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Persistence of an Alfalfa Strain of *Verticillium albo-atrum* in Soil

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ABSTRACT

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The potential of an alfalfa strain of *Verticillium albo-atrum* to survive in the absence of alfalfa (*Medicago sativa*) was tested in unsterile soil collected from a field of alfalfa severely affected with Verticillium wilt. Propagules added to soil were distinguished from indigenous propagules of *Verticillium* spp. by use of a benomyl-tolerant isolate. Dark mycelium, conidiophores, and conidia were produced on alfalfa stems buried in soil in petri plates at −0.01, −0.3, or −3.0 bars ψ_m and 6 and 21 C, but production was reduced in soil at −0.01 bar. The number of conidiophores decreased significantly ($P < 0.05$) between 4 and 16 wk after burial. Numbers of conidia declined after 5 wk in soils held at $\psi_m \geq 1.0$ bar. Saprophytic capability of *V. albo-atrum* in unsterile soil was limited. The distance that *V. albo-atrum* grew through soil was 5 mm and <12% of available healthy tissue was colonized. The pathogen persisted in infected alfalfa stems in a field from November to October, but only 3% of the tissue remaining after 11 mo yielded *V. albo-atrum*.

In New York State, alfalfa (*Medicago sativa* L.) usually is grown in rotation with corn (*Zea mays* L.) (2 yr) followed by oats (*Avena sativa* L.) or wheat (*Triticum aestivum* L.) (1 yr). Stands of alfalfa commonly remain productive for 4–5 yr. Verticillium wilt, caused by *Verticillium albo-atrum* Reinke et Berth., was discovered in New York in 1981 (R. L. Millar, *unpublished*); some affected stands became uneconomical to maintain by the second or third year. A concern of growers has been whether the crop rotation currently practiced is an effective control measure. Therefore, persistence and activity of *V. albo-atrum* in soil were investigated.

Based on investigations done in other areas with *V. albo-atrum* on potatoes (16,18), persistence of *V. albo-atrum* in soil for longer than 3 yr is unlikely if the soil is cropped in that period to monocotyledonous species and dicotyledonous weeds are suppressed. However, isolates of *V. albo-atrum* from cultivated hosts other than alfalfa are not pathogenic to alfalfa (5,7) and isolates from alfalfa are only weakly virulent on other suscepts (3,5,8,13,19). Most reports on the ecology of *V. albo-atrum* in soil have not involved the alfalfa strain (12,16–18,20,21,25); in only two studies (1,5) was either the alfalfa strain or alfalfa used. Therefore, it was necessary to obtain information specific to the alfalfa strain. The specific objectives were to determine the persistence of the alfalfa strain of *V. albo-atrum* in soil; the extent to which colonized alfalfa tissue serves as a source of *V. albo-atrum* for saprophytic colonization of other alfalfa tissue in soil or for infection of alfalfa plants; and the effect of temperature, soil moisture, or depth of burial on survival of *V. albo-atrum* in soil. A preliminary report has been presented (9).

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MATERIALS AND METHODS

Soil. Soil was from an alfalfa field severely affected with Verticillium wilt in Tompkins County, NY. The soil was a Valois gravelly silt loam (42.1% sand, 47.6% silt, and 10.3% clay) with the following characteristics: pH 5.9 (determined in water), 11 meq/100 g soil exchange acidity (pH 7.0), 4.5% organic matter; and by elemental analysis 9.2 ppm P, 235 ppm K, 225 ppm Mg, 1,200 ppm Ca, 110 ppm NO_3-N, and 5 ppm NH_3-N. Soil was collected in autumn, passed through a 6-mm-mesh screen into galvanized trash cans, and stored in an unheated shed for up to 6 mo, then in a cold room at approximately 4 C.

A soil moisture release curve was generated using 600-ml Büchner funnels with fritted glass bottoms (Corning Glass

Works, Corning, NY) (4), and a soil pressure plate (Soil Moisture Co., Santa Barbara, CA). Moisture content was determined gravimetrically (Ohaus Moisture Determination Balance, Ohaus Scale Corp., Union, NJ). When the soil was collected, soil moisture content was approximately 26.5% (−0.3 bar [30 kPa] matric potential [ψ_m]); it remained at this level during storage. For the experiments, a ψ_m of −0.01 bar (1 kPa) (50% moisture) was obtained by evenly adding water to soil in petri plates. Soil was air-dried for 18 hr to obtain a ψ_m of −1.0 bar (100 kPa), or 24 hr for a ψ_m of −3.0 bars (300 kPa).

Infected plant material. Single-conidium isolates of *V. albo-atrum* were maintained on prune-lactose-yeast agar (24) and transferred bimonthly. Isolate 78-I was obtained from diseased alfalfa from Wyoming County, NY, in 1981. This isolate was like other New York isolates in morphology, growth, and virulence (R. L. Millar, *unpublished*). A benomyl-tolerant selection (hereafter BT) was obtained by culturing isolate 78-I in Czapek Dox broth amended with benomyl (Benlate 50 WP), 50 mg/L. Isolates 78-I and BT were compared on the basis of sporulation, growth, virulence, and persistence in soil by methods described below. After 4 days of growth in Czapek Dox broth or 20 days on prune-lactose-yeast agar, neither the numbers of conidia per milliliter nor the colony diameters differed significantly (t test, $P < 0.05$). Virulence of the two isolates on Iroquois alfalfa was not different (Mann-Whitney test, $P < 0.05$). After 8 wk in soil at 21 C, the isolates did not differ appreciably in persistence, formation of dark mycelium, and production of conidiophores at any ψ_m (−0.01, −0.3, and −3.0 bars).

To obtain inoculum, *V. albo-atrum* was grown in Czapek Dox broth on a rotary shaker at 150 rpm for 3–4 days. The culture was filtered through three layers of cheesecloth and centrifuged at 12,000 *g* for 10 min; the conidia were suspended in demineralized water, counted in a hemacytometer, and diluted to obtain the desired spore concentration. Roots from 5- to 6-wk-old plants (cultivar Iroquois) grown in 1:1:1 (v/v) compost:peat:sand were washed and immersed in a conidial suspension (8×10^6 conidia per milliliter) for 10 min. The plants were transplanted into individual pots and the stems were pruned to 8–10 cm high. It was later found that stem inoculation was equally effective for production of infected plant material. Stems of alfalfa plants, approximately 10 wk old, in individual pots were pruned to just above the first node and the cut surface was brushed with a paintbrush dipped in a suspension of 10^5 conidia per milliliter. Plants were kept at 22 C either in the greenhouse or growth chamber, depending on the time of year, for 1–3 mo.

Selective medium. Ethanol-streptomycin agar (ESA) (14) was used to recover *V. albo-atrum* from infected alfalfa stems but was unsatisfactory for infected tissue that had been buried in soil, because streptomycin sulfate (Sigma Chemical Company, St. Louis, MO) at 100 mg/L did not inhibit gram-negative bacteria. Therefore, ESA was modified (MESA) by adding 100 mg of potassium penicillin-G (The Upjohn Company, Kalamazoo, MI), 125 mg of chloramphenicol (Sigma), and 10 mg of 75% a.i. pentachloronitrobenzene per liter; MESA amended with 25 mg benomyl/L (MESA-B) was used for the selective recovery of isolate BT of *V. albo-atrum*.

Persistence of *V. albo-atrum* in stems buried in soil. Unsterile field soil (60 g) was weighed into 9-cm-diameter petri plates. Dried stems infected with isolate BT were cut into 2-cm lengths after the leaves had been removed. The surface of stem segments was examined to ensure absence of dark mycelium and sporulation, and then five segments were arranged symmetrically on the soil surface and covered with 30 g of soil. Five plates per treatment were used. Groups of plates with the same ψ_m were sealed in double plastic bags and placed in incubators.

Stems recovered from soil were examined with a stereoscopic microscope at ×60 for sporulation. The number of conidiophores was rated using the scale: 0 = none, 1 = 1–5, 2 = 5–10, 3 = 10–50, 4 = 50–100, and 5 = 100 conidiophores per stem. The stems were then washed with a stream of water to remove adhering soil particles, blotted dry, and rated visually for the amount of dark mycelium according to the following scale: 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–99%, and 5 = > 100% of the stem covered by dark mycelium. Often dark mycelium appeared only on one side of the stem, possibly corresponding to the distribution of *V. albo-atrum* in only one portion of the vascular tissue. The stems were placed on MESA-B for recovery of isolate BT. After 4 and 7 days at 22 ± 2 C, the plates were examined with a stereoscopic microscope for growth of *V. albo-atrum*. No naturally occurring benomyl-tolerant isolates were recovered from 90 healthy stems buried as controls in two experiments.

The percentage of segments yielding isolate BT of *V. albo-atrum* for each treatment was transformed by taking the arcsin of its square root. Analysis of variance was performed for the transformed data. Mean ratings for dark mycelium and sporulation were calculated for each plate and ranked; the ranks were used for an analysis of variance or Mann-Whitney tests, respectively. For analyses of variance, orthogonal polynomials were calculated to partition sums of squares into single-degree-of-freedom contrasts where appropriate (23).

The effect of depth of burial on persistence, formation of dark mycelium, and sporulation of *V. albo-atrum* was examined for infected stems. Because soil structure might have affected persistence, intact, 10-cm-diameter soil cores were collected in August 1984 from the field (planted to corn) from which soil had been collected previously. At each of 10 sites, cores at depths of 0–6, 6–12, and 12–17 cm were obtained in sequence by using a golf green cup-cutter. Five stems infected with isolate BT were placed at two depths: between the upper and middle and between the middle and lower cores. The cores ($\psi_m \simeq -2.0$ bars) were placed in glazed ceramic crocks and held in place with a small amount of sieved soil ($\psi_m \simeq -0.3$ bars) previously collected from the same field. The crocks were enclosed in plastic bags and placed in a growth chamber at 21 C. Twenty-five stems were recovered after 6 and 12 wk, rated for dark mycelium and sporulation, and placed on MESA-B.

Persistence of *V. albo-atrum* in field sites. Alfalfa stems and roots naturally infected with *V. albo-atrum* were placed in the field from which the soil had been collected. Tissue (approximately 30 stems or three roots) was placed in bags constructed from 3-mm-mesh nylon and the bags were filled with soil from the field. At each of four sites in November 1983, three bags each of stems and roots were buried 15 cm deep or left on the surface. In May 1984, four bags of stems, infected with isolate BT, were buried 15 cm deep at each of four sites in the same field.

At recovery, stems and roots were washed and placed on MESA or MESA-B for recovery of *V. albo-atrum* or isolate BT, respectively. Tissue fragments were floated off the soil and collected on a 0.84-mm-mesh sieve. The pieces were distributed evenly on the agar surface.

Occurrence of propagules of *V. albo-atrum* in soil. Unsterile field soil in petri plates from which infected stems had been removed was sampled to determine the population level of *V. albo-atrum* in the soil. The equivalent of 1.0 g of air-dry soil was added to 9 ml of 0.1% (w/v) of water agar and vigorously mixed for 15 sec with a Vortex mixer. Two serial dilutions were made by using 0.1% water agar and mixing for 10 sec. Three 0.5-ml samples of the 10^{-3} dilution were dispersed evenly over the surface of MESA-B plates. Plates were held at 21 C for 5–6 days. Colonies were identified and counted by using a stereoscopic microscope at ×25.

Because it usually was not possible to determine what type of propagules produced the colonies recovered from soil, conidia were added to soil to learn if they declined in numbers in a manner similar to that observed for propagules produced in situ. Ten milliliters of conidial suspension (5×10^5/ml) produced on alfalfa stems infected with isolate BT were atomized onto each of two 250-g portions of unsterile field soil. The resulting moisture levels were approximately −0.6 and −2.0 bars. Soil (15 g) was weighed into 5-cm-diameter petri plates and immediately sampled by dilution plating on MESA-B. The soil plates were enclosed in plastic bags, placed in incubators at 6 and 21 C, and resampled after 1 or 3 wk. Four replicate plates were used per treatment combination.

Colonization of alfalfa tissue in soil. To determine the ability of *V. albo-atrum* to colonize alfalfa tissue in soil, a modification of the method of Stack and Millar (22) was used. A root (1 cm long) or stem segment (2 cm long) infected with isolate BT was positioned at the center of a 9-cm-diameter petri plate that contained 60 g of soil. This segment served as the source of *V. albo-atrum*. Five healthy root or stem segments (1 cm long) from greenhouse-grown plants were positioned 5 or 10 mm away from the source to serve as baits, and 30 g of soil was added to cover the segments. Ten replicate plates were used. The plates were sealed in double plastic bags and kept in incubators. At recovery, sources were removed from the soil before the baits; then individual sources and baits were washed with a stream of water, blotted dry, and placed on MESA-B for recovery of *V. albo-atrum*. In three experiments, stem segments were used as sources and baits; root segments were used as sources and baits in two additional experiments.

Infection of seedlings from colonized stem segments. Whether stems colonized by *V. albo-atrum* could serve as a source of inoculum for infection of plants was tested in a growth chamber (20 C day, 18 C night, 16-hr photoperiod with approximately 18,000 lux, 70–80% RH). Stem segments (2 cm long) infected with isolate BT and covered with dark mycelium were buried 2 cm from the bottom of individual compartments (4 × 4 × 12 cm) of folding polypropylene trays (Spencer-Lemaire Industries, Edmonton, Alberta, Canada) filled with unsterile field soil. Five Iroquois alfalfa seeds treated with captan (50% a.i.) were planted in each compartment. Seedlings were thinned after emergence to one per compartment. Two groups of 64 and 63 plants were set up with infected stem segments collected from diseased plants; for another group of 59 plants, infected stem segments which previously had been buried in unsterile field soil for 11 wk were used to determine if burial in soil had any effect on the infection potential of the pathogen in stem segments.

After 8 wk, only stems of the plants were harvested. They were surface-disinfested in 5.25% sodium hypochlorite for 1 min, rinsed in sterile water, and a 1.5-cm-long segment from the base of each stem was placed on ESA amended with benomyl (ESA-B). Plates were observed after 4 days for growth of *V. albo-atrum* from the ends of the segments. The plants were allowed to regrow and the entire plant was harvested after another 9 wk. Segments excised from the stems were placed on ESA-B; source segments were also recovered and placed on MESA-B.

RESULTS

Persistence of *V. albo-atrum* in buried alfalfa tissue. Infected stems buried in unsterile field soil at −0.01, −0.3, or −3.0 bars ψ_m were kept at 6 or 21 C for 4, 8, or 16 wk. After 16 wk, ψ_m was approximately −0.03, −0.5, and −6.0 bars, respectively. In all three experiments, *V. albo-atrum* was recovered from 72–100% of the stems, regardless of the treatment. Neither time, temperature, nor the interaction of time, temperature, and moisture was statistically significant ($P < 0.10$). The effect of moisture was significant ($P < 0.10$), with an approximately linear increase in recovery as soil moisture decreased. The lowest average recovery was from stems buried in soil at −0.3 bar.

Dark mycelium formed on buried stems under all combinations of moisture and temperature. By 4 wk, the number of stems with dark mycelium differed only slightly among treatments; however, the amount of dark mycelium per stem increased nonlinearly as soil moisture decreased ($P < 0.01$) (Fig. 1). Both temperature and time affected the response of the fungus to moisture. There was a larger difference in formation of dark mycelium between moisture levels at 6 C than at 21 C. Over time, the largest difference between moisture levels was at 4 wk and the smallest was at 16 wk.

The number of conidiophores produced on buried stems was significantly less (Mann-Whitney test, $P < 0.05$) for soil initially at −0.01 bar compared to both −0.3 and −3.0 bars for both temperatures at each time interval (Fig. 2). The number of conidiophores present decreased significantly ($P < 0.05$) between 4 and 16 wk at −3.0 bars and 6 C and at all moisture levels at 21 C. In most treatments, there was no adverse effect of low temperature, and the number of conidiophores produced per segment in soil was significantly greater at 6 C ($P < 0.05$) than at 21 C after 8 wk for soil at −0.3 and −3.0 bars, and after 16 wk for soil at −0.3 bar.

The presence or absence of clusters of spores also was noted. Generally, more spore clusters were observed on stems from wet soil than on stems from dry soil. The number of spore clusters observed decreased over time for all soils, but the decrease was greater for the drier soils. The number of conidiophores was not significantly correlated with the amount of dark mycelium on a stem.

Recovery of *V. albo-atrum* from stems buried between intact soil cores was 76 and 64% for stems at 6 and 12 cm, respectively, after 6 wk and 56 and 48%, respectively, after 12 wk. Mean ratings for the amount of dark mycelium and the number of conidiophores after 6 wk were 2.7 and 1.8, respectively, for stems buried at 6 cm and 2.2 and 1.1 for stems at 12 cm. The corresponding values after 12 wk were 1.6 and 0.1 for stems buried at 6 cm and 1.3 and 0.0 for stems at 12 cm.

Persistence of *V. albo-atrum* in tissue buried in the field. Infected tissue placed in the field in November 1983 was recovered from two of four sites in May 1984. At both depths, *V. albo-atrum* was recovered from stems (Table 1) but not from roots. The stems were broken but only slightly decomposed. The remaining material was collected in October 1984. Approximately two-thirds of the original material had decomposed, and only one-third of the

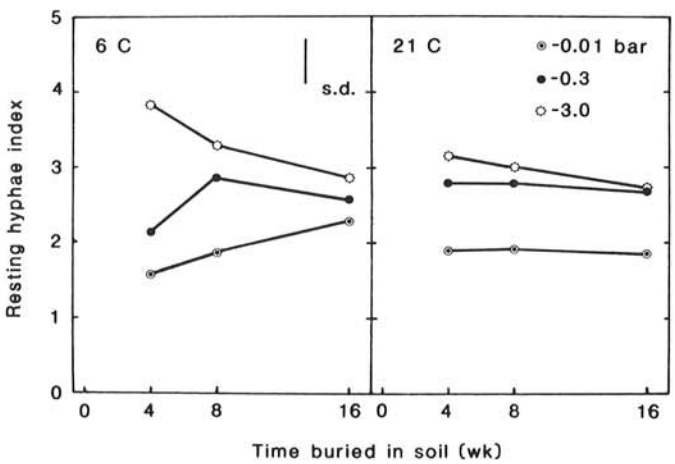

Fig. 1. Average amount of dark mycelium produced by *Verticillium albo-atrum* on alfalfa stems during 16 wk in unsterile field soil in petri plates. Amount of dark mycelium per stem was rated on a scale of 0 (no dark mycelium) to 5 (100% of stem covered). Abbreviation s.d. = pooled standard deviation.

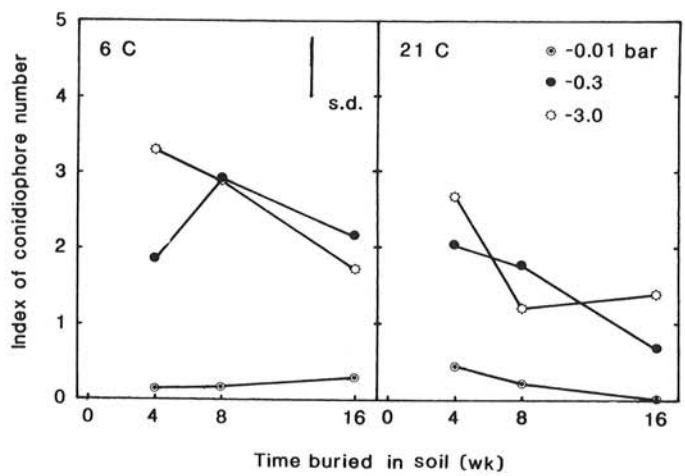

Fig. 2. Production of conidiophores by *Verticillium albo-atrum* on alfalfa stems during 16 wk in unsterile field soil in petri plates. The number of conidiophores per stem segment (2 cm) was rated (0 = none, 5 = 100) at recovery. Abbreviation s.d. = pooled standard deviation.

remaining material was present as segments 2 cm or longer. *V. albo-atrum* was recovered from an average of 2.7% of the stems. The debris, other than segments 2 cm long, yielded between zero (45 of 64 plates) and four colonies per plate.

A second group of stems, infected with isolate BT, was buried in May 1984 and recovered in September 1984. These stems had decomposed to a degree similar to that of material collected after 11 mo of burial. *V. albo-atrum* was recovered from an average of 10% of the stem segments (Table 1). The debris yielded between zero (eight of 41 plates) and 24 colonies per plate. The number of colonies from debris buried 11 mo was significantly less (Mann-Whitney test, $P < 0.05$) than the number of colonies arising from debris buried only 4 mo.

Occurrence of propagules of *V. albo-atrum* in soil. After stems with conidiophores were removed from soil in petri plates, soil from the sites of these stems was sampled for propagules of *V. albo-atrum*. In general, samples from soil at 6 C yielded more colonies than did those from 21 C, and drier (−1.0 or −3.0 bars) soils usually yielded more colonies than did moist (−0.3 bar) soils. After stems had been buried 4 wk, the number of colonies ($\times 10^3$) per gram of dry soil ranged from four to 181. The number of propagules recovered was not correlated with the number of conidiophores that had been observed per stem. Soils were transferred to 21 C after the initial sampling. There was no effect at the second sampling of the previous incubation at 6 C on the number of colonies recovered from soil subsequently held at 21 C. In soil at −3.0 bars, the number of colonies recovered increased slightly after 5 wk, whereas at −1.0 and −0.3 bar, recovery decreased to less than 10^3 colonies per gram dry soil.

When conidia were added directly to soil, the number of colonies ($\times 10^2$) per gram of dry soil recovered at zero time was 236 ± 7; after 1 wk, the number had declined significantly ($P < 0.05$) to 132 ± 4, and after 3 wk it was 120 ± 5. The decrease was the same regardless of soil moisture and temperature conditions tested. The number of colonies recovered stabilized at a level similar to that for propagules recovered from soil after sporulating stems had been removed. These data, and observations of the plates, indicated that conidia were responsible for most of the colonies that had developed in both cases.

Saprophytic colonization of alfalfa tissue from infected tissue segments. *V. albo-atrum* was recovered infrequently from stem and root baits that had been placed 5 mm from sources of *V. albo-atrum*. In two experiments in which stems were used as sources and baits, frequency of colonization ranged from 0 to 12% (50 baits per treatment), depending on the treatment. Frequency of colonization either remained constant or was less after 4 or 8 wk than at 2 wk. On analysis of the transformed frequency data (arcsin-square root percentage) no statistically significant effect of time, temperature (8 and 21 C), or ψ_m (−0.01 and −0.3 bar) on colonization was detected (analysis of variance, $P < 0.10$). At −1.0 bar, frequency of colonization was as low as in the moister soils.

When stem segments with dark mycelium of isolate BT were used as sources and segments of field-grown roots as baits, frequency of colonization after 2 wk at 21 C and −0.3 bar was 34%; for all other treatments, it was 12% or less. When the data for −0.3 bar from three experiments with stem segments as sources were combined and analyzed, neither time nor temperature significantly affected colonization. When root segments were used as sources and baits, frequency of colonization at 21 C was 14% after 6 days and 2% after 15 days.

To determine whether *V. albo-atrum* could grow from a colonized bait to another uncolonized bait in unsterile field soil, in the first two experiments with stems, two series of baits were buried, one placed as a ring 5 mm from the source at the center, and another series located as a ring 5 mm beyond the first. Although four of 900 baits in the outer ring of baits yielded *V. albo-atrum*, none of the 900 baits in the inner ring yielded the fungus. Therefore, bait-to-bait spread of *V. albo-atrum* was negligible. In a separate experiment, stem segments placed as baits 10 mm from the sources were not colonized during 8 wk, regardless of soil temperature (6 or 21 C) or ψ_m (−0.01 or −0.3 bar).

Infection of seedlings from colonized stem segments. Alfalfa plants became infected when *V. albo-atrum*-colonized stems (not previously buried) were used as the source of inoculum in a greenhouse experiment. Based on two platings of stems after 8 and 17 wk, 25% of 127 plants were infected. Many infected plants were symptomless. Of the stem segments used as sources of inoculum, 93% of 109 that were recovered yielded *V. albo-atrum* after burial for 17 wk.

When colonized stem segments that previously had been buried 11 wk in unsterile field soil were used as sources of inoculum, 17% of 59 plants became infected. Of the stem segments used as sources of inoculum, 80% of 49 recovered yielded *V. albo-atrum*. Prior burial of sources had no significant effect (chi-square analysis, $P < 0.10$) on the number of infected plants.

DISCUSSION

Fungicide tolerance has been used relatively infrequently to "mark" isolates of fungi introduced into soil (15,22). By using isolate BT of *V. albo-atrum*, it was possible to determine the persistence and activity of the pathogen in unsterile soil from a field of alfalfa affected with Verticillium wilt. Because isolate BT could be recovered selectively from soil on a benomyl-amended medium, its activity could be monitored without interference from an indigenous population of the alfalfa strain, other *Verticillium* spp., or fungi resembling *Verticillium*.

V. albo-atrum persisted in alfalfa tissue buried 15 cm deep in the field for 11 mo (Table 1), which was within the range of values (4, 9, and 17 mo) reported by others (12, 5, and 20, respectively). Differences in persistence reported by different investigators may be due to the size of tissue fragments used and the conditions that prevailed. In regions where the soil does not freeze, some microbial activity throughout the period may hasten the decline of *V. albo-atrum* (12), whereas in regions like New York State where soil can remain frozen for several months, depending on the snow cover, decay may be slowed and persistence extended.

Recovery of *V. albo-atrum* from stems buried in the field between May and September (16 wk) averaged only 10%, whereas recovery was 72% or greater after 16 wk in soil in the laboratory. In addition, stem tissues underwent more decay in the field than in the laboratory and less dark mycelium formed on stems buried in the field than on stems buried in soil in petri plates. Fluctuating

TABLE 1. Recovery of *Verticillium albo-atrum* from infected alfalfa stems buried in the field

Time buried Field site[a]	Recovery from segments (%)[b] Buried 15 cm	 Surface	Total colonies from debris[c]
Nov 1983–Nov 1984			
Site 1	n.d.[d]	8.2[e]	n.d.
Site 2	15.6	14.1	n.d.
Nov 1983–Oct 1984			
Site 3	4.5	n.d.	16
Site 4	2.9	0.6	8
May 1984–Sept 1984			
Site 5	8.8	n.d.	139
Site 6	3.6	n.d.	36
Site 7	20.1	n.d.	40
Site 8	8.2	n.d.	77

[a] Approximately 30 stems were placed in a 3-mm-mesh nylon bag filled with a Valois gravelly silt loam soil. Three bags were buried at each of four sites in November 1983; four bags were buried at each of four additional sites in May 1984. The sites were located in the field from which soil used in the study had been collected.

[b] Stems were washed, placed on modified ethanol-streptomycin agar (with benomyl on September 1984), and examined for growth of *V. albo-atrum* after 5 days.

[c] Stem fragments less than 2 cm long were floated off the soil by wet-sieving, collected on a 0.84-mm-mesh sieve, and evenly distributed on the surface of agar plates.

[d] Abbreviation n.d. = not done.

[e] Values are mean percent recovery per site from three (sites 1–4) or four (sites 5–8) samples calculated with [(dry weight of stems yielding *V. albo-atrum*)/(dry weight of all stems)] × 100.

moistures and temperatures and a potentially more diverse microbial community in the field could account for these differences.

Persistence, formation of dark mycelium, and sporulation for stems buried between intact cores of field soil were lower than those for stems buried in sieved field soil. The amount of dark mycelium was much closer to that formed on stems buried in the field than that on stems buried in sieved soil. This suggests that intact soil cores may simulate the field environment better than sieved soil and that soil structure may be an important factor affecting the ecology of soilborne pathogens, perhaps because of its effect on soil aeration and on distribution of microorganisms according to depth, either of which may have been markedly different in sieved soil.

Moist soil ($\psi_m \geqslant -0.3$ bar) was unfavorable for formation of dark mycelium, production of conidiophores, and persistence of propagules in soil, whereas drier soil was not; moisture was the only factor adversely affecting persistence of the pathogen in stems buried in soil in the laboratory. Many New York soils on which alfalfa is grown have high water-holding capacity and are poorly drained. Frequently, in spring and autumn, these soils are near saturation, which would promote decline of *V. albo-atrum*.

Temperature had no effect on persistence and relatively little effect on formation of dark mycelium and production of conidiophores and conidia. That activity of *V. albo-atrum* was not reduced at 6 C indicates the fungus is tolerant of low temperature and may be more able to compete with other soil organisms at low temperatures.

Formation of dark mycelium and persistence of *V. albo-atrum* apparently are associated. Between 72 and 100% of stem fragments with dark mycelium also yielded *V. albo-atrum*. Dark mycelium apparently functions well as a resistant structure, either in association with debris or free in soil. However, since at −0.3 bar formation of dark mycelium was high and persistence was low, presence of dark mycelium is not always positively correlated with persistence.

The abundance of conidiophores on buried stems decreased between 4 and 16 wk (Fig. 2). Aftr 16 wk at −0.3 and −3.0 bars ψ_m, the remains of the lower portions of conidiophores, which are melanized, were visible. Degradation of the upper fertile, hyaline portion of the conidiophores had occurred. However, the rate of degradation was not measured, and production of new conidiophores while degradation was occurring would have reduced the apparent rate of degradation. Sewell (17) reported that sporulation of *V. albo-atrum* on debris in soil continued 2–7 wk after conidiophores first were observed.

It is possible that the procedure used to determine the saprophytic colonization of plant tissue might not have detected all instances of colonization. However, the same experimental procedure (22) has shown a high degree of competitive saprophytic capability for *Phytophthora megasperma* Drechs. f. sp. *medicaginis* Kuan and Erwin in natural soil. Thus, the small amount of colonization determined for *V. albo-atrum* apparently is an accurate reflection of its limited saprophytic capability in soil. Limited saprophytic ability of *Verticillium* spp. in soil has been reported previously (2,11,17,26), although Slattery (21) reported that a potato isolate of *V. albo-atrum* colonized buried tissues of field crops. The ability of *V. albo-atrum* to colonize tissue was not significantly affected by the type of tissue (stem or root), soil moisture, temperature, or the length of time in soil. Soil properties, such as pH or bulk density, are unlikely to have been factors that limited colonization of baits by *V. albo-atrum* since the soil pH was 5.9, well within the pH range for *V. albo-atrum* (6), and bulk density was approximately 1.2 g/cm^3, which is in the range of an average value for silt loams.

It seems that *V. albo-atrum* has limited saprophytic capability to colonize new substrates in soil and to retain possession of a colonized substrate. Because colonized alfalfa debris undergoes decay, for the pathogen to persist, it would have to grow from colonized tissue to new tissue. Since under laboratory conditions *V. albo-atrum* grew infrequently from colonized to uncolonized baits, it may also be unlikely to do so under field conditions.

Stem segments colonized by *V. albo-atrum* were effective as sources of inoculum for infecting susceptible alfalfa plants. Until infected tissue decays, it apparently can serve as a source of inoculum. Since growth of *V. albo-atrum* through soil is infrequent and limited to 5 mm, growth of the roots toward infected debris probably is necessary for infection (25).

This investigation has provided information on the potential of *V. albo-atrum* to persist in unsterile field soil under a range of environmental conditions. Based on the rate of decline of the pathogen in soil and its limited saprophytic capabilities, the following practices may be effective in reducing the incidence of Verticillium wilt of alfalfa: the current crop rotation with nonhosts such as corn and small grains (10) for 2–3 yr to provide sufficient time for infected debris to decay, rigorous control of broadleaf weed hosts (13,19), and cultivation during the rotations to hasten the decomposition of alfalfa debris.

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