Preliminary Studies on the Incidence of Needle Endophytes in some European Conifers

By F. E. Carroll, E. Müller and B. C. Sutton

University of Oregon, Dept. of Biology, Eugene, Oregon 97403, USA
Institut für Spezielle Botanik, ETH, CH 8092 Zürich, Schweiz
Commonwealth Mycological Institute, Kew, England

Summary

Symptomless fungal infections such as that discussed by Bernstein (1974) in Oregon (USA) in *Pseudotsuga menziesii* are also common in diverse European native and introduced conifers. The identification of the fungi can be done from cultures. Surface sterilized needle segments were planted on nutrient agar and the fungi were isolated from the growing colonies. It was not always possible to identify the fungi either because they would not sporulate or because they were not mentioned in the available literature. The frequency of occurrence of the common fungi is presented in charts.

Although the experimental work was done over a single year, and the results must be considered approximate, definite trends have been observed. The division of needles into segments has enabled us to differentiate fungi associated with the petiole from those associated with the needle proper. Special attention was given to the relationship between the age of the needle and the number and genera of fungi isolated. The frequency of occurrence was low in very young needles and increased with age, an indication that direct infection occurs from normal spore fall. On the other hand, a single example of possible systemic infection was encountered (i. e. *Guignardia philoprina* in *Taxus* needles).

The significance of the needle endophytes is not yet clear. Possible hypotheses must be further tested.

Zusammenfassung

Symptomlose pilzliche Infektionen, wie sie von Bernstein (1974) in Oregon für Nadeln von *Pseudotsuga menziesii* nachgewiesen wurden, sind auch in Europa bei verschiedenen autochtonen und eingeführten Koniferen weit verbreitet. Der Nachweis derartiger Pilze ist durch die Kultur möglich. Äusserlich sterilisierte Nadelstücke wurden auf sterile Agarnährböden ausgelegt und die herauswachsenden Pilzmyzelien isoliert. Soweit möglich wurden die Pilze bestimmt, doch war

dies nicht immer möglich, weil sie zum Teil nicht sporulierten, oder weil sie mit der zur Verfügung stehenden Literatur nicht erfasst werden konnten. Das Vorkommen einer grösseren Zahl von Pilzarten ist in Tabellen zusammengefasst.

Obschon die Untersuchungszeit nur ein Jahr umfasste und deshalb die Verhältnisse nur angenähert erfasst werden konnten, liessen sich aus den Ergebnissen einige allgemeine Tendenzen herauslesen. Die Zerteilung der Nadeln in einzelne Abschnitte liess einen Unterschied in der Zusammensetzung der Endophyten-Flora der Nadelbasis gegenüber den übrigen Abschnitten erkennen: Arten der Nadelbasis treten nur ausnahmsweise in den übrigen Nadelteilen auf. Besondere Aufmerksamkeit wurde der Beziehung des Nadelalters zur Zahl und zu den Arten der isolierten Pilze geschenkt. Ganz junge Nadeln enthalten nur wenige Pilze; die Artenzahl nimmt mit dem Alter sukzessive zu, was den Schluss zulässt, dass direkte Infektion durch Sporen den Normalfall darstellt. Anderseits scheinen auch vereinzelt systemische Infektionen möglich (z. B. *Guignardia philoprina* in *Taxus*-Nadeln).

Die Bedeutung der Nadelpilze ist noch nicht klar; die möglichen Hypothesen müssen noch genauer geprüft werden.

Introduction

During the past 50 years sporadic papers in the phytopathological and botanical literature have reported the presence of symptomless, endophytic fungal infections in the leaves and twigs of a variety of plants. Such infections were first discussed on the basis of microscopic examination of stained leaf and twig tissue in *Picea* and *Larix* (Lewis, 1924), in several grasses (Sampson, 1933, 1935, 1938, 1939; Neill, 1940), in *Casuarina* (Bose, 1947) and in *Helianthemum* (Boursnell, 1950). Subsequently culture studies have revealed the presence of latent fungal infections in the leaves and twigs of a great variety of evergreen tropical fruit plants (Rayner, 1948; Tokunaga and Yokohama, 1955; Schüepp, 1961; Tokunaga and Ohira, 1973). Most recently research by Bernstein (1974) in a stand of old-growth Douglas fir (*Pseudotsuga menziesii*) has demonstrated the almost ubiquitous presence of endophytic needle fungi in *Pseudotsuga menziesii*. The present investigation was carried out in an attempt to extend Bernstein's observations to other coniferous species.

Material and Methods

A. Species List and Collecting Sites

Branchlets of *Abies alba* Mill., *Picea excelsa* Link, *Pinus silvestris* L., *Pseudotsuga menziesii* (Mirb.) Franco and *Taxus baccata* L. were obtained from several sites in the vicinity of Zürich, Switzerland. One sample of *Picea excelsa* was collected at Ochsenalp (Schanfigg,

Graubünden) in Switzerland. Other specimens of *Pinus nigra* ARNOLD, *Pinus silvestris*, *Pseudotsuga menziesii* and *Sequoia sempervirens* (LAMB.) ENDL. were collected in the Fontainebleau forest near Paris, France.

B. Collecting Methods

Samples were collected by pruning branches from standing trees or from trees just felled to the ground. In this case, samples were separated into 3 groups: top, middle and bottom parts of the canopy. Sample branchlets were tagged for identification purposes, taken back to the laboratory and kept in the cold room at 4° C for 1 to 4 days.

C. Culture Work

Needles from each age class were chosen arbitrarily from several branchlets from a given tree. Needles were dipped briefly in 90% ethanol to wet the surface, and surface sterilized for 10 minutes in a solution of 2 parts Chlorox: 1 part water. The needles were cut in 2, 4 or 8 segments depending on needle size and the segments were transferred in serial order to 120 mm Petri plates containing 2% malt extract agar. The plates were incubated under fluorescent lights in a culture room at 21° C. Periodically they were checked for fungal growth. Fungal presence on each individual segment was recorded on appropriate data sheets and arbitrary numbers beginning with 1 were serially assigned to the fungi which appeared. When there was only vegetative growth, fungal mycelium was isolated from the bottom of the agar plates and transferred to 2% malt extract agar slants. When fruiting structures were present isolation was done by direct transfer of conidia or ascospores. Isolates which failed to sporulate after 4 weeks at 21° C were incubated for 4 to 8 weeks at 16° C under fluorescent lights.

Results

A. *Abies alba* MILL.

All samples were taken from felled trees (50—75 yrs old) in the Boswil forest (Aargau), Switzerland. Samples 1 and 2 were collected on Nov. 8, 1973, samples 3 and 4 on Dec. 20, 1973. Samples 1 and 3 came from the top part of the canopy; for sample 2, branchlets were taken from the top, middle and lower part of the canopy; sample 4 came from the middle part of the canopy.

Fungi commonly found are entered in charts; the rare isolates are listed and indexed for origin (petiole P vs. needle proper N) and age (number between two dots). Unidentified fungi are listed by their isolate code letter and number, e. g. A 42 isolate # 42 from *Abies alba*.

1. *Abies alba* # 1

(i) see chart

(ii) Rare isolates: *Geniculosporium* sp. (P. 4.), *Sclerophoma pythiophila* (Corda) v. Höhn. = *Dothichiza pithiophila* (Corda) Petr. (P. 1.).

2. *Abies alba* # 2

(a) Top — Rare isolates only: *Phomopsis occulta* Trav. (P. 5.), *Sclerophoma pythiophila* (P, N. 3.), *Acremonium* sp. (N. 1.), *Cladosporium* sp. (P, N. 2.)

(b) Middle

(i) see chart

(ii) sporadic occurrence: *Sclerophoma pythiophila* (P. 8—10., N. 1. 2. 4. 6.), *Geniculosporium* sp. 1. (P. 3. 5. 6. 8., N. 1.), *Geniculosporium* sp. 2. (P. 3. 6. 8.).

(c) Bottom

(i) see chart

(ii) rare isolates: *Geniculosporium* sp. 1 (P. 5.), *Geniculosporium* sp. 2 (P. 7. 8.).

3. *Abies alba* # 3

(i) see chart

(ii) sporadic occurrence: Imperfect state *Xylaria* (P. 1. 5., N. 6.), *Geniculosporium* sp. 1 (P. 6., N . 2. 5.), *Sclerophoma pythiophila* (P. 3., N. 2. 4.).

(iii) rare isolates: *Phomopsis* sp. (P. 1.), *Neohendersonia kickxii* (Westd.) Sutton & Pollack (P. 4.), *Asterosporium strobilorum* Roum. & Fautrey (P. 4.), *Phaeosphaeria* sp. (N. 2.).

4. *Abies alba* # 4

(i) see chart

(ii) sporadic occurrence: Imperfect state *Xylaria* P. 8., N. 4.), *Geniculosporium* sp. 1 (P. 7., N. 4.), *Microsphaeropsis* sp. 1 A 39 (N. 2.), *Microsphaeropsis* sp. 2 A 42 (N. 4.), *Pyrenochaeta* sp. (P. 5.), *Phlyctaena* sp. (N. 7.), *Cytosporella* sp. (P. 4.).

B. *Picea excelsa* Link.

All samples were collected in Switzerland: sample 1 in the Zürichberg forest (Zürich) on Oct. 29, 1973; sample 2 in the Boswil forest (Aargau) on Dec. 20, 1973; sample 3 at Ochsenalp, elevation 1600 m (Graubünden) on Jan. 13, 1974. Samples 1 and 2 were taken from felled trees, sample 3 was obtained by pruning off branchlets of the bottom part of the canopy.

1. *Picea excelsa* # 1

(i) sporadic occurrence: *Sirococcus strobilinus* PREUSS (P. 1. 2.), Imperfect state *Xylaria* (N. 3.).

(ii) rare isolates: *Codinaea simplex* HUGHES & KENDRICK.

2. *Picea excelsa* # 2

(i) see chart

(ii) sporadic occurrence: *Sclerophoma pythiophila* (P. 1. 3.), *Phlyctaena* sp. (P. 4.), Pc 31 = Sphaeropsidales (P. 3.).

(iii) rare isolates: *Alternaria* sp. (P. 2.), *Naemacyclus* sp. (P. 4.), *Epicoccum purpurascens* EHRENB. ex SCHLECHT. (P. 5.), *Phomopsis occulta* (P. 5.), Imperfect state *Xylaria* (P. 5.), *Geniculosporium* sp. (P. 6.), *Cytosporella* sp. (P. 5.), *Cryptocline conigena* (SACC. & ROUM.) ARX. (P. 6.).

3. *Picea excelsa* # 3

(i) see chart

(ii) sporadic occurrence: Pc 20 = Sphaeropsidales (P. 3. 4.), *Atichia* sp. (P. 3. 4.), *Sporonema* sp. (P and N. 5.), Pc 43 sterile (P. 7.), Pc 47 sterile (P. 7. 9.).

(iii) rare isolates: *Naemacyclus* sp. (P. 11.), Pc 44 sterile (P. 8.), Pc 46 sterile (P. 8.).

C. *Pinus nigra* ARNOLD

Two samples were collected by pruning off branchlets from the lower part of the canopy of standing trees in the Fontainebleau forest (Seine et Marne) France. Sample 1 was collected on Jan. 5, 1974, sample 2 on May 4, 1974.

1. *Pinus nigra* # 1

(i) see chart. Note: The isolate of *Geniculosporium serpens* produced stromata and perithecia of *Hypoxylon serpens* var. *serpens* J. H. MILLER in culture.

(ii) sporadic occurrence: *Sclerophoma pythiophila* (N. 1. 2.), *Sporonema* sp. (P. 4., N. 2.), *Pragmopycnis* sp. (P. 2. 4.).

(iii) rare isolates: PnN 5 sterile (N. 2.), PnN 8 sterile (N. 2.).

2. *Pinus nigra* # 2

(i) see chart

No other fungal growth was observed besides that reported on the chart.

D. *Pinus silvestris* L.

The first sample was taken from a felled tree in the Boswil forest (Aargau) Switzerland, on Nov. 8, 1973. The second one was obtained from the lower part of the canopy of a standing tree in the Fontainebleau forest (Seine et Marne) France on Jan. 6, 1974.

1. *Pinus silvestris* # 1

(i) sporadic occurrence: Pn 8 = *Lophodermium pinastri* (Schrad. ex Fr.) Chev. (P. 1. 2. 3.), *Sclerophoma pythiophila* P. 2. 3., N. 1. 2. 3.), Imperfect state *Xylaria* (P. 2. 3.), *Naemacyclus minor* Butin (N. 2. 3.), Pn 11 = Sphaeropsidales (N. 2. 3.). (ii) rare isolate: *Geniculosporium* sp. (P. 3.).

2. *Pinus silvestris* # 2

(i) see chart

(ii) sporadic occurrence: *Sclerophoma pythiophila* (P. 1. 3., N. 2.), Pn 17 = Sphaeropsidales (P. 1. 3., N. 3.).

(iii) rare isolates: *Naemacyclus minor* (N. 3.), *Atichia* sp. (P. 2.), *Coryneum brachyurum* Lk. state of *Pseudovalsa lanciformis* (Fr.) Ces. and de Not. (P. 1.), *Endomelanconium pini* (Corda) Petrak (P. 3.).

E. *Pseudotsuga menziesii* (Mirb.) Franco

All samples were obtained by pruning off branches from the lower part of the tree canopy. Two samples were collected in young plantations in Switzerland: sample 1 was taken from 6—7 yr old trees in the Boswil forest (Aargau) on Dec. 20, 1973 and sample 2 was taken from 10—12 yr old trees in the Adlisberg forest (Zürich) on the same day. Sample 3 was collected from an isolated 20 yr old tree in the Chenoise farm (Marne) France on Jan. 5, 1974. Samples 4 and 5 were collected from 50—60 yr old trees in the Fontainebleau forest (Seine et Marne) France on Jan. 6, 1974 and May 4, 1974, respectively.

1. *Pseudotsuga menziesii* # 1

(i) rare isolates only: *Phomopsis occulta* (N. 2.); *Hemidothis* sp. (P. 3.); *Cytospora* sp. (N. 2.); *Tripospermum myrti* (Lind) Hughes (N. 1.), *Sclerophoma pythiophila* (N. 3.); DF 6 sterile (N. 3.), DF 10 Sphaeropsidales (N. 3.).

2. *Pseudotsuga menziesii* # 2

(i) see chart

(ii) rare isolates: *Phomopsis occulta* (P. 4.), *Geniculosporium* sp. (P. 4.), DF 12, Sphaeropsidales (N. 2.).

3. *Pseudotsuga menziesii* # 3
(i) rare isolates only: DF 17 sterile (P. 1.), *Cryptocline* sp. (P. 1.), *Macroventuria wentii* v. der Aa (P. 2.), *Phlyctaena* sp. (N. 2.).

4. *Pseudotsuga menziesii* # 4
(i) see chart
(ii) rare isolates: DF 21 Sphaeropsidales (N. 2. 7.), *Phialophora* sp. (P. 3.), *Naemacyclus* sp. (N. 7.).

5. *Pseudotsuga menziesii* # 5
(i) rare isolates only: *Hemidothis* sp. (P. 7.).

F. *Sequoia sempervirens* (Lamb.) Endl.

Two samples were obtained by pruning off branchlets from the lower part of the canopy of standing trees in the Fontainebleau forest (Seine et Marne) France. Sample 1 was collected on Jan. 5, 1974, sample 2 on May 4, 1974.

1. *Sequoia sempervirens* # 1
(i) see chart
(ii) sporadic occurrence: *Aureobasidium pullulans* (de Bary) Arnaud. (P. 4. 5., N. 4.), *Pseudopatellina* sp. (P. 7., N. 10.).
(iii) rare isolates: S 20 Sphaeropsidales (P. 5.), *Microsphaeropsis* sp. S 11 (N. 10.), *Phomopsis* sp. (P. 6.).

2. *Sequoia sempervirens* # 2
(i) sporadic occurrence: *Cryptosporiopsis abietina* Petrak (P. 6.), *Trimmatostroma salicis* Corda (N. 6.).
(ii) rare isolates: *Hemidothis* sp. (P. 3.).

G. *Taxus baccata* L.

The samples were obtained from old-growth stands in the vicinity of Zürich (Switzerland). Sample 1 was collected at Uetliberg on Nov. 13, 1973, sample 2 at Felsenegg on Nov. 18,1973.

1. *Taxus baccata* # 1
(i) see chart
(ii) sporadic occurrence: *Geniculosporium* sp. (P. 3., N. 2.).
(iii) rare isolates: *Oedocephalum* sp. (N. 2.), T 4 Sphaeropsidales (N. 3.).

2. *Taxus baccata* # 2
(i) see chart
(ii) rare isolates: T 5 sterile (N. 4.), *Cryptosporiopsis abietina* (N. 5.).

Abies Alba # 1 top of canopy

	Yr.	1	2	3	4
	S	20	15	15	12
Phomopsis occulta Trav.	P		20%	20%	
	N				
Cytosporella sp.	P	15%	20%	40%	8.3%
	N				

Abies alba # 2 bottom of canopy

	Yr.	1	2	3	4	5	8—9
	S	10	10	5	5	6	6
Phomopsis occulta Trav.	P	30%	30%	40%			
	N						
Cytosporella sp.	P		10%			83.3%	50%
	N						

Abies alba # 2 middle of canopy

	Yr.	1	2	3	4	5	6	7	8	9	10
	S	15	10	13	12	15	10	5	5	5	5
Phomopsis occulta Trav.	P	13.3%									
	N										
Cytosporella sp.	P	6.7%	30%		33.3%		10%	40%			40%
	N										

Abies alba # 3

	Yr.	1	2	3	4	5	6
	S	12	13	16	10	6	8
Phomopsis occulta Trav.	P	16.7%		12.5%			
	N						
Cytosporella sp.	P	25%	30.8%	6.3%	40%	16.7%	25%
	N						

Abies alba # 4

	Yr.	1	2	3	4	5	6	7	8
	S	15	16	20	40	30	30	20	10
Phomopsis occulta Trav.	P	13.3%	6.2%		5%	6.7%		5%	5%
	N								
Cryptosporiopsis sp.	P					3.3%			
	N					11.1%	11.1%	1.7%	20%

Picea excelsa # 2

	Yr.	1	2	3	4	5	6
	S	35	37	26	26	28	18
Dothistroma sp. 1	P	8.6%	32.4%	19.2%	38.5%	10.7%	11.1%
	N	0.9%					
Dothistroma sp. 2	P		5.4%	11.5%	3.8%	3.6%	
	N						
Plectophomella	P			3.8%			
sp.	N	0.9%		3.8%	2.6%	1.2%	6.2%

Pinus nigra # 1

	Yr.	1	2	3	4
	S	9	6	6	6
Naemacyclus niveus	P				16.7%
(Pers. ex Fr.) Fuck.	N	11.1%	22.5%	23.8%	71.4%
ex Sacc.					
Geniculosporium serpens	P	11.1%	16.6%	33.3%	16.7%
Chesters and Greenhalgh	N	1.6%			

Pinus nigra # 2

	Yr.	1	2	3	4
	S	6	6	6	8
Naemacyclus niveus	P				
(Pers. ex Fr.) Fuck.	N	19%	64.3%	78.6%	98.2%
ex Sacc.					

Pinus silvestris # 2

	Yr.	1	2	3	4
	S	20	22	14	6
Rhinocladiella sp.	P				
	N	1.7%	4.5%	11.9%	38.9%
Leptostroma pinastri	P				
Desm.	N		4.5%	28.6%	5.6%
Hemidothis sp.	P		18.2%	14.3%	66.6%
	N				
Pragmopycnis sp.	P	25%	4.5%		
	N				

Picea excelsa # 3

	Yr.	1	2	3	4	5	6	7	8	9	10	11	12
	S	26	24	28	24	22	13	23	19	19	11	14	7
Microsphaeropsis sp.	P			14.3%	12.5%	4.5%			26.3%	5.3%			14.3%
	N												
Phlyctaena sp.	P				4.2%		15.4%	8.7%		15.8%		7.1%	
	N												
Libertella sp.	P					13.6%	30.8%	13%		15.8%			
	N					1.5%							
Myriellina sp.	P				8.3%	4.5%	15.4%	30.4%	5.3%	10.5%	18.2%	7.1%	28.5%
	N												
Plectophomella sp.	P									5.3%		14.3%	
	N							1.4%	8.8%	1.7%		14.3%	

Sequoia sempervirens # 1

	Yr.	1	2	3	4	5	6	7	8	9	10
	S	30	30	32	28	18	12	11	8	7	8
Hemidothis sp.	P			6.2%	32.1%	16.7%					
	N			2.4%		8.3%	2.8%	9.1%			
Cryptosporiopsis abietina Petrak	P							63.6%	87.5%	71.5%	75%
	N						2.8%				
Trimmatostroma salicis Corda	P										
	N				3.6%			9.1%	50%	14.3%	
Plectophomella sp.	P					5.6%		9.1%		14.3%	25%
	N			1.2%				36.4%	37.5%		11.1%

Pseudotsuga menziesii # 2

	Yr.	1	2	3	4	5
	S	25	25	35	14	16
Imperfect state	P	4%	4%	11.4%	28.6%	37.5%
Xylaria	N	1.3%		0.9%	14.3%	14.6%

Pseudotsuga menziesii # 4

	Yr.	1	2	3	4	5	6	7	8
	S	20	30	30	30	28	20	15	6
Hemidothis sp.	P			6.7%		14.3%	15%	20%	50%
	N								

Taxus baccata # 1

	Yr.	1	2	3	4	5
	S	5	12	8	5	5
Phyllosticta	P	80%	100%	100%	100%	100%
concentrica Sacc.	N	80%	46.9%	70.8%	100%	86.7%

Taxus baccata # 2

	Yr.	1	2	3	4	5
	S	10	5		6	5
Phyllosticta	P		20%		100%	80%
concentrica Sacc.	N	6.6%	13.3%		77.8%	40%

Captions for Charts

Yr: needle age classes
S: size of sample in number of needles (S box empty = no sample)
P: percentage of petiole segments on which fungus X was observed
N: percentage of needle segments (whole needles without petiole end) on which fungus X was observed
(P and/or N box empty = 0% occurrence)

A *Abies alba*
Pc *Picea excelsa*
Pn N *Pinus nigra*
Pn *Pinus silvestris*
DF *Pseudotsuga menziesii*
S *Sequoia sempervirens*
T *Taxus baccata*

Discussion

Since this study was limited to a single calendar year (1973—74) the results reported do not provide information on seasonal variation. The sample size was small and therefore inadequate for statistical analysis. However, certain trends have been observed. The division of the needles into segments before culturing gives some information on the location of the fungus within the needles. This procedure has enabled us to distinguish the petiole-associated fungi from those which appear on the rest of the needle. But for a few exceptions, petiole-associated fungi are not found on the lamina of the needle and vice versa.

In *Abies alba* the most common petiole-associated fungi are a species of *Cytosporella* and *Phomopsis occulta*. Bose (1947) has observed a symbiotic relationship between *Phomopsis casuarinae* F. Tassi and *Casuarina equisetifolia* Forst. Rayner (1948) notes the presence of *Phomopsis* sp. in *Coffea arabica* L. with no obvious symptoms. Bolay et al. (1968) report that *Phomopsis viticola* Sacc. is responsible for the dead-arm disease of grape. Other species of *Phomopsis* have been found in needles of *Sequoia sempervirens* and *Picea sitchensis* (Bong.) Carr. in the Pacific Northwest (F. Carroll, unpublished). The role of *Phomopsis* as a symbiont, a weak parasite or a serious pathogen in some conifers has yet to be determined.

Only one species, A 43, was commonly found in the *Abies* needle proper. Although its taxonomic position is still undecided, it should be stressed that it was present only on needles 5—8 years old.

An extensive study of the mycoflora of *A. alba* needles has been published by Gourbiere (1974a, 1974b, 1975). Due to the methodology used, a clear distinction between the epiphytic and endophytic flora cannot be made and direct comparison of his and our results is impossible. However, it is interesting to note that *Cytospora* sp. found by Gourbiere in the Mont Pilat forest in France, was never isolated from the Swiss stand.

A variety of fungi were isolated from petioles of *Picea excelsa*, while only one (*Plectophomella* sp.) was commonly found associated with the needle proper.

In the spring, the *Pinus nigra* needle-associated mycoflora consisted exclusively of *Naemacyclus niveus*. A few other isolates were obtained from the petiole and needles in the fall samples from the same area. According to Butin (1973) *N. niveus* is considered saprophytic by some authors and responsible for needle-cast by others.

The *Pinus silvestris* samples had both petiole- and needle-associated fungi. One of the common needle fungi, *Leptostroma pinastri* [= st. imp. *Lophodermium pinastri* (Schrad. ex Fr.) Chev.] is known to cause needle cast and to occur in living needles in its spermogonial form

(GREMMEN, 1959). KENDRICK and BURGES (1962) report that in Delaware Forest (Cheshire, England) 40% of living *P. silvestris* needles are infected by *L. pinastri* in the spring; at this stage there are no obvious symptoms.

For *Pseudotsuga menziesii*, the mycoflora varied greatly with collecting sites. Both samples from the Foret de Fontainebleau had the same petiole associated fungus, *Hemidothis* sp. The frequency of occurrence in spring was greatly reduced when compared to that of the winter. A fungus, tentatively called the imperfect state of a *Xylaria*, was commonly isolated from the petiole as well as the needles of 10—12 yr old trees in the Swiss plantation. None of the fungi isolated during this study fits the descriptions given by BERNSTEIN (1974) for endophytes found in old-growth Douglas firs in the Oregon Cascades. However, recent work done by one of us (FEC unpublished) shows that the imperfect state of the *Xylaria* is also present in the needles of mature trees along the Oregon coast.

Direct comparison of the data obtained from the *Sequoia sempervirens* samples can be made since they both came from the same locality. The frequency of occurrence of endophytes is higher in winter than in spring. Some of the fungi are present in the petiole as well as the needle proper (*Hemidothis* sp. and *Plectophomella* sp). *Hemidothis* sp. is restricted to needles 1—7 yrs old while *Cryptosporiopsis* sp. appears in those 7—10 yrs old. This suggests that there is a succession in the petiole endophytic flora of *Sequoia*. *Trimmatostroma salicis* occurs only in older needles. According to MILLAR (1974) *T. salicis* grows and sporulates on living coniferous needles. MILLAR states that as the needles age, the cuticular waxes weather, leading to a decrease in resistance to pathogens. Such a phenomenon could explain the endophytic presence of *T. salicis* in the older needles.

In *Taxus baccata*, *Phyllosticta concentrica*, the conidial state of *Guignardia philoprina* (BERK. et COURT.) van der AA, represents the single widespread endophyte with a frequency of occurrence in petiole and needle often reaching 100%. *G. philoprina* is commonly found in *Taxus* litter (MÜLLER, unpublished). HUDSON (1962) reports the presence of *G. citricarpa* Kiely in the leaf blades of *Saccharum officinarum* L. "with symptoms that are either inconspicuous or virtually absent." *G. citricarpa* is also the cause of systemic infections in *Citrus* sp. (SCHÜEPP, 1961). Since the fungus is present in petioles as well as laminae, we believe that the infection in *Taxus* is systemic. This could be proved by direct staining techniques and/or culture of other parts of the tree.

During this study fungi known to be common members of the phyllosphere epiflora (see PUGH and BUCKLEY, 1971a) were occasionally isolated. Several isolates of *Aureobasidium pullulans* were cultured and

could be distinguished from one another by different growth rates, pigmentation and aspect of the colonies. After several weeks of incubation most of the isolates were determined as *Sclerophoma pythiophila* sensu BUTIN (1963) and scored as such. The occasional presence of *A. pullulans* in conifer needles can be interpreted as a contamination from the epiflora. In pine needles, however, BATKO et al. (1958) find that *S. pythiophila* colonizes injuries caused by insects and can lead to early defoliation.

While the nature and role of the phyllosphere epiflora is fairly well documented (see PREECE and DICKINSON, 1971), little is known about the phyllosphere endophytic mycoflora. For several hosts a symbiosis or latent infection not restricted to leaves but present in the whole plant has been demonstrated (SAMPSON, 1933, 1935, 1938, 1939; NEILL, 1940; BOSE, 1947; BOURSNELL, 1950; PUGH and BUCKLEY, 1971b). In those cases, the fungus was shown to be present from the seed stage to the mature plant. For other hosts, spreading via mycelial growth was proved by grafting experiments (SCHÜEPP, 1961; BOLAY et al., 1968).

BERNSTEIN (1974) has studied the microbial epi- and endoflora of Douglas fir living needles. She has shown that an alga *Protococcus viridis*, several yeasts, *A. pullulans* and *Atichia* sp. are common surface dwellers; the interior of the needles is commonly inhabited by one or two fungi, neither of which has been identified although they sporulate readily in culture. BERNSTEIN notes that the fungal endophytes become established during the first year of the needle life with 0% occurrence at 5 months, 10—30% at 10—12 months, and 60% at 17 months. Her results strongly suggest that the infection is not systemic but is initiated by spores. We believe this process occurs for fungi associated with the needle proper (e. g. *Cryptosporiopsis* sp., *Plectophomella* sp., *Naemacyclus niveus*). Overwintering stages are probably present in the tree canopy within the lodged litter, thus providing an inoculum easily dispersed by air currents or rain.

Petiole-associated fungi are assumed to be also twig-dwellers. Indeed, culture of surface-sterilized twigs have shown some common petiole fungi to be associated with the cortex of the twigs but not with the vascular system (F. CARROLL, unpublished). The distinction between the petiole and the needle endophytic mycoflora has been further demonstrated by substrate utilization tests performed on the most common isolates (G. CARROLL, unpublished). These show that pectin is utilized by almost all of the fungi, lignin is utilized to a limited extent by needle fungi but not by petiole fungi, and the ability to break down cellulose, xylan, mannan, and galactan is restricted to the petiole fungi. These results suggest that needle fungi are dependent on the host for simple carbon sources while petiole fungi are more active decomposers.

The phyllosphere fungi, epiphytes and endophytes play an important role in coniferous litter decomposition (see KENDRICK and BURGES, 1962; HARLEY, 1971; MILLAR, 1974). The presence of endophytic fungi in living needles may protect them against infection by active needle parasites, allow the reabsorption of organics and minerals from leachates, and lead to the redistribution of nutrients prior to needle fall. Some of these hypotheses are being currently investigated as part of an intensive study on the incidence and significance of coniferous needle and twig endophytes in the Pacific Northwest.

Acknowledgments

We wish to thank the director and colleagues of the Swiss Forest Research Institute, Birmensdorf, Switzerland, especially Dr. G. BAZZIGHER and Mr. F. HUMM from the Institute of Special Botany, Swiss Federal Institute of Technology, for their cooperation during this project.

References

BATKO, S., MURRAY, J. S. and PEACE, T. R. (1958). *Sclerophoma pithyophila* associated with needle cast of pines and its connexion with *Pullularia pullulans*. — Trans. Br. Mycol. Soc. **41**: 126—128.

BERNSTEIN, MARY E. (1974). Microbial populations of Douglas fir needles. — Ph. D. Thesis, University of Oregon, Eugene.

BOLAY, A., SEMECNIK, A. and DUCROT, V. (1968). L'excoriose de la vigne. — Agriculture romande VII (4) série A 45—50.

BOSE, S. R. (1947). Hereditary (seed-borne) symbiosis in *Casuarina equisetifolia* Forst. — Nature (London) **159**: 512—514.

BOURSNELL, J. G. (1950). The symbiotic seed-borne fungus in the Cistaceae. — Ann. Bot. (London) N. S. **14**: 217—243.

BUTIN, H. (1973). Morphologische und taxonomische Untersuchungen an *Naemacyclus niveus* (PERS. ex FR.) FUCK. ex SACC. und verwandten Arten. — Eur. J. For. Path. **3**: 146—163.

GOURBIERE, F. (1974a). Les champignons microscopiques liés aux aiguilles de sapin (*Abies alba* MILL.). 1. Premiers résultats. — Bull. Soc. Mycol. Fr. **90**: 89—96.

— (1974b). Les champignons microscopiques liés aux aiguilles de sapin (*Abies alba* MILL.). 2. Variations saisonnières de la microflore des aiguilles tombantes. — Bull. Soc. Mycol. Fr. **90**: 325—333.

— (1975). Les champignons microscopiques liés aux aiguilles de sapin (*Abies alba* MILL.). 3. Microflore des aiguilles vivantes. — Bull. Soc. Mycol. Fr. **91**: 429—441.

GREMMEN, J. 1959. A contribution to the mycoflora of pine forests in the Netherlands. — Nova Hedwigia **1**: 251—288.

HARLEY, J. L. (1971). Fungi in ecosystems. — J. Ecol. **59**: 653—668.

HUDSON, H. J. (1962). Succession of microfungi on aging leaves of *Saccharum officinarum*. — Trans. Br. Mycol. Soc. **45**: 395—423.

KENDRICK, W. B. and BURGES, A. (1962). Biological aspects of the decay of *Pinus sylvestris* leaf litter. — Nova Hedwigia **4**: 313—342.

LEWIS, F. J. (1924). An endotropic fungus in the Coniferae. — Nature (London) **114**: 860.

MILLAR, C. S. 1974. Decomposition of coniferous leaf litter. pp. 105—128, In: Biology of Plant Litter Decomposition, Vol. I. C. H. DICKINSON and G. J. F. PUGH, eds. Academic Press, London and New York.

NEILL, J. C. (1940). The endophyte of Rye-grass (*Lolium perenne*). — N. Z. J. Sci. Tech. **21**: 280—291.

PUGH, G. J. F. and BUCKLEY, N. G. (1971a). The leaf surface as a substrate for colonization by fungi. pp. 431—445, In: Ecology of Leaf Surface Microorganisms. T. F. PREECE and C. H. DICKINSON, eds. Academic Press, London and New York.

— — (1971b). *Aureobasidium pullulans*: An endophyte in sycamore and other trees. — Trans. Br. Mycol. Soc. **57**: 227—231.

RAYNER, R. W. (1948). Latent infection in *Coffea arabica* L. — Nature (London) **161**: 245—246.

SAMPSON, K. (1933). The systemic infection of grasses by *Epichloe typhina* (PERS.) TUL. — Trans. Br. Mycol. Soc. **18**: 30—47.

— (1935). The presence and absence of an endophytic fungus in *Lolium temulentum* and *L. perenne*. — Trans. Br. Mycol. Soc. **19**: 337—343.

— (1938.) Further observations on the systemic infection of *Lolium*. — Trans. Br. Mycol. Soc. **21**: 84—97.

— (1939). Additional notes on the systemic infection of *Lolium*. — Trans. Br. Mycol. Soc. **23**: 316—319.

SCHÜEPP, H. (1961). Untersuchungen über *Guignardia citricarpa* KIELY, den Erreger der Schwarzfleckenkrankheit auf *Citrus*. — Phytopathol. Z. **40**: 258—271.

TOKUNAGA, Y. and OHIRA, I. (1973). Latent infection of anthracnose on *Citrus* in Japan. — Rept. Tottori Mycol. Inst. (Japan) **10**: 693—702.

— and YOKOHAMA, M. (1955). Latent infections associated with some fruit diseases. In: Jubilee Publication in Commemoration of the Sixtieth Birthday of Professor Tochinai and Professor Fukushi. pp. 249—254. Minato-ku, Tokyo.