# CHARACTERISTICS AND DEVELOPMENT OF NECROPHYLACTIC PERIDERMS IN MATURE BARK OF AMERICAN BEECH[1,2]

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Abstract.--Investigations were made of necrophylactic periderms which were found to delimit natural and experimentally induced cankers in American beech. Anatomical evidence is presented which supports the hypothesis that the necrophylactic periderm is generated from recent derivatives of the vascular cambium as well as from living cells of the bark tissues present at the time of wounding or infection.

## INTRODUCTION

Woody plant periderms which arise as the result of mechanical injuries, insect, or pathogen attack have been referred to as wound periderms (Bramble 1936, Tainter 1970) or necrophylactic periderms (Mullick 1977, Soo 1977). As a general rule, such a periderm provides a barrier which effectively limits the spread of pathogenic fungi through the bark tissues and prevents them from reaching the vascular cambium. As a result of periderm development in response to bark injuries, a certain degree of resistance to pathogen invasion is imparted to the plant (Horsfall and Cowling 1980).

Necrophylactic periderms have been shown or implicated to play a role in tree diseases such as chestnut blight (Bramble 1936), white pine blister rust (Struckmeyer and Riker 1951), Nectria canker of hardwoods (Grant and Spaulding 1939), yellow-laminated root rot of Douglas-fir (Mullick 1977) and the beech bark disease of European beech (Bazzigher 1957, Braun 1976, Braun 1977, Fink and Braun 1980). Braun (1976) and Fink and Braun (1980) have suggested that the bark damage in European beech resulting from the feeding activity of the scale insect *Cryptococcus fagisuga* Lind. allows the bark to dry and crack along the sclerified phloem rays to the vascular cambium. The presence of the rays themselves may also prevent the wound periderm from forming an uninterrupted barrier to the subsequent invasion of the beech bark by *Nectria coccinea* (Pers. ex Fr.) Fries.

Necrophylactic periderm development has been generally accepted as a major defense mechanism in woody plants (Horsfall and Cowling 1980, Mullick 1977), but few studies have been made of the ontogeny of the process. Temperature (Marks and Minko 1970), light conditions (Borger and Kozlowski 1972a), growth regulating substances (Borger and Kozlowski 1972b), plant water relations (Butin 1952, Puritch and Mullick 1975), and season of wounding (Grant and Spaulding 1939, Mullick and Jensen 1976) influence necrophylactic periderm development. Differences in these environmental conditions may often account for the success or failure of the plant to defend itself against pathogen ingress. With the exception of a study conducted by Ehrlich (1934), the role of the first exophylactic periderm in canker development, and the development of necrophylactic periderms in American beech (*Fagus grandifolia* Ehrh.) have not been reported.

The objectives of the present investigation were to (i) describe canker formation on American beech resulting from mechanical injury and inoculation with *Nectria coccinea* var. *faginata* Loh., Wats. & Ayers during each of the four

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seasons, (ii) compare necrophylactic periderm development in trees which are susceptible with that in trees apparently resistant[4] to the beech bark disease, and (iii) describe two canker types of the beech bark disease resulting from natural infections on American beech.

## MATERIALS AND METHODS

### *Seasonal inoculation study*

Twenty-four American beech, each 5 to 10 cm in diameter, were selected at the Kingman Research Farm in Madbury, New Hampshire. One of four groups of six trees each was selected for treatment in each season. Treatments were initiated in the summer (8/23/79), fall (10/23/79), winter (1/16/80), and spring (4/1/80). Trees for each treatment were harvested for anatomical study two years from the time of treatment initiation.

Five whorls of 12 mm diameter wounds were inflicted on each of five trees in each group. Each whorl consisted of two wounds inoculated with a three week old malt agar culture of a single-ascospore isolate of *Nectria coccinea* var. *faginata*, a wound left uninoculated, and an area of the bark inoculated but left unwounded. The wounds were inflicted by the removal of a 12 mm diameter disc of periderm tissue. Wounds were approximately 0.5 mm in depth. All wounds were inflicated the same day in a given season. Wounds to be inoculated were so treated from the top whorl to the bottom whorl after 0, 2 days, 1, 2, and 4 weeks had passed, respectively, since the time of injury. These five trees were designated as the treatment trees.

The remaining tree in each group served as a control for time of inoculation. This tree was wounded, by whorl, at the corresponding treatment time intervals. All wounds were inoculated immediately after being inflicted. This tree was designated as the control tree.

Trees were inspected regularly for canker development over a period of two years, after which time they were harvested. Representative samples of uninoculated wounds, inoculated wounds, and healthy bark were sectioned to a thickness of 10 to 20 µm on a cryostat set at approximately -25 C. Sections were either stained with Toluidine Blue O or left unstained, and mounted in glycerol on standard glass microscope slides. Small samples (1 X 1 X 2 mm) of bark tissues were also fixed, dehydrated, infiltrated, and embedded in Spurr's epoxy resin (Kosakai 1973). Epoxy embedded tissues were sectioned at 3 to 5 µm with an ultramicrotome equipped with a glass knife. Sections were stained with Toluidine Blue O or left unstained, and examined microscopically.

### *Development of Necrophylactic periderm in American beech either susceptible or apparently resistant to the beech bark disease*

Four pairs of American beech, each pair consisting of one tree susceptible and one tree apparently resistant to the beech bark disease, were selected for study. Two pairs were located at the Hubbard Brook Experimental Forest near West Thornton, New Hampshire, and two were located at the Bartlett Experimental Forest near Bartlett, New Hampshire.

Trees selected as pairs were a maximum of 2 m apart. Susceptible trees had obvious delimited cankers on which evidence of fruiting by *Nectria coccinea* var. *faginata* could be found. The scale insect *Cryptococcus fagisuga* was present in moderate to low populations. Apparently resistant trees had no evidence of cankering or fruiting by *N. coccinea* var. *faginata*. An occasional individual scale insect was found on some of the apparently resistant trees. All trees were between 19.6 and 30.0 cm in diameter (dbh).

Four whorls of eight injuries per whorl were made between 0.5 and 2 m above ground on the bole of each tree. Wounds were made by removing a 10 mm diameter disc of tissue approximately 0.5 mm in thickness from the bark. Susceptible trees were wounded where the bark was free of both primary causal agents of the beech bark disease. Wounds were inflicted on 18 and 20 September, 1981.

Wounds of the top two whorls of each tree were left uninoculated; wounds of the bottom two whorls were inoculated with a 10 mm diameter malt extract agar disc from a three-week old single-ascospore isolate of *N. coccinea* var. *faginata*. Inoculations were made the same day as wounds were inflicted. Bark samples of wounds of both treatments from all trees were collected at approximately bimonthly intervals and analyzed for anatomical changes. The cryostat was used to prepare fresh tissue sections, and the ultramicrotome was used to

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[4] In this study "apparently resistant" is used to describe healthy beech trees occurring in forest stands which have been heavily damaged by the beech bark disease. The term should not be taken to imply actual resistance or tolerance.

prepare epoxy-embedded tissues, as described previously.

*Two canker types of the beech bark disease on American beech*

A large number of American beech growing in natural forest stands at the Hubbard Brook and Bartlett Experimental Forests were inspected over a three-year period. Both research areas are classified as aftermath zones (Shigo 1970) with respect to the beech bark disease. The disease has been present in both areas for approximately thirty years.

Bark samples were collected from healthy trees and from trees exhibiting various degrees of cankering by *N. coccinea* var. *faginata*. Sections of bark samples were prepared for microscopic examination as described previously, and anatomical characteristics were related to general canker appearance.

## RESULTS

*Seasonal inoculation study*

*Nectria coccinea* var. *faginata* was able to incite cankers on American beech stems when applied to bark tissues from which only the first exophylactic periderm had been removed (Fig. 1). Although many cankers developed, none grew larger than 3.5 cm in length or 2.2 cm in width. The slowest cankers to develop reached a maximum size within 12 months after initiation.

Figure 1.--Canker development around wounds 18 months after being inoculated. Arrow indicates erumpent sporodochia of the fungus. Scale bar represents 5 mm.[5]

Frequency of canker development was influenced by the season in which the trees were wounded and inoculated (Table 1). Twenty-two of the fifty wounds inflicted and inoculated in the fall resulted in cankers, compared with 0, 1, and 4 of those inoculated in winter, spring, or summer, respectively.

Table 1.--Effect of season of wounding and inoculation with *Nectria coccinea* var. *faginata* on canker development on American beech. Wounds were areas of the bark from which only the first exophylactic periderm had been removed.

| Season of wounding and inoculation | Number of inoculated wounds forming cankers | |
|---|---|---|
| | Treatment trees[a] | Control trees[b] |
| Spring | 1 | 0 |
| Summer | 4 | 1 |
| Fall | 22 | 7 |
| Winter | 0 | 0 |

[a]Each value represents the number of wounds forming cankers from a total of 50 inoculated wounds. Wounds were inflicted the same day in a given season but inoculated after various time intervals of up to four weeks.

[b]Each value represents the number of wounds forming cankers from a total of 10 inoculated wounds. Wounds were inflicted at various time intervals but inoculated immediately after being inflicted.

The time interval between wounding and inoculation influenced canker development (Table 2). The longer the time interval between wounding and inoculation, the fewer cankers developed. No wounds left uninoculated resulted in cankers, nor were any cankers formed when inoculum was applied to nonwounded bark.

Canker development on trees used as controls for the time intervals was also affected by season of inoculation. Again, most cankers occurred on wounds inflicted in the fall. However, canker development became less frequent as the fall season progressed, even when wounds were inoculated immediately after being inflicted. Of the seven fall wounds resulting in cankers, only one of four wounded and inoculated after 15 November resulted in canker formation, compared with six of six wounds inflicted and inoculated prior to 2 November. No cankers developed from wounds inflicted and inoculated during the winter season.

[5]For all Figures: FEP = First exophylactic periderm, NP = Necrophylactic periderm, NZ = Necrotic zone, P = Phloem, PH = Phellem, S = Sclerified parenchyma, SPR = Sclerified phloem ray.

Table 2.--Effect of various time intervals between wounding and inoculation of *Nectria cocoinea* var. *faginata* on canker development of American beech. Wounds were areas of the bark from which only the first exophylactic periderm had been removed.

| Days between wounding and inoculation | Number of inoculated wounds forming cankers | |
|---|---|---|
| | Treatment trees[a] | Control trees[b] |
| 0 | 10 | 3 |
| 2 | 7 | 2 |
| 7 | 3 | 2 |
| 14 | 4 | 1 |
| 28 | 3 | 0 |

[a]Each value represents the number of wounds forming cankers from a total of 40 inoculated wounds. A total of 10 wounds were inflicted in a given season and inoculated after each given time interval.

[b]Each value represents the number of wounds forming cankers from a total of 8 inoculated wounds. Two wounds were inflicted in a given season and inoculated after each given time interval.

Examination of tissue sections cut from two-year-old wounds which had resulted in cankers revealed that a well-developed necrophylactic periderm had formed. This periderm consisted of a phellem from twenty to twenty-five cell layers in thickness (Fig. 2). A phelloderm could not be distinguished. The necrophylactic periderm was never observed to be interrupted by the sclerified phloem rays. Rather, it always formed a continuous barrier between healthy and necrotic tissue, with the sclerified phloem rays separated completely from healthy tissues (Fig. 2). Large groups of sclerified parenchyma were commonly observed as short bands centripetal to the sclerified phloem rays which had been separated from healthy tissue by the necrophylactic periderm.

Healthy bark tissue was composed of long files of phloem cells (Fig. 3). The cells were uniform in size and shape. Bands of sclerified parenchyma were a common characteristic of this bark tissue.

*Development of necrophylactic periderm in susceptible and apparently resistant American beech*

The general appearance of one pair of American beech selected for study is shown in Figure 4. On close examination, many small delimited cankers were apparent on the susceptible tree. The bark of some cankers shows deep splits indicating infestation by the scale insect *Xylococculus betulae* (Perg.). Even though the trees are touching at the base, one tree of the pair has no cankers and is considered to be apparently resistant to the beech bark disease.

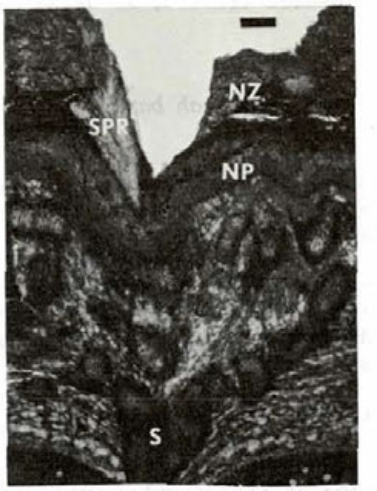


Figure 2.--Necrophylactic periderm delimiting the canker shown in Figure 1. The wound was inflicted in the fall and inoculated one week later. Note the shape of the sclerified phloem ray which has been delimited to the necrotic zone. Scale bar represents 40 µm; transverse section.

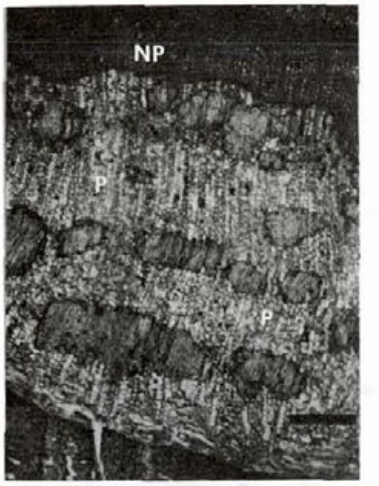


Figure 3.--Appearance of phloem centripetal to the necrophylactic periderm. Note the long, even radial files of vascular cambium derivatives. Scale bar represents 20 µm; transverse section.

Bark tissues behind wounds of both susceptible and apparently resistant trees eight weeks after injury appeared slightly discolored. Healthy bark tissues appeared light brown or tan, while tissues abutting the wound surface were dark red-brown. Tissues of wounds which had been inoculated appeared even darker, and the extent of the discolored tissues was larger than of wounds left uninoculated. No

differences in wounds between susceptible and apparently resistant trees were evident.

Figure 4.--Susceptible (right) and apparently resistant (left) American beech at the Hubbard Brook Experimental Forest. Scale bar represents 15 cm.

The bark surface around wounds 20 weeks after wounds had been inflicted was smooth, with no evidence of cankering (Fig. 5). Fruiting structures of *N. coccinea* var. *faginata* were not observed when inoculated wounds were examined. Microscopic examination of wounded tissues revealed that the extent of the discolored zone had increased only slightly from that of the eight week old wounds. No anatomical changes were apparent in bark tissues at this time.

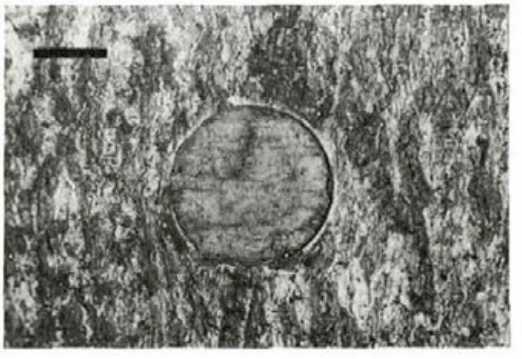

Figure 5.--The appearance of a wound twenty weeks after being inflicted on the bark of a susceptible tree and inoculated with *Nectria coccinea* var. *faginata*. No cankering is yet apparent. Scale bar represents 5 mm.

Significant changes in canker appearance and bark anatomy had occurred in tissues collected and examined 38 weeks after injuries had been inflicted. Abundant sporodochia of *N. coccinea* var. *faginata* were observed (Fig. 6). Anatomical changes in bark tissues behind both inoculated and uninoculated wounds were also evident (Fig. 7). A necrophylactic periderm was confluent with the first exophylactic periderm, and formed a continuous barrier between healthy and necrotic tissues. Neither sclerified phloem rays nor large groups of sclerified parenchyma interrupted the necrophylactic periderm (Fig. 8).

Figure 6.--Canker appearance 38 weeks after a wound was inflicted and inoculated with *Nectria coccinea* var. *faginata*. Sporodochia of the fungus are present (arrows). Scale bar represents 5 mm.

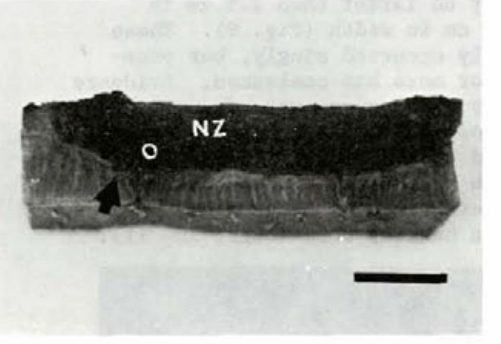


Figure 7.--Bark sectioned through a wound to show canker extent. The necrotic zone which has developed is delimited from the healthy tissue by a necrophylactic periderm, seen here as a narrow band (arrow). Scale bar represents 5 mm; transverse section.

*Two canker types of beech bark disease on American beech*

Two distinct types of bark cankers were evident on infected trees. Cankers restricted in size and defined in cross section by a distinct necrophylactic periderm were designated as delimited cankers. Cankers not defined in cross section by a necrophylactic

periderm were designated as diffuse cankers. Diffuse cankers were not restricted in size, and usually resulted in large necrotic areas. Populations of *C. fagisuga* were low on trees exhibiting either canker type. This does not necessarily reflect past scale populations on those trees.

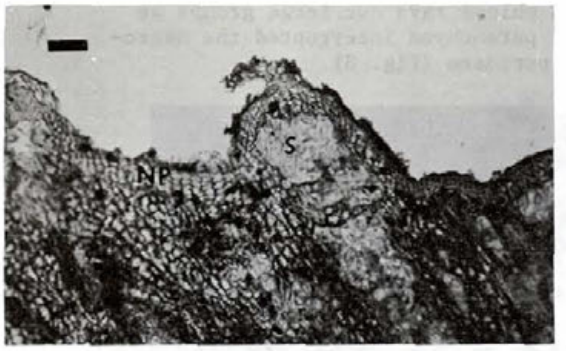


Figure 8.--Section of tissue from the canker shown in Figure 7. Note that the groups of sclerified cells do not interrupt the continuity of the necrophylactic periderm. Scale bar represents 100 µm; transverse section.

Characteristics of delimited cankers, the most common type encountered, are shown in Figures 9 through 14. Delimited cankers were small, usually no larger than 1.5 cm in length by 2.5 cm in width (Fig. 9). These cankers usually occurred singly, but occasionally two or more had coalesced. Evidence of fruiting by *N. coccinea* var. *faginata* was observed on most delimited cankers. Cankers were delimited by a distinct necrophylactic periderm which apparently always formed a continuous protective sheath between the necrotic tissue and the healthy bark (Figs. 10, 11).

Figure 9.--Appearance of typical delimited cankers. Scale bar represents 15 mm.

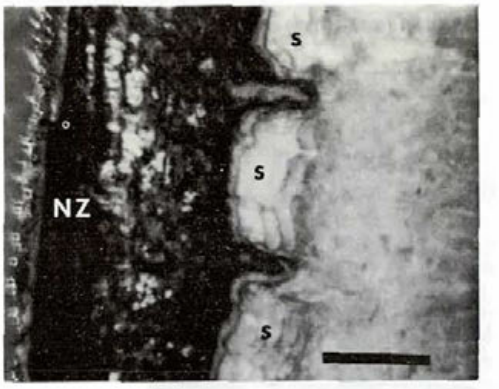


Figure 10.--Well defined necrophylactic periderm delimiting necrotic tissue from healthy bark. Note the bands of sclerenchyma developing behind this periderm. Scale bar represents 1 mm; transverse section.

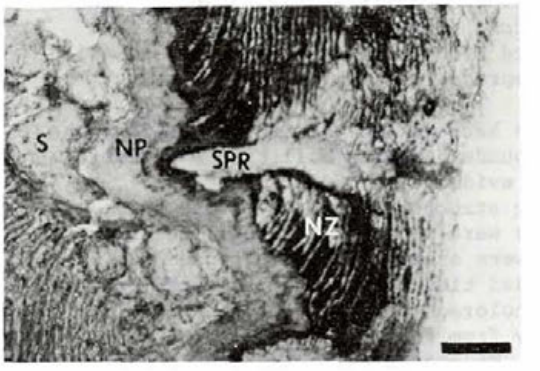


Figure 11.--Exclusion of the sclerified phloem rays from the healthy bark tissues by the necrophylactic periderm. Scale bar represents 300 µm; transverse section.

Evidence that the necrophylactic periderm of delimited cankers was continuous was demonstrated by autoclaving bark samples containing cankers for ten minutes. After autoclaving, the entire canker, consisting of the necrotic bark tissue and the phellem of the necrophylactic periderm, could be easily separated as a single piece from the healthy bark tissue (Fig. 12). The necrophylactic periderm was not interrupted either by large groups of sclerenchyma or by the sclerified phloem rays. This periderm included a well-developed phellem twenty-five to thirty-five cell layers in thickness, and a single phellogen layer (Fig. 13). A phelloderm was apparently lacking.

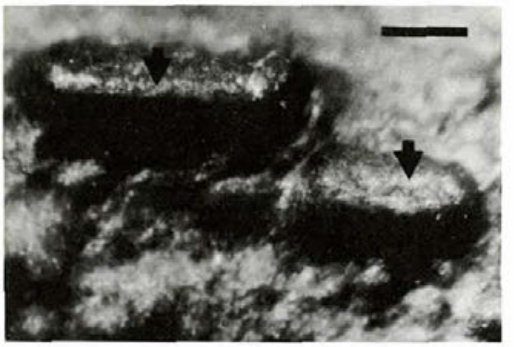

Figure 12.--Appearance of the necrophylactic periderm entirely ensheathing the necrotic zone, or canker. Arrows indicate where the necrophylactic periderm formed centripetal to the sclerified phloem rays. Scale bar represents 0.5 mm.

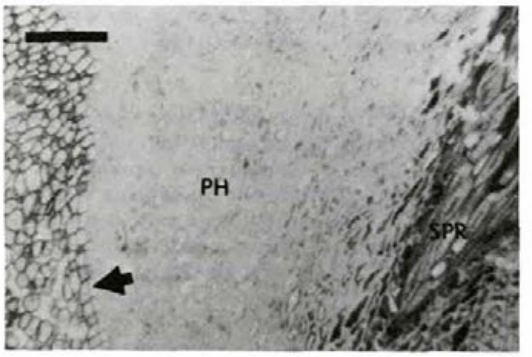


Figure 13.--Necrophylactic periderm showing sclerified phloem ray excluded from healthy tissue, a well developed phellem, and the phellogen (arrow). Scale bar represents 50 µm; transverse section.

Bark tissues centripetal to the necrophylactic periderm appeared similar to those of other healthy bark tissues, with the exception of the arrangement of sclerenchyma. Often, bands of sclerified parenchyma were arranged so that they formed one to several sheets of tissue immediately centripetal to the necrophylactic periderm (Figs. 10, 11). Other large groups of sclerenchyma were located in alignment with the sclerified phloem rays which had been excluded from the living bark by the necrophylactic periderm (Fig. 14).

Diffuse cankers were observed on only a few trees. This canker type is more common in the killing front zone of the disease which is now located in New York and Pennsylvania.

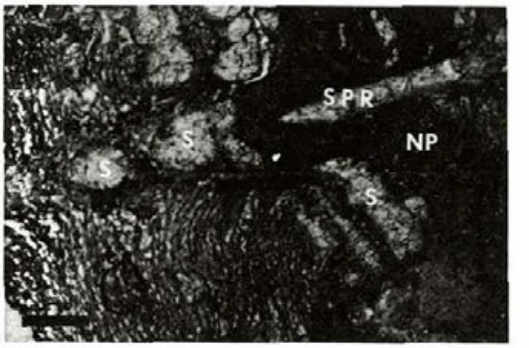


Figure 14.--Appearance of sclerenchyma centripetal to the necrophylactic periderm, and the sclerified phloem rays. Compare the general shape of the sclerified phloem ray to that shown in Figure 2. Scale bar represents 300 µm; transverse section.

Active diffuse cankers may be recognized by the relatively large numbers of perithecia of *N. coccinea* var. *faginata* on extensive areas of the bark, although the bark is not disrupted. Two tree boles were completely covered with perithecia, and the canker presumably involved the entire tree stem. In both instances, tree mortality had occurred within a few months after the initial observation.

A necrophylactic periderm had not developed in diffuse cankers (Figs. 15, 16). The bark area between healthy and necrotic tissues was often discolored in a random pattern, with no distinct demarcation between these tissues. The exophylactic periderm, parenchyma, and phloem cells were killed before an anatomical response to the advancing pathogen had occurred (Fig. 16).

Figure 15.--General appearance of bark at the canker margin. Perithecia of *Nectria coccinea* var. *faginata* (arrow) are present. Scale bar represents 1 mm; transverse section.

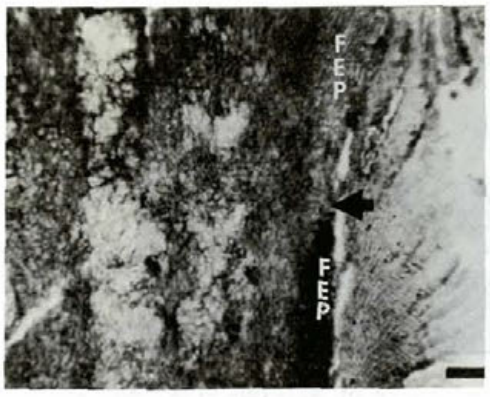


Figure 16.--Extent of necrosis in the first exophylactic periderm (arrow). Note that no apparent anatomical barriers occur in the region between the discolored and healthy areas. Scale bar represents 200 µm; transverse section.

## DISCUSSION

The seasonal inoculation study demonstrated that the fungus *N. coccinea* var. *faginata* can grow through healthy bark tissues and induce cankers, thereby acting as a parasite, but the fungus cannot directly penetrate the phellem. The fungus was most successful when wounds were inflicted and inoculated in the early fall. This agrees with the findings of Grant and Spaulding (1939), who studied the effects of season of wounding on canker development resulting from inoculation with another species of *Nectria* on various species of hardwoods. Injuries made by the feeding activity of *C. fagisuga* may be more receptive to infection in the early fall, after the eggs have been laid and the adults have died. Ascospore and conidia discharge by *N. coccinea* var. *faginata* is also likely to occur during the fall, when moist climatic conditions are prevalent.

For these reasons, it appears likely that early fall is critical in terms of beech bark disease development. Wounds have been made by the insect, the fungus is disseminating spores, and necrophylactic periderm is slow to develop in response to injury (Mullick and Jensen 1976). Cankers developed as a result of inoculating wounds, but they had become delimited within two growing seasons by a well-developed necrophylactic periderm. Perrin (1979) and Lonsdale (1980) studied canker development on European beech resulting from inoculation of *N. coccinea* on trees stressed by high populations of *C fagisuga*, and reported less severe cankering when the insect was not present or was removed. Perrin (1979) suggested that it was the continued presence of relatively high populations of *C. fagisuga* that ultimately resulted in the inability of the tree to limit canker size. European beech is apparently capable of limiting damage caused by *N. coccinea* alone (Bazzigher 1957). The present study supports this concept. Inoculated trees were free of *C. fagisuga*, and were not under the influence of any apparent stress factor. Damage resulting from infection by *N. coccinea* var. *faginata* was limited and localized.

A necrophylactic periderm was initiated between 30 and 38 weeks after fall wounding and inoculation. This provided a long period of time for the pathogen to become well established in bark tissues. However, it is unlikely that the fungus was active during this entire period, since cankers failed to develop from wounds inoculated in the late fall or winter.

No apparent difference was observed in rate of necrophylactic periderm development between susceptible and apparently resistant trees. Necrophylactic periderm development is influenced by genetic processes, but it was not possible to relate genetic differences to susceptibility to the beech bark disease in this study.

There was a substantial difference in canker characteristics between inoculated and uninoculated wounds. A clearly discernable canker developed around inoculated wounds, and the necrotic region of these cankers were approximately 1.5 times the diameter of those resulting from uninoculated wounds. Also, sporodochia of *N. coccinea* var. *faginata* developed in abundance around inoculated wounds. No differences in characteristics between the necrophylactic periderms of inoculated and uninoculated wounds were apparent.

The characteristics of the naturally induced delimited cankers were similar to those of cankers induced by mechanical wounding and inoculation. Sheets of sclerified parenchyma were arranged in layers centripetal to the necrophylactic periderm, and large groups of sclerenchyma developed in alignment with sclerified phloem rays now included with the necrotic tissue. The necrophylactic periderm had developed as a continuous protective sheath, and was not interrupted by sclerified phloem rays. This observation is not consistent with those of Braun (1976), but perhaps the European beech responds differently with respect to necrophylactic periderm formation. Specific comparative studies are needed.

These investigations have led to the hypotheses that the necrophylactic periderms produced in response to mechanical injury or natural infections in American beech are generated from recent derivatives of the vascular cambium as well as from living cells of the bark already present at the time of wounding. This hypothesis is supported by two key anatomical observations. First, the sclerified phloem rays

present in the necrotic zone centrifugal to the necrophylactic periderm were shaped as those in healthy bark (Figs. 11, 13). That is, the ray end closest to the vascular cambium was sharply pointed or tapered. Sclerified phloem rays were generally uniform in width except in this region. Secondly, the radial files of cells centripetal to bark cankers likely resulted from a rapid and uniform development of the vascular cambium. Stimulation of the vascular cambium to produce substantially different xylem cells in response to injury has been well explored (Mulhern, Shortle and Shigo 1979, Shigo and Marx 1977, Tippett and Shigo 1981). A different response, one involving the phloem, is also apparently stimulated by certain specific conditions resulting from bark injury.

The development of a necrophylactic periderm from existing cells in the bark tissue is also evident from Mullick's investigations (Mullick 1977, Mullick and Jensen 1973) and the present study (Figs. 7 and 9), but the development of an uninterrupted necrophylactic periderm in American beech may still depend on the ability of the vascular cambium to produce phloem derivatives capable of differentiating into a new phellogen. However, these events may only occur in those localized areas where sclerified phloem rays are found within a few cells of the vascular cambium (Fig. 7).

In light of the work by Perrin (1979) and Lonsdale (1980), it may be rewarding to investigate the influence of high populations of *C. fagisuga* on the vascular cambium. The insect may be capable of preventing the vascular cambium from producing phloem derivatives which are in turn capable of differentiating into a necrophylactic periderm. Bark invasion by *N. coccinea* var. *faginata* may then be anatomically unopposed by the host. Once the fungus reaches and kills the vascular cambium, its further progress in the bark tissue is usually not interrupted.


## SUMMARY

Necrophylactic periderms provide a barrier which can effectively limit the spread of pathogenic fungi through bark tissues of woody plants. Knowledge of the characteristics and development of necrophylactic periderms in American beech (Fagus grandifolia) would provide insight into the etiology of the beech bark disease, and may help to explain reported differences in susceptibility of American beech to this disease. The objectives of this study were to (i) describe canker formation on American beech resulting from mechanical wounds followed by inoculations with Nectria coccinea var. faginata during each of the four seasons, (ii) compare necrophylactic periderm development in trees which are susceptible with that in trees apparently resistant to the beech bark disease, and (iii) describe two canker types of the beech bark disease resulting from natural infections. Nectria coccinea var. faginata was able to incite cankers on American beech stems when applied to bark tissues from which only the first exophylactic periderm had been removed. Most cankers developed from wounds inflicted and inoculated in the fall. Two years after wounds had been inflicted, the necrophylactic periderm which had developed consisted of a phellem twenty to twenty-five cell layers in thickness and a single layer of phellogen. A phelloderm could not be distinguished. The necrophylactic periderm was never observed to be interrupted by sclerified phloem rays. Anatomical changes in bark tissues in response to wounds inflicted in the fall on susceptible or apparently resistant trees did not occur until after thirty weeks. No difference in time of initiation of the necrophylactic periderm was observed between susceptible and apparently resistant trees, or between inoculated and uninoculated wounds. Two distinct types of natural bark cankers were evident on infected trees. The majority of trees examined had numerous small (2 cm diam.) cankers which had become effectively delimited by a necrophylactic periderm. A few trees had large, diffuse cankers which were not delimited by a necrophylactic periderm. The characteristics of the naturally induced delimited cankers were similar to those of cankers induced by mechanical wounding and inoculation. Sheets of sclerified parenchyma were arranged in layers centripetal to the necrophylactic periderm. The necrophylactic periderm developed as a continuous sheath and was not interrupted by sclerified phloem rays. These observations have led to the hypothesis that the necrophylactic periderms produced in response to mechanical injury or natural infections are generated from recent derivations of the vascular cambium as well as from living cells of the bark already present at the time of wounding.



## RÉSUMÉ

Les péridermes nécrophylactiques sont une barrière efficace pour limiter le développement des champignons pathogènes dans les tissus de l'écorce des plantes ligneuses. La connaissance des propriétés et du développement des péridermes nécrophylactiques chez le hêtre à grandes feuilles (Fagus grandifolia) nous aiderait à comprendre l'étiologie de la maladie de l'écorce du hêtre, et peut aider à comprendre les differences connues dans la susceptibilité du hêtre à cette maladie. Les objectifs de la présente étude sont: 1) de décrire la formation de chancres sur le hêtre par suite

d'une blessure mécanique suivie d'inoculations avec le Nectria coccinea var. faginata, à chacune des quatre saisons, 2) de comparer le développement de péridermes nécrophylactiques chez les arbres qui sont susceptibles aveccelui chez les arbres apparamment résistants à la maladie, et 3) de décrire deux types de chancres de la maladie de l'écorce du hêtre issus d'infections naturelles. Le Nectria coccinea var. faginata produisait des chancres sur les troncs de hêtres seulement lorsqu'appliqué sur de l'écorce dont le premier périderme exophylactique avait été enlevé. La majorité des chancres se développaient de blessures infligées et inoculées à l'automne. Deux ans après le moment de la blessure, le périderme nécrophylactique qui s'était développé consistait en une assise de tissus subéreux de 20 à 25 cellules d'épaisseur et d'une seule couche de phellogène. On n'a pu distinguer de phelloderme. On n'a noté aucune interruption du préiderme nécrophylactique par des rayons de phloème à cellules épaissies. Aucun changement anatomique dans les tissus de l'écorce ne sont apparus durant les trois semaines suivant l'apparition d'une blessure faite à l'automne sur les arbres susceptibles ou apparamment résistants. Aucune différence ne fut observée dans le moment de l'initiation du périderme nécrophylactique entre les arbres susceptibles ou apparamment résistants, ou entre les blessures inoculées ou non. Deux types distincts de chancres de l'écorce d'origine naturelle étaient évidents chez les arbres infectés. La majorité des arbres examinés avaient de nombreux petits chancres (2 cm diamètre) qui étaient effectivement délimités par un périderme nécrophylactique. Quelques arbres montraient de gros chancres diffus qui n'étaient pas délimités par un tel périderme. Les caractères des chancres délimités et d'origine naturelle étaient similaires à ceux des chancres par blessure mécanique et inoculation. Des assises de parenchyme à cellules épaisses étaient disposées en couches centripètes par rapport au périderme nécrophylactique. Celui-ci se développait en une assise continue et n'était pas interrompue par des rayons de phloème à cellules épaisses. Ces observations nous amènent à l'hypothèse de la formation de péridermes nécrophylactiques en réponse à une blessure mécanique ou à des infections naturelles et à son développement à partir de formations récentes du cambium vasculaire ainsi que de cellules vivantes dans l'écorce, déjà présentes au moment de la blessure.


ZUSAMMENFASSUNG

Wundperidermien können die Ausbreitung pathogener Pilze im Rindengewebe von Holzpflanzen wirkungsvoll begrenzen. Die Kenntnis der Eigenschaften und der Entwicklung von Wundperidermien bei Fagus grandifolia könnte mithelfen, die Ätiologie der Buchen-Rindennekrose zu verstehen und gleichzeitig Resistenzunterschiede gegenüber dieser Krankheit zu erklären. Die Ziele der vorliegenden Untersuchung waren: 1. Die Beschreibung der Krebsentwicklung bei Fagus grandifolia nach künstlicher Verwundung und nachfolgender Inokulation mit Nectria coccinea var. faginata zu allen vier Jahreszeiten, 2. ein Vergleich der Wundperidermentwicklung bei anfälligen und offensichtlich gegen die Krankheit resistenten Buchen und 3. die Beschreibung von zwei Krebstypen im Zusammenhang mit der Buchen-Rindennekrose und natürlichen Infektionen. Nectria coccinea var. faginata verursachte Krebse an Buchen, bei denen nur das äußerste Rindenperiderm entfernt worden war. Die meisten Krebse entwickelten sich nach Verwundung und Inokulation im Herbst. Zwei Jahre nach der künstlichen Verwundung war ein Wundperiderm aus einem Phellogen und 20 bis 25 Phellemzellschichten entstanden. Ein Phelloderm war nicht zu beobachten. Das Wundperiderm war in keinem Falle von sklerotischen Baststrahlen unterbrochen. Anatomische Veränderungen im Rindengewebe anfälliger und offenbar resistenter Buchen als Folge der Verwundungen im Herbst traten erst nach 30 Wochen auf. Hinsichtlich des zeitlichen Beginns der Wundperidermbildung waren zwischen anfälligen und offenbar resistenten Buchen keine Unterschiede festzustellen. Auch beim Vergleich infizierter und nicht infizierter Wunden waren keine Unterschiede zu beobachten. An natürlich infizierten Bäumen traten zwei deutlich verschiedene Krebstypen auf. Die Mehrzahl der untersuchten Bäume wies viele kleine (2cm Ø) Krebse auf, die jeweils von einem Wundperiderm wirksam abgegrenzt waren. Wenige Bäume zeigten große ausufernde Krebse, die nicht von Wundperidermien begrenzt waren. Das Aussehen der natürlichen abgegrenzten Krebse ähnelte dem der Krebse, die nach künstlicher Verwundung und Inokulation entstanden waren. Sklerenchymatische Zellschichten traten zentripetal zum Wundperiderm auf. Wundperiderm entwickelte sich in einer geschlossenen, nicht von sklerotischen Baststrahlen unterbrochenen Schicht. Die Beobachtungen führten zu der Hyothese, daß Wundperidermien als Folge mechanischer Verletzungen oder natürlicher Infektionen aus jungen Abkömmlingen des Kambiums wie aus lebenden Rindenzellen hervorgehen können, die zum Zeitpunkt der Verwundung schon vorhanden sind.

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