Aphanomyces Blight of Amazon Sword Plants

W. H. Ridings and F. W. Zettler

Plant Pathologist, Division of Plant Industry, Florida Department of Agriculture and Consumer Services; and Associate Professor, respectively, Department of Plant Pathology, University of Florida, Gainesville 32601.

Contribution No. 318, Bureau of Plant Pathology, Florida Agricultural Experiment Station Journal Series Paper No. 4449.

The authors appreciate the advice and assistance of R. A. Gasser, Stuart, Florida, who furnished us with most of the specimens of disease-free amazon sword plants used throughout this study. The authors are also grateful to J. W. Kimbrough and John Beckner, Botany Department, University of Florida, for mycological advice and for assistance rendered in identifying the alismataceous plants tested in this study, respectively. Theresa E. Hoff and Helen F. Hayslip provided excellent technical assistance.

This investigation was supported in part by U.S. Army Corps of Engineers Contract No. DACW 73-71-C 0002, Florida Department of Natural Resources Contract No. 3, and by the United States Department of the Interior, Office of Water Resources Research, as authorized under the Water Resources Research Act of 1964.

Accepted for publication 6 September 1972.

ABSTRACT

An *Aphanomyces* sp. was frequently isolated from tissues of amazon sword plants (*Echinodorus brevipedicellatus*) affected by a previously unrecognized disease found at an aquatic plant nursery in South Florida. Suspensions of zoospores or mycelial fragments infected 28 of 45 inoculated plants of *E. brevipedicellatus*. Symptoms were the same as those observed under field conditions and on plants inoculated with water taken from containers with infected plants. The following alismataceous species did not become infected when inoculated: *Echinodorus* 'Rangerii', *E. grisebachii, E. longistylis, E. martii, Sagittaria lorata*, or *S. sinensis*. Likewise, inoculated specimens of *Hydrilla verticillata, Myriophyllum spicatum*, and *Alternanthera philoxeroides* remained healthy. The morphology of the oogonia, oospores, antheridia, and primary and secondary zoospores of this fungus was the same as that of published descriptions of *A. euteiches* and the two isolates of this species used for comparison. The amazon sword plant isolate infected seedlings of *Pisum sativum, Beta vulgaris, Raphanus sativum, Vicia faba*, and *Vigna unguiculata (sinensis)*. Inoculated seedlings of *Lycopersicon esculentum* and *Avena sativa* did not become infected. The two *A. euteiches* isolates infected seedlings of *P. sativum*, but failed to infect *B. vulgaris, R. sativum, A. sativa, L. esculentum*, or *E. brevipedicellatus*.

Phytopathology 63:289-295

The amazon sword plant (*Echinodorus brevipedicellatus* Buch.) is among several species of Alismataceae grown in Florida and shipped throughout the USA and Canada as ornamental plants for aquariums. This plant is a native of the American tropics, and is usually cultivated as a submersed plant, although it often occurs as an emersed bog plant in its native habitat (5, 16). Economically, the amazon sword plant is among the most important of aquarium plants, and currently retails at $0.75 to $3.50/plant, depending upon its size when sold. In 1968, the aquarium plant industry was estimated at ca. $2,000,000; 90% of the aquarium plants were produced in Florida (18).

Little is known about diseases of *Echinodorus* spp., although the fungi, *Burrillia echinodori, Cercospora echinodori, Doassansia alismatis, D. morotiana*, and *Phyllosticta echinodoricola* have been reported to cause leaf spots on these plants (1, 3, 17, 19, 20).

In October 1970, a disease of amazon sword plants was observed in outdoor tanks at an aquatic plant nursery in South Florida. Leaves of infected plants were necrotic at the base, and frequently were detached from the petiole, resulting in their floating to the surface of the water. Severely infected plants were chlorotic and appeared blighted. The disease spread rapidly to other amazon sword plants at this nursery and, by January 1971, had caused total losses in 11 of 27 propagating tanks, each with an estimated 1,000 to 1,500 plants.

This study was designed to identify the agent responsible for this disease of amazon sword plants and to determine whether it was also pathogenic to other aquatic plant species.

MATERIALS AND METHODS.—Aquatic plants used in these experiments were obtained from nursery stock tanks in South Florida or from waterways in Central Florida. None of the plants used in these experiments developed symptoms of blight prior to inoculations or when used as control plants during an experiment. The following alismataceous plants were tested: amazon sword (*E. brevipedicellatus*); dwarf amazon sword (*E. grisebachii* Small); melon sword (*E. longistylis* Buch.); ruffled sword (*E. martii* Micheli); broadleaf sword (*Echinodorus* 'Rangerii'); *Sagittaria lorata* Small; and *S. sinensis* Sims. Nonalismataceous aquatic plants tested were: Eurasian water milfoil (*Myriophyllum spicatum* L.); hydrilla [*Hydrilla verticillata* (L.f.) Royle]; and alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.].

The binomials given above for the alismataceous plants are those used by aquarists, and may not be necessarily botanically correct. Specimens of these and other aquarium plants are commonly collected in

their native habitats and distributed in this country without ever having been identified by plant taxonomists. The most recent and authoritative taxonomic work on the genus *Echinodorus* appears to be that by Fassett (5). In our study, only specimens of amazon sword plants flowered but, upon examination, a species could not be determined from any of those listed by Fassett (J. Beckner, *personal communication*). Accordingly, voucher specimens of all above-listed alismataceous plants used in this study have been deposited for future reference in the University of Florida Herbarium (FLAS accession numbers 109516-109524).

Experimental aquatic plants were planted ca. 10 days before inoculation in 3.8-liter (1 gal) wide-mouth glass jars loosely capped with metal lids (Fig. 1-C). These bottles, which contained 5 to 6 cm of fine grade unwashed quartz sand, were filled to ca. 0.75 capacity (2,500 ml) with tap water aged 1 to 2 weeks. The water used in these experiments ranged in degrees of hardness from 100 to 292 mg of calcium carbonate/liter, and from pH 7.9 to 8.3. Jars containing the plants were maintained at 20 to 24 C and were provided with 500 to 1,000 ft-c of light (recorded at surface of jar lids) from incandescent and fluorescent bulbs.

Isolations from infected plant material were made from segments of infected tissues rinsed in sterile tap water, dipped briefly in 70% ethanol, submersed for 2 min in 0.1% sodium hypochlorite, rinsed in sterile tap water, and blotted dry. Bits of excised tissue from the margins of healthy and infected tissue were then placed on potato-dextrose agar (PDA) in petri dishes. Cultures of fungi growing from the plant tissues were subsequently transferred to test tube slants containing either PDA or Difco cornmeal agar.

A fungus was consistently isolated from naturally infected plants. It was designated as 948-A, and tentatively identified as *Aphanomyces euteiches* Drechs. Accordingly, two isolates of *A. euteiches* were obtained from G. C. Papavizas of the USDA at Beltsville, Md., for comparison. These two isolates are designated herein as AE-6 and AE-7. For future reference, cultures of these fungi have been deposited in the Florida Type Culture Collection maintained at the Division of Plant Industry in Gainesville.

The inoculum used in pathogenicity studies consisted of suspensions of zoospores or mycelial fragments. Zoospores of 948-A were produced only by flooding 5-day-old water agar cultures at 24 C with sterile tap water for 18 to 24 hr at the same temp. Zoospores of AE-6 and AE-7 were produced only by culturing these isolates for 3 days in a glucose-peptone medium and washing the mycelial mats according to the procedure of Mitchell & Yang (11). Final zoospore counts were determined with a haemocytometer. Mycelial fragments were produced from 3- to 6-day-old cultures in the Mitchell and Yang medium by decanting the liquid medium, rinsing the mycelial mats with sterile tap water, and comminuting two mycelial mats/100 ml of sterile tap water in a blender for 15 to 20 sec.

Inoculations of aquatic plants.—In preliminary trials, one to three healthy, actively growing amazon sword plants were planted in jars and inoculated by the addition of either water from nursery tanks containing infected plants or an infected plant which was planted adjacent to healthy ones in the same jar containing aged tap water. In inoculation studies using fungus cultures, suspensions of zoospores or mycelial fragments of either 948-A, AE-6, or AE-7 were added to jars containing one to three plants, or to wax cups containing either two-three excised leaves or five-ten 1-cm^2 leaf pieces. The cup method was convenient for using a high inoculum density in a small volume. Zoospore suspensions were added to the jars to give a final concentration of 1 to 2 $\times$ 10^3/ml in a 2,500-ml total volume and to cups to give a final concentration of 5 $\times$ 10^4/ml in a 100-ml total volume. Suspensions of mycelial fragments were used at the rate of two comminuted cultures/jar or cup.

Inoculation of terrestrial plants.—Seeds of oat (*Avena sativa* L. '501'); beet (*Beta vulgaris* L. 'Early Wonder', 'Ruby Queen', 'Detroit Dark Red'); tomato (*Lycopersicon esculentum* Mill. 'Bonny Best'); pea (*Pisum sativum* L. 'Little Marvel', 'Alaska'); radish (*Raphanus sativum* L. 'White Icicle', 'Cherry Belle'); broadbean (*Vicia faba* L. 'Longpod'); and cowpea (*Vigna unguiculata* [L.] Walp. [*sinensis*] 'Ramshorn Blackeye') were germinated in vermiculite in 10.2 cm (4-inch) clay pots. Newly emerged seedlings were drenched with a zoospore suspension (50,000/ml in 50 ml) or removed from the vermiculite and the roots soaked in a zoospore suspension (50,000/ml in 100 ml) for 30 min prior to repotting in a sand-peat mixture. Isolate 948-A was inoculated to all the terrestrial plant species, whereas isolates AE-6 and AE-7 were inoculated only to oat, beet, tomato, pea, and radish. Controls were treated the same as the inoculated seedlings except that tap water alone was substituted for the zoospore suspension. Inoculated seedlings were incubated in the greenhouse at ca. 25 C. Twenty-five seedlings/treatment were used in each of three experiments.

RESULTS.—In four experiments, 23 of 28 healthy amazon sword plants became infected when planted adjacent to diseased plants or when planted in jars containing water from infected plant sources (Table 1). None of 25 amazon sword plants used as controls in these trials became infected. The symptoms of infected plants were readily apparent and appeared identical to those observed in nursery tanks in South Florida. Symptoms of necrosis appeared on the leaves developing from the shoot apex ca. 7-10 days after inoculation. Necrosis usually developed at the base of the young leaves and spread acropetally primarily along the veins. Infected tissues, at first water-soaked, soon became skeletonized through lamellar decomposition (Fig. 1-B). Such symptoms rarely developed on leaves that were fully expanded at the time of inoculation. In many instances, complete yellowing and death of the entire plant resulted within 4 weeks after inoculation (Fig. 1-A). In six experiments using suspensions of

TABLE 1. Susceptibility of aquatic plants to various inocula of isolate 948-A of *Aphanomyces euteiches*

Aquatic plant			
Family	Genus and species	Inoculum[a]	Infected plants[c]
Alismataceae	*Echinodorus brevipedicellatus* (Source A)[b]	W/P	18/18
		Z	17/23
		M	7/12
	E. brevipedicellatus (Source B)[b]	W/P	5/10
		Z	4/10
	E. grisebachii	W/P	0/8
		Z	0/22
	E. longistylis	Z	0/4
	E. martii	W/P	0/2
		Z	0/3
	E. 'Rangerii'	W/P	0/4
		Z	0/3
	Sagittaria lorata	W/P	0/2
		Z	0/4
	S. sinensis	W/P	0/2
		Z	0/8
Amaranthaceae	*Alternanthera philoxeroides*	W/P	0/4
		M	0/7
Haloragaceae	*Myriophyllum spicatum*	W/P	0/4
		M	0/7
Hydrocharitaceae	*Hydrilla verticillata*	W/P	0/4
		M	0/7

[a] W/P = plants inoculated either by planting infected amazon sword plants adjacent to healthy test plants or by adding water from containers with diseased plants to jars with healthy test plants. Z = zoospores of 948-A added to jars to a final concentration of 1 to 2×10^3 zoospores/ml. M = mycelial fragments of 948-A added to jars at a rate of two comminuted cultures/jar.

[b] Plants from two commercial sources were used. Although sold as one species, they represent two separate shipments from South America.

[c] Number of infected plants over number inoculated.

zoospores or mycelial fragments of 948-A, 28 of 45 inoculated amazon sword plants developed identical symptoms to those observed in the aquatic plant nursery, indicating that this fungus was the agent responsible for the disease (Table 1). Moreover, the fungus was repeatedly isolated from infected tissues. None of 18 noninoculated plants used as controls in these trials ever became infected.

Zoospores and mycelial fragments of 948-A also proved pathogenic to amazon sword plants in trials with excised whole leaves and leaf pieces floating in wax cups. In two experiments, leaf tissues in a total of 14 cups became infected 6-8 days after inoculation. None of the leaf tissues in 14 cups used as controls became infected. As before, the fungus was readily reisolated.

Echinodorus brevipedicellatus was the only aquatic plant susceptible to 948-A. None of the other aquatic species developed symptoms when inoculated with suspensions of zoospores or mycelial fragments of 948-A or water from containers with infected amazon sword plants (Table 1). The results from inoculations of *Echinodorus* 'Rangerii' supported observations by the grower that plants of this species did not become infected when present in tanks containing infected amazon sword plants. Pathogenicity studies with excised whole leaves or leaf pieces in wax cups produced the same results as those obtained with intact plants in jars. In one experiment, leaf pieces of *E. brevipedicellatus* inoculated with 2×10^3 zoospores/ml became infected, whereas leaf tissues of *Echinodorus* 'Rangerii', *E. grisebachii, E. martii, S. lorata*, and *S. sinensis* remained healthy in three replicated trials for each plant species.

Zoospores of 948-A proved pathogenic with both inoculation techniques to all tested cultivars of pea, beet, cowpea, radish, and broadbean. Infected seedlings of beet and radish were damped off, whereas pea seedlings showed a root rot and wilt. Infection of cowpea and broadbean seedlings resulted in a root rot, progressive necrosis of the hypocotyl, and a retardation of growth. In most instances, symptoms were noted 2-7 days after inoculation. Inoculated seedlings of tomato and oat remained healthy and showed no symptoms of infection. In one additional trial, the pathogenicity of isolate 948-A to seedlings of beet, radish, and cowpea was further confirmed by inoculations with zoospores from each of five single zoospore cultures.

Isolates AE-6 and AE-7 were pathogenic only to pea. Seedlings of beet, radish, tomato, and oat cultivars did not show symptoms. Isolations from infected pea roots or hypocotyls consistently resulted in the recovery of the isolate used for inoculation.

The AE-6 and AE-7 isolates of *A. euteiches* did

not infect amazon sword plants. In two experiments involving intact amazon sword plants in jars, no infection occurred in 13 and 17 plants inoculated with mycelial fragments plus zoospores of AE-6 and AE-7, respectively, whereas 7 of 12 amazon sword plants inoculated with 948-A became infected after 12 days. The 14 noninoculated amazon sword plants used as controls in these experiments never became infected. Similar results of no infection were obtained in an experiment involving leaf pieces of amazon sword plants placed into 11 cups containing a suspension of mycelial fragments of AE-6 or AE-7. The leaves in the 12 cups used as controls likewise remained uninfected.

The cultural characteristics and morphology of 948-A, AE-6, and AE-7 were similar to one another and were generally consistent with those described for *A. euteiches* (7, 13). Mycelium of 948-A, like that of AE-6 and AE-7, was hyaline and showed an arachnoid growth habit on water agar and PDA. The mycelial growth rate of 948-A (30 mm diam/24 hr) exceeded that of AE-6 (20 mm diam/24 hr) and AE-7 (21 mm diam/24 hr) on PDA contained in petri dishes at room temperature (22 to 25 C). The growth rate of 948-A on PDA was optimal at 30 C (42 mm diam/24 hr).

Hyphae of 948-A were coenocytic, and ranged from 5.8 to 13.8 μ in diam. Flooding of 5-day-old water agar cultures resulted in the formation of primary zoospores which were liberated individually through evacuation hyphae after 12 hr at 24 C. These primary zoospores encysted at the orifices of the evacuation hyphae to form clusters of spherical, smooth-walled cysts 9.2 to 9.6 μ in diam (Fig. 2-A). Approximately 12 to 24 hr after flooding, these cysts either germinated directly or gave rise to single reniform secondary zoospores by papillate discharge. Observations of secondary zoospores of 948-A with a phase contrast microscope showed them to be laterally biflagellate.

The sexual stages of all three isolates were produced 2 to 3 days after flooding 5-day-old water agar cultures. Considerably fewer oospores were produced by 948-A, however, than by AE-6 and AE-7 under the same conditions. The oogonia of all three isolates were spherical, contained solitary oospores, and were borne terminally from short lateral branches of the mycelium. Most were surrounded by one-three curved, clavate, and apparently diclinous antheridia that were contiguous to the outer oogonial wall (Fig. 2-B). The outer walls of the oogonia were smooth, whereas the inner walls were sinuous. Oogonia of 948-A measured 27.0 to 35.7 μ (mean, 31.4 μ) in diam, whereas the oogonia of AE-6 measured 26.0 to 33.6 μ (mean, 29.6 μ); and those of AE-7 were 21.4 to 29.6 μ (mean, 25.5 μ) in diam. The oospores of 948-A measured 22.0 to 27.0 μ in diam (mean, 24.0 μ); whereas the oospores of AE-6 measured 19.4 to 27.5 μ (mean, 22.8 μ), and those of AE-7 were 16.0 to 22.5 μ (mean, 19.3 μ) in diam. The range and mean size were taken from 25 oogonia and oospores for each isolate.

DISCUSSION.—This study showed that the blight of *E. brevipedicellatus* observed in South Florida was caused by a species of *Aphanomyces*. In numerous experiments, inoculation with suspensions of zoospores or mycelial fragments of 948-A resulted in disease symptoms the same as those observed at the

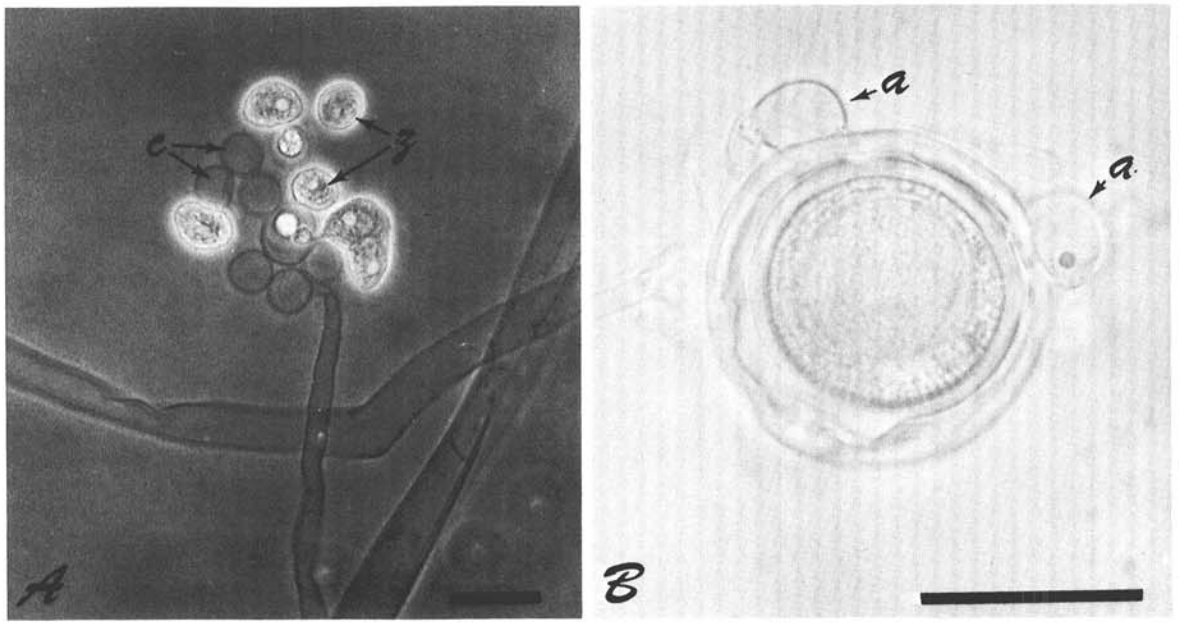

Fig. 2. A) Evacuation hypha of isolate 948-A of *Aphanomyces* sp. with a cluster of encysted primary zoospores (c) and secondary zoospores (z). **B)** Oogonium of isolate 948-A with smooth outer wall, sinuous inner wall, two clavate antheridia (a), and a single oospore. Scale line for A and B is 20.0 μ.

Fig. 1. A) Submersed healthy (left) and blighted (right) plants of *Echinodorus brevipedicellatus*; the latter were inoculated with zoospores of isolate 948-A of *Aphanomyces* sp. **B)** Leaves of infected *E. brevipedicellatus* with symptoms typical of early stages of blight; note the basal water-soaked tissues which ultimately become skeletonized through lamellar decomposition. **C)** 3.8-liter (1 gal) wide-mouth glass jar containing a single healthy specimen of *E. brevipedicellatus* rooted in unwashed quartz sand.

aquatic plant nursery. Moreover, this organism was repeatedly reisolated from diseased tissues, but never from healthy tissues. The identification of 948-A as a species of *Aphanomyces* was based primarily on the observation of filiform zoospores emerging from an evacuation hypha in a single file and encysting at the orifice, and on the presence of uniovulate oogonia (15). This appears to be the first report of any species of *Aphanomyces* infecting an alismataceous plant as well as the first report of a species of this genus infecting an aquatic phanerogam. Other species of *Aphanomyces* are reported as root pathogens of terrestrial phanerogams or parasites of aquatic cryptogams (13).

Of the species of *Aphanomyces* listed by Scott (13) and Drechsler (4), 948-A best fits *A. euteiches* in that it (i) is pathogenic to pea and other legumes; (ii) has relatively large spherical oogonia (mean 31.4 μ in diam) with smooth outer walls and sinuous inner walls; and (iii) has one to three clavate and mostly diclinous antheridia per oogonium. This isolate differed from the AE-6 and AE-7 isolates in that it (i) had a faster rate of mycelial growth on PDA at room temperatures; (ii) produced relatively few oospores on water agar; (iii) produced zoospores from water agar cultures; (iv) was pathogenic to the amazon sword plant; and (v) was pathogenic to beet and radish seedlings. This extended host range which includes beet and radish appears to be inherent to this isolate, since all five single zoospore isolates tested gave the same pathogenicity results. However, we consider these differences to be insufficient at this time to consider 948-A as a new species of *Aphanomyces*. Indeed, similar reports of variability among isolates of *A. euteiches* have been published (2, 6, 8, 10, 12). Moreover, although considered primarily a pathogen of legumes (7, 9, 14), isolates of *A. euteiches* have been reported to infect species in such nonleguminous genera as *Apium, Hordeum, Spinacea, Linum*, and *Amaranthus* (14).

This appears to be the first report of *A. euteiches* in Florida. It is not known how widespread this fungus is in Florida among plantings of amazon sword plants, since no concerted efforts were made in this study to survey these plants. *Aphanomyces euteiches* is a common pest of peas in more northerly latitudes, thus arousing speculation as to the origin of this pathogen on amazon sword plants. It is possible that it was introduced from abroad, perhaps from the Amazon Basin, on shipments of aquarium plants. Perhaps it is an exotic strain of this species better adapted to warmer climates than isolates heretofore studied. The rapid growth rate of this organism at 30 C favors this hypothesis. Alternatively, this organism may have evolved locally, perhaps on a weed species of legume, only to have manifested itself as a pathogen of the amazon sword plant through accidental contamination.

Diseases of aquarium plants have been sadly neglected and may be much more significant than is generally recognized. The aquarium industry is rapidly expanding, and merits greater attention by plant pathologists.

LITERATURE CITED

1. BATISTA, A. C., & A. F. VITAL. 1952. Phyllosticta echinodoricola. Bol. Secretaria Agr. Pernambuco 19:28.
2. BEUTE, M. K., & J. L. LOCKWOOD. 1967. Pathogenic variability in Aphanomyces euteiches. Phytopathology 57:57-60.
3. CHUPP, C. 1953. A monograph of the fungus genus Cercospora. Ithaca, N.Y. 667 p.
4. DRECHSLER, C. 1929. The beet water mold and several related root parasites. J. Agr. Res. 38:309-361.
5. FASSETT, N. C. 1955. Echinodorus in the American tropics. Rhodora 57:133-156.
6. HAGLUND, W. A., & T. H. KING. 1961. Inoculation technique for determining tolerance of Pisum sativum to Aphanomyces euteiches. Phytopathology 51:800-802.
7. JONES, F. R., & C. DRECHSLER. 1925. Root rot of peas in U.S. caused by Aphanomyces euteiches. J. Agr. Res. 30:293-325.
8. KING, T. H., & H. BISSONETTE. 1954. Physiologic specialization in Aphanomyces euteiches. Phytopathology 44:495 (Abstr.).
9. LINFORD, M. B. 1927. Additional hosts of Aphanomyces euteiches, the pea root rot fungus. Phytopathology 17:133-134.
10. LLANOS, M. CARMEN, & J. L. LOCKWOOD. 1960. Factors affecting zoospore production by Aphanomyces euteiches. Phytopathology 50:826-830.
11. MITCHELL, J. E., & C. Y. YANG. 1966. Factors affecting growth and development of Aphanomyces euteiches. Phytopathology 56:917-922.
12. SCHAREN, A. L. 1960. Germination of oospores of Aphanomyces euteiches embedded in plant debris. Phytopathology 50:274-277.
13. SCOTT, W. W. 1961. A monograph of the genus Aphanomyces. Virginia Agr. Exp. Sta. Tech. Bull. 151. 95 p.
14. SHERWOOD, R. T., & D. J. HAGEDORN. 1962. Studies on the biology of Aphanomyces euteiches. Phytopathology 52:150-154.
15. SPARROW, F. K. 1960. Aquatic phycomycetes. Univ. Michigan Press, Ann Arbor. 1187 p.
16. STODOLA, J. 1967. Encyclopedia of water plants. TFH Publ., Inc. Jersey City, N.J. 368 p.
17. UNITED STATES DEPARTMENT OF AGRICULTURE RESEARCH SERVICE. 1960. Index of plant diseases in the United States. USDA Handbook 165. 531 p.
18. UNIVERSITY OF FLORIDA INSTITUTE OF FOOD AND AGRICULTURAL SCIENCES. 1969. 1969 DARE Rep. Publ. No. 7. 192 p.
19. WHETZEL, H. H., & F. D. KERN. 1926. The smuts of Puerto Rico and the Virgin Islands. Mycologia 18:114-124.
20. ZUNDEL, G. L. 1951. Notes on the Ustilaginales of the world. V. Mycologia 43:267-270.