Morphology, Pathogenicity, and Host Range of *Phytophthora megasperma*, *P. erythroseptica*, and *P. parasitica* from Arrowleaf Clover

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The author is grateful to N. Edwards, Brown Loam Branch Experiment Station, Raymond, MS, for assistance in locating diseased plants in the field; to K. Athow, C. Grau, and K. Roy for supplying isolates of *P. megasperma* var. *sojae* and soybean cultivars; and to J. Irwin for supplying an isolate of *P. megasperma*.

Accepted for publication 2 July 1980.

ABSTRACT

Pratt, R. G. 1981. Morphology, pathogenicity, and host range of *Phytophthora megasperma, P. erythroseptica,* and *P. parasitica* from arrowleaf clover. Phytopathology 71:276-282.

Phytophthora megasperma, P. parasitica, and a species provisionally identified as *P. erythroseptica* were isolated from arrowleaf clover plants with symptoms of root rot in Mississippi. *P. erythroseptica* also was isolated from hop clover and *P. megasperma* from a cruciferous weed. Problems in identification of isolates and the validity of taxonomic criteria are discussed. *P. megasperma* was highly virulent on arrowleaf clover and less virulent on subterranean, crimson, and white clovers. *P. erythroseptica* was highly virulent on crimson clover and less virulent on arrowleaf. *P. parasitica* was weakly virulent on all species tested. Red, berseem, and alsike clovers and alfalfa were resistant to isolates of the three *Phytophthora* spp. from clovers. Generally isolates of *P. megasperma* from arrowleaf clover, alfalfa, and soybean were pathogenically specific to original hosts. These results support the use of a forma specialis concept with *P. megasperma.* A forma nova, *Phytophthora megasperma* f. sp. *trifolii* f. nov. is proposed for isolates pathogenic to arrowleaf clover but not to alfalfa and soybean.

Additional key words: Trifolium agrarium, Trifolium alexandrinum, Trifolium hybridum, Trifolium incarnatum, Trifolium pratense, Trifolium repens, Trifolium subterraneum, Trifolium vesiculosum, Glycine max, Medicago sativa, Rorippa sp.

Arrowleaf clover (*Trifolium vesiculosum* Savi) is an annual species of Mediterrean origin introduced to the USA in 1956 (15). It is primarily grown in mixed pastures with ryegrass and small grains to increase soil nitrogen and to provide forage for late autumn and spring grazing. Three cultivars that were developed in Alabama, Georgia, and Mississippi are being increasingly grown throughout the southeastern USA (15). However, growers have often observed failure of established stands of arrowleaf clover, and causes of these failures are generally unknown. In some instances, dying out of arrowleaf clover stands has been attributed to disease (11,22).

Phytophthora root diseases of alfalfa (*Medicago sativa* L.) and soybean (*Glysine max* (L.) Merr.) caused by *Phytophthora megasperma* Drechs. (8) (*P. megasperma* f. sp. *medicaginis* [16]) and *P. megasperma* Drechs. var. *sojae* A. A. Hildeb. (10) (*P. megasperma* f. sp. *glycinea* [16]), respectively, have been recognized throughout the USA during the last 25 yr. No similar diseases have been reported to occur on clover species in the field. Red (*T. pratense* L.), white (*T. repens* L.) and crimson (*T. incarnatum* L.) clovers are resistant to *P. megasperma* isolates from alfalfa (7,9), and red and white clovers are also resistant to isolates from soybean (14,28). In one report (12), a fungus identified as *P. megasperma* was isolated from discolored roots of subterranean clover (*T. subterraneum* L.); however, no disease symptoms were described and pathogenicity of the isolate was not assessed on clover plants beyond the seedling stage.

During 1978 and 1979, symptoms suggestive of Phytophthora root rot were observed in field-grown arrowleaf clover at two locations in Mississippi. Three *Phytophthora* species were isolated from diseased plants. The purpose of this report is to describe identities, morphological characteristics, virulence, and host ranges of *Phytophthora* isolates. Host specificity of *P. megasperma* isolates from arrowleaf clover, alfalfa, and soybean also is evaluated.

MATERIALS AND METHODS

Isolation and identification of *Phytophthora* spp. Three to five sections of diseased root tissue (~0.5 cm diameter) were excised from each plant with a flamed scalpel and maintained on moist filter paper for up to 3 hr. Sections were surface sterilized in 1%

sodium hypochlorite for 30 sec, rinsed in sterile distilled water, briefly blotted on sterile filter paper, and plated on 2% cornmeal agar (CMA) (23) amended with pimaricin (20 mg/L). Colonies developing after 3–5 days at 24–26 C were transferred to additional CMA plus pimaricin. If bacteria were present, cultures were transferred to 2% water agar to promote aseptic growth, and additional transfers were made from uncontaminated sectors of colonies. Pure cultures were stored in slants of CMA at 4 and 12 C.

Colony morphology, radial growth rates, and cardinal growth temperatures of *Phytophthora* isolates were determined from colonies grown on Difco potato-dextrose agar (PDA), CMA, and clarified V-8 juice agar (V-8A) (23). Oogonia, antheridia, and oospores were observed on CMA, V-8A, and in pea broth (PB) cultures. Sporangia and zoospores were observed in cultures grown in PB and flooded with soil-extract water. Pea broth was prepared by boiling 75 g of dried split peas in 600 ml of distilled water for 20 min, filtering the broth through cheesecloth, adjusting it to 500 ml with additional water, and autoclaving. Soil-extract water was prepared by mixing equal quantities of field soil and distilled water to an even suspension and incubating for 24 hr without stirring. The water was poured through double-layer filter paper and added to colonies from PB after the broth was poured off.

Isolates were identified according to the key of Waterhouse (32) and by comparisons with original (33) and additional species descriptions.

Inoculation of plants and evaluation of disease. Plants of clover species and alfalfa were grown for 6–7 wk prior to inoculation on a growth bench or in the greenhouse in 10.5-cm-diameter clay pots that contained a mixture of clay loam soil, sand, and peat (1:1:1, v/v), either untreated or autoclaved at 105 C for 12 hr. Plants on the growth bench were illuminated by Gro-lux fluorescent (Sylvania, Danvers, MA 01923) and incandescent bulbs with a combined illuminance of $7-12 \times 10^3$ lux during a 14-hr photoperiod. Ambient temperatures ranged from 25 to 29 C. Daylengths in the greenhouse ranged from 10.5 to 12.5 hr and ambient temperatures ranged from 24 to 30 C. Commercially produced inoculum of *Rhizobium* strains compatible with each plant species was dusted around seedlings and watered into soil 10 days after planting.

Inoculum of *Phytophthora* isolates was produced on mixtures of cornmeal and sand (25). Inoculum of single or composited isolates was blended into soil mixture to give a ratio of 1/32 (inoculum to soil, v/v) before transplanting of plants, or inoculum was added to fill centerwells (2.4 cm in diameter, 6 cm deep) dug into pots with plants growing around the rim. Four plants were grown in or transplanted into each pot. Soil was alternately flooded to saturation from beneath and watered with drainage unimpeded every 2 days for 4 wk. Roots of plants were then washed free of soil and scored for disease.

Host reactions and disease severity in clover and alfalfa plants were evaluated as follow: 0 = immune = no symptoms of disease; 1 = highly resistant = occasional feeder roots rotted, but no lesions elsewhere; 2 = moderately resistant = frequent rotting of feeder roots plus small lesions on taproot or large lateral roots, but no enlarged or coalesced lesions; 3 = moderately susceptible = extensive rotting of feeder roots, plus large lesions on taproot and large lateral roots, but with new roots developing; 4 = highly susceptible = all roots extensively rotted, plants frequently killed, no new roots present.

Soybeans were inocululated by the hypocotyl-insertion method of Laviolette and Athow (17). Seedlings were grown in 10.5-cm-diameter pots (five seedlings per pot) for 9–10 days on the growth bench before inoculation and for 6 days after inoculation. Host reactions were evaluated as resistant if tissue did not darken or if darkening did not extend more than 2 mm beyond cut surfaces, and as susceptible if rot extended up and down hypocotyls 1–2 cm beyond the cut or if hypocotyls were girdled and the plants collapsed.

RESULTS

Symptoms of disease and isolation of Phytophthora spp. Symptoms of Phytophthora root diseases were observed in arrowleaf clover at Raymond and Starkville, Mississippi, in May and June, 1978. At Raymond, plants grown in mixed pastures with ryegrass had symptoms very similar to those that occur in alfalfa (7). Most plants in one low, poorly drained pasture were killed. Among survivors, lateral roots were frequently destroyed, and disease symptoms extended up the taproots and into the crowns. Sharp borderlines were usually evident between healthy and red-brown, diseased tissue in longitudinal sections (Fig. 1). These plants often were as tall as healthy plants, but stems were reddened and largely defoliated, and the remaining leaves were either chlorotic or necrotic. Similar, but less severe, symptoms occurred in arrowleaf clover plants grown in adjacent pastures that were higher and better drained. Plants of hop clover (*T. agrarium* L.) in the low pasture often had reddened stems, chlorotic leaves, and lesions on roots. Subterranean clover plants had lesions on roots, and foliage was sometimes stunted. No symptoms were observed in red clover. Lesions were also present on taproots of a cruciferous weed, *Rorippa* sp.

Four isolates identified as *P. megasperma* were obtained from five diseased arrowleaf clover plants from the low pasture and 10 isolates were obtained from 23 plants from the high pastures at Raymond. One isolate of *P. megasperma* also was obtained from *Rorippa*. Eight isolates provisionally identified as *P. erythroseptica* Pethybr. were obtained from 15 plants of hop clover. No *Phytophthora* isolates were obtained from seven plants of subterranean clover.

Arrowleaf clover plants in a nursery and pasture at Starkville also had symptoms of root disease, but these were not similar to the symptoms observed at Raymond. Many plants were stunted, wilted, and had reddened leaves, but extensive defoliation usually did not occur even in plants that were killed. Small roots frequently were rotted, and lesions were present on lateral roots. Taproots were not completely rotted, but internal vascular tissues often were discolored up to crowns. Only five *Phytophthora* isolates were obtained from 74 plants. However, isolations were attempted during an extended period of drought when conditions were unfavorable for disease development. Two isolates were provisionally identified as *P. erythroseptica* and three were identified as *P. parasitica* Dastur.

Morphology of *Phytophthora* spp. Isolates of *P. megasperma*

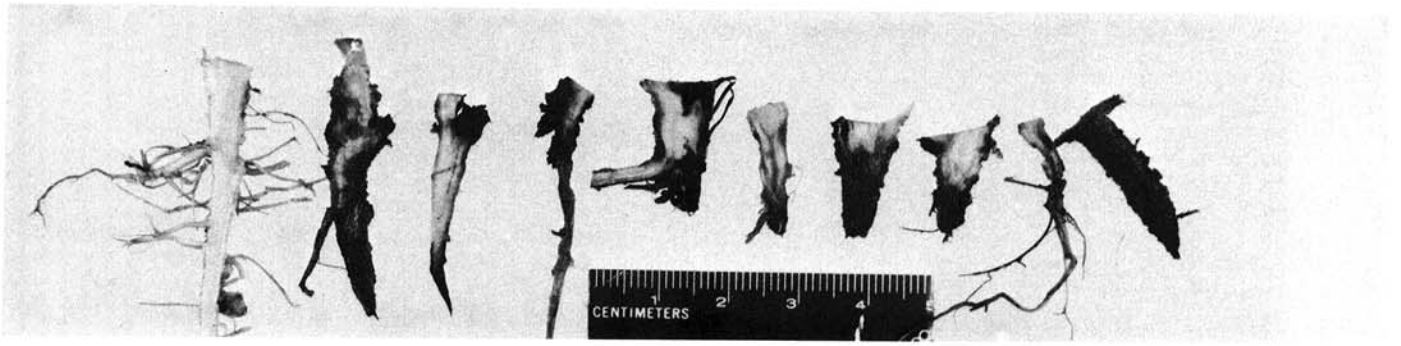

Fig. 1. Longitudinally sectioned taproots of field-grown arrowleaf clover showing symptoms of root rot caused by *Phytophthora megasperma*. Root on the left is healthy, other roots are diseased.

from arrowleaf clover were slow growing (radial growth of ~0.5 cm/day on V-8A at 25 C). Colonies were sparse on CMA; dense, appressed, and homogeneous on PDA; and dense, weakly to moderately aerial, and homogeneous on V-8A. Colony margins were rough or irregular on CMA and PDA. No growth occurred at 30 C. Oogonia and oospores were few to frequent on CMA and PB and usually numerous on V-8A. Fifty oogonia of each of two isolates on V-8A were 21–51 μm in diameter (means 39 and 43 μm, respectively); oospores were 19–46 μm in diameter (means 33 and 37 μm, respectively). A majority of antheridia were paragynous on V-8A and PB and a minority were amphigynous. Sporangia were usually ovoid-to-ellipsoid, proliferous, and without apical thickening. Isolates from arrowleaf clover and alfalfa were very similar in most characteristics, but the latter grew more slowly on V-8A, formed more aerial mycelium on PDA and V-8A, and formed more irregular shaped sporangia.

The isolate from *Rorippa* differed from the arrowleaf isolates. Colonies on PDA were very dense and formed coarsely radiate patterns. Oogonia and oospores were extremely numerous on V-8A and PB. Sporangia were few and irregular in shape.

Isolates provisionally identified as *P. erythroseptica* were fast growing (radial growth of ~1.0 cm/day on V-8A at 25 C). On CMA, colonies were finely radiate. On PDA and V-8A, colonies were dense and moderately aerial with faint petal- or star-shaped patterns. Colony margins were even or slightly undulating. Slight growth occurred at 4 and 34 C. Production of oogonia and oospores varied among isolates from none, to occasional, to very numerous on CMA, V-8A, and PB, respectively. Fifty oogonia of each of three isolates on CMA were 22–49 μm in diameter (means 39–41 μm); oospores were 20–42 μm diameter (means 34–35 μm). Antheridia were mainly paragynous on CMA, but amphigynous on PB. Sporangia were consistently ovoid to slightly obpyriform. Masses of small, interconnected, spherical hyphal swellings were common in some isolates on PB, but absent in others. Five isolates that did not form oogonia were intercrossed in all combinations on V-8A. No oogonia were produced in crossing plates. Ten single-zoospore cultures were obtained from one isolate which produced numerous oogonia and oospores. All of these cultures produced the sexual structures as frequently as did the parental isolate. Identification of these isolates is provisional on account of their paragynous antheridia, which are not commonly accepted as a characteristic of *P. erythroseptica* (32).

Isolates of *P. parasitica* from arrowleaf clover were similar in morphology to *P. nicotianae* B. de Haan var. *nicotianae sensu* Waterhouse (32). Hyphalike prolongations often radiated from sporangia and hyphal swellings in flooded cultures from PB. These isolates were considered to be *P. parasitica* because characteristics used to separate varieties of *P. parasitica* or *P. nicotianae* are often inconsistent (29).

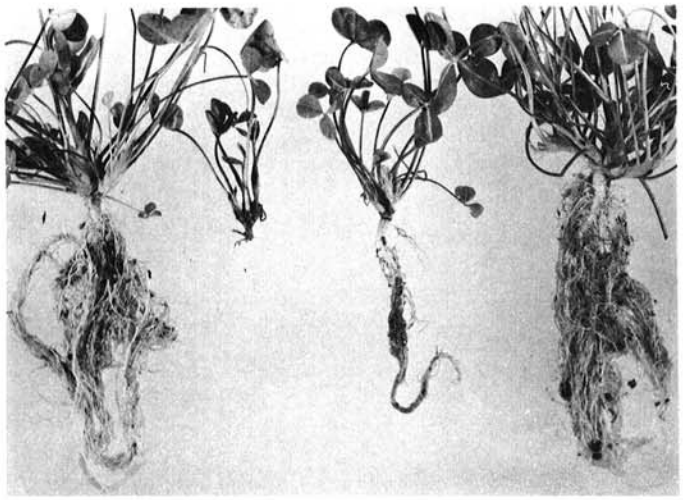

Fig. 2. Roots of 10-wk-old arrowleaf clover plants grown in soil (left to right) noninfested and infested with *Phytophthora megasperma, P. erythroseptica,* and *P. parasitica.*

Pathogenicity of *Phytophthora* spp. to clovers and alfalfa. Pathogenicity of the *Phytophthora* spp. from different sources was evaluated on seven species of clover and on alfalfa in replicated experiments on the growth bench and in the greenhouse. *P. megasperma* was highly virulent on arrowleaf clover (Fig. 2) and alfalfa (Table 1). Most plants of arrowleaf clover cultivars Meechee and Yuchi, and alfalfa cultivars Vernal and Apollo, were killed by *P. megasperma* isolates from arrowleaf clover and alfalfa, respectively. Arrowleaf clover isolates of *P. megasperma* also caused severe disease on subterranean clover; foliage was severely stunted and cortical tissues of taproots were extensively rotted. Disease symptoms occurred in crimson and white clovers inoculated with *P. megasperma* from arrowleaf, but severity of disease varied greatly among plants of the same cultivars (Table 1). Alfalfa isolates of *P. megasperma* caused slight symptoms in crimson and subterranean clovers. The *P. megasperma* isolate from *Rorippa* caused little damage to roots of any plant species.

P. erythroseptica caused severe disease in crimson clover (Table 1) and many plants were killed. Disease was less severe on arrowleaf clover; however, even though few plants were killed, nearly all had very small root systems because of extensive rotting of lateral roots (Fig. 2). The one isolate tested from arrowleaf clover was slightly more virulent to arrowleaf clover and alfalfa than were the three composited isolates from hop clover.

P. parasitica caused few or no disease symptoms on any clover species or on alfalfa.

Alsike, berseem, and red clovers generally were highly resistant or immune to all *Phytophthora* isolates. Only occasional plants became diseased. White clover also was highly resistant to *Phytophthora* isolates from all sources except *P. megasperma* from arrowleaf clover.

Yuchi arrowleaf clover was damaged less severely than Meechee by *P. erythroseptica* and by *P. megasperma* from alfalfa ($P=0.01$). Yuchi is an earlier maturing cultivar and may have had more secondary development in roots. Both cultivars were equally and extremely susceptible to *P. megasperma* from arrowleaf clover.

Results generally were similar for experiments conducted on the growth bench with untreated soil and in the greenhouse with steamed soil. However, *P. erythroseptica* frequently was reisolated from plants in the untreated soil to which it had not been added. This suggested that *P. erythroseptica* was present at low levels in untreated soil and may have caused some disease attributed to *P. megasperma* and *P. parasitica*. When the experiment was repeated in the greenhouse with steamed soil, all cultures reisolated were of the same species that had been added as inoculum (Table 1).

Host specificity of *P. megasperma*. Four cultivars of arrowleaf clover, alfalfa, and soybean each were inoculated with three individual isolates of *P. megasperma* from arrowleaf clover, three isolates from alfalfa, and four isolates from soybean. In each cultivar, severe disease symptoms consistently developed only in plants inoculated with isolates originally obtained from that same host (Table 2). In most plants of arrowleaf clover and susceptible alfalfa inoculated with virulent isolates of *P. megasperma* from the same hosts, roots were either completely rotted 4 wk after inoculation, or most lateral roots were rotted and large progressive lesions were present on taproots. In contrast, plants inoculated with isolates from the opposite hosts, or with isolates from soybean, usually had no symptoms or few lateral roots rotted and only small, nonprogressive lesions on taproots. In stems of susceptible soybean plants inoculated with *P. megasperma* from soybean, large, sunken, dark lesions developed and usually girdled and caused collapse of stems. Soybean plants inoculated with isolates of *P. megasperma* from arrowleaf clover and alfalfa, however, did not develop any symptoms of disease.

Occasional plants of alfalfa and arrowleaf clover became severely diseased after inoculation with isolates of *P. megasperma* from other hosts. Atypical disease reactions also occurred in a few soybean plants of the cultivars Mukden and Altona after inoculation with *P. megasperma* isolates from soybeans (Table 2).

One isolate of *P. megasperma* from arrowleaf clover was significantly less virulent ($P=0.01$) than were the other two isolates

TABLE 1. Severity of disease caused by three species of *Phytophthora* from different hosts in seven species of clover and in alfalfa[a]

Phytophthora species and source host[b]	Plant species, cultivars, and disease scores[c]													
	Arrowleaf clover		Subterranean clover		Crimson clover		Red clover		White clover		Alsike clover	Berseem clover	Alfalfa	
	Meechee	Yuchi	Mt. Barker	Woogenellup	Chief	Tibbee	Kenland	Kenstar	Lucky	Regal	(unknown)	(unnamed)	Vernal	Apollo
P. megasperma														
Arrowleaf clover	4.0	4.0	3.7	3.0	2.5	2.9	0.4	0.4	2.2	2.0	0.9	0.6	0.9	0.7
	4–4	4–4	3–4	2–4	1–4	2–4	0–4	0–2	1–4	0–4	0–2	0–1	0–3	0–2
	(+)	(+)	(−)	(+)	(+)	(+)	(−)		(+)	(+)			(+)	
Alfalfa	1.2	0.2	1.7	1.1	1.7	1.1	0.1	0.3	0.0	0.0	0.0	0.7	3.8	3.4
	0–3	0–1	1–3	0–2	1–4	0–3	0–1	0–2	0–0	0–0	0–0	0–4	3–4	2–4
	(+)		(+)		(+)	(+)						(+)	(+)	(+)
Rorippa sp.	0.1	0.0	0.1	0.0	0.5	1.6	0.1	0.0	0.0	0.0	0.0	0.4	0.7	0.3
	0–1	0–0	0–1	0–0	0–2	0–3	0–1	0–0	0–0	0–0	0–0	0–1	0–3	0–2
						(+)							(+)	
P. erythroseptica														
Arrowleaf clover	3.0	2.1	0.8	1.3	3.3	3.8	0.7	0.3	0.1	0.1	0.2	0.8	1.7	2.0
	2–4	1–3	0–2	0–3	3–4	3–4	0–1	0–1	0–1	0–1	0–1	0–1	1–2	1–3
	(+)	(+)		(+)	(+)	(+)								(+)
Hop clover	2.2	1.7	0.3	0.2	3.1	3.5	0.2	0.1	0.2	0.1	0.2	0.4	0.2	0.8
	2–3	1–2	0–1	0–2	2–4	3–4	0–1	0–1	0–1	0–1	0–1	0–1	0–1	0–2
	(+)				(+)	(+)								
P. parasitica														
Arrowleaf clover	0.3	0.1	0.0	0.0	1.4	1.1	0.0	0.1	0.0	0.0	0.1	0.6	0.4	0.3
	0–4	0–1	0–0	0–0	1–3	0–3	0–0	0–1	0–0	0–0	0–1	0–1	0–1	0–1
	(−)				(−)	(+)								
None (control)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Plants grown for 6 wk in the greenhouse in steamed soil were inoculated by adding an infested mixture of cornmeal and sand to centerwells in pots. Plants were grown for 4 wk after inoculation with alternate flooding and draining of soil.

[b] Three isolates were composited in inoculum of *P. megasperma* from arrowleaf clover, *P. erythroseptica* from hop clover, and *P. parasitica*. Two isolates were composited in inoculum of *P. megasperma* from alfalfa. Single isolates of *P. megasperma* from *Rorippa* sp. and *P. erythroseptica* from arrowleaf clover were used for inoculum.

[c] The upper value is the mean disease score of 12 plants, with 0 = no disease symptoms, 1 = slight disease, and 4 = most severe disease (plants usually dead). The middle value is the range of disease scores of 12 plants. (+) = pathogen reisolated from one to three plants with disease scores of 3 or 4, (−) = pathogen not reisolated.

(Table 2). One isolate from soybean was also less virulent on susceptible cultivars than were three other isolates. No clear differences in virulence were apparent among three isolates from alfalfa.

Seven additional isolates of *P. megasperma* from arrowleaf clover were each inoculated onto Meechee arrowleaf clover and Vernal alfalfa by the centerwell-inoculation technique (four pots of each cultivar per isolate) and onto Harosoy soybean by the hypocotyl-insertion technique (five plants per isolate). Mean disease scores obtained with these isolates ranged from 3.9 to 4.0 on arrowleaf clover and from 0.3 to 0.6 on alfalfa. No symptoms of disease occurred in any soybean plants.

DISCUSSION

Results of this study suggest that Phytophthora root diseases may become important limiting factors for production of arrowleaf clover and possibly other annual species in the southeastern USA. Disease caused by *P. megasperma* on arrowleaf in the field was as severe as that which occurs on alfalfa (7). In greenhouse trials the incidence, progression, and severity of symptoms were similar in arrowleaf clover and alfalfa. Most plants of three American cultivars and one experimental line of arrowleaf clover, and of two susceptible cultivars of alfalfa, were dead within 4 wk after inoculation with appropriate isolates of *P. megasperma.*

Although *P. megasperma* was the most virulent pathogen on arrowleaf clover, *P. erythroseptica* also caused significant damage. Few plants were killed by *P. erythroseptica* in the greenhouse, but nearly all were severely stunted because of extensive destruction of lateral and feeder roots (Fig. 2). It is doubtful that these plants could have survived under field conditions. In the nursery where *P. erythroseptica* occurred, numerous arrowleaf clover plants were killed or severely stunted from root rot, but *P. megasperma* was not isolated at that location. These results and observations suggest that arrowleaf clover is susceptible to two different Phytophthora diseases, and that either of these could cause failure of stands. These diseases may account for failure of arrowleaf clover to thrive on low-lying, poorly drained, or heavy-textured soils (15).

Other annual clovers grown in the Southeast may also be damaged by Phytophthora root diseases. Growth of subterranean clover was significantly retarded by *P. megasperma* in the greenhouse. Plants of crimson clover were frequently killed by *P. erythroseptica.* Damage was sufficient to suggest that productivity of these species might be decreased by Phytophthora diseases in the field. In contrast, most plants of alsike, berseem, and red clovers were highly resistant or immune to the three *Phytophthora* spp. Overall, these species did not sustain sufficient damage to suggest that Phytophthora diseases might limit their productivity in the field.

The isolates of *P. megasperma* from arrowleaf clover, alfalfa, and soybean studied here were consistently pathogenic only on hosts from which they were originally isolated. Host specificity was clearly evident because in compatible host-pathogen combinations most plants were killed or severely diseased, while in incompatible combinations most inoculated plants did not differ significantly from controls or were only slightly diseased (Tables 1 and 2).

Kuan and Erwin (16) recently proposed the use of formae speciales to describe isolates of *P. megasperma* from alfalfa and soybean that were host-specific in pathogenicity, but not distinguishable by sizes of oogonia and oospores (8,10,24,32). They designated isolates pathogenic to alfalfa as "*P. megasperma* f. sp. *medicaginis*" and isolates pathogenic to soybean as "*P. megasperma* f. sp. *glycinea.*" Results of this study support the proposal of Kuan and Erwin for alfalfa and soybean isolates. These results further indicate that isolates of *P. megasperma* from arrowleaf clover, which cannot be distinguished from alfalfa and soybean isolates by sizes of sexual structures (16), are equally host-specific in pathogenicity. Therefore, it is proposed that isolates of *P. megasperma* that are pathogenic to Amclo, Meechee, and Yuchi arrowleaf clover, but not to alfalfa or soybean, to be designated "*Phytophthora megasperma* forma specialis *trifolii.*"

Although *P. megasperma* f. sp. *trifolii* is most virulent on arrowleaf clover, it also causes significant disease in subterranean clover and some disease in crimson and white clovers. Previous reports indicated that *P. megasperma* isolates from soybean and alfalfa may also cause disease in other hosts (9,13). If numerous additional hosts of the formae speciales of *P. megasperma* are

TABLE 2. Disease severity and host reactions of arrowleaf clover, alfalfa, and soybean cultivars inoculated with isolates of *Phytophthora megasperma*[a]

Phytophthora megasperma			Plant species, cultivars, and disease scores[b] or host reactions[c]											
			Arrowleaf clover				Alfalfa				Soybean			
Source	Race	Isolate	Amclo	Meechee	Yuchi	RRPS-3	Vernal	Saranac	Apollo	Agate	Harosoy	Mukden	Sanga	Altona
Arrowleaf clover	···	1	4.0	4.0	3.9	3.7	0.1	0.5	0.1	0.2	R[c]	R	R	R
			4–4	4–4	3–4	1–4	0–1	0–3	0–2	0–1	R–R	R–R	R–R	R–R
	···	2	3.9	4.0	3.8	3.9	0.9	0.0	0.3	0.2	R	R	R	R
			3–4	4–4	2–4	3–4	0–3	0–0	0–3	0–2	R–R	R–R	R–R	R–R
	···	3	1.7	2.0	1.0	1.8	0.3	0.6	0.2	0.5	R	R	R	R
			0–4	0–4	0–2	0–4	0–1	0–2	0–1	0–2	R–R	R–R	R–R	R–R
Alfalfa	···	1	0.0	0.0	0.1	0.1	4.0	4.0	3.8	2.8	R	R	R	R
			0–0	0–0	0–1	0–1	4–4	4–4	3–4	1–4	R–R	R–R	R–R	R–R
	···	2	0.7	0.3	0.1	0.1	3.5	3.5	3.3	2.5	R	R	R	R
			0–3	0–2	0–1	0–1	2–4	1–4	0–4	1–4	R–R	R–R	R–R	R–R
	···	3	0.1	0.5	0.0	0.2	3.7	4.0	2.3	2.6	R	R	R	R
			0–1	0–3	0–0	0–1	3–4	4–4	0–4	1–4	R–R	R–R	R–R	R–R
Soybean	1	1	0.1	0.7	0.7	0.0	1.4	0.4	0.8	0.5	S	R	R	R
			0–1	0–2	0–1	0–0	0–3	0–2	0–4	0–2	S–S	R–R	R–R	S–R
		2	0.4	0.0	0.0	0.0	0.5	0.9	0.8	0.8	S	R	R	R
			0–2	0–0	0–0	0–0	0–1	0–2	0–2	0–2	S–S	R–R	R–R	R–R
	3	1	0.0	0.1	0.0	0.0	0.8	0.6	0.3	0.6	S	S	R	R
			0–0	0–1	0–0	0–0	0–2	0–3	0–2	0–3	S–S	S–S	R–R	S–R
		2	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.8	S	S	R	R
			0–0	0–0	0–0	0–0	0–3	0–0	0–0	0–2	S–S	S–R	R–R	R–R
None (control)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	R	R	R	R

[a] Clover and alfalfa plants grown for 6 wk on a growth bench in steamed soil were inoculated by adding an infested mixture of cornmeal and sand to centerwells in pots. Plants were grown for 4 wk after inoculation with alternate flooding and draining of soil. Soybean plants were grown for 9 days, inoculated by inserting mycelium into wounds in the stems, and grown for an additional 6 days.

[b] The upper value is the mean disease score of 10–12 plants, with 0 = no disease, 1 = slight disease and 4 = most severe disease (plants usually dead). The lower value is the range of disease scores of 10–12 plants.

[c] The upper value is the predominant host reaction of 12 plants, with R = resistant = no lesions on stems and S = susceptible = lesions extending up and down stems or stems girdled. The lower value is the range of host reactions of 12 plants.

discovered, and especially if host ranges are found to overlap, then it may be desirable to further describe isolates according to a system of primary and secondary hosts. Such a system has been used with certain formae speciales of *Fusarium oxysporum* Schlecht. that cause disease in many plant species in addition to their primary hosts (1,2). By these standards (1), arrowleaf clover would be considered the primary host of *P. megasperma* f. sp. *trifolii*, and subterranean clover would be considered a secondary host.

Isolates of *P. erythroseptica* and *P. megasperma* described here are apparently different from other isolates from Mississippi that were previously identified as those species (12,19). Isolates that caused root and crown rot of vetch were identified as *P. erythroseptica* (19). However, these isolates were very slow growing, and their optimal and maximal growth temperatures were lower than those reported for *P. erythroseptica* (30,31,32) and those observed here. Another isolate from discolored roots of subterranean clover in Mississippi, designated *P. megasperma* (12), may be *P. megasperma* f. sp. *glycinea* (*P. megasperma* var. *sojae* [10]), because of its pathogenicity to soybean. The authors were reluctant to consider this isolate as *P. megasperma* var. *sojae* partly because it also caused damping-off of seedlings of alfalfa, arrowleaf clover, crimson clover, and pea. They concluded that the clover isolate had a wider range of pathogenicity than was recognized for *P. megasperma* var. *sojae*. However, this conclusion does not appear warranted, because damping-off reactions often are not suitable criteria for determining host ranges of pathogens or susceptibility of hosts. Other investigators also have observed damping-off of seedlings of nonhost plants by *P. megasperma* f. sp. *medicaginis* (5,7) and *P. megasperma* f. sp. *glycinea* (14).

Identification of *P. erythroseptica, P. megasperma,* and other *Phytophthora* spp. with nonpapillate, proliferous sporangia depends largely upon whether oogonia and oospores are present in single cultures and upon whether antheridia are predominately amphigynous or paragynous (32). Results of this study suggest that both of these criteria are of limited taxonomic value. Some isolates of the species considered to be *P. erythroseptica* produced few or no sexual structures, some produced occasional sexual structures, and others consistently produced abundant sexual structures. All isolates were obtained from plants of the same two species with similar symptoms and were collected at the same two locations. The sexual isolates corresponded to *P. erythroseptica* in that colonies were fast growing (4,18,30) and developed floral or stellate patterns with moderate aerial mycelium (21,30,32), and maximal growth temperatures were near 34 C (30–32). Sizes of oogonia and oospores were within the range described for *P. erythroseptica* (3,26,31,32). The fact that single-zoospore cultures from sexual isolates were consistently sexual suggests that these isolates are of a homothallic species; the occurrence of paragynous antheridia precludes their identification as a heterothallic species (27). The asexual isolates cannot be identified as *P. erythroseptica* by the key of Waterhouse because the species is stated to always form oogonia in culture (32). However, because the asexual isolates were identical to the sexual isolates in all other morphological features, they also are provisionally identified as *P. erythroseptica*. Cairns and Muskett (3) also observed an isolate of *P. erythroseptica* that did not produce the sexual stage in culture.

Identification of *P. erythroseptica* and *P. megasperma* is based partly upon whether antheridia are paragynous or amphigynous. *P. megasperma* has both amphigynous and paragynous antheridia, while in *P. erythroseptica* the antheridia are described as only amphigynous (32). Four isolates of *P. megasperma* from arrowleaf clover and alfalfa produced a majority of paragynous antheridia in both agar and broth cultures and in two of these paragyny occurred more frequently in broth than in agar. However, among three isolates of *P. erythroseptica,* the majority of antheridia were paragynous in agar cultures, but amphigynous in broth. These isolates cannot be considered to be *P. erythroseptica* by the key of Waterhouse (32) on account of their frequent paragynous antheridia. However, they clearly differ from *P. megasperma* in features of colony morphology, growth rates, temperature-growth relations, and pathogenicity. Therefore, these isolates are provisionally considered to be *P. erythroseptica* in spite of the occurrence of paragynous antheridia.

Converse and Schwartz (6) also observed paragynous antheridia in isolates identified as *P. erythroseptica*. Savage et al (27) accepted paragynous antheridia as a characteristic of *P. erythroseptica*. Mundkur (20) observed lateral papillae on amphigynous antheridia of *P. himalayensis* (*P. erythroseptica sensu* Waterhouse [32]) that fertilized oogonia in the manner of paragynous antheridia; for this reason, he considered amphigynous and paragynous antheridia to be fundamentally similar.

The variability in occurrence of sexual structures and nature of antheridia of isolates of *P. erythroseptica* and *P. megasperma* observed here indicates that additional taxonomic criteria are needed for identification of these and similar *Phytophthora* spp.

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