# Infection of Defoliated Sugar Maple Trees by Armillaria mellea

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ABSTRACT

Manually-defoliated and insect-defoliated trees were inoculated with *Armillaria mellea* to determine the influence of time of season and frequency of defoliation on root invasion by the fungus. Insect populations collapsed and caused only light defoliation. This resulted in a low incidence of infection of trees by *A. mellea* and low tree mortality. Manual defoliation in June or July for 1 or 2 yr resulted in more infections and higher mortality than did manual defoliation in August. The incidence of infection was higher when trees were defoliated for two consecutive years than when they were defoliated once. Not all dead trees were infected by *A. mellea*. There was evidence that *Stegonosporium ovatum* may hasten death of stem tissues severely stressed by defoliation.

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Increased tree mortality after defoliation by insects has been associated with *Armillaria mellea* (Vahl ex Fr.) Kummer (3, 4, 8). Defoliation can induce chemical changes in roots of sugar maple, *Acer saccharum* Marsh. These changes favor the growth of *A. mellea*, and may influence susceptibility of the tree to attack by the fungus (5, 9). The magnitude of some of these changes is related to the degree, frequency (10), and timing of defoliation (5, 9). This study was conducted to determine the influence of these factors on root invasion by *A. mellea*.

MATERIALS AND METHODS.—*Manual defoliation.*—Understory sugar maple trees, 2-5 cm in

diam at 1.4 m above ground and 5-8 m tall, growing in southern Connecticut were bent over and completely defoliated; the leaf blades were removed, but the petioles were left intact. Twenty-five trees were defoliated in June; 10 in 1969, 5 in 1970, and 10 in both 1969 and 1970. Twenty trees each were treated in July and August; 10 in 1969, 5 in 1970, and 5 in both 1969 and 1970. And 25 trees were defoliated in June and again in August; 20 in 1969 and 5 in 1970. All defoliations were done between the 14th and 18th day of the month.

*Insect defoliation.*—In May 1969, three plots were established in north-central New York State, where stands of sugar maple and American beech (*Fagus grandifolia* Ehrh.) had been completely defoliated by the saddled prominent caterpillar, *Heterocampa guttivitta* Wlkr., in 1968. The major portion of foliage was consumed between mid-July and early August. Thirty trees similar to those used in the manual defoliation study were randomly selected in each plot. One plot was in a stand that had been aerially sprayed with insecticide in 1968 to control the insects and prevent defoliation and was scheduled to be sprayed again in 1969; the second plot was in an adjacent stand that had been severely defoliated in 1968 and was to be sprayed in 1969; and the third plot

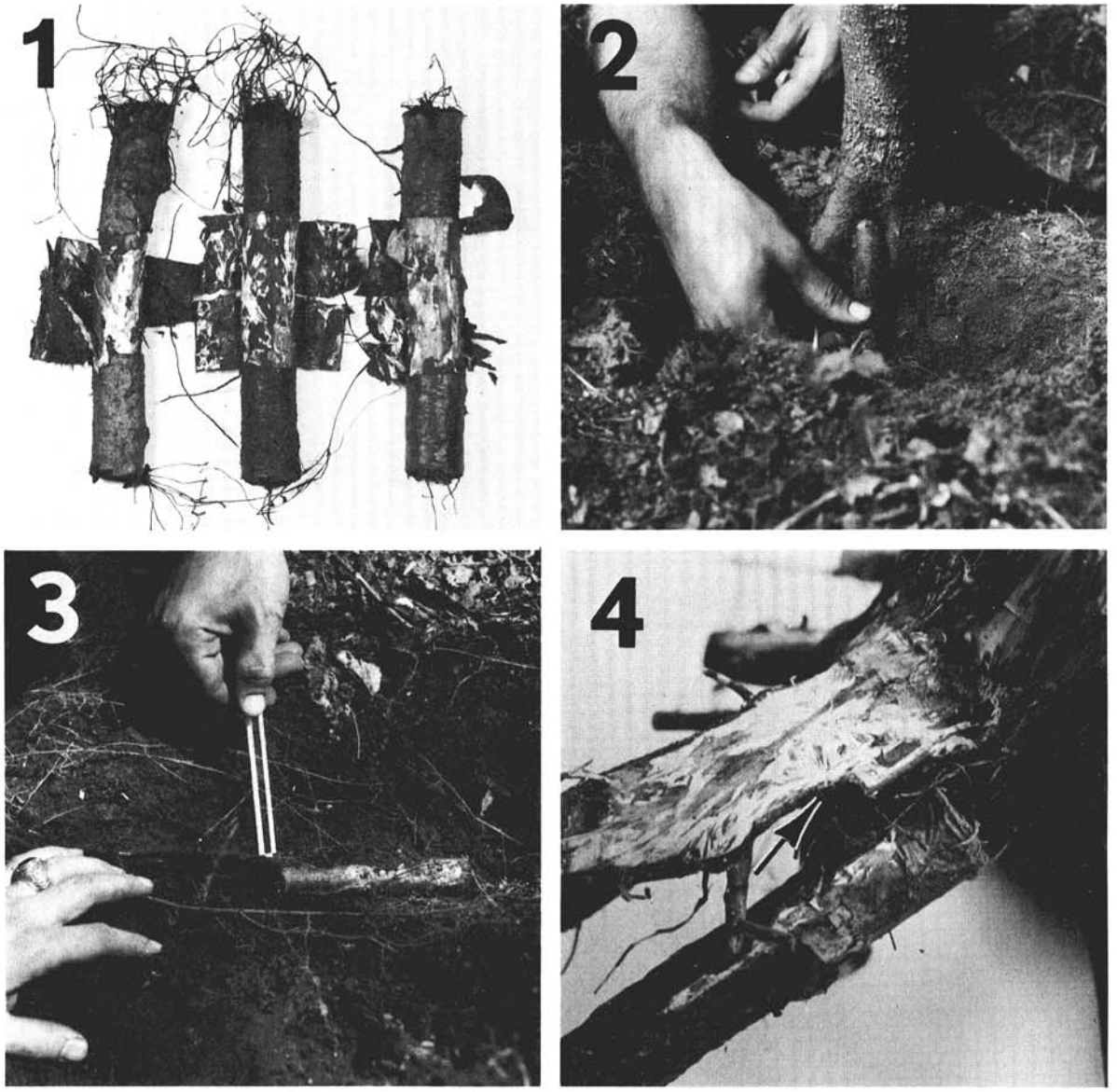


**Fig. 1-4.** **1)** Stem sections of *Alnus rugosa* colonized by *Armillaria mellea* showing mycelial fans and rhizomorphs after approximately 90 days of incubation. **2)** Position of inoculum at root collar. **3)** Position of inoculum along lateral root. **4)** Attachment of rhizomorphs from inoculum to main root at juncture with root collar (arrow) and subsequent mycelial fan development (bark removed).

was in a nearby stand that had been severely defoliated in 1968. This latter stand was not scheduled to be sprayed, and heavy defoliation was expected there in 1969.

*Preparation of inoculum.*—The inoculum was prepared as follows: stems of speckled alder, *Alnus rugosa* (Du Roi) Spreng (7), were cut into sections 12.5 cm long and 1.5-2.5 cm in diam. Twenty sections were tied together, packed with moistened peat moss in cans 17.5 cm high and 15.0 cm diam, covered with aluminum foil, and autoclaved for 80 min at 125 C. A 9.5-cm diam disk of a 3-wk-old malt agar culture of *A. mellea* was placed on top of the bundle of stems and then was covered with sterilized peat moss. The cans were covered with foil and placed in polyethylene bags. The stems were then incubated for at least 3 mo at 25 ± 2 C. When the inoculum was used in the field, subcortical mycelial fans were well developed and abundant rhizomorphs had grown from the ends of the stem sections (Fig. 1).

*Inoculation schedule and technique.*—All the trees were inoculated in May 1969. This was prior to the first manual defoliation of the trees in Connecticut but the season after the first heavy insect defoliation of the trees in New York. Twenty of the 25 trees manually defoliated in June or June-August were inoculated. The other five trees were noninoculated check trees. All trees defoliated in July or August were inoculated. In addition, 20 nondefoliated trees were inoculated as check trees and 10 trees that were neither inoculated nor defoliated were selected as control trees. In the insect defoliation plots, 20 of the 30 trees were inoculated; and the other 10 were used as noninoculated controls.

Trees were prepared for inoculation by carefully removing the soil from around the root collar and along one lateral root. Inoculum sections were abutted to the root collar (Fig. 2) and placed adjacent to the lateral root, 45-60 cm from the base of the tree (Fig. 3) and covered with soil.

*Observations and harvest.*—All trees were examined in autumn 1969, 1970, and 1971; leaf condition and extent of refoliation were recorded. In spring 1970 and 1971, leaf condition and extent of crown dieback (light, moderate, severe) were noted. Manually-defoliated trees were also checked periodically during the summer.

In the manual defoliation plot, five inoculated trees defoliated in June, July, August, or June-August 1969 and 10 inoculated nondefoliated trees were harvested in autumn 1970. Half of the inoculated trees in each insect-defoliation plot were removed in autumn 1970. All remaining trees in both areas were harvested in autumn 1971. Trees that died during the study were harvested immediately, regardless of when they were scheduled to be removed.

When trees were harvested, the root collar and the lateral roots within a 45-cm radius of the trunk were removed, placed in large polyethylene bags, and refrigerated at 5 C in the laboratory until examined. The inoculated lateral root was sampled to include an additional portion 24 cm distal to the inoculum. Inoculum sections were wired in place before the roots were removed from the soil.

In the laboratory, inoculum sections were carefully removed; and rhizomorph development and attachment to the roots were noted. The inoculum sections were checked for viability of *A. mellea* by reisolating the fungus on malt agar. Root collars and lateral roots were washed; and the density of external rhizomorphs was estimated as heavy, moderate, or light. Infection of roots by *A. mellea* was determined by the presence of mycelial fans beneath the bark. Suspected invasion sites where

TABLE 1. Incidence of infection by *Armillaria mellea* and mortality of manually-defoliated sugar maple trees

| Treatment | Infected | | Noninfected | | Total |
|---|---|---|---|---|---|
| | Dead | Living | Dead | Living | |
| Defoliated-inoculated[a] | 23[b] | 5 | 9 | 43 | 80 |
| Nondefoliated-inoculated | 0 | 0 | 0 | 20 | 20 |
| Defoliated-noninoculated[c] | 3 | 0 | 5 | 2 | 10 |

[a]Defoliated in June, July, August, or June-August for one or two consecutive years.
[b]Total number of trees during a 2.5-yr period.
[c]Defoliated in June or June-August for one or two consecutive years.

TABLE 2. Effect of time and frequency of manual defoliation on infection by *Armillaria mellea* and mortality of sugar maple trees

| Defoliation | | Dead trees | | Living trees | | |
|---|---|---|---|---|---|---|
| Time | Frequency (years) | Inf.[a] | Noninf. | Inf. | Noninf. | Total |
| June | 1 | 4[b] | 0 | 0 | 11 | 15 |
| June | 2 | 2 | 0 | 1 | 2 | 5 |
| June[c] | 2 | 3 | 1 | 0 | 1 | 5 |
| July | 1 | 3 | 2 | 1 | 9 | 15 |
| July | 2 | 2 | 0 | 0 | 3 | 5 |
| August | 1 | 0 | 1 | 1 | 13 | 15 |
| August | 2 | 1 | 1 | 1 | 2 | 5 |
| June-August | 1 | 11 | 5 | 1 | 3 | 20 |
| June-August[c] | 1 | 0 | 4 | 0 | 1 | 5 |
| Nondefoliated | ... | 0 | 0 | 0 | 20 | 20 |
| Nondefoliated[c] | ... | 0 | 0 | 0 | 10 | 10 |

[a]Inf. = Infected by *A. mellea*, Noninf. = not infected by *A. mellea*.
[b]Total number of trees during a 2.5-yr period.
[c]Trees were not inoculated.

mycelium was not evident were cultured to reisolate the fungus.

RESULTS.—*Manual defoliation.*—

—1. Infection and mortality–overall effect of defoliation.—Defoliation predisposed sugar maple trees to attack by *A. mellea* (Table 1). Only those trees that were defoliated were infected by the fungus. In trees that were killed by *A. mellea*, the root collar and at least the inoculated lateral root were completely girdled subcortically by mycelium of the fungus; and rhizomorphs were usually firmly attached to the tree (Fig. 4). In the five trees living when harvested but infected by *A. mellea*, fungal development ranged from partial or complete girdling of small roots (1.0 cm diam) to one-third girdling of the root collar. No evidence of successful infection was observed on root systems of any nondefoliated tree. Although the fungus was found on roots from these nondefoliated trees, it was associated with wound sites and seemed to be walled off from living tissues.

Trees usually died in the growing season immediately following the season of defoliation. Trees that survived the first growing season after defoliation did not die unless they were defoliated again.

Not all trees that succumbed were killed by *A. mellea* (Table 1). Five of the nine defoliated and inoculated trees and four of the five defoliated but noninoculated trees that died and were not infected by *A. mellea* had been defoliated twice in one season, June and August. These trees were extensively colonized by *Stegonosporium ovatum* (Pers. ex Merat) Hughes, as indicated by the fruiting bodies on the main stem. The trees had died from the top down, and a gray-green discoloration was advancing downward into the root collar area. Root systems seemed healthy.

—2. Infection and mortality–effect of time and frequency of defoliation.—Infection and death of defoliated trees were influenced by the time of defoliation (Table 2). Trees defoliated for 1 or 2 yr in June or July seemed to be more susceptible to attack by *A. mellea* than were trees defoliated for 1 or 2 yr in August (Table 2).

The frequency of defoliation increased the incidence of infection (Table 2). Two defoliations (June and August) in the same season resulted in the most infected trees and greatest number of tree deaths. Nine of the 45 trees (20%) defoliated in June, July, or August for 1 yr only were infected compared to 10 trees infected out of 20 trees (50%) defoliated in June, July, or August for two consecutive years.

The effects of time and frequency of defoliation were also evident in the amount of crown dieback (Table 3). Dieback ranged from light to severe for all defoliation treatments, but trees defoliated in August for 1 or 2 yr suffered the least damage. In trees with living crowns at the time of harvest, dieback was moderate to severe in 50% of those defoliated in June, July, or August for 2 yr, compared to 31% for trees similarly defoliated for only 1 yr.

*Growth and survival of inoculum.*—Rhizomorph density on the roots and root collar was estimated as moderate to heavy on 50%, light on 30%, and absent on 20% of all trees. There were no differences in rhizomorph density on the roots among the treatments, but 26 of the 31 trees infected by *A. mellea* had moderate to heavy rhizomorphs. The fungus was not recovered from 5 of 200 inoculum sections, but in no case were both sections from the same tree nonviable. Rhizomorphs were growing from 84% of the inoculum sections and were found on the surface of 83% of the inoculated root systems.

*Infection of defoliated trees by naturally occuring A. mellea.*—When we originally established the manual-defoliation plot, some trees defoliated in June and others defoliated in June-August were not inoculated to serve as check trees (Table 1). Earlier defoliation studies in this same area resulted in no mortality in noninoculated trees (5). However, in our study, four out of five noninoculated, June-defoliated trees died; and of these, three were infected and girdled by *A. mellea.*

To determine the infectivity of *A. mellea* occurring naturally, we defoliated additional groups of 10 trees each in mid-June, mid-July, or mid-August 1971. One tree from the June series and one from the July series died during the winter of 1972. Most of their root systems were dead or dying, but there was no evidence of infection by *A. mellea.* Bark of the crown and upper portion of the main stem was well colonized by *S. ovatum*, but the fungus had not completely colonized the main stem bark as noted in twice-defoliated trees. By September 1972, no additional trees had died; and twig dieback was light or absent.

*Insect defoliation.*—Only one of the 60 inoculated trees died during the experiment. This tree, located in the plot defoliated in 1968 but not thereafter, was dead by autumn 1969. The root collar and inoculated lateral root were completely girdled subcortically by mycelium of *A. mellea*, similar to that observed in the manual defoliation plots (Fig. 4). Ten of the 19 inoculated trees in this plot were harvested in autumn 1970. No additional infection by *A. mellea* was observed or determined by reisolation attempts. Tree crowns had only light twig dieback, and leaf condition was good. In autumn 1971, two of the nine remaining trees were infected by *A. mellea.* In both trees,

TABLE 3. Number of trees with different classes of crown dieback at time of harvest in trees manually-defoliated at different times during the growing season for one or two consecutive years

| Date defoliated | Dieback of crown | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Light | | Moderate | | Severe | | Complete | |
| | 1 yr | 2 yr | 1 yr | 2 yr | 1 yr | 2 yr | 1 yr | 2 yr |
| June | 7 | 1 | 2 | 2 | 2 | 1 | 4 | 6 |
| July | 6 | 1 | 1 | 1 | 3 | 1 | 5 | 2 |
| August | 11 | 3 | 1 | 0 | 2 | 0 | 1 | 2 |
| June & August | 1 | ... | 0 | ... | 4 | ... | 20 | ... |
| Total | 25 | 5 | 4 | 3 | 11 | 2 | 30 | 10 |

wound sites on lateral roots near the inoculum were invaded, and mycelium was beginning to advance around the roots. No noninoculated trees died.

In the plot defoliated severely in 1968 and not sprayed in 1969, the insect population collapsed; defoliation was light in 1969 and absent in 1970 and 1971. None of the 10 trees harvested in autumn 1970 was infected by *A. mellea*. Two of 10 trees harvested in autumn 1971 were infected. Infection and fungus development were similar to that previously described. No noninoculated trees died in this plot.

In the plot where trees were never defoliated, no infection by *A. mellea* was observed. The fungus was observed in association with root wounds; but, as in the manual defoliation study, the fungus was prevented from invading living tissues.

The range of rhizomorph density on the roots and root collars was similar to that observed in the manual-defoliation plots. The fungus was recovered from all inoculum sections, but no rhizomorph growth was observed from the ends of 20% of the sections. There seemed to be no difference in rhizomorph density on the roots among the three plots.

DISCUSSION.—Mortality of trees after insect defoliation must be considered in control decisions. It is necessary not only to identify what factors cause mortality, but also to understand what controls the action of these factors.

Our results show that pathogenicity of *A. mellea* on defoliated sugar maple trees is influenced by when and how often trees are defoliated. Defoliation in June or July resulted in more infections by the fungus than defoliation in August, and a higher percentage of trees were infected after two consecutive years of defoliation than after only 1 yr.

Defoliation in June or July can lower total cellular root extractives (food reserve) (5) and increase the reducing sugars and amino acids that can influence the growth of the fungus (9). The lower incidence of invasion of August-defoliated trees by *A. mellea* may be related to the physiological reaction of trees defoliated late in the growing season. For example, on some of these trees, only a few scattered terminal buds leafed out, and on others no bud break occurred at all; the buds were apparently in the resting stage. The effect of defoliation may be greatest when it causes the tree to refoliate heavily in the same season it is defoliated (10), and it is the refoliation process that results in a weakened tree. Thus, one would expect that two defoliations in one season would severely weaken a tree and result in the most damage; and that is what we observed.

Our results differ somewhat from those of Houston and Kuntz (3) who showed that, in Wisconsin, the highest sapling and bud mortality resulted from complete defoliation in early to mid-August. In those studies, refoliation was marked on August-defoliated trees. In our studies in Connecticut, trees defoliated just at mid-August refoliated slightly or not at all, which caused little adverse effect on tree vigor.

We anticipated the additional effect of a second year of defoliation on infection by *A. mellea*. Concurrent studies on the physiology of defoliated sugar maple indicated that tree vigor measured as food storage (starch) was severely reduced by two consecutive years of defoliation (10).

*A. mellea* was neither observed nor isolated from all dead trees. However, 64% of the dead trees not invaded by *A. mellea* had been defoliated twice in a single season. In other studies on sugar maple (3), most trees that were defoliated twice in one season were dead the following year; but the cause of death was not determined. In the present study, all of the crown and main stem of these trees not invaded by *A. mellea* were extensively colonized by *S. ovatum*. A *Stegonosporium* sp. has been associated with sugar maples showing dieback, but it was considered to be saprophytic (2). Our results suggest that *S. ovatum* can attack severely stressed living tissues, thereby hastening tissue death. In the case of those trees defoliated twice in one season, perhaps crown death occurred faster and before root invasion by *A. mellea*. The root systems of all but one tree seemed healthy.

The low incidence of *A. mellea* infection in the insect-defoliated plots, especially in the plot that was not sprayed, can be attributed to little additional defoliation due to collapse of the insect population. Thus the trees were able to recover. Our physiological studies confirmed that if trees are not defoliated or if defoliation is light the year following the first defoliation, trees can partially recover from the effects of a defoliation (10).

Results from the manual-defoliation study suggest that some of the inoculated trees in the insect-defoliated plots may have been naturally tolerant of the effects of defoliation. In the manual-defoliation study, trees that survived the first growing season after defoliation usually did not die unless they were defoliated again. All of the trees in the insect-defoliation plots were inoculated in the spring after the year of defoliation, and we purposely selected trees with no advanced decline symptoms. It is possible that the trees chosen for the insect-defoliation series were relatively tolerant of defoliation and that, in the absence of a second heavy defoliation, they were resistant to invasion by the fungus.

Although *A. mellea* has been shown to invade nonstressed trees (6), the fungus is often considered a weak pathogen (1). Our results show clearly that nonstressed sugar maple trees were not invaded by the fungus even though numerous rhizomorphs were in contact with the root system. However, trees stressed by defoliation were predisposed to invasion by the fungus. Characterization of those physical and chemical changes induced by stress that lower resistance of root tissue to *A. mellea* may permit us to control infection by *A. mellea*–perhaps by ameliorating the changes or by preventing them from occurring. Some of the effects of stress on carbohydrates and amino acids have already been elucidated (9).

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