Biological Species of *Armillaria* Isolated from Sour Cherry Orchards in Michigan

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ABSTRACT

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In Michigan, Armillaria root rot severely affects Montmorency sour cherry trees growing in sandy soils. Haploid, single basidiospore cultures were established from basidiocarps of *Armillaria* collected from 56 sour cherry, two sweet cherry, four peach, one apple, and five oak trees. A total of 77 trees from 20 orchards and from one nonorchard site were sampled. Where basidiocarps or viable basidiospores were absent, haploid isolates were recovered from diploid isolates by inducing somatic segregation. Based on the sexual compatibility of paired isolates, three intersterile groups of *Armillaria* were identified among the collected isolates. These intersterility groups were compatible with North American intersterility groups I, III, and VI of *Armillaria* as designated by Anderson and Ullrich. From the 72 orchard trees sampled, groups I, III, and VI were collected from 58, 3, and 11 trees, respectively. Group VI was also collected from five oaks at one location. North American group I, which corresponds with the taxonomic species *A. ostoyae*, was widely distributed in Michigan orchards. Group VI, *A. mellea* sensu stricto, and group III were restricted in their distribution. All three biological species should be considered pathogens of orchard crops.

Additional key word: *Prunus cerasus*.

Armillaria root rot has been reported as a disorder of deciduous fruit trees in many orchard-growing areas worldwide (5,6,8,10). In Michigan, Armillaria root rot has been observed in orchards for several years and is a widespread problem on sour cherry trees (*Prunus cerasus* L.) growing in sandy soils (7). Traditionally, isolates of *Armillaria* associated with orchard trees have been classified as *A. mellea* (Vahl ex Fr.) Kummer (15). However, recent genetic studies of *Armillaria* from forest trees indicate that *A. mellea* is made up of a complex of several reproductively isolated, intersterile groups (3,9,11). Although these groupings are currently referred to as biological species, some European and North American biological species correspond to accepted taxonomic species (2,11,12).

The objective of this research was to determine the occurrence and distribution of different biological species of *Armillaria* in Michigan orchards. As recommended by Wargo and Shaw (15), we used the Roman numeral classification system of Anderson and Ullrich (3) when referring to biological species groups.

MATERIALS AND METHODS

Isolate collection. In September and October 1985, 127 basidiocarps of *Armillaria* were collected from trees in 17 of 20 orchard sites in Michigan (Fig. 1). Where possible, basidiocarps were collected from different infection foci within each orchard. Basidiocarps were collected from 58 Montmorency sour cherry trees (*P. cerasus* L.), two sweet cherry trees (*P. avium* L.), one apple tree (*Malus pumila* Mill.), and four peach trees (*P. persica* (L.) Batsch). Most of the trees were dead at the time of sampling. Basidiocarps were also collected from five oak stumps at a location outside the fruit-growing area.

To obtain single basidiospore isolates, 12-mm-diameter plugs were cut from the pileus of each basidiocarp with a cork borer. The upper surface of the pileus was coated with petroleum jelly and stuck to the bottom of a large, 14-cm deep paper cup. Each cup was inverted over a 100- × 15-mm petri plate containing 1% malt extract agar. Basidiospores were allowed to discharge for 3–5 hr or until several basidiospores could be observed scattered on the surface of the medium with a dissecting microscope. After 12–24 hr at about 21 C, individual germinated basidiospores were selected and transferred with a fine metal needle to 60- × 15-mm petri plates containing the enriched malt extract medium of Shaw and Roth (SR medium) (13). Approximately 8–12 single-spore isolates were obtained from each basidiocarp for study by this method. Two basidiocarps collected from sour cherry failed to release viable basidiospores. Diploid isolates were established for these two basidiocarps by aseptically placing pieces of stipe tissue onto SR medium.

No basidiocarps were present in three orchards, so isolations were made directly from infected woody tissue. Pieces of infected host material were aseptically removed and placed onto 2% malt extract agar and subsequently subcultured and grown on SR medium. Diploid isolates were obtained in this manner from seven sour cherry trees. Haploid isolates were produced from these diploid isolates and from the stipe isolates by inducing somatic segregation (1,4). The diploid isolates were grown on SR medium amended with 25 μg/ml of benomyl for 6 wk. Portions of mycelium from the margins of the colonies were ground in 7-ml glass tissue grinders with 2.5 ml of sterile distilled water. Portions of the resultant mycelial suspension were diluted in series, and 0.2-ml aliquots were plated onto the surface of 100- × 15-mm petri plates containing SR medium. Any discrete colonies with fluffy white mycelial growth, typical of haploid strains, were transferred for further study.

Interfertility studies. Interfertility, or sexual compatibility, among the isolates was tested by pairing haploid tester isolates as described by Anderson and Ullrich (3) and by Korhonen (9). Sexual compatibility in *Armillaria* is tetrapolar (14). A single basidiocarp can produce basidiospores in four sexual compatibility groups or mating types. Haploid isolates representing the possible mating types from a given basidiocarp were selected by pairing all of the haploid, single basidiospore isolates obtained from a basidiocarp and recording the compatible and incompatible responses. A total of 33 basidiocarps was examined in this manner, at least one from each sampling site where basidiocarps were present. All pairings were made on SR medium in 60- × 15-mm petri plates at 21 C. The responses were

scored after 4–6 wk. Interfertile and intersterile relationships between isolates were recorded and tester isolates representative of the different mating types were chosen. The tester isolates obtained from each of the 33 basidiocarps were then paired with two to four tester isolates from each of the other basidiocarps. Interfertile or intersterile responses between isolates from different basidiocarps were scored. In this manner, intersterile groups were established among the 33 sampled basidiocarps. Representative haploid tester isolates from each of the intersterile groups were then paired with one or two randomly selected haploid isolates from each tree sampled. The haploid isolates came from either single basidiospores or were produced through somatic segregation. Intersterile and interfertile responses were scored and isolates were placed into the appropriate intersterile group.

Biological species determinations. Representative tester isolates from the intersterile groups in our pool of Michigan isolates were crossed with tester strains provided by J. B. Anderson for North American Biological Species I, II, III, V, VI, VII, IX, and X of *Armillaria*. Intersterile and interfertile responses were scored.

RESULTS

Tetrapolar sexual compatibility, or bifactorial heterothallism, was confirmed for 32 of the 33 basidiocarps examined. Among the single basidiospore isolates obtained from each tested basidiocarp, three or four mating types were represented. In the one case, where bifactorial heterothallism was not confirmed, only two mating types were represented in the 11 single basidiospore isolates available for testing.

Three intersterile groups were found among the collection of haploid isolates of *Armillaria* established from orchard trees (Table 1). These three intersterile groups were compatible with tester isolates of North American groups I, III, and VI. From the 72 orchard trees sampled, groups I, III, and VI were collected from 58, 3, and 11 trees, respectively. Tester isolates of *Armillaria* collected from oak stumps were compatible with testers of group VI. Group I was detected in each of the six counties surveyed. In Leelanau, Grand Traverse, and Benzie counties, group I was the only biological species of *Armillaria* collected. In Manistee, Mason, and Oceana counties, groups III and VI were collected in addition to group I. Excluding the oak isolates, group VI was collected only on orchard trees in southern Oceana County (Table 1). Except for site L, in Manistee County, where groups I and III were recovered from different sour cherry trees, only one intersterility group of *Armillaria* was collected from a single orchard or site (Table 1).

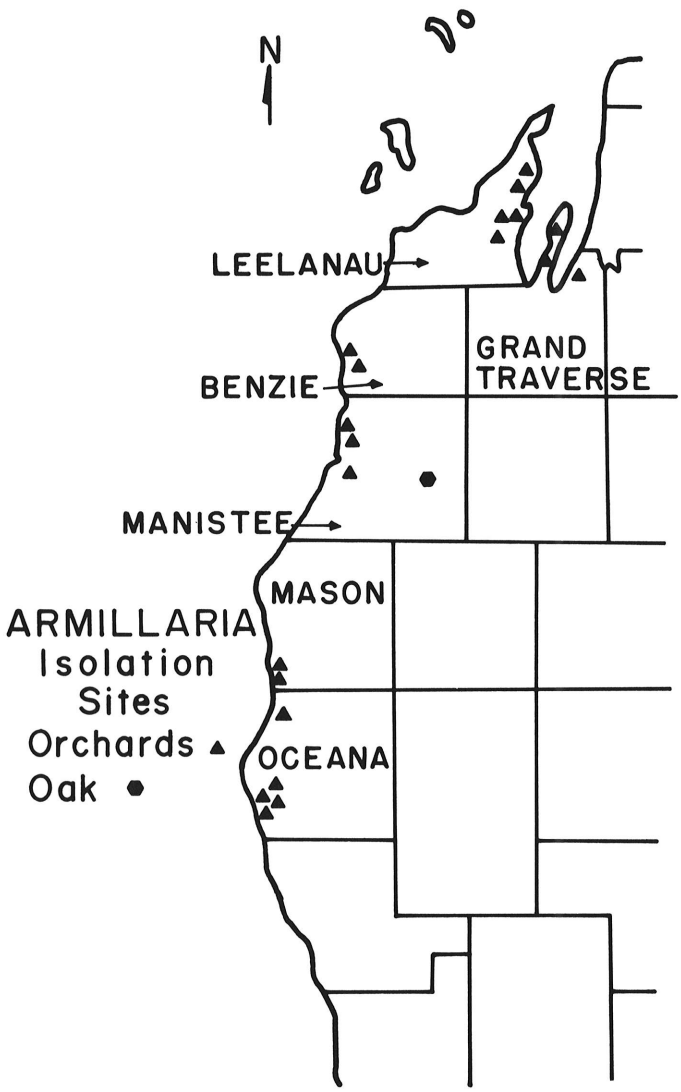

Fig. 1. Location by county of 20 orchard sites and one nonorchard site in Michigan where *Armillaria* was collected for determination of biological groups. The six counties surveyed were in the lower peninsula bordering Lake Michigan.

DISCUSSION

This study supports the observation (7) that among the tree fruit crops grown in Michigan, Armillaria root rot occurs most commonly on sour cherry. We sampled sour cherry orchards almost exclusively because these were the only problem sites known to the cooperative extension horticultural and fruit agents who helped us locate orchards for study. Most sour cherry trees are grafted on Mahaleb rootstock. Sweet cherry orchards or blocks growing adjacent to diseased sour cherry orchards are not seriously affected by Armillaria root rot. Most sweet cherries are grafted on Mazzard rootstock. The two infected sweet cherry trees found in this survey were interplanted with sour cherry and were grafted on Mahaleb rootstock. The four infected peach trees were located in an old sour cherry site where, before its removal and planting with peach, Armillaria root rot was identified by A. L. Jones and L. J. Bradford. The infected apple tree was adjacent to infected sour cherry trees. Except for two sites, all orchards were planted on old cherry orchard sites. Thus cherry, not newly cleared forest land,

TABLE 1. Distribution pattern of biological species of *Armillaria* isolated from orchard sites in Michigan

County	Site	Host	Trees (no.)	North American biological species[a]
Leelanau	A	sour cherry	7	I
	B	sour cherry	3	I
	C	sour cherry	3	I
	D	sour cherry	5	I
		sweet cherry	2	I
	E	sour cherry	1	I
Grand Traverse	F	sour cherry	6	I
	G	sour cherry	5	I
	H	sour cherry	6	I
Benzie	I	sour cherry	5	I
	J	sour cherry	1	I
Manistee	K	sour cherry	1	III
	L	peach	4	I
		sour cherry	1,1	I,III
	M	sour cherry	2	I
	N	oak	5	VI
Mason	O	sour cherry	1	III
	P	sour cherry	2	I
		apple	1	I
Oceana	Q	sour cherry	5	VI
	R	sour cherry	1	VI
	S	sour cherry	1	VI
	T	sour cherry	4	VI
	U	sour cherry	4	I

[a] North American biological species of *Armillaria* based on the classification system of Anderson and Ullrich (3).

was involved in most of the outbreaks of Armillaria root rot investigated in this study.

Basidiospores appear to play a limited role in the spread of *Armillaria*, as suggested by Wargo and Shaw (15). Basidiocarps of *Armillaria* were abundant in 1985, and they are common to abundant in most years. If spore dispersal played a major role in the spread of *Armillaria*, there should be a greater mix of biological species within and between orchards in northern Michigan. However, group I was concentrated primarily in areas north of Oceana County, whereas group VI was concentrated in the southern part of Oceana County. Studies examining the distribution of individual clones, within biological species, would provide additional data as to the role of basidiospores in the dissemination of the fungus within the orchards.

The sexual compatibility studies show, as reported for *Armillaria* on forest trees (3,9,11), that Armillaria root rot on orchard crops should not be attributed solely to *A. mellea*. Three intersterile groups of *Armillaria* were collected from orchard trees in Michigan, and these groups corresponded to North American groups I, III, and VI of Anderson and Ullrich (3). Interfertility studies had previously identified isolates in groups I and VI as *A. ostoyae* (Romagn.) Herink. and *A. mellea* sensu stricto, respectively (2,11,12). *A. ostoyae* was isolated from 14 orchards in different parts of the state and from four species of fruit trees. This species should be considered an important pathogen of orchard trees in Michigan, particularly sour cherry. Previously, *A. ostoyae* was described as a pathogen of conifers and of deciduous trees growing among conifers (11,12). In addition to *A. ostoyae* and *A. mellea*, group III of *Armillaria*, which has not been given a species designation, should be considered as a threat to sour cherry and potentially other orchard trees.

The pathogenic behavior of *A. ostoyae* and *A. mellea* on sour cherry is similar to the pathogenic behavior of these species on forest trees (15). In the forest, both species are capable of killing vigorously growing trees. To bring orchards into production early, cultural practices that reduce stress and promote rapid growth are used. In many of our orchard sites *Armillaria* has killed such vigorous trees in the second or third year after planting and continues to kill trees, forming expanding centers of infection.

The susceptibility of sour cherry rootstocks to a variety of biological species of *Armillaria* may complicate efforts to select and develop clones that are resistant to this serious pathogen. Our results suggest that at least three biological species of *Armillaria* should be used when evaluating the resistance of cherry rootstocks to *Armillaria*. Among the three biological species recovered, no pathogenic specialization was observed. All three biological species were capable of killing vigorous sour cherry trees. However, the predominance of *A. ostoyae* (group I) in Leelanau, Grand Traverse, and Benzie counties, where cherry production is greatest and the pathogen is widespread, indicates that a concentrated effort is needed to find rootstocks resistant to this species.

LITERATURE CITED

1. Anderson, J. B. 1983. Induced somatic segregation in *Armillaria* mellea diploids. Exp. Mycol. 7:141-147.
2. Anderson, J. B., Korhonen, K., and Ullrich, R. C. 1980. Relationships between European and North American biological species of *Armillaria mellea*. Exp. Mycol. 4:87-95.
3. Anderson, J. B., and Ullrich, R. C. 1979. Biological species of *Armillaria mellea* in North America. Mycologia 71:402-414.
4. Anderson, J. B., and Yacoob, R. 1984. Benomyl-induced somatic segregation in diploid *Armillaria mellea*. Phytopathology 74:612-615.
5. Bliss, D. E. 1951. The destruction of *Armillaria mellea* in citrus soils. Phytopathology 41:665-683.
6. Childs, L., and Zeller, S. M. 1929. Observations on Armillaria root rot of orchard trees. Phytopathology 19:869-873.
7. Jones, A. L., and Sutton, T. B. 1984. Diseases of tree fruits. North Cent. Regional Ext. Pub. 45. 60 pp.
8. Kable, P. F. 1974. Spread of *Armillariella* sp. in a peach orchard. Trans. Br. Mycol. Soc. 62:89-98.
9. Korhonen, K. 1978. Interfertility and clonal size in the *Armillariella mellea* complex. Karstenia 18:31-41.
10. Marsh, R. W. 1952. Field observations on the spread of *Armillaria mellea* in apple orchards and a black currant plantation. Trans. Br. Mycol. Soc. 35:201-207.
11. Morrison, D. J., Chu, D., and Johnson, A. L. S. 1985. Species of *Armillaria* in British Columbia. Can. J. Plant Pathol. 7:242-246.
12. Roll-Hansen, F. 1985. The *Armillaria* species in Europe: A literature review. Eur. J. For. Path. 15:22-31.
13. Shaw, C. G., III, and Roth, L. F. 1976. Persistence and distribution of a clone of *Armillaria mellea* in a ponderosa pine forest. Phytopathology 66:1210-1213.
14. Ullrich, R. C., and Anderson, J. B. 1978. Sex and diploidy in *Armillaria mellea*. Exp. Mycol. 2:119-129.
15. Wargo, P. M., and Shaw, C. G., III. 1985. Armillaria root rot: The puzzle is being solved. Plant Dis. 69:826-832.