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PLANT PATHOLOGY AND NEMATOLOGY

Method for Rapid Production of *Fusarium oxysporum* f. sp. *vasinfectum* Chlamydospores

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ABSTRACT

A soil broth made from the commercial potting mix SuperSoil® induced rapid production of chlamydospores in several isolates of *Fusarium oxysporum*. Eight of 12 isolates of *F. oxysporum* f. sp. *vasinfectum* produced chlamydospores within five days when grown in SuperSoil broth. The chlamydospore-producing isolates included four known races and four genotypes of *F. oxysporum* f. sp. *vasinfectum*. The SuperSoil broth also induced rapid chlamydospore production in three other *formae speciales* of *F. oxysporum*: *lycopersici, lactucae,* and *melonis*. No change in chlamydospore production was observed when variations of the SuperSoil broth (no glucose added, no light during incubation, and 60-min autoclave times) were tested on six isolates of *F. oxysporum* f. sp. *vasinfectum*. Although chlamydospore yields in SuperSoil broth without glucose were comparable to a published soil broth, SuperSoil broth was easier to make and its yields were more consistent. One isolate of *F. oxysporum* f. sp. *vasinfectum* race 4 consistently produced greater than 10 million chlamydospores per 50 ml of inoculated SuperSoil broth. SuperSoil broth may be useful for generating chlamydospores for studies of *F. oxysporum* and similar species in approximately the same time period as production of conidia in liquid or agar media.

Many plant pathogenic fusaria, including *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *vasinfectum* (Atk.) Snyd. & Hans., produce chlamydospores upon host death (Nelson, 1981). The thick-walled, asexually-produced chlamydospores persist in soil until a host is encountered (Baker, 1953; Nash et al., 1961). Despite the fact that chlamydospores are the primary soilborne propagule of *F. oxysporum*, conidia are frequently used in pathogenicity assays (Elgersma et al., 1972; Garibaldi et al., 2004; Ulloa et al., 2006) because mass quantities of conidia are easily generated. However, conidia may be inappropriate substitutes for chlamydospores for studies involving pathogen survival in soil. Conidia may be less resistant than chlamydospores to adverse environmental conditions (Baker, 1953; Freeman and Katan, 1988; Goyal et al., 1974).

Various methods for chlamydospore production in the lab have been described, but many have limitations that preclude rapid production of large quantities of chlamydospores. Some methods are best suited for small-scale production, such as those using size-exclusion filters (Hsu and Lockwood, 1973). Other methods take weeks for the fungus to convert to chlamydospores (Castillo et al., 1998; Locke and Colhoun, 1974; Singleton et al., 1992). Preliminary experiments showed that conidia of *F. oxysporum* f. sp. *vasinfectum* took longer than two weeks to convert to chlamydospores using the sterile talc method of Locke and Colhoun (1974; R. Bennett, unpublished data). Others have produced chlamydospores in media made from celery extracts (Huang et al., 1983), agricultural by-products (Ciotola et al., 2000; Hebbar et al., 1996), and acidic synthetic components (Cochrane and Cochrane, 1971). A 1% sorghum straw broth (Ciotola et al., 2000) induced chlamydospore production in an isolate of *F. oxysporum* f. sp. *vasinfectum* within one week, but the straw fragments made it difficult to quantify the chlamydospores (R. Bennett, unpublished data). In the end, a soil broth approach was favored due to its simplicity in preparation and ecological relevance to a soilborne fungus. Frequently-cited, soil-based methods for producing chlamydospores (e.g., Dhingra and Sinclair, 1995; Leslie and Summerell, 2006; Singleton et al., 1992; Summerell et al. 2003) include the soil agar method of Klotz et al. (1988), and the soil broth methods of

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Alexander et al. (1966) and Short and Lacy (1974). The size exclusion filters required by some of these methods (Alexander et al., 1966; Short and Lacy, 1974) are expensive. The soil agar method (Klotz et al., 1988) generates chlamydospores that are difficult to quantify visually because of the presence of agar and soil particles (R. Bennett, unpublished data). The soil agar and filter-based methods seem better suited for providing morphological characteristics than for producing large, quantifiable numbers of chlamydospores.

A method for producing chlamydospores without such limitations was reported by Goyal et al. (1973). Using a broth made from organic soil, they reported up to 8×10^4 chlamydospores per ml of *F. oxysporum* f. sp. *melonis* were produced after 7 days. Initial attempts to obtain similar results with *F. oxysporum* f. sp. *vasinfectum* and local field soil were unsuccessful, but modifications of the Goyal et al. method using SuperSoil®, a commercial potting mix, were promising. The objectives of this study were to develop and evaluate SuperSoil-based broths for generating large numbers of *F. oxysporum* chlamydospores within one week. Variations of the SuperSoil broth were evaluated, and a final protocol was compared to published soil broth methods.

MATERIALS AND METHODS

1:4 (weight/volume) SuperSoil broth with glucose. The soil broth of Goyal et al. (1973) was modified by changing the ratio of soil to water from 1:3 to 1:4 (w/v) and by using SuperSoil Potting Soil (Scotts Miracle-Grow, Marysville, OH) in place of "greenhouse organic soil". Air-dried SuperSoil (125 g) in 500 ml of reverse-osmosis water (Titan 500, RO Ultratech, Fallbrook, CA; total dissolved solids < 50 ppm/TDS) was agitated at 90 rpm for 1 h on an orbital shaker (model DS-500, VWR, West Chester, PA). The mixture was poured through a 1.7-mm mesh sieve to remove large particles, and filtered again through either eight layers of cheesecloth or a fine mesh sieve (0.25- or 0.15-mm). To every 50 ml of filtered soil broth, 0.025 g of glucose was added before autoclaving at 121°C for 20 min. The soil broth was autoclaved a second time the following day. Sediment remaining in the sterilized broth was allowed to settle 2-3 h before approximately 50 ml of the liquid fraction was decanted into sterile 125-ml flasks.

The clear soil broth was inoculated with 400 µl of a conidial suspension made by adding 1.5 ml of sterile water to the surface of a 5- to 10-d-old culture grown on carnation leaf agar (Leslie & Summerell, 2006) or ¼-strength potato dextrose agar (Difco, BD, Franklin Lakes, NJ) in 60-mm Petri plates. Spores were dislodged with a sterile glass rod to make a suspension of approximately 2.5×10^5 conidia per ml. Flasks of inoculated soil broth were agitated at room temperature (24-27°C) on a benchtop-orbital shaker at 90 rpm. Natural lighting was provided by a nearby window with a northern exposure (11.5- to 14.5-h photophase). After 5 and 10-12 d, a sterile wooden applicator (Puritan Medical Products, Guilford, ME) was used to transfer samples of the mycelium onto microscope slides. Mycelium aggregates were examined under the microscope at 200x magnification for chlamydospores. Chlamydospore production was subjectively rated (high, medium, or low/none) by visually rating the quantity of mycelium converted to chlamydospores (Figure 1). Twelve isolates of *F. oxysporum* f. sp. *vasinfectum*, including races 1, 2, 4, and 8, and six additional genotypes, were used to evaluate SuperSoil broth with glucose (Table 1). Chlamydospore production in SuperSoil broth with glucose was also tested in three additional *formae speciales*: one isolate representing each of races 2 and 3 of *F. oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hans., two race 1 isolates of *F. oxysporum* f. sp. *lactucae* Matuo & Motohashi, and one isolate (race 2) of *F. oxysporum* f. sp. *melonis* Snyder & Hans. (Table 1). Experiments evaluating chlamydospore production in 1:4 (w/v) SuperSoil broth with glucose were conducted three times. Chlamydospore production was qualitatively evaluated in one flask per isolate in each of the three repetitions of the experiments.

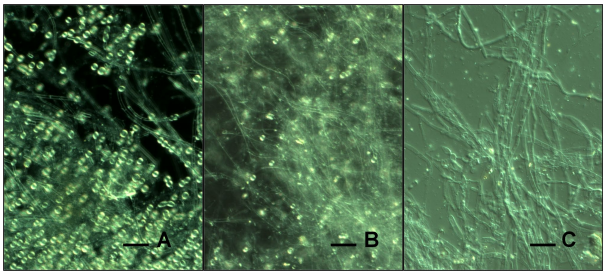

Fig. 1. Levels of chlamydospore production in SuperSoil® broth seven days after inoculation as shown by three isolates: A) High, CA-14; B) Medium, FOV21; C) Low, 140. Bar = 50 µm.

Table 1. Strains of *Fusarium oxysporum* used to test 1:4 (w/v) SuperSoil® broth with glucose.

Isolate[z]	Race-Genotype	Origin	Source[y]	Chlamydospore Production[x]
F. oxysporum* f. sp. *vasinfectum				
ATCC 16421	race 1	SC, USA	G. M. Armstrong[1,2]	high
ATCC 16611	race 2	USA	G. M. Armstrong[1,2]	high
CA14	race 4	CA, USA	R. M. Davis[1]	high
ATCC 16613	race 4	India	G. M. Armstrong[1,2]	low/none
NRRL 31665	race 8	China	K. O'Donnell[3]	high
CA-1	race 8	CA, USA	R. M. Davis[1]	low/none
FOV18	A1	Australia	R. M. Davis	low/none
FOV21	A2	Australia	R. M. Davis	medium
108	108	GA, USA	P. D. Colyer[4]	high
110	110	AR, USA	P. D. Colyer[4]	high
112	112	AR, USA	P. D. Colyer[4]	high
140	140	AR, USA	P. D. Colyer[4]	low/none
F. oxysporum* f. sp. *lycopersici				
F702	race 2		R. M. Davis	high
1002	race 3		R. M. Davis	high
F. oxysporum* f. sp. *melonis				
Fom	race 2		R. M. Davis	high
F. oxysporum* f. sp. *lactucae				
GL 1110	race 1	CA, USA	T. R. Gordon[5]	high
GL 1112	race 1	CA, USA	T. R. Gordon[5]	high

[z] Culture collection abbreviations: ATCC, American Type Culture Collection, Manassas, VA; NRRL, ARS Culture Collection, Peoria, IL.

[y] Source and references, if applicable: [1] (Kim et al. 2005); [2] (Skovgaard et al. 2001); [3] (O'Donnell et al. 2009); [4] (Holmes et al. 2009); [5] (Scott et al. 2010).

[x] Chlamydospore production in SuperSoil broth with glucose (125 g SuperSoil, 500 ml water, 0.025 glucose per 50 ml filtered broth) was qualitatively estimated based on the amount of mycelium converted to chlamydospores in three experiments.

Variations of the 1:4 (w/v) SuperSoil broth with glucose. Three variations of the 1:4 (w/v) SuperSoil broth were also tested: no glucose added, incubation without light (flasks covered with foil), and autoclaving time of 60 min instead of 20 min on two consecutive days (Table 2). SuperSoil broth components and incubation conditions were identical to the protocol described above except for the variable tested. Samples of mycelium were qualitatively rated for chlamydospore production after 5 d. A subset of six isolates of *F. oxysporum* f. sp. *vasinfectum* (ATCC 16421, ATCC 16611, CA14, NRRL 31665, FOV21, and 110) was used to test the three variations of the SuperSoil broth with glucose. For the SuperSoil variation excluding glucose, seven additional isolates (108, 112, F702, 1002, Fom, GL 1110, GL 1112) were also tested (Table 2). Experiments evaluating chlamydospore production in the three variations of SuperSoil broth were conducted three times, with one flask per isolate × broth combination.

Chlamydospore production in 1:4 (w/v) SuperSoil broth without glucose compared to other soil broths. One isolate of *F. oxysporum* f. sp. *vasinfectum* race 4, CA14, was used to compare chlamydospore yield among soil broth recipes (Table 3). Chlamydospore production was quantified in SuperSoil broth without glucose (1:4, w/v, soil:water), and the soil broths of Goyal et al. (1973; 1:3, w/v, soil:water) and Alexander et al. (1966). The 1:4 (w/v) SuperSoil broth was made as previously described but without the addition of glucose. In addition, sediment was removed from the broth by centrifuga-

tion to avoid the 2-3 hr required to settle sediment. After the broth was filtered through cheesecloth or fine-mesh sieves, the liquid was centrifuged in 50-ml aliquots in Falcon tubes for 5 min at 3000 *g* (Eppendorf 5810R; rotor, F-34-6-38). The total volume of the clear broth was recorded. Two versions of soil broth using the Goyal et al. (1973) 1:3 soil to water ratios were made: one was made with field soil (Wasco sandy loam; coarse-loamy, mixed, superactive, nonacid, thermic Typic Torriorthents) and another was made with SuperSoil Potting Soil. A total of 250 g of SuperSoil or field soil in 750 ml of reverse-osmosis water was agitated for 2 h on an orbital shaker at 90 rpm. The mixture was filtered through a 1.7-mm mesh sieve and filtered again through glass wool. Sediment was removed by centrifugation as for the SuperSoil broth. The total volume of cleared broth collected was recorded and 0.025 g of glucose was added for every 50 ml. Three 125-ml flasks (subsamples) were filled with 50 ml of each of the cleared broths and autoclaved for 20 min. When cooled, each flask was inoculated with 400 µl of a conidial suspension (ca. 2.5×10^5 conidia/ml) and incubated at room temperature on an orbital shaker (90 rpm).

The soil broth of Alexander et al. (1966) was made by mixing 500 g of field soil in 500 ml of reverse-osmosis water. The mixture was allowed to settle for 3 h, filtered through glass wool, and filter-sterilized using a 0.22-µm vacuum filter (250-ml capacity; Corning Inc., Corning, NY). Three ml of the sterile extract was placed in a 60-mm Petri plate and inoculated with 200 µl of a conidial suspension (ca. 2.5×10^5 conidia/ml). Three Petri dishes of inoculated soil extract were used in each experiment and incubated without agitation at room temperature.

Table 2. Variations of the 1:4 (w/v) SuperSoil® broth with glucose evaluated for chlamydospore production by isolates of *Fusarium oxysporum*.

Variations of SuperSoil Broth with Glucose [z]	Isolates Used [y]	Chlamydospore Production [x]
Incubated without light [w]	Subset A	high
Autoclaved 60 min [v]	Subset A	high
No glucose added	Subset A + B	high

[z] SuperSoil broth with glucose: 125 g air-dried SuperSoil, 500 ml reverse-osmosis water, and 0.025 g glucose for every 50 ml of filtered soil broth. Broth was autoclaved 20 min on two consecutive days, and inoculated broth was incubated with natural lighting for five days.

[y] Subset A isolates: ATCC 16421, ATCC 16611, CA14, NRRL 31665, FOV 21, 110; Subset B isolates: 108, 112, F702, 1002, Fom, GL 1110, GL 1112.

[x] Chlamydospore production in SuperSoil broth was qualitatively estimated based on the amount of mycelium converted to chlamydospores in three experiments.

[w] Flasks covered with foil.

[v] Broth autoclaved 60 min instead of 20 min on two consecutive days.

Table 3. Broth yield and chlamydospore production of CA14, a race 4 isolate of *Fusarium oxysporum* f. sp. *vasinfectum*, among soil broth recipes.

Composition of Soil Broth [z]	Broth Yield (ml) [y]	No. Chlamydospores per ml Broth [x]	Total No. Chlamydospores per L recipe [w]	No. Chlamydospores per g Substrate [v]
Field soil:water (1:3 w/v) + glucose	783**	2.3×10^{5} ns	1.8×10^{8} *	5.4×10^{5} ns
SuperSoil:water (1:3 w/v) + glucose	211**	5.7×10^{5} **	1.2×10^{8} ns	3.6×10^{5} *
SuperSoil:water (1:4 w/v), no glucose	429	3.0×10^{5}	1.3×10^{8}	5.1×10^{5}

[z] Broths using 1:3 (w/v) soil to water ratios (250 g substrate per 750 ml H_2O; Goyal et al., 1973) were compared to a 1:4 (w/v) SuperSoil broth without glucose (250 g substrate per L H_2O). Broths with glucose had 0.025 g glucose for every 50 ml of filtered soil broth. Experiments were conducted four times, and means were analyzed using Dunnett's adjustment for multiple comparisons, significant at $P \leq 0.05$ (**), $P \leq 0.10$ (*), or not significant (ns).

[y] Mean broth yield for recipes scaled to 1 L of water (SE = ±19 ml).

[x] Mean concentration of chlamydospores in flasks/subsamples (SE = $\pm 6.3 \times 10^4$).

[w] Total number of chlamydospores obtained for recipe scaled to 1 L of water (SE = $\pm 2.1 \times 10^7$).

[v] Total number of chlamydospores obtained per gram of soil/substrate (SE = $\pm 6.8 \times 10^4$).

Chlamydospores were quantified seven days after inoculation. The concentration of chlamydospores was quantified for individual flasks of the 1:4 (w/v) SuperSoil and 1:3 (w/v) field soil and SuperSoil broths by transferring the contents of one flask to a 360-ml-capacity sterile stainless steel blender container (Eberbach Corp., Ann Arbor, MI). Contents of the three Petri dishes containing the inoculated broth of Alexander et al. (1966) were combined into a sterile stainless steel mini container (Waring Laboratory, Torrington, CT). Mycelium and chlamydospore aggregates were broken into small clusters (1-3 chlamydospores each) with five, 30-sec blending sessions using a heavy-duty blender (Waring). Blender containers were chilled in ice water after every two blending sessions. The concentration of chlamydospores was determined using an improved Neubauer hemacytometer (Hausser Scientific, Horsham, PA). Experiments comparing chlamydospore production in 1:4 (w/v) SuperSoil broth with the 1:3 (w/v) field soil and SuperSoil broths of Goyal et al. (1973) were conducted four times. The soil broth of Alexander et al. (1966) was evaluated three times. Because the three Petri dishes were combined for blending, data for the Alexander et al. broth lacked subsamples and were not included in the analyses with the other soil broths.

Data were analyzed by one-way ANOVA using PROC GLIMMIX of SAS (SAS, ver. 9.2, SAS Institute, Cary, NC). Analyses were conducted on the following response variables: broth yield per liter of recipe, concentration of chlamydospores in broth, total number of chlamydospores obtained per liter of recipe, and the number of chlamydospores obtained per gram of substrate used in recipe. In all analyses, soil broth method was included as a fixed effect and repetition of experiment was considered a random effect. Denominator degrees of freedom were corrected using the Kenward-Rogers option. When analyses indicated a significant effect of soil broth method, the 1:3 (w/v) field soil and SuperSoil broths with glucose were compared to the 1:4 (w/v) SuperSoil broth without glucose using the ADJUST=DUNNETT option of the LSMEANS statement, which controlled type I experiment-wise error rate at $\alpha = 0.05$ within each set of comparisons. The three analyses of chlamydospore yield were based on counts from three subsamples (flasks) of the soil broths. Consequently, the random effect of experiment×method was used as the error term for testing the fixed effect of soil broth method in these analyses. Because significant heterogeneity of variance and non-normality were not observed, untransformed data were used in all analyses.

RESULTS AND DISCUSSION

Chlamydospore production in 1:4 (w/v) SuperSoil broth with glucose and variations. Eight of the twelve isolates of *F. oxysporum* f. sp. *vasinfectum* formed chlamydospores in 1:4 (w/v) SuperSoil broth with glucose within five days of inoculation (Table 1). Chlamydospore-producing isolates included races 1, 2, 4, 8, and genotypes A2, 108, 110, and 112 (Table 1). All chlamydospore-producing isolates produced copious quantities of chlamydospores (Figure 1a) except isolate FOV21, which produced about half as many as the other chlamydospore-producing isolates (Figure 1b). Four isolates, including races 4, 8, Australian isolate FOV18, and genotype 140, produced few or no chlamydospores in 1:4 (w/v) SuperSoil broth with glucose at 10-12 d after inoculation (Table 1; Figure 1c). All isolates of *F. oxysporum* ff. spp. *lycopersici*, *melonis*, and *lactucae* also produced high numbers of chlamydospores in the SuperSoil broth within five days. Chlamydospores were intercalary or terminal on hyphae, and were produced singly or in short chains (Figure 1a) typical of *F. oxysporum* (Leslie and Summerell, 2006). Most chlamydospores were produced in mycelia grown from the inoculated conidia (Figure 1); few of the inoculated conidia converted conidial cells directly into chlamydospores.

Chlamydospore production in the 1:4 (w/v) SuperSoil broth with glucose did not appear to be related to race of *F. oxysporum* f. sp. *vasinfectum* or the age of the culture, at least within this small sample of isolates. An isolate of each race 4 and race 8 produced copious chlamydospores, while the other isolate of each race did not. Two of the ATCC isolates (16421 and 16611), which have been cultured at least 27 years (Kappelman, 1983), produced chlamydospores. Isolates producing low quantities of chlamydospores have been in culture a minimum of five years (genotype 140; Holmes et al. 2009), and a maximum of 37 years (CA-1; Kim et al. 2005). A possible explanation for these discrepancies may be cultural degeneration, a phenomenon commonly observed in lab-maintained cultures of *Fusarium* (Leslie and Summerell, 2006).

Isolate CA14 consistently produced an average of 3×10^5 chlamydospores per ml ($>10^7$ chlamydospores per flask) of inoculated 1:4 (w/v) SuperSoil

broth within 7 d (Table 3). Spore concentrations in flasks varied from 1.9-4.6 × 10^5 chlamydospores per ml. Yield in other high chlamydospore-producing isolates of *F. oxysporum* were similar to CA14, as estimated from the size of the chlamydospore-mycelium aggregates (data not shown).

Variations of the 1:4 (w/v) SuperSoil broth (no glucose, incubation in dark, 60-min autoclave times) did not affect chlamydospore production in the six isolates tested (ATCC 16421, ATCC 16611, CA14, NRRL 31665, FOV21, and 110; Table 2). Light was influential in methods using media made from other substrates (Ciotola et al., 2000; Elzein and Kroschel, 2004), but it does not appear to be a requirement for chlamydospore production in SuperSoil broth. Bacterial contamination occasionally occurred in flasks autoclaved 20 min, and general plant pathology protocols have recommended soil be autoclaved 60 min on two consecutive days (Dhingra and Sinclair, 1995). No changes in chlamydospore production were observed when the SuperSoil broth was autoclaved for 60 min instead of 20 min. Chlamydospore production was also unaffected in the seven additional isolates (108, 112, F702, 1002, Fom, GL 1110, GL 1112) grown in 1:4 (w/v) SuperSoil broth without glucose. Chlamydospores were produced within 5 d after inoculation in all isolates and were not visibly different in quantity or appearance from the spores produced in 1:4 (w/v) SuperSoil broth with glucose.

Chlamydospore production in 1:4 (w/v) SuperSoil broth without glucose compared to other soil broths. Because chlamydospore production was unaffected by absence of glucose, the simpler protocol without glucose was used for comparisons with other soil broths. Broth yield of the Alexander et al. (1966) method was limited by the sterilizing filter. Less than 30 ml of sterile broth was obtained after 3 h of vacuum filtration in each of three of experiments. Significant differences in broth yield were found among the other soil broth methods (F = 1118.23; df = 2, 6; $P < 0.01$). Broth yield of the 1:4 (w/v) SuperSoil broth without glucose (429 ml) was intermediate to, and significantly different from ($P < 0.01$), broth yields of the 1:3 (w/v) field soil broth with glucose (783 ml) and the 1:3 (w/v) SuperSoil broth with glucose (211 ml; Table 3). Broth yield of the 1:3 (w/v) SuperSoil broth was about 25% that of the 1:3 (w/v) field soil broth, probably due to water absorption by peat in the SuperSoil Potting Soil.

Significant differences in chlamydospore concentration were also found among the soil broth methods (F = 22.3; df = 2, 6; $P < 0.01$). The concentration of chlamydospores in the1:4 (w/v) SuperSoil broth without glucose (3.0 × 10^5 chlamydospores/ml) differed from that of the 1:3 (w/v) SuperSoil broth (5.7 × 10^5 chlamydospores/ml; $P < 0.01$), but not of the 1:3 (w/v) field soil broth (2.3 × 10^5 chlamydospores/ml; P = 0.43; Table 3). Considerable variation among experiments was observed in the concentration for the 1:3 (w/v) field soil broth. Although the mean concentration for the 1:3 (w/v) field soil broth was not significantly different from that of the 1:4 (w/v) SuperSoil broth without glucose, the mean concentration in one experiment was 1.2 × 10^5 chlamydospores/ml. Few chlamydospores (2.6 × 10^4 chlamydospores/ml) were found in the Alexander et al. (1966) broth relative to the other soil broths.

Significant differences were found among soil broth methods when yield was adjusted to the total number of chlamydospores per liter of recipe (F = 5.32; df = 2, 6; P = 0.05). The concentration of chlamydospores in the 1:3 (w/v) SuperSoil broth with glucose was nearly twice that of the 1:4 (w/v) SuperSoil broth without glucose, but the total number of chlamydospores did not differ between these methods (P = 0.95; Table 3). The difference between 1:4 (w/v) SuperSoil broth without glucose and the 1:3 (w/v) field soil broth in chlamydospore yield per liter of recipe was significant (P = 0.06; Table 3). The number of chlamydospores produced per gram of substrate differed among broth methods (F = 4.63; df = 2, 6; P = 0.06). The 1:4 (w/v) SuperSoil broth without glucose was similar to the 1:3 (w/v) field soil broth (P = 0.84), but different from the 1:3 (w/v) SuperSoil broth with glucose (P = 0.10; Table 3).

The 1:4 (w/v) SuperSoil broth without glucose was a simple and reliable method for generating large quantities of chlamydospores of *F. oxysporum*. Initial attempts at generating chlamydospores using field soil with the 1:3 (w/v) Goyal et al. (1973) protocol produced few chlamydospores. Higher numbers of chlamydospores were obtained in these experiments comparing the soil broth methods, but broth yields of 1:3 (w/v) field soil method varied among experiments. Others also reported variability in chlamydospore production when extracts were made from soil collected at one location but during different times of the year (Ford et al., 1970). A commercially available potting mix such as SuperSoil should provide more consistent results than using field soils of variable composition. Chlamydospores of isolate CA14 were consistently obtained from a minimum of ten

different 1-cu.-ft. bags of SuperSoil purchased over a four-year period (Bennett et al., 2011).

SuperSoil may not be available in all areas, but other commercial organic potting mixes may yield similar results. The SuperSoil label lists peat, spaghnum peat, compost, urea, ureaform (synthetic product of urea and formaldehyde), monoammonium phosphate, potassium nitrate, ferrous sulfate, a wetting agent, and a NPK ratio of 0.14-0.09-0.02. Because peat is a major component in SuperSoil, the 1:4 (w/v) SuperSoil broth has more water than the original Goyal et al. (1973) recipe to compensate for absorption and to facilitate broth collection. Consequently, broth is more easily extracted from the 1:4 (w/v) SuperSoil mix than from the 1:3 (w/v) Goyal recipe. The 1:4 (w/v) SuperSoil broth procedure is also simpler than the Goyal broths because glucose is not required. The total number of chlamydospores obtained per liter of recipe did not differ between 1:4 (w/v) SuperSoil broth without glucose and the 1:3 (w/v) field and SuperSoil broths with glucose. In addition, the 1:4 (w/v) SuperSoil broth without glucose was slightly more efficient ($P = 0.10$) than the 1:3 (w/v) SuperSoil broth with glucose in the number of chlamydospores produced per gram of potting mix. If large quantities of chlamydospores are needed, the 1:4 (w/v) SuperSoil broth without glucose can be scaled up by increasing the number of flasks. The method of Alexander et al. (1966), which yields little broth and few chlamydospores, is clearly not suitable for large-scale production of chlamydospores.

In conclusion, the 1:4 (w/v) SuperSoil broth without glucose consistently and rapidly induced chlamydospore production by many isolates of *F. oxysporum* f. sp. *vasinfectum* and several other *formae speciales*. Chlamydospores produced in the 1:4 (w/v) SuperSoil broth without glucose can be generated in the time required to produce conidia and have been used successfully in virulence assays (R. Bennett, unpublished). This method should be useful for studies of pathogenicity and pathogen survival of *F. oxysporum*.

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DISCLAIMER

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