22.1	2.37	15.9	1.96
	0	50	0	50	0	60	0	50
Vaccinium angustifolium	28.6	2.52	35.5	2.72	33.4	2.84	40.3	2.91
	0	80	0	90	0	75	0	80
Juniperus communis	4.1	1.17	1.8	0.64	9.1	1.92	0	0
	0	30	0	20	0	40	0	0
Juniperus horizontalis	0	0	2.3	1.05	0	0	0	0
	0	0	0	35	0	0	0	0
Lichen spp.	28.3	2.42	42.6	3.07	36.2	2.67	23	3.1
	0	60	5	90	0	80	0	80
Potentilla tridentata	3.9	1.17	0.3	0.31	4	0.83	4.4	0.69
	0	50	0	15	0	25	0	15
Empetrum nigrum	1.3	0.5	0.5	0.43	2.9	1.5	4.4	1.98
	0	15	0	20	0	60	0	65
Maianthemum canadensis	1.8	0.53	2.1	0.42	1.1	0.31	0.5	0.22
	0	15	0	10	0	5	0	5
Festuca ovina	3.6	1.21	2.9	0.65	2.1	5.33	1.7	0.67
	0	40	0	15	0	15	0	30
Sphagnum spp.	4.6	1.23	1.7	0.69	4.1	1.39	21.8	2.73
	0	40	0	20	0	50	0	60
Kalmia angustifolium	2.1	2.08	2.9	1.13	2.9	2.05	7	2.4
	0	100	0	30	0	95	0	90
Ledum groenlandicum	0.9	0.67	0.5	0.37	0.3	0.31	1.1	0.69
	0	25	0	15	0	15	0	30
Cornus canadensis	0	0	6	0.73	0.3	0.18	0.9	0.35
	0	0	0	15	0	5	0	10
Sorbus canadensis	0.5	0.37	0.1	0.1	0	0	0	0
	0	15	0	5	0	0	0	0
Bare ground	2.6	0.83	0.3	0.23	0.4	0.29	2.6	1.25
	0	25	0	10	0	10	0	40
Epilobium angustifolium	1.4	0.83	0.5	0.27	0	0	0	0
	0	30	0	10	0	0	0	0
Gaultheria hispidula	0.8	0.37	2.5	0.65	0.2	0.15	0.1	0.1
	0	10	0	20	0	5	0	5

Appendix C3: Mean and range of percent coverage for vegetation recorded during the 1998, 1999 and 2000 field seasons at the Little Catalina site. 2000A denotes a normal larval correlation grid, 2000B denotes a mass trapping grid.

	Mean % coverage (+/- SEM) (Range) Minimum-Maximum							
Plant Species	1998		1999		2000A		2000B	
Vaccinium vitis-idaea	25.8	1.18	21.4	1.81	10.9	0.68	9.8	0.84
	5	35	5	80	5	25	5	40
Vaccinium angustifolium	13.9	1.27	10.8	2.24	10	1.3	10.2	1.2
	0	40	0	65	0	50	0	40
Juniperus communis	0	0	0	0	0.4	0.25	0	0
	0	0	0	0	0	10	0	0
Juniperus horizontalis	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
Lichen spp.	22.5	1.8	36.3	2.41	55.8	2.51	56.6	2.36
	0	50	0	65	15	90	15	85
Potentilla tridentata	3.9	1.16	4.3	0.95	4.1	0.78	2.6	0.47
	0	40	0	30	0	25	0	15
Empetrum nigrum	6.2	1.34	9.2	1.85	5.1	1.3	19.2	2.41
	0	30	0	45	0	40	0	70
Maianthemum canadensis	0	0	1.5	0.87	0	0	0	0
	0	0	0	40	0	0	0	0
Festuca ovina	0.2	0.21	0.8	0.27	0.3	0.18	0	0
	0	10	5	40	0	5	0	0
Sphagnum spp.	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
Kalmia angustifolium	10.9	2.08	4.6	1.15	14.8	1.79	1.1	0.4
	0	50	0	35	0	60	0	10
Ledum groenlandicum	8.2	1.03	6.7	1.62	1.9	0.47	15.4	2.1
	0	20	0	50	0	10	0	70
Cornus canadensis	3.9	0.91	2	0.44	2.2	0.39	1.6	0.34
	0	20	0	10	0	10	0	5
Sorbus canadensis	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
Bare ground	3.5	1.48	11.1	1.99	14	2.13	5.21	1.11
	0	55	0	55	0	60	0	35
Epilobium angustifolium	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
Gaultheria hispidula	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Appendix C4: Mean and range of percent coverage for vegetation recorded during the 1998, 1999 and 2000 field seasons at the Freshwater site. 2000A denotes a normal larval correlation grid, 2000B denotes a mass trapping grid.

	Mean % coverage (+/- SEM) (Range) Minimum-Maximum							
Plant Species	1998		1999		2000A		2000B	
Vaccinium vitis-idaea	30.1	2.64	29.7	3.03	32.3	2.67	30.3	3.29
	0	80	0	80	0	65	0	85
Vaccinium angustifolium	17.7	1.38	23.8	2.55	30.3	2.2	30.5	2.28
	0	40	0	80	5	65	5	70
Juniperus communis	22.9	2.92	0	0	18.3	3.07	17.7	3.16
	0	75	0	0	0	80	0	85
Juniperus horizontalis	0	0	22.6	2.96	0	0	0.3	0.31
	0	0	0	60	0	0	0	15
Lichen spp.	3.5	1.23	7	2.26	7.1	1.83	15.3	2.79
	0	40	0	60	0	45	0	70
Potentilla tridentata	2.9	0.65	1.5	0.36	2.4	0.51	2.5	0.45
	0	20	0	10	0	15	0	10
Empetrum nigrum	2.2	1.01	1.5	0.36	7.1	2.31	5.2	2.6
	0	40	0	35	0	60	0	90
Maianthemum canadensis	1.4	0.41	0.9	0.27	0.7	0.26	2.1	0.39
	0	10	0	5	0	5	0	10
Festuca ovina	1.3	0.57	2.1	0.63	1.4	0.36	1.5	0.47
	0	20	0	20	0	10	0	15
Sphagnum spp.	14.5	1.74	34.7	2.89	22.1	2.44	19.5	2.39
	0	40	0	80	0	60	0	60
Kalmia angustifolium	1.7	0.97	2.4	1.36	0.5	0.31	2.5	1.72
	0	40	0	60	0	10	0	80
Ledum groenlandicum	4.7	1.49	0	0	0	0	0	0
	0	50	0	0	0	0	0	0
Cornus canadensis	0.5	0.22	0	0	0	0	0.3	0.18
	0	5	0	0	0	0	0	5
Sorbus canadensis	0.2	0.21	0	0	0.3	0.18	0	0
	0	10	0	0	0	5	0	0
Bare ground	1.6	0.67	0.4	0.33	2.2	0.8	3.1	1.15
	0	20	0	15	0	20	0	40
Epilobium angustifolium	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
Gaultheria hispidula	0.8	0.48	0	0	0	0	0.2	0.15
	0	20	0	0	0	0	0	5

Appendix D1: Weather summary for St. John's Airport with Pouch Cove adult catch per trap per night, 1996, 1998-2000.

Year	Month	Day	Maximum temperature (°C)	Minimum temperature (°C)	Mean temperature (°C)	Precipitation (mm)	Average Wind Speed (km/hr)	Adult catch per trap/night
1996	June	24	14.6	3.6	9.1	1.9	21.5	0.0
		27	12.9	4.7	8.9	7.6	19.8	0.0
	July	2	19.5	12.6	16.1	4.0	22.6	0.2
		5	24.3	16.4	20.3	11.7	29.8	0.1
		8	21.9	10.2	16.1	3.2	25.0	0.2
		11	20.4	12.3	16.4	1.3	24.5	0.6
		15	20.3	9.3	14.8	14.7	23.6	0.3
		18	21.7	10.9	16.3	3.6	28.2	0.3
		21	23.3	13.1	18.2	3.0	25.7	0.3
		24	16.7	10.2	13.5	0.6	15.1	0.5
		28	20.9	11.1	16.0	0.5	27.2	0.2
	August	31	20.8	10.6	15.7	0.0	11.9	0.2
		4	22.7	15.1	18.9	0.1	15.7	0.1
		7	26.6	15.5	21.1	0.0	18.3	0.0
		11	24.3	14.8	19.5	2.2	26.2	0.0
			21.4	12.3	16.9	5.8	37.4	0.0
		19	20.8	12.8	16.8	0.2	21.8	0
1998	June	24	15.4	5.6	10.5	0.0	17.2	0.0
	July	1	18.3	8.4	13.4	12.2	24.5	0.1
		7	19.2	12.3	15.8	11.5	18.1	0.7
		14	16.8	10.0	13.5	0.1	14.8	0.2
		20	25.1	14.6	19.9	4.2	18.3	0
		27	23.0	11.9	17.5	5.8	20.0	0
1999	July	1	19.6	10.2	14.9	15.7	17.6	0.1
		7	13.8	6.2	10.1	4.5	12.7	1.5
		14	19.8	10.5	15.2	4.6	13.7	0.7
		21	17.3	9.5	13.4	1.3	15.6	0.3
		28	18.3	12.9	15.6	9.4	13.9	0
	August	7	19.7	13.3	16.5	1.5	15.1	0
2000	July	1	18.9	10.4	14.7	5.2	11.9	0.0
		10	23.2	13.3	18.3	1.3	21.8	0.1
		19	21.5	12.7	17.1	2.3	21.2	0.1
		26	17.6	11.5	14.6	1.0	9.9	0.1
		31	16.5	10.1	13.3	9.2	18.6	0

Appendix D2: Weather summary for Victoria weather station with Freshwater and Bryant's Cove adult catch per trap per night, 1996-2000.

Year	Month	Day	Maximum temperature (°C)	Minimum temperature (°C)	Mean temperature (°C)	Precipitation (mm)	Adult catch per trap/night	
							Freshwater	Bryant's Cove
1996	June	24	15.7	2.5	9.1	0.0	0.0	-
		27	14.5	4.0	9.3	10.3	0.0	-
	July	2	20.2	10.2	15.2	10.3	0.2	-
		5	24.7	16.0	20.4	1.7	0.3	-
		8	20.8	12.0	16.4	0.0	0.3	-
		11	20.7	11.0	15.8	3.5	0.5	-
		14	22.7	13.3	18.0	12.7	0.3	-
		18	21.2	10.2	15.7	3.9	0.2	-
		22	21.8	14.5	18.2	2.3	0.3	-
		25	15.3	10.0	12.7	0.3	0.1	-
		29	21.5	12.8	17.2	2.7	0.1	-
	August	1	23.5	11.5	17.5	0.5	0.1	-
		5	23.8	14.5	19.2	0.3	0.0	-
		8	28.3	15.3	21.9	0.0	0.0	-
		12	25.0	15.5	20.3	8.0	0.1	-
		15	21.0	14.2	17.6	5.8	0.0	-
1997	July	2	12.0	7.8	12.6	0.0	-	0.0
		6	18.8	11.0	16.1	6.7	-	0.1
		10	26.2	12.5	19.2	0.0	-	0.6
		13	22.8	13.3	17.2	1.9	-	0.8
		17	18.0	7.7	12.7	7.9	-	0.6
		20	24.5	13.7	19.3	0.4	-	0.3
		24	19.7	10.0	14.9	0.7	-	0.3
		27	21.8	9.2	14.9	0.0	-	0.1
		31	20.5	8.7	15.5	0.4	-	0.2
	August	3	16.5	12.8	17.9	1.2	-	0.0
		7	22.0	13.3	17.8	8.3	-	0.0
		10	22.3	11.2	17.3	3.2	-	0.1
		14	25.7	12.8	16.6	1.2	-	0.0
		17	16.7	9.8	13.9	1.3	-	0.0
		21	16.3	6.0	11.3	0.0	-	0.0
		24	20.2	8.3	15.0	4.3	-	0.0
		27	20.0	10.2	14.4	0.3	-	0.0
		31	19.2	10.3	13.8	20.1	-	0.0
1998	July	1	23.0	3.2	15.1	7.1	1.0	3.4
		7	19.7	10.5	16.4	8.5	6.1	0.4
		13	14.8	7.3	11.6	0.3	2.1	0.1
		20	24.3	16.3	19.5	10.9	0.1	0.0
		27	24.3	10.3	17.4	0.3	0.0	0.1
1999	July	1	22.0	8.0	17.0	3.3	0.2	1.6
		7	16.2	7.8	12.2	4.3	4.3	1.0
		15	21.3	11.2	16.8	6.2	0.4	0.4
		22	22.2	10.0	15.5	0.0	0.4	0.0
		29	19.7	11.5	15.6	10.7	0.0	0.0
2000	July	1	19.2	8.7	13.9	5.1	0.0	0.0
		10	23.0	13.0	18.0	1.7	0.0	0.0
		18	23.7	10.3	17.0	0.7	0.0	0.0
		26	18.5	11.5	15.0	0.7	0.0	0.0

Appendix D3: Weather summary for Bonavista weather station with Little Catalina adult catch per trap per night, 1996-2000.

Year	Month	Day	Maximum temperature (°C)	Minimum temperature (°C)	Mean temperature (°C)	Precipitation (mm)	Adult catch per trap/night
1996	June	24	11.8	3.1	7.5	0.8	0.0
		27	14.8	5.9	10.4	5.7	0.0
	July	2	22.6	11.3	17.0	0.0	0.6
		5	23.1	13.1	18.1	4.7	3.5
		8	20.2	10.6	15.4	0.0	4.9
		11	20.5	11.7	16.1	0.7	4.7
		15	19.9	9.2	14.6	10.3	6.5
		18	21.4	11.7	16.5	3.7	3.8
		22	21.0	11.7	16.4	1.5	4.9
		25	16.7	9.4	13.1	0.0	1.6
		29	17.9	10.0	13.9	5.7	2.0
	August	1	25.1	12.8	19.0	0.0	2.1
		5	23.9	13.9	18.9	3.5	1.2
		8	25.0	16.3	20.7	0.0	1.0
		12	22.4	13.9	18.1	5.5	0.9
		15	19.4	12.7	16.0	0.7	0.4
		19	21.9	13.1	17.5	2.9	0.2
1997	June	30	10.6	6.4	8.5	0.0	0.0
	July	3	17.1	8.0	12.6	0.3	0.0
		7	21.6	11.5	16.5	3.3	0.1
		10	24.9	11.1	18.0	0.2	0.9
		14	19.8	11.2	15.6	1.5	1.8
		17	14.0	7.7	10.8	16.5	1.4
		21	21.4	12.3	16.9	0.0	1.3
		24	17.8	9.9	13.9	0.0	1.3
		28	15.4	5.5	10.5	0.2	0.6
		31	20.5	10.8	15.7	4.0	0.4
	August	4	21.6	13.5	17.6	3.2	0.1
		7	19.5	11.4	15.5	7.8	0.0
		11	23.3	13.0	18.2	0.3	0.1
		14	18.8	12.8	15.8	0.3	0.4
		18	17.9	12.3	15.1	5.2	0.1
		21	15.2	8.8	12.0	0.3	0.1
		25	16.1	10.3	13.2	0.3	0.0
1998	July	1	17.8	9.1	13.5	3.5	1.0
		7	17.9	11.6	14.8	4.7	2.2
		13	13.1	8.1	10.6	0.2	3.2
		20	25.2	14.8	20.0	2.5	0.0
		29	20.9	11.9	16.4	0.0	0.0
1999		1	17.6	10.2	13.9	2.3	0.0
		7	12.3	7.2	9.8	0.2	0.2
		14	18.6	10.4	14.5	6.5	1.5
		21	19.2	11.0	15.1	3.8	2.2
		28	20.7	12.5	16.5	3.0	1.5
	August	2	20.1	12.0	17.1	0.2	0.0
2000	July	1	16.8	8.6	12.8	1.9	0.0
		10	23.0	12.1	17.5	1.0	0.0
		19	21.6	13.0	17.3	4.2	0.0
		26	17.5	11.2	14.4	0.2	0.0
		31	19.0	12.0	15.5	0.0	0

Appendix D4: Weather summary for Long Harbour weather station with Chance Cove adult catch per trap per night, 1997.

Year	Month	Day	Maximum temperature (°C)	Minimum temperature (°C)	Mean temperature (°C)	Precipitation (mm)	Adult catch per trap/night
1997	June	30	12.5	6.8	9.7	0.1	0.0
	July	5	18.2	11.2	14.7	7.2	0.0
		10	22.5	10.8	16.7	4.6	0.4
		14	20.2	10.8	15.5	3.6	0.6
		17	17.3	9.8	13.6	1.9	0.5
		20	19.0	10.8	14.9	0.4	0.5
		23	17.3	11.2	14.3	0.0	0.3
		27	17.7	11.5	14.6	0.0	1.0
		30	18.2	11.2	14.7	4.0	1.2
	August	3	18.8	14.7	16.8	2.4	0.8
		6	21.0	14.0	17.5	5.1	0.3
		9	20.3	12.3	16.3	3.3	0.1
		13	20.3	15.0	17.7	0.0	0.0
		16	17.0	11.8	14.4	2.3	0.0
		19	16.7	12.5	14.6	0.7	0.0
		22	18.0	6.7	12.3	0.0	0.0
		25	19.2	12.3	15.8	2.4	0.0