Fungal Flora of Crambe Seeds and Virulence of Alternaria brassicicola

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ABSTRACT

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Fifty-one samples of crambe seed from Illinois, Indiana, Maryland, and Ohio were plated on PDA and the associated fungi were identified. *Alternaria* spp. comprised 86% of the isolates. *Alternaria tenuis* was most commonly isolated (47%) followed by *A. brassicicola* (39%). No difference was noted in the kind of fungi isolated among sources, states, or years, but a greater array of fungi was isolated from poor-quality seed. Crambe plant selections and miscellaneous crucifers varied in susceptibility to *A. brassicicola*. Symptom expression and disease severity were influenced by inoculum density, postinoculation drying of leaves, duration of incubation period, and the leaf surface inoculated. Virulence of the organism was retained over one year when isolates were cultured on filter paper and stored in petri dishes at 5 C.

Additional key words: Alternaria tenuis, crucifers, positional leaf susceptibility.

Crambe (*Crambe abyssinica* Hochst. ex. R. E. Fries) is a relatively new oil crop within the United States. Most of the research on crambe diseases has been done in eastern and northern Europe (8). Fusarium wilt is the only fungus disease of crambe on which research has been reported from the United States (1). Leppik (8) compiled a list of diseases that affect crambe. According to G. A. White *(personal communication)* Alternaria leaf spot is the most important disease of *C. abyssinica*. The objective of this study was to identify fungi from crambe seed grown in different states in different years, and to study the virulence of *Alternaria brassicicola* (Schw.) Wiltsh. [*A. circinans* (Berk. & Curt.) Bolle]. Preliminary results have been reported (6).

MATERIALS AND METHODS

Seed from 51 locations within Illinois, Indiana, Maryland, and Ohio were received and threshed. Seed from each source was placed in a small, plastic screen basket (30 × 45 mm) to assure uniform surface sterilization. Each lot of seed was submerged in 95% alcohol for 30-45 seconds, then transferred to 0.1% mercuric chloride for 1 minute. Seeds then were washed in four changes of sterile distilled water, and transferred to dishes containing potato-dextrose agar (PDA) and the sodium salt of 2,4-D acid (4). The latter delayed seed germination, but had no detrimental effect on microflora. Each dish contained eight seeds. Seeds then were incubated under cool-white fluorescent light (463-1,045 lux) at room temperatures (23-27 C) for 7 days prior to identification of fungi. The total number of seeds plated was 2,479. The number of seeds plated per location varied from 42 to 84, depending on kind and number of fungi isolated.

Optimum temperatures for radial growth of *A. brassicicola* on PDA were determined. Small disks of agar (7 mm in diameter) containing the fungus were transferred to sterile PDA in petri dishes. Dishes were incubated in lighted chambers at 18, 23, and 28 C. Dishes were replicated 5 times, and the test was repeated. Radial growth was measured 8 days later.

Cultures of *A. brassicicola* were maintained on filter paper (5). Nonsterile filter paper in petri dishes was moistened with distilled water. Several plugs of PDA (approximately 10-15 mm in diameter) containing the fungus were transferred to different locations within the petri dishes. Dishes were incubated at 25 C for 3-4 weeks, then transferred to 5 C storage where they remained until ready for use.

Fresh cultures always were initiated by using spores from the filter paper. Spores were transferred to dishes containing lukewarm PDA, agitated gently, and then allowed to solidify. Dishes were incubated under fluorescent light at 23-27 C for 4-6 days. Contents of dishes (1 dish/100 ml of water) were comminuted in a Waring Blendor. This, generally, resulted in a suspension of approximately 30,000 spores/ml.

The method of inoculation was standardized by utilizing a spore-mycelial suspension of approximately

TABLE 1. Kind and relative prevalence of fungi isolated from crambe seed, 1973-74[a]

Fungi	Isolates (% of total)
Alternaria tenuis	47
A. brassicicola	39
Penicillium sp.	4
Aspergillus flavus	3
Mucor sp.	3
Rhizopus nigricans	1
Miscellaneous fungal species[b]	3

[a]Based on 2,479 seeds plated, yielding 1,235 fungus isolates.
[b]Included *Alternaria* spp., *Aspergillus niger,* bacterial spp., *Chaetomium* sp., *Circinella* sp., *Cladosporium* sp., *Fusarium* sp., and other unidentified fungi.

2,000-3,000 spores/ml with 5 drops of Tween-20/200 ml of suspension. Both the adaxial and abaxial leaf surfaces of 4- to 6-week-old plants were inoculated with a Schrader paint spray gun (7). Following inoculation, plants were permitted to dry, placed in a moist chamber, and atomized with water. Temperatures in the chamber fluctuated between 22 and 26 C. After 72 hours, the plants were placed on a greenhouse bench. One to four days later, the plants were rated on a scale of 0 = no disease to 9 = severely diseased.

RESULTS

Fungi associated with seed.—*Alternaria* spp. comprised 86 percent of the isolates (Table 1). *Alternaria tenuis* C. G. Nees was more abundant than *A. brassicicola*. The remaining genera of fungi and bacteria comprised only 14 percent of the isolates. Poor quality seed (shriveled, and 2 years old) yielded a greater array of fungi *(Penicillium, Aspergillus, Cladosporium, Mucor,* and *Rhizopus)* than the good quality seed. There was essentially no difference in the genera of fungi isolated among seed from different states, locations, or year of production.

Radial growth.—The maximum radial growth (63 mm) of *A. brassicicola* occurred at 23 C. At 18 C radial growth measured 53 mm, whereas at 28 C only 43 mm was recorded. Subsequent observations indicate that little or no growth occurs at 33 C. However, the fungus is capable of renewing growth on appropriate media if the temperature is lowered from 33 to 25 C.

Symptoms on greenhouse-grown plants.—Small, black, linear lesions occurred on the petioles, stems, and veins of the abaxial leaf surfaces, and on the seed pods.

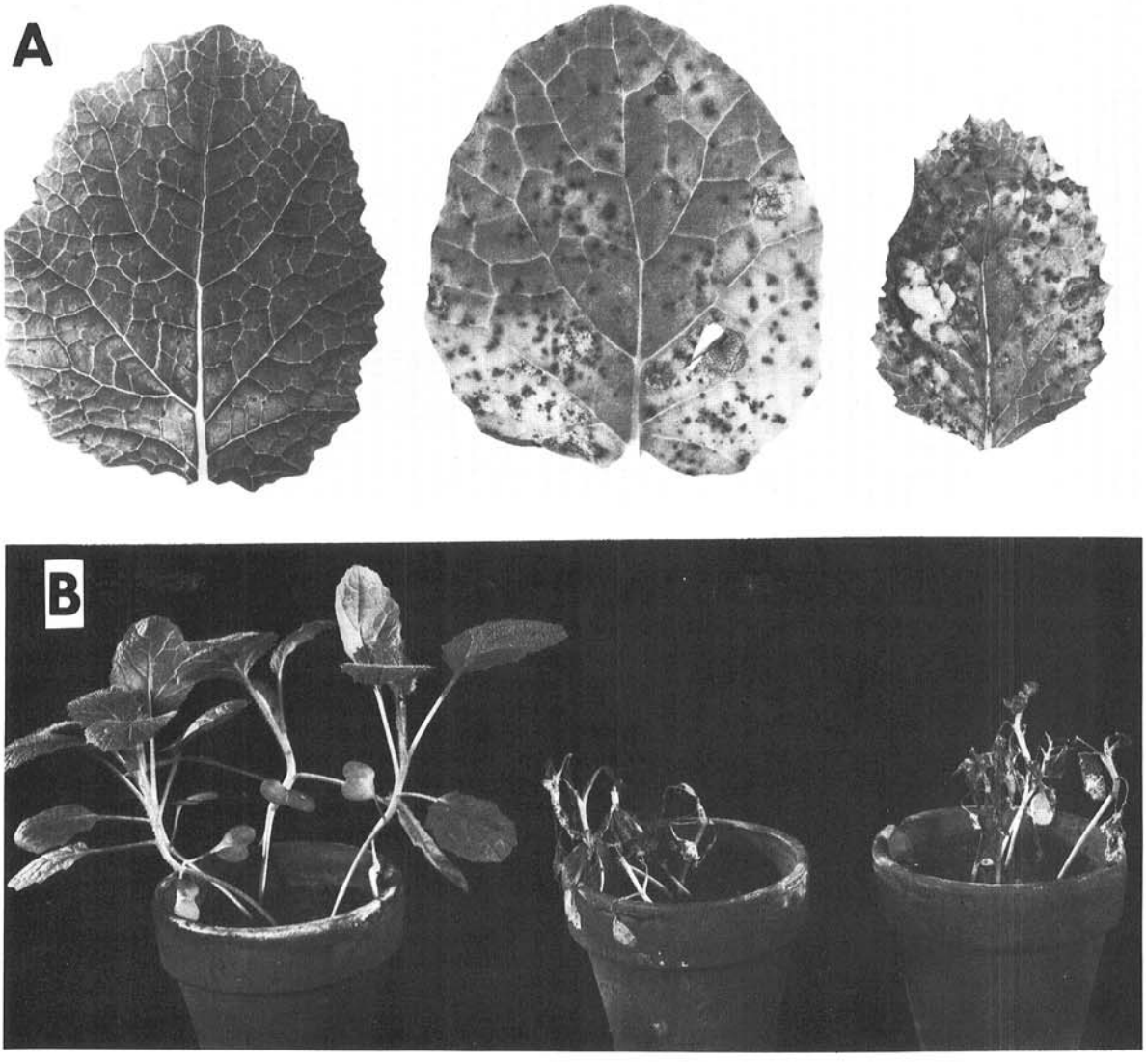

Fig. 1-(A, B). *Alternaria brassicicola* on crambe plants: **A)** leaf lesions; **B)** seedling stem tip girdling, defoliation, and death of plants. Noninoculated leaf and plants on the left.

horseshoe beginning at the point of entry into a field. Ten cores taken 0-30 cm deep and 10 steps apart were bulked into a composite sample for each side of the horseshoe pattern. The soil samples were air-dried 2 days at 21-24 C, ground in a soil mill to pass a 2-mm screen, and mixed well (1). By using the procedures reported earlier (5, 15), an assay was made on each of the three composite samples per field at each sampling date. When appropriate, assay samples were treated for 10 seconds with sodium hypochlorite and desorbed with potassium hydroxide to overcome dormancy of microsclerotia (2).

RESULTS

In our original survey, high inoculum levels of *Verticillium* in soils usually were associated with fields having an intensive cotton-cropping history (Table 1, last four entries). However, in many fields (Table 1, first 12 entries) there appeared to be little correlation between *Verticillium* inoculum levels and previous cropping history (e.g., field 30 vs. 72).

To evaluate further the influence of crop rotation on pathogen survival, eight fields were selected for continuous monitoring over an extended period. Changes in inoculum densities from month to month generally were gradual whether they were ascending or descending in response to cropping practices, as illustrated in the preceding report (2). Maximum annual inoculum densities, determined in July-September, for eight fields with different cropping histories are presented in Fig. 1. The excellent agreement between month-to-month assay results [see Fig. 1 in (2)] assured us that the values given in Fig. 1 represent seasonal inoculum changes rather than sampling error. In fields repeatedly cropped to a susceptible crop (cotton), high inoculum densities (20-60 microsclerotia/g soil) are built up and persist, as seen in field 89 and the 1971 value for field 77 (Fig. 1). Inoculum density increased rapidly following only 1 year of a susceptible crop with the increase appearing in the year following this crop. This delayed increase occurred regardless of whether the subsequent crop was susceptible (Fig. 1, fields 88, 42A, and 77) or nonsusceptible (Fig. 1, fields 42, 79, and 86). Once soils were infested, the rate of decrease in inoculum, even in the presence of immune crops, was low (Fig. 1, fields 42, 77, 86, and 86A). Seasonal decreases in inoculum in continuous susceptible culture (Fig. 1, fields 42A and 88) were equal to any seasonal decreases in immune-crop culture (Fig. 1, fields 42, 77, 79, and 86).

DISCUSSION

Microsclerotia are the principal persistent propagules of *Verticillium* spp. in soil (12, 16, 21). That these microsclerotia originate in infested host tissue was suggested (6, 8) and confirmed (16). The finding that the rapid buildup of *V. albo-atrum* in soil does not become evident until the year following the planting of a susceptible crop (Fig. 1) is consistent with this view. Infested host tissue first must be incorporated in the soil and decomposed before the inoculum buildup is detectable (3, 21). This time lag in inoculum release must be taken into consideration in any efforts to manipulate pathogen populations in soils.

Our data suggest that *V. albo-atrum* can survive in soils for long periods. An extrapolation of attrition rates in fields 77, 86, and 86A (Fig. 1) indicates that from 10 to over 20 years are required for the pathogen population to drop to near zero. These estimates agree with the findings of others (13, 23, 24, 27) that *Verticillium* spp. persist in soils for many years and can induce severe wilt in susceptible crops even after 6-12 years of nonhost cropping.

The rapid buildup and slow attrition of propagules of *V. albo-atrum* in soil limits the value of crop rotation for controlling this soil-borne pathogen. Within 1-2 years the fungus can increase to 30-40 microsclerotia/g soil in fields with initially relatively low (1-5 microsclerotia/g soil)

TABLE 1. Inoculum densities of *Verticillium albo-atrum* in variously cropped soils of the San Joaquin Valley of California

Field	County	Inoculum density[a] 1971	Crop histories					
			1971	1970	1969	1968	1967	1966
81	Merced	0.03	cot[b]	wh-mi	cot	fal	fal	cot
82	Merced	0.03	cot	alf	alf	alf	alf	. . .
83	Merced	0.05	cot	cant	cot	cant	cot	. . .
36	Kings	0.08	cot	ba-wh	alf	alf	alf	ba-co
88	Merced	0.20	cot	alf	alf	alf	cot	cot
30	Kings	0.48	cot	cot	cot	cot	alf	alf
80	Madera	0.93	cot	cot	corn	cot	corn	. . .
87	Merced	1.15	cot	ba	cot	wh	tom	alf
73	Kings	3.50	cot	wh	wh	cot	cot	alf
72	Tulare	3.90	cot	b.e.	s.b.	cot	alf	alf
78	Kings	4.10	cot	cot	wh	cot	cot	. . .
42	Kings	4.70	cot	cot	corn	cot	cot	cot
74	Tulare	24.60	cot	s.b.	cot	cot	cot	cot
75	Tulare	28.00	cot	cot	cot	cot	cot	cot
89	Merced	38.10	cot	cot	cot	cot	alf	alf
77	Tulare	47.00	cot	cot	cot	cot	alf	alf

[a] Microsclerotia per gram dry soil.

[b] Abbreviations for crops: cotton, cot; wheat-milo, wh-mi; fallow, fal; alfalfa, alf; cantaloupe, cant; barley-wheat, ba-wh; barley-corn, ba-co; barley, ba; blackeye cowpea, b.e.; sugar beet, s.b.

population levels (Fig. 1, fields 42A, 86, and 88). These values are over 10-fold the level (about four microsclerotia/g soil) needed to cause 100% infection in a cotton crop (4). Although attrition occurs during nonsuscept culture (Fig. 1), the observed rate is far too slow to make short-term crop rotation meaningful as a control measure. In field 86A (Fig. 1) microsclerotia of *V. albo-atrum* had not dropped sufficiently after 6 years of nonhost cropping to make it safe again for cotton.

Crop rotations, however, can be useful under certain circumstances. If the rotation is initiated when the inoculum is close to the minimum level needed for 100% infection in a susceptible crop, the attrition could be sufficient to drop the inoculum to a level where disease incidence in a subsequent susceptible crop would be reduced significantly. This situation is evident in field 79 (Fig. 1). However, such a situation would probably be the exception in the San Joaquin Valley since the inoculum buildup is so rapid. This also may explain some of the conflicting reports on the results of crop rotation in controlling *Verticillium* spp. Depending on inoculum level of an experimental field when a rotation regime was initiated, the attrition rate may or may not be sufficient to reduce disease incidence. Thus, it is important to know inoculum levels in experimental fields. The occasional large decreases in inoculum level observed in most fields (Fig. 1) also may figure in the conflicting reports on crop rotation, in that their unpredictable and short-term nature would necessarily lead to inconsistent results.

In most rotation studies, pathogen attrition has not been measured. Since inoculum buildup in continuous host culture progresses steadily while that in rotation plots is disrupted during the nonhost cycle, a direct plot comparison at the end of the rotation sequence may be misleading. With the possible exception of the report by McKay (20), crop rotations, when data permit evaluation, have not caused a reduction of the wilt pathogen. Although disease incidence was lower in rotation plots than in continuous cotton plots in the rotation trials of Hinkle and Fulton (14), at best no reduction in disease incidence occurred whereas in almost all cases Verticillium wilt incidence was significantly higher in each plot at the end of the 5th year than at the start of the trial. A similar situation is observable in data reported by George et al. (9). In reports of shorter studies, the observed differences in wilt incidence between continuous host and rotation plots have not been drastic (7, 17, 26). This suggests that rotations merely stretch out the time involved in inoculum buildup, but are unable to avert it. In California where wilt reductions have been reported in crop rotation studies with cotton (7, 9, 19, 26), the corresponding yield increases have averaged only 10% (our own calculations of reported data).

Although we do not wish to detract from the agronomic benefits of crop rotation, we conclude that rotations per se have little effect on *V. albo-atrum*

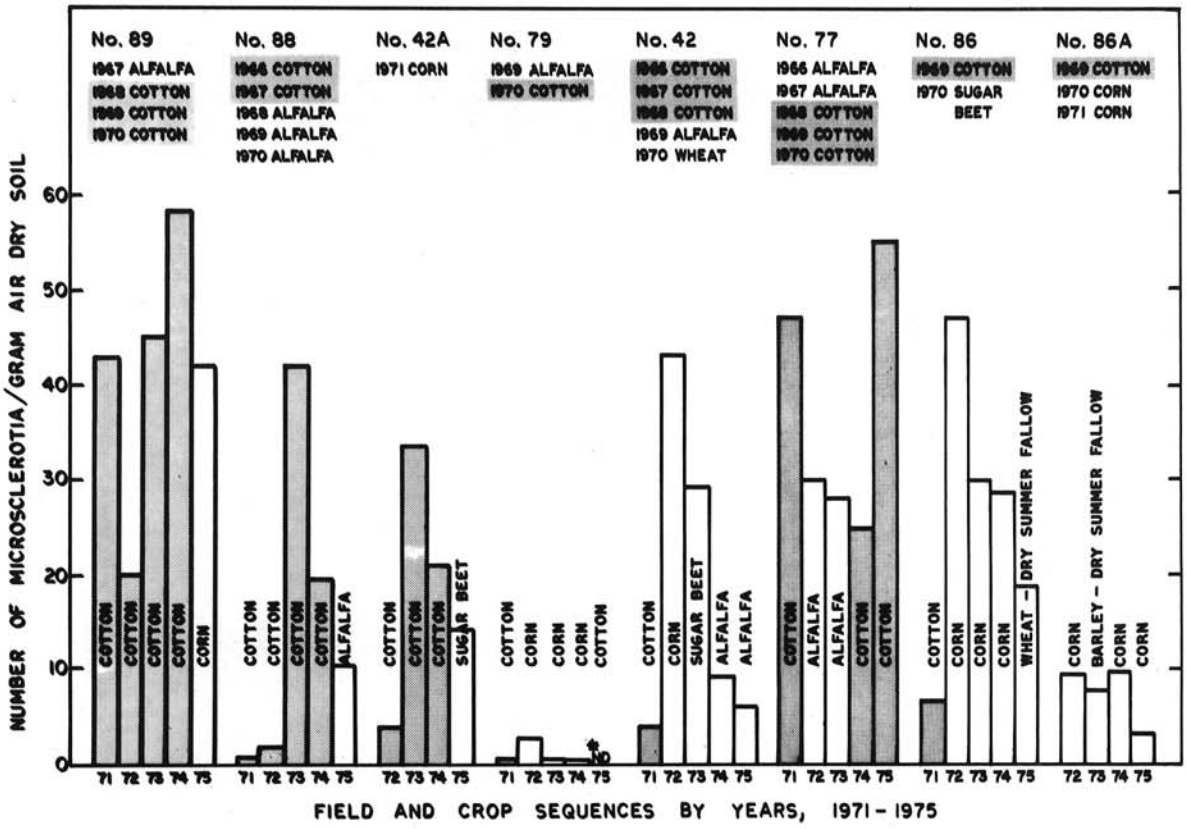

Fig. 1. Influence of cropping sequences on inoculum densities of *Verticillium albo-atrum*. Shading indicates a *Verticillium*-susceptible crop.

survival in soils and are ineffective in the long run as control measures for this important soil-borne pathogen.

Of particular importance is our observation that significant attrition of microsclerotia does occur in field soils. However, such attrition occurs independently of the crop grown. Since reduction in inoculum in continuous, susceptible culture was equal to any seasonal decreases in immune-crop culture, reductions in fields 42, 77, 79, and 86 (Fig. 1) cannot be attributed to the presence of sugar beets, alfalfa, or corn. Apparently factors independent of the crop grown are involved in these reductions in inoculum density. It is important to identify the variables both for understanding the biology of this pathogen in the soil and for developing control measures. Preliminary data indicate that soil temperature-moisture interactions are important in survival of the fungus.

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