Beech bark mycoflora and its distribution in relation to the presence of the scale insect, *Cryptococcus fagisuga* Lind.

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Beech trees in several locations in the Toronto area were examined for bark fungi and their distribution in relation to diameter of trees and scale insect ***(Cryptococcus fagisuga*** Lind.) infestation classes. The distribution of some of these fungi indicated a possible role in the development of infestations of beech trees by the scale insect.

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On a examine des bouleaux situés à differents endroits de la region de Toronto afin de déceler la presence de champignons corticoles et de determiner leur distribution en fonction du diamètre des arbres et des categories d'infestation par la cochenille ***(Cryptococcusfagisuga*** Lind.). La distribution de certains champignons indique que ceux-ci favorisent peut-être I'infestation du bouleau par la cochenille.

Introduction

Fernandez and Boyer (1988) reported on the presence and distribution of one of the known causal organisms of Beech Bark Disease, the scale insect *Cryptococcus* fagisuga Lind., in the Toronto area. Assays for the presence of bark fungi yielded no evidence of the presence of the other causal agent, the fungus ***Nectria coccinea*** var. *faginata* Lohman, Watson & Ayres (Shigo, 1963).

Houston ***et al.*** (1979) found the patterns of colonization of individual beech trees by *C.* fagisuga to be markedly influenced by the bark flora, and reported positive and negative associations with respect to different organisms. Given the potential of the bark microflora to influence the development and course of disease (Bier, 1963; Bier and Rowat, 1962) it would be interestingto determine whether the distribution of the beech scale insect reported by Fernandez and Boyer (1988) is correlated with the presence of any inhabitant of the bark which could potentially be considered as biological control agent. This represents a preliminary survey of the bark fungi of *C.* fagisuga-free and infested beech trees in the Toronto area.

Materials and methods

A total of six stands, in mixed maple beech communities around the Toronto region, were examined in the summer of 1982. Trees were classified according to diameter class (3-10, 10-17, 17-24, 24-31, 31-38, 38-45, 45-55, and > 55 cm) and infestation with C. fagisuga (0, 1-25, 25-50, 50-75, and 75-110 colonies/25 cm^2) as reported by Fernandez and Boyer (1988).

Within each stand, trees representative of diameter and scale insect infestation classes were sampled for epiphytic fungi, with a total of 47 trees being examined. A 5 cm^2 portion of the bark was rubbed with a moist sterile cotton pad, which was then gently pressed on Oxoid malt agar (1.2%) plates amended with streptomycin. To obtain an estimate of the abundance of the fungi, the swab was placed in 75 cc of sterile distilled water, and after shaking for 5 minutes, 0.5 ml was spread on the same medium, each sample being replicated four times. Plates were incubated in the dark at 25°C for 7 days.

Most fungi were identified to genera or species. Frequency of isolation was calculated as the percentage number of trees from which a fungus was isolated at least once, and abundance as the average number of colonies of that fungus per plate.

Results

Thirty-four species of fungi were isolated from the bark of the 47 beech trees examined (Table 1). The most frequent and abundant fungi were ***Aureobasidium pullulans*** (de Bary) Arnaud, ***Cladosporium herbarum*** (Pers.) Link ex S.F. Gray, and a 'White Yeast'. Other frequently isolated and/or abundant fungi were: ***Alternaria alternata*** (Fr.) Keissler, ***Aposphaeria*** sp., ***Gliocladium roseum*** Bain., ***Fusarium*** sp., ***Papulospora*** sp., ***Trichoderma viride*** Pers. ex S.F. Gray, and the unidentified fungi BC-13, OP-23, and a 'Pink Yeast'.

Chi-square tests for number of species present (Table 2) indicated that there was no significant difference in the mean number of species among diameter classes (χ^2 (.05), df: 7, 6.81, $.3 < P < .5$). Any attempt to correlate the presence of bark fungi with scale insect infestation classes is limited by the small sample size of the two highest infestation classes (Table 3). However, some trends were apparent. Examination of the distribution of these fungi among trees in the different scale insect infestation classes revealed that some were more frequently isolated from trees in the lower than the higher infestation classes, or non-infested trees (Table 3). (Chi-square on contingency table: χ^2 (.05), df: 3, 13.18, $.001 < P < .01$; Mann-Whitney test indicated the difference lay between the 1-25 class and the rest).

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Table 1. Percent isolation and abundance of bark fungi from beech trees in the Toronto area.

Fungus	Percent Isolation	Abundance No, Colonies/ Plate)
Alternaria alternata (Fr.) Keissler	44.4	1.0
Aposphaeria **sp.**	23.8	58.2
Arthrinum **sp.**	1.6	6.5
Aspergillum niger van Tieghem	7.9	2.2
Aureobasidium pullulans (de Bary) Arnaud	61.9	15.0
Camarosporium **sp.**	1.6	1.0
Cladosporium herbarum (Pers.) L. ex S.F. Gray	84.1	13.2
Coniothyrium **sp.**	4.8	1.0
Cytospora sp.	3.2	1.0
Epicoccum nigrum Link.	1.6	1.0
Fusicoccum **sp.**	9.5	7.5
Fusarium **sp.**	4.8	14.6
Cliocladium roseum Bain.	23.8	20.8
Mortierella ramanniana (Moller) Linnem.	3.2	2.0
Mortierella **sp.**	6.4	1.0
Mucor sp.	39.7	1.3
Cylindrocarpon **sp.**	18.0	2.0
Nematogonium **sp.**	3.2	2.5
Nigrospora sp.	7.9	1.0
Papulospora **sp.**	36.5	4.3
Penicillium **sp.**	30.2	3.0
Pestalotia **sp.**	3.2	1.0
Phoma **sp.**	1.6	2.0
Trichoderma viride Pers. ex S.F. Gray	31.8	3.5
Unidentified filamentous fungi		
BC-13	17.5	1.8
DW-8	3.2	1.0
GC-34	3.2	1.0
MS-1	3.2	1.0
OP-23	38.1	28.0
PC-35	7.9	42.2
Yeasts		
Black Yeast	9.5	6.0
Pink Yeast	34.9	9.0
White Yeast	85.7	26.2

Table 2. Richness of bark fungi **by** diameter class.

Diameter Class (cm)	No. Trees Sampled	Mean No. Fungal Species +SE
3 - 10	5	5.8 + 0.8
10+ - 17	12	7.1 + 0.9
17+ - 24	9	7.0 + 1.0
24+ - 31	7	7.8 + 0.9
31+ - 38	6	8.2 + 0.7
38+ - 45	5	6.0 + 0.8
45+ - 55	2	10.0 + 1.0
>55	1	7.5

Table 3. Richness of bark fungi by scale insect infestation class.

Infestation Class (colonies/25 cm^2)	No. Trees Sampled	Mean No. Fungal Species +SE
0	16	6.3 + 0.8
1 - 25	22	8.3 + 0.4
25+ - 50	6	7.0 + 0.5
75+ - 110	3	4.5 + 0.8

Fungi most frequently isolated from non-infested or lightly-infested trees (Table 4), and thus could possibly play a role in development of infestations, were ***A.*** *pullulans*, *G.* ***roseum*** and *T.* ***viride.*** C. *herbarum* and the 'White Yeast' seemed to be isolated with a high frequency regardless of the level of scale infestation of the tree.

Discussion

This survey of beech bark fungi revealed a large mycoflora present in trees of different sizes and stands, but with a very limited number of frequently isolated fungi (*i.e.* 'residents'). Despite the fact that there was a higher number of fungi isolated from lightly-infested trees than from non-infested ones, the majority of the most abundant and/or frequent fungi were present in both scale-infested and non-infested trees. Cotter and Blanchard (1982) isolated similar genera from American beech trees in New Hampshire, but reported that **most** of them were also isolated with similar frequencies from trees with than without Beech Bark Disease. In our study, the higher number of fungi isolated from lightly-infested trees than from non-infested ones may reflect the utilization of habitats created by the effects of colonization by the insect. Age, however, does not seem to give a similar increase on the number of bark fungi present.

Table 4. Percent isolation of bark fungi by scale insect infestation class of beech trees.

Fungus	Infestation Class (colonies/25 cm^2)			
	0	1-25	25+-50	50+-100
A. alternata	33	50	17	25
A. pullulans	76	68	50	0
Aposphaeria sp.	13	38	17	0
C. herbarum	83	83	100	100
Fusarium sp.	13	46	50	0
G. roseum	57	42	28	0
Papulospora sp.	13	40	33	25
T. viride	50	50	17	0
BC-13	33	13	17	25
OP-23	57	40	33	25
White Yeast	a3	100	100	75

Fungi that were not isolated from heavily infested trees but were present in relatively high frequency in non-infested and lightly-infested trees were ***A. pullulans***, G. ***roseum*** and ***T. viride.*** The antagonistic nature of the latter two fungi has been widely documented (Barnett, 1963; Barnett and Lilly, 1962; Bell ***et al.*** 1982; di Menna, 1962; Dubos and Bulit, 1981; Reinecke, 1981; Shigo, 1958; Skidmore, 1976; Wood, 1951). The antagonism of ***A. pullulans*** towards pathogenic organisms has also been reported in several studies (Deo Bhatt and Vaughan, 1963; Fokkema, 1973; Fokkema and Lorbeer, 1974; Pace and Campbell, 1974; Warren, 1972).

Future work on the possible role played by these fungi in the establishment of the insect should concentrate on 'resident' fungal species which were most frequently isolated from non-infested trees, such as ***A. pullulans.*** The feasibility of manipulating the environment to increase populations of other potential antagonists present in lower frequencies should also be investigated. In any case, selection and manipulation of potential antagonists should foremost take into account the period of dissemination of the insect (June to November).

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