Fungicide Sensitivity of *Pythium* spp. Associated with Cavity Spot of Carrot in California and Michigan

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Abstract

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The identity of 172 isolates of *Pythium* spp. from cavity spot lesions on carrot produced in California and Michigan was determined, and their sensitivity to three fungicides was examined. *Pythium violae* accounted for 85% of California isolates, with *P. irregulare*, *P. dissotocum* (the first report as a carrot pathogen in the United States), *P. ultimum*, and *P. sulcatum* making the balance. *P. sulcatum*, *P. sylvaticum*, and *P. intermedium* were the most commonly recovered (85%) species in Michigan; others from Michigan included *P. intermedium*, *P. irregulare*, and an unclassified strain, M2-05. On fungicide-amended media, 93% of isolates were sensitive to mefenoxam (inhibition of mycelial growth was >60% at 10 µg active ingredient [a.i.]/ml); however, two of five isolates of *P. irregulare* from California were highly resistant

(≤60% inhibition at 100 µg a.i./ml); about half of the isolates of *P. intermedium* and *P. sylvaticum* and a single isolate of *P. violae* were highly or intermediately resistant to mefenoxam (>60% inhibition at 100 µg a.i./ml, or ≤60% inhibition at 10 µg a.i./ml). *P. dissotocum*, *P. irregulare*, *P. sulcatum*, M2-05, and three of seven isolates of *P. intermedium* were insensitive to fluopicolide (effective concentrations for 50% growth inhibition [EC₅₀] were >50 µg a.i./ml), while *P. sylvaticum*, *P. ultimum*, *P. violae*, and some isolates in *P. intermedium* were sensitive (EC₅₀ < 1 µg a.i./ml). All isolates were sensitive to zoxamide (EC₅₀ < 1 µg a.i./ml). Sensitivity baselines of *P. violae* to zoxamide and fluopicolide were established.

Carrot (*Daucus carota* L.) is planted on over 35,000 ha in the United States, with an approximate value of \$600 million (3). California and Michigan are the two largest carrot-producing states for the fresh market, accounting for 89% of U.S. production (5). Carrot cavity spot (CCS), an economically important soilborne disease, is one of the limiting factors in the production of high-quality roots (33). Disease symptoms are characterized by sunken elliptical lesions on the surface of the tap root. The lesions darken in color and increase in size as the disease progresses (20,27,38).

Many *Pythium* spp. have been reported to cause CCS but the profile of species varies from region to region (1,12,15,27). In Japan, Australia, certain European countries, and some regions of North America, the slow-growing species *Pythium violae* and *P. sulcatum* are the most important causal agents of CCS (17,20,42), whereas the fast-growing species *P. sylvaticum*, *P. ultimum*, and *P. intermedium* are the most common species associated with CCS in Canada (2), Norway (19), and the United Kingdom (41). Other *Pythium* spp., including *P. coloratum* (14), *P. rostratum* (41), *P. dissotocum* (2), *P. macrosporum* (2,22), *P. irregulare* (20), and *P. aphanidermatum* (40), also have been associated with CCS. At least four *Pythium* spp. associated with CCS in California have been reported (18,38) but the causal agents of CCS in Michigan have not been well documented (31).

Management of CCS is difficult. Crop rotation is often ineffective because *Pythium* spp. generally have broad host ranges (12,13,21,32). Partial resistance to CCS is limited to a few carrot cultivars (7,8,20), and minimizing the disease with irrigation management may moderate the disease to a small extent (28,38). Most growers judiciously use fungicides to minimize the impact of CCS but the performance of fungicides has been variable. Metalaxyl or

its enantiomer mefenoxam has long been used (10,11,20) but its efficacy has been reduced by enhanced microbial degradation (15), reduced persistence of the chemical in soil (11), and emerging resistant pathogen populations (2,22). Zoxamide (FRAC Code 22), which is not currently registered for use on carrot, effectively inhibited in vitro mycelial growth of *P. sulcatum*, *P. sylvaticum*, and *P. macrosporum* in laboratory conditions, and provided significant and consistent control of CCS caused by *P. sulcatum* in greenhouse assays (22). However, the impact of zoxamide on other *Pythium* spp. remains unknown. In most states, the fungicide fluopicolide (FRAC Code 43) is registered for use on vegetables, except carrot. In general, fluopicolide effectively reduces the impact of diseases caused by oomycete pathogens, including some *Pythium* spp. (36), but no data are available for CCS control. Although both zoxamide and fluopicolide inhibit mitosis and cell division (4), the cellular targets differ and the spectrum might be different. Thus, zoxamide and fluopicolide represent new fungicides with the potential to manage CCS, and their registration for use on carrot in California and Michigan is anticipated. For these reasons, the objectives of this study were to identify the pathogen complex and determine the fungicide sensitivity of the pathogen complex of CCS in California and Michigan.

Materials and Methods

Isolation and identification of *Pythium* spp. Freshly harvested carrot roots with cavity spot symptoms were collected in 2010 from four commercial carrot fields in Michigan, including three in Pentwater County and one in Fremont County, and from four fields in Kern County, CA. The samples were processed immediately after shipment to the laboratory. Carrot roots were washed in running tap water and blotted dry. Tissue from the margin of cavity spot lesions was excised, surface disinfested with 10% bleach (0.62% NaClO) for 3 min, rinsed in sterile distilled water three times, air dried, and cut into about 5-mm (in length) pieces. The tissue was placed on water agar (WA) media amended with carben-dazim at 10 µg /ml, ampicillin at 50 µg /ml, rifampicin at 50 µg /ml, and PCNB at 10 µg /ml (CARP). When mycelial growth from the diseased tissue was observed 3 to 5 days later, hyphal tips were

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transferred to potato dextrose agar (PDA; EMD Chemicals Inc., Gibbstown, NJ) plates amended with CARP for later use. For culture storage, all isolates were cultured on WA for a week, then cut into small mycelial plugs (4 mm in diameter) and placed in a 15-ml test tube containing 10 ml of sterile distilled water and five sterilized hemp seeds. Cultures were stored at room temperature ($22 \pm 1^\circ\text{C}$).

The identification of each isolate was determined by morphological characteristics and DNA sequences. For morphological identification, isolates were cultured in sterilized, distilled water with grass (*Festuca arundinacea*) blades at $22 \pm 1^\circ\text{C}$ under fluorescent light, with four replicates per species, as described by Waterhouse (39), and examined based on the key of van der Plaats-Niterink (37). DNA was extracted from the isolates using the following procedure. A mycelial culture plug (4 mm in diameter) of a *Pythium* sp. was placed on a disc (8 mm in diameter) of cellophane (Fisher Scientific Co., Pittsburgh, PA) overlaid on PDA-CARP, and incubated for 2 to 5 days. When the colony nearly reached the edge of the cellophane, the mycelium on the cellophane disc was scraped off and DNA was extracted using a cetyltrimethylammonium bromide procedure (30). The extracted DNA was used as a template for polymerase chain reaction (PCR) to amplify the rDNA sequences of the internal transcribed spacer (ITS) with primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (43). The thermal cycler setting was an initial denaturation at 94°C for 4 min; followed by 36 cycles of 30 s at 94°C , 30 s at 58°C , and 45 s at 72°C ; and an extension at 72°C for 10 min. The PCR products were purified with the IsoPure PCR Purification Kit (Denville Scientific Inc., South Plainfield, NJ) and submitted to Michigan State University Genomics Core Facility for sequencing (ABI 3730xl; Applied Biosystems, Inc. Foster, CA). Both DNA strands of the ITS region were sequenced. The resulting ITS sequences were compared with sequences of closely related species and deposited at the National Center for Biotechnology Information GenBank database.

Virulence of *Pythium* spp. Harvested carrot roots were inoculated following a previously described method (19). Briefly, mature carrot roots ('Nantindo') were gently washed with tap water, surface sterilized in 10% bleach for 3 min, rinsed with sterile distilled water three times, and placed on a plastic grid in a plastic box with water below the level of the grid to maintain high humidity. Inoculum consisted of mycelial plugs (4 mm in diameter) cut with a cork borer from the margin of actively growing colonies of *Pythium* spp. on PDA-CARP. Four mycelial plugs were placed linearly on one carrot root at 3-cm intervals, with the mycelial side facing down on the carrot. On each root, two agar plugs (without mycelia) were used as controls. Three replicate carrot roots were inoculated with each isolate. The inoculated roots were incubated in darkness at room temperature ($22 \pm 1^\circ\text{C}$). During the incubation period, sterile distilled water was gently sprayed daily over the roots to maintain high moisture. The diameters of the lesions were measured perpendicularly 1 week after inoculation, and the two measurements were averaged. Four independent experiments were conducted for all *Pythium* spp. but *P. ultimum*, *P. sylvaticum*, and M2-05 were tested two times independently.

Fungicide sensitivity of *Pythium* spp. Sensitivity to mefenoxam was examined as described (29), with slight modification.

Mefenoxam (47.6% active ingredient [a.i.], Ridomil Gold EC; Novartis Crop Protection, Inc. Greensboro, NC) was dissolved in dimethyl sulfoxide (DMSO) to make a stock solution ($5 \times 10^4 \mu\text{g}$ a.i./ml). Mefenoxam was added to PDA to final concentrations of 0, 10, or $100 \mu\text{g}$ a.i./ml and final DMSO concentration was adjusted to 0.1% in PDA. Agar plugs (4 mm in diameter) covered with actively growing mycelium of the different isolates were cut and transferred to plates containing mefenoxam. Triplicate plates were incubated for each isolate per concentration at $22 \pm 1^\circ\text{C}$ in the dark. Colony diameters were measured in two perpendicular directions for all plates when the diameters of the controls were at least 30 mm (the incubation time for this measurement varied depending on the species). The sensitivity to fungicides was expressed as percent inhibition of mycelial growth [$100 \times (\text{control} - \text{treated})/(\text{control} - 4 \text{ mm})$]. Isolates were categorized as sensitive when the inhibition was $>60\%$ with mefenoxam at $10 \mu\text{g}$ a.i./ml, highly resistant when the inhibition was $\leq 60\%$ at $100 \mu\text{g}$ a.i./ml; and intermediately resistant when the inhibition was $\leq 60\%$ at $10 \mu\text{g}$ a.i./ml and $>60\%$ at $100 \mu\text{g}$ a.i./ml. These thresholds provided excellent resolution of resistant and sensitive isolates in previous studies (2,29,34).

Fungicide-amended agar with various concentrations (0, 0.01, 0.1, 1, and $10 \mu\text{g}$ a.i./ml) of fluopicolide (Presidio, 39.5% a.i.; Valent BioScience Co., Libertyville, IL) or zoxamide (80% a.i.; Gowan Co., Yuma, AZ) were tested in pilot experiments. If the growth of isolates on plates with fungicides at $10 \mu\text{g}$ a.i./ml was more than 50% of controls, two concentrations of fungicides were used (10 and $50 \mu\text{g}$ a.i./ml); if the growth of isolates on plates with fungicides at $10 \mu\text{g}$ a.i./ml was less than 50% of controls, then five concentrations were used (Table 1). To do this, the fungicide was dissolved in DMSO to make a stock solution ($1 \times 10^4 \mu\text{g}$ a.i./ml). Cultural plugs of *Pythium* spp. were transferred to the fungicide-amended medium for incubation as described above and three replicate plates for each isolate. The effective concentration of the fungicide to inhibit 50% of mycelial growth (EC_{50}) was determined as described above. All fungicide sensitivity studies were repeated.

Statistical analysis. Data were analyzed with SAS software (version 9.2; SAS Institute Inc. Cary, NC). Procedure GLM was used for an analysis of variance, and Fisher's least significant difference multiple range test was performed for separating means. EC_{50} values were calculated using regression analysis (using procedure REG) of percentage of growth inhibition against the logarithmic values of fungicide concentrations to the base 10.

Results

Isolation and distribution of *Pythium* spp. In total, 172 *Pythium*-like isolates were obtained from 169 isolations from carrot roots with cavity spot symptoms. In most cases, one species was obtained per lesion. More than one *Pythium*-like species was obtained from three lesions. All *Pythium* isolates were classified into nine species based on morphological characteristics and DNA sequences. These included *P. violae* and *P. dissotocum* only from California, and *P. intermedium*, *P. periplocum*, and *P. sylvaticum* only from Michigan (Table 2). *P. irregulare*, *P. sulcatum*, and *P. ultimum* were isolated from both California and Michigan (Table 2). The sexual stage of strain M2-05 was not observed due to a single isolate obtained; therefore, the morphological characters

Table 1. Concentrations of fluopicolide and zoxamide used for determining *Pythium* spp. sensitivity

<i>Pythium</i> spp.	Concentration (μg active ingredient/ml)	
	Fluopicolide	Zoxamide
<i>Pythium dissotocum</i>	0, 10, 50	0, 0.03, 0.06, 0.09, 0.18, 0.4
<i>P. intermedium</i>	0, 10, 50	0, 0.05, 0.1, 0.2, 0.4, 1
<i>P. irregulare</i>	0, 10, 50	0, 0.05, 0.1, 0.5, 1, 4
<i>P. sulcatum</i>	0, 10, 50	0, 0.02, 0.04, 0.08, 0.1, 0.4
<i>P. sylvaticum</i>	0, 0.05, 0.1, 0.2, 0.4, 1	0, 0.05, 0.1, 0.2, 0.4, 1
<i>P. ultimum</i>	0, 0.4, 0.6, 0.8, 1.2, 2	0, 0.005, 0.01, 0.025, 0.05, 0.1
<i>P. violae</i>	0, 0.4, 0.6, 0.8, 1.2, 2	0, 0.008, 0.012, 0.024, 0.036, 0.048
Strain M2-05	0, 10, 50	0, 0.05, 0.1, 0.2, 0.4, 1

were not fully described, and its ITS sequence was 100% identical to *P. recalcitrans*.

Virulence of *Pythium* spp. The size of the lesions varied depending on *Pythium* spp. ($P < 0.0001$), and there was a difference in lesion size between isolates within some species ($P < 0.0001$). The size of the lesions caused by the various isolates also varied by trial. Both *P. intermedium* and *P. ultimum* caused the largest lesions except, in one trial, strain M2-05 and *P. violae* were the least virulent species. Small lesions were observed in two of four trials when carrot was inoculated with *P. periplocum* but *P. periplocum* was

reisolated in none of the trials, indicating that it was not pathogenic to carrot (Table 3).

Fungicide sensitivity of *Pythium* spp. Mefenoxam. Most (93%) isolates of *Pythium* spp. were sensitive to mefenoxam. However, 3 of 7 Michigan isolates of *P. intermedium*, 5 of 10 Michigan isolates of *P. sylvaticum*, and a single California isolate of *P. violae* had an intermediate level of resistance. Two of eight isolates of *P. irregulare* from California were highly resistant to mefenoxam (Table 4).

Fluopicolide. All isolates of *P. dissotocum*, *P. irregulare*, *P. sulcatum*, and strain M2-05 were insensitive to fluopicolide ($EC_{50} >$

Table 2. *Pythium* spp. recovered from carrots with cavity spot symptoms in California and Michigan in 2010 and identified using morphological characteristics and sequence of the internal transcribed sequences regions of rDNA

Locations ^w	N ^x	Number of isolates									Total
		<i>Pythium dissotocum</i>	<i>P. intermedium</i>	<i>P. irregulare</i>	<i>P. periplocum</i>	Strain M2-05	<i>P. sulcatum</i>	<i>P. sylvaticum</i>	<i>P. ultimum</i>	<i>P. violae</i>	
California											
Kern County 1*	70	3 ^y	...	4	...	...	3	...	1	61 ^y	72
Kern County 2	46	1	...	1	...	...	...	...	...	44	46
Kern County 3	17	...	...	...	...	...	...	...	...	17	17
Kern County 4	3	...	...	...	...	...	...	...	3	...	3
Subtotal	136	4	...	5	...	...	3	...	4	122	138
Michigan											
Pentwater County 1*	5	...	2 ^z	...	...	...	4 ^z	...	...	...	6
Pentwater County 2	12	...	...	1	...	...	6	5	...	...	12
Pentwater County 3	3	...	...	1	...	...	2	...	...	...	3
Fremont County	13	...	5	1	1	1	...	5	...	...	13
Subtotal	33	...	7	3	1	1	12	10	...	...	34
Total	169	4	7	8	1	1	15	10	4	122	172

^w Asterisks (*) represent different fields.

^x Number of isolations.

^y Two isolations were a combination of *P. dissotocum* and *P. violae*.

^z One isolation was a combination of *P. intermedium* and *P. sulcatum*.

Table 3. Virulence of *Pythium* spp. on carrot roots as expressed by lesion size

<i>Pythium</i> spp.	Lesion diameter (cm) ^z							
	N	Trial 1	N	Trial 2	N	Trial 3	N	Trial 4
<i>Pythium ultimum</i>	16	1.7 a	16	1.9 a	—	—	—	—
<i>P. intermedium</i>	20	1.7 a	28	1.3 bc	24	1.6 a	28	1.5 a
<i>P. sylvaticum</i>	40	1.6 a	40	1.2 bc	—	—	—	—
<i>P. sulcatum</i>	40	1.6 ab	38	1.5 b	60	0.8 b	44	1.1 ab
<i>P. irregulare</i>	28	1.1 b	32	1.1 c	32	1.0 b	28	0.8 b
<i>P. dissotocum</i>	12	1.0 b	12	1.2 c	8	1.0 b	6	0.7 bc
<i>P. violae</i>	48	0.6 c	56	0.4 d	56	0.3 c	56	0.3 c
Strain M2-05	4	0.6 c	4	0.5 d	—	—	—	—
<i>P. periplocum</i>	4	0.0 c	4	0.1 d	4	0.0 d	4	0.3 c

^z Lesion diameters were measured perpendicularly and averaged. Mean values followed by the same letter are not significantly different in the same column according to Fisher's least significant difference method at $\alpha = 0.05$. N = number of observations and — = not tested.

Table 4. In vitro sensitivity of *Pythium* spp. to mefenoxam, zoxamide, and fluopicolide

<i>Pythium</i> spp.	Mefenoxam		Fluopicolide		Zoxamide	
	N ^x	Sensitivity ^y	N	EC ₅₀ (μg a.i./ml) ^z	N	EC ₅₀ (μg a.i./ml)
<i>Pythium dissotocum</i>	3	S	4	> 50	4	0.09 ± 0.03
<i>P. intermedium</i>	4	S	4	< 10	7	0.08 ± 0.02
<i>P. irregulare</i>	3	IR	3	> 50	...	...
	6	S	7	> 50	8	0.52 ± 0.24
	2*	HR	...	...	...	...
<i>P. sulcatum</i>	12	S	15	> 50	15	0.03 ± 0.02
<i>P. sylvaticum</i>	5	S	10	0.16 ± 0.06	11	0.18 ± 0.05
	5	IR	...	...	...	...
<i>P. ultimum</i>	3	S	3	0.40 ± 0.05	4	0.021 ± 0.001
<i>P. violae</i>	115	S	107	0.90 ± 0.27	110	0.024 ± 0.008
	1*	IR	...	...	...	...
Strain M2-05	1	S	1	> 50	1	0.06

^x N = number of isolates tested and asterisks (*) indicate that isolates tested were from California.

^y Sensitivity to mefenoxam was determined by the inhibition of mycelial growth on potato dextrose agar amended with mefenoxam at 10 and 100 μg a.i./ml relative to that on the nonamended plate. S = sensitive, >60% on 10 μg a.i./ml; IR = intermediately resistant, ≤60% on 10 μg a.i./ml and >60% on 100 μg a.i./ml; and HR = resistant, ≤60% on 100 μg a.i./ml.

^z EC₅₀ = the effective concentration for 50% mycelial growth inhibition.

50 µg a.i./ml; Table 4). Four of seven isolates of *P. intermedium* were sensitive to fluopicolide, with mycelial growth inhibition >77% at 10 µg a.i./ml of fluopicolide ($EC_{50} < 10$ µg a.i./ml); the radial growth of the other three isolates was inhibited <30% with fluopicolide at 50 µg a.i./ml ($EC_{50} > 50$ µg a.i./ml; Table 4). *P. ultimum* (mean value of $EC_{50} = 0.4$ µg a.i./ml), *P. sylvaticum* ($EC_{50} = 0.16$ µg a.i./ml), and *P. violae* ($EC_{50} = 0.9$ µg a.i./ml) were all sensitive to fluopicolide. The EC_{50} baseline for 107 isolates of *P. violae* ranged from 0.4 to 1.7 µg a.i./ml and was distributed as a unimodal curve (Fig. 1). The results of the two trials were combined.

Zoxamide. All eight *Pythium* spp. were sensitive to zoxamide, although their sensitivities varied (Table 4). EC_{50} ranged between 0.52 µg a.i./ml for *P. irregulare* and 0.021 µg a.i./ml for *P. ultimum*. Strain M2-05 was not compared because only one isolate was examined. A sensitivity baseline was established using 110 *P. violae* isolates, with EC_{50} values of 0.01 to 0.06 µg a.i./ml, which were distributed in a unimodal curve (Fig. 2).

Discussion

In this survey of fields in California and Michigan, multiple *Pythium* spp. were associated with CCS, as suggested by others (17,20,42). The species distribution and frequency, however, were dependent on the location of the fields. *P. violae* was the primary cause of CCS in California, consistent with historical reports (17,20,42). In contrast, *P. sulcatum*, *P. sylvaticum*, and *P. intermedium* accounted for the majority of isolations from CCS in Michigan. *P. sulcatum* and *P. irregulare* were recovered in both California and Michigan. Surprisingly, *P. violae*, *P. dissotocum*, and *P. ultimum* were not recovered in Michigan, and *P. sylvaticum*, *P. intermedium*, and M2-05 were not isolated from California carrot. The different distributions of *Pythium* spp. associated with CCS may reflect different climates, soil types, or other factors of the two states (6). This is the first report of *P. dissotocum* from carrot in the United States, following a report in the United Kingdom (41) and Canada (2).

The ITS sequence of strain M2-05 was 100% identical to the ITS sequence of *P. recalcitrans*, reported as a pathogen of grapevine and common beet (25) (GenBank accession number EF195139), but their β -tubulin sequences were only 87% identical (X. H. Lu, unpublished). Because this is the first report in carrot, additional studies need to be conducted to determine the taxonomy compared with the known *P. recalcitrans* from other crops. The only isolate of *P. periplocum* examined in this study was not pathogenic to carrot. This species could be an opportunistic organism, because it has been reported as a mycoparasite of *Botrytis cinerea* (26), *Fusarium culmorum* (9), and *P. ultimum* (1). Its ecological role in carrot fields needs to be determined.

Fig. 1. Distribution of effective concentrations of fluopicolide at which 50% of mycelial growth was inhibited (EC_{50}) for 107 isolates of *Pythium violae*. The frequency (%) is the proportion of isolates with EC_{50} values in the range provided.

Mefenoxam-resistant isolates of *P. dissotocum*, *P. irregulare*, *P. sylvaticum*, *P. sulcatum*, and *P. ultimum* have been reported from carrot (2,22) and other crops (16,23,24,29,34,35). Similarly, two *P. irregulare* isolates from California were highly resistant to mefenoxam, and approximately half of the isolates of *P. intermedium* and *P. sylvaticum* from Michigan were intermediately resistant to mefenoxam. This is the first report of resistance to mefenoxam within a population of *P. intermedium* and *P. violae*, which all were collected from the field. Thus, mefenoxam may not provide satisfactory CCS control in all areas as resistant populations develop. Because of this, new fungicides are urgently needed for the carrot industry. In this study, zoxamide reduced mycelial growth of all *Pythium* spp. tested, and should be considered a candidate for CCS control as an alternative to mefenoxam.

The sensitivity baselines of zoxamide and fluopicolide for *P. violae*, the most important cause of CCS in California, were established here. These baselines will be helpful for monitoring sensitivity shifts within a population of a pathogen, and may provide useful information on the management of fungicide resistance if fluopicolide and zoxamide are adopted by the carrot industry as expected. Although baseline sensitivities for other *Pythium* spp. are also important, they could not be established here because few isolates were available. All isolates of *P. violae*, *P. ultimum*, and *P. sylvaticum* were sensitive to fluopicolide, indicating that fluopicolide may be another alternative to mefenoxam, especially in California, where *P. violae* is the primary casual agent of CCS. Because *P. sulcatum*, *P. irregulare*, *P. dissotocum*, and strain M2-05 are apparently insensitive to fluopicolide, this fungicide probably shouldn't be recommended for use on carrot in Michigan. Among the seven *P. intermedium* isolates, three were sensitive and four were resistant to fluopicolide. Our study on homological analysis of ITS sequences of these isolates showed that the sensitive and insensitive isolates can be classified into two different clusters (X. H. Lu, unpublished), suggesting independent subgroups.

The composition of species of *Pythium* from carrot in California and Michigan is different; therefore, the choice of fungicides will need to be based on fungicide sensitivities. For example, fluopicolide might be used in California where *P. violae* is the major concern, and zoxamide may be recommended for the management of CCS in both states. Established sensitivity baselines of these fungicides will help monitor fungicide sensitivity shifts in the field.

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Fig. 2. Distribution of effective concentrations of zoxamide at which 50% of mycelial growth was inhibited (EC_{50}) for 110 isolates of *Pythium violae*. The frequency (%) is the proportion of isolates with EC_{50} values in the range provided.

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