

Identification of *Fusarium solani* f. sp. *cucurbitae* Race 1 and Race 2 with PCR and Production of Disease-Free Pumpkin Seeds

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ABSTRACT

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Fusarium solani f. sp. *cucurbitae* causes a fruit rot of cucurbits and is classified into two races that are actually distinct species: *F. solani* f. sp. *cucurbitae* race 1 (Fsc1) and race 2 (Fsc2). Because Fsc1 and Fsc2 are not easily distinguished morphologically, we developed a polymerase chain reaction (PCR) assay for rapid identification. Taxon-specific primers were designed from translation elongation factor 1- α sequences. Because clean seed is critical for disease control, we conducted experiments to determine if we could predict whether seed would be infected based on a visual rating of the fruit at harvest. In two trials in commercial pumpkin fields, eight fruit in each of four categories were selected: asymptomatic fruit, mildly infected fruit, severely infected fruit but without lesions extending into the seed cavity, and severely infected fruit with at least one lesion extending into the seed cavity. Isolates from both lesions and seed were identified as Fsc1 based on the PCR assay. No infected seed were recovered from fruit in which the surface was lesion-free or in which a lesion extended less than midway through the fruit flesh. Consequently, a rapid, visual inspection and exclusion of symptomatic fruit should be sufficient to obtain uninfected seed, even in infested fields.

Additional keywords: *Cucurbita maxima*, *C. pepo*, *Nectria haematococca* mating population I

Fusarium fruit rot (FFR) is caused by two "races" of *Fusarium solani* f. sp. *cucurbitae*: race 1 and race 2. All cucurbits tested are susceptible, but generally only pumpkin (*Cucurbita pepo* L.) and winter squash (*C. pepo* L., *C. moschata* Duchesne, and *C. maxima* Duchesne) are affected in the field (3,17); the disease is an economic problem for pumpkin growers on California's central coast, with at least 30% of the pumpkin fruit infected in some fields.

F. solani f. sp. *cucurbitae* race 1 (Fsc1), teleomorph *Nectria haematococca* mating population I, causes crown, fruit, and root rots of cucurbits (15). *Fusarium* crown and root rot can develop as a cortical rot of the crown and roots of the plant. Sporodochia form at the soil line on the infected plant, and macroconidia from sporodochia then infest the surrounding soil (14,17). In soil, Fsc1 conidia often form chlamydospores, which can survive for several years (12). Chlamydospores and conidia germinate to infect the fruit rind in contact with moist

soil, causing fruit infections characterized by dry rot and sporodochia formation. After Fsc1 penetrates the fruit rind and then the flesh, it grows into the seed cavity. Once in the seed cavity, the fungus penetrates the seed and remains between the seed coat and the cotyledons; *F. solani* f. sp. *cucurbitae* generally does not invade the cotyledons or reduce seed viability (17).

Toussoun and Snyder (17) identified a related fungus, *F. solani* f. sp. *cucurbitae* race 2 (Fsc2), during a survey for the causal agent of *Fusarium* crown and root rot. Initially, Fsc2 was classified as a different race because it only causes a fruit rot, whereas Fsc1 affects the fruit, crown, and roots (8,9,14,17). However, Fsc1 and Fsc2 are in distinct mating populations, and Fsc2 was given the teleomorphic name *N. haematococca* mating population V (11). In concordance with both taxonomic characters and mating data, subsequent DNA sequence analyses support the contention that Fsc1 and Fsc2 are related but distinct species (7,13,16). Because there are few morphological features that distinguish Fsc1 from Fsc2 (11), field diagnosis of the disease has been inferred based on whether or not crown and root rot is present.

California produces 25% of the pumpkins in the United States and is the largest supplier of the nation's fresh-market pumpkin. Approximately 90% of the pumpkins grown in California is for the Halloween market and, at some farms,

pumpkin fruit are sold directly to consumers (6). Consequently, much of the pumpkin production occurs near population centers where farmland is expensive and limited. Growers also are limited in their selection of pumpkin cultivars because the primary demand is for large Jack-O-Lantern pumpkin fruit. Howden, the leading cultivar in pumpkin production (6), is highly susceptible to fruit infection by *F. solani* f. sp. *cucurbitae*. On ornamental pumpkin, any lesion renders the fruit unmarketable.

Recommendations for control of FFR include a 3-year rotation without cucurbits if a field is infested and use of clean seed (3,12,17). Commercially produced seed usually is coated with a contact fungicide (e.g., thiram), but this does not prevent the transmission of *F. solani* f. sp. *cucurbitae*-infected seed because the pathogen remains viable inside the seed coat. Therefore, only pathogen-free seed can prevent the transmission of *F. solani* f. sp. *cucurbitae*. However, in the United States, cucumber seed are sold to growers without any guarantee that the seed are free of *F. solani* f. sp. *cucurbitae*.

For seed producers, the infection level in seed and the level of field infestation are unknown. Here, we propose that a simple visual inspection of fruit before harvest provides a rapid method for seed companies to evaluate whether or not seed harvested from those fruit might be infected. The objectives of this study were to (i) develop a polymerase chain reaction (PCR) assay that rapidly identifies and distinguishes between race 1 and 2 of *F. solani* f. sp. *cucurbitae* and other non-*F. solani* f. sp. *cucurbitae* isolates or *Fusarium* spp., (ii) determine the relationship between the spatial position of *F. solani* f. sp. *cucurbitae* in the flesh of the pumpkin fruit tissue and the likelihood of seed infestation and infection, and (iii) determine whether a rapid inspection for FFR lesions on the surface of pumpkin fruit before harvest provides a cultural control method to select seed that is free of *F. solani* f. sp. *cucurbitae*.

MATERIALS AND METHODS

Primer design. The translation elongation factor 1- α (TEF) gene commonly is used for DNA sequence-based identification in *Fusarium* spp. because there frequently is insufficient variation in the internal transcribed spacer (ITS) region (7). Consequently, this region was selected for

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the design of taxon-specific primers for Fsc1 and Fsc2. Seventeen *F. solani* sequences obtained from GenBank (accession numbers AF178327 to AF178330, AF178337, AF178343 to AF178347, AF178353, and AF178355 to AF178360) were aligned, and portions of the sequences specific to Fsc1 and Fsc2 were used to select potential primers using the primer design function in Seqweb (CGC Wisconsin Package). Potential Fsc1 and Fsc2 primers were BLAST searched using the National Center for Biotechnology Information sequence database. Primer pairs Fsc1-EF1 (5'-GCTAACAATCATCTACAGAC-3')/Fsc1-EF2 (5'-GACGGA TGAGAGAAC-3') and Fsc2-EF1 (5'-GTTGGTGACATATCTCCC-3')/Fsc2-EF3 (5'-GAGTGAGAGACATGACGG-3') did not have any significant homology with other sequences in the database.

PCR and primer specificity. The specificity of the Fsc1 and Fsc2 primers was tested against isolates of Fsc1, Fsc2, other *F. solani*, and other *Fusarium* spp. that were either obtained from culture collections or isolated as part of another study (Table 1). Isolates were single-spore purified, cultured on V8 juice agar, and then stored as conidia at -80°C in 25% glycerol. Isolates were cultured on either potato dextrose agar (PDA) or V8 juice agar. DNA was extracted from isolates by incubating a small amount of fungal material in 40 µl of Bio-Rad InstaGene Matrix at 95°C for 10 min, vortexing for 10 s, and centrifuging at 12,000 rpm for 3 min. In addition to the Fsc1-specific and Fsc2-specific primers, DNA was amplified with the universal fungal primers ITS1-f (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (5,18) to confirm that fungal DNA was present in the samples. PCR was performed in a Biometra UNO II Thermocycler (Biometra Inc., Tampa, FL). A total reaction volume of 50 µl contained 1× PCR Buffer II (Applied Biosystems, Foster City, CA), 2 mM MgCl₂, 0.2 mM dNTP mix (Applied Biosystems), 1× bovine se-

rum albumen (New England Biolabs, Ipswich, MA), 0.1 µM each of forward and reverse primers, 1 unit of AmpliTaq Gold DNA Polymerase (Applied Biosystems), and 2 µl of template DNA. Reaction conditions were 94°C for 10 min followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The final extension was at 72°C for 10 min. PCR products were loaded on a 1.5% agarose gel with 0.1% ethidium bromide in Tris-acetate EDTA buffer, separated for 30 min at 100 V, and visualized under ultraviolet light.

Field sites. Samples were collected from pumpkin fields with FFR in Pescadero, CA in October 2004 and 2005. For the 2004 study, the field had a previous history of pumpkins and FFR in 2000, and then a 3-year cereal rotation before it was planted with *C. pepo* cv. Howden in the spring. For the 2005 study, the field similarly had a history of pumpkin production and FFR, a 4-year flower rotation starting in 2001, and then was planted with *C. pepo* cv. Expert in the spring. Disease incidence on fruit was estimated in both fields by randomly selecting 100 pumpkin fruit and rating them for disease as described below. No weeds in the family Cucurbitaceae were observed while the crop was being grown or in the surrounding area.

Sample collection. Pumpkin fruit were examined for the presence of lesions typical of FFR. Eight fruit in each of the following categories were selected: asymptomatic fruit, mildly infected fruit, severely infected fruit with lesions that did not extend into the seed cavity, and severely infected fruit with at least one lesion that extended into the seed cavity (Fig. 1). After selecting a fruit, the fruit circumference and lesion diameters were measured. Adhering soil was wiped off and the pumpkin was cut in half, from the blossom end to the stem end, allowing collection of seed in the fruit section touching the ground. Seed were gathered from the lower half of the pumpkin and placed in a plastic bag. Two samples of infected fruit tissue then were taken from the edge of the lesions

using a cork borer. In the case of asymptomatic pumpkin, fruit tissue was taken from the surface that was in contact with the soil. Multiple cuts were made perpendicular to the lesions, and the maximum lesion depth and its proximity to the seed cavity were measured.

***F. solani* f. sp. *cucurbitae* isolation from tissue and seed.** Plugs of diseased flesh from pumpkin fruit were surface-disinfested by swirling briefly in 70% ethanol, followed by immersion in 1% sodium hypochlorite for 1 min. Tissue then was rinsed with sterile DI water, patted dry with sterile paper towels, and placed on Fusarium selective medium (FSM). FSM contained the following per liter: 15 g of Bacto Peptone, 1 g of KH₂PO₄, 0.5 g of MgSO₄·7H₂O, 20 g of agar, 0.2 g of pentachloronitrobenzene, and 0.3 g of streptomycin sulfate. For asymptomatic pumpkins, epidermal tissue in contact with the soil was cleaned, plugs of tissue were cut, and plugs were surface disinfested and similarly plated. Plates were incubated at approximately 22°C under lights. After 5 days, plates were evaluated for the presence of *F. solani* f. sp. *cucurbitae*-like colonies.

Pumpkin seed were rinsed with water to remove adhering debris. Seed were plated with and without surface disinfestation, allowing the comparison of the number of seed with *F. solani* f. sp. *cucurbitae* inside the seed coat (infected) versus the number of seed with *F. solani* f. sp. *cucurbitae* only on the surface (infested). The method for surface disinfesting seed was tested by artificially infesting clean seed by swirling 100 seeds for 30 min in a solution of 10² Fsc1 conidia ml⁻¹. Then, 50 seeds each were plated on FSM with and without a surface-disinfestation treatment. Seed were surface disinfested by swirling briefly in 70% ethanol, soaking in 0.5% sodium hypochlorite for 2 min, and rinsing with sterile DI water. In 2004, seed from multiple samples were processed concurrently whereas, in 2005, in order to eliminate the possibility of infesting seed in the labora-

Table 1. The specificity of translation elongation factor (TEF) 1- α primers that were designed to amplify *Fusarium solani* f. sp. *cucurbitae* race 1 (Fsc1) and race 2 (Fsc2)

Species	Origin of isolates	No. of isolates	Positive PCR ^a	Source of isolates
<i>F. solani</i> f. sp. <i>cucurbitae</i> race 1	<i>Cucurbita pepo</i>	7	Fsc1	M. Jones, B. Aegerter, H. Mehl
<i>F. solani</i> f. sp. <i>cucurbitae</i> race 2	<i>C. pepo</i> , <i>Cucumis sativus</i>	6	Fsc2	K. O'Donnell, H. Mehl
<i>F. solani</i>	<i>Cucurbita pepo</i>	2	-	H. Mehl
<i>F. solani</i> f. sp. <i>batatas</i>	<i>Ipomoea batatas</i>	2	-	K. O'Donnell
<i>F. solani</i> f. sp. <i>eumartii</i>	<i>Solanum tuberosum</i> , <i>S. lycopersicum</i>	13	-	M. Romberg
<i>F. solani</i> f. sp. <i>glycines</i>	<i>Glycine max</i>	2	-	K. O'Donnell
<i>F. solani</i> f. sp. <i>mori</i>	<i>Morus alba</i>	1	-	K. O'Donnell
<i>F. solani</i> f. sp. <i>pisii</i>	<i>Pisum sativum</i> , <i>G. max</i>	6	-	K. O'Donnell, M. Jones
<i>F. solani</i> f. sp. <i>piperis</i>	<i>Piper nigrum</i>	1	-	K. O'Donnell
<i>F. solani</i> f. sp. <i>robiniae</i>	<i>Robinia</i> sp.	2	-	K. O'Donnell
<i>F. solani</i> f. sp. <i>xanthoxyli</i>	<i>Xanthoxylum piperitum</i>	1	-	K. O'Donnell
<i>F. oxysporum</i>	<i>C. pepo</i>	3	-	S. VanTuyl
<i>F. proliferatum</i>	<i>C. pepo</i>	1	-	H. Mehl
<i>Fusarium</i> spp.	<i>C. pepo</i> , <i>Cucumis sativus</i>	5	-	H. Mehl

^a Results of DNA amplification with Fsc1-specific primers Fsc1-EF1/Fsc1-EF2 and Fsc2-specific primers Fsc2-EF1/Fsc2-EF3. Isolates with negative polymerase chain reaction (PCR) results to both primers are designated with a (-).

tory, each sample was processed completely independently. For each sample, 25 surface-disinfested seeds and 25 seeds that were not surface disinfested were plated on

FSM. Plates were incubated at approximately 22°C under lights and checked periodically for fungal growth for 7 days. Each seed was scored positive or negative

for production of *F. solani* f. sp. *cucurbitae*-like colonies.

Confirmation of *F. solani* f. sp. *cucurbitae* identification from FSM by PCR. Colonies on FSM were scored as having a macroscopic appearance of *F. solani* f. sp. *cucurbitae* or not. Each year, FSM-based diagnosis was confirmed by PCR using the Fsc1-specific and Fsc2-specific primers on at least one flesh lesion isolate per infected fruit ($n = 24$ per year). Similarly, for both years, if *F. solani* f. sp. *cucurbitae* was identified on FSM on any seed in a sample, at least one isolate from the non-surface-disinfested and the surface-disinfested sample ($n = 92$) was confirmed with PCR. The ITS and TEF regions of five *F. solani* f. sp. *cucurbitae* isolates from the study sites (GenBank accession no. DQ913771, DQ913772, DQ913774 to DQ913776, DQ913801, DQ913802, and DQ91384 to DQ913806) and four isolates collected from pumpkin elsewhere in California (GenBank accession no. DQ913764 to DQ913767 and DQ913794 to DQ913797) were sequenced to confirm that the primers were, indeed, species specific. In addition, isolates that were scored as not *F. solani* f. sp. *cucurbitae* on FSM also were screened with the taxon-specific primers to make certain that a negative identification on FSM was accurate.

Data analysis. In the text, medians are used rather than means because the percentage data are not normally distributed (10). The hypotheses that seed were infected or infested only when the lesion penetrated to the margin of the seed cavity were evaluated by categorical data analysis with tests of independence. *P* values were determined by Fisher's exact test in SAS (SAS Institute, Cary, NC). We used log-linear models (1) in data analysis and presentation (DAP; GNU/Linux, Free Software Foundation, Boston) to test whether the association between penetration to the seed cavity and seed infection was reproducible in the 2 years (4). As recommended (1) for analyses with zero values, 0.5 was added to all cells.

RESULTS

PCR primer specificity. We developed primers to identify and differentiate between Fsc1 and Fsc2 because both cause FFR on pumpkins and are difficult to differentiate morphologically. Primer pairs Fsc1-EF1/Fsc1-EF2 and Fsc2-EF1/Fsc2-EF3 specifically amplified DNA from seven isolates of Fsc1 and six isolates of Fsc2 that were identified by DNA sequences (Table 1; Fig. 2). All PCR products were of the predicted sizes of 505 bp for Fsc1 and 425 bp for Fsc2 (Fig. 2). The TEF and ITS regions of five isolates from pumpkins in this study and four isolates obtained previously from pumpkins that were amplified with the Fsc1-specific primers were sequenced. Sequences from the TEF region confirmed that the Fsc1-

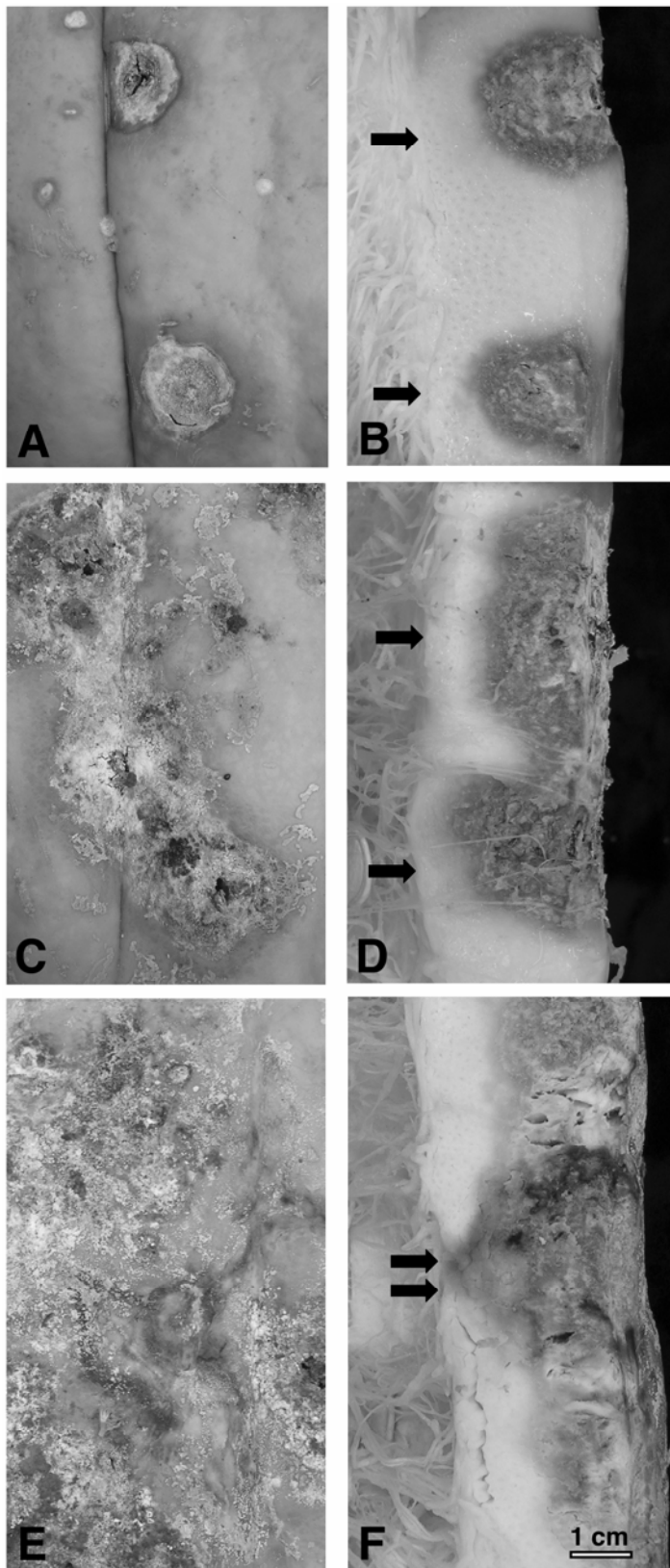


Fig. 1. Photographs of **A**, **C**, and **E**, fruit surfaces and **B**, **D**, and **F**, cross-sections of lesions caused by *Fusarium solani* f. sp. *cucurbitae* race 1 in three infection severity categories: **A** and **B**, mildly infected; **C** and **D**, severely infected but with no lesions that extended into the seed cavity; and **E** and **F**, severely infected with a lesion that extended into the seed cavity. Single arrows indicate the margin between the fruit flesh and seed cavity. **F**, Double arrows indicate a lesion that extends into the seed cavity.

specific primers amplified DNA from the expected region, and both TEF and ITS sequences confirmed that, indeed, isolates were Fsc1. In a similar manner, we demonstrated that the Fsc2-specific primers amplified the expected DNA from 12 Fsc2 isolates (GenBank accession no. DQ913750 to DQ913761 and DQ913780 to DQ913791).

Neither primer pair amplified DNA from a total of 28 isolates from eight non-cucurbit formae speciales of *F. solani*. In addition, neither primer amplified DNA from three *F. oxysporum* isolates, one *F. proliferatum* isolate, five *Fusarium* spp. that could not be identified to species or species complex based on sequencing data, and two *F. solani* isolates that were neither Fsc1 or Fsc2 that were isolated from the cucurbits listed in Table 1. Also, six isolates identified morphologically as *Fusarium* spp. other than *F. solani* were tested with the PCR assay, and the Fsc1-specific and Fsc2-specific primers did not produce an amplicon from any of these isolates.

Isolation and identification of field isolates. For this study, we examined pumpkin fruit throughout two fields in California's central coastal region. FFR incidence in the field was 64 and 39% in 2004 and 2005, respectively. The entire field was examined, and no signs or symptoms of *Fusarium* crown and foot rot were observed in either year. Eight fruit in each severity category were selected per year, and *F. solani* f. sp. *cucurbitae* was isolated from all lesions. No *Fusarium* spp. were isolated from asymptomatic controls. In both 2004 and 2005, isolates from fruit lesions that were identified as *F. solani* f. sp. *cucurbitae* on the semiselective medium ($n = 24$ per year) were identified as Fsc1 with the PCR assay. In addition, *F. solani* f. sp. *cucurbitae* was isolated from pumpkin seed and identified as Fsc1 by PCR ($n = 92$) in both years. In nine FFR fruit lesions from the 2 years, other *Fusarium* spp. were isolated, but no single species was isolated consistently, and Fsc1 always was co-isolated. On seed, a *Fusarium* sp. other than *F. solani* was isolated only from non-surface-disinfested seed from five fruit in 2004. In one severely infected fruit in 2005, a *Fusarium* sp. was co-isolated with Fsc1 from both non-surface-disinfested and surface-disinfested seed and from the fruit lesion. Fsc2 was not isolated in this study.

Association of the depth of lesion in the fruit flesh with seed infection. The surface-disinfestation method was tested on artificially infested pumpkin seed, and Fsc1 grew from all 50 infested seeds on FSM but not from the surface of 50 disinfested seeds. In 2004 and 2005, 100 and 75%, respectively, of the pumpkin fruit with a lesion extending to the margin of the seed cavity had at least one infected seed. In contrast, only one of eight asymptomatic fruit had 1 of 25 seeds that were

infected in 2004 and no asymptomatic fruit had any infected seed in 2005. In 2004, a median of 0% (95% confidence interval [CI]: 0 to 0.8%) of the seed in the eight asymptomatic fruit were infected, and none of the seed in the eight asymptomatic fruit were infected in 2005 (95%CI_{median}: 0 to 0%). In the 16 symptomatic fruit in which a lesion was present but did not penetrate to the margin of the seed cavity, a median of 0% (95%CI: 0 to 5%) of the seed were infected in 2004 and no seed were infected in 2005 (95%CI_{median}: 0 to 0%). In fruit in which a lesion appeared to penetrate to the cavity margin, a median of 68% (95%CI: 53 to 97%) and 66% of seed in eight fruit were infected in 2004 and 2005, respectively.

We used two-by-two contingency tables of one or more seed infected or not and 100% penetration or not to evaluate the

association of fruit without a lesion extending to the seed cavity with a fruit having no infected seed. Pumpkin fruit without an Fsc1 lesion penetrating the seed cavity were significantly less likely to have any infected seed (Fisher's exact test, $P = 0.0006$ and $P < 0.0001$) than pumpkin fruit with a lesion extending to the seed cavity in 2004 and 2005, respectively. Similarly, a two-by-two-by-two contingency table with both years analyzed by a log-linear model of the data indicated that 100% penetration and seed infection were significantly associated ($P = 0.0001$) and that the effect of year was not significant ($P = 0.60$; i.e., that the results were reproducible).

Because seed can be both superficially infested and internally infected, we estimated the percentage that were infested but not infected (Table 2). In 2005, similar to the infection data, pumpkin fruit without

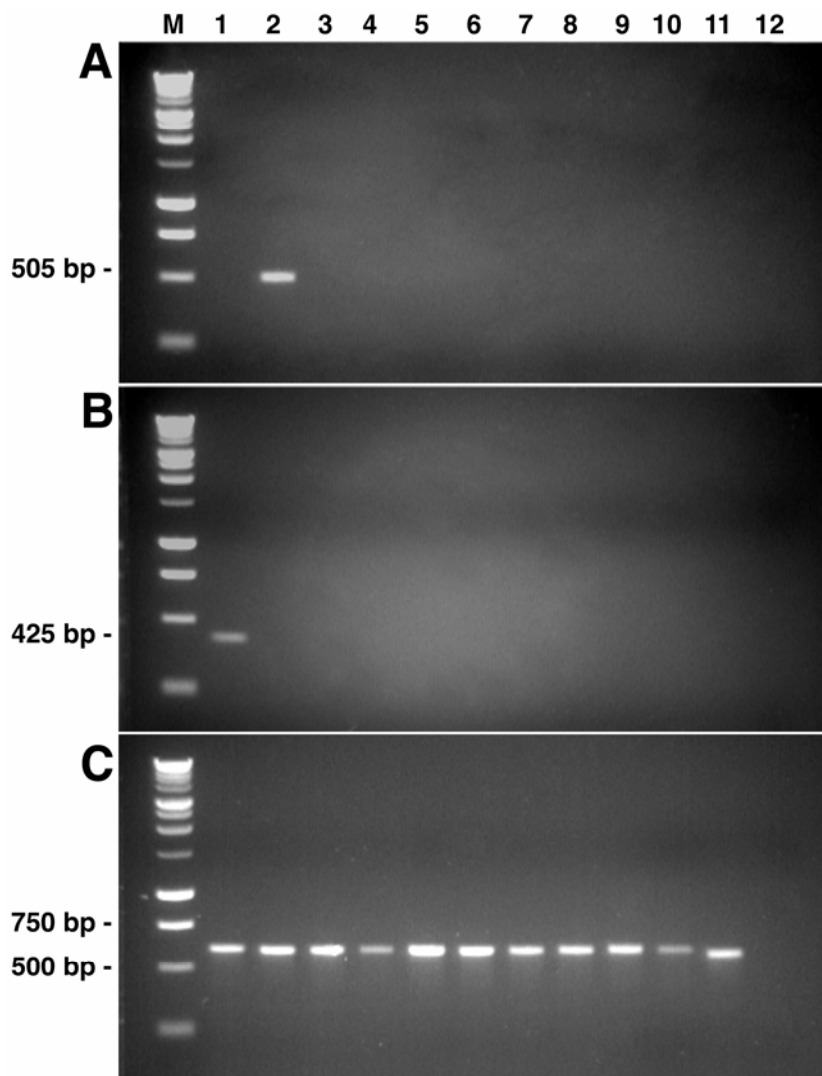


Fig. 2. Gels demonstrating the specificity of the primers **A**, Fsc1-EF1/Fsc1-EF2 and **B**, Fsc2-EF1/Fsc2-EF3 for *Fusarium solani* f. sp. *cucurbitae* race 1 (Fsc1) and *F. solani* f. sp. *cucurbitae* race 2 (Fsc2), respectively. **C**, Polymerase chain reaction with fungal-specific internal transcribed spacer (ITS) region primers was run concurrently to verify that amplifiable DNA was present and that there were no false negatives. Lane M, 1-kb ladder (Promega); lane 1, Fsc2; lane 2, Fsc1; lane 3, *F. solani* f. sp. *batatas*; lane 4, *F. solani* f. sp. *mori*; lane 5, *F. solani* f. sp. *xanthoxyli*; lane 6, *F. solani* f. sp. *pisi*; lane 7, *F. solani* f. sp. *robiniae*; lane 8, *F. solani* f. sp. *piperis*; lane 9, *F. solani* f. sp. *eumartii*; lane 10, *F. solani* f. sp. *glycines*; lane 11, *F. oxysporum* f. sp. *cucumerinum*; and lane 12, negative control.

Table 2. Estimated percentage of pumpkin seed that were either infected or infested but not infected with *Fusarium solani* f. sp. *cucurbitae* race1 (Fsc1) in each of four different fruit rot lesion severity categories in 2005^a

Category of fruit lesion	Mean ± SEM		
	Penetration (%) ^b	Infected seed (%) ^c	Infested, not infected (%) ^d
Asymptomatic	0 ± 0	0 ± 0	0 ± 0
Mildly infected	52 ± 6	0 ± 0	0 ± 0
Severely infected but with no lesions that extended into the seed cavity	73 ± 6	0 ± 0	3 ± 3
Severely infected with a lesion that extended into the seed cavity	100 ± 0	52 ± 14	22 ± 7

^a There were eight replicate fruit in each category. Statistical analyses are shown in the text. SEM = standard error of the mean.

^b Lesion penetration. Data for the lesion that penetrated the furthest across the fruit flesh is shown. For example, a fruit that had a lesion that penetrated to the margin of the seed cavity had a flesh penetration of 100%.

^c Estimated as the percentage of seed with Fsc1 after surface disinfection.

^d Infested but not infected seed. Estimated as the percentage of seed with Fsc1 before surface disinfection minus the percentage of seed with Fsc1 after surface disinfection.

a lesion that penetrated into the seed cavity were significantly less likely to have any seed that were infested but not infected (Fisher's exact test, $P = 0.0002$) than fruit with a lesion that extended to the seed cavity. In the eight fruit in which the lesion penetrated to the seed cavity margin, a median of 98% of the seed (95%CI: 6 to 100%) was infested. All (100%) of the non-surface-disinfested seed produced Fsc1 colonies in four of the pumpkin fruit. Collectively, in 2005, in the 10 fruit in which a lesion penetrated 95% or more of the pulp depth, 8% or more of seed were infested in eight of the fruit. In 2004, seed from most of the pumpkin fruit were infested whether or not the pumpkin had a lesion present (*data not shown*). Because the seed most likely were infested during sample processing, we changed our processing procedures for 2005.

In 2005, 100 of the grower's seeds that were used to establish that year's crop were plated on FSM. *F. solani* f. sp. *cucurbitae* was not isolated from any seed, indicating that the inoculum for the current year's infection may not have originated from an infested seed lot.

DISCUSSION

The 2004 and 2005 studies demonstrated that Fsc1 does not infect pumpkin seed unless there are visible lesions on the fruit. The data from 2005 demonstrate that once the pathogen penetrates the seed cavity, seed infestation and infection occur rapidly. In 16 fruit in which a lesion extended to the seed cavity, percentage of infection ranged from 0 to 100%.

Toussoun and Snyder (17) were the first to demonstrate that *F. solani* f. sp. *cucurbitae* is seed transmitted and the importance of using uninfested seed for disease control. However, this is the first study in which the relationship between the position of *F. solani* f. sp. *cucurbitae* in the pumpkin fruit tissue and the likelihood of seed infection has been addressed. Once the lesion extends to the margin of the seed cavity, seed infestation and infection occurs. Consequently, a rapid visual inspection and exclusion of fruit with lesions is sufficient to obtain uninfested seed. This inexpensive management practice should

be used by seed producers given that available control measures for growers such as fumigation and rotations as long as 3 years are both expensive and less than fully effective in preventing disease.

In 2005, the grower had a small batch of seed left over from that year's planting. Preliminary analyses of those seeds failed to support the hypothesis that Fsc1 within seed contaminated the field in 2005. A larger number of seed is required to confirm this result; however, if confirmed, this would indicate that survival in at least some California coastal soils can be longer than the previous estimate of less than 3 years (12).

This is the first time Fsc1 has been distinguished from Fsc2 and other *Fusarium* spp. with a PCR assay. Previously, researchers differentiated Fsc1 and Fsc2 by either mating tests (9,11) or pathogenicity tests (2,9,17), which are time consuming. Consequently, in California, field diagnoses of Fsc2 have been based primarily on FFR without obvious crown or foot rot; however, this is not a reliable diagnostic criterion. Toussoun and Snyder (17) concluded that Fsc1 was a more common cucurbit pathogen in California than Fsc2 and our survey data in 2004 (*data not shown*) support that conclusion.

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LITERATURE CITED

- Agresti, A. 1990. Categorical Data Analysis. John Wiley & Sons, New York.
- Boughalleb, N., Armengol, J., and El Mahjoub, M. 2005. Detection of races 1 and 2 of *Fusarium solani* f. sp. *cucurbitae* and their distribution in watermelon fields in Tunisia. *J. Phytopathol.* 153:162-168.
- Davis, R. M., Turini, T. A., Gubler, W. D., and Stapleton, J. J. 2005. UC IPM Pest Management Guidelines: Cucurbits. Univ. Calif. Agric. Nat. Resour. Publ. 3445.
- Epstein, L., Beede, R., Kaur, S., and Ferguson, L. 2004. Rootstock effects on pistachio trees

grown in *Verticillium dahliae*-infested soil. *Phytopathology* 94:388-395.

- Gardes, M., and Bruns, T. D. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol. Ecol.* 2:113-118.
- Gaskell, M., and Smith, R. 1997. Pumpkin production in California. Vegetable Production Series. Univ. Calif. Agric. Nat. Resour. Publ. 7222.
- Geiser, D. M., Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T. J., Zhang, N., Kuldau, G. A., and O'Donnell, K. 2004. FUSARIUM-ID v. 1.0: A DNA sequence database for identifying *Fusarium*. *Eur. J. Plant Pathol.* 110:473-479.
- Hawthorne, B. T., Ball, R. D., and Rees-George, J. 1994. Genetic analysis of variation of pathogenicity in *Nectria haematococca* (*Fusarium solani*) on *Cucurbita* sp. *Mycol. Res.* 98:1183-1191.
- Hawthorne, B. T., Rees-George, J., and Broadhurst, P. G. 1992. Mating behaviour and pathogenicity of New Zealand isolates of *Nectria haematococca* (*Fusarium solani*). *N.Z. J. Crop Hortic. Sci.* 20:51-57.
- Huston, A. D. 1999. Calculating nonparametric confidence intervals for quantiles using fractional order statistics. *J. Appl. Stat.* 26:343-353.
- Matuo, T., and Snyder, W. C. 1973. Use of morphology and mating populations in the identification of formae speciales in *Fusarium solani*. *Phytopathology* 63:562-565.
- Nash, S. M., and Alexander, J. V. 1965. Comparative survival of *Fusarium solani* f. *cucurbitae* and *F. solani* f. *phaseoli* in soil. *Phytopathology* 55:963-966.
- O'Donnell, K. 2000. Molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex. *Mycologia* 92:919-938.
- Samac, D. A., and Leong, S. A. 1989. Disease development in *Cucurbita maxima* (squash) infected with *Fusarium solani* f. sp. *cucurbitae*. *Can. J. Bot.* 67:3486-3489.
- Snyder, W. C., and Hansen, H. N. 1941. The species concept in *Fusarium* with reference to Section Martiella. *Am. J. Bot.* 28:738-742.
- Suga, H., Hasegawa, T., Mitsui, H., Kageyama, K., and Hyakumachi, M. 2000. Phylogenetic analysis of the phytopathogenic fungus *Fusarium solani* based on the rDNA-ITS region. *Mycol. Res.* 104:1175-1183.
- Toussoun, T. A., and Snyder, W. C. 1961. The pathogenicity, distribution, and control of two races of *Fusarium* (*Hypomyces*) *solani* f. *cucurbitae*. *Phytopathology* 51:17-22.
- White, T. J., Bruns, T., Lee, S. B., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: *PCR Protocols: a Guide to Methods and Applications*. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, San Diego, CA.