SPECIFICITY OF CRYPTOCOCCUS FAGISUGA AND NECTRIA COCCINEA ASSOCIATION IN BEECH BARK DISEASE IN EUROPE[1]

R. Perrin[2]

Abstract.--The specificity of the Cryptococcus fagisuga and N. coccinea association is studied by artificial inoculation of 4 species of Nectria: N. coccinea, N. ditissima, N. galligena and N. cinnabarina on bark infested with beech scale. N. coccinea appears to be the most efficient species for producing necrosis at the cambial level. The composition of the bark, in relation to induced pectinase activity gives a good explanation of the specificity recorded here.

INTRODUCTION

During the last 10 years European beech trees have been seriously affected by two diseases both involving Nectria species. Dense young stands arising from natural beech regeneration are affected by a canker disease caused by Nectria ditissima Tul. Nectria coccinea is associated with Cryptococcus fagisuga Lind. in the beech bark disease (Parker 1974, Perrin 1974). Other Nectria species are known only as saprophytes on beech (Perrin 1976), Nectria cinnabarina (Tode ex Fr.) Fries being undoubtedly the most widespread among them. This latter species is sometimes recorded as a pathogen on other trees e.g. elm. The well known agent of European canker of apple also belongs to the genus Nectria: N. galligena Bres has been implicated in beech bark disease in North America (Cotter 1977) and by mistake in Danish forests (Thomsen et. al. 1949). Despite the great number of Nectria species associated with beech trees from the most specific one N. ditissima, to the most common saprophyte N. cinnabarina, N. coccinea is the only fungus which has been isolated with any obvious consistency from necroses on trees carrying heavy infestations of the beech scale. These observations suggest a close specificity of the association C. fagisuga/ N. coccinea. Very frequently both N. ditissima forming cankers on twigs and N. coccinea causing necrosis on the trunk can be observed on the same tree in French forests but N. ditissima has never been found following Cryptococcus fagisuga attack. Nevertheless some bark necroses may be produced by N. coccinea alone without previous infestation of the scale insect (Leibundgut and Frick 1943) or perennate after disappearance of the insect ('T disease', 'cankered necrosis' (Perrin 1979) drought damage (Lonsdale 1980)). *It seems that the specificity of the* association is the result of a special advantage given to N. coccinea after physiological alteration of the bark by C. fagisuga.

The purpose of this investigation was to study the specificity of the association C. fagisuga/N. coccinea, a key feature of the disease process, by artificial inoculations.

MATERIALS AND METHODS

Ten beech trees, heavily infested by beech scale, between 50-80 years old were selected at four different sites in the forest of Amance (north-east France). The degree of infestation by C. fagisuga was recorded using the scoring system of Parker (1974). Infestation varied between 3 and 5 at the site of inoculation (Table 1). These sites were randomly distributed along the most infested side of each tree. Wound inoculations were made according to the method previously described (Perrin 1980) with four different species of Nectria; N. coccinea, N. ditissima, N. cinnabarina and N. galligena and a control

[1]Paper presented at the I.U.F.R.O. Working Party Conference, Hamden C T., U.S.A. 27 September to 7 October, 1982.

[2]Forest Pathologist, I.N.R.A. Station de Recherches sur la Flore Pathogène dans le Sol, 17, Rue Sully 21034 - Dijon Cedex, France.

Table 1.--Bark necrosis incited by four different species of Nectria eleven months after artificial inoculations. N. coccinea = N.C., N. galligena = N.G., N. ditissima = N.D., N. cinnabarina = N.C.$_i$

Tree	Size of the necrosis mm²								External appearance
	Phelloderm level				Cambium level				
	N.C.	N.G.	N.D.	N.C.$_i$	N.C.	N.G.	N.D.	N.C.$_i$	
1	375.0 b (4)	110.1 (4)	104.3 (5)	44.0 (3/4)	15.2	0	86.2	0	N.C. dark depressed spot
2	85.5 (5)	8.7 (4/5)	71.7 (5)	10.9 (5)	16.7	6.5	0	0	-
3	25.4 (5)	39.1 (5)	12.3 (5)	0 (5)	0	28.3	0	0	N.G. dark depressed spot
4	31.9 (5)	30.4 (4/5)	27.5 (4/5)	8.7 (5)	4.3	0	0	0	-
5	66.7 (4/5)	30.4 (4)	45.6 (4/5)	15.2 (5)	12.3	6.5	0	2.9	-
6	10.9 (4)	13.8 (4)	2.0 (4)	0 (4)	0	13.8	0	0	-
7	287.0 (5)	70.3 (5)	60.9 (5)	10.1 (4)	37.7	40.6	0	0	N.C./N.G. dark depressed spots and slime fluxes
8	- (5)	- (5)	- (5)	- (5)	-	-	-	-	Numerous slime fluxes natural necrosis
9	39.1 (3)	39.1 (3)	30.4 (3)	4.3 (3)	42.7	5.1	3.0	0	N.C./N.G. dark depressed spots and slime fluxes
10	28.3 (4)	26.7 (4)	32.6 (4)	3.1 (4)	29.1	2.3	16.2	0	-
Mean	105.5^{a}_{1}	40.9_1	43.0_1	10.7_2	32.7_1	11.5_2	11.7_2	0.3_3	

a Means followed by the same number are not significantly different according to the Mann-Whitney 'u' test (P = 0.05)

b Numbers in brackets are degree of C. fagisuga infestation

treatment (without any fungus) on each tree. The first three were mycelial strains freshly isolated from beech material in the forest of Amance, the last one was isolated from an active canker on an apple tree. Inoculation was done on 13th November, 1979 and protection devices were removed approximately one month later.

Monthly observations were made until October 1980. At the end of October the necrotic area was assessed at the phelloderm level after removing the suberized layer and at the cambium level giving an idea of the depth extension of the necrosis. The size of the necrosis was recorded by tracing off the discoloured zone around the wound. Identification of the organism causing necrosis was made by placing a piece of aseptically sampled bark on malt agar.

RESULTS

No change in the external appearance of the bark occurs during the dormant period. Frost which was especially severe because of the artificial wet conditions under protective devices appeared to induce a large decrease in the density of beech scale.

Reddish black depressed spots appeared externally around some inoculation wounds (Table 1) during the last fortnight of April and slime fluxes occurred early in May at the time of flushing. The occurrence and size of the necrotic patches (Plate 1) are gathered in Table 1.

For all of the control treatments no necrosis developed either at the phelloderm or cambium level. Healing over of the wound was always effective. The same result was obtained for two inoculations with N. cinnabarina.

The size of the necrosis shows a great variability from one tree to another for each fungal species and also between species on the same tree. Data collected on tree 8 cannot be taken into consideration because of natural infection occurring in the vicinity of inoculation sites. The ranking of trees according to the size of the necrosis at the phelloderm level did not differ greatly from one Nectria species to another. Tree 1 allowed the greatest and tree 6 the least extension of the necrosis whichever fungus was inoculated. Some trees seemed to be very sensitive, others more resistant to attack by any species of Nectria. No relationship was found between the sensitivity of a tree and degree of C. fagisuga infestation (Table 1) but this may be due to the limited range of scale insect infestation levels on the trees. Despite an extension of N. coccinea induced necrosis of more than twice those of N. ditissima and N. galligena this was not significant (Mann and Whitney 'u' test, Siegel 1956). On the other hand the necrosis caused by N. cinnabarina was significantly smaller than those caused by the other fungi.

At the cambial level the frequency and size of the necrosis allows a better distinction between the species. Only one inoculation with N. cinnabarina led to a cambial necrosis, restricted to a point, from which the fungus could not be re-isolated.

N. ditissima produced a cambial necrosis on only three trees and there was great variation in the size of the necroses. There were seven successful inoculations for both N. coccinea and N. galligena. The necroses generated by N. coccinea were on average 3 times larger than those resulting from N. ditissima or N. galligena inoculation. Since necroses cannot be induced on uninfested bark (Perrin 1979, 1980), the significant differences indicated by the 'u' test express the specific advantage to N. coccinea of changes in C. fagisuga infested bark.

DISCUSSION AND CONCLUSIONS

A phelloderm lesion can be easily overcome by the tree, whereas a cambial necrosis indicates that the organism causing the necrosis has overcome host defence mechanisms and will have a long term effect on tree health. The specificity of the association between N. coccinea and C. fagisuga observed in natural conditions could be explained by the advantage which it showed over other Nectria species in the present inoculation experiments on infested bark.

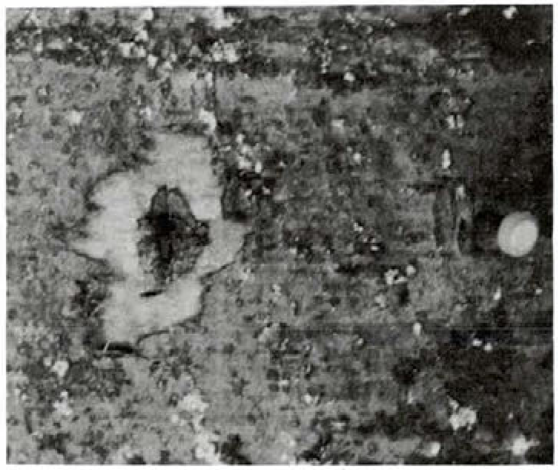

Plate 1--Nectria coccinea necrosis at the phelloderm level.

Flack and Swinburne (1977) did cross-inoculation experiments with N. galligena, N. ditissima and N. coccinea on various hosts. They reported that the wide host-range of N. galligena which included beech was in contrast to the close specificity shown by N. ditissima and N. coccinea which are restricted to beech. From our results and observations N. galligena and N. ditissima are able to destroy trunk bark of scale infested beech but not as efficiently as N. coccinea. In addition penetration courts may not have the same receptivity for all pathogens. By comparison of the pathogenicity of N. coccinea and N. ditissima we can distinguish another level of specialisation. Nectria ditissima seems to be well adapted for parasitising juvenile bark whereas N. coccinea has a greater ability to destroy the old trunk bark. Many authors have described the composition of the bark of beech. All of them revealed substantial differences between the trunk and the twigs. The most important variation concerned the proportion of calcium present. Kreutzer (1976) indicated 0.9% Ca^{2+} for the trunk and 0.19% for the twigs. Pathogenicity of N. ditissima decreases rapidly when the proportion of Ca^{2+} increases in the bark (Perrin unpublished data). In addition some pectinase activity of N. coccinea like endopolygalacturonase, essential for pathogenicity are specially and strongly stimulated in vitro when the fungus uses the bark of the trunk as a carbon source (Perrin this volume). The pectinase pool of N. coccinea differs from those of N. ditissima by an intense endopolymethyltranseliminase activity (R. Perrin, In Prep.).

In conclusion differences in the composition of the bark in relation to variation in the potential pectinase arsenal of the pathogens induced by the bark give a good explanation of parasitic specialisation and subsequent specific localisation or association of the pathogens.

ZUSSAMMENFASSUNG

Das charakteristische gemeinsame Auftreten von Cryptococcus fagisuga und Nectria coccinea wurde mit Hilfe von Infektionsversuchen untersucht. Von der Buchenwollaus befallene Rinde wurde mit Nectria coccinea, N. ditissima, N. galligena und N. cinnabarina infiziert. Von den 4 Pilzen scheint N. coccinea für die Entstehung von Nekrosen im Kambiumbereich am wichtigsten zu sein. Die Besonderheit des Laus-Pilz-Komplexes lässt sich an Hand der Zusammensetzung der Rinde im Hinblick auf die Pectinaseaktivität erklären.

RÉSUMÉ

La spécificité de l'association Cryptococcus fagisuga et Nectria coccinea est étudiée à l'aide d'inoculations artificielles de 4 espèces de Nectria: N. coccinea, N. ditissima, N. galligena et N. cinnabarina, sur de l'écorce infestée par la cochenille du hêtre. N. coccinea semble être l'espèce la plus efficace à produire une nécrose au niveau du cambium. La composition de l'écorce en relation avec l'activité pectinolytique résultante donne une bonne explication de la spécificité exprimée ici.

LITERATURE CITED

Cotter, H.V.J. 1977. Beech bark disease: Fungi and other associated organisms. M.S. Thesis University of New Hampshire, Durham.

Flack, N.J., and T.R. Swinburne. 1977. Host range of Nectria galligena Bres and the pathogenicity of some northern Ireland isolates. Trans. Br. mycol. Soc. 68: 185-192.

Kreutzer, 1976. Effect on growth in next rotation (regeneration). In Symposium on the harvesting of a larger part of the forest biomass FAO/CEE/OII. IUFRO 14-16 June 1976. Hyuinkaa (Finland).

Leibundgut, H. and L. Frick. 1943. Eine Bunchenkrankheit im schweizerischen Mitteland. Schweiz. Z. Forstwes. 94: (10) 297-306.

Lonsdale, D. 1980. Nectria coccinea infection of beech bark: variations in disease in relation to predisposing factors. Ann. Sci. Forest. 37: 307-318.

Parker, E.J. 1974. Beech bark disease. Ph.D. Thesis. University of Surrey, November 1974, 161 pp.

Perrin, R. 1974. Le dépérissement du hêtre en France. Eur. J. For. Path. 4: 249-251.

Perrin, R. 1976. Clef de détermination des Nectrias d'Europe. Bull. Soc. Mycol. France, 2: 335-347.

Perrin, R. 1979. Contribution à la connaissance de l'étiologie de la maladie de l'écorce du hêtre. I - Etat sanitaire des hêtraies. Rôle de Nectria coccinea (Pers ex. Fries) Fries. Eur. J. For. Path. 9: 148-166.

Perrin, R. 1980. 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: 319-332.

Siegel, S. 1956. Non parametric statistics for the behavioural sciences. 312 pp. McGraw-Hill N.Y. Toro. Lond.

Thomsen, M., N.F. Buchwald and P.A. Hauberg. 1949. Angreb af Cryptococcus fagi, Nectria galligena og andre parasiter paa bøg i Danmark 1939-43 Forstlige Forsogsvaesen i Danmark 18: (157) 97-326.