Verticillium Wilt of Velvetleaf (*Abutilon theophrasti*)

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ABSTRACT

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Since 1975, symptoms typical of a vascular wilt disease have been observed on velvetleaf (*Abutilon theophrasti*) within a weed nursery at the University of Wisconsin Experimental Farm, near Arlington. *Verticillium dahliae* was recovered from symptomatic plants, and Koch's postulates were completed to determine the causal agent. Leaves show mottling initially, and irregular chlorotic areas coalesce and progressively become necrotic; interveinal necrosis becomes pronounced and major veinal tissues remain green. In symptomatic plants, there is a darkening of the steele of the roots and stems. Plants with advanced symptoms of the disease were observed to have reduced seed production and a general decline in height and vigor. Field experiments showed that the indigenous soil population of *V. dahliae* was specific for velvetleaf and failed to induce symptoms in and was not isolated from pea, potato, tomato, sunflower, tobacco, soybean, cotton, cabbage, pepper, peppermint, or alfalfa. *V. dahliae* was isolated from 92% of the velvetleaf plants sampled by the end of the growing season (2 September). The number of free microsclerotia per gram of soil varied from 2,300 to 13,400 and 800 to 7,800 for total *Verticillium* spp. and *V. dahliae*, respectively. About 59% of the *Verticillium* species recovered from the Arlington Plano silt loam soil were identified as *V. dahliae*. *V. dahliae* was not isolated from seeds of naturally infected or artificially inoculated velvetleaf plants.

Numerous weed hosts are involved in the survival, reproduction, and spread of soilborne plant pathogens (7). As hosts, weeds may be a reservoir for plant pathogens in the absence of a susceptible crop. Historically, specific weeds are known to be natural reservoirs for *Verticillium* species (3,10,19,26). Velvetleaf (*Abutilon theophrasti* Medic.) was reported to be a symptomless carrier of *V. albo-atrum* (Reinke & Berth.) by Carpenter (5) in 1914. Rudolph (23) and others (3,9) later discovered this weed could host *V. dahliae* Kleb. Moreover, the passage of isolates of *V. dahliae* or *V. albo-atrum* through a weed did not alter virulence on the host from which it was originally isolated (10,12).

Although Kirkpatrick and Harrison (17) have described the effects of *V. dahliae* on the emergence, survival, and reproduction of velvetleaf, a comprehensive description of this wilt disease in velvetleaf is lacking. Therefore, we report our findings of disease occurrence in stems, symptomology, host specificity, seed infection, and the number of free microsclerotia in naturally infested soil associated with Verticillium wilt of velvetleaf.

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MATERIALS AND METHODS

Isolation and identification of *V. dahliae*. Velvetleaf plants with symptoms of a vascular wilt were collected from several locations in Wisconsin during the summers of 1977 and 1978, placed in polyethylene bags, and stored at 3 C until isolations were made. Stem segments (5 cm) were surface-disinfested for 10 sec, rinsed in sterile distilled water, and plated on acidified potato-dextrose agar (PDA). Verticillate-type conidiophores were observed 24–36 hr after incubation. Single-spored cultures were transferred to prune-lactose yeast agar (PEA) for identification (25). Conidiophores and conidia were measured after growth on PEA and incubation in the dark at 24 C for 4–6 days. Cultures were maintained on PDA slants at 10 C until pathogenicity was evaluated.

Pathogenicity tests. Heat-treated velvetleaf seeds (70 C, 15 min) were planted in flats of vermiculite and grown in a growth chamber at 24 C, 143 $\mu E^{-2} s^{-1}$ of light and 14-hr days. Simultaneously, *V. dahliae* isolates, obtained by procedures described, were grown on PDA containing 200 ppm streptomycin (SPDA) at 24 C for 10 days. Individual mycelial disks were removed from SPDA plates with a 12-mm cork borer and aseptically transferred to 500-ml Erlenmeyer flasks containing 100 ml of Difco Czapek-Dox broth (CZB) (6). Seeded flasks were placed on a rotary shaker at 22 C. Seven to 10 days later, conidial suspensions were prepared by filtering flask contents through double layers of cheesecloth. The final inoculum concentrations were estimated by a hemacytometer and varied from 1.5 to 8.6 $\times$ 10^6 conidia per milliliter.

Two-week-old plants were removed from the flats, the vermiculite washed from their roots, and the root systems dipped for 5 min in inoculum. Inoculated

plants were transplanted to pasteurized soil (2:1 compost/sand mix) and grown in 15-cm-diameter (1,000 cm^3) pots, three plants per pot. The experiment consisted of a randomized complete block design with each isolate replicated three times. Control plants were handled identically but dipped in sterile CZB. Plants were incubated in an air-conditioned greenhouse at 24 C under fluorescent light for 16 hr/day.

Three weeks after inoculation, disease ratings were recorded for velvetleaf plants as follows: 0 = no visible symptoms; 1 = slight wilting, chlorosis, or necrosis on 1% of the leaves; 2 = chlorosis, necrosis, and wilting on 1–19% of the leaves; 3 = up to 80% of the leaves chlorotic, necrotic, and wilted; 4 = ⩾80% of the leaves chlorotic, necrotic, and wilted; and 5 = dead plant. After disease severity was estimated, plants were harvested and 10 stem sections per plant were plated on an ethanol-streptomycin agar (ESA) medium (21) to determine the presence of *V. dahliae*.

Host specificity. In 1978 and 1979, a number of horticultural and agronomic crops were evaluated for susceptibility to *V. dahliae* in a naturally infested plot located at the University of Wisconsin Experimental Farm near Arlington. The plot was situated within the weed nursery where Verticillium wilt of velvetleaf was first observed.

In 1978, the following crops were grown and observed for foliar and stem symptoms: alfalfa (*Medicago sativa* L.), cabbage (*Brassica oleracea* L.), cotton (*Gossypium hirsutum* L.), pea (*Pisum sativum* L.), pepper (*Capsicum frutescens* L.), potato (*Solanum tuberosum* L.), soybean (*Glycine max* (L.) Merr.), sunflower (*Helianthus annus* L.), tobacco (*Nicotiana tabacum* L.), and tomato (*Lycopersicon esculentum* Mill.).

In 1979, the following crops were grown in the infested site and observed for symptoms:. tobacco (cultivar Havanna 500), sunflower (cultivars Cargill 204 and 205, DO 843 and 704, 4-W 900 and 1100-C, GH 10, 20, and 30, Sungro 342, 372A, 378, and 380A, Sunbred 212 and 254, Sunhi 304 and 325, PAG 101, and Sigro 894-A and 454) squash (*Cucurbita maxima* Dcne. cvs. Buttercup 406, Table Queen 409, Table King 409^{H}, Golden Hubbard 413, and 294), tomato (cultivars Beef Master 431, Beefsteak 45, Burgess Early 456, Burpee's Big Boy 451, Glamour 438, Ground Cherry 455, Heinz 1350, Jubilee Yellow 459, Oxheart 441, Patio Hybrid 427, Roma Paste 436, White Beauty 442, Wisconsin 55, Wisconsin 453, and Wisconsin Chief 426), potato (cultivars Nordsman and Russet Burbank), and mint (*Mentha piperita* L. var. *officinalis* Sole).

Plants were assayed for *V. dahliae* in the fall of 1979. Five 1-cm pieces were cut from stems, surface-disinfested in 0.5% sodium hypochlorite for 1 min, and plated on water agar. Stem pieces were incubated for 4 wk at 22–25 C and examined for the presence of *Verticillium* spp. Isolations were made from five plants of tobacco, five plants per cultivar of sunflower, and one plant per cultivar of squash and tomato. Also, isolations were made from the stem, tuber, and root tissue of 42 plants of potato. Additional isolations were made from 50 velvetleaf plants collected at random throughout the nursery site.

Field occurrence. Velvetleaf plants were observed for symptoms of Verticillium wilt at the Arlington weed nursery. The occurrence and extent of *V. dahliae* infection in stems was determined by randomly sampling plants at 2-wk intervals. At each survey, two velvetleaf plants were removed from 60 sampling sites, labeled, and stored in polyethylene bags at 3 C until isolations could be made. Four surveys were made on the following dates: 23 July, 6 August, 20 August, and 2 September.

To assay for *V. dahliae*, five consecutive 3-cm pieces were cut from each stem, surface-disinfested in 0.5% sodium hypochlorite solution for 60 sec, and plated on SPDA. Stem pieces were incubated for 2 wk at 22–25 C. The number of infected plants was recorded (as a percentage of the total sample population) for each survey.

Propagule enumeration of *V. dahliae* within naturally infested soil. Soil from the Arlington weed nursery was sampled to determine the population density of *V. dahliae* relative to the total *Verticillium* spp. population. Twenty-five composite samples, consisting of three smaller samples, were collected at random from a 0.4-ha area. At each sample site, a trowel was used to loosen and mix the soil in 10-cm deep hole and about 200 cm^3 of soil was collected.

Composite soil samples were air-dried for 105 days to eliminate short-lived propagules (1,23). Samples were screened through 100-mesh screen to remove plant debris and large soil particles, then through 500-mesh screen to separate individual microsclerotia (50–200 mm) from soil particles (11).

A modified Menzies-Greibel agar (20) was used to assay for *Verticillium*. Chlortetracycline was omitted from the medium, and 1 M guanidine (3 ml/L) and polygalacturonic acid (2 g/L) were added according to Huisman and Ashworth's (11) preparatory methods. Polygalacturonic acid enhances *Verticillium* spp. identification by causing subsurface colonies to form in the pectate substrate, thus allowing extended incubation periods (27). The medium was buffered to pH 7.0 before adding agar (20).

The population of free microsclerotia was estimated by the direct plating techniques of Johnson and Curl (16). Four dilution plates, prepared from each of the 25 soil composites, were treated as one sample and incubated at 22–24 C. After 2 wk of incubation, the soil residue and most microbial colonies were removed from the agar surface by washing under a gentle stream of tap water. Discrete colonies of microsclerotia-forming species of *Verticillium* were identified with a dissecting microscope along with total number of *Verticillium* spp.

Seed infection. To determine whether *V. dahliae* is seedborne, seed was harvested from both diseased and symptomless velvetleaf plants. Seed was collected from symptomless plants found near Oregon, WI, and from symptomatic velvetleaf plants collected from the Arlington weed nursery.

Laboratory experiments were conducted with 2,000 seeds from each location. One thousand of the 2,000 seeds were mechanically scarified to enhance germination. Scarified and unscarified seeds were surface-disinfested 30 sec in a 0.5% sodium hypochlorite solution and placed on ESA, 10 seeds per plate. Seed treatments were replicated 100 times; each plate was a replicate. Agar plates were stored at room temperature (22–24 C) in polyethylene bags. Over a 4-wk period, germinated and ungerminated seeds were examined microscopically for the presence of *V. dahliae*.

Seed obtained from symptomless and symptomatic velvetleaf plants was planted in naturally infested Arlington soil that was heat-sterilized or kept in its natural state. Velvetleaf seeds were surface-disinfested with a 0.25% sodium hypochlorite solution for 10 min followed by a rinse of sterile distilled water. From the two seed lots, five pots (replicates), with five seedlings per pot, were established for each of the soil treatments. Plants were grown for 8 wk under greenhouse conditions described previously. Ten stem sections per plant stem were surface-disinfested for 30 sec in a 0.5% sodium hypochlorite solution and rinsed in sterile distilled water before plating. Stem sections were incubated on ESA at 24 C for 2 wk and examined for *V. dahliae*.

RESULTS

Symptoms. Diseased velvetleaf plants were randomly distributed throughout a field, regardless of topography. Symptom expression was related to the maturity of infected plants. Therefore, various stages of symptom development could be detected in velvetleaf at any one time. Symptoms first appeared in young plants as the primary leaves began to wilt with warm midday temperatures, regaining turgidity only at night. When about 5–6 wk old, diseased velvetleaf plants showed a characteristic mottling of the leaves, with pale irregular areas developing interveinally (Fig. 1). These areas of chlorosis coalesced, turned brown, and later enlarged to envelope the veinal

areas, giving a "burnt" appearance to leaves. Infected leaf margins curled upward and inward, occasionally twisting to form a spiral. After infection, plants became progressively less vigorous and recovered more slowly from wilt until plants became permanently flaccid.

Chlorosis and necrosis of the leaves was most pronounced in mature plants by mid-July. A decline in plant vigor was noted near the end of the weed's life cycle. In advanced stages of the disease, plants show a darkening of the steele of the roots and stems. Stems of severely diseased plants bend in a characteristic shepherd's crook. A reduction in seed pod size and number was observed; many pods produced aborted seeds.

Isolation and identification of *V. dahliae*. *Verticillium dahliae* was consistently recovered from lower portions of velvetleaf stems bearing leaves with symptoms. *Verticillium*-like conidiophores were produced at the ends of the cut stems 24–36 hr after incubation on ESA. Microsclerotia developed 10–14 days later. Isolates varied in their morphological characteristics with respect to profuseness of the mycelial mat, colony color, and ability to form microsclerotia when transferred to SPDA.

The fungus produced conidia and dark brown to black microsclerotia (20–51 μm [$x = 33$] × 46–109 μm [$x = 93$]) typical of *V. dahliae*. Hyaline mycelia produced conidiophores on which single aggregates of conidia were produced in a gelatinous matrix. Conidiophores and conidia of isolates recovered from diseased velvetleaf stems were measured. The lack of darkened, widened, conidiophore bases, the conidial size and shape, and the conidiophore morphology were consistent with descriptions by Smith (24) and Isaac (14) for *V. dahliae*.

Pathogenicity tests. Each inoculated velvetleaf plant developed symptoms similar to those observed in the field, whereas the uninoculated plants remained symptomless. *V. dahliae* was isolated readily from diseased velvetleaf seedlings. Mean *Verticillium* disease severity and percentage stem colonization for 10 *V. dahliae* isolates is summarized in Table 1. Isolates G-2b, G-4, and G-7 were similar, being less pathogenic than all others; however, G-2b differed from the CZB control, whereas G-4 and G-7 did not. Isolates G-2b and G-7 colonized host stem tissue significantly less than all other isolates. Isolate G-4 colonized stem tissues readily, yet caused less severe foliar symptoms than other isolates with comparable stem colonization values.

Host specificity. Field trials in 1978 and 1979 indicated that *V. dahliae* failed to induce symptoms in alfalfa, cabbage, cotton, pea, pepper, peppermint, potato, soybean, squash, sunflower, tobacco, and tomato, whereas velvetleaf was severely diseased at the Arlington site. *V. dahliae* was not isolated from any plant species other than velvetleaf in 1979.

Field occurrence. In 1980, *V. dahliae* was recovered from 7% (8/116), 0% (0/105), 75% (83/110), and 92% (56/61) of the velvetleaf plants sampled on 23 July, 6 August, 20 August, and 2 September, respectively. The percentages of individual stem pieces per plant from which *V. dahliae* was recovered were 1.4, 0, 66, and 93% for sampling dates of 23 July, 6 August, 20 August, and 9 September, respectively.

Propagule enumeration for *V. dahliae*. The plating of four replicates per composite soil sample provided an indication of variation among replicates, whereas the 25 composite samples indicate variation between samples. The mean numbers of free microsclerotia per plate were 14.7 and 8.6 for total *Verticillium* spp. (*V. nigrescens, V. tricorpus,* and *V. dahliae*) and *V. dahliae*, respectively. Variation among replicates was not statistically significant compared with variation between composite soil samples ($F = 1.99$ and 1.87 for *Verticillium* spp. and *V. dahliae*, respectively). The number of free microsclerotia per gram of soil ranged from 2,300 to 13,400 and 800 to 7,800 for total *Verticillium* spp. and *V. dahliae*, respectively; means were 5,840 and 3,440, respectively. *V. dahliae* constituted 59% of the total *Verticillium* spp. colonies recovered from the Arlington Plano silt loam soil.

Seed infection. *V. dahliae* could not be isolated from seed (scarified or unscarified) obtained from either symptomatic or nonsymptomatic velvetleaf plants.

Greenhouse experiments, with sterilized and nonsterilized soil, indicated that velvetleaf seedlings were free of *V. dahliae* when grown in sterilized soil regardless of seed origin. Yet, all seed gave rise to diseased velvetleaf plants when grown in nonsterilized field soil. *V. dahliae* was consistently isolated from diseased seedlings. Greenhouse and laboratory experiments provided evidence that velvetleaf seed is not infected by *V. dahliae*.

DISCUSSION

Biological strains with restricted host ranges are known to exist for *V. dahliae*.

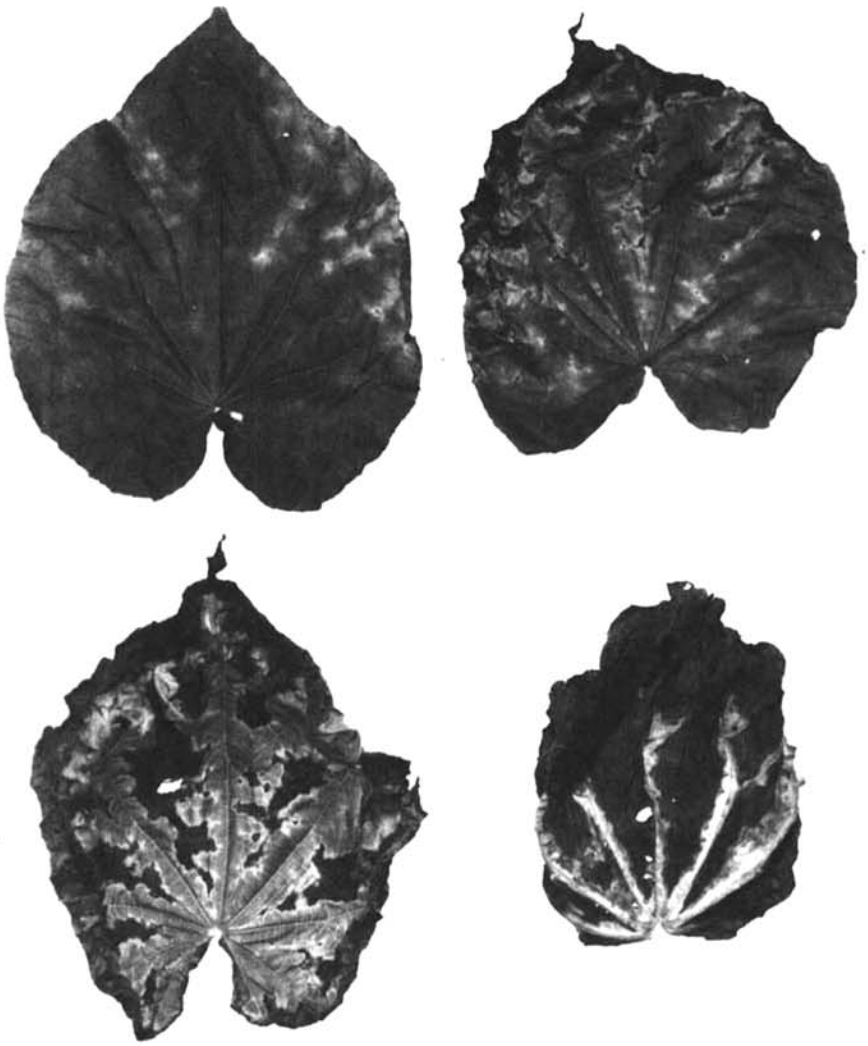

Fig. 1. Progression of foliar symptoms caused by *Verticillium dahliae* on velvetleaf.

Table 1. Disease severity and stem colonization of velvetleaf by isolates of *Verticillium dahliae* originating from velvetleaf

Isolate	Disease severity[y] (0–5)	Stem colonization[z] (%)
Uninoculated	0.0 a	0 a
G-2b	0.8 b	39 b
G-4	0.5 ab	100 c
G-7	0.6 ab	53 b
G-8	4.0 c	100 c
G-11	4.2 c	93 c
G-15	3.5 c	89 c
G-21	4.1 c	95 c
G-26	4.2 c	100 c
G-29	4.2 c	95 c
G-33	3.5 c	100 c

[y] Disease severity is based on an index scale of 0–5, where 0 = no symptoms; 1 = slight wilting, chlorosis, or necrosis on 1% of leaves; 2 = chlorosis, and wilting on 1–19% of leaves; 3 = 20–80%; 4 = 80%; and 5 = dead plant. Mean separation within the column by Duncan's multiple range test at $P = 0.05$.

[z] Percentages based on the average of three replicates, three plants per replicate and 10 stem sections per plant. Mean separation within the column by Duncan's multiple range test at $P = 0.05$.

Isaac (13) and Nelson (22) demonstrated that *V. dahliae* isolates from Brussel sprouts (*Brassica oleracea* var. *gemmifera* DC.) and mint, though pathogenic to their respective hosts, were nonpathogenic to a wide range of plant species with known susceptibility to *Verticillium*. In our field experiments, *V. dahliae* failed to induce disease symptoms in pea, potato, tobacco, sunflower, cotton, soybean, cabbage, pepper, peppermint, and alfalfa. At the same site, *V. dahliae* caused severe symptoms in velvetleaf and reduced plant size, vigor, and seed production. Infected velvetleaf plants showed wilt symptoms similar to those described for cotton (8). In 1979, *V. dahliae* was readily isolated from velvetleaf; 92% of the plants sampled were infected by the end of the growing season (2 September). In contrast, *V. dahliae* could not be isolated from peppermint, tobacco, tomato, potato, squash, and sunflower grown in a naturally infested field plot. Such preliminary results suggest a degree of host specificity between *V. dahliae* and velvetleaf.

The role of velvetleaf as an inoculum reservoir for agronomic crops remains unknown. However, soil populations of *V. dahliae*, higher than previously associated with cotton and potato production (4,15), were associated with a high incidence of diseased velvetleaf plants. Crop rotation is suggested for control of diseases caused by *V. dahliae* (8,18), but a weed host such as velvetleaf could negate the benefits of crop rotation. However, the apparent host specificity *V. dahliae* expressed for velvetleaf in our field experiments leads us to speculate that this host-pathogen association is not important as an inoculum reservoir for cultivated crops. In contrast, the personally observed decline of velvetleaf populations in many fields suggests that *V. dahliae* may be acting as a naturally occurring biological agent.

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LITERATURE CITED

1. Ashworth, L. J., Jr., and Huisman, O. C. 1972. Influence of desiccation on viability of microsclerotia of *Verticillium albo-atrum*. Phytopathology 62:744.
2. Baker, K. F., Snyder, W. C., and Hansen, H. N. 1940. Some hosts of *Verticillium* in California. Plant Dis. Rep. 24:424-425.
3. Brown, F. H., and Wiles, A. B. 1970. Reaction of certain cultivars and weeds to a pathogenic isolate of *Verticillium albo-atrum* from cotton. Plant Dis. Rep. 54:508-512.
4. Butterfield, E. J., and DeVay, J. E. 1977. Reassessment of soil assays for *Verticillium dahliae*. Phytopathology 67:1073-1078.
5. Carpenter, C. W. 1914. The Verticillium wilt problem. (Abstr.) Phytopathology 4:393.
6. Christen, A. A., and Peadon, R. N. 1981. Verticillium wilt in alfalfa. Plant Dis. 65:319-321.
7. Dinoor, A. 1974. Role of wild and cultivated plants in the epidemiology of plant diseases in Israel. Annu. Rev. Phytopathol. 12:413-436.
8. El-Zik, K. M. 1985. Integrated control of Verticillium wilt of cotton. Plant Dis. 69:1025-1032.
9. Engelhard, A. 1957. Host index of *Verticillium albo-atrum* Reinke and Berth. (including *V. dahliae* Kleb.). Plant Dis. Rep. Suppl. 244:23-49.
10. Heale, J. B., and Isaac, I. 1963. Wilt of lucerne caused by species of *Verticillium*. IV. Pathogenicity of *V. albo-atrum* and *V. dahliae* in soil and in weeds; effect upon lucerne production. Ann. Appl. Biol. 52:439-451.
11. Huisman, O. C., and Ashworth, L. J., Jr. 1974. Quantitative assessment of *V. albo-atrum* in field soils: Procedural and substrate improvements. Phytopathology 64:1043-1046.
12. Isaac, I. 1949. Comparative study of pathogenic isolates of *Verticillium*. Br. Mycol. Trans. 32:127-157.
13. Isaac, I. 1957. Verticillium wilt of Brussels sprouts. Ann. Appl. Biol. 45:276-283.
14. Isaac, I. 1967. Speciation in *Verticillium*. Annu. Rev. Phytopathol. 5:207-222.
15. Isaac, I., Fletcher, P., and Harrison, J. A. C. 1971. Quantitative isolation of *Verticillium* spp. from soil and moribund potato haulm. Ann. Appl. Biol. 67:177-183.
16. Johnson, L. F., and Curl, E. A. 1972. Methods for Research on the Ecology of Soil-Borne Mint Pathogens. Burgess Publishing, Minneapolis, MN.
17. Kirkpatrick, B. L., and Harrison, H. F. 1979. Effect of *Verticillium* on velvetleaf (*Abutilon theophrasti* Medic.) and its theoretical implications. (Abstr.) Pages 76-77 in: Weed Sci. Soc. Am., San Francisco, CA.
18. Leyendecker, P. J., Jr. 1950. Effects of certain cultural practices on Verticillium wilt of cotton in New Mexico. N.M. Agric. Exp. Stn. Bull. 356.
19. Martinson, C. A., and Horner, C. E. 1962. Importance of nonhosts in maintaining the inoculum potential of *Verticillium*. (Abstr.) Phytopathology 52:742.
20. Menzies, J. D., and Greibel, G. E. 1967. Survival and saprophytic growth of *V. dahliae* in uncropped soil. Phytopathology 57:703-709.
21. Nadakavukaren, M. J., and Horner, C. E. 1959. An alcohol agar medium selective for determining *Verticillium* microsclerotia. Phytopathology 49:527-528.
22. Nelson, R. 1950. Verticillium wilt of peppermint. Mich. Agric. Exp. Stn. Bull. 221:1-259.
23. Rudolph, B. A. 1931. *Verticillium hadromtcosis*. Hilgardia 5:201-353.
24. Smith, H. C. 1965. The morphology of *V. albo-atrum*, *V. dahliae*, and *V. tricorpus*. N.Z. J. Agric. Res. 8:450-478.
25. Talboys, P. W. 1960. A culture-medium aiding identification of *Verticillium albo-atrum*. Plant Pathol. 9:57-58.
26. Woolliams, G. E. 1966. Host range and symptomatology of *Verticillium dahliae* in economic, weed, and native plants in interior British Columbia. Can. J. Plant Sci. 46:662-669.
27. Zehsazian, H. 1966. An improved selective medium for the isolation of *V. dahliae* Kleb. M.S. thesis. Oregon State University, Corvallis.