Vegetative Compatibility Among *Fusarium oxysporum* f. sp. *basilicum* Isolates Recovered from Basil Seed and Infected Plants

WADE H. ELMER, Department of Plant Pathology and Ecology, Connecticut Agricultural Experiment Station, Box 1106, New Haven 06504, and ROBERT L. WICK and PATRICIA HAVILAND, Department of Plant Pathology, University of Massachusetts, Amherst 01003

ABSTRACT

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Forty-five isolates of *Fusarium oxysporum* f. sp. *basilicum* were used in heterokaryon tests to classify isolates into vegetative compatibility groups (VCGs). Nitrate nonutilizing mutants were selected from isolates obtained during 1991–1993 from diseased basil plants and infested seeds from California, Colorado, Connecticut, Massachusetts, Nevada, New York, South Carolina, and Italy. An Italian isolate of *F. o. basilicum* (ATCC 38560) deposited by researchers in 1975 was included also. All isolates except NY63 from New York were vegetatively compatible with each other and were virulent on susceptible basil in the greenhouse. Because isolates of *F. o. basilicum* may represent a unique VCG, heterokaryon tests could replace the lengthy pathogenicity tests and reduce the amount of time necessary to distinguish nonpathogenic isolates of *F. oxysporum* from isolates of *F. o. basilicum*. The rapid spread of this disease in the United States is likely caused by dissemination of infested seeds.

Additional keywords: Fusarium wilt, heterokaryosis

Fusarium wilt of basil (*Ocimum basilicum* L.) was first reported in the Soviet Union in 1956 (19) and in 1957 (13). The first mention of a forma specialis was in 1968 by Dzidzariya (6), who referred to the causal agent as *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *basilicum*. The disease was then reported in Italy in 1975 (9) and in France in 1982 (15). In 1992, losses attributed to Fusarium wilt on basil were reported in Massachusetts (20), Nevada (K. Kosta, *personal communication*), New Mexico (J. Rodebaugh, *personal communication*), New York (D. Lukens, *personal communication*), Colorado (J. Meneley, *personal communication*), and several counties in California (5; A. Gabrick, *personal communication*). In 1993, the disease was reported in South Carolina (11), Connecticut (W. H. Elmer, *unpublished*), and Israel (T. Katan, *personal communication*).

Martini and Gullino (14) suggested that the rapid spread of this disease into noninfested areas in Italy was a result of growers using infested seed. They found that 0.4% of nondisinfested commercial seed and 0.2% of disinfested seed harbored *F. o. basilicum*. The recent outbreak of Fusarium wilt of basil in the United States may also be a result of growers and garden centers buying and selling infested seed and transplants.

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Vegetative compatibility in fungi is a genetic trait controlled by *vic* or *het* loci (1), and identical alleles at each loci must be present in two compatible hyphae before anastomosis can occur. Compatible isolates form vegetative compatibility groups (VCGs). Since gene transfer via parasexuality requires anastomosis, isolates of an asexual fungal species within a VCG are genetically isolated from isolates of the same species in other VCGs.

Heterokaryon tests involving nitrate nonutilizing (*nit*) mutants have been used extensively to classify pathogenic formae of *F. oxysporum* into VCGs (2–4,7,8, 10,17,18). Heterokaryon tests demonstrate whether or not two isolates of *F. oxysporum* are vegetatively compatible and thus genetically similar (18). Puhalla (18) hypothesized that in fungi like *F. oxysporum*, which has no telemorph, there would be a correlation between VCGs and formae speciales. These correlations have been demonstrated for a number of formae speciales in *F. oxysporum* (2,4,18), but exceptions occur (7,8). Our objectives were to collect isolates of *F. o. basilicum* from different locales and from commercial seed sources and classify them into VCGs using *nit* mutants.

MATERIALS AND METHODS

Collection of isolates. Isolates of *F. o. basilicum* were collected from several sources in the United States and from one known Italian source (Table 1). Monosporic cultures from each isolate were stored in short-term culture on potato-dextrose agar (PDA) slants and long-term culture in 15% glycerol at −40 C.

Isolations from infected stem tissue and seeds were done at the Connecticut Agricultural Experiment Station in New Haven and the University of Massachusetts in Amherst. Infected material was obtained from our respective diagnostic clinics and by site visits. Small pieces of discolored stem tissue were surface-disinfested for 1 min in 10% household bleach, rinsed in distilled water, and placed on acidified PDA, PDA with streptomycin (100 μg/ml), or Komada's selective medium (12). Colonies of *F. oxysporum* were established from single spores and subcultured to PDA or minimal medium (MM) (3).

During the course of our experiments, two seed sources (seed lot ST159C, origin Italy, and seed lot Mar. 1993 159, Stokes Seed, origin United States) were found to be infested with *F. oxysporum*. Seeds from both sources were assayed for incidence of infestation. Isolations were done by placing 25 seeds per plate on acidified PDA, PDA with streptomycin, and Komada's selective medium. At least 200 seeds were examined from each seed lot. Single spores from recovered isolates of *F. oxysporum* were cultured, stored, and then subjected to the heterokaryon and pathogenicity tests described below.

Heterokaryon tests. Methods for the media and procedures for selecting, characterizing, and pairing the *nit* mutants in heterokaryosis tests have been described in detail by Correll et al (3); a brief description is provided here. Each isolate was grown on MM (3) for 3–4 days, then four colonized agar plugs were transferred to plates filled with PDA supplemented with 15 g of $KClO_4$ (PDC medium). After 7–14 days, rapidly growing sectors were transferred to MM, nitrite agar (MM in which the $NaNO_3$ was replaced by $NaNO_2$, 0.5 g/L), and hypoxanthine agar (MM in which the $NaNO_3$ was replaced by hypoxanthine, 0.2 g/L). On the basis of their growth characteristics, described by Correll et al (3), they were assigned to phenotypic class *nit1*, *nit3*, or NitM. As these heterokaryosis tests became more routine, only *nit1* phenotypes were selected from test isolates. Heterokaryon tests were conducted by pairing colonized agar plugs of a NitM mutant from a known tester strain with a *nit1* mutant of a test strain 2 cm apart on MM. Plates were incu-

bated at 20–24 C and examined daily for 2 wk. The appearance of aerial mycelium at the point of hyphal contact was evidence of heterokaryosis and vegetative compatibility.

Pathogenicity tests. Pathogenicity tests were conducted on susceptible basil cv. Spicy Globe or sweet basil transplants that were grown in 10-cm plastic pots filled with commercial potting mix. Inoculum was increased on PDA plates incubated for 10–14 days, and conidia were washed from the plates with sterile distilled water and filtered through sterile cheesecloth. A pipette was used to place 20 ml of approximately 10^6 conidia per milliliter into a hole 1 cm deep and 1 cm away from the base of the plant; control plants received 20 ml of distilled water (three replicate plants per test isolate). Plants were randomly placed on the greenhouse bench or in a growth chamber set at 25 C for 14–21 days, whereupon they were rated for the presence or absence of wilting and vascular discoloration at the base of the stem. An isolate was considered virulent if at least one of the three inoculated plants developed symptoms, and the fungus was then reisolated.

RESULTS

Isolations and pathogenicity. All symptomatic basil plants received through the diagnostic clinics or during site visits had vascular discoloration at the stem base. Colonies of *F. oxysporum* grew readily from surface-disinfested tissue on the various isolation media. Seed isolations from Spicy Globe lot ST159C and sweet basil lot Mar. 1993 159 yielded *F. oxysporum* at 12 and 5%, respectively. Contamination by dematiaceous hyphomyceteous fungi hindered recovery of *F. oxysporum* on PDA with streptomycin but not on Komada's selective medium.

All isolates of *F. oxysporum* received abroad or isolated by us were virulent on sweet basil seedlings in the greenhouse except for one isolate from New York, NY63. Symptoms varied from moderate stunting to death of the plant and included wilting, defoliation of basal leaves, and dark lesions on the stems with vascular discoloration. The Italian isolate (ATCC 38560) did not cause wilt, defoliation, or vascular discoloration in the stem but did induce a slight stunting of the plants, and the fungus was reisolated from the stems. All of the noninoculated control plants remained healthy, and no *F. oxysporum* was recovered from the stems.

Heterokaryon tests. The number of chlorate-resistant sectors, percent *nit* mutants, and their classification as *nit1, nit3*, or NitM mutant phenotypes did not vary much among four isolates of *F. o. basilicum* that were examined in detail. In all, approximately two or three sectors were recovered per colony, and between 81 and 100% (mean = 91%) were *nit* mutants. Of the *nit* mutants, 3–23% (mean = 12%) were the NitM phenotype. After pairing the NitM mutants with other *nit1* mutants from the four isolates, the heterokaryotic growth between *nit1* mutants and the NitM mutant called MA48-16M were consistently the most robust in all intra- and interisolate pairings (*data not shown*). This NitM mutant (MA48-16M) and a *nit1* mutant were designated as tester strains and used in all subsequent heterokaryosis tests.

The *nit1* mutants from all the test isolates formed a clear line of aerial heterokaryotic growth with MA48-16M, but there were differences in the time required for the aerial mycelium to develop and in the overall robustness of the growth. Most pairings yielded a dense heterokaryotic growth 1–2 days after hyphal contact, but a few pairings took as long a 4–5 days following hyphal contact for the heterokaryon to form.

Table 1. Origins of *Fusarium oxysporum* f. sp. *basilicum* isolates used in vegetative compatibility tests

No. of isolates	Origin	Year isolated	Source[v]	Basil tissue	Pathogenic on sweet basil[w]	Vegetatively compatible MA48-16M[x]
1	Italy	1975	a	Stem	Yes/no[y]	Yes
4	Massachusetts	1991	b	Stem	Yes	Yes
3	California	1992	d	Stem	Yes	Yes
1	California	1992	e	Stem	Yes	Yes
1[z]	New York	1992	b	Stem	No	No
3	Colorado	1993	f	Stem	Yes	Yes
4	Connecticut	1993	g	Stem	Yes	Yes
4	South Carolina	1993	c	Stem, root	Yes	Yes
4	Nevada	1993	i	Stem	Yes	Yes
5	New York	1993	h	Stem	Yes	Yes
7	United States	1993	b,g	Seed	Yes	Yes
8	Italy	1993	b	Seed	Yes	Yes

[v] a = ATCC 38560, deposited by G. M. Armstrong from D. S. Grasso, University of Catania, Italy; b = isolates from R. L. Wick; c = isolates from A. Keinath, Clemson University; d = isolates from R. Davis, University of California, Davis; e = isolates from A. Gabrick, Soil and Plant Laboratory, Orange, California; f = infected plants from J. Meneley, Ag Bio Development Inc., Westminster, Colorado; g = W. H. Elmer; h = infected plants from D. Lukens, Camp Venture, Inc., Nanuet, New York; i = infected plant from K. Kosta, Nevada State Department of Agriculture, Reno.

[w] Yes = isolates were capable of causing Fusarium wilt on sweet basil transplants in the greenhouse; no = isolates did not cause wilting, stunting, leaf defoliation, dark basal stem lesions, or vascular discoloration in the stem.

[x] Vegetative compatibility tests conducted by pairing *nit1* mutants with the NitM test strain from MA48 as described by Correll et al (3).

[y] Only slight stunting was observed and no vascular discoloration.

[z] Isolate NY63.

DISCUSSION

Over 40 isolates of *F. o. basilicum* collected from several locales were all vegetatively compatible with the isolate (ATCC 38560) of *F. o. basilicum* from Italy. This pathogenic group may represent a new and unique VCG, but extensive testing with known VCGs would be required. Basil reportedly originated in Asia/Africa, and citations in the literature would lead us to suspect the original point of dissemination was somewhere in the Soviet Union (6,13,19), and from there it possibly spread to Italy (9,14), France (15), and eventually the United States (5,11,20). Infested seed is the logical means for such rapid dissemination, and our studies provide persuasive evidence to support the conclusion that infested seed is spreading a clonal population of *F. o. basilicum* in Europe and the United States.

The inability of ATCC 38560 to cause significant disease on basil may be the result of its long-term storage, since it is common for isolates of *F. oxysporum* to lose virulence after prolonged storage (16). All of the other vegetatively compatible isolates that were recently isolated from infected tissue were virulent on basil.

Given the long-term soil persistence of pathogenic formae of *F. oxysporum*, growers with noninfested soils should exercise caution when purchasing seed and transplants from areas where the disease has been reported. Although the need for seed disinfestation procedures is urgent, methods have not been developed for basil seed. Historically, cultivar resistance has proved to be the most effective strategy for control of Fusarium wilt diseases, but no highly resistant cultivars have yet been identified (*unpublished*).

The pattern reported in this study is consistent with Puhalla's (18) model that VCGs and formae speciales are correlated. The sudden appearance of this disease and the apparent homogeneity of the pathogenic population may present a rare opportunity to study factors affecting the epidemiological spread of a Fusarium wilt disease. The use of *nit* mutants to distinguish the pathogen from nonpathogens may allow researchers to

forgo pathogenicity tests and enumerate larger numbers of seedborne isolates than before. A wild-type isolate of *F. o. basilicum* has been deposited at the Fusarium Research Center, Pennsylvania State University, University Park.

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