

Distributions and Hosts of *Armillaria* Species in New York

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ABSTRACT

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A statewide investigation was conducted to identify the species of *Armillaria* and determine their geographic distributions and host/substrate relationships in New York. At 273 sites, samples of rhizomorphs, wood, and/or basidiomata were collected from 414 trees, snags, stumps, or logs that were colonized by *Armillaria*. Field isolates were identified to species by mating the unknown isolates with previously identified haploid isolates. Six species were identified in New York forests. *A. calvescens*, the most common species, was widely distributed and had a broad host/substrate range. It occurred mainly on hardwoods (86% of collections), commonly causing butt rot. *A. gallica*, the second most common species, also was found primarily on hardwoods (86% of collections), especially oaks, and often caused butt rot. It was widespread but was not found in the Adirondack region. *A. ostoyae*, the third most common species, was found predominantly in the Adirondack region. It was the only species found principally on conifers (89% of collections). *A. gemina* was scattered throughout the state and was found almost always on hardwoods (96% of occurrences). *A. sinapina*, like *A. ostoyae*, was found mainly in the Adirondack region but was found mostly on hardwoods (60% of occurrences). *A. mellea* sensu stricto was found only once on Long Island on *Quercus alba* and once in central New York on an unidentified stump. All species except *A. mellea* were found infecting cambial tissue of living hosts. There was no significant difference among *Armillaria* species in the frequencies of cambial infections.

Armillaria spp. cause root diseases on a large number of woody and herbaceous species throughout the world (15). They are common root-rotting fungi on conifers in the Northeast (20-22) and are associated with mortality of many hardwoods (11,12). Recent work indicates that several taxonomically and biologi-

cally distinct species exist within the formerly broadly conceived single species, *A. mellea* (Vahl:Fr.) P. Kumm. (1,3,4). Nine species are currently recognized in North America, six of which have been collected in the Northeast (1,3,4). There is limited information on geographic distributions of these important species, their host ranges, and pathogenicity.

The objectives of this study were to identify *Armillaria* species found in New York forests and to determine their geographic distributions and host/substrate relationships. Host/substrate relationships included host ranges, occurrence

of cambial infection, occurrence of butt rot, crown health, crown position, the diameter at breast height, and obvious stress factors.

MATERIALS AND METHODS

Site selection. Two hundred and seventy-three sites were selected from New York State-owned forest lands in which *Armillaria* occurred. An attempt was made to nonrandomly select sites beforehand that were distributed evenly throughout the state's subregions (19). This was done to obtain a sampling distribution that was more regular than that of state-owned forest lands. To include the less common tree species, different forest types were sampled within the state's subregions (6). Sampling occurred during September 1986, from May to September 1987, from June to September 1988, and from May to July 1989.

Host/substrate. Rhizomorphs, wood samples, and/or basidiomata were collected from 180 living trees and from 234 snags, stumps, or logs that were colonized by *Armillaria*. The first living host found to be colonized by *Armillaria* was preferentially selected at a site. If no live trees were found to be colonized, then the first substrate found to be colonized by *Armillaria* was selected. In some cases, only one isolate was collected at a site, and in others, multiple isolates were collected. Random sampling of hosts/substrates was not attempted.

Live trees were examined by removing soil from around the root collar. If rhizo-

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morphs were present at the root collar, major roots also were excavated. When potential signs or symptoms of cambial infection were observed on the living trees (rhizomorphs penetrating the bark, sunken areas in the bark, or discolored or darkened areas), small areas of bark were removed to inspect for cambial infection.

For each host/substrate from which *Armillaria* was collected, observations were made on the geographic location, tree species, and condition (live tree, stump, snag, or log) of the substrate. For live trees, cambial killing (mycelial fan penetrating into or infecting live cambium of the root collar and/or roots) and/or the occurrence of butt rot (rotting

the central xylem at the base of a live tree) were noted. Butt rot was noted when decay of the central xylem could be observed through cracks, basal lesions, decayed roots, stilt roots, or by digging up from below a tree. All trees could not be examined for butt rot because the trees were not dissected.

The crown position (dominant, co-dominant, intermediate, or suppressed), the diameter at breast height, and crown health were noted for 131 of the 180 live trees sampled. Crowns were ranked as healthy (0-20% crown dieback), declining (21-80% crown dieback), or almost dead (80-99% crown dieback). Obvious stress factors affecting the 131 host trees were recorded, including insect damage, mechanical damage, cankers, suppression (suppressed crown position), overmaturity, and water stress. Overmature trees were those with diameters greater than 250% of mature trees as defined by Harlow et al (9). Water stress was noted on sites with standing water at least part of the year (such as swamps where roots were underwater) or sites with greater than 50% slope and coarse-textured soils (steep coarse-textured sites).

Isolation and identification. Isolations from field samples were made on benomyl streptomycin malt agar (18). For rhizomorph isolations, 1-cm rhizomorph sections were surface-disinfested in a solution of 1.05% NaOCl and 20% etha-

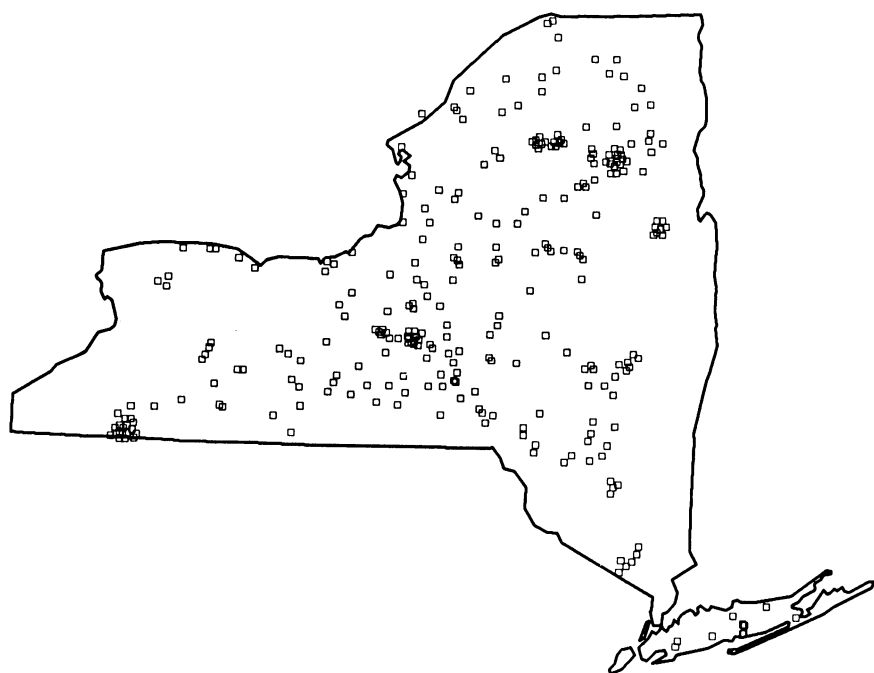


Fig. 1. Distribution of sites in New York State in which *Armillaria* species were found.

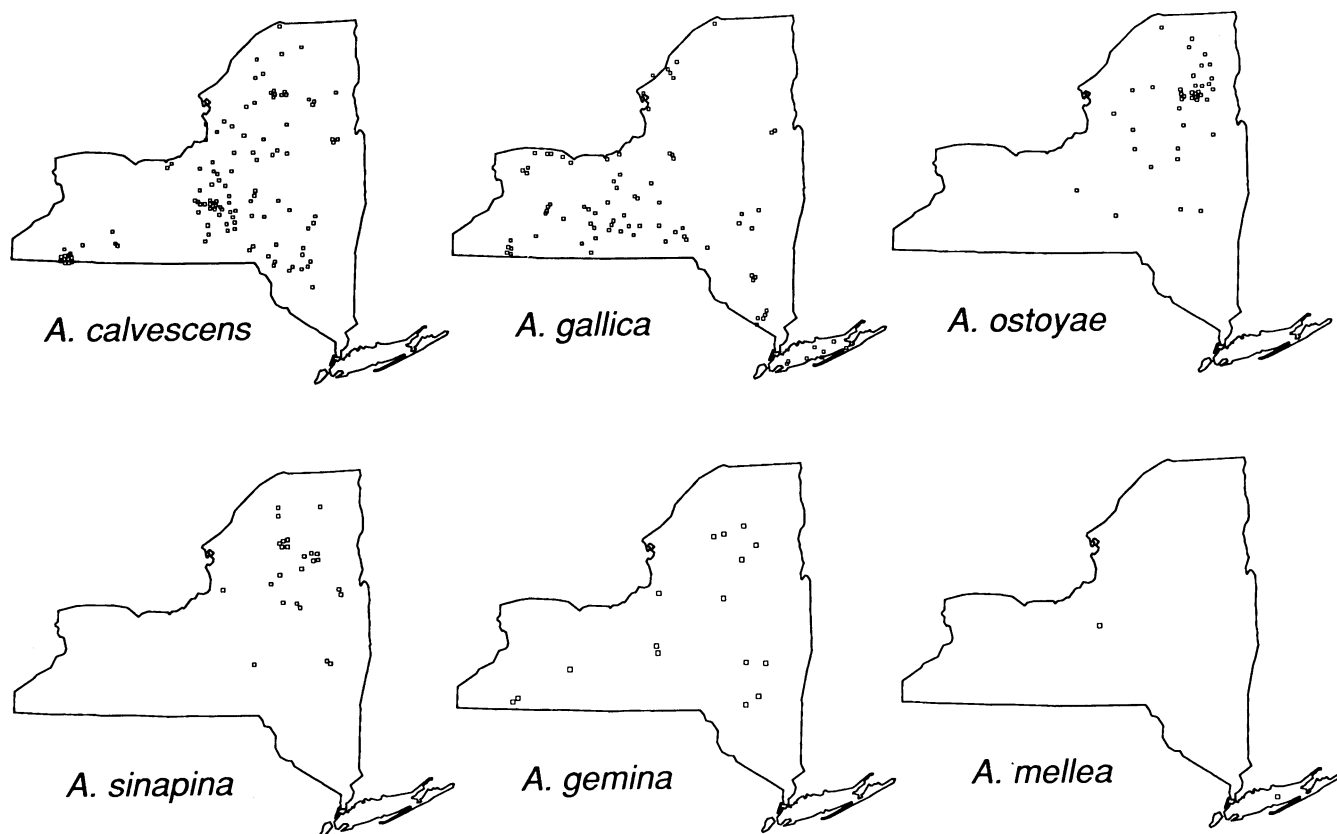


Fig. 2. Distribution of sites in New York State in which *Armillaria calvescens*, *A. gallica*, *A. ostoyae*, *A. sinapina*, *A. gemina*, and *A. mellea* were found.

nol for approximately 3 min before plating. For wood isolations, the wood was split and small chips were removed from the newly exposed wood surface before plating. Basidiome isolations were made from context tissues. Cultures were transferred to malt extract agar (MEA) (1.5% malt extract and 1.5% agar) after 3–6 wk. All cultures were incubated at room temperature in the dark. Stock cultures were maintained on MEA at 4 C in the dark.

Isolates were identified to species by matings with haploid tester isolates from known species (10) on MEA. Putatively diploid unknown isolates (7) were paired with two to three haploid tester isolates of each of the six *Armillaria* species reported in the Northeast (*A. calvescens* Bérubé & Dessureault, *A. gallica* Marxmüller & Romagn. [= *A. bulbosa* (Barla) Kile & Watling, = *A. lutea* Gillet], *A. ostoyae* (Romagn.) Herink., *A. sinapina* Bérubé & Dessureault, *A. gemina* Bérubé & Dessureault, and *A. mellea*). Five-millimeter plugs of mycelium (cut from MEA plates) were placed approximately 5 mm apart. After 6 wk, three plugs were taken from the haploid tester side of the confrontation line (5 mm past the confrontation line, on the far edge of the

culture, and halfway between) for subculturing (10). Morphological characteristics of the subcultures were observed after another 10 days. Fluffy, white subcultures were recorded as incompatible; flat, darkened subcultures were recorded as compatible (10). For reference, the morphological characteristics were compared with the unpaired tester isolates. All matings and subcultures were incubated at room temperature in the dark.

Analysis. For the quantitative variable (diameter at breast height), one-way analysis of variance was used followed by Fisher's protected least significant difference range test at the 95% confidence level. For frequencies, the chi-square goodness-of-fit procedure was used. The expected frequencies used were $(S \cdot C) / N$, where S is the number of observations of an *Armillaria* species, C is the total number of observations of the category of interest, and N is the total number of observations. Unless otherwise noted, differences were considered significant at probabilities of 0.05 or less.

RESULTS

Species identification. *Armillaria* was isolated from 414 different host/substrates at 273 sites (Fig. 1). *Armillaria*

was successfully isolated from approximately 95% of the host/substrates sampled. The two most abundant species were *A. calvescens* (found 164 times at 117 sites) and *A. gallica* (found 100 times at 85 sites). Less prevalent were *A. ostoyae* (found 85 times at 39 sites), *A. sinapina* (found 26 times at 25 sites), and *A. gemina* (found 31 times at 16 sites). *A. mellea* was the least common species (found two times at two sites).

Six isolates, collected from different sites, could not be identified as any of the six tested species. Three of the unidentified isolates gave unclear results, two reacted with more than one tester species, and one did not react with any of the testers.

Distribution. *A. calvescens* was found predominantly in the western and eastern Appalachian Highlands, Adirondack, and Catskill regions (Fig. 2). *A. gallica* was found in all major regions except the Adirondack region. *A. ostoyae* and *A. sinapina* were found mainly in the Adirondack region. *A. gemina* was found throughout the state. *A. mellea* was found on Long Island and in central New York at an experimental forest.

Host relationships. *Armillaria* spp. were isolated from 34 tree species (Table

Table 1. Observations of *Armillaria* species on tree species in New York forests

Host	<i>Armillaria</i> species										Total no.
	<i>calvescens</i>		<i>gallica</i>		<i>ostoyae</i>		<i>sinapina</i>		<i>gemina</i>		
	No. isolated ¹	Condition ^u	No. isolated	Condition	No. isolated	Condition	No. isolated	Condition	No. isolated	Condition	
<i>Acer saccharum</i>	56	s,i,b	4	s,i	3	s,i	0	...	10	s,i,b	73
<i>Fagus grandifolia</i>	19	s,i,b	6	s,i,b	1	s	2	s,i,b	9	s,i,b	37
<i>Acer rubrum</i>	19	s,i,b	11	s,i,b	1	i	4	s,i,b	1	s	36
<i>Picea rubens</i>	4	s,i	0	...	23	s,i,b	4	s,i,b	0	...	31
<i>Abies balsamea</i>	3	s	2	s,i	17	s,i,b	1	s	0	...	23
<i>Quercus rubra</i>	2	s,i,b	16	s,i,b	0	...	0	...	1	s	19
<i>Pinus sylvestris</i>	2	s,i	3	s,i	13	s,i	0	...	1	i	19
<i>Pinus strobus</i>	4	s,i	3	s,i	7	s,i	2	s,i,b	0	...	16
<i>Betula alleghaniensis</i>	7	s,i,b	1	s	2	s	4	s	1	s	15
<i>Prunus serotina</i>	5	s,i,b	3	s,b	0	...	2	s	2	s	12
<i>Quercus alba</i>	0	...	12	s,i,b	0	...	0	...	0	...	12
<i>Tsuga canadensis</i>	4	s	2	s	3	s	2	s	0	...	11
<i>Fraxinus americana</i>	3	s,b	6	i,b	0	...	1	s	0	...	10
<i>Pseudotsuga menziesii</i>	1	s	0	...	8	s,i	0	...	0	...	9
<i>Pinus resinosa</i>	1	s	1	s	4	s,i	1	s	0	...	7
<i>Tilia americana</i>	6	s,i,b	1	s	0	...	0	...	0	...	7
<i>Betula papyrifera</i>	1	s	2	s	1	s	0	...	1	s	5
Unidentified hosts	19	s	9	s	0	...	1	s	4	s	33
Other hardwoods ^v	6	s,i,b	16	s,i,b	1	...	2	s,i,b	1	s	26
Other conifers ^w	2	s	2	s	1	s	0	...	0	...	5
Total	164		100		85		26		31		406
Hardwoods ^x	124		78		9		15		26		252
Conifers ^y	21		13		76		10		1		121
P^z	<0.01		<0.01		<0.01		0.42		<0.01		

¹ Total number of times isolated from a tree species.

^u Found as: s = sap-rot of a stump, snag, or log; i = infection of the cambium of a live tree (mycelial fan penetrating into live cambium of the root collar and/or roots); b = butt rotter of a live tree (rotting the central xylem at the base of a live tree).

^v Other hardwoods are those that make up a total for all species of *Armillaria* of less than five including: *Carya cordiformis*, *Populus deltoides*, *Liriodendron tulipifera*, *Populus grandidentata*, *Quercus prinus*, *Ulmus americana*, *Carpinus caroliniana*, *Juglans cinerea*, *Acer pensylvanicum*, *Quercus bicolor*, *Populus tremuloides*, *Fraxinus nigra*, *Acer saccharinum*, *Crataegus* spp., and *Carya ovata*.

^w Other conifers are those that make up a total for all species of *Armillaria* of less than five including *Picea abies* and *Larix* spp.

^x Total number of collections from identified hardwood species.

^y Total number of collections from identified conifer species.

^z Probability that there is no difference in frequencies of host types (hardwood or conifer) for each species of *Armillaria* based on chi-square tests.

1). Statistically significant differences in relative frequencies among the *Armillaria* species were found among tree types (hardwood and conifer) based on chi-square tests ($P < 0.01$). *A. calvescens*, *A. gallica*, and *A. gemina* were found more frequently than expected by chance on hardwoods. *A. calvescens* commonly was found on maples and *A. gallica* on oaks. *A. ostoyae* was found more frequently than expected by chance on conifers. *A. sinapina* showed no tree preference. *A. mellea* sensu stricto was found once on *Quercus alba* L. and once on an unidentified stump.

Significant differences were found among the *Armillaria* species in frequency of association with butt rot (Table 2). *A. calvescens* and *A. gallica* both caused butt rots more frequently than expected by chance.

Armillaria was found infecting the live cambium of 32% of the host/substrates sampled (Table 2). *A. ostoyae* was found infecting live cambium on a greater proportion of its hosts than were other species, but there was no significant difference among *Armillaria* species in the frequencies of cambial infections. No significant difference among *Armillaria* spp. was observed in the frequencies of live vs. dead hosts colonized (*data not shown*).

No significant difference among *Armillaria* spp. was found in the following host characters: stress, diameter at breast height, crown position, and crown health. No significant difference was observed among the *Armillaria* species in the relative number of isolations from stumps, snags, or logs (*data not shown*).

DISCUSSION

The presence of several distinct species has explained some of the variation in basidiome features, host range, pathogenicity, symptom expression, geographic distribution, and cultural characteristics previously attributed to a single species of *Armillaria* (20). Although no differences were found among the *Armillaria* species in frequencies of live trees infected, their distributions and hosts

varied. This indicates that site conditions and hosts play an important role in determining the presence and potential pathogenicity of a species.

A. calvescens previously was reported exclusively in the Northeast, as a minor species, typically found on dead hardwood materials (2,4). In the current study, it was abundant on a large number of host species, most commonly maples and other hardwoods. It was commonly associated with butt rot.

A. gallica was reported as a species common in southern latitudes and at low altitudes in the northern temperate hemisphere (8). This seems to hold true in New York. *A. gallica* is reportedly most common on dead hardwoods and is considered a weak pathogen of hardwoods and conifers in the northern temperate hemisphere (8,16,17). In the current study, it was found frequently on oaks and other hardwoods. It also was commonly associated with butt rot, which supports earlier observations in England (16,17).

A. ostoyae was reported as a species common in northern latitudes in the northern temperate hemisphere (8), which is the case in New York. This may be related to the distribution of conifers. *A. ostoyae* previously had been found on conifers as an aggressive pathogen in England (16,17) but also has been found as a pathogen of hardwoods (14). In the current study, it was found principally on conifers in the Adirondack region. Although *A. ostoyae* was reported as a more aggressive pathogen compared with other *Armillaria* species, except *A. mellea* (20), pathogenic differences were not indicated in this study.

A. sinapina is considered a relatively northern species (8). It was previously reported as a weak pathogen, often on hardwoods but also on conifers (5,13). It was common in the northern part of New York and had no strong relationship to either host/substrate type.

A. gemina has been found rarely and only in the Northeast, commonly on dead hardwoods (1,4). In the current study, it was found throughout New York as

an infrequent species, almost exclusively on hardwoods.

A. mellea sensu stricto was reported as an aggressive pathogen of hardwoods in England (16,17). It was previously found to have a southern distribution (8). *A. mellea* may be more common south of New York. In this study, it was found once on *Q. alba* on Long Island and once at an experiment station in central New York, where it may have been introduced.

The identification and characterization of *Armillaria* species are critical starting points in making management recommendations to avoid or reduce losses attributable to the disease. Differences in host and site preferences among the *Armillaria* species may help in deciding which tree species to grow in an area. However, long-term studies of losses attributable to a particular *Armillaria* species would be required for management recommendations.

The six *Armillaria* species identified in New York forests differ in geographic distributions and host/substrate relationships. Although there is overlap among the species, these differences can help in distinguishing among them and in understanding variation in this important group of pathogens.

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Table 2. Number of observations of butt rot and cambial infection of hosts in which each *Armillaria* species was isolated

<i>Armillaria</i> spp.	No. observed ^w	Butt rot ^x	Cambial infection ^y
<i>A. calvescens</i>	164	39	54
<i>A. gallica</i>	100	23	33
<i>A. ostoyae</i>	85	4	32
<i>A. sinapina</i>	26	5	6
<i>A. gemina</i>	31	3	6
Total	406	74	131
P^z		<0.01	0.54

^wTotal number of observations for each species of *Armillaria*.

^xIsolates from live trees that were rotting the central xylem at the base of the trees.

^yIsolates from live trees that were penetrating into the cambium of the root collar and/or roots.

^zProbability that there is no difference among frequencies of *Armillaria*, within columns, based on chi-square tests.

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