Susceptibility of Fraser Fir to *Phytophthora capsici*

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ABSTRACT

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Phytophthora cinnamomi, *P. drechsleri*, *P. citricola*, and *P. cactorum* limit Fraser fir production, whereas *P. capsici* affects Solanaceous, Cucurbitaceous, and Fabaceous crops. Some vegetable growers in Michigan plant conifers for the Christmas tree market in fields infested with *P. capsici*. To determine the susceptibility of Fraser fir to *P. capsici*, stems (no wound or 1- or 3-mm-diameter wound) or roots (2 or 4 g of infested millet seed or $2 \text{ or } 5 \times 10^3$ zoospores/ml of a zoospore suspension) of seedlings were inoculated with each of four *P. capsici* isolates and incubated in growth chambers (20 or 25°C). In addition, Fraser fir seedlings were planted in two commercial fields naturally infested with *P. capsici*. All *P. capsici* isolates tested incited disease in the seedlings regardless of incubation temperature or inoculation method. Seedlings (72%) planted in *P. capsici*-infested fields developed disease symptoms and died. Most of the *P. capsici* isolates obtained from the Fraser fir seedlings infected while in the field were recovered from root tissue. Identification was confirmed by species-specific direct colony polymerase chain reaction. The pathogen was successfully recovered from stems of all stem-inoculated seedlings, and from roots and stems of all root-inoculated seedlings; the phenotype of the recovered isolate matched the phenotype of the inoculum. This study suggests that planting Fraser fir in fields infested with *P. capsici* could result in infection and that adjustments in current rotational schemes are needed.

Additional keywords: Christmas tree diseases, etiology, soilborne diseases

Each year, approximately 33 to 36 million Christmas trees are produced in North America with an estimated farm gate value of \$462 million (5). Michigan ranks third in the production of Christmas trees, with 17,000 ha and an annual value of \$4 million (2,5). Nationally, the most common species used in plantations include Douglas fir (*Pseudotsuga* sp.), Fraser fir (*Abies fraseri*), Noble fir (*A. procera*), and Balsam fir (*A. balsamea*) (5). With its natural Christmas tree shape and excellent post-harvest quality, Fraser fir is one of the most valued trees in the Christmas tree industry (5), rapidly becoming the most popular tree among growers in Michigan. In 2005, 3,000 ha were planted to Fraser fir in Michigan (2).

Phytophthora root rot and shoot blight can limit the production and marketability of Fraser fir (5,6), as reported in North Carolina (3) and Michigan (1). With the exception of Michigan, *Phytophthora cinnamomi* (11) is typically the causal organism. However, *P. drechsleri* (4), *P. citricola* (35), and *P. cactorum* (1) have also been associated with root rot and shoot blight

symptoms in Fraser fir and other *Abies* and conifer species grown for Christmas trees (1,4,13,14,21,30). Plant death ranging from 30 to 75% can occur when conditions are favorable (5). Fraser fir is especially susceptible to *P. cinnamomi* and other *Phytophthora* species; symptoms including characteristic reddish-brown needles develop rapidly, and most trees die in 4 to 5 weeks (5,6,17).

To prevent Phytophthora root rot and shoot blight in Christmas trees, it is important that growers plant pathogen-free seedlings into well-drained fields without a history of root rot organisms (3). In some growing regions, uninfested fields are becoming increasingly scarce, limiting the expansion of production (5). Michigan vegetable growers are diversifying their crops and including Fraser fir plantings in soils infested with *Phytophthora capsici* Leonian. *P. capsici* has a broad host range including Solanaceous, Cucurbitaceous, and Fabaceous crops (8). Crop rotation is used to manage *P. capsici*, but this strategy is limited by the long-term survival of oospores in soil (27) and an increasing list of *P. capsici*-susceptible hosts (10). The objective of this study was to determine whether *P. capsici* is pathogenic to Fraser fir. Specifically, we sought to determine the following: (i) whether or not incubation temperature, inoculation method, or *P. capsici* isolates influence infection and disease development, and (ii) whether or not Fraser fir seedlings could become in-

fectured when planted in a field naturally infested with *P. capsici*. A preliminary report of these findings has been published (32).

MATERIALS AND METHODS

Isolate selection, maintenance, and inoculum preparation. *P. capsici* isolates collected in Michigan from infected Cucurbitaceous and Solanaceous crops were selected from the culture collection maintained in the laboratory of M. K. Hausbeck at Michigan State University (MSU). They were phenotypically characterized according to mating type (MT) and sensitivity to the fungicide mefenoxam (24). Isolate OP97 (A1 MT) was isolated from pickling cucumber and SP98 (A2 MT) from pumpkin, and both are sensitive to mefenoxam. Isolate SFF3 (A2 MT) originates from pickling cucumber, and isolate 12889 (A1 MT) was obtained from pepper; both are insensitive to mefenoxam.

Actively growing cultures of each isolate were obtained by transferring agar plugs from long-term stock cultures (stored at 20°C in sterile microcentrifuge tubes with 1 ml of sterile water and one sterile hemp seed) onto V8 juice agar (16 g of agar, 3 g of CaCO₃, 160 ml of unfiltered V8 juice, and 840 ml of distilled water). Cultures were maintained at room temperature (21 ± 2°C) under fluorescent light. To ensure that isolates were virulent, cucumber fruits were inoculated with each isolate. Cucumbers were washed with water, disinfested for 5 min in a 5% sodium hypochlorite solution, and dried at room temperature. A small superficial wound was made with a sterile needle in the center of each cucumber. Agar plugs (7 mm diameter) from the margins of actively growing colonies were placed upside down on top of the wound. A sterile microcentrifuge tube (with the cap removed) was placed over each agar plug and fixed to the fruit with petroleum jelly. Control cucumbers were inoculated as described above using a sterile 7-mm-diameter plug of V8 agar. Cucumbers were placed in aluminum trays with wet paper towels and covered with plastic wrap to maintain high relative humidity. Trays were incubated at room temperature (21 ± 2°C). Symptomatic cucumber tissue (5 mm) was excised and transferred to V8 juice agar and maintained under the same conditions described above. Clean cultures of each isolate were obtained from the infected cucumber tissue.

Bare-root Fraser fir seedlings were purchased from a nursery in Holland, MI that

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obtained the seed from Roan Mountain, NC (lat. 36°09'N, long. 82°05'W). Seedlings were grown in a greenhouse and then transplanted into a field nursery bed for one growing season at the nursery in Holland. Seedlings used in all experiments were 0.15 to 0.3 m in height with 6- to 10-mm-diameter stems and grown in 3-liter pots containing soilless medium (Baccto Professional Planting Mix, Michigan Peat Company, Houston, TX).

Three types of inoculum were prepared and included agar plugs, a zoospore suspension, and infested millet seed. Agar plugs (7 mm diameter) of actively growing *P. capsici* containing mycelium and sporangia were used for stem inoculations. Zoospore inoculum was prepared by pouring 10 ml of sterile distilled water into a petri plate with actively growing and sporulating *P. capsici* on V8 agar. The plates were moved to a 4°C environment for 30 min and then returned to 25°C for 30 min to trigger zoospore release. The number of zoospores in a 1-ml aliquot of the suspension was determined by using a hemacytometer. To facilitate counting, zoospores were first encysted by spinning the suspension at full speed on a vortex mixer for 1 min. Inoculum concentration was adjusted to 2×10^3 and 5×10^3 zoospores/ml. Viability was confirmed after mixing with a vortex by transferring drops of zoospore suspension onto V8 agar and observing colony growth. *P. capsici*-infested millet seed was also prepared. Millet seed (100 g) was mixed with asparagine (0.08 g) and water (72 ml) in a 500-ml Erlenmeyer flask. The flask was capped with aluminum foil and autoclaved twice consecutively. The flask was shaken to homogenize the mixture. The seeds were inoculated with four 7-mm-diameter agar plugs from an actively growing *P. capsici* V8 culture. The inoculated millet seeds were incubated at room temperature ($21 \pm 2^\circ\text{C}$) under constant fluorescent light for 4 weeks.

Stem inoculation. Three different methods were used to inoculate each of

four Fraser fir seedlings using isolate OP97. Inoculations without wounding involved placing an agar mycelial plug on the stem 2 cm above ground level and covering the plug with Parafilm to retain moisture. A second inoculation method included making a small wound (1 mm diameter) in the stem (2 cm above ground level) using a sterile syringe needle, placing the agar mycelial plug over the wound, and covering the plug with Parafilm. A third inoculation method included making a larger wound (3 mm diameter) in the stem (2 cm above ground level) using a sterile cork borer, placing the agar plug over the wound, and covering the plug with Parafilm. As a control, four seedlings were each inoculated using the three inoculation methods previously described and sterile 7-mm-diameter V8 agar plugs. The inoculated and control seedlings were placed in a randomized arrangement in each of two growth chambers maintained at 20 or 25°C with fluorescent lighting (14/10 day/night photoperiod and 95 mE of light intensity). Seedlings were watered once a week if the soil was dry. This experiment was conducted twice.

Additional studies were conducted using a small wound inoculation method or no wounding. Four isolates of *P. capsici* (OP97, SFF3, SP98, and 12889) were used as inoculum with four replicate plants used per isolate. Four control plants were inoculated using V8 agar plugs. The seedlings were placed randomized in a growth chamber at 25°C and maintained and watered as previously described. This experiment was conducted twice.

Root inoculation. Roots were inoculated by dispensing 10 ml of a suspension containing 2×10^3 or 5×10^3 zoospores/ml on the soil surface at the base of each seedling or by mixing the soil with 2 or 4 g of infested millet seed. Approximately 50 g of soil surrounding the stem was removed, mixed with the millet seed, and replaced around the stem. Four Fraser fir seedlings were inoculated with *P. capsici* OP97, and four control seedlings were inoculated

with sterile distilled water or millet seed inoculated with sterile V8 agar plugs. The seedlings were placed in a randomized arrangement in a growth chamber at 25°C and maintained and watered as previously described. This experiment was conducted twice.

Millet seed infested with one of four isolates of *P. capsici* (OP97, SFF3, SP98, and 12889) was used to inoculate seedlings after transplanting as previously described. Four replicate seedlings were used per isolate. Four control plants were inoculated with millet seed with sterile V8 agar plugs. The seedlings were placed randomized in a growth chamber at 25°C and maintained and watered as previously described. This experiment was conducted twice.

Disease assessment and data analysis.

Disease assessment was initiated 1 week following inoculation and continued at weekly intervals for 2 months or until all seedlings were dead. Disease progression was recorded for each seedling as the percentage of branches with bronze coloration, and the area under the disease progress curve (AUDPC) was calculated. The AUDPC values were averaged and used for all statistical analyses, and their residuals followed the assumptions of all statistical tests performed. The experiment was arranged in a split-plot with whole plots in a randomized complete block design. Data were subjected to analysis of variance using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC), and multiple comparisons among the means were conducted using *t* tests (LSD) when effects were found to be statistically significant at 0.05 levels.

Field experiment. In 2007, seedlings were planted in two commercial fields in Michigan known to be infested with *P. capsici*. Previously, the fields had been cropped primarily to cucurbits, including summer and winter squash and cucumber, that had become infected by *P. capsici*. Field 1 was located in Cass County and had sandy loam soil, and field 2 was located in Oceana County and had Benona sand soil. The study was conducted in each field from 29 June to 31 August and repeated from 2 September to 9 November 2007. Soil and air temperatures were obtained from Michigan Automated Weather Network (MAWN) for the Lawton Station located 14.5 km away from field 1 and from the Hart Station located 8 km away from field 2.

One hundred and fifty Fraser fir seedlings were planted in each field with 0.6 m spacing between seedlings within a row and centered in 10 beds that were 18 m long and 0.6 m wide with spacing of 1.5 m between beds. Yellow squash, a known *P. capsici*-susceptible host, was established in the plant beds between the Fraser fir seedlings. Each row contained approximately 15 Fraser fir and yellow squash seedlings. Eight asymptomatic or eight

Fig. 1. Symptoms of *Phytophthora capsici* root rot on Fraser fir. **A**, Normal appearance of foliage of a control seedling. **B**, Bronzing of needles starting in lower part of seedling that progresses upward to the rest of the foliage. **C**, Complete bronzing of all foliage of the seedling.

symptomatic Fraser fir seedlings were sampled randomly from each field every week for 9 weeks, beginning 1 week after planting. When symptoms were not observed, only asymptomatic seedlings were collected and sampled. Toward the end of the study when symptoms were observed in most of the planted seedlings, only symptomatic seedlings were collected. Yellow squash showing crown rot was also sampled. The incidence of *P. capsici* recovered from each tissue type (root, stem, branch, and needle) was determined for Fraser fir. The incidence of *P. capsici* from each tissue type was used for all statistical analyses, and their residuals followed the assumptions of all statistical tests performed. The experiment was arranged in a split-plot with whole plots in a randomized complete block design. Data were subjected to analysis of variance using the PROC MIXED and PROC GLIMMIX procedures of SAS (SAS Institute), and multiple comparisons among the means were conducted using *t* tests (LSD) when effects were found to be statistically significant at 0.05 levels.

Pathogen isolation. Control and inoculated seedlings from the growth chamber experiments were washed with water to remove the soilless growing medium particles and other debris, dipped into 70% ethanol for 1 min to surface disinfect all tissues, and air-dried. The washing and surface sterilization step was accomplished prior to making an incision in the sample to avoid exposing the tissues inside and potentially compromising the success of pathogen isolation. Tissues from root, stem, branch, and needle were excised and cut in half with a sterile scalpel and plated onto BARP-(benomyl, ampicillin, rifampicin, and pentachloronitrobenzene) amended, unclarified V8 agar (27). This technique facilitated the growth of the pathogen from the infected tissue into the growth media. The same procedure was used to isolate *P. capsici* from asymptomatic and symptomatic Fraser fir and yellow squash seedlings planted in the field.

Cultures that were suspected to be *P. capsici* based on morphological characteristics were transferred to new BARP plates. Axenic cultures were incubated for 7 days on V8 agar with continual fluorescent lighting under ambient laboratory conditions ($21 \pm 2^\circ\text{C}$). Cultures were positively identified as *P. capsici* based on morphological characteristics described by Waterhouse (37). Isolates obtained from seedlings in the growth chambers were characterized for MT and mefenoxam resistance as previously described (24) to compare the phenotype of the isolate obtained with the original inoculum. Isolates obtained from the field were also characterized for MT and mefenoxam resistance.

Molecular confirmation of field isolates. Field isolates suspected to be *P.*

capsici and positively identified by morphological characteristics were further confirmed by direct colony polymerase chain reaction (PCR) (23). Mycelium was prepared by inoculating 1-ml aliquots of cucumber extract amended with antibiotics in sterile 1.5-ml microcentrifuge tubes. Cucumber extract was prepared by blending 2 kg of fresh cucumbers in 1 liter of water, filtering through cheesecloth, centrifuging the juice at $15,550 \times g$ for 20 min, and transferring the supernatant to a clean flask. The supernatant was autoclaved and the top layer of the extract was aspirated into a new flask without transferring any precipitate from the bottom. Antibiotics (50 ppm vancomycin, 25 ppm pimaricin, and 50 ppm ampicillin) were added to the extract, and it was stored in 50-ml falcon tubes for later use. Cultures were grown overnight on a shaker at room temperature ($21 \pm 2^\circ\text{C}$) under constant fluorescent light. Cultures were centrifuged at $21,130 \times g$ for 2 min, and the supernatant was removed. Mycelium was rinsed 3 times with 1 ml of sterile water, centrifuged at $21,130 \times g$ for 1 min, and the supernatant was removed in each washing step. A small amount of mycelium was transferred to PCR tubes and macerated with a sterile

micropipette tip. The PCR mix was directly added to the mycelium.

Two specific primers for *P. capsici* were used for PCR: one forward primer (CAPFW; 5'TTAGTTGGGGTCTTGT ACC3'), and one reverse primer (CAPRV2; 5'TACGGTTCACCAGCCCAT CA3') that were designed by Silvar et al. (36). Direct colony PCR reactions were performed in a total volume of 25 μl containing mycelium, 2.5 μl 10 $\times$ PCR reaction buffer (Invitrogen, Carlsbad, CA), 14.3 μl sterile water, 1 μl 10 μM MgCl_2 (Invitrogen), 4 μl 1.25 mM dNTP mix (Invitrogen), 1 μl each primer 10 μM (MSU Macromolecular Structure Facility, East Lansing, MI), and 0.2 μl Taq DNA polymerase (Invitrogen). The PCR reaction was performed in a programmable Eppendorf mastercycler ep systems thermal cycler (Eppendorf, Westbury, NY) starting with 5 min at 95°C to release DNA from mycelium, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 60 s, with a final extension step of 5 min at 72°C . PCR products were analyzed by electrophoresis in 1% (wt/vol) agarose gel in 1 $\times$ Tris-acetate-EDTA buffer (29), stained with ethidium bromide (5 $\mu\text{g}/\text{ml}$) for visualiza-

Table 1. Effects of inoculum, pathogen isolate, incubation temperature, and inoculation method on the area under the disease progress curve (AUDPC) values for disease incidence on Fraser fir seedlings caused by *Phytophthora capsici*

Treatment main effect (P value)	AUDPC mean ^a
Stem inoculation using OP97	
Temperature (0.5644)	
20°C	219.3 a
25°C	290.7 a
Inoculation method (0.0081)	
No wound	196.8 a
1-mm wound	322.5 b
3-mm wound	245.7 b
Temperature $\times$ inoculation method (0.2568)	
Stem inoculation using one of four isolates	
Inoculation method (0.0138)	
No wound	239.6 a
1-mm wound	293.9 b
Isolate (0.4847)	
OP97	249.5 a
SP98	279.5 a
SFF3	266.9 a
12889	271.3 a
Inoculation method $\times$ isolate (0.4617)	
Soil infestation using OP97	
Inoculation method (0.0168)	
2×10^3 zoospores/ml	61.3 a
5×10^3 zoospores/ml	101.9 a
2 g of infested millet seed	375.3 b
4 g of infested millet seed	380.8 b
Soil infestation using one of four isolates	
Inoculation method (0.9603)	
2 g of infested millet seed	285.7 a
4 g of infested millet seed	281.3 a
Isolate (0.1974)	
OP97	205.8 a
SP98	322.8 a
SFF3	314.1 a
12889	291.4 a
Inoculation method $\times$ isolate (0.9802)	

^a Means within a column for each treatment main effect followed by the same letter are not significantly different (LSD).

tion, and compared with a 100-bp ladder (Invitrogen). A control with extracted DNA was used to ensure that detection of the pathogen was possible using direct colony PCR.

RESULTS

All Fraser fir seedlings inoculated with *P. capsici* developed symptoms and died; wounding was not required. The first disease symptoms occurred approximately 2 weeks after inoculation. Overall disease

symptoms included reddish-brown foliage or bronzing and needle death that began in the lower portion of the seedling and progressed upward until all of the foliage exhibited symptoms; dark, discolored, and rotted roots along with seedling death were also observed (Fig. 1).

Significant differences occurred among the stem inoculation methods (ANOVA, $P = 0.0081$, Table 1). Wounded seedlings developed symptoms sooner than unwounded seedlings (Fig. 2A and B) when

inoculated with *P. capsici* OP97 according to AUDPC data. There was not a significant difference in disease resulting from the 1- and 3-mm-diameter wounds. However, they were both different from the inoculation method without wounding.

Although seedlings incubated at 20°C died 3 to 4 weeks later than seedlings incubated at 25°C (Fig. 2A and B), AUDPC means did not differ significantly between incubation temperatures (ANOVA, $P = 0.5644$, Table 1). Interaction effects were not found between temperature and the inoculation method (ANOVA, $P = 0.2568$, Table 1). An incubation temperature of 25°C was used in subsequent growth chamber experiments.

Significant differences were observed among the methods used to infest the soil (ANOVA, $P = 0.0168$, Table 1). According to AUDPC data, a zoospore suspension was significantly less effective than using infested millet seed. Most of the seedlings (97%) inoculated with millet seed became infected and died (Fig. 2E). In contrast, 87% of the seedlings inoculated with a zoospore suspension became infected as determined by pathogen isolation at the conclusion of the experiment, but only 2% died (a maximum of 26.8% showed symptoms). The suspension containing a low (2×10^3 /ml) or high (5×10^3 /ml) zoospore concentration was significantly different from soil infestation using 2 or 4 g of millet seed (Table 1). Seedlings inoculated using millet seed died 4 to 6 weeks after inoculation, similar to that observed for the stem inoculation experiments. No significant differences were found among zoospore concentrations or millet seed quantities used for soil infestation (Table 1).

When four *P. capsici* isolates (OP97, SP98, SFF3, or 12889) were used to inoculate wounded (1 mm diameter) or unwounded seedling stems, significant differences among the isolates were not observed (ANOVA, $P = 0.4847$, Table 1). Similarly, significant differences were not found among *P. capsici* isolates (ANOVA, $P = 0.1974$, Table 1) when infested millet was used as inoculum. All isolates were capable of producing disease and death in seedlings regardless of whether they were inoculated via the stem (Fig. 2C and D) or soil infestation (Fig. 2F and G) method. There were significant differences between the no-wound and 1-mm-diameter wound stem inoculation methods according to AUDPC data (ANOVA, $P = 0.0138$, Table 1), as observed in the previous experiment for isolate OP97. No interaction effects were found between the isolate and the agar plug inoculation method (ANOVA, $P = 0.4617$) or the infested millet inoculation method (ANOVA, $P = 0.9802$, Table 1). No significant differences were observed between the two millet seed quantities (ANOVA, $P = 0.9603$, Table 1) used to infest the soil.

The pathogen was isolated only from inoculated symptomatic seedlings in all

Fig. 2. Progression of disease caused by *Phytophthora capsici* in inoculated Fraser fir seedlings. Points represent mean values of eight replicates with the standard deviation. **A and B,** Comparison of inoculation of *P. capsici* OP97 onto unwounded (none) or wounded (1- or 3-mm wounds) stem and grown at **A,** 20°C and **B,** 25°C. **C and D,** Comparison of inoculations of four isolates of *P. capsici* onto **C,** unwounded or **D,** wounded (1 mm) stems and grown at 25°C. **E,** Comparison of rates of *P. capsici* OP97-infested millet seeds inserted into the growing media near the stem and concentrations of *P. capsici* OP97 zoospore suspension poured into the growing media near the stem and grown at 25°C. **F and G,** Comparison of inoculum of four isolates of *P. capsici* prepared as **F,** 2 g infested millet seed and **G,** 4 g infested millet seed inserted into the potting media near the stem and grown at 25°C.

growth chamber experiments. The phenotype (MT and mefenoxam resistance) was confirmed and matched the phenotype of the isolate used as inoculum. The control seedlings remained asymptomatic.

Over 70% of the total number of seedlings planted in both fields naturally infested with *P. capsici* developed disease symptoms and died. Root rot symptoms were observed approximately 2 to 3 weeks after planting in fields 1 and 2 at both planting times. Foliar disease symptoms included bronzing and death of the needles similar to that observed on inoculated plants in the growth chamber experiments. There was not a significant difference in seedling death due to variability between the field sites (ANOVA, $P = 0.4678$) or the planting times (ANOVA, $P = 0.5277$). Seedlings died quickly, 3 to 5 weeks after planting in the first field experiment; temperatures were higher in the first experiment relative to the second field experiment (Table 2). In the second experiment, Fraser fir seedlings died 4 to 6 weeks after planting. All yellow squash seedlings died 4 to 5 weeks after planting in both experiments, and planting times and all isolations made from roots and stems were positive for *P. capsici* growth. Growth of *P. capsici* was observed in all isolations from symptomatic Fraser fir seedlings (Table 3), but significant differences in pathogen incidence were found among different plant parts (PROC GLIMMIX, $P < 0.0001$). Field 1 yielded a total of 84 *P. capsici* isolates for both planting times (Table 3) obtained from Fraser fir root (87%) and stem (13%) tissue (Table 3). Field 2 yielded a total of 87 *P. capsici* isolates for both planting times (Table 3) obtained from root (90%) and stem (10%) tissue (Table 3). There was not a significant difference in the number of isolates obtained from roots or stems due to variability between the field sites (ANOVA, $P = 0.3440$ and $P = 0.2271$, respectively) or the planting times (ANOVA, $P = 0.3390$ and $P =$

0.3733, respectively). Although the branches and needles exhibited bronzing in both fields (Fig. 1), the pathogen was not easily recovered from branches and was never recovered from the needles. The majority of the *P. capsici* isolates collected were sensitive to the fungicide mefenoxam, and approximately 50% was of the A2 MT (Table 4). Clean cultures were obtained from 60% of the original cultures obtained directly from Fraser fir tissue. The pathogen was not isolated from tissue of asymptomatic Fraser fir seedlings.

P. capsici was positively identified based on morphological characteristics and was confirmed by direct colony PCR amplification with *P. capsici*-specific primers. The PCR product obtained for all isolates had the expected size of 594 bp (36) when compared with the 100-bp ladder (Fig. 3). Direct colony and control DNA PCR reactions produced the expected band for positive identification of *P. capsici* (Fig. 3). Other organisms that were isolated from symptomatic and asymptomatic seedlings included *Pythium* spp., *Alternaria* spp., and *Fusarium* spp. *Pythium* spp. were particularly common from root and stem tissue isolations, and *Alternaria* spp. were commonly obtained from branch and needle tissue.

DISCUSSION

Phytophthora root rot and blight incited by *P. cinnamomi* (11), *P. drechsleri* (4), *P. citricola* (35), or *P. cactorum* (1) causes needles and branches of Fraser fir to turn reddish-brown in color (5). Similar symptoms were observed in seedlings infected with *P. capsici*. A wound was not required for infection; however, wounding significantly increased disease incidence. All *P. capsici* isolates tested incited disease in the seedlings regardless of temperature (20 or 25°C) or inoculation method, indicating that *P. capsici* can infect Fraser fir.

Most of the *P. capsici* isolates obtained from the Fraser fir seedlings infected while

in the field were recovered from root tissue. In addition, the pathogen was successfully recovered from stems of all stem-inoculated seedlings, and from roots and stems of all root-inoculated seedlings; the phenotype of the recovered isolate always matched the phenotype of the inoculum.

In our study, *P. capsici* was more difficult to recover from Fraser fir seedlings than from the yellow squash. *P. capsici* can be difficult to isolate from certain tissue types such as mature pepper stems, but is readily isolated from symptomatic pepper fruits (M. K. Hausbeck, unpublished data). Other *Phytophthora* spp. are not readily isolated from the fibrous tissue of asparagus crowns and fern (9,34) or the woody tissue of oak (7) or various ornamentals (12).

Direct colony PCR was a fast and reliable way to verify the identity of *P. capsici* isolated from plant tissue and allowed the processing of a high number of isolates by eliminating the DNA extraction step (Fig. 3). Other organisms that were isolated from both symptomatic and asymptomatic Fraser fir seedlings included *Pythium* spp., *Alternaria* spp., and *Fusarium* spp. *Pythium* spp. were particularly common from root and stem tissue and *Alternaria* spp. from branch and needle tissue. *Alternaria* spp. are among the most common needle phylloplane fungi and are commonly isolated from needles of other Pinaceae members (31).

Fraser fir seedlings planted in the *P. capsici*-infested fields died quickly in the first experiment that was conducted during a time of relatively high temperatures compared to the second experiment. However, there was no significant difference in seedling death between fields at different planting times, and when inoculated plants were incubated in growth chambers at 25 or 20°C there was no significant difference in disease. The minimum, optimum, and maximum temperatures for growth of *P. capsici* are 10, 28, and >35°C, respectively (8). The temperatures used in the growth chamber experiments are close to the optimum temperature for *P. capsici* growth, in the first field experiment the average soil temperature was also close to the optimum growth temperature for *P. capsici*. In the second field experiment, the average soil temperature was close to the minimum growth temperature for *P. capsici*. Since Fraser fir is sensitive to sustained high temperatures (day temperatures >30°C)

Table 2. Soil and air temperatures of two commercial fields in Michigan naturally infested with *Phytophthora capsici* during the months from June to November 2007

Field	Months	Air temperature (°C)			Soil temperature (°C)		
		Average	Minimum	Maximum	Average	Minimum	Maximum
1	Jun - Aug	21.6	3.9	35.0	24.3	19.2	29.4
	Sep - Nov	12.3	-8.2	32.6	15.8	12.6	19.0
2	Jun - Aug	19.9	1.6	33.2	26.1	18.5	33.7
	Sep - Nov	10.9	-8.4	32.1	13.3	8.3	18.2

Table 3. Incidence of *Phytophthora capsici* and isolates obtained from different parts of the symptomatic Fraser fir seedlings sampled from naturally infested fields and both planting dates

Field	Symptomatic seedlings sampled	Total number of isolates	Isolates obtained ^a (%)		Incidence of <i>P. capsici</i> ^b (%)			
			Root	Stem	Root	Stem	Branch	Needle
1	144	84	73 (87)	11 (13)	83 (57)	53 (37)	8 (6)	0 (0)
2	144	87	78 (90)	9 (10)	80 (55)	55 (38)	9 (7)	0 (0)

^a As determined by axenic pathogen isolation at the conclusion of the experiment.

^b As determined by the total number of tissue pieces that presented *P. capsici* growth.

(16), the relatively high temperatures in the first field experiment may have stressed the seedlings, increasing their susceptibility to *P. capsici*. Fraser fir naturally occurs on highly acidic soils (pH 3.5) at elevations between 1,100 and 2,000 m (16,18) in the Appalachian Mountains (33) where temperatures range from -12 to 26°C depending on the season (16,28). Day temperatures of 22 to 27°C and night temperatures of 13 to 19°C appear optimum for producing containerized Fraser fir (16).

Once soils become infested with *P. capsici*, oospores allow overwintering and persistence of the pathogen for several years (15,27). Oospores form when both mating types come into contact; fields in Michigan infested with *P. capsici* have been found to have both mating types present (24,26). Oospores in soil and plant debris are considered primary inoculum (15), which may directly infect the plant or develop a sporangium that is capable of releasing 20 to 40 motile zoospores when water is present. Mycelium can develop on host tissue under favorable conditions, forming secondary inoculum (sporangia, zoospores) that can be produced repeatedly during the growing season (8). The agar plugs and millet seed used as inoculum in the growth chamber experiments contained mycelium and sporangia, and since only single *P. capsici* isolates were used, sexual reproduction was prevented. The zoospore suspension did not contain other inoculum types. In the field experiment, the Fraser fir seedlings could have been exposed to oospores, mycelium, sporangia, and/or

zoospores since both *P. capsici* mating types were found and sexual reproduction was likely. In the growth chamber experiments, the zoospore inoculum did not appear to be as effective as the other inoculum types used in our study. Viability of the zoospore suspension was checked after mixing with a vortex, and it was found to be satisfactory; however, it is possible that the zoospores did not remain viable. This is possibly because the zoospore inoculum did not have an associated food base, as did the agar plugs and millet seed inoculum. The agar plug and millet seed inoculum could produce new growth to infect the seedlings when conditions were favorable.

There were no significant differences in plant death among the *P. capsici* isolates used as inoculum. The *P. capsici* isolates from seedlings planted into *P. capsici*-infested fields exhibited variation in mating type and resistance to mefenoxam. Field populations of *P. capsici* from Solanaceous and Cucurbitaceous hosts are reported to have variation in virulence and other phenotypic characteristics (19,20). Historically, field 2 has had *P. capsici* populations that were primarily sensitive to mefenoxam (M. K. Hausbeck, unpublished data); however, in the present study we recovered 42 isolates that were fully insensitive to the fungicide. Field 1 has a history of a heterogeneous *P. capsici* population with sensitive and insensitive isolates (M. K. Hausbeck, unpublished data), which was also found in this study. The recovery of A1 and A2 mating types in a ratio close to 1:1 from the seedlings is expected from

Michigan vegetable fields (26) and suggests that sexual recombination is likely. Therefore, genetic diversity is being maintained within field populations and isolates have an increased chance of gaining new traits such as mefenoxam resistance or increased virulence.

Planting Fraser fir for Christmas tree production is increasing in the northern Great Lakes region due to the tree's desirable traits of foliage color, shape, fragrance, and excellent needle retention (33). It is important to use locally or regionally developed guidelines to grow Fraser fir because of the specific environmental conditions they require for good performance. Christmas tree growers in Michigan perceive Fraser fir to be more sensitive to soil conditions, more likely to experience nutrient disorders, and more responsive to fertilization than other Christmas tree species, restricting production to only optimum sites (33).

The number of fields historically used for vegetable production in Michigan that have become infested with *P. capsici* is increasing (25,26), and growers are planting Fraser fir as a rotational crop. Infested acreage and urban pressure is increasing across vegetable production areas in Michigan, limiting growers' ability to avoid the pathogen (15). *P. capsici* has been documented in many vegetable production regions (15), and Michigan grows over 33,000 ha of susceptible crops (22). Other species of *Phytophthora* are known to occur on Fraser fir in Michigan, such as *P. cactorum* (1) and *P. citricola* (D. W. Fulbright, unpublished data). However, only *P. capsici* was recovered from the infested field sites, probably because they were historically cropped to vegetables, not woody ornamentals.

With the identification of Fraser fir as a newly recognized host for *P. capsici*, it is important to elucidate the etiology of the pathogen on Christmas trees for the development of appropriate crop rotation strategies. The present study of *P. capsici* on Fraser fir indicates that adjustments in current rotational schemes are needed, as planting Fraser fir in fields infested with *P. capsici* could result in infection and should be avoided.

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Table 4. Mating type and mefenoxam sensitivity of *Phytophthora capsici* isolates from symptomatic Fraser fir seedlings grown in two commercial fields in Michigan naturally infested with *P. capsici*

Field	Total number of isolates	MT ^a	Mefenoxam sensitivity ^b (%)			Total (%)
			I	IS	S	
1	84	A1	3 (3.6)	4 (4.7)	32 (38.0)	39 (46.3)
		A2	10 (12.0)	4 (4.7)	31 (37.0)	45 (53.7)
2	87	A1	29 (33.3)	2 (2.3)	11 (12.6)	42 (48.2)
		A2	13 (15.0)	4 (4.6)	28 (32.2)	45 (51.8)
Total	171		55 (32.0)	14 (8.0)	102 (60.0)	171

^a Indicates mating type (A1 or A2) of isolate.

^b Indicates isolate sensitivity to the fungicide mefenoxam: insensitive (I), intermediately sensitive (IS), and sensitive (S).

Fig. 3. Polymerase chain reaction (PCR) confirmation of isolate identification with *Phytophthora capsici*-specific primers: M, 100-bp ladder, with uppermost band 1,000 bp; 1, 2, 3, 4, PCR product of direct colony PCR of isolates obtained from different symptomatic seedlings sampled in the field experiment; 5, PCR product from DNA extracted from mycelium of *P. capsici* OP97; 6, PCR product from DNA extracted from mycelium of *P. capsici* SP98; and 7, negative control where water was added to the PCR reaction instead of mycelium or DNA.

LITERATURE CITED

1. Adams, G. C., and Bielenin, A. J. 1988. First report of *Phytophthora cactorum* and *P. citricola* causing crown rot of fir species in Michigan. Plant Dis. 72:79.
2. Anonymous. 2006. Nursery and Christmas Trees 2004-2005. USDA National Agricultural Statistics Service. Online publication.
3. Benson, D. M., and Grand, L. F. 2000. Incidence of *Phytophthora* root rot of Fraser fir in North Carolina and sensitivity of isolates of *Phytophthora cinnamomi* to metalaxyl. Plant Dis. 84:661-664.
4. Benson, D. M., Grand, L. F., and Suggs, E. G. 1976. Root rot of Fraser fir caused by *Phytophthora drechsleri*. Plant Dis. Rep. 60:238-240.
5. Chastagner, G. A., and Benson, D. M. 2000. The Christmas tree: Traditions, production, and diseases. Plant Health Progress doi: 10.1094/PHP-2000-1013-01-RV. Online publication.
6. Chastagner, G. A., Riley, K. L., and Hamm, P. B. 1990. Susceptibility of *Abies* spp. to seven *Phytophthora* spp. (Abstr.) Phytopathology 80:887.
7. Davidson, J. M., Werres, S., Garbelotto, M., Hansen, E. M., and Rizzo, D. M. 2003. Sudden oak death and associated diseases caused by *Phytophthora ramorum*. Plant Health Progress doi: 10.1094/PHP-2003-0707-01-DG. Online publication.
8. Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society, St. Paul, MN.
9. Falloon, P. G., and Grogan, R. G. 1988. Isolation, distribution, pathogenicity and identification of *Phytophthora* spp. on asparagus in California. Plant Dis. 72:495-497.
10. Gevens, A. J., and Hausbeck, M. K. 2005. *Phytophthora capsici* isolated from snap bean is pathogenic to cucumber fruit and soybean. (Abstr.) Phytopathology 95:S162.
11. Grand, L. F., and Lapp, N. A. 1974. *Phytophthora cinnamomi* root rot of Fraser fir in North Carolina. Plant Dis. Rep. 58:318-320.
12. Hahn, R., and Werres, S. 1997. Development of a dot immunobinding assay to detect *Phytophthora* spp. in naturally dark-rooted woody plants. Ann. Appl. Biol. 130:453-466.
13. Hamm, P. B., and Hansen, E. M. 1982. Pathogenicity of *Phytophthora* species to Pacific Northwest conifers. Eur. J. For. Pathol. 12:167-174.
14. Hamm, P. B., and Hansen, E. M. 1987. Identification of *Phytophthora* spp. known to attack conifers in the Pacific Northwest. Northwest Sci. 61:103-109.
15. Hausbeck, M. K., and Lamour, K. H. 2004. *Phytophthora capsici* on vegetable crops: Research progress and management challenges. Plant Dis. 88:1292-1303.
16. Hinesley, L. E. 1981. Initial growth of Fraser fir seedlings at different day/night temperatures. For. Sci. 27:545-550.
17. Hinesley, L. E., Parker, K. C., and Benson, D. M. 2000. Evaluations of seedlings of Fraser, Momi, and Siberian fir for resistance to *Phytophthora cinnamomi*. HortScience 35:87-88.
18. Hinesley, L. E., and Saltveit, M. E. J. 1980. Ethylene adversely affects Fraser fir planting stock in cold storage. Southern J. Appl. Forestry 4:188-189.
19. Hwang, B. K., and Kim, C. H. 1995. *Phytophthora* blight of pepper and its control in Korea. Plant Dis. 79:221-227.
20. Islam, S. Z., Babadoost, M., Lambert, K. N., Ndeme, A., and Fouly, H. M. 2005. Characterization of *Phytophthora capsici* isolates from processing pumpkin in Illinois. Plant Dis. 89:191-197.
21. Kenerley, C., and Bruck, R. I. 1981. *Phytophthora* root rot of Balsam fir and Norway spruce in North Carolina. Plant Dis. 65:614-615.
22. Kleweno, D. D. 2007. Michigan 2006-2007 highlights. Michigan Department of Agriculture, Lansing. Online publication.
23. Kong, P., Hong, C., Richardson, P. A., and Gallegly, M. E. 2003. Single-strand-conformation polymorphism of ribosomal DNA for rapid species identification in genus *Phytophthora*. Fungal Genet. Biol. 39:238-249.
24. Lamour, K. H., and Hausbeck, M. K. 2000. Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. Phytopathology 90:396-400.
25. Lamour, K. H., and Hausbeck, M. K. 2001. Investigating the spatiotemporal genetic structure of *Phytophthora capsici* in Michigan. Phytopathology 91:973-980.
26. Lamour, K. H., and Hausbeck, M. K. 2001. The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. Phytopathology 91:553-557.
27. Lamour, K. H., and Hausbeck, M. K. 2003. Effect of crop rotation on the survival of *Phytophthora capsici* in Michigan. Plant Dis. 87:841-845.
28. Leffler, R. J. 1981. Estimating average temperatures on Appalachian summits. J. Appl. Meteorol. 20:637-642.
29. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
30. McCain, A. H., and Scharpf, R. F. 1986. *Phytophthora* shoot blight and canker disease of *Abies* spp. Plant Dis. 70:1036-1037.
31. Miller, J. D., Strongman, D., and Whitney, N. J. 1985. Observations on fungi associated with spruce budworm infested balsam fir needles. Can. J. For. Res. 15:896-901.
32. Quesada-Ocampo, L. M., Fulbright, D. W., and Hausbeck, M. K. 2007. Susceptibility of Fraser fir to *Phytophthora capsici*. (Abstr.) Phytopathology 97:S95.
33. Rothstein, D. E., and Lisuzzo, N. J. 2006. Optimal nutrition and diagnosis for *Abies fraseri* Christmas trees in Michigan. NJAF 23:106-113.
34. Saude, C., Hurtado-Gonzalez, O. P., Lamour, K. H., and Hausbeck, M. K. 2008. Occurrence and characterization of *Phytophthora* sp. pathogenic to asparagus (*Asparagus officinalis*) in Michigan. Phytopathology 98:1075-1083.
35. Shew, H. D., and Benson, D. M. 1982. Fraser fir root rot induced by *Phytophthora citricola*. Plant Dis. 65:688-689.
36. Silvar, C., Duncan, J. M., Cooke, D. E. L., Williams, N. A., Diaz, J., and Merino, F. 2005. Development of specific PCR primers for identification and detection of *Phytophthora capsici* Leon. Eur. J. Plant Pathol. 112:43-52.
37. Waterhouse, G. M. 1963. Key to the Species of *Phytophthora* de Bary. Commonwealth Mycological Society, Kew, Surrey, UK.