Abundance and distribution of rhizomorphs of *Armillaria* spp. in defoliated mixed oak stands in western Maryland

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The abundance and distribution of rhizomorphs of *Armillaria* spp. in the soil were quantified in undisturbed stands and in stands defoliated 1 and 5 years previously by insects. Although the species of *Armillaria* was not determined, similar mixed oak forests in south central Pennsylvania contain North American biological species VII (*Armillaria bulbosa* Barla.). Several analysis techniques were tested for sensitivity to differences in distribution of rhizomorphs. Rhizomorph distribution within the 0.04-ha study plots was uniform in the undisturbed stands, but was significantly greater near dead trees in the defoliated stands. Total rhizomorph abundance was greater on plots defoliated 5 years before sampling than on more recently defoliated plots, and it was least on undefoliated plots. Rhizomorph density near dead trees was highly correlated with overall rhizomorph density. Greater rhizomorph abundance near recently dead trees or stumps may have important implications for management decisions in the presence of gypsy moth (*Lymantria dispar* L.) infestations.

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La fréquence et la distribution des rhizomorphes d'armillaire (*Armillaria* spp.) dans le sol ont été quantifiées dans des peuplements non perturbés et dans des peuplements qui avaient été défoliés 1 et 5 ans auparavant par des insectes. Bien que l'espèce d'armillaire n'ait pas été identifiée, des forêts mélangées de chêne semblables à celles qui furent étudiées contiennent l'espèce biologique nord-américaine VII (*Armillaria bulbosa* Barla.). Plusieurs techniques d'analyses furent testées pour leur capacité à faire ressortir les différences dans la distribution des rhizomorphes. La présence des rhizomorphes était uniforme dans les placettes d'échantillonnage de 0,04 ha des forêts non perturbées, mais était significativement plus élevée près des arbres morts dans les peuplements défoliés. La quantité totale de rhizomorphes était plus grande dans les placettes défoliées 5 ans avant l'échantillonnage que dans celles où la défoliation était plus récente, et elle était la plus faible dans les placettes non défoliées. La densité des rhizomorphes près des arbres morts était fortement corrélée avec la densité générale des rhizomorphes. Une plus grande quantité de rhizomorphes près des souches et des arbres morts récemment peut avoir des conséquences importantes sur les décisions d'aménagement lorsqu'on est en présence d'une infestation par la Spongieuse (*Lymantria dispar* L.).

[Traduit par la revue]

Introduction

The root disease fungus *Armillaria* is one of the most prominent killers and decayers of deciduous and coniferous trees and shrubs in natural forest stands, plantations, orchards, and gardens throughout the world (Wargo and Shaw 1985). In coniferous forests of the northern Rocky Mountains, where the fungus aggressively attacks pines (*Pinus* spp.), true firs (*Abies* spp.), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), pathogenicity of *Armillaria* is inversely related to primary productivity (McDonald *et al.* 1987*a*, 1987*b*). It attacks, colonizes, and kills apparently healthy trees of all ages in enlarging groups of diseased trees. Rotting lateral roots of killed trees serve as inoculum sources and avenues for spreading the fungus into adjacent trees, and the infection process is repeated (Shaw and Roth 1976).

The primary role of *Armillaria* in the predominantly deciduous forests of the eastern United States is that of a secondary pathogen, attacking trees weakened by biotic or abiotic stresses (Wargo 1984). The fungus colonizes and kills deciduous and coniferous trees that have been weakened by such agents as defoliation by insects or frost, foliage diseases, stem cankers, bark-sucking insects, drought, soil saturation, soil compaction, or air pollution (Wargo 1980). Weakened trees are colonized by *Armillaria* (*i*) from rhizomorphs that are already present on the root surfaces or that grow from roots of previously colonized and killed trees or (*ii*) from mycelia, through contact with diseased roots or quiescent lesions that are reactivated by stress. Rhizomorphs are commonly present on the surfaces of lateral roots of healthy trees, especially in stands where disturbance has provided stumps as food bases for rhizomorph production (Pronos and Patton 1978; McDonald *et al.* 1987*a*). However, rhizomorphs occur less frequently in new-growth forests on recently reforested arable land where tree mortality is low, the amount of substrate for colonization and rhizomorph

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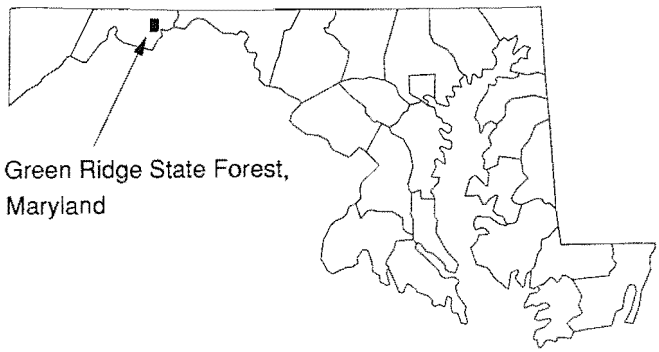

FIG. 1. Map of Maryland showing study location on the Green Ridge State Forest.

development in the soil is limited, and inoculum from old-growth trees is not present.

Even though individual trees of the same species may receive similar stress and have rhizomorphs along their root systems, many are not attacked successfully by *Armillaria* (Wargo 1977, 1983). Differences in site, disturbance history, soil factors, and tree vigor are complicating influences (Singh 1981). Tree mortality after defoliation by the gypsy moth (*Lymantria dispar* L.) has been observed to be greater in stands that have been disturbed by partial cuttings (Dr. Barry Towers, Pennsylvania Bureau of Forestry, personal communication). *Armillaria* rhizomorphs and mycelial fans are commonly observed on recently dead trees. Presumably the greater mortality is associated with greater and more aggessive inoculum buildup on the stumps and root systems of the cut trees (Pronos and Patton 1978). If this buildup does occur, it is a strong argument for allocating limited resources to protect against defoliating insects in stands with recent cutting. To attribute increased tree mortality to increased abundance of *Armillaria*, a satisfactory means for estimating abundance of *Armillaria* per unit area must be developed; the present studies were conducted to develop a sampling procedure that could provide such an estimate. Because rhizomorphs are a major means by which *Armillaria* attacks trees, we sought to determine factors that affect differences in distributions of rhizomorphs in mixed oak stands.

Methods

Study area

The studies were conducted in mixed oak stands in the Ridge and Valley province on the Green Ridge State Forest in Maryland (Fig. 1). Predominant species included northern red oak (*Quercus rubra* L.), white oak (*Q. alba* L.), black oak (*Q. velutina* Lam.), chestnut oak (*Q. prinus* L.), and scarlet oak (*Q. coccinea* Muenchh.). We sampled in previously established study plots with a known defoliation history, so we were able to avoid duplicating the collection of some overstory data. Sampling of rhizomorphs for the initial study was done in the winter of 1985–1986 and sampling for the second study, in the winter of 1986–1987. Sampling sites represented three disturbance histories: (*i*) stands defoliated in 1981 by a complex of spring-defoliating insects, reportedly including linden loopers (*Erranis tiliaria* Harris), (*ii*) stands defoliated in 1985 by gypsy moth, and (*iii*) stands showing no recent disturbances. Study areas were selected for similarity of site quality, stocking level, aspect, slope position, and species composition (Table 1). Complete cutting histories were not known, but no cutting had occurred in any of the stands since 1981, and all stumps present appeared to be at least 15 to 20 years old.

No attempt was made to identify the species of *Armillaria* in the forests sampled in this study. However, concurrent work by P.M. Wargo (unpublished data) in similar mixed oak forests in south central Pennsylvania indicates that North American biological species VII (*Armillaria bulbosa* Barla., synonyms *A. gallica* Marxm. and *A. lutea* Gillet) is the most common species of *Armillaria* in these mixed oak stands. This species of *Armillaria* is characterized as a stress-induced pathogen and a prolific producer of rhizomorphs (Rishbeth 1982, 1985).

Initial study

Within the stands characterized, 85 sample plots were located in 1985. There were 29 plots where no defoliation had occurred (controls), 30 plots where gypsy moths had defoliated the stand in 1985, and 26 plots where linden loopers had defoliated the stands in 1981. At each plot three soil samples were taken at random and three were taken within 30–50 cm of a dead tree or stump to determine rhizomorph content. (These samples are termed biased samples in the sections that follow.) Individual samples were collected using a procedure established by Wargo *et al.* (1987). The unincorporated litter was brushed away, and a 15 × 15 cm square template was placed on the forest floor. We used a sharpened army bivouac shovel to cut around the template, carefully shearing any roots that crossed the boundary of the sample. The sample was removed and separated into two portions, the organic layer and the A-horizon, to determine any differences in rhizomorph distribution between soil horizons. The thickness of each layer was recorded, and the samples were placed in separate plastic bags for transport to the laboratory.

In the laboratory each sample was carefully dissected to separate the rhizomorph segments from the soil and roots. Total length and total weight of rhizomorph segments in each sample were measured, and volume of soil for each sample was calculated. Rhizomorph length per unit volume of soil was then calculated for the organic layer and A-horizon for each sample. The three random and three biased samples were averaged separately for each plot to yield one value for randomly placed samples and one for biased samples. Ovendry weight of rhizomorphs in each sample from the initial experiment was determined and calculated per unit soil volume.

Second study

The initial study provided information regarding abundance of rhizomorphs near dead trees relative to random samples, but was not designed to facilitate estimates of overall density per unit area. Therefore a second study was initiated to examine distribution of rhizomorphs at a scale larger than a single tree's root system.

Study plots were replicated five times in the stands with the three defoliation histories described earlier, for a total of 15 plots. Each plot was 40 × 40 m. All living trees, dead trees, and stumps over 7.5 cm on the plot were mapped. Stumps were recorded only if they were still firmly anchored in the soil. Species and diameter at breast height were recorded for each stem. Centered within the 40 × 40 m plot was a 20 × 20 m plot that was systematically gridded into 121 subplots (1.8 × 1.8 m). The number of plots was a compromise between the detail of information needed and the effort required to collect and process the samples. A soil sample for determining rhizomorph content was taken down to the top of the B-horizon at the center of each subplot. Soil samples were collected during the winter of 1986–1987 and frozen for up to several months until processed in the laboratory. Both field and laboratory procedures were the same as described for the initial study.

Results and discussion

Analysis of variance of rhizomorph abundance in the first experiment showed no significant difference ($p < 0.05$) between the organic and mineral horizons. Variation was great among samples from both horizons (Fig. 2). In some

TABLE 1. Characteristics of sample stands by treatment category

Years after defoliation	Forest type	Site index red oak	Basal area (m^2/ha)	Mean dbh (cm)	Mortality (%)*	Elevation (m)
5	52†	64	28	26	13	330–350
1	52	62	23	25	12	275–300
—‡	52	58	23	29	7	305–325

*Basal area of dead stems as a proportion of total basal area.
†Society of American Foresters cover type 52: white oak, northern red oak, black oak.
‡No known defoliation.

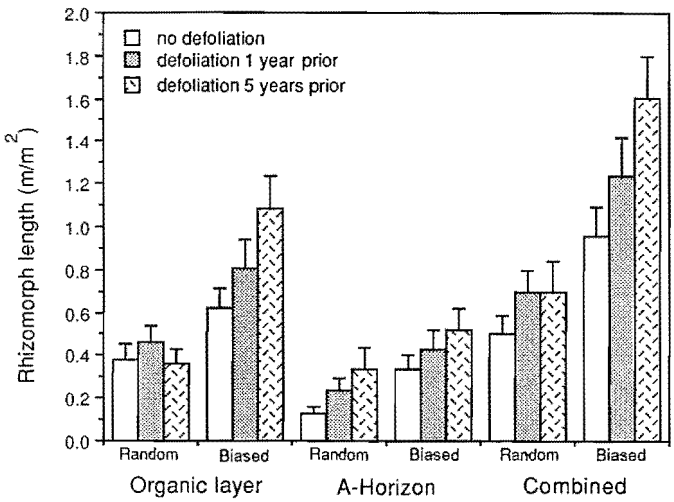

FIG. 2. Rhizomorph length in organic and mineral horizons in soil samples taken randomly and near dead trees.

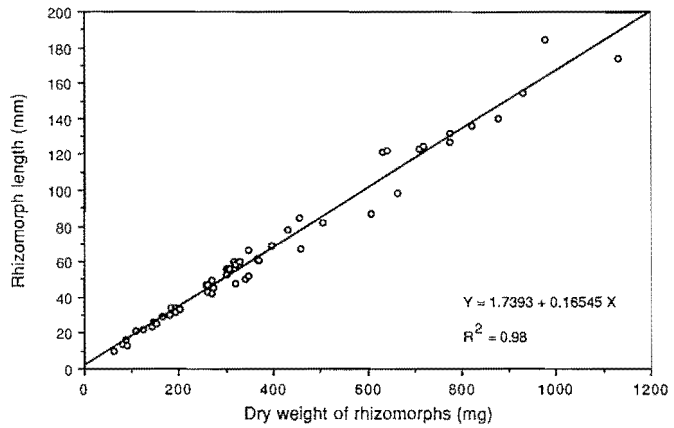

FIG. 3. Dry weight of *Armillaria* rhizomorphs predicted from rhizomorph length in each soil sample. (Only samples weighing over 1 mg were included, due to sensitivity of scale.)

samples the greater quantity was found in the organic layer, in others in the mineral soil, with no apparent consistency. The analyses that follow were performed on the sum of the two measurements.

Dry weight analyses produced no improvement in variation over total length or length per unit volume within biased samples, within random samples, or between the two groups. Overall, weight was closely correlated (R^2 = 0.98) with total length in samples that weighed at least 1 mg, and the biomass of rhizomorphs in a sample could be estimated accurately from the length (Fig. 3).

TABLE 2. Abundance of *Armillaria* rhizomorphs in sample plots following defoliation

Area	Years after defoliation	Total length* (m/m^2)
1	5	12.137*a*
2	1	7.959*b*
3	—†	4.545*c*

*Means followed by different letters are significantly different at the 0.10 level.
†No known defoliation.

TABLE 3. Frequency of occurrence of rhizomorphs in soil samples from a systematic grid in areas with different defoliation histories

Area	Years after defoliation*	Present	Absent	Total
1	5	372	233	605
2	1	310	295	605
3	—†	236	369	605
Total		918	897	1815

*Frequencies are highly dependent (χ^2 = 61.311, $p < 0.001$) on time since defoliation.
†No known defoliation.

Because a few very thick rhizomorphs may affect dry weight in a greater proportion than they affect inoculum potential, we reasoned that the pathologically relevant variable was total length. All future analyses were thus performed only on total length and length per unit volume.

In the second experiment analysis of rhizomorph length in the organic and A-horizons showed no difference between the two layers. Total length per sample, converted to length per unit area (m/m^2), was chosen for further analysis as representative of abundance of rhizomorphs of *Armillaria*. Overall abundance of rhizomorphs of *Armillaria* was significantly greater in the defoliated stands (Table 2). The stands with recent defoliations also had more dead trees than the undisturbed stand. However, this study did not determine whether the increased abundance of *Armillaria* resulted in greater mortality or simply reflected the increased available substrate.

Distribution of the rhizomorphs was not uniform throughout the plots. Many soil samples contained no rhizomorphs, especially in the undefoliated stand (Table 3). The first year's sampling and analysis suggested that both the likelihood of finding rhizomorphs and the quantity present, when found, were much higher in the samples taken within 50 cm from a dead tree (Fig. 2).

TABLE 4. Abundance of *Armillaria* rhizomorphs in soil samples within different radii around dead trees, live trees, and stumps across all defoliation histories

Radius from tree	Tree status	No. of trees	Mean samples per tree*	Mean length of rhizomorphs (m/m^2)†
1.8 m	Live	359	2.73 (0.05)	8.32*a*
	Dead	67	2.82 (0.14)	19.15*b*
	Stump	62	2.60 (0.14)	9.53*a*
3.0 m	Live	445	6.25 (0.14)	7.98*a*
	Dead	84	6.06 (0.33)	12.99*b*
	Stump	70	6.51 (0.33)	9.20*a*
Crown radius	Live	505	8.89 (0.29)	9.26*a*
	Dead	92	7.25 (0.53)	13.41*b*
	Stump	81	8.15 (0.54)	9.08*a*

*Standard error is given in parentheses.
†Means of rhizomorph lengths followed by the same letter are not significantly different at the 0.05 level, compared within each radius level.

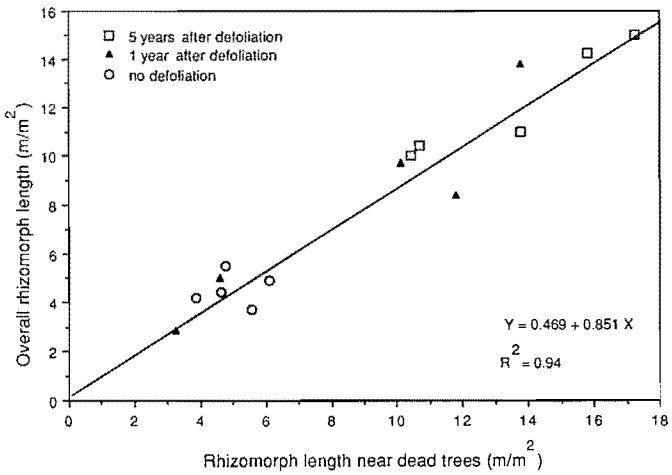

FIG. 4. Overall abundance of *Armillaria* rhizomorphs on 15 sample plots predicted from samples within the radius of influence of dead trees in areas with three different defoliation histories.

The correlation of rhizomorph abundance with distance to the nearest dead tree proved highly significant but too low ($p < 0.01, r = 0.14$) to draw any inferences. However, samples that were within the influence of dead trees showed significantly higher densities of rhizomorphs (Table 4). One satisfactory measure of "radius of influence" for dead trees was crown radius, computed for each dead tree using equations published by Lamson (1987). The estimate of rooting area as equivalent to crown size is probably an underestimate for stand-grown trees, but may provide a good representation of those roots large enough to persist and provide a sufficient substrate for rhizomorph production. The overall abundance on a plot is indeed highly correlated ($R^2 = 0.94$) with the abundance within the radius of influence of dead trees, as illustrated by Fig. 4.

The data in Table 4 indicate the generally greater abundance of rhizomorphs around recently dead trees compared with either live trees or stumps. The lack of difference between live trees and stumps indicates that the food base of stumps and their root systems was becoming exhausted. Age of the stumps was not known, but they all appeared to be at least 15 to 20 years old.

Conclusions

Sampling implications

Great variability between samples of rhizomorphs of *Armillaria* in forest stands is apparent. The irregular distribution requires intensive sampling to obtain a reasonable amount of confidence in an estimate. In part, this is due to the growth habits of tree roots from which the rhizomorphs grow. Tree roots distribute themselves nonuniformly throughout the forest floor and upper layers of mineral soil. The number of roots from a given tree may increase with distance from the base of the tree, but the size and persistence of the roots decrease (Stout 1956). Thus the amount of substrate made available to *Armillaria* when a tree dies is likely to be inversely related to the distance from the tree (Singh 1981).

If density of rhizomorphs is indeed higher near dead trees, as our data indicate, then sampling near such dead trees will produce fewer samples with no rhizomorphs present. It is desirable, for estimating an overall population, to have few samples with zero counts, because the distribution of samples will be more nearly normal (Steel and Torrie 1980). The high correlation of rhizomorph densities near dead trees with densities found throughout the study plots indicates that sampling predominantly near dead trees will produce a good estimate of overall rhizomorph density. The estimate must, however, be weighted to account for the different probabilities of sampling and the proportion of area represented by each sample class.

Management implications

The data from these studies show that *Armillaria* rhizomorphs are present in large quantities after defoliation episodes. Rhizomorph abundance is correlated with mortality of trees in defoliated stands. *Armillaria* rhizomorphs are more abundant 5 years after defoliation than in the 1st or 2nd year after defoliation. Because cutting activity increases the substrate available for *Armillaria* to colonize, it may serve to increase the inoculum potential in a stand. Trees weakened by defoliation are known to have increased vulnerability to *Armillaria* attack (Wargo 1977). A close examination of Table 4 supports this conclusion. The dead trees have a high abundance of rhizomorphs, whereas the stumps (all from older cutting activity long prior to defolia-

tions) are returning to levels similar to those around living trees. Thus high-value stands with recent management activity need to be given high priority for protection from defoliation until abundance of *Armillaria* has again decreased.

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