Translocation of Oxathiapiprolin in Bell Pepper Plants and Systemic Protection of Plants Against Phytophthora Blight

Tianli Qu, Chemistry and Pharmacy College, Qingdao Agricultural University, Shandong 266109, China; and Department of Plant Pathology, University of Georgia, Tifton 31794; Timothy L. Grey, Department of Crop and Soil Sciences, University of Georgia; and Alexander S. Csinos, and Pingsheng Ji, Department of Plant Pathology, University of Georgia, Tifton 31794

Abstract

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Production of bell pepper is seriously affected by *Phytophthora capsici*, the causal agent of Phytophthora blight. Limited approaches are available for effective management of the disease. Oxathiapiprolin is a fungicide recently registered in the United States that suppressed *P. capsici* and reduced Phytophthora blight on bell pepper significantly in our previous studies. It is unknown whether oxathiapiprolin translocates in bell pepper plants systemically after application. Experiments were conducted to determine uptake of oxathiapiprolin by bell pepper plants and its systemic movement in the plant. Quantification of oxathiapiprolin in plant tissues was conducted by high-performance liquid chromatography (HPLC) that detected the compound sensitively and selectively. Percentage of recovery of oxathiapiprolin from plant tissues was calculated by comparing the quantities in plant tissues determined by HPLC with known quantities of the compound added to the plant tissues. Recovery rates of oxathiapiprolin from pepper plant tissues

ranged from 87.0 to 119.3%. When oxathiapiprolin was applied to roots of bell pepper plants grown in hydroculture, the compound was detected in the root within 4 h and in the cotyledon, first true leaf, and second true leaf within 8 h. It was detectable in the top new leaf 48 h after application to the root. In greenhouse studies with bell pepper plants grown in pots, oxathiapiprolin was applied as a soil drench at 100 and 400 $\mu\text{g/ml}$. The compound was detected in the root within 3 days and in the stem and first true leaf within 6 days when applied at 100 $\mu\text{g/ml}$. It was detected in the root, stem, first true leaf, and top new leaf within 3 days when applied at 400 $\mu\text{g/ml}$. Phytophthora blight on pepper foliage was significantly reduced when oxathiapiprolin was applied as a soil drench at 100 or 400 $\mu\text{g/ml}$ under greenhouse conditions. This is the first report indicating systemic movement of oxathiapiprolin in pepper plants that provides useful information for designing fungicide application programs for effective management of the disease.

Phytophthora blight, caused by *Phytophthora capsici*, is responsible for significant yield and quality reduction in the production of vegetable crops worldwide (Erwin and Ribeiro 1996; Hausbeck and Lamour 2004; Sanogo and Ji 2013). The pathogen has a wide host range and vegetable crops seriously affected in the southeastern United States include squash (*Cucurbita pepo*), pepper (*Capsicum annuum*), watermelon (*Citrullus lanatus*), zucchini (*Cucurbita pepo* var. *cylindrica*), cucumber (*Cucumis sativus*), and eggplant (*Solanum melongena*). Bell pepper is among the most susceptible crop that is affected by the disease in both spring and fall growing seasons in Georgia. In addition to root and crown rot, pepper foliage is commonly affected, causing stem lesions and leaf blight that may result in significant growth and yield reduction.

P. capsici is a soilborne pathogen that is difficult to control. Host resistance and crop rotation are recommended strategies for control of soilborne diseases. Some bell pepper cultivars with moderate levels of resistance to Phytophthora blight are commercially available but they usually produce smaller fruit and are not commonly adopted by Georgia growers (Dunn et al. 2013; Foster and Hausbeck 2010; Sanogo and Ji 2012). A challenge in managing Phytophthora blight is that the pathogen survives in irrigation water sources and may produce oospores that can survive in soil for years (Erwin and Ribeiro 1996; Gevens et al. 2007; Krober 1980; Wang et al. 2009). Both mating types of the pathogen have been discovered in major vegetable-growing areas in Georgia; therefore, oospores are likely produced in the fields (Yin et al. 2012). Contaminated irrigation water and long-term survival of oospores in the absence of hosts of the pathogen reduce efficacy of crop rotation for managing the disease.

Application of fungicides is the most common method used by vegetable producers to manage Phytophthora blight. A few new fungicides used for control of *P. capsici* include fluopicolide, mandipropamid, and dimethomorph. Isolates of *P. capsici* from different vegetable production areas are sensitive to fluopicolide, and application of fluopicolide reduced Phytophthora blight on pepper, squash, cucumber, tomato (*S. lycopersicum*), and watermelon under greenhouse and field conditions (Cerkaskas et al. 2015; Foster and Hausbeck 2010; Jackson et al. 2010; Keinath and Kousik 2011; Kousik et al. 2011; Matheron and Porchas 2015; Meyer and Hausbeck 2013). Fluopicolide is a fungicide in the benzamide and pyridine class that delocalizes spectrin-like proteins (Toquin et al. 2008).

Mandipropamid is another commonly used fungicide for control of *P. capsici*, which is in the mandelamide class and inhibits synthesis of lipids and cell membrane (Kuck and Gisi 2008). Mandipropamid inhibited different asexual life stages of *P. capsici* and was effective in reduction of Phytophthora blight on pepper, tomato, and cucurbits (Cerkaskas et al. 2015; Foster and Hausbeck 2010; Jackson et al. 2012; Ji et al. 2011; Kousik et al. 2011; Matheron and Porchas 2015; Meyer and Hausbeck 2013). Dimethomorph belongs to the mandelamide class and has a mode of action similar to that of mandipropamid (Kuck and Gisi 2008; Kuhn et al. 1991). A number of studies indicated that dimethomorph was active in suppression of *P. capsici* and reduction of Phytophthora blight on pepper and other vegetable crops (Jackson et al. 2012; Keinath 2007; Kousik et al. 2011; Matheron and Porchas 2000a, 2000b, 2015; Meyer and Hausbeck 2013; Tamietti and Valentino 2001).

Although resistance in *P. capsici* populations to the above mentioned newer fungicides has not been documented, it is known that this pathogen has the ability to develop fungicide resistance. Resistance to mefenoxam has been reported in various vegetable-producing regions (Café-Filho and Ristaino 2008; Dunn et al. 2010; Gevens et al. 2007; Jackson et al. 2010; Keinath 2007; Lamour and Hausbeck 2000), and more recent research has documented *P. capsici* isolates resistant to cyazofamid in the southeastern United States (Jackson et al. 2012; Kousik and Keinath 2008). Hence, it is highly desirable to identify

Corresponding author: P. Ji; E-mail: pji@uga.edu

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new fungicides with different mode of actions to be used in integrated fungicide programs. Oxathiapiprolin is a new fungicide in the isoxazoline class recently developed by DuPont (Wilmington, DE) that inhibits oxysterol-binding proteins (Cohen 2015; Shepherd et al. 2013). It suppressed asexual life stages of *P. capsici* at lower concentrations than other fungicides targeting oomycete pathogens and reduced Phytophthora blight on bell pepper significantly under field conditions in Georgia (Ji and Csinos 2015). It is unknown whether oxathiapiprolin translocates in pepper plants and provides systemic protection of pepper foliage when applied to the root system. The objectives of this research were to develop a sensitive method to detect oxathiapiprolin in pepper plant tissues and determine whether the compound could move from pepper roots to the foliage. Understanding properties of the fungicide such as its movement in pepper plants will provide valuable information guiding design of application methods of the fungicide for more effective disease management.

Materials and Methods

Detection of oxathiapiprolin by HPLC. Oxathiapiprolin ($\geq 99\%$ purity), provided by DuPont, was dissolved in 1% formic acid in acetonitrile (vol/vol) at concentrations of 1, 5, 10, 25, 50, 100, and 500 $\mu\text{g/ml}$. The solutions were placed in 1.5-ml screw vials (Thermo Scientific, Waltham, MA), with three replicates for each concentration. Analysis was performed by a high-performance liquid chromatography (HPLC) system (Waters 2695; GMI Inc., Ramsey, MN) with a diode-array detector (Waters 2996), an autosampler, and an Empower 3 analytical workstation. Separation was conducted using a XTerra RP C₁₈ column (5 μm , 4.6 by 150 mm; Restek Corp., Bellefonte, PA). Mobile phases A (acetonitrile) and B (0.1% formic acid in water) (45:55, vol/vol) were used with a flow rate of 0.8 ml/min in the isocratic elution. Analytical wavelengths of 240, 260, and 280 nm were compared in preliminary studies, and 260 nm, which yielded the best detection, was used in the following studies. Concentrations of oxathiapiprolin were calculated based on the areas of chromatographic peaks, as described below. Limits of detection and quantification were calculated using the methods reported previously (Luo et al. 2015).

Detection of oxathiapiprolin in plant tissues. To determine whether oxathiapiprolin could be selectively detected in plant tissues, bell pepper ('Aristotle') was grown from seed in 8.5-cm pots containing a commercial potting mix (Scotts Miracle-Gro Co., Marysville, OH) under greenhouse conditions. A randomized complete block design was employed with three replicates. Seedlings at the six- to seven-leaf growth stage, three from each replicate, were removed from pots to be used for treatment with oxathiapiprolin, and three for untreated control. Roots were washed under running tap water to remove soil, rinsed three times with sterile distilled water (SDW), and dried with sterile paper towels. Plant tissues, including root, stem (1 cm from the soil line), cotyledon, and leaf of the three plants from each replicate, were mixed, and approximately 1.0 g of each plant tissue was weighed and placed in a mortar. Oxathiapiprolin was dissolved in 1% formic acid in acetonitrile at 12 $\mu\text{g/ml}$ and 0.25 ml of the solution was added to plant tissue in the mortar and ground in liquid nitrogen. Plant tissues were transferred to 15-ml plastic tubes and extraction of oxathiapiprolin followed the methods described previously, with minor modifications (Wu et al. 2014). Briefly, 0.75 ml of formic acid aqueous solution (1%, vol/vol) and 2 ml MeCN were added in each tube and vortexed for 3 min. NaCl (0.6 g) was added to the tube 30 min later, and the tubes were vortexed for 1 min and centrifuged at 4,000 rpm for 10 min. The supernatant (1.5 ml) was transferred to 2.0-ml dispersive solid-phase extraction tubes (Fisher Scientific, Milford, MA) containing 50 mg of C₁₈, 50 mg of graphitized carbon black, 50 mg of primary-secondary amine, and 150 mg of MgSO₄. The tubes were vortexed for 1 min and centrifuged at 10,000 rpm for 15 min. The supernatants were filtered through 0.45- μm nylon syringe filter (Restek Corp.) and analyzed by HPLC, as described above.

Determination of recovery and stability of oxathiapiprolin. Determination of recovery and stability of oxathiapiprolin was conducted using the methods by Wu et al. (2014), with modifications. Pepper seedlings were grown in the greenhouse as mentioned above

and a randomized complete block design with three replicates was employed. Plant tissues were taken as described above and approximately 1.0 g of tissue was placed in a mortar. Solutions of oxathiapiprolin at 12, 120, and 1,200 $\mu\text{g/ml}$ were prepared as above and 0.25 ml of the solution was added to plant tissues in the mortar and ground in liquid nitrogen. Oxathiapiprolin was extracted from plant tissues and analyzed by HPLC as described above, with six injections of each sample. Percentage of recovery of oxathiapiprolin was calculated by comparing the quantities in plant tissues determined by HPLC with the known quantities of the compound added to the plant tissues. Relative standard deviation of the recovery rates was calculated as reported previously (Zhang et al. 2016). To determine stability of the compound in extracted samples, the samples were kept at 4°C for 5 days. Samples were analyzed by HPLC every day on five consecutive days and percentage of recovery was calculated as described above.

Translocation of oxathiapiprolin from roots to foliage of pepper in hydroculture. Pepper plants were grown and prepared as described above. QGU42 (active ingredient = 10% oxathiapiprolin) was provided by DuPont and a solution of oxathiapiprolin was prepared at 100 $\mu\text{g/ml}$ in SDW. The roots of a seedling were placed in a 250-ml plastic bottle containing 125 ml of oxathiapiprolin solution, with roots submerged in the solution. Roots placed in bottles containing 125 ml of SDW were used as a control. The plants were incubated in a growth chamber at $23 \pm 1^\circ\text{C}$ with a 12-h photoperiod. A randomized complete block design was employed with five replicates. A pepper seedling from each replicate was taken for analysis at 0, 4, 8, 12, 24, 48, 72, 96, 120, and 144 h after placing roots in the fungicide solution. Plant tissues taken for analysis included the root, stem, cotyledon, first leaf, second leaf, and top leaf. Approximately 1.0 g of each plant tissue was taken, weighed, and ground separately in a mortar with liquid nitrogen. Extraction of oxathiapiprolin followed the methods described above and extracted samples were kept at 4°C and analyzed by HPLC within 3 days.

Translocation of oxathiapiprolin from roots to foliage of pepper under greenhouse conditions. Pepper seedlings (Aristotle) were grown from seed in pots containing a potting mix in a greenhouse, as described earlier. QGU42 was diluted in SDW and 20 ml of fungicide solution (100 or 400 $\mu\text{g/ml}$) was applied by soil drench to a pepper plant at the six- to seven-leaf stage. Plants receiving 20 ml of SDW were used as a control. A randomized complete block design was employed with four replicates.

To prepare zoospores of *P. capsici*, isolate PPC7 (Yin et al. 2012) was grown on V8 juice agar plates sealed with parafilm at 25°C for 5 days. The plates with parafilm removed were placed under continuous light at 25°C for 48 h, and 10 ml of SDW was added to each plate. Sporangia were induced to release zoospores by chilling at 4°C for 20 min and sitting at room temperature ($22 \pm 1^\circ\text{C}$) for 20 min. Zoospore suspension was adjusted to 5×10^2 spores/ml by observing with a hemocytometer.

Three days after fungicide application, zoospore suspension was applied to foliage of pepper plants (10 ml/plant) with a handheld sprayer. Soil in the pots was covered by sterile aluminum foil to prevent contact of zoospore suspension with the soil. The plants were incubated in a moisture chamber ($>95\%$ relative humidity) for 24 h, aluminum foil was removed, and the plants were then kept in a greenhouse at 20°C (night) to 28°C (day). Three plants were taken from each replicate for each treatment 3, 6, 14, and 21 days after fungicide application. Approximately 1.0 g of each plant tissue (i.e., root, stem, first leaf, and top leaf) was used for extraction and analysis of oxathiapiprolin using the methods described above.

Evaluation of Phytophthora blight severity. Development of Phytophthora blight was assessed 3, 6, 10, 14, 17, and 21 days after fungicide application. Disease incidence was quantified as percent diseased plants. Disease severity was evaluated using a 0-to-5 scale (Yin et al. 2012), where 0 = no visible symptoms; 1 = $\leq 50\%$ of stem with lesions or $\leq 50\%$ of leaves wilted; 2 = $>50\%$ of stem with lesions or $>50\%$ of leaves wilted; 3 = $\leq 50\%$ of stem with lesions and $>50\%$ of leaves wilted, or $>50\%$ of stem with lesions and $\leq 50\%$ of leaves wilted; 4 = $>50\%$ of stem with lesions and $>50\%$ of leaves wilted; and 5 = dead plant. Area under the disease progress

curve (AUDPC) was calculated based on disease severity using the method published previously (Shaner and Finney 1977). Diseased plants from each treatment were taken for isolation of causal agent on PARPH medium, and isolates were identified by morphological characteristics and polymerase chain reaction analysis using *P. capsici*-specific primers (Yin et al. 2012).

Statistical analysis. All experiments were conducted twice under the same conditions. In the lab experiments to detect different concentrations of oxathiapiprolin (1 to 500 µg/ml) by HPLC, linear regression analysis was conducted using Microsoft Excel (v15.0) to determine linearity of calibration curves of the various concentrations of oxathiapiprolin and coefficient of determination (R^2). Linear regression equation was established to describe the relationship between concentrations of oxathiapiprolin and areas of chromatographic peaks. Data from the hydroculture and greenhouse experiments were analyzed using the general linear model procedures of the Statistical Analysis System (SAS Institute, Cary, NC), and differences among the means of the treatments were separated by Fisher's protected least significant difference test at $P < 0.05$.

Results

Detection, recovery, and stability of oxathiapiprolin. The relationship between concentrations of oxathiapiprolin (y) and areas of chromatographic peaks (x) was best described by the linear regression equation $y = 19,423x + 1,370.2$, in an oxathiapiprolin concentration range from 1 to 500 µg/ml ($R^2 = 0.9999$). Limits of detection and quantification were 0.001 and 0.15 µg/ml, respectively. No interferences appeared around the retention time of oxathiapiprolin (approximately 7.1 min) in the untreated control pepper samples (roots, stems, and leaves) or pepper samples with oxathiapiprolin added (Fig. 1). Oxathiapiprolin was not detected in the untreated control samples. Recovery rates of oxathiapiprolin from plant tissues

were calculated by comparing the quantities in plant tissues determined by HPLC with known quantities of the compound added to the plant tissues. Percent recovery of oxathiapiprolin from treated samples ranged from 97.0 to 118.7% and 87.0 to 119.3% when the samples were analyzed within a day and daily for 5 days, respectively (Table 1). Relative standard deviations of the recovery rates were 1.1 to 5.5%, which were regarded as satisfactory (Zhang et al. 2016).

Translocation of oxathiapiprolin from roots to foliage of pepper in hydroculture. Movement of oxathiapiprolin from root to foliage was assayed at different time intervals after treatment. The compound was detected in the roots 4 h after treatment with an amount of 18.5 µg/g of root (Table 2). The quantity was the highest at 48 h (120.1 µg/g of root) and it was still detectable at 144 h (23.2 µg/g of root). Oxathiapiprolin was detected in the stem,

Table 1. Recovery and stability of oxathiapiprolin added to pepper plant tissues^w

Conc (µg/ml) ^z	Analysis within a day ^x		Analysis in 5 days ^y	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
1	97.0	4.0	87.0	2.9
10	118.7	1.1	93.6	2.1
100	100.8	5.5	119.3	3.1

^w Percent recovery of oxathiapiprolin was amount detected in the samples in comparison with the amount added to the plant tissues. RSD = relative standard deviation of the means calculated as reported previously (Zhang et al. 2016).

^x Samples were treated with oxathiapiprolin and analyzed in the same day.

^y Samples were treated with oxathiapiprolin and analyzed daily on five consecutive days (kept at 4°C).

^z Concentrations of oxathiapiprolin added to pepper plant tissues.

Fig. 1. High-performance liquid chromatography chromatograms of oxathiapiprolin extracted from pepper plant. **A**, Untreated control plant without oxathiapiprolin and **B**, plant treated with oxathiapiprolin.

cotyledon, first true leaf, and second true leaf 8 h after treatment with lower amounts compared with the root, that ranged from 0.6 $\mu\text{g/g}$ of cotyledon to 1.7 $\mu\text{g/g}$ of stem. The compound was detectable in all four foliar tissues within 144 h after treatment, with relatively higher amounts in the stem, first true leaf, and second true leaf at 48 h. The compound was not detected in the top leaves until 48 h after treatment and was detected from 48 to 144 h, ranging from 1.0 to 3.9 $\mu\text{g/g}$ of tissue. Oxathiapiprolin was not detected in the untreated control samples.

Translocation of oxathiapiprolin from roots to foliage of pepper under greenhouse conditions. Oxathiapiprolin was detectable in the roots 3, 6, 14, and 21 days after soil drench with the product in greenhouse. The amounts in the roots did not change over the 21-day period, ranging from 0.6 to 0.8 $\mu\text{g/g}$ of root when oxathiapiprolin was applied at 100 $\mu\text{g/ml}$, and from 0.7 to 0.8 $\mu\text{g/g}$ of root when applied at 400 $\mu\text{g/ml}$ (Table 3). The compound was detected in the stem and first true leaf 6 days after soil drench, with an amount of 0.6 to 0.9 $\mu\text{g/g}$ in stem tissue and 0.6 $\mu\text{g/g}$ in leaf tissue at 6 to 21 days when applied at 100 $\mu\text{g/ml}$. It was detected in the top leaves only at day 21 (0.6 $\mu\text{g/g}$ of leaf) when applied at 100 $\mu\text{g/ml}$. When oxathiapiprolin was applied at 400 $\mu\text{g/ml}$, it was detected in the stem, first leaf, and top leaf within 3 days of application and was still detectable 21 days after application, with amounts ranging from 0.8 to 1.2 $\mu\text{g/g}$ of stem and 0.6 to 1.1 $\mu\text{g/g}$ of leaf. No oxathiapiprolin was detected in the untreated control plants.

Evaluation of Phytophthora blight severity. Microorganisms isolated from symptomatic plants on PARPH were morphologically identified as *P. capsici*, with confirmation by molecular identification. Application of oxathiapiprolin by soil drench reduced Phytophthora blight on pepper foliage significantly when zoospore suspension of *P. capsici* was applied to the foliage. Disease incidences were 22.2 and 16.7% when the product was applied at 100 and 400 $\mu\text{g/ml}$, respectively, which were significantly lower compared with the untreated control (Table 4). AUDPC values were also reduced significantly when it was applied at 100 or 400 $\mu\text{g/ml}$ compared with the control. There was no significant difference in disease reduction when oxathiapiprolin was applied at 100 or 400 $\mu\text{g/ml}$.

Discussion

Oxathiapiprolin is a new fungicide for control of oomycete pathogens and was first registered by Syngenta Crop Protection for commercial use in the United States in 2015 (trade name: Orondis). This compound inhibited mycelial growth, sporangial production, and zoospore germination of *P. capsici* isolates at low concentrations (Ji and Csinos 2015). In field studies conducted in Georgia, oxathiapiprolin applied through drip irrigation or by soil drench plus foliar sprays reduced Phytophthora blight on bell pepper significantly (Ji and Csinos 2015). It was unknown if this compound could translocate systemically in pepper plants. Experiments conducted in this report indicated that oxathiapiprolin moved from roots to stems and

leaves of pepper plants, and application of the compound to pepper roots reduced Phytophthora blight on the foliage significantly.

Previous studies indicated that oxathiapiprolin was effective in suppression of oomycete pathogens. It inhibited zoospore release, cystospore germination, sporangiophore development, and sporangial production of *Pseudoperonospora cubensis*, causal agent of downy mildew of cucurbits (Cohen 2015). In assays using detached leaves of cucurbits, oxathiapiprolin inhibited formation and expansion of lesions on the leaves caused by *P. cubensis*. In another study, foliar applications of oxathiapiprolin reduced downy mildew on basil caused by *Peronospora belbahrii* under greenhouse and field conditions (Patel et al. 2015). In greenhouse studies, seed treatment with oxathiapiprolin significantly reduced downy mildew severity on cucumber and basil (Cohen 2015; Patel et al. 2015). Field studies conducted in 2011 to 2013 in Georgia indicated that oxathiapiprolin

Table 3. Translocation of oxathiapiprolin in pepper plants following soil drench in greenhouse studies

Conc ($\mu\text{g/ml}$) ^y	Days ^z	Oxathiapiprolin in plant tissue ($\mu\text{g/g}$) ^x			
		Root	Stem	First leaf	Top leaf
100	3	0.7 $\pm$ 0.0 fghi	ND	ND	ND
	6	0.8 $\pm$ 0.1 efg	0.6 $\pm$ 0.0 i	0.6 $\pm$ 0.0 i	ND
	14	0.6 $\pm$ 0.0 hi	0.9 $\pm$ 0.3 de	0.6 $\pm$ 0.0 i	ND
	21	0.6 $\pm$ 0.0 i	0.6 $\pm$ 0.0 i	0.6 $\pm$ 0.0 i	0.6 $\pm$ 0.0 i
400	3	0.7 $\pm$ 0.1 fgh	1.1 $\pm$ 0.5 bc	0.6 $\pm$ 0.0 i	0.6 $\pm$ 0.0 i
	6	0.8 $\pm$ 0.0 efg	1.2 $\pm$ 0.4 ab	1.0 $\pm$ 0.3 cd	0.6 $\pm$ 0.0 i
	14	0.8 $\pm$ 0.1 ef	1.2 $\pm$ 0.6 a	1.1 $\pm$ 0.3 bc	0.6 $\pm$ 0.0 i
	21	0.8 $\pm$ 0.1 ef	0.8 $\pm$ 0.1 ef	0.6 $\pm$ 0.0 i	0.6 $\pm$ 0.0 i

^x Values are means of four replicates $\pm$ standard errors of the means. Values followed by the same letters indicate no significant difference ($P = 0.05$). ND = not detected in samples ($<0.41 \mu\text{g/g}$).

^y Concentrations of oxathiapiprolin used for soil drench.

^z Time (days) after application of oxathiapiprolin.

Table 4. Efficacy of soil drench with oxathiapiprolin for reduction of Phytophthora blight on pepper foliage under greenhouse conditions^w

Concentration ($\mu\text{g/ml}$) ^x	Incidence (%) ^y	AUDPC ^z
0	86.1 $\pm$ 6.3 a	717 $\pm$ 38 a
100	22.2 $\pm$ 7.0 b	84 $\pm$ 29 b
400	16.7 $\pm$ 7.5 b	52 $\pm$ 18 b

^w Values are means of four replicates $\pm$ standard errors of the means. Values followed by the same letters indicate no significant difference ($P = 0.05$).

^x Concentrations of oxathiapiprolin used for soil drench.

^y Final disease incidence (percent diseased plants).

^z Area under the disease progress curve.

Table 2. Translocation of oxathiapiprolin in pepper plant in hydroculture after application to the root

Time (h) ^z	Oxathiapiprolin in plant tissue ($\mu\text{g/g}$) ^y					
	Root	Stem	Cotyledon	First leaf	Second leaf	Top leaf
0	ND	ND	ND	ND	ND	ND
4	18.5 $\pm$ 1.3 f	ND	ND	ND	ND	ND
8	30.4 $\pm$ 9.2 de	1.7 $\pm$ 0.2 i	0.6 $\pm$ 0.4 i	0.9 $\pm$ 0.1 i	0.8 $\pm$ 0.1 i	ND
12	37.7 $\pm$ 5.0 d	3.0 $\pm$ 1.4 hi	1.0 $\pm$ 0.7 i	0.9 $\pm$ 0.2 i	0.9 $\pm$ 0.1 i	ND
24	86.7 $\pm$ 8.5 b	3.1 $\pm$ 1.2 hi	1.6 $\pm$ 1.3 i	1.4 $\pm$ 0.1 i	1.2 $\pm$ 0.2 i	ND
48	120.1 $\pm$ 13.5 a	7.5 $\pm$ 1.0 g	1.8 $\pm$ 0.7 i	2.5 $\pm$ 1.3 hi	4.4 $\pm$ 1.6 h	3.9 $\pm$ 2.1 hi
72	54.6 $\pm$ 15.8 c	2.9 $\pm$ 1.3 hi	1.8 $\pm$ 0.1 i	1.1 $\pm$ 0.0 i	1.1 $\pm$ 0.2 i	1.2 $\pm$ 0.1 i
96	37.9 $\pm$ 6.5 d	3.0 $\pm$ 2.8 hi	1.4 $\pm$ 0.2 i	1.2 $\pm$ 0.1 i	1.7 $\pm$ 0.8 i	1.4 $\pm$ 0.4 i
120	31.3 $\pm$ 10.5 d	1.3 $\pm$ 0.0 i	1.1 $\pm$ 0.1 i	1.0 $\pm$ 0.1 i	1.4 $\pm$ 0.7 i	1.7 $\pm$ 0.7 i
144	23.2 $\pm$ 11.7 ef	2.3 $\pm$ 0.9 i	1.2 $\pm$ 0.1 i	1.0 $\pm$ 0.2 i	1.0 $\pm$ 0.1 i	1.0 $\pm$ 0.5 i

^y Values are means of five replicates $\pm$ standard errors of the means. Values followed by the same letters indicate no significant difference ($P = 0.05$). ND = not detected in samples ($<0.41 \mu\text{g/g}$).

^z Time (h) after application of oxathiapiprolin.

applied through transplant water and directed sprays significantly reduced black shank of tobacco caused by *Phytophthora nicotianae* (Ji et al. 2014). In a study on chili pepper, plant mortality caused by *P. capsici* was significantly decreased in greenhouse trials with soil applications of oxathiapiprolin; however, oxathiapiprolin did not significantly reduce final plant mortality in field trials (Matheron and Porchas 2015). Ineffective disease suppression observed in the field may be due to lack of efficient movement of oxathiapiprolin in the soil to the root zone when applied by furrow irrigation (Matheron and Porchas 2015).

Systemic movement of oxathiapiprolin in plants was assayed indirectly in cucumber (Cohen 2015). In studies with detached cucumber leaves, oxathiapiprolin applied to one side of the leaf reduced disease on the other side of the leaf. On cucumber grown in pots in a greenhouse, oxathiapiprolin spray applied to the first true leaf reduced downy mildew caused by *P. cubensis* on the second true leaf, and the compound applied as a soil drench to the roots of cucumber provided complete protection of the leaves against the disease (Cohen 2015). These studies indicated that oxathiapiprolin might translocate from leaves to leaves and from the root to the foliage of cucumber plants. The indirect method is easier and more convenient to use than direct detection by HPLC. However, the indirect method could not quantify how much oxathiapiprolin was translocated from one plant tissue to another. In addition, disease reduction by application of chemical compounds might be due to mechanisms such as induced plant systemic resistance; hence, it was uncertain whether oxathiapiprolin translocated in the plants in studies using the indirect methods. An analytical method for direct detection of oxathiapiprolin in plants was developed by Wu et al. (2014) using liquid chromatography coupled with tandem mass spectrometry. In the seven crop plants studied (cucumber, grape [*Vitis vinifera*], potato [*S. tuberosum*], tomato, watermelon, watermelon peel, and wheat [*Triticum aestivum*]), the limit of detection for oxathiapiprolin was 0.5 µg/liter. Percent recoveries of oxathiapiprolin from tissues of the seven crops ranged from 82.0 to 110.7%. The analytical method also successfully detected two metabolites of oxathiapiprolin, IN-E8S72 and IN-WR791, in the plant tissues (Wu et al. 2014). IN-E8S72 and IN-WR791 did not show activities to suppress oomycete pathogens (C. P. Shepherd, personal communication).

Although direct detection of movement of oxathiapiprolin in plants was not reported previously, movement of some other fungicides used for control of oomycete pathogens was studied in a few crops. Fluopicolide could be absorbed by cucumber roots from aqueous solutions but it was not detected in cucumber roots after spray application to the leaves (Rupprecht 2007). In a study on tomato, fluopicolide applied to soil was absorbed by tomato roots and then translocated to the stems and leaves. It was detected in tomato roots for more than 30 days and in leaves and stems 20 days after application (Jiang et al. 2015). In tomato, 20% fosetyl-Al translocated from the roots to the foliar parts 3 h after application, and the quantities of the fungicide in roots and foliar parts were similar after 72 h (Leconte et al. 1988). In translocation research using ¹⁴C-metalaxyl in tomato and other plants, the fungicide moved from lower to upper leaves and vice versa (Zaki et al. 1981). It was translocated laterally and upward to the leaves after the fungicide was absorbed by the stem. A minor proportion of the labeled fungicide was detected in the root after application to the leaf or stem (Zaki et al. 1981).

The present study is the first report to directly detect translocation of oxathiapiprolin in plants. Wu et al. (2014) conducted research to recover oxathiapiprolin from a few plants other than pepper but did not detect translocation of the compound in the plants. The limit of detection was 0.001 µg/ml and average recoveries from pepper plant samples ranged from 87.0 to 119.3% in the present study, which were similar to the detection limit and recovery rates in other plants studied (Wu et al. 2014). The detection method was able to sensitively detect oxathiapiprolin when it was applied to pepper plant tissues in lab studies or applied to the roots of potted pepper plants under greenhouse conditions. In greenhouse studies, oxathiapiprolin was detected in pepper stems and leaves at 0.6 to 1.1 µg/g of tissue after application of the compound to the roots. The amount of oxathiapiprolin in the

pepper plant tissues should be high enough to suppress asexual life stages of *P. capsici* since the dose of the compound at which 50% inhibition occurs for inhibiting mycelial growth, sporangial production, and zoospore germination of *P. capsici* isolates averaged 0.001, 0.0003, and 0.54 µg/ml, respectively (Ji and Csinos 2015). This coincided with the significant disease reduction on the foliage when oxathiapiprolin was applied to the soil and root in the greenhouse studies. In the studies with potted plants, oxathiapiprolin was detected at lower quantities in plant tissues than in the hydroculture studies. This was probably due to absorption of oxathiapiprolin by the potting mix used in the studies. Further studies could be conducted in greenhouse and field settings to evaluate the effects of different soil types on absorption and translocation of oxathiapiprolin. In research to determine translocation of oxathiapiprolin from roots to foliage of pepper in hydroculture, oxathiapiprolin at 1.8 to 120 µg/g of tissue was detected in plant samples 48 h after application of the compound to the roots. Quantities of oxathiapiprolin in plant tissues declined after 48 h. Although this may be related to plant metabolism, the reason for the decline is uncertain and remains to be elucidated. Further research can also be conducted to detect whether oxathiapiprolin moves from pepper foliage to the roots, and its translocation in other crops susceptible to *P. capsici*. These studies will provide a more comprehensive understanding of oxathiapiprolin plant activity, which can be valuable for developing more effective or economical fungicide application methods for managing *Phytophthora* blight on vegetables.

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