Host Range and Phylogeny of *Fusarium solani* f. sp. *eumartii* from Potato and Tomato in California

M. K. Romberg and R. M. Davis, Department of Plant Pathology, University of California, Davis 95616

ABSTRACT

Romberg, M. K., and Davis, R. M. 2007. Host range and phylogeny of *Fusarium solani* f. sp. *eumartii* from potato and tomato in California. Plant Dis. 91:585-592.

Fusarium solani f. sp. *eumartii*, historically considered solely a pathogen of potato (*Solanum tuberosum*), was associated with tomato plants (*Lycopersicon esculentum*) exhibiting foot rot symptoms in California. The pathogenicity of California isolates of *F. solani* f. sp. *eumartii* from potato plants with Eumartii wilt symptoms and tomato plants with foot rot symptoms was determined on potato, tomato, pepper (*Capsicum annuum*), and eggplant (*S. melongena*). Isolates from both potato and tomato caused dry rot symptoms on potato tubers and root or collar rot on all four host species in the greenhouse. In field trials, isolates from both tomato and potato were pathogenic on tomato, potato, and pepper, confirming that the host range of *F. solani* f. sp. *eumartii* is not limited to potato. The phylogeny of isolates from potato and tomato was determined based on sequences of two DNA fragments: rDNA internal transcribed spacer regions and partial sequences of elongation factor 1- α . All of the California isolates of *F. solani* f. sp. *eumartii* from tomato and potato formed a single monophyletic clade distinct from other formae speciales and mating populations of *F. solani*. The results of this study demonstrate that Eumartii wilt and tomato foot rot in California both are caused by *F. solani* f. sp. *eumartii*.

Eumartii wilt of potato (*Solanum tuberosum*), caused by *Fusarium solani* f. sp. *eumartii* (C. Carpenter) W.C. Snyder & H.N. Hans., was first described by Carpenter in 1915 in Pennsylvania (5). Since then, it has been reported from various locations in the United States as well as Canada, Argentina, and Brazil (3,14,17,22). Symptoms of the disease include dry rot of the stem end of potato tubers and general wilt of foliage. The appearance of *F. solani* f. sp. *eumartii* in seed-growing regions in Brazil in 1996 was considered to be a potentially devastating threat to potato production in that country (22). In September 2004, potato plants exhibiting symptoms of Eumartii wilt were found in fields in San Joaquin County, CA. In addition to Eumartii wilt, isolates of *F. solani* can cause other diseases, including foot rot of tomato (*Lycopersicon esculentum*). Foot rot of tomato first was reported in 1975 in Australia (39) and was observed in California in 1991 (10). The disease is also present in Brazil (41). A survey of infested fields in 2000–01 in California indicated that the disease is now present in at least five counties (Yolo, Sutter, San Joaquin, Sacramento, and Stanislaus) in the Central Valley (31).

Based on preliminary morphological and DNA sequence data, isolates of *F. solani* obtained from potato plants exhibiting Eumartii wilt and tomato plants exhibiting foot rot appeared to be nearly identical. In order to test the relationship between isolates causing these two diseases, we determined their cross-pathogenicity on several solanaceous crops. In addition, we determined the phylogeny of isolates identified as *F. solani* f. sp. *eumartii* from potato and tomato within the *F. solani*–*Nectria haematococca* species complex based on sequences of two DNA fragments: rDNA internal transcribed spacer (ITS) regions and partial sequences of the elongation factor (EF) 1- α gene. Isolates from the two hosts and various geographical areas in which this fungus occurs in northern California also were categorized into vegetative compatibility groups (VCGs).

MATERIALS AND METHODS

California and NRRL potato isolates. Isolates from potato tissue were obtained between September and November 2004 from symptomatic tubers and plants from commercial fields in San Joaquin County, CA. Isolates Fs301, Fs302, and Fs303 were obtained from lesions on underground stems of potato plants (cv. Provento) in a field exhibiting late-season wilt symptoms. Isolates Fs304, Fs305, and Fs306 were obtained from tubers exhibiting stem-end rot and vascular discoloration (cvs. Cal White, Chieftan, and ZM13-4, respectively). The latter three isolates were from three separate fields in San Joaquin

County. To obtain isolates from underground stem lesions, tissue from the margin of the lesions was surface sterilized in 0.6% NaOCl, blotted dry, and placed on acidified potato dextrose agar (APDA). To obtain isolates from rotting tubers, the periderm surrounding symptomatic tissue was aseptically removed. A 0.5-cm² section of discolored vascular tissue and adjacent parenchyma tissue then was plated directly on APDA. Pure isolates of the fungus were subcultured once onto PDA. Pure isolates initially were identified as *F. solani* f. sp. *eumartii* based on colony and macroconidia morphology on PDA and carnation leaf agar (26). Single-spore isolates then were obtained from the axenic subculture. Mycelia obtained from single-spore isolates were stored on sterilized air-dried filter paper (Whatman 1) at 4°C. The following isolates of various other potential pathogens of potato were obtained from the Agricultural Research Service Culture Collection (NRRL), Peoria, IL: *F. oxysporum* f. sp. *tuberosi* (NRRL 22555), *F. coeruleum* (NRRL 20434), *F. avenaceum* (NRRL 25476), *F. sambucinum* (NRRL 22187), and *F. eumartii* (NRRL 13997).

Tomato isolates. Isolates of *F. solani* from tomato were cultured from root lesions on tomato plants grown in the Sacramento Valley and northern San Joaquin Valley of California. The pathogenicity of all tomato isolates was confirmed previously on tomato plants (cv. Early Pak 7) in the greenhouse (31). Briefly, roots of seedlings at the two-leaf stage were dipped in spore suspensions and maintained in the greenhouse for 4 weeks. At least one tomato isolate from each county in which foot rot had been noted in a previous study (31) was selected for phylogenetic and VCG analysis (Fs102, Fs107, Fs112, Fs120, Fs122, and Fs123). The two isolates (Fs102 and Fs107) previously used for host range studies (31) were used for pathogenicity tests on pepper, eggplant, and potato plants in this study.

Spore suspensions. For the tuber, greenhouse, and field pathogenicity tests, spore suspensions were created in a similar manner. Macro- and microconidia were harvested from 2-week-old cultures grown on PDA at 25°C by adding 3 ml of sterile water to the plates and scraping the surface of the agar plate with a sterile glass slide. The resulting conidial or mycelial suspension was filtered through eight layers of cheesecloth to remove mycelial fragments.

Corresponding author: M. K. Romberg
E-mail: mkromberg@gmail.com

Accepted for publication 1 December 2006.

Conidia concentrations were calculated using a hemacytometer and diluted in water to a concentration of 10^7 macro- and microconidia/ml for the tuber and pathogenicity tests. For the field tests, conidia were diluted to a concentration of 1.6×10^6 micro- and macroconidia/ml. Suspensions for the tuber pathogenicity tests consisted of single isolates. Inoculum for the greenhouse and field host range tests consisted of combined suspensions of Fs301 and Fs306 or Fs102 and Fs107.

Pathogenicity tests. All of the potato and tomato isolates as well as the five NRRL isolates listed previously were tested for pathogenicity on potato tubers. Tubers (cv. Provento) were scrubbed with antibacterial soap (Dial, Scottsdale, AZ), immersed in 0.6% NaOCl for 10 min, rinsed in sterile distilled water, and air dried. The tubers then were cut into two to four pieces (approximately 25 g each) with a flame-sterilized knife and placed on moist paper towels in clear plastic containers. A flame-sterilized no. 2 cork borer was used to punch a well in each tuber section approximately 10 mm deep by 5 mm in diameter. Then, 100 μ l of a 10^7 macro- and microconidia/ml suspension of each isolate was pipetted into the well. An equal volume of sterile water was used as a control. Three tuber sections were inoculated with each isolate or water. At the end of a 2-week incubation period at room temperature, tuber lesions were rated visually on a 0-to-3 scale, where 0 = no rot; 1 = fungal growth extending beyond borer hole, no vascular discoloration, no tissue breakdown; 2 = extensive mycelial growth, vascular discoloration, or tissue breakdown; and 3 = extensive mycelial growth, vascular discoloration, and tissue breakdown.

The pathogenicity of all isolates from potato also was determined on healthy 8-cm-tall potato seedlings (cv. Red La Soda) separated from their seed tubers. After roots were trimmed, the entire root system was immersed in a 10^5 micro- and macroconidia/ml suspension of each isolate. Each seedling was transplanted into a separate 15-cm-diameter pot containing University of California (UC) greenhouse potting mix (50/50 mixture of washed sand and sphagnum peat moss amended with 16.5 kg of dolomite lime, 5.5 kg of calcium carbonate lime, 5.5 kg of single superphosphate, 112 g of potassium sulfate, and 112 g of potassium nitrate/m³). Each treatment was replicated three times. Plants were maintained in the greenhouse at 25 to 28°C and 40 to 60% relative humidity for 50 days and then removed from the pots for symptom evaluation (i.e., vascular discoloration, crown rot, and stem lesions). The pathogenicity tests on tubers and potato seedlings each were repeated once.

Greenhouse and field host range experiments. *F. solani* f. sp. *eumartii* isolates Fs301 and Fs306 from potato and isolates

Fs102 and Fs107 from tomato were included in greenhouse and field experiments.

Greenhouse. In the greenhouse host-range experiments, potato seedlings (cv. Provento), tomato seedlings (cv. Early Pak 7), pepper (*Capsicum annuum*) seedlings (cv. Cal Wonder), and eggplant (*S. melongena*) seedlings (cv. Black Beauty) were inoculated at the two-leaf stage. After the seedlings were removed from the growing medium (1:1 UC potting mix and vermiculite), their trimmed roots were submerged in a conidial suspension for 3 min. Six plants of each species were inoculated per treatment (water control, Fs102 and 107 combined, or Fs301 and 306 combined). Each seedling then was transplanted into a separate 15-cm-diameter pot and placed in a greenhouse in a completely randomized design. Greenhouse conditions were the same as for pathogenicity tests. The experiment was repeated once.

To evaluate the susceptibility of six different potato cultivars to *F. solani* f. sp. *eumartii*, six seedlings each of cvs. Cal White, Chieftan, Paloma Blanca, Provento, Red La Soda, and ZM8-2 were inoculated as above with isolates Fs301 and 306 combined. Seedlings were produced from potato seed pieces (25 to 50 g each) planted in a mixture of 1:1:2 vermiculite:perlite:UC soil mix in 30-cm-diameter pots. Twelve seed pieces were placed on the surface of the soil mix about 10 cm from the rim of the pot and then covered with more of the same mix. Pots were watered daily. Once the potato seedlings were approximately 8 to 10 cm tall, their roots were trimmed, rinsed in distilled water, and submerged in water or a 10^7 macro- and microconidia/ml spore suspension for 3 min. Each seedling then was transplanted into a 15-cm-diameter pot and placed in a greenhouse in a completely randomized design. All plants were maintained in the greenhouse for 8 weeks. The experiment was repeated once.

Plant tops and root systems were separated 2.5 cm above soil line. Root systems were trimmed to remove the finer roots. Visible lesions on the washed taproots were rated on a 0-to-5 scale, where 0 = no lesion, 1 = lesion <3 mm in diameter, 2 = lesion 3 to 5 mm, 3 = lesion 5 to 10 mm, 4 = lesion >1 cm, and 5 = 100% necrotic or girdled. Each taproot then was cut longitudinally and observed for presence or absence of vascular discoloration. Dry weights of the harvested plants in the host range experiments were determined after 4 to 5 days in a 50°C oven.

To compare the effect of *F. solani* f. sp. *eumartii* on different potato cultivars, the dry weight of the foliar portion of all the potato plants was obtained as above. Before drying, foliar symptoms were rated on a scale of 0 to 4, where 0 = no foliar symptoms, 1 = some spotted bronzing on <25% of the leaves, 2 = bronze spots on 25 to

50% of the leaves, 3 = bronze spots on 25 to 50% of the leaves and interveinal or marginal necrosis, and 4 = spotting and necrosis on >50% of the leaves. The main stem of each plant was removed from its pot, trimmed to remove the roots, and rinsed clean. Lesions on the main stem were rated on a 0-to-5 scale as described above. Presence or absence of vascular discoloration was observed by cutting each stem longitudinally.

The average dry weights of the inoculated plants were compared with that of the control plants for each species and potato cultivar using Dunnett's method in JMP5.1 (SAS Institute, Cary, NC). Average disease ratings were compared between each treatment and the control using χ^2 analysis.

Field. The pathogenicity of *F. solani* f. sp. *eumartii* on field-grown tomato, pepper, and potato was determined at the UC Davis Plant Pathology Armstrong field station. Seedlings were inoculated and planted in a randomized complete block design (six blocks with 10 plants per treatment) on 11 May 2005. Pepper (cv. Cal Wonder) and tomato (cv. Early Pak) seed were sown in UC potting mix in styrofoam seedling trays and maintained in a greenhouse for approximately 8 weeks prior to transplanting. Potato (cv. Provento) seed pieces (25 to 50 g each) were cut 1 week before planting and cured at room temperature in paper bags. A spore suspension of 1.6×10^6 micro- and macroconidia/ml was made as described above. A separate spore suspension of each isolate pair (Fs102 and Fs107 from tomato or Fs301 and Fs306 from potato) was generated for each block. The roots of 10 tomato and 10 pepper seedlings were submerged in water or each spore suspension for 3 min and then transplanted. To inoculate potato seed, seed pieces were placed in a polyvinyl bag with 400 ml of the spore suspension for 3 min.

The inoculated potato plants began to show symptoms at 30 days and were rated before they became entirely necrotic. Non-inoculated potato plants and the tomato and pepper plants were rated at 90 days after planting. Aboveground symptom expression was rated on a 0-to-4 scale as for the greenhouse. Plants then were removed from the ground and the roots and underground stems were rated separately for lesions on a 0-to-5 scale as described previously. Ratings for foliar symptoms were grouped into three categories for improved statistical analysis: none = rating 0, moderate = ratings 1 and 2, and severe = ratings 3 and 4. Belowground symptoms were similarly grouped into categories (i.e., none = rating 0, moderate = ratings 1 and 2, and severe = ratings 3 and 4). The association between inoculation and symptom category was analyzed with χ^2 analysis in JMP5.1.

DNA extraction and amplification. DNA was extracted from 4- to 5-day-old

mycelia grown in liquid media (20.7 g of D-glucose, 1.2 g of DL-asparagine, 1.2 g of $K_2HPO_4 \cdot 3H_2O$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of yeast extract, and 0.1 g of NaCl per liter). Genomic DNA of all isolates was extracted using the Dneasy Plant Tissue Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions and eluted in a total of 200 µl of EB buffer. All DNA samples were stored at -20°C.

Two fragments were amplified: the ribosomal DNA ITS region (40) and the translation EF 1-α gene (20). Amplification was carried out in 50-µl reactions containing approximately 60 ng of template DNA, 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, 200 µM each dATP, dCTP, dGTP, and dTTP, 2 mM $MgCl_2$, 100 µM each primer, and 0.1 unit of Taq polymerase.

Thermocycler (PTC-100; MJ Research, Watertown, MA) conditions were 94°C for 1 min, 55°C for 2 min, and 72°C for 2.5 min for 40 cycles, plus a final extension of 10 min at 72°C for ITS primers ITS4 and ITS5 (40); and 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min for 40 cycles, plus a final extension of 5 min at 72°C for EF primers EF1 and EF2 (20). PCR products were purified using QIAquick PCR Purification Kits according to the manufacturer's instructions (Qiagen Inc.). The purified amplicons of the ITS and EF were sequenced with forward and reverse primers using automated sequencing at the UC Davis DBS Automated DNA Sequencing Facility. Primers used for sequencing were the same as those used for amplification. Sequences were viewed and edited using

Chromas 2.3 (Technelysium PTY Ltd., Tewantin, Australia) before phylogenetic analysis.

Sequence analysis. Sequences were obtained from both ITS and EF regions for all California potato and tomato isolates used in this study as well as the NRRL isolates of *F. coeruleum* and *F. eumartii*. Sequences of ITS and EF regions of other isolates and species were obtained directly from GenBank (Table 1). Sequenced regions were aligned with ClustalW in Vector NTI (InFormax, Frederick, MD) and manually adjusted with MacClade 4.03 (Sinauer Associates Inc., Sunderland MA). Sequence analysis was performed using PAUP 4.0b10 (Sinauer Associates Inc.). *F. staphyleae* was used as the outgroup for the EF and ITS analyses to facilitate com-

Table 1. Sequences and isolates used in phylogenetic analyses in this study

Taxon ^b	GenBank accession number ^a		Other ID ^c	Host	Source ^d
	ITS	EF			
<i>Fusarium ambrosium</i>	AF178397	AF178332	NRRL 20438 = IMI 296597	<i>Xyleborus fornicatus</i>	27
<i>F. coeruleum</i>	DQ164858	DQ164859	NRRL 20434	Unknown	**
<i>F. illudens</i>	AF178393	AF178326	GJS 82-98 = BBA 67606 = NRRL 22090	<i>Beilschmiedia tawa</i>	27
<i>F. martii-phaseoli</i>	AF178396	AF178331	ATCC 60860 = NRRL 22158	<i>Phaseolus vulgaris</i>	27
<i>F. solani</i>					
f. sp. <i>batatas</i> MP11	AF178407	AF178343	BBA 64683 = Clark S565 = NRRL 22400	<i>Ipomoea batatas</i>	27
f. sp. <i>cucurbitae</i> MPV	AF178411	AF178329	PBG-153 = NRRL 22141	<i>Cucurbita</i> sp.	27
f. sp. <i>cucurbitae</i> MPI	AF178410	AF178346	ATCC 18099 = NRRL 22153	<i>Cucurbita</i> sp.	27
f. sp. <i>cucurbitae</i> MPI	...	AF178327	NRRL 22098	<i>Cucurbita</i> sp.	27
f. sp. <i>eumartii</i>	AY043470	...	CNPH31	<i>Solanum tuberosum</i>	GB
f. sp. <i>eumartii</i>	AY043474	...	CNPH45A	<i>S. tuberosum</i>	GB
f. sp. <i>eumartii</i>	AY043477	...	CNPH80	<i>S. tuberosum</i>	GB
f. sp. <i>eumartii</i>	AY043469	...	IMI263815	<i>S. tuberosum</i>	GB
f. sp. <i>eumartii</i>	AY043471	...	CNPH34	<i>S. tuberosum</i>	GB
f. sp. <i>eumartii</i>	DQ164843	DQ164847	Fs306	<i>S. tuberosum</i>	**
f. sp. <i>eumartii</i>	DQ164844	DQ164848	Fs112	<i>Lycopersicon esculentum</i>	**
f. sp. <i>eumartii</i>	...	...	NRRL 13997	Unknown	**
f. sp. <i>eumartii</i>	AF150468	...	SUF1223	<i>S. tuberosum</i>	35
f. sp. <i>glycines</i>	AF178419	AF178357	IN-F2X-11A = NRRL 22825	<i>Glycine max</i>	27
f. sp. <i>mori</i> MP111	...	AF178359	NRRL 22157	Unknown	27
f. sp. <i>mori</i> MP111	AF178420	AF178358	ATCC 18699 = NRRL 22230	<i>Morus alba</i>	27
f. sp. <i>mori</i> MP111	AF129105	...	MAFF 840046	Unknown	GB
f. sp. <i>phaseoli</i>	AY220236	AY220186	NRRL 31156	Unknown	1
f. sp. <i>piperis</i>	AF178422	...	GJS 89-14 = NRRL 22570	<i>Piper nigrum</i>	27
f. sp. <i>pisi</i> MPV1	AF130142	...	MAFF 840047	<i>Pisum sativum</i>	GB
f. sp. <i>pisi</i> MPV1	...	AF178337	NRRL 22278	Unknown	27
f. sp. <i>piperis</i>	...	AF178360	NRRL 22570	Unknown	27
f. sp. <i>radicicola</i>	AF150466	...	SUF209	<i>S. tuberosum</i>	35
f. sp. <i>radicicola</i>	AF150467	...	SUF210	<i>S. tuberosum</i>	35
f. sp. <i>robineae</i> MPV11	...	AF178330	ATCC 18692 = NRRL 22161	<i>Robinea pseudoacacia</i>	27
f. sp. <i>robineae</i> MPV11	AF178416	AF178353	GJS 91-122 = BBA 67586 = NRRL 22586	<i>R. pseudoacacia</i>	27
f. sp. <i>xanthoxyli</i> MP1V	AF178401	AF178336	ATCC 42366 = NRRL 22277	<i>Xanthoxylum</i> sp.	27
<i>F. staphyleae</i>	AF178423	AF178361	GJS 92-125 = NRRL 22316	<i>Staphylea trifolia</i>	27
<i>Nectria borneensis</i>	AF178415	AF178352	GJS 85-19 7= BBA 65095 = NRRL 22579	Bark	27
<i>N. ipomoeae</i>	AF178398	AF178335	NRRL 22101	Unknown	27
<i>N. plagianthi</i>	AF178417	AF178354	GJS 83-146 = BBA 68511 = NRRL 22632	<i>Hoheria galbrata</i>	27
<i>Neocosmospora africana</i>	AF178412	AF178348	ATCC 16238 = NRRL 22436	Soil	27
<i>N. ornamentata</i>	AF178413	AF178349	CBS 562.70 = NRRL 22468	Nut of <i>Arachis hypogaea</i>	27
<i>N. vasinfesta</i>	...	AF178350	NRRL 22166	Unknown	27

^a ITS = internal transcribed spacer and EF = elongation factor.

^b MP = mating population.

^c ATCC = American Type Culture Collection, Manassas, VA; BBA = Biologische Bundesanstalt für Land- und Forstwirtschaft Institut für Mikrobiologie, Berlin, Germany; CBS = CentralBureau voor Schimmelcultures, Baarn, The Netherlands; Clark = C. Clark, Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge; CNPH = National Center for Horticulture, Brasilia, Brazil; FRC = Fusarium Research Center, Pennsylvania State University, University Park; GJS = G. J. Samuels, United States Department of Agriculture-Agricultural Research Service (ARS), BARC-West, Beltsville, MD; IMI = International Mycological Institute, Egham, England; IN = S. Abney, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN; MAFF = Ministry of Agriculture, Forestry and Fisheries, Seoul, Korea; PBG = Brian T. Hawthorne, DSIR Plant Protection, Private Bag, Auckland, New Zealand; SUF = culture collection of Fusarium in Sinshu University, Japan; and QM = Quartermaster Culture Collection, now housed at the ARS culture Collection (NRRL), NCAUR, Peoria, IL.

^d Symbol: ... = unknown.

^e Source number refers to citation number in literature cited. GB = GenBank, unpublished and ** = this study.

parison with previous studies. Most parsimonious trees for both regions were found with a heuristic search with tree-bisection-reconnection (TBR) branch swapping, starting trees gained by stepwise addition, and 1,000 addition-sequence replicates. Ambiguous regions from the sequences were removed for the final alignment and trees obtained were deposited in TreeBASE (accession no. SN2811).

Nitrate nonutilizing mutants. Nitrate nonutilizing (*nit*) mutants were generated by inoculating PDA plates from single-spore cultures stored on filter paper discs (29). Four agar sections (9 mm²) were removed at the margin of fungal growth on the PDA plates and placed equidistant on PDA + 4% KClO₃. When mutant production from PDA + 4% KClO₃ was not adequate, PDA + 5% KClO₃ was used. The margin of each fast-growing sector arising on PDA + KClO₃ was transferred to Czapek agar (Sigma-Aldrich, St. Louis) by stab inoculation using twice-autoclaved toothpicks. Cultures were evaluated for typical flat growth which indicated the formation of a *nit* mutant. The *nit* mutants

then were transferred to Czapek agar + hypoxanthine (0.163 g/liter) to determine *nit1* or *nitM* status (8). Reciprocal pairings between *nitM* and *nit1* mutants from both sets of isolates were made by stabbing each mutant pair onto a single plate of Czapek + 20% soluble starch (Sigma-Aldrich) at a distance of approximately 1 cm between each isolate (9). Growth at the intersection of each pair was rated as non-existent = 0, spotty = 1, or strong = 2. Pairings were repeated once.

RESULTS

Pathogenicity tests on potato. All potato and tomato isolates of *F. solani* f. sp. *eumartii* from California caused sunken dry lesions and vascular discoloration on tubers of cv. Provento (average rating = 3 for all isolates). NRRL isolates *F. sambucinum* (22187) and *F. avenaceum* (25476) caused sunken lesions on the tubers (average rating = 3 for both isolates), but *F. oxysporum* f. sp. *tuberosi* (22555), *F. coeruleum* (20434), and *F. eumartii* (13997) did not cause lesions on any of the inoculated tubers and showed minimal

growth on tuber tissue (average rating = 0 for all three isolates). All potato isolates of *F. solani* f. sp. *eumartii* were pathogenic on Red La Soda plants in the greenhouse. Symptoms included crown rot, vascular discoloration, and wilt. *F. solani* f. sp. *eumartii* was reisolated from the vascular tissue of all inoculated plants.

Greenhouse and field host-range experiments. In greenhouse trials, isolates from both tomato and potato were pathogenic on tomato, potato, eggplant, and pepper. Symptoms on all species included crown rot, vascular discoloration, and lesions on the tap root or, for potato, lesions on the underground stem. No foliar symptoms were observed on pepper or eggplant; however, infected tomato and potato plants exhibited leaf necrosis and small bronze irregular spots on the leaves. A significant reduction of the weights of tomato, eggplant, or pepper plants was not observed when these plants were inoculated with isolates from either potato or tomato, and the isolates from potato also did not significantly affect the dry weight of inoculated potato plants (*data not presented*). These results were the same for both trials. However, the isolates from tomato significantly ($P = 0.05$) reduced the dry weights of potato plants by 67% in one trial and 65% in a second trial (inoculated mean/noninoculated mean was 3.5/10.6 g for trial 1 and 11.3/32.1 g for trial 2).

Plants of all six potato cultivars were susceptible to *F. solani* f. sp. *eumartii*. Large necrotic lesions developed on the underground stems of all cultivars, but foliar responses were variable. Bronze spotting typical for Eumartii wilt, in addition to marginal leaf necrosis and wilting, developed on the leaves of Provento, Chieftan, and ZM8-2. No foliar symptoms developed on Cal White plants. The response of cv. Paloma Blanca to *F. solani* f. sp. *eumartii* infection was variable. Some plants showed no foliar symptoms; on others, symptoms were limited to bronze spotting. Leaves of cv. Red La Soda were wilted but showed no marginal necrosis and only minor leaf spotting. Plant dry weights of cvs. Red La Soda, ZM8-2, and Chieftan were reduced by at least 25%, but dry weights of Cal White, Paloma Blanca, and Provento were not significantly reduced (*data not shown*). Results were the same in both trials.

Crown or root lesions caused by *F. solani* f. sp. *eumartii* developed on potato, tomato, and pepper plants in the field (Table 2). Symptoms on inoculated potato plants appeared as early as 30 days after transplanting and the potato plants were rated at 30 and 90 days after transplanting to include the plants showing early symptoms. By 90 days, the early-symptomatic potato plants were entirely dead. At 30 days, the non-inoculated potato plants showed no symptoms, whereas inoculated plants showed moderate to severe symptoms. By 90 days,

Table 2. Effect of *Fusarium solani* f. sp. *eumartii* isolates on tomato, potato, and pepper in the field^a

Host, source ^d	Disease	Foliar symptoms ^b			Belowground symptoms ^c		
		No. of plants	χ^2	<i>P</i>	No. of plants	χ^2	<i>P</i>
Pepper							
None	None	57	...	...	57	...	...
	Moderate	0	...	...	0	...	...
	Severe	0	...	...	0	...	...
Tomato	None	54	...	...	35	...	...
	Moderate	0	...	...	19*	24.20	<0.0001
	Severe	0	...	...	0	...	...
Potato	None	51	...	...	39	...	...
	Moderate	0	...	...	12*	15.09	<0.0001
	Severe	0	...	...	0	...	...
Tomato							
None	None	51	...	...	51	...	...
	Moderate	0	...	...	0	...	...
	Severe	0	...	...	0	...	...
Tomato	None	8	...	...	0	...	...
	Moderate	35*	62.93	<0.0001	18*	69.0	<0.0001
	Severe	8	...	...	33*	84.0	<0.0001
Potato	None	8	...	...	5	...	...
	Moderate	34*	61.84	<0.0001	20*	55.37	<0.0001
	Severe	10	...	...	27*	63.78	<0.0001
Potato							
None	None	7	...	...	6	...	...
	Moderate	18	...	...	17	...	...
	Severe	11	...	...	13	...	...
Tomato	None	0	...	...	0	...	...
	Moderate	4	...	...	2	...	...
	Severe	42*	18.49	<0.0001	44*	11.08	0.0046
Potato	None	0	...	...	0	...	...
	Moderate	4	...	...	0	...	...
	Severe	36*	16.09	0.0002	40*	10.12	0.0060

^a Field in a randomized complete block design, with six plots per treatment and 10 plants per plot. Counts for all plants remaining at 90 days shown; * = significantly different ($P < 0.01$) from the control (no inoculum) when analyzed by χ^2 analysis.

^b Foliar symptoms: none = no disease symptoms, moderate = bronze spotting on 25 to 50% of leaves and interveinal or marginal leaf necrosis, severe = spotting and necrosis on >50% of leaves.

^c Belowground symptoms: none = no disease symptoms, moderate = lesions 3 to 10 mm in diameter, severe = lesions >1 cm to belowground portion 100% necrotic or girdled.

^d Source = *Fusarium solani* f. sp. *eumartii*, tomato isolates Fs102 and 107, and potato isolates Fs301 and 306. Seedlings (tomato and pepper) and seed tubers were inoculated by immersing roots in a 10⁶ macro- and microconidia/ml suspension for 3 min immediately before transplanting. Potato plants were rated at 30 and 90 days after transplanting, pepper and tomato at 90 days after transplanting.

the symptoms on all potato plants were difficult to distinguish from typical senescence, accounting for the ratings of moderate to severe for noninoculated plants. Root symptoms did not develop on all pepper plants, and those that did develop symptoms were rated as moderate (lesions 3 to 10 mm in diameter). Root lesions, if present, on the tomato plants were either moderate or severe (lesions >1 cm or stem girdled), whereas root lesions on nearly all potato plants were >1 cm in diameter. Foliar symptoms developed on potato and tomato, but there were no foliar symptoms on pepper. The majority of inoculated tomato plants had a foliar disease rating of moderate (bronze spotting on 25 to 50% of leaves and interveinal or marginal leaf necrosis), and the majority of potato plants received a disease rating of severe (severe = spotting and necrosis on >50% of leaves).

Phylogenetic analysis. For the sake of clarity, only representative isolates are shown in Figures 1 and 2 and listed in Table 1. When all six potato and six tomato isolates were included, the trees were identical, with all isolates from potato and tomato from California in exactly the same location as the representative isolates.

Parsimony analysis of the EF sequences yielded 14 most parsimonious trees, for which a consensus tree is shown in Figure 1 (tree length 443, CI = 0.7359, RI = 0.7462). The EF sequences of the potato and tomato isolates formed part of a clade which included *F. solani* f. sp. *batatas*, *F. solani* f. sp. *xanthoxyli*, and *N. ipomoeae*, but separation of *F. solani* f. sp. *eumartii* from these three species was supported in 100% of the trees. The sequence of the NRRL isolate of *F. solani* f. sp. *eumartii* was in a clade consisting of *F. solani* f. spp. *mori*, *pisi*, *robiniae*, and *cucurbitae* mating population V (MPV).

Parsimony analysis of the ITS sequences yielded 267 equally parsimonious trees. A consensus tree for the ITS is shown in Figure 2 (tree length 242, CI = 0.6777, RI = 0.7562). Sequences of *F. solani* f. sp. *eumartii* were found in several of the clades. A previous study of the phylogeny of *F. solani* based on the rDNA ITS region (35) included only one isolate of *F. solani* f. sp. *eumartii*. In this study, the sequence of that isolate was found in the same clade as *F. coeruleum*. An isolate previously identified as *F. eumartii* (NRRL 13997) did not group with the potato and tomato isolates of *F. solani* f. sp. *eumartii* used in this study but, instead, showed highest identity to an isolate of *F. solani* f. sp. *cucurbitae* MPV. Two of the sequences from Brazil (GenBank accession nos. AY043471 and AY043474), which represent isolates that caused little or no disease on potato (25), did not group with the potato and tomato isolates of *F. solani* f. sp. *eumartii* in this study.

VCGs. The *nit1* mutants were generated from all six of the potato isolates. Despite

repeated attempts, *nitM* mutants were generated only from potato isolate Fs302. The *nit1* and *nitM* mutants were obtained from tomato isolates Fs120 and Fs123, *nit1* mutants only from tomato isolate Fs122, and *nitM* mutants only from tomato isolate Fs107. All *nit1* mutants were paired with all *nitM* mutants, although it was not possible to pair a *nit1* and *nitM* mutant from each isolate.

Compatible mutants generated dense aerial mycelium along the line of contact between two colonies. The growth of complemented mycelia was strong for all pairings except those involving isolates Fs122

and 306, which only showed spotty growth. The majority of isolates from potato and tomato composed two distinct VCGs. All of the tomato isolates belonged to one VCG, but there was intraspecific variation in the potato isolates. One potato isolate (Fs304 from cv. Cal White) showed complementary pairing only with tomato isolates and one potato isolate (Fs305 from cv. Chieftan) and was not compatible with any other isolates.

DISCUSSION

F. solani (sensu lato, teleomorph *N. haematococca* Berk. & Broome) is found

Fig. 1. One of 267 most parsimonious trees of internal transcribed spacer (ITS) sequences of *Fusarium* and *Nectria* spp.; numbers indicate bootstrap values greater than 50. Taxa followed by GenBank accession number or ID as given in Table 1.

throughout the world as a pathogen on a variety of hosts (11,12). For the plant-pathogenic members, at least 19 formae speciales have been proposed based on hosts (2). Seven mating populations (biological species) were identified, which largely correspond to formae speciales and phylogenetic species (23,27). A recent three-gene phylogenetic study revealed that *F. solani* comprises 45 phylogenetic species, and isolates from various habitats often have identical genetic makeup (43). These results indicate cross-pathogenicity; however, no pathogenicity tests were included (43). In the present study, we demonstrated that *F. solani* f. sp. *eumartii* was pathogenic on pepper, eggplant, and tomato in addition to potato, which is a wider host range than previously reported for this forma specialis (15).

Worldwide, at least two formae speciales of *F. solani* in addition to *N. haematococca* have been identified as causal agents of tuber dry rot. The causal agent of dry rot

of potato tubers in Serbia, the United Kingdom, and the United States (Illinois) was identified as *F. solani* f. sp. *coeruleum* (4,33,34). In one of these reports, *F. solani* f. sp. *coeruleum* was referred to as *N. haematococca* (anamorph *F. solani* f. sp. *coeruleum*) (33), although no production of perithecia on diseased tissue was reported. In addition to these reports, *N. haematococca* associated with dry rot of potato in Venezuela apparently readily produced perithecia in culture (37).

In addition to causing potato tuber dry rot, *F. solani* and *N. haematococca* are the causal agents of a variety of wilt and root rot diseases of eggplant, pepper, and tomato. *F. solani* caused a wilt and root rot in eggplant in the former Soviet Union (30), and also caused root rot of eggplant in India (16). In neither location was the *F. solani* on eggplant identified to forma specialis. *N. haematococca* caused stem and fruit rot of greenhouse-grown sweet pepper in Canada in 1994 (18) and in New

Zealand in 2001 (38). In Venezuela, *N. haematococca* was identified as the causal agent of a collar and root rot of sweet pepper cultivated under the shade of passion fruit plants (6). *N. haematococca* produced perithecia on infected pepper tissue in all three locations. *F. solani* also was associated with a root rot of pepper grown in Bulgaria, but no perithecia on infected plants were reported (19). Foot rot of tomato caused by *F. solani* has been reported in Australia (39), the United States (California; 10), and Brazil (41). In India, *F. solani* causes a pre- and postemergence damping-off of tomato seedlings and caused wilt of tomato in Turkey and along the Ivory Coast (7,13,28). In all of these locations, the causal agent was identified simply as *F. solani* and was not further characterized to forma specialis.

It is difficult to determine whether all of these reports refer to a genetically similar *F. solani* without access to the isolates from each region and host species. In California, *F. solani* f. sp. *eumartii* causes both foot rot in tomato and *Eumartii* wilt of potato. It may be that the causal agent of these various diseases of different hosts throughout the world is the same organism. However, the isolates from potato and tomato in this study did not readily produce perithecia in culture or on host tissue, and it is possible that the occurrence of *N. haematococca* reported on pepper and potato involved a different organism. Genetic analysis involving isolates from various geographic areas and hosts would help answer this question. If the same special form of *F. solani* caused disease on these various hosts, management strategies such as seed sanitation and attempts to restrict the movement of the pathogen to areas not yet infested would be impacted. Because pepper plants do not display foliar symptoms, yet develop root lesions, pepper may lead to an increase in inoculum levels without any obvious foliar symptoms.

The ability of one of the potato isolates to form heterokaryons with the tomato isolates is further evidence that the same fungus causes disease in both hosts. However, the isolates of *F. solani* f. sp. *eumartii* from potato are not strictly clonal and compose at least two or three separate VCGs. This suggests that either recombination or mutation occurred at some point in the history of this fungus in northern California. All of the tomato isolates were represented by a single VCG. Production of both *nit1* and *nitM* mutants from all isolates involved proved difficult. Further research involving more isolates from both hosts and mutants of both types from each isolate is needed to clarify VCG status of isolates of *F. solani* f. sp. *eumartii* from potato and tomato. All of the potato isolates came from a single county, whereas those of tomato came from several counties in northern California. In the area where tomato foot rot has been identified,

Fig. 2. One of 14 most parsimonious trees of elongation factor 1- α (EF) sequences of *Fusarium* and *Nectria* spp.; numbers indicate bootstrap values greater than 50. Taxa followed by GenBank accession number or ID as given in Table 1.

tomato and potato are grown in proximity in only one county. The variability of the potato isolates suggests that the population of *F. solani* f. sp. *eumartii* on potato may be an older population, and the appearance of *F. solani* f. sp. *eumartii* on tomato may represent a single introduction of the fungus into tomato fields.

The taxonomy of *Fusarium* spp. causing disease on potato as well as other solanaceous crops has proved difficult. Taxonomic studies using morphological features consistently place the fungus identified here as *F. solani* f. sp. *eumartii* in the section Martiella of the genus *Fusarium*, usually as a variety or forma specialis of *F. solani*. Wollenweber (32) included *F. solani* var. *eumartii* as a variety of *F. solani*. Booth (2) listed *F. solani* f. sp. *eumartii* (C. Carpenter) W. C. Snyder & H. N. Hans. (syn. *F. eumartii*, *F. solani* var. *eumartii* (C. Carpenter) Wr.) as a forma specialis of *F. solani*. Gerlach and Nirenberg, however, considered *F. eumartii* to be a separate species in the section Martiella, differentiated from *F. solani* on the basis of macroconidial morphology (11). The teleomorph of *F. solani*, *N. haematococca*, typically is listed as the probable teleomorph of *F. solani* f. sp. *eumartii*, though there is little evidence for a sexual state of this fungus. The most recent description of the teleomorph state of *F. solani* f. sp. *eumartii* is from 1926 (11).

In 1972, Matuo and Snyder studied the relationship between mating populations and formae speciales of *F. solani*. They stated that most of the formae speciales or races of *F. solani* contained a single type of macroconidia based on morphology, but isolates of *F. solani* f. sp. *eumartii* belonged to two groups, each with a different morphological type of macroconidia. Thus, morphology by itself was undependable for classification of strains. Molecular characters provide a clearer picture of the relationship between various mating populations and formae speciales in the *N. haematococca*-*F. solani* species complex (21,27,35,36).

F. solani f. sp. *eumartii* has been included in only a few of the more recent molecular and taxonomic studies of *F. solani*. No isolates of *F. solani* f. sp. *eumartii* were included in the molecular phylogeny of the *N. haematococca*-*F. solani* species complex published by O'Donnell in 2000. Pathogenic *F. solani* f. sp. *eumartii* isolates from potato in Brazil were compared with *F. oxysporum* and *F. solani* potato isolates from Brazil and Argentina using restriction fragment length polymorphisms (25). All of the *F. solani* f. sp. *eumartii* isolates that caused wilt symptoms of potato composed a single unique restriction digest group, whereas nonpathogenic isolates composed two separate groups and those causing only dry rot composed a fourth, unique group. A phylogenetic tree generated from sequences of the rDNA ITS region placed a single *F. solani* f. sp.

eumartii isolate (GenBank accession no. AF150468, SUF1223) with one type of macroconidium from Japan in a group separate from all other *F. solani* formae speciales (35). It is not known whether recent pathogenicity tests were performed on this isolate, SUF1223 from the Shinsu University culture collection included in the studies by Suga et al. (35,36), which bore little identity to other isolates of *F. solani*. In this study, it was more closely related to *F. coeruleum* (Fig. 2). Pathogenicity tests based solely on tuber dry rot would not be able to differentiate *F. solani* f. sp. *eumartii* from *F. coeruleum*. Hence, determination of the ability of this isolate to produce wilt symptoms in potato plants also would have been necessary.

Isolates of *F. solani* associated with potato tuber dry rot have been identified as various species and formae speciales, including *F. solani* (Mart.) App. & Wr. emend. W.C. Snyder & H.N. Hans. 'Coeruleum' (syn. *F. solani* f. sp. *coeruleum* and *F. coeruleum*), *F. eumartii* C. Carpenter (syn. *F. solani* f. sp. *Eumartii*), and *F. solani* (Mart.) App. & Wr. (syn. *F. solani* f. sp. *eumartii*, teleomorph *N. haematococca*) (15). Although the teleomorph of both *F. coeruleum* and *F. solani* f. sp. *eumartii* typically is identified as *N. haematococca*, production of a sexual state for either one is not well documented. Phylogenetic analysis in this study of gene fragments of the EF1- α gene and ITS rDNA region of *F. coeruleum* and *F. solani* f. sp. *eumartii* showed that *F. coeruleum* formed part of a monophyletic clade distinct from the *F. solani*-*N. haematococca* clade which included *F. solani* f. sp. *eumartii*. This evidence supports the hypothesis that *F. coeruleum* should not be included in the *F. solani*-*N. haematococca* complex. The placement of *F. solani* f. sp. *eumartii*, however, in the *F. solani*-*N. haematococca* complex is well supported.

Using sequences in the EF 1- α gene and rDNA ITS regions, nine formae speciales (*cucurbitae* race 1 and 2, *batatas*, *mori*, *xanthoxyli*, *pisi*, *robineae*, *glycines*, and *piperis*) in the *F. solani* complex were resolved as phylogenetically distinct species, in agreement with prior mating population data which resolved seven of the formae speciales into distinct, biologically separate mating populations (23,24,27). Because at least two of the formae speciales (*glycines* and *piperis*) appear to represent strictly mitosporic species, it appears that meiosis was lost more than once in the evolution of *F. solani* (27). Previous attempts to mate isolates of *F. solani* f. sp. *eumartii* produced no sexual structures (23). In the present study, none of the isolates produced sexual structures when paired (*data not shown*), and it is likely that *F. solani* f. sp. *eumartii* represents another strictly mitosporic species in addition to *F. solani* f. sp. *glycines*, *F. martii-phaseoli*, and *F. ambrosium* (27).

The use of molecular techniques for identification and characterization of various *F. solani* species found on solanaceous crops should help clarify the taxonomic standing of these various species and shed light on the possible movement of this pathogen between tomato, potato, pepper, and eggplant. Further characterization of the populations of *F. solani* f. sp. *eumartii* from California, Brazil, Argentina, and elsewhere could lead to information on the origin, evolution, and spread of this fungus in the Americas, leading in turn to improved monitoring and control of the pathogen.

ACKNOWLEDGMENTS

We thank Kerry O'Donnell for generously providing isolates of *F. oxysporum* f. sp. *tuberosi* (NRRL 22555), *F. coeruleum* (NRRL 20434), *F. avenaceum* (NRRL 25476), *F. sambucinum* (NRRL 22187), and *F. eumartii* (NRRL 13997).

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