Characterization of Foliar Web Blight of Spinach, Caused by *Pythium aphanidermatum*, in the Desert Southwest of the United States

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Abstract

A unique foliar disease of spinach, determined to be caused by *Pythium aphanidermatum*, was observed on spinach in Yuma County, AZ and Imperial County, CA desert spinach production areas in both 2015 and 2016. The foliar symptoms of the disease included water-soaked foliage, rapid collapse of young plants, and white, aerial, cottony mycelia. The disease was associated with hot (27 to 42°C) and wet conditions associated with overhead irrigation under high-density plantings (>8.0 million seeds/ha). Isolations were performed on symptomatic tissue, and DNA was recovered from pure culture of the isolates recovered and sequenced using the internal transcribed spacer (ITS) ribosomal DNA (rDNA) primers ITS1/ITS4 and gene *cytochrome oxidase I* (COXI) primers FM55 and FM59. BLAST searches in GenBank indicated that the isolates were *P. aphanidermatum* based on 99 to 100% homology of ITS rDNA. Moreover, the DNA sequences of the ITS and COXI were identical for the five representative isolates. The objective of this research was to determine whether *P. aphanidermatum* recovered from symptomatic spinach tissue

was able to cause foliar web blight and damping-off of spinach and other crops. In addition to spinach, other hosts evaluated included cotton, soybean, pepper, tomato, cucumber, melon, squash, lettuce, corn, wheat, and rice in greenhouse trials. Inoculations were performed by either foliar inoculations or infesting the soil with plugs of potato dextrose agar colonized by the *P. aphanidermatum*. Web blight symptoms were severe on spinach and all other dicotyledonous hosts tested, except lettuce. No web blight symptoms were observed on corn or rice, and only minor symptoms were observed on 10-day-old seedlings of wheat. *P. aphanidermatum* caused severe preemergence damping-off of all dicotyledonous plant species tested but only caused limited seedling size reduction in corn and wheat. Mefenoxam treatment of spinach seed provided complete protection against preemergence damping-off of spinach at both low (0.15 g a.i./kg of seed) and high (0.70 g a.i./kg of seed) rates of application, and the high rate of the application resulted in complete protection against web blight of spinach for 10 to 20 days after planting.

Pythium aphanidermatum can cause seed rot, seedling damping-off, or root and stem rot of a wide range of crops (Sutton et al. 2006). *P. aphanidermatum* can also cause foliar diseases such as cottony blight or cottony leak on various plants such as cucumber fruit (Drechsler 1925), snap bean (Damicone et al. 2012), and turf grass (Allen et al. 2004). These foliar and fruit diseases are often associated with a proliferation of white, cottony mycelium on diseased tissue under favorable environmental conditions (Drechsler 1925).

P. aphanidermatum and *P. dissotocum* have been reported as damping-off and seedling pathogens of spinach in Arizona (Bates and Stanghellini 1984; Gold and Stanghellini 1985; Stanghellini et al. 1984). However, foliar web blight of spinach has not been observed until recently and web blight has not been reported on spinach. A unique foliar disease of spinach, suspected of being caused by *Pythium* spp., was repeatedly observed on spinach in the Yuma County, AZ and Imperial County, CA spinach production areas in October 2015 (Fig. 1) and again in January to March 2016. The foliar symptoms of the disease included water-soaked foliage and rapid collapse of young plants, with luxuriant, white, cottony mycelia on the plant leaves. The disease was associated with hot (27 to 42°C) conditions under sprinkler irrigation with high-density plantings (>8.0 million seeds/ha) commonly used for fresh-market spinach production.

The objectives of this research were to identify the causal organism of foliar web blight of spinach and determine the host range of the spinach foliar web blight pathogen. Foliar web blight and pre- and post-emergence damping-off pathogenicity tests were performed on spinach, cotton, soybean, pepper, tomato, cucumber, melon, squash, lettuce, corn, wheat, and rice in greenhouse trials. The fungicide mefenoxam was evaluated as a seed treatment for the management of foliar web blight

and damping-off of spinach. A preliminary report has been published (Feng et al. 2016).

Materials and Methods

Pathogen isolation, production of reproductive structures, and species identification. Seven different spinach production areas were sampled in Arizona and California from October 2015 to March 2016. Pure *Pythium* cultures were isolated from symptomatic spinach leaves cultured on potato dextrose agar (PDA) (Difco Laboratories, Detroit) amended with antibiotics: chloramphenicol (50 µg/ml), ampicillin (25 µg/ml), and rifampin (10 µg/ml). Five isolates from different fields in Arizona (AZ1, AZ2, AZ3, AZ4, and AZ5) were obtained by transferring the mycelium from the edge of a colony on the modified PDA plates.

The five isolates were examined for sporangia, antheridia, oogonia, and oospore production following the procedures outlined below. Plugs were obtained from the edge of a 3-day-old corn meal agar culture of each isolate. Production of typical asexual and sexual structures was obtained with the grass leaf water culture technique (<http://www.apsnet.org/edcenter/intropp/LabExercises/Pages/OomycetesInstructorNotes.aspx>). Four agar plugs of each isolate with mycelium (diameter = 0.5 cm and height = 0.25 cm) were placed in a sterile petri dish. A shallow layer (20 ml) of sterile deionized water was added to the petri dish, and 10 to 12 leaf pieces (each 0.5 to 1 cm) of autoclaved grass leaves were placed in contact with the colonized agar plugs. Petri dishes containing the water-grass culture were incubated at 22°C under continuous fluorescent light for 5 to 30 days. After sporangia, antheridia, oogonia, and oospores were produced, the asexual and sexual structures, size of reproductive structures, and colony morphology used to identify the species of each isolate were based on the key and descriptions of van der Plaats-Niterink (1981) and Dick (1990) and the monographs and keys of Waterhouse (1967, 1968).

The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) and the mitochondrial encoded *cytochrome oxidase I* (COXI) gene for five isolates (AZ1, AZ2, AZ3, AZ4, and AZ5) were amplified by polymerase chain reaction (PCR) with the universal primers of ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATAGC-3') (White et al. 1990) and primers

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of FM55 (5'-GGCATACCAGCTAAACCTAA-3') and FM59 (5'-TTTATGGTCAATGTAGTGA-3') (Martin 2000). The DNA was extracted from *Pythium* spp. using the DNeasy Plant Mini Kit following the manufacturer's instructions (Qiagen, Valencia, CA). The PCR products amplified with the ITS and COXI primers were purified using a Quick PCR purification kit (Qiagen, Valencia, CA) and directly sequenced using the ITS rDNA and COXI primers at the DNA Resource Center at the University of Arkansas, Fayetteville. Sequencing reactions were carried out with the ABI PRISM Dye Terminator Cycle Sequence Ready Reaction Kit (Applied Biosystems Inc., Foster City, CA).

Web blight test on spinach. Initially, isolates AZ1, AZ2, and AZ3 were used to inoculate on 30-day-old spinach (*Spinacia oleracea*) (Viroflay; Pop Vriend Seed Co., Ankijk, The Netherlands). Twenty spinach seeds were planted in each pot (diameter = 10 cm and height = 10 cm) with potting mix LC1 (Sungro Horticulture Distribution Inc., Agawam, MA). Spinach plants were inoculated with four plugs (0.5 cm in diameter) of 3-day-old PDA colonies of each isolate by placing one plug on the top leaf of each plant. Plants were incubated in a mist chamber for 6 days at 19 to 22°C and a relative humidity (RH) between 90 and 100%. Web blight was rated at 4 to 6 days after inoculation. PDA plugs without *Pythium* colonization were inoculated as the control. The experiment was set up in a completely randomized design with three replications (pots) per treatment, and 16 to 18 plants were grown in each pot. The experiment was repeated once.

Additionally, plants of two commercial spinach cultivars (Viroflay and Carmel; Pop Vriend Seed Co.) and two University of Arkansas spinach breeding lines (08-03 and 04-103) were used to evaluate the potential for these Arizona isolates of *Pythium* to cause foliar web blight on spinach. Spinach plants that were 10, 20, and 30 days old were each inoculated with four colonized PDA plugs (0.5 cm in diameter) of a single isolate (AZ3) taken from 3-day-old PDA colonies by placing the plugs on plant leaves. PDA plugs without *Pythium* colonization were inoculated as a control treatment. Plants were incubated in a mist chamber for 6 days at 19 to 22°C and 90 to 100% RH. Web blight was rated four to 6 days after inoculation. The experiment was set up in a completely randomized design with three replications (pots) per treatment and 16 to 18 plants were grown in each pot. The experiment was repeated once.

Web blight test on different hosts. The potential for the Arizona isolates of *Pythium* to cause foliar web blight signs and symptoms was evaluated on multiple plant species, including spinach (Viroflay), cotton (*Gossypium* sp. 'Arkot 0316'), soybean (*Glycine max* 'Hutchson'), pepper (*Capsicum annuum* 'Tabasco'), tomato (*Solanum lycopersicum* 'Bradley'), cucumber (*Cucumis sativus* 'Alibi' pickling cornichons), melon (*C. melo* 'Roadside'), squash (*Cucurbita pepo* 'Daisy'), lettuce (*Lactuca sativa* 'Iceberg' and 'Romaine'), corn (*Zea mays*, line 203-8, wheat (*Triticum aestivum*, line HUS116), and rice (*Oryza sativa*, line URRN Ent158). Seed were planted in each pot with the same dimensions noted above and in the same potting mix. A different number of seed was planted in each pot depending on seed size: 10 seeds were planted for corn, cucumber, and cotton; 20 seeds were planted for wheat, rice, spinach, soybean, tomato, pepper, and lettuce; and 5 seeds were planted for melon and squash. The seedlings were each inoculated with four colonized PDA plugs of 3-day-old *Pythium* (AZ3) cultures by placing a plug on the leaf surface. Noninoculated plants were used as a control treatment. Plants were incubated in a mist chamber for 6 days, as previously described. Web blight was rated 4 to 6 days after inoculation, as previously described. The experiment was set up in a completely randomized design with three replications (pots) per treatment. The experiment was repeated once.

Disease rating. Web blight disease severity was scored on a disease scale of 0 to 3, where 0 = no symptoms on leaves; 1 = leaves with wilting but without visible white mycelium; 2 = all leaves wilted, with minimum white mycelium; and 3 = leaves completely wilted, with extensive white cottony mycelium.

Damping-off test. Pathogenicity tests were conducted to determine pre- and postemergence damping-off caused by the Arizona

Pythium isolate AZ3 on multiple plant species: spinach (Viroflay), cotton (Arkot 0316), soybean (Hutchson), pepper (Tabasco), tomato (Bradley), cucumber (Alibi pickling cornichons), melon (Roadside), squash (Daisey), lettuce (Iceberg and Romaine), corn (203-8), wheat (HUS116), and rice (URRN Ent158). Ten plugs (diameter = 0.5 cm) of 3-day-old PDA colonized by the *Pythium* isolate were incorporated into the same potting mix used as described above. Seed of the appropriate host plant were then planted in the infested soil. The number of seed planted in each pot was the same as described above. Noninfested soil planted with each of the same host species (cultivars) was used as a control treatment. Plants were watered daily. Seedling counts were recorded 15 days after planting. Symptomatic seed and roots of multiple plants were washed with distilled water, surface sterilized with 70% alcohol for 1 min, then cultured on PDA to verify the presence of the pathogen. The plant height of corn, wheat, and rice was measured at 10, 20, and 30 days after planting. The experiment was set up in a completely randomized design with three replications (pots) per treatment. The experiment was repeated once.

Evaluation of mefenoxam on foliar web blight and damping-off. The effect of mefenoxam spinach seed treatment on web blight and damping-off of spinach was evaluated. The spinach cultivars Banjo and Tambourine (Pop Vriend Seed Co.) were used as described above. The seed were treated with high (0.70 g a.i./kg) and low (0.15 g a.i./kg) rates of mefenoxam to reflect rates commonly used in the European Union and United States, respectively (<http://seminisus.s3.amazonaws.com/wp-content/uploads/2015/09/Seed-Treatment-Tech-Sheets-Spinach-8-18-15.pdf>). Nontreated seed of each cultivar was used as a control treatment. Seedlings were inoculated when they were either 10, 20, or 30 days old. The incubation conditions were as previously described. Web blight ratings were recorded 4 days postinoculation, as described above.

The effects of mefenoxam seed treatment on damping-off tests also were evaluated for Banjo and Tambourine spinach. The treatments included the high and low rates of mefenoxam described above along with nontreated seed of each cultivar as a control treatment. Twenty seeds of each cultivar were planted in each pot. Percent stands (seed germination rates) were recorded 15 days after planting. The experiment was set up in a completely randomized design with three replications (pots) per treatment. The experiment was repeated once.

Statistical analysis and experimental design. All analyses were performed using Statistical Analysis Systems software (PC-SAS 8.0; SAS Institute, Cary, NC). A general linear model (Proc GLM) was used to analyze foliar web blight severity, stand count, and plant heights (tallest leaf of each plant in each pot; all of the plants were measured). Data from the repeated experiments, including the web blight and damping-off experiments, were not significantly different based on a test of homogeneity of variance; therefore, the data were

Fig. 1. Foliar symptoms of web blight were observed on spinach in baby leaf fields in Arizona caused by *Pythium aphanidermatum*, which included water-soaked foliage, rapid collapse of young plants, and white, aerial cottony mycelia.

combined across repeats of an experiment for statistical analyses. Disease ratings were transformed using $R^* = (R1.5 - 1)/1.5$ to obtain a linear scale and normally distributed variable with constant variance (Liu et al. 2016). The result of the analysis of variance ($P > 0.05$) was not different for the transformed data compared with the nontransformed data; hence, analyses of the nontransformed data are presented.

Results

Pathogen isolation, production of reproductive structures, and species identification. Hyphae produced in grass water culture were hyaline, septate, and 2 to 10 μm (average $6.2 \pm 3.8 \mu\text{m}$) wide. Oogonia were terminal, globose, smooth, and 20 to 25 μm (average $22.5 \pm 2.5 \mu\text{m}$) in diameter.

Antheridia were barrel-shaped, intercalary or terminal, while oospores were globose, and 10–14 μm long and 10 to 14 μm wide. Oospores were aplerotic, 17 to 21 μm (average $19 \pm 2.0 \mu\text{m}$) in diameter, and the wall was 2 μm thick. Sporangia were inflated, consisting of terminal complexes of swollen hyphal branches. Encysted zoospores were 11 to 12 μm (average $11.5 \pm 0.05 \mu\text{m}$) in diameter. These morphological characters were consistent with those of *P. aphanidermatum* (Dick 1990; van der Plaats-Niterink 1981; Waterhouse 1967, 1968).

Based on molecular analysis for genus and species identification, BLAST searches (Altschul et al. 1997) showed 100% homology to the ITS rDNA sequences of all five Arizona isolates with the ITS rDNA sequences of *P. aphanidermatum* deposited in GenBank (KT336808.1, KR095341.1, KP063124.1, KP063123.1, KJ755088.1, KJ162355.1, KF561235.1, AY598622.2, JN695784.1, JF775594.1, JF775593.1, EU162763.1, AF452151.1, AF452149.1, and AF452148.1). The GenBank accession number for the ITS rDNA sequences of the *P. aphanidermatum* isolate (AZ3) used in this study was KX397624. BLAST searches (Altschul et al. 1997) showed 99% homology of the COXI sequence of AZ3 with COXI sequences of *P. aphanidermatum* deposited in GenBank (AY129164, HQ708485, and AY564163). The GenBank accession number for the COXI sequences of *P. aphanidermatum* (AZ3) is KY689037.

Web blight test. The pathogenicity tests of AZ1, AZ2, and AZ3 on Viroflay spinach indicated that the three isolates of *P. aphanidermatum* can cause severe web blight symptoms within 2 days of inoculation (data not shown). Inoculation tests on the spinach cultivars (Viroflay, Carmel, line 08-03, and line 04-103) indicated that signs and symptoms of infection were evident after 2 days in the mist chamber following inoculation. Symptoms included water-soaked foliage and the signs included white cottony mycelium on the surface

of infected plants 4 days after inoculation. There were no significant differences in severity of foliar web blight among spinach cultivars (Viroflay, Carmel, line 08-03, and line 04-103) (data not shown) or among plants inoculated at different ages (10, 20, or 30 days old) (Fig. 2, data for Viroflay).

Web blight test on different hosts. Of 13 crop types tested, foliar web blight symptoms were most severe on spinach seedlings that were 10, 20, or 30 days old, followed by pepper, tomato, cucumber, cotton, soybean, melon, and squash (Fig. 3). Symptoms of web blight appeared on spinach leaves (Viroflay) 2 days after inoculation in the mist chamber. Seedlings collapsed in 4 days after inoculation, with abundantly white cottony mycelia visible. However, web blight symptoms were not observed on lettuce (cultivars Iceberg and Romaine) after 6 days in the mist chamber. There was no significant difference in web blight severity ratings among seedlings of spinach, pepper, tomato, cucumber, cotton, melon, and squash inoculated when seedlings were 10, 20, or 30 days old. However, web blight was significantly less severe on 30-day-old soybean seedlings than 10- or 20-day-old soybean seedlings (Fig. 3). No web blight was observed on corn or rice seedlings inoculated when they were 10, 20, or 30 days old. Minor leaf wilting without white mycelium appeared on 10-day-old wheat seedlings (Fig. 3).

Damping-off test on different hosts. *P. aphanidermatum* AZ3 caused severe preemergence damping-off on all dicotyledonous plants tested (spinach, soybean, tomato, cucumber, cotton, squash, pepper, melon, and lettuce) but not on any of the monocotyledonous plants (corn, wheat, and rice) (Fig. 4). *P. aphanidermatum* caused a 100% preemergence damping-off on spinach, soybean, and tomato. Stands of cucumber, cotton, and squash were greatly reduced compared with stands of the control plants of each plant type growing in noninfested potting medium (1.8, 1.7, and 4.3%, respectively). Stands of pepper, melon, and both types of lettuce (Iceberg and Romaine) were reduced as well (16.4, 25, 28, and 39%, respectively) (Fig. 4). *P. aphanidermatum* only caused minor seedling size reduction (not significantly different at $P = 0.05$) to corn and wheat plants but not to rice plants.

Evaluation of mefenoxam for management of web blight and damping-off of spinach. Mefenoxam seed treatment provided complete protection against foliar web blight of spinach when 10- to 20-day-old plants were grown from seed treated at the high rates of mefenoxam and planted into potting medium infested with the AZ3 isolate of *P. aphanidermatum*. The average disease ratings was 0.1 (standard error [SE] 0.096) for both 10- and 20-days seedlings for both Banjo and Tambourine treated with high fungicide rates compared with the disease rating of 3.0 (SE 0.01) for the control

Fig. 2. Web blight severity of 10-, 20-, and 30-day-old seedlings of spinach (cultivar Viroflay) 4 days after inoculation with isolates of *Pythium aphanidermatum* obtained from symptomatic spinach plants in Arizona, and incubated in a mist chamber. Means with different letters are significantly different at $P < 0.05$ based on Fisher's protected least significant difference. Data represent the means of two separate runs of the experiment.

Fig. 3. Web blight severity of 10-, 20-, and 30-day-old seedlings of different hosts 4 days after inoculation with isolates of *Pythium aphanidermatum* obtained from symptomatic spinach plants in Arizona, and incubated in a dew chamber. Means with different letters are significantly different at $P < 0.05$ based on Fisher's protected least significant difference. Data represent the means of two separate runs of the experiment.

plants. Although web blight was observed on spinach plants that grew from seed treated with mefenoxam at the lower rate, web blight severity was significantly less than that observed on spinach plants that grew from the nontreated control seed (Fig. 5). The average disease ratings were 1.83 (SE 0.096), 0.83 (SE 0.095), and 3 (SE 0.01) for Banjo and 1.83 (SE 0.096), 0.67 (SE 0.12), and 3 (SE 0.01) for Tambourine compared with the disease rating of 3.0 (SE 0.01) of the control plants. Leaves of 30-day-old spinach plants that grew from seed treated with mefenoxam at the lower rate of application showed no foliar blight control; however, there was a significant reduction in web blight severity on inoculated spinach plants that grew from seed treated with mefenoxam at the higher rate (Fig. 5). There was no significant effect for the cultivars ($P = 0.25$) and cultivar-seed treatment interaction ($P = 0.71$); however, the effect of low and high rates of fungicide was significantly different ($P = 0.03$). Mefenoxam treatment provided complete protection against damping-off when spinach seed was treated at either the high or low rates of application evaluated. Spinach stands in pots in which the mefenoxam-treated seed was sown into soil infested with *P. aphanidermatum* were 100% those of the spinach stands in pots in which the seed was planted into noninfested soil, when seed was treated with the fungicides at the low or high rates of application (Fig. 6). There was a complete stand loss when nontreated control seed spinach was planted in infested soil (Fig. 6).

Discussion

Based on inoculations of spinach plants in this study, it was evident that the organism recovered from spinach field samples in Arizona with spinach web blight symptoms, identified as *P. aphanidermatum*, was able to cause web blight. The *P. aphanidermatum* was also able to cause severe pre- and postemergence damping-off spinach. In addition, the *P. aphanidermatum* isolates caused severe foliar web blight symptoms on other dicotyledonous plants, including pepper, tomato, cucumber, cotton, soybean, melon, and squash, but not lettuce. Moreover, the severity of web blight symptoms varied among these dicotyledonous plants, with much greater severity observed on spinach, pepper, tomato, cucumber, cotton, melon, and squash, as well as younger (10- and 20-day-old) seedlings of soybean, and with much lower disease severity on 30-day-old soybean plants. *P. aphanidermatum* caused minor web blight symptoms on the leaf of 10-day-old wheat seedlings but did not cause any web blight symptoms on 20- or 30-day-old wheat seedlings (age of plants at the time of inoculation) or on corn or rice seedlings (10-, 20-, or 30-day-old seedlings of either species).

The significant differences in severity of web blight observed on dicotyledonous versus monocotyledonous plants suggest a potential major difference in the resistance mechanisms to web blight between

Fig. 4. Percent stand counts of different plant hosts grown in *Pythium aphanidermatum*-infested soil with the isolates obtained from baby leaf spinach crops in Arizona that displayed symptoms of web blight. Means with different letters are significantly different at $P < 0.05$ based on Fisher's protected least significant difference. Data represent the means of two separate runs of the experiment.

these two groups (Parker 2009). The isolates of *P. aphanidermatum* from Arizona did not cause web blight on the dicotyledonous lettuce (Iceberg and Romaine), which indicates that lettuce plants might have different resistance mechanisms to infection by *P. aphanidermatum* as a web blight pathogen compared with the other dicotyledonous plants evaluated in this study. Understanding the mechanisms of resistance to web blight might provide important information for effective management of *Pythium* web blight on spinach and other host plants.

P. aphanidermatum is considered a broad-host-range species favored by moderate or high temperatures (23 to 27°C) under high RH (Garibaldi et al. 2015; Gold and Stanghellini 1985; Stanghellini et al. 1984). This study showed the same trend of a broad host range for the Arizona isolates obtained from baby leaf spinach crops, and that web blight developed more rapidly and was more severe on spinach than on the other host plants identified under higher temperatures (data not shown). The Arizona isolates of *P. aphanidermatum* caused severe web blight symptoms on spinach in as little as 2 days after inoculation in a mist chamber, with the spinach leaves collapsing and the presence of luxuriant, cottony mycelia. Consequently, *P. aphanidermatum* can cause considerable damage to spinach leaves and lead to yield reduction if environmental conditions are conducive.

Fig. 5. Web blight disease severity of spinach seedlings at 10, 20, and 30 days at 4 days after inoculation. Spinach seed treatments tested include nontreated (a) and low (L = 0.15 g a.i./kg of seed) (b) and high (H = 0.70 g a.i./kg of seed) (c) rates of mefenoxam. Means with different letters are significantly different at $P < 0.05$ based on Fisher's protected least significant difference. Data represent the means of two separate runs of the experiment.

Fig. 6. Percent stand of spinach cultivars Banjo and Tambourine in *Pythium aphanidermatum*-infested soil. Means with different letters are significantly different at $P < 0.05$ based on Fisher's protected least significant difference. Data represent the means of two separate runs of the experiment.

The results of this study indicated that *P. aphanidermatum* can cause severe pre- and postemergence damping-off of all dicotyledonous plants tested in this research, including spinach, soybean, tomato, cucumber, cotton, squash, pepper, melon, and lettuce, but not on the monocotyledonous plants tested, including corn, wheat, and rice. Therefore, crop rotation could potentially be used to reduce soilborne inoculum of *P. aphanidermatum*, although the efficacy of this cultural practice on soilborne inoculum in Arizona and California remains to be evaluated.

Mefenoxam seed treatment provided complete protection against foliar web blight of spinach when 10- to 20-day-old plants were inoculated that grew from seed treated at the high rate of mefenoxam (0.70 g a.i./kg seed). These data indicated that mefenoxam treatment of seed is an effective approach for controlling *Pythium* web blight. Because the Yuma County, AZ and Imperial County, CA areas are the primary winter spinach production areas (October to March) in the United States, mefenoxam seed treatment could be an effective management option for conventional spinach production. For organically grown spinach, web blight management is more of a challenge. For organic production, practices such as crop rotation (to a monocotyledonous crop or a nonhost crop such as lettuce), reduced plant densities, and irrigation practices that allow the plant leaves to dry rapidly between irrigations would likely be helpful at reducing web blight development (<http://anrcatalog.ucanr.edu/pdf/7212.pdf>).

Mefenoxam seed treatment provided complete protection against preemergence damping-off of spinach and protected older plants from web blight. Although the fungicide mefenoxam provided complete protection against both foliar web blight and damping-off of spinach when applied as a seed treatment at a high rate of application in this study, the sensitivity of *P. aphanidermatum* to the fungicide should be monitored for the development of fungicide resistance, because *Pythium* spp. easily develop fungicide resistance to mefenoxam on a large number of ornamentals and horticultural crops (Moorman and Kim 2004). Thus far, there are no reports of mefenoxam resistance with *P. aphanidermatum* on spinach; however,

resistant strains of *P. aphanidermatum* were isolated frequently from floriculture crops in recent years (Lookabaugh et al. 2015).

Integrated disease management is important for reducing the potential for damage caused by *P. aphanidermatum*. Spinach seed treatment at low and high rates of mefenoxam demonstrated the effectiveness of this management approach for both foliar web blight and preemergence damping-off. Additional management approaches could include reduced plant densities to improve air circulation between plants and crop rotation with crops with reduced susceptibility to the web blight pathogen (<http://anrcatalog.ucanr.edu/pdf/7212.pdf>).

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