SOME ASPECTS OF THE ECOLOGY OF *NECTRIA* ON BEECH[1]

David Lonsdale[2] and Christine Sherriff[3]

Abstract. --Observations of the mycoflora of beech bark infested with *Cryptococcus fagisuga* suggested that *Nectria coccinea* can colonise sites on and in the outer tissues, and that invasion of inner bark could later develop. Although these sites harboured fungi antagonistic to *N. coccinea*, experiments suggested that it is well adapted under some circumstances to escape serious competition.

INTRODUCTION

Despite the prominence of canker diseases in forest pathology research, little attention has been paid to the pre-infection ecology of the organisms involved. In most cases, our knowledge corresponds to an idealised cycle whose most significant stages are the initiation of the infection and the production of infective propagules. This simple concept is valid only where these propagules can alight on a site where susceptible host tissues are available for infection during the life of these propagules, and there are clearly many cases where these conditions are not fulfilled. Their fulfilment may be obstructed by several possible barriers and, in *Nectria* diseases of beech, at least three such barriers can be recognised.

Before defining these barriers it is important to bear in mind the relative parasitic strengths of *Nectria* species, as well as their relative fitnesses to exist outside the parasitic niche. For example, *N. ditissima* seems able to invade unstressed beech bark more aggressively than *N. coccinea* (Parker 1974), while *N. cinnabarina* apparently includes strains ranging from those which can cause perennating cankers on hardwoods to others which are purely saprophytic.

The most obvious barrier to infection is the intact outer bark of the stem. For the more parasitic *Nectria* species, leaf scars may be a point of weakness in the barrier through which infection may occur. This certainly applies to twig canker of apple caused by *N. galligena* (Wiltshire 1921) and may apply in the case of *N. ditissima* on the twigs of beech. No evidence exists for this mode of entry for the less specialised parasites, *N. coccinea* and *N. cinnabarina*, suggesting that some form of injury is necessary for the barrier to be breached. For *N. coccinea*, this is apparently achieved by the wounds caused by the beech scale, *Cryptococcus fagisuga* (Ehrlich 1934).

Once in direct contact with the inner bark tissue, the fungus encounters additional barriers formed by the living cells in response to attack. For *N. ditissima* and possibly for some strains of *N. cinnabarina*, this resistance can probably be overcome, at least at certain times of the year. For *N. coccinea*, however, it seems that invasion of the tissues is soon delimited by host response (Parker 1974, Perrin 1979) unless the tree is infested to the point of stress by *C. fagisuga* (Houston 1973, Lonsdale 1980a, Perrin 1980) or is otherwise stressed (Lonsdale *op cit.*).

Thus, the relative parasitic weakness of *N. coccinea* imposes on it a dependence on agents which can not only break down the tree's pre-existing barriers but can also impair its ability to form barriers in response to attack. In these respects, *C. fagisuga* seems to be a dual agent. Heavy infestations of *C. fagisuga*, however, generate a third possible kind of barrier: an ecological barrier, or more precisely an ecosystem which must be negotiated by *N. coccinea*. The commonly observed discoloration of the insects' waxy secretion attests to the presence of a dense microflora and microfauna, an ecosystem which will be encountered by the propagules of any canker fungus alighting on the bark. In other words, it seems unlikely that

[1] Paper presented at the IUFRO Beech Bark Disease Working Party Conference, Hamden, CT. USA, 27 September to 7 October 1982.

[2] Forest Pathologist, Forestry Commission, Forest Research Station, Alice Holt Lodge, Farnham, Surrey, England.

[3] Former industrial trainee in Applied Biology Undergraduate course, University of Bath, Avon, England. Present address: Dept. of Botany, University Park Nottingham, England.

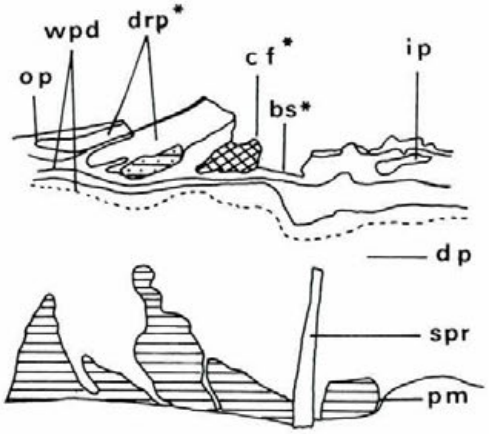
beech bark disease is consequent upon the simple process of *Nectria* spores alighting on the bark surface and immediately giving rise to invasion of the tissues. The corollary is that this disease involves some form of persistence, saprophytic or otherwise, on or near the bark surface.

These arguments prompt the questions: 'Where is *N. coccinea* before it invades the bark tissues; how does it survive out of the parasitic environment, and why is it the characteristic invader of beech bark rather than other weak parasites? It is to these questions that the present work was directed.

EXPERIMENTAL

Characterisation of the bark mycoflora

Preliminary observation of beech bark, heavily infested by *Cryptococcus*, suggested that three main possible niches of latent colonisation by *N. coccinea* could be characterised. They were: a) colonies of the insect itself (i.e. insects plus wax) b) the bark surface around the colonies and c) superficial necroses produced within the bark as a result of mass feeding by the insects. These sites are illustrated in Figure 1.



op: original phellem, wpd : wound periderm, drp: disrupted necrotic parenchyma, cf: C. fagisuga wax, bs: bark surface near wax, ip: 'island' of parenchyma, dp: deep tissue of cortex & phloem, pm: phloem, spr: sclerotic phloem ray

Figure 1.--Illustration of possible sites of latent colonisation by *N. coccinea* on beech bark undergoing prolonged and severe *Cryptococcus*-attack (drawn from a t.s).

Small fragments of insect colony material, of the bark surface layers and of necrotic phelloderm tissue were plated out on 2% malt extract agar (MA) and on tap water agar containing 250 µM vinclozolin, a fungicide which can to some extent select for *Nectria* (D. Lonsdale & M. Turton unpublished[1]). The samples were collected from thirty to forty-five-year-old beech plantations in Alice Holt and Queen Elizabeth Forests in Hampshire, southern England. Care was taken to avoid sampling any material which might have come from typical *Nectria* lesions of the inner bark. Fungi growing on the isolation plates were identified at least to genus wherever possible, and a full list will be published elsewhere.

In order to examine the fungus flora more sensitively, individuals of *C. fagisuga* from trees with various intensities of infestation were plated on 0.01% yeast extract agar (YEA). The insects were either removed from colony debris using a wet sieving technique (D. Wainhouse personal communication) or individually picked from the trees using a tripod-mounted dissecting microscope. The agar plating of bark and insect material was felt to be a limited method of gaining insight into the nature of the beech bark microflora, and so direct observations of fungal development on infested bark were made. The bark was taken in July 1982 from trees at Queen Elizabeth and Marden Forests in southern England, representing a range of infestation catagories, and 25 mm discs were incubated on moist tissue paper at room temperature (19 - 27^{0}C for three weeks. During this period the dominant fungi developing from the insect colonies were examined using the high power of a dissecting microscope. The individual insect plating and the bark disc incubation are reported in more detail elsewhere (Lonsdale, these Proc.). An additional survey of dominant species of the mycoflora was carried out by microscopic examination of fungal fruiting masses which had developed under natural conditions on infested bark at Alice Holt, Queen Elizabeth and Marden Forests.

For present purposes, the direct observations and the isolation data are summarised in a descriptive form (Table 1). This excludes the many genera of hyphomycetes which seemed to occur only occasionally, and it also excludes yeasts and bacteria. It must also be emphasised that the role of the bark microfauna seemed to be very important in relation to grazing of the fungal colonies and dissemination of propagules, but no quantitative data could be gathered concerning these effects.

---

[1] University of Bath undergraduate industrial placement report.

Among the fungi listed in Table 1, *Cladosporium cladosporioides* (Fres.) de Vries was usually the most frequent and abundant of the dematiaceous species which caused blackening of the insect wax, although *Ramichloridium subulatum* de Hoog was dominant on some trees. It may be noted here that blackened wax was more wettable than fresh wax, a characteristic which could influence the micro-environment of the bark. *Verticillium lecanii* Viegas, a known entomophagous species (Petch 1948) was also a very dominant component of the mycoflora but was dependent on the presence of massed insect colonies (Lonsdale, these Proc.). *Nectria viridescens* Booth was also noteworthy for its frequency. *Nectria coccinea*, however, was infrequent, yet it was one of only three species found in all the three niches: insect colony material, the bark surface and necrotic flecks in the phelloderm. The other species were *C. cladosporioides* and *V. lecanii*. It was also found in preliminary work that *N. coccinea* artificially introduced on to infested bark readily grew and formed micro-conidia (Plate 1).

Table 1.--Summary of the apparent ecological status of the more frequent fungi of *Cryptococcus*-infested beech bark

Cryptococcus colonies	Bark surface	Phelloderm necroses
Ecological Status		
Cladosporium cladosporioides		
Dominant on old colonies and present on live insect bodies, especially those in young colonies.	Largely confined to sites under or near insect colonies.	Occasionally present.
Pyrenochaeta sp.		
Occasionally present.	Not detected.	Frequent.
Ramichloridium subulatum de Hoog		
Frequently present, and occasionally more dominant than *Cladosporium*.	Confined to sites near insect colonies.	Not detected.
Verticillium lecanii Viegas		
Very frequent on live insect bodies and dominant on colonies, but only where the colonies are large.	Much of the bark surface around colonies may be colonised.	Occasionally present.
Nectria viridescens Booth		
Frequent on live insect bodies and on colonies at all stages of development.	Often growing extensively around insect colonies.	Not detected.
Mucor spp.		
Sometimes dominant colonists of single or very small massed colonies.	Rarely present beyond margins of insect colonies.	Not detected.
Acremonium spp.		
Occasionally present on live insect bodies and may overgrow colonies.	Common on general surface.	Not detected.
Penicillium spp		
Never dominant but frequently present.	Rarely dominant, but frequently present.	Occasionally present but sp. not same as insect associate.
Fusarium lateritium Nees		
As *Penicillium* spp.	Not detected, though isolated from uninfested bark.	Frequently present.
Nectria coccinea		
Infrequently detected.	Infrequently detected	Infrequently detected.

Many other fungi which were observed occurred with too low a frequency or abundance to be associated with a particular niche. Most common among these were *Trichoderma viride*, *Trichothecium* sp., *Fusarium avenaceum*, *Alternaria alternata*, *Stemphylium botryosum*, *Pyrenochaeta* sp., (an un-named species), *Coniothyrium* sp., *Epicoccum* sp., and several yeasts and yeast-like fungi including *Aureobasidium pullulans*. Bacteria were frequently isolated, but were visually inconspicuous on the natural substrates.

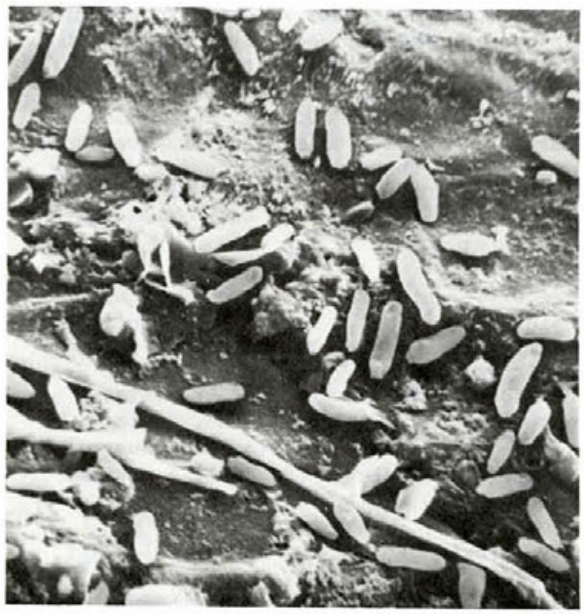


Plate 1. --*N. coccinea* growing and forming micro-conidia on *Cryptococcus*-infested beech bark, following artificial inoculation (scanning electron micrograph, X 2 250) (copyright: Long Ashton Research Station)

In vitro interactions of some beech bark fungi

The recognition of those fungi which seemed to be dominant colonisers of scale-infested bark suggested two lines of enquiry into possible fungal interactions. These were: a) interactions between *N. coccinea* and dominant or frequent bark fungi and b) between *C. cladosporioides* (apparently the most dominant fungus) and other bark fungi. These other fungi were to include *N. coccinea* and several of the commoner species in the mycoflora.

The *in vitro* interactions were observed on agar challenge plates, with the opposing inocula on 6mm diameter blocks cut from young cultures on 2% MA placed 4cm apart on 15cm^3 85mm diameter plates. The initial medium used

was 2% MA, but other media were used to represent a wider range of nutrient concentrations, perhaps bearing some relationship to the variations in nutritional status occurring on the bark surface. These media contained a mineral/amino-acid/thiamine base (Elliott 1972) with the addition of 0.2, 0.4, 0.6, 0.8 and 1.0% sucrose of 1% tyndallised citrus pectin (Sigma Chemical Co. Inc., St Louis, MO, U.S.A.) as carbon source. Pectin was felt to be of some interest in relation to colonisation of internal niches in the bark tissues.

The results of these challenge tests are summarised in Table 2. The strong inhibition of *N. coccinea* by *F. lateritium* on malt and sucrose agars was noteworthy, as was the total compatibility of *N. coccinea* with *C. cladosporioides*. It was also of interest that *V. lecanii*, a dominant fungus on mass *C. fagisuga* colonies, was able to cause lysis of *N. coccinea*, and that the degree of lysis was dependent upon the concentration of sucrose in the basal agar medium. To examine the effect further, a series of similar challenges was set up between these two fungi using sucrose concentrations of 0, 0.5, 1.0, 1.5, and 2.0%. Five replicates were set up for each concentration and incubated at 20^0C.

Three different effects were evident after the opposing mycelia met. Most striking of these was virtually total lysis of the *Nectria* colony (Plate 2a). Individual hyphae of *V. lecanii* grew alongside those of *N. coccinea* and the latter became devoid of contents (Plate 2b). This extreme lysis only occurred on the 0.5% sucrose medium as shown in Table 3. Secondly a partial lysis occurred at 0 and 1.0% sucrose and on one replicate at 2.0%. The third effect was a surface overgrowth of *N. coccinea* by *V. lecanii*, this occurring at all sucrose concentrations, even where lysis was only partial or absent.

Table 3.--Lysis of *N. coccinea* by *V. lecanii* on agar challenge plates

	Sucrose concentration in basal medium				
	0.0	0.5	1.0	1.5	2.0 %
Mean radius of lytic zone 25 days after meeting of colonies (mm)	0.0	20.0	0.0	0.0	0.0

The above data refer only to the intense form of lysis

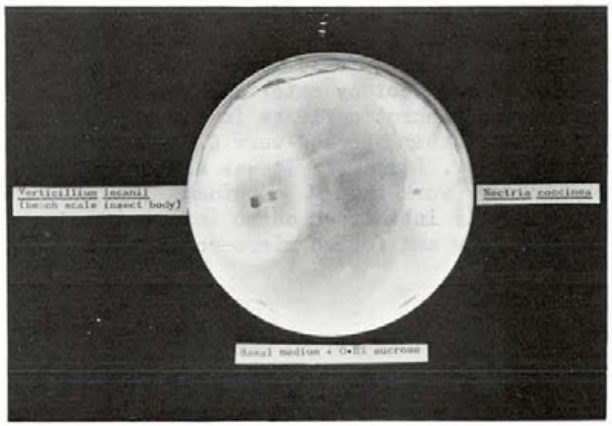


Plate 2a.--Lysis of a culture of *N. coccinea* by *V. lecanii*

Table 2.--Summary of agar challenge tests between *N. coccinea* and other fungi

Test fungus	Degree of inhibition of *N. coccinea*							Interaction in contact zone between colonies
	Malt	Basal medium plus sucrose					Pectin	
	0.2%	0.2%	0.4%	0.6%	0.8%	1.0%	1.0%	
N. coccinea	0	-	-	-	-	-	-	free intergrowth
Acremonium sp.	+	+	+	+	+	+	+	slight intergrowth
F. lateritium	+++	+	++	+	+	+	0	no intergrowth
Pyrenochaeta sp.	+	+	+	+	+	+	+	no intergrowth (sucrose)
V. lecanii	NT	+	++	++	++	+++	++	*Nectria* lysed and overgrown on sucrose
C. cladosporioides	0	0	0	0	0	0	0	free intergrowth

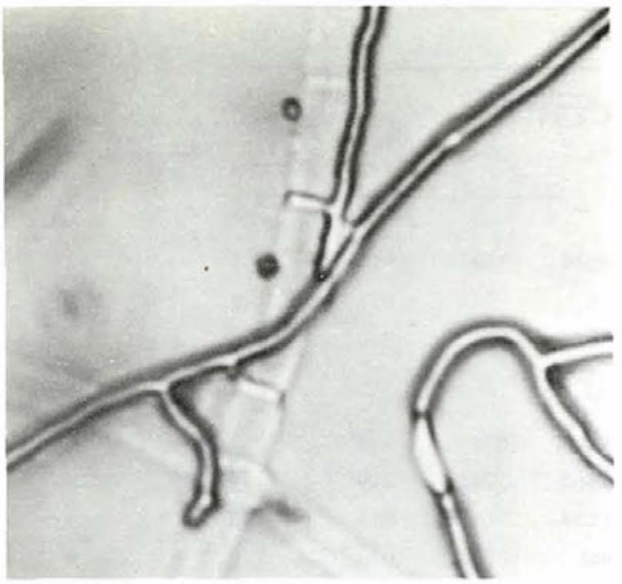


Plate 2b.--Hyphal interaction between *N. coccinea* (wider hyphae) and *V. lecanii* showing loss of content from the hyphae of *N. coccinea* (x 3250)

Comparative compatibility of *N. coccinea* and other bark fungi with *C. cladosporioides*

The challenge tests and much preliminary work had shown that *N. coccinea* and *C. cladosporioides* could intergrow freely on a range of agar media, including the basal medium supplemented with several sucrose concentrations. The dominance of *Cladosporium* on *Cryptococcus*-infested beech bark and its presumably intense competition for nutrients may favour the growth of fungi which are compatible with it at the expense of their potential antagonists. In order to compare the success of *N. coccinea* with that of other bark fungi in the presence of *C. cladosporioides* at low nutrient concentrations, two series of challenge inoculations were set up. The first series involved 'colony opposition' challenges on 0.1 and 0.01% yeast extract (Difco Laboratories, Detroit, U.S.A.) agar (YEA), 5mm diameter inoculum discs being placed 4cm apart on the plates, before incubation at 20°C. The second series involved the inoculation of the test fungi on to 'lawn' cultures of *C. cladosporioides* on 0.01% YEA and on 0.2% *Cryptococcus* colony agar which contained a suspension of insect bodies plus wax. The 'lawn' cultures were inoculated after three different establishment periods.

The results of the 'colony opposition' challenges are summarised in Table 4 and show that, of the test fungi chosen *N. coccinea* was the only one which was completely uninhibited and which freely intergrew with *C. cladosporioides* at both nutrient levels.

In the 'pre-establishment' challenges on 0.01 YEA and on 0.2% CfA the growth of the test fungi was measured by microscopic inspection of the plates at intervals of 16, 25 and 30 days. Four different *Nectria* species were included in this experiment. The results are shown in Table 5 and represent the reduction of growth rate of each test fungus, as compared with its growth in axenic culture. The growth rate of *F. lateritium* was reduced significantly more than the rates of all three British *Nectria* sp. This was of particular interest in view of the potentially antagonistic effect of *F. lateritium* upon *N. coccinea*. *Verticillium lecanii* was also of interest in this respect, and it was more strongly inhibited than *N. coccinea*, at least on the six-day-old lawns. Its growth in axenic culture was in any case much slower than that of *N. coccinea*. The growth of the N. American isolate of *N. coccinea* var. *faginata* was reduced more than that of the British *Nectria* spp. with most combinations of lawn age and nutrient medium. Over the entire range of combinations, the only fungus which tolerated the presence of *Cladosporium* nearly as well as the British *Nectria* isolates was the *Ceratocystis* sp.

Table 4.--Inhibition of the growth of fungi challenged against C. cladosporioides in low nutrient conditions

Test fungus	% YEA	Inhibition score (0,+,++,+++)	Presence of colony contact (+/-)	Presence of intergrowth (+/-)
N. coccinea ACR	0.01	0	+	+
	0.1	0	+	+
F. lateritium S2	0.01	+	+	-
	0.1	+	-	-
F. lateritium ED	0.01	+	+	-
	0.1	+	+	-
F. lateritium EL	0.01	+	-	-
	0.1	+	-	-
V. lecanii F3	0.01	+	-	+
	0.1	+	+	-
Pyrenochaeta F3A	0.01	0	+	+
	0.1	+	+	-
Acremonium A	0.01	+	+	+
	0.1	0	+	-

Table 5.--Growth of some beech bark fungi through *Cladosporium* lawn cultures in low nutrient conditions

Age of lawn (days) at introduction of test fungus	Growth rate: reduction (percent) of axenic rate						Axenic growth rate (mm day^{-1})	
	0.01% YEA+			0.2% CfA†				
	6	13	20	6	13	20	YEA+	CfA†
Test fungus								
N. coccinea ACR	20c	33ab	51bc	9a	39bcd	14a	4.5	3.4
N. viridescens F3	19b	24a	43bc	13b	9a	9a	2.1	1.9
N. ditissima EJP	15a	28a	43a	27cd	33b	7a	3.9	3.5
N. coccinea var. *faginata* 33A	45e	75d	56cd	62e	65de	76c	3.2	2.7
F. lateritium EL	81g	86e	86d	79f	84e	82c	6.5	5.7
Pyrenochaeta F3A	48f	63cd	74cd	51e	46bcd	50bc	1.9	1.7
V. lecanii CF1	44e	56bcd	52bcd	38d	37bcd	51c	2.5	2.3
Ceratocystis PCM	36d	38abc	32b	20bc	24ab	27ab	6.3	4.8

+ 0.01% yeast extract agar † *Cryptococcus* agar

Analysis of variance, following angular transformation of the data for each lawn age, showed overall significant differences between test species ($p < 0.005$) within each lawn age, values followed by the same letter did not differ at the 5% level in Duncan's New Multiple Range Test (Steel & Torrie 1960).

Comparative roles of different *Nectria* spp.

The above data suggested that *N. coccinea* may be ecologically favoured on bark dominated by *C. cladosporioides*, and the more limited data for *N. viridescens* and for *N. ditissima* provided similar evidence for these species. The log inoculations, described below, indicated that *N. viridescens* has no ability to parasitise beech bark and that its presence in natural lesions is probably due to secondary colonisation. *Nectria ditissima*, however, is a more aggressive parasite in unstressed bark than is *N. coccinea* (Parker 1974) and if it is ecologically favoured on *Cryptococcus*-infested beech bark, it should be expected to occur as a major cause of beech bark disease.

In order to study further the interactions between *N. ditissima* and *C. cladosporioides* a series of challenge plates was set up on the basal medium agar amended with 1% autoclaved pectin or sucrose at 0.2, 0.6 and 1.0%. In all these challenge tests *N. ditissima* suppressed the growth of *C. cladosporioides* and, on the pectin agar, it overgrew the latter. These findings indicated that *N. ditissima* and *N. coccinea* differ in their interaction with *C. cladosporioides*, but they gave no reason to assume that *N. ditissima* might thus be at a relative disadvantage.

Fungal interactions in the phytoparasitic environment

The typically dominant status of *N. coccinea* in beech bark lesions indicated that this fungus is well adapted to the parasitic invasion of stressed host tissues. A number of other fungi have, however, also been isolated from the bark lesions, some of them frequently, and three of these were selected for a series of bark inoculations designed to test a) their abilities to invade stressed tissue in their own right and b) their interactions with *N. coccinea* in lesions. At the same time a number of isolates of *N. coccinea* from niches in or on the outer bark were tested for their ability to form lesions. In order to standardise the plant material, detached logs were used in preference to standing *C. fagisuga*-infested trees.

The logs, one metre in length, were obtained from a forty-nine year-old beech plantation. In order to minimise difference in rates of drying-out and tissue senesence, they were selected for similarity of diameter and their cut ends were sealed with a latex emulsion.

Inoculation was performed using a bolt wound and cling-film wrapping technique as described by Lonsdale (1980b), using in each case approx. 0.1 g of an inoculum consisting of a 3% maizemeal/sand culture (Butler 1953) of the test fungus. The cultures had been grown at 20^0C in $250cm^3$ Ehrlenmeyer flasks over a period of two to four weeks, depending on the growth rate of the fungus.

Each log received inoculations at four points around its circumference at each of either two or three positions along its length, and each treatment was replicated between six logs and between all longitudinal inoculation positions. Incubation took place out-of-doors during the winter and spring of 1980/81, with the logs held in a near-vertical position and supported above a concrete surface by nails driven into one of the cut ends.

The treatments and the fungi used were as follows:-

a) *N. coccinea* pathogenicity tests with four 'outer bark' isolates of the fungus, and a mixture of isolates obtained from inner bark lesions:

   BC1 from the bark surface of a *C. fagisuga*-infested beech.

   CW2 from a colony of *C. fagisuga*.

   F2 from a necrotic fleck in the phelloderm of an infested beech.

   F3 as F2, from another beech tree.

   LM4/2/1 lesion mixture containing three isolates.

b) Mixed inoculations (i.e. at one wound site):

   N. coccinea LM4/2/1, mixture of three bark lesion isolates in combination with each of the following fungi -

   N. viridescens L1, a lesion isolate of this frequently occurring bark surface fungus.

   Fusarium lateritium C, a bark lesion isolate of this ubiquitous beech bark fungus and known antagonist of *N. coccinea*.

   Pyrenochaeta sp. F3, an isolate from a necrotic fleck of the type possibly utilised by *N. coccinea* during latent infections.

c) 'challenge' inoculations between *N. coccinea* LM/4/2/1 and each of the fungi named in (b), with the inoculation points 4cm longitudinally apart.

('Controls' were set up using a sterilised maizemeal/sand mixture).

The assessment of each log took place when at least one lesion could be detected by preliminary probing, a stage reached by all the logs within a four-week period in May-June 1981. The measurements of lesion lengths and breadths (Table 6) showed that all the *N. coccinea* isolates, whether obtained from bark lesions, necrotic flecks, the bark surface or from *C. fagisuga* colonies, were able to invade stressed beech bark tissue. None of the other fungi had this ability.

Table 6.--Lesion development following single-species inoculations on beech logs

Inoculant fungus	Lesion length (mm)	Lesion width (mm)
N. coccinea LM4/2/1	61.7 ± 29.5	19.9 ± 12.7
N. coccinea BC1	60.8 ± 25.6	16.5 ± 8.1
N. coccinea CW2	70.8 ± 46.7	12.6 ± 5.6
N. coccinea F2	70.4 ± 34.6	18.2 ± 10.1
N. coccinea F3	56.4 ± 27.7	12.9 ± 3.9
N. viridescens L1	9.2 ± 2.6	5.1 ± 1.6
F. lateritium C	9.0 ± 6.3	6.5 ± 2.9
Pyrenochaeta sp F3	9.3 ± 4.4	6.3 ± 3.4
control	8.0 ± 2.7	5.0 ± 2.7

mean values are followed by 95% confidence intervals.

At the time when the lesions were measured, a series of bark plugs was removed from all zones of each lesion and plated out on 2% MA for isolation of micro-organisms. The re-isolation frequencies of the inoculant fungi in the mixed species and 'challenge' inoculations are shown in Figures 2a and 2b together with mean lesion dimensions. These date show that despite the inability of *F. lateritium*, *N. viridescens* and *Pyrenochaeta* sp. to colonise the bark tissues as primary invaders, they were able to colonise tissue freshly invaded by *N. coccinea*. They did not reduce the invasive ability of *N. coccinea* but they did reduce the frequency with which it was re-isolated from colonised tissue. Thus, in the lesions arising from mixed inoculations of *N. coccinea* and *F. lateritium*, only 17% of attempted re-isolations from the central zones yielded *N. coccinea*, compared with 42% for *F. lateritium*. It could not be judged whether this reduced recovery of *N. coccinea* was partly the result of competition on the isolation plates.

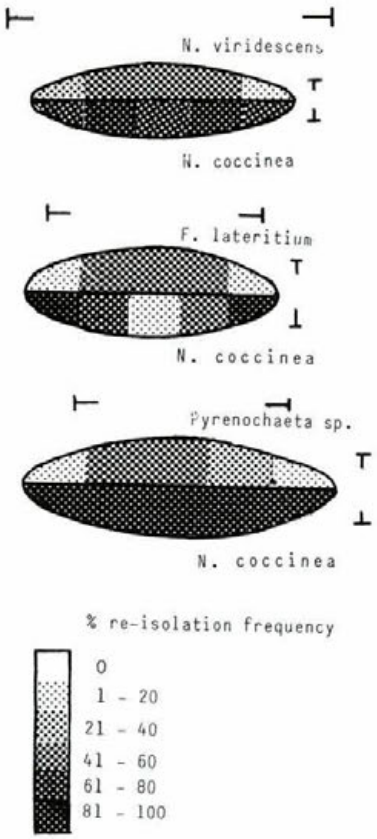


Figure 2a.-- Lesion size and re-isolation of inoculant fungi in mixed inoculations on beech logs. The diagrams show mean lesion lengths and breadths (less 10mm for the inoculation holes; bars indicate 95% confidence limits). Each diagram is split to show re-isolation frequencies for N. coccinea (below) and for the other inoculant fungus (above) as indicated by labels.

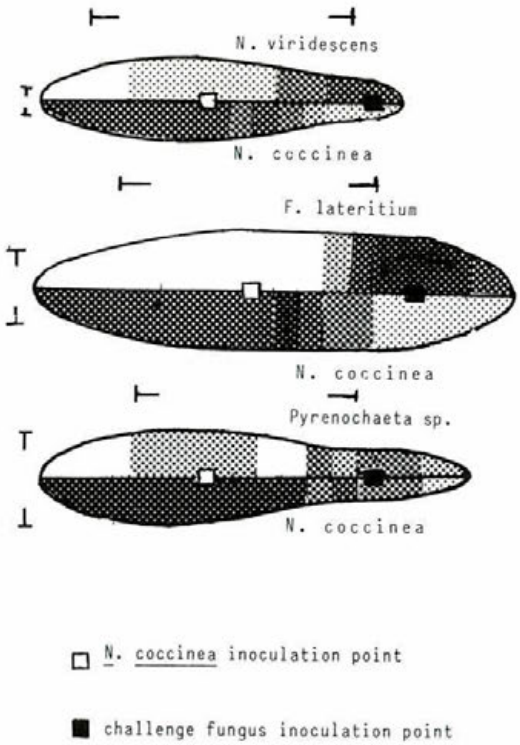


Figure 2b.-- Lesion dimensions and re-isolation of inoculant fungi in challenge inoculations on beech logs. Data are presented as for Figure 2a with the addition of markers for the two inoculation sites. Confidence intervals for lesion breadth refer only to the 'N. coccinea' ends of the lesions.

DISCUSSION

The detection of *Nectria coccinea* on the surface of scale-infested beech bark in the small necrotic flecks within such bark supports the idea that latent colonisation is involved in beech bark disease. The fact that isolates taken from these outer bark sites were pathogenic on logs also supports this view. It seems unlikely, however, from the sampling and observational data, that *N. coccinea* is a major component of the cauloplane microflora, and further work will be necessary to determine whether a link exists between its colonisation of the outer bark and its invasion of deeper tissues. In particular, the development of better selective isolation methods would show whether the apparent infrequency of occurrence of *N. coccinea* is due in part to competition on the culture medium from *Fusarium lateritium* or other antagonists. Cotter (1977) found that a *Fusarium* sp. was much more readily isolated

from perithecia of *N. coccinea* var. *faginata* on *Fagus grandifolia* than was *Nectria* itself. The present work represents a preliminary characterisation of the ecosystem which *N. coccinea* must negotiate in order to gain access to inner bark tissue. It is interesting that the fungus, at least *in vitro*, seems to grow much better than its most obvious competitors in the presence of *Cladosporium cladosporioides*, a very dominant member of this ecosystem. It remains unclear to what extent these 'most obvious competitors', the antagonistic *F. lateritium* and the mycoparasitic *Verticillium lecanii*, may suppress *N. coccinea* on the bark. The effects of sugar concentration on the mycoparasitic ability of *V. lecanii* were of particular interest, and helped to emphasise the need to understand the temporal and spatial nutrient gradients that occur on the bark.

The log inoculations drew a distinction between those fungi which can invade stressed host tissues and those which are secondary colonists of lesions. Although some secondary colonists may have a powerful inhibitory effect on the growth of other fungi, as is the case with *F. lateritium*, there seems little doubt that the ability of *N. coccinea* to act as a pioneer invader must often enable it to escape such inhibition. This can also be concluded from the findings of Gotwols, Blanchard & Shortle (1980). Whether secondary colonisation of lesions could reduce the ability of *N. coccinea* to persist to the fruiting stage is not clear but it has often been isolated from old lesions.

The present findings are consistent with the idea that *N. coccinea* occurs on the bark, that it is ecologically favoured in this environment and that it can readily exploit the inner bark when stress in the tree becomes sufficiently severe. The rarity of *N. ditissima* and of the more pathogenic strains of *N. cinnabarina* or of other canker fungi in the necrotic bark of scale-infested beech is not, however, explained by these data. The explanation might be simple; for example, the reluctance of *N. ditissima* to form micro-conidia in culture may suggest also a lack of ability to achieve adequate dispersal on the bark in conditions where it cannot produce sporodochia or perithecia. It is worth noting that infection of apple leaf scars by the similar twig canker fungus *N. galligena* can be inhibited by saprophytes in the outer scar tissue (Swinburne 1973), an effect which can be used in biocontrol procedures (Corke & Hunter 1979). These outstanding questions clearly demand more detailed observational and experimental work and they highlight the importance of understanding not only the processes of pathogenesis but also the ecological interactions upon which pathogenesis may often depend.

ACKNOWLEDGEMENTS

We thank Mr M. Turton and Mrs Jane Holmes for technical assistance and Drs C.M. Braser and J.N. Gibbs for discussions. We also thank Drs P.C. Mercer and D.R. Houston for fungal cultures and the Commonwealth Mycological Institute for assistance with identifications. One of us (D. Lonsdale) gratefully acknowledges grants from the Stanley Smith Horticultural Trust and the Thomas Phillips Price Trust

RÉSUMÉ

Une étude de mise en culture de l'écorce du hêtre envahie par la cochenille Cryptococcus fagisuga a révélé la présence du Nectria coccinea dans les trois niches suivantes: (1) les colonies du Cryptococcus, (2) la surface de l'écorce à proximité de ces colonies, et (3) des points nécrotiques dans l'écorce extérieure des arbres envahis depuis longtemps. Ceci indique la possibilité que les maladies de l'écorce du hêtre comportent un élément d'infection latente, bien que le N. coccinea n'ait pas souvent été isolé pendant cette étude.

Parmi les nombreux autres champignons présents sur ou dans l'écorce, seules les deux espèces suivantes étaient présentes dans toutes les susdites niches: Cladosporium cladosporioides, dominant dans de vieilles colonies de Cryptococcus, et Verticillium lecanii, abondant parmi les fortes infestations d'insectes. D'autres champignons souvent isolés sont: Ramichloridium subulatum, Pyrenochaeta sp., Nectria viridescens, Mucor spp., Acremonium spp., Penicillium spp. et Fusarium lateritium.

Des essais de provocation in vitro et à différentes concentrations de sucre, entre N. coccinea et des champignons ordinaires de l'écorce démontrent que deux espèces, V. lecanii et F. lateritium, sont antagonistes à N. coccinea; la première serait fortement mycoparasitaire. Un essai de taux de croissance sous conditions de faible nutrition démontre que la croissance de trois espèces britanniques de Nectria obtenues de l'écorce du hêtre, à travers les cultures "en pelouse" de C. cladosporioides est moins inhibée que celles de F. lateritium et de V. lecanii. La variété nord-américaine, N. coccinea var. faginata est plus fortement inhibée que les isolats britanniques.

L'inoculation de bûches de hêtre démontre que les isolats de N. coccinea obtenus des susdites niches dans l'écorce extérieure sont capables de provoquer la nécrose de l'écorce, contrairement à F.

lateritium, N. viridescens et Pyrenochaeta sp. Toutefois, ces autres champignons sont capables de coloniser les tissus envahis par N. coccinea et de réduire la fréquence avec laquelle cette espèce est ré-isolée.

ZUSAMMENFASSUNG

Isolierungen von Buchenrinde, die von der Buchenwollschildlaus (Cryptococcus fagisuga) befallen waren, ergaben, daß Nectria coccinea in den folgenden Nischen anzutreffen war: 1. Lauskolonien, 2. Rindenoberfläche in der Umgebung von Lauskolonien, 3. Nekrosen in der äußeren Rinde von länger befallenen Bäumen. Dies deutet darauf hin, daß, obwohl N. coccinea während der Untersuchung nicht häufig isoliert werden konnte, der Pilz latent vorhanden und Teil des Krankheitskomplexes ist.
Von den vielen in und auf der Rinde vorhandenen Pilzen waren nur die folgenden zwei Arten in allen drei Nischen anzutreffen: Cladosporium cladosporioides, ein Pilz der hauptsächlich von alten Lauskolonien isoliert wurde, und Verticillium lecanii, der in großem Umfang in Bereichen mit intensiver Verlausung anzutreffen war. Andere häufig isolierte, aber nicht in allen drei Nischen vertretenen Pilze sind: Ramichloridium subulatum, Pyrenochaeta sp., Nectria viridescens, Mucor spp., Acremonium spp., Penicillium spp. und Fusarium lateritium.
In Dualkulturen zwischen N. coccinea und häufig vorkommenden Rindenpilzen auf Nährböden mit unterschiedlichem Zuckergehalt ergab sich, daß sich V. lecanii und F. lateritium antagonistisch zu N. coccinea verhielten. V. lecanii wirkt offenbar als starker Mykoparasit. Wachstumsversuche bei niedrigem Nährstoffgehalt zeigten, daß drei englische Nectria spp. von Buchenrinde beim Durchwachsen von C. cladosporioides-Kulturen weniger gehemmt wurden als F. lateritium und V. lecanii. Nectria coccinea var. faginata aus Nordamerika wurde stärker gehemmt als die Isolate aus Großbritannien.
Inokulationsversuche an Stammabschnitten zeigten, daß N. coccinea-Isolate aus den obenerwähnten Nekrosen in der äußeren Rinde im Gegensatz zu F. lateritium, N. viridescens und Pyrenochaeta sp. Rindennekrosen hervorrufen konnten. Allerdings waren die letztgenannten Pilze in der Lage, von Nectria coccinea befallenes Gewebe zu besiedeln. Die Reisolierbarkeit von N. coccinea ging dadurch zurück.

LITERATURE CITED

Butler, F.C. 1953. Saprophytic behaviour of some cereal root-rot fungi. I. Saprophytic colonization of wheat straw. Ann. appl. Biol. 40:284-297.

Corke, A.I.K. and Hunter, I. 1979. Biocontrol of *Nectria galligena* infection of pruning wounds on apple shoots.

Cotter, H.V.T. 1977. Beech bark disease: fungi and other associated organisms. Master's Thesis, University of New Hampshire, Durham, 142 p.

Ehrlich, J. 1934. The beech bark disease. A *Nectria* disease of *Fagus*, following *Cryptococcus fagi* (Baer.). Can. J. Res. 10:594-692. (spec. no.).

Elliott, C.G. 1972. Calcium chloride and growth and reproduction of *Phytophthora cactorum*. Trans. Br. mycol. Soc. 58:169-172.

Gotwols, T.A., Blanchard, R.O. and Shortle, W.C. 1980. Some factors affecting canker formation in American beech inoculated with *Nectria coccinea* var. *faginata*. Eur. J. For. Path. 10:365-370.

Houston, D.R. 1973. Diebacks and declines: diseases initiated by stress, including defoliation. International Shade Tree Conference Proc. 49:73-76.

Lonsdale, D. 1980a. *Nectria coccinea* infection of beech bark: variations in disease in relation to predisposing factors. Ann. Sci. forest. 37:307-317.

Lonsdale, D. 1980b. *Nectria* infection of beech bark in relation to infestation by *Cryptococcus fagisuga* Lindiger. Eur. J. For. Path. 10:161-168.

Parker, E.J. 1974. Some investigations with beech bark disease *Nectria* in southern England. Eur. J. For. Path. 5:118-124.

Perrin, R. 1960. Contribution à la connaissance de l'étiologie de la maladie de l'écorce du hêtre. II. Etude expérimentale de l'association *Cryptococcus fagisuga* Lind. -*Nectria coccinea* (Pers. ex Fries) Fries. Rôle respectif des deux organismes. Ann. Sci. forest. 37:319-331.

Petch, T. 1948. A revised list of British entomogenous fungi. Trans. Br. mycol. Soc. 31:286-304.

Steel, R.G.D. and Torrie, J.H. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company Inc., 481 p.

Swinburne, T.R. 1973. Microflora of apple leaf scars in relation to infection by *Nectria galligena*. Trans. Br. mycol. Soc. 3:389-403.

Wiltshire, S.P. 1921. Studies on the apple canker fungus. I. Leaf scar infection. Ann. appl. Biol. 8:182-192.