Effects of Propamocarb Hydrochloride on Mycelial Growth, Sporulation, and Infection by *Phytophthora nicotianae* Isolates from Virginia Nurseries

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ABSTRACT

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Propamocarb hydrochloride is a systemic fungicide commonly used for control of *Phytophthora* diseases of nursery crops. Here we report on the effect of this compound on different growth stages of *Phytophthora nicotianae*, a major pathogen of numerous herbaceous and some woody ornamental plants. A total of 71 isolates were assayed for sensitivity to propamocarb at two concentrations of 1.8 mg/ml (label rate) and 10 mg/ml using clarified V8 agar as a base medium. All isolates grew at 10 mg/ml with the most sensitive isolate having 34.8% relative growth compared with growth on nonamended medium. Nine isolates were selected and further tested for mycelial growth at 0, 1, 10, 25, 50, and 100 mg/ml, and for sporangium production, zoospore motility, and germination at 0, 5, 50, 500, 5,000, and 50,000 µg/ml. EC₅₀ values ranged from 2.2 to 90.1 mg/ml for mycelial growth, 133.8 to 481.3 µg/ml for sporangium production, 88.1 to 249.8 µg/ml for zoospore motility, and 1.9 to 184.6 µg/ml for zoospore germination, respectively. In addition, 17 selected isolates were evaluated for propamocarb sensitivity on *Pelargonium × hortorum* cv. White Orbit. Two days after seedlings were treated with propamocarb at 3.6 mg/ml, they were inoculated by either inverting one 5-mm-diameter plug of a 3-day-old culture or applying a 10-µl drop containing 20 zoospores onto each cotyledon. Propamocarb hydrochloride provided good protection of geranium seedlings from zoospore infections but not from mycelial infections. These results suggest that this fungicide must be used preventively for *Phytophthora* disease management and that mycelial growth may not be the most reliable measurement to determine the development of fungicide resistance to this compound in *Phytophthora* species at production facilities and in the landscape.

Additional keywords: fungicide resistance, IPM

Phytophthora species are a group of common and destructive pathogens of a large number of agriculturally and ecologically important plants (11,12). They cause rots of roots, crown, stems, leaves, and fruits and often result in severe plant losses. This is particularly significant for nursery crops (19,20,22,25) because many ornamental plant species are grown close together and each may host several *Phytophthora* species. For example, rhododendron is susceptible to at least 17 different species of *Phytophthora* (17,23,39, 41,46). *Phytophthora* species may produce chlamydospores that allow survival under harsh environments for a long period of time. They also produce flagellate zoospores that disseminate through irrigation

systems (21). In addition, they can be easily spread to other production facilities and landscapes through movement of contaminated nursery stock (27). Thus, it is difficult to keep *Phytophthora* diseases under control once plants and/or growing media are contaminated within a production facility or landscape.

Chemical control is a current approach for management of *Phytophthora* diseases of nursery crops. Commonly used compounds include mefenoxam, propamocarb hydrochloride (hereafter referred to as propamocarb), fosetyl aluminum, and etridiazole. Each of these fungicides has a unique mode of action and supposedly has good protective and curative activity. However, mefenoxam resistance has been documented in several *Phytophthora* species at nurseries in California (14) and North Carolina (25). Resistance to fosetyl-Al also was found in *Bremia lactucae* Regel, a close relative to *Phytophthora* species (4). There is a growing concern over whether *Phytophthora* species at nurseries and in landscapes have developed resistance to other major compounds.

Investigation into this question is of critical importance to formulate effective disease and fungicide resistance management programs locally and nationally.

Propamocarb was first introduced into European markets for control of oomycete pathogens in ornamental crops and certain vegetables in 1978 (33). It has since been widely used as a soil drench against *Phytophthora* and *Pythium* diseases of numerous crops (9,10,13,33,35,36). Specifically, it has been used successfully to control potato late blight where metalaxyl-resistant populations presented an increasingly serious problem (2,3). It has been speculated that the mode of action of propamocarb is through selectively interfering with the biosynthesis of oomycete membranes (32), although this has not been proven. Due to its lack of adverse effects on beneficial microorganisms such as mycorrhizae and *Trichoderma* species, propamocarb has been considered as a good component of IPM programs (29,47).

Propamocarb sensitivity has been assessed on a variety of *Phytophthora* species at several stages of their life cycles. It varied among species of *Phytophthora* and among isolates of the same species (35). It also differed with growth stages of individual isolates. For example, EC₅₀ values for mycelial growth were 29.3 µg/ml for *P. cactorum* (Lebert & Cohn) J. Schrot. (43), 111 µg/ml for *P. palmivora* (E.J. Butler) E.J. Butler (42), >1,000 µg/ml for *P. drechsleri* Tucker (8) and *P. nicotianae* Breda de Haan (syn: *Phytophthora parasitica* Dastur) (16), and 6,246 µg/ml for *P. infestans* (Mont.) de Bary (37). The EC₅₀ values for sporangium production had a similar magnitude of variation (25 to 4,000 µg/ml) among *P. infestans* (37), *P. palmivora* (7,42), and *P. nicotianae* (16). Oospore formation by *P. infestans* was found to be very sensitive to propamocarb, with complete inhibition at 100 µg/ml (1,37).

In spite of varying in vitro activity, propamocarb often provided considerable protection of plants from infection by *Phytophthora* spp. For example, this fungicide provided good control of pepper blight caused by *P. capsici* Leonian (47), potato late blight by *P. infestans* (2,3,26,37,38), tobacco black shank by *P. parasitica* var. *nicotianae* (30,36), and cucumber damping-off by *P. drechsleri* (8). This com-

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pound is used by the turf and ornamental industries in Pennsylvania and Virginia for control of oomycete diseases (18,31), especially where mefenoxam resistance increasingly has become problematic (14,15,24,25). Growers want to know how effective propamocarb is for control of *Phytophthora* diseases and whether similar fungicide resistance problems have arisen with this compound. However, little is known about propamocarb sensitivity of *Phytophthora* species in ornamental plants in the United States.

This study used *P. nicotianae* as a model system to determine its propamocarb sensitivity in media and geranium seedlings. *P. nicotianae* is one of the most common and damaging *Phytophthora* pathogens in numerous herbaceous annuals and perennials as well as some woody ornamental plants (11,12). We have frequently isolated this pathogen from an array of diseased nursery crops (19,20,22) and in irrigation water over the past 6 years (5,6), and have accumulated a number of isolates. This study was aimed at assessing these isolates for their sensitivity to propamocarb to fill a major knowledge gap in fungicide resistance. In addition, we examined several growth stages in the life cycle of *P. nicotianae* and infection of geranium seedlings by two types of inoculum of selected isolates in an attempt to understand the inconsistencies between in vitro and in vivo efficacy data in the literature and to evaluate the reliability of using mycelial growth alone for propamocarb resistance screening.

MATERIALS AND METHODS

Isolate origin and identity confirmation. A total of 71 isolates of *P. nicotianae* were included in this study. The majority of these isolates were recovered from plant tissues of diverse nursery crops and irrigation water in Virginia. Several isolates from other crops and locations, kindly provided by James Adaskaveg (University of California, Riverside), Mannon Gallegly (West Virginia University), David Shew (North Carolina State University), and Sharon von Broembsen (Oklahoma State University) were included for comparison. Most isolates were recovered since 2000; however, two isolates (23A8 and 23A9) were recovered in 1968, before propamocarb was marketed for use on ornamental crops. The identity of all isolates was verified using a single-strand conformation polymorphism analysis of the ribosomal DNA internal transcribed spacer 1 (27).

Population sensitivity. Propamocarb (propyl [3-(dimethylamino) propyl] carbamate monohydrochloride; Bayer Environmental Science, Research Triangle Park, NC) was assessed at concentrations of 1.8 mg/ml (equivalent to label rate: 3 fl. oz./10 gal) and 10 mg/ml. Clarified V8 (CV8) agar (pH = 6.3) was used as a base medium. The fungicide was diluted in sterile distilled water then added to the

autoclaved medium at 50°C. Mycelial plugs (5 mm diameter) were cut from the margin of 4- to 5-day-old CV8 agar cultures. One plug was placed in the center of a 9-cm petri dish with the mycelia in contact with the medium. Three replicate dishes per treatment were inoculated for each isolate. The inoculated dishes were then incubated at 23–26°C for 5 to 7 days in darkness. When colonies on nonamended control dishes had reached the edge, colony diameters were measured in two perpendicular directions for all treatments. The diameter of the mycelium plug was subtracted; the two measurements were averaged. Relative growth of an isolate on amended media was calculated by dividing colony diameter in amended dishes by that in the nonamended control dishes, and expressed as a percentage.

Effective concentration of propamocarb against mycelial growth. A subset of nine isolates representing different levels of sensitivity to propamocarb based on the sensitivity assays described above was selected to determine their EC₅₀ values. These isolates were assessed using the same medium amended with propamocarb at 1, 10, 25, 50, and 100 mg/ml plus a nontreated control. Three replicate petri dishes were used for each treatment. The dishes were inoculated, incubated, and colony growth was determined as described above. The data of percent inhibition of mycelial growth was transformed to probits based on a normal distribution.

Effective concentration against sporangium production. The same nine iso-

lates were tested at five concentrations of 5, 50, 500, 5,000, and 50,000 µg/ml plus a nontreated control. Sterile soil water extract (SSWE) was used as a medium to induce sporangia formation (11). SSWE was prepared by mixing 10 g (fresh weight) of a sandy loam soil in 1 liter of distilled water on a magnetic stirrer overnight at room temperature (23–25°C), settling for 8 h, then filtering through a Whatman paper (no. 1). Propamocarb was added to SSWE at the designated concentrations. Four mycelial disks (5 mm diameter) were removed from the edges of 5-day-old CV8 agar cultures and placed in petri dishes containing 8 ml of SSWE without or with propamocarb at a designated concentration. Three replicate dishes were used for each concentration per isolate. After incubation under fluorescent light for 10 h at 23–26°C, SSWE was removed and replaced with 5 ml of 10% bleach solution to stop formation of new sporangia. The number of sporangia along the margins of each mycelial disk was counted under a compound microscope. The percent reduction in sporangium production was computed by dividing the average number of sporangia in a treatment by the corresponding number in the nontreated control.

Effective concentration against zoospore motility and germination. Sporangia were produced in SSWE as described above, and zoospore release was triggered by replacing with prechilled sterile distilled water (at 4°C) and then warming for 20 min at room temperature. Zoospore

Fig. 1. Distribution of 71 isolates of *Phytophthora nicotianae* in response to 1.8 and 10 mg/ml of propamocarb incorporated into 20% clarified V8 agar.

concentrations were adjusted to about 1,800 per ml. To assess zoospore motility, 5 ml of an adjusted zoospore suspension was added to each of three replicate petri dishes containing equal volumes of propamocarb solutions at 0, 10, 100, 1,000, 10,000, or 100,000 µg/ml to give a final concentrations of 0, 5, 50, 500, 5,000, or 50,000 µg/ml. Zoospore suspensions were maintained at 25°C and observed microscopically to determine the time required for complete cessation of motility (zoospore movement time in minutes). Observations were made at 1-min intervals for the first 15 min, then at 15-min intervals for the next 45 min, and finally at 1-h intervals until no zoospore movement was

detected. Relative motility was calculated by dividing zoospore movement time in a treatment by that in the nontreated control. To assess zoospore germination, 100 µl of an adjusted zoospore suspension was spread onto each of three replicate dishes of CV8 agar containing propamocarb at a concentration of 0, 5, 50, 500, 5,000, or 50,000 µg/ml. After a 2-day incubation at 25°C in the dark, the number of *Phytophthora* colonies appearing on each petri dish was recorded. Relative germination was computed by dividing the number of colonies in a treatment by that in the nontreated control.

In vivo efficacy tests. Seventeen resistant isolates were further tested for

propamocarb sensitivity using seedlings of geranium (*Pelargonium × hortorum* cv. White Orbit). Seedlings were treated with propamocarb then challenged with *P. nicotianae* as described by Moorman and Kim (31) with some modifications. A plastic container (9 cm in diameter and 4.5 cm deep) was used to hold a smaller petri dish (6 cm diameter) with a filter paper (Whatman no. 4) on its top. Twenty-five ml of water-soluble fertilizer (N at 400 µg/ml; 20% N, 20% P₂O₅, 20% K₂O soluble fertilizer; Olympic Chemical Co., Mainland, PA) was added to each container to keep the filter paper moist. Five geranium seeds were placed on the filter paper. The containers were placed inside a tray sealed with a transparent plastic cover and incubated for 5 days in a growth chamber with fluorescent lights at 23–25°C. Once the cotyledons were fully expanded, the fertilizer solution in each container was replaced with the same amount of propamocarb solution at 3.6 mg/ml (twice the label rate). Two days after replacement, the plants were inoculated on both cotyledons with either mycelial plugs or zoospores. Experiments with mycelial inoculum used five plants per replicate. Five-mm-diameter mycelial plugs were taken from the margin of a 3-day-old culture. One plug was placed on the upper surface in each leaf after seedlings were sprayed with SSWE to facilitate plug adhesion. The experiment with zoospore inoculum used 10 plants per replicate. Zoospore suspensions were prepared as described above. A 10-µl drop containing 20 zoospores was placed on the upper surface of each leaf. All inoculated plants were incubated in a moist chamber in the dark for 24 h and grown under fluorescent light at 23–25°C. Four to 6 days after inoculation, the number of seedlings with blackened stem, leaf necrosis, or wilted appearance was counted.

Experiment design and data analysis.

Each experiment described above was conducted twice on different dates. Treatments within each repeating experiment were arranged in a completely randomized design. Data from two experiments were pooled together according to homogeneity of variance. Analysis of variance and multiple range tests were performed with SAS (Proc GLM, SAS Institute, Cary, NC). Linear regression lines of logit-transformed percentage against the log₁₀ of the propamocarb concentration were fitted using Proc REG of SAS. The resulting equations were used to calculate EC₅₀ values. The Mann-Whitney *U* test (49) was performed to determine the differences in mortality of geranium seedlings between the fungicide treatment and nontreated control for each isolate and inoculation experiment. Percent disease control was calculated by comparing the seedling mortality in propamocarb treatment with that in nontreated control for individual iso-

Fig. 2. Impact of propamocarb dose on **A**, mycelial growth and **B**, sporangia production of five representative isolates of *Phytophthora nicotianae*, expressed as relative growth compared with that of the control medium. Clarified V8 agar was used as a base medium for mycelial growth and sterile soil water extract for sporangium production assays. Isolate 26E7 was recovered from *Nicotiana tabacum* in North Carolina in 2003, 16C8 and 25J8 from irrigation water in central and southwestern Virginia (2000), 2C10 from *Buxus sempervirens* in central Virginia (2000), and 49J9 from *Daphne* sp. in eastern Virginia (2001). Each number is a mean of six replicates from two experiments.

lates. Analysis of variance then was performed to determine the differences in disease control among the 17 isolates by type of inoculum.

RESULTS

Isolate identity confirmation. All isolates tested yielded ca. 300 bp amplicon and produced an SSCP banding pattern characteristic of *P. nicotianae* on 8% polyacrylamide gels.

Population sensitivity. All isolates grew substantially in the presence of propamocarb at both concentrations (1.8 and 10 mg/ml). Approximately 88% of isolates had over 50% relative growth of the non-amended controls at both concentrations. No isolates exhibited more than 100% or less than 30% relative growth (Fig. 1).

Effective concentration against mycelial growth. The relative sensitivity of five isolates of *P. nicotianae* to propamocarb was shown in Figure 2A. Estimated EC_{50} values for nine isolates ranged from 2.2 to 90.1 mg/ml, depending on the host plant/substrate and geographic origin. Six isolates had EC_{50} values between 2.2 and 10 mg/ml; one isolate had EC_{50} values between 10 and 30 mg/ml. Two isolates had EC_{50} values of over 30 mg/ml (Table 1); these two isolates were recovered from boxwood and nursery irrigation water. The overall mean EC_{50} value was 20.6 mg/ml.

Effective concentration against sporangium production. Nine isolates in nontreated control petri dishes produced an average of 82 sporangia per agar disk (along the perimeter) after 10-h incubation in SSWE under fluorescent light. All isolates exhibited a similar pattern in response to propamocarb. Over 60% reduction in sporangium production was observed at 500 μ g/ml for seven isolates and at 5 mg/ml for the two remaining isolates (Fig. 2B). No sporangia production was seen at 50,000 μ g/ml. Little inhibitory effect on sporangium production was observed at 50 μ g/ml or less. The EC_{50} values of this compound against sporangium production by nine isolates ranged from 133.8 to

Fig. 3. Impact of propamocarb dose on **A**, zoospore motility and **B**, zoospore germination of six isolates of *Phytophthora nicotianae*, expressed as relative zoospore motility or germination in comparison with that in the nontreated control. Sterile soil water extract was used to induce sporangium production then replaced with prechilled at 4°C to trigger zoospore release. Zoospore motility was determined by microscopic examination after mixing equal amounts of spore suspension and propamocarb solution and measured as time (min) required for complete cessation of all spores. Zoospore germination was determined by spreading aliquots of spore suspension in petri dishes with clarified V8 agar amended with propamocarb then counting the emerging *Phytophthora* colonies. Isolates 25J8 and 16C8 were recovered in 2000 from irrigation water in southwestern and central Virginia, respectively, 2C10 from *Buxus sempervirens* in central Virginia (2000), 31A1 from *Petunia* sp. from eastern Virginia (2004), 26E7 from *Nicotiana tabacum* in North Carolina (2003), and 4J9 from *Daphne* sp. in eastern Virginia (2001). Each number is a mean of six replicates from two experiments.

Table 1. Effective concentration (EC_{50}) of propamocarb for 50% reduction in mycelial growth, sporangium production, zoospore germination, and duration of zoospore motility of *Phytophthora nicotianae* isolates

Isolate	Host/substrate	Geographic origin	Year	EC_{50} (μg/ml) ^y			
				Mycelial growth	Sporangium production	Zoospore germination	Zoospore motility
25J8	Irrigation water	Virginia	2001	90,100	481.3	184.6	249.8
16C8	Irrigation water	Virginia	2000	3,500	218.2	1.9	88.1
1E3	Irrigation water	Oklahoma	...	3,200	198.4	1.9	104.5
2C10	<i>Buxus sempervirens</i>	Virginia	2000	80,100	442.1	131.4	215.6
31A1	<i>Petunia</i> sp.	Virginia	2004	5,500	204.2	6.9	121.2
31D5	<i>Petunia</i> sp.	Virginia	2004	10,100	217.2	4.1	117.7
4J9	<i>Daphne</i> sp.	Virginia	2001	5,400	133.8	8.8	100.7
28B5	<i>Lavandula angustifolia</i>	Virginia	2002	6,900	200.6	39.1	134.1
26E7	<i>Nicotiana tabacum</i>	North Carolina	2003	2,200	162.0	30.8	98.8

^y EC_{50} values were estimated by fitting a regression line of the logit-transformed percent inhibition against the log-transformed concentration of propamocarb. These values are means of six replicates from two repeating tests.

^z Unknown.

481.3 µg/ml with a mean of 250.9 µg/ml (Table 1).

Effective concentration against zoospore motility and germination. All isolates responded to propamocarb similarly in terms of zoospore motility and germination (Fig. 3). Zoospores retained their motility in SSWE for an average of 401 min in the absence of this fungicide. EC₅₀ values for reduction of zoospore motility ranged from 88.1 to 249.8 µg/ml among the nine isolates (Table 1). All isolates completely lost their motility within 10 min at the concentration of 5,000 µg/ml or above. Germination of zoospores was more sensitive to propamocarb than motility. On average, 32 *Phytophthora* colonies were recovered from the control petri dish (without propamocarb). The EC₅₀ values for zoospore germination ranged from 1.9 to 184.6 µg/ml. Less than 20% of zoospores germinated at 500 µg/ml.

In vivo efficacy tests. When the plants were inoculated with mycelial plugs, colonization first took place at the inoculation site then rapidly spread either up to the growing tip or down the stem, causing stem rot (blackened). Eventually the plants collapsed. The majority of isolates infected and killed 80% or more geranium seedlings that had been pretreated with propamocarb as well as the control plants (Table 2). When inoculated with zoospores, geranium seedlings developed similar symptoms but at lesser degrees. Hypersensitive lesions occasionally occurred in some inoculated leaves. Generally, longer periods of time were needed for symptom development and mortality

was lower for seedlings challenged with zoospore inoculum than with mycelial plugs. The average mortality rate caused by zoospore inoculum was less than 50% in control plants. Propamocarb exhibited 60 to 100% protection of geranium seedlings against zoospore infections, depending on isolate (Table 2). There were significant differences in propamocarb protection of geranium seedlings from zoospore infection ($P < 0.001$) but not from mycelial infection ($P = 0.3239$) among the isolates tested.

DISCUSSION

This study assessed the propamocarb sensitivity in 71 isolates of *P. nicotianae* at four different growth stages and its efficacy for reducing geranium mortality caused by two types of inoculum of the 17 selected isolates. This fungicide provided little or no inhibition of mycelial growth nor any control of mycelial infection. But it consistently suppressed all other growth stages assessed in vitro and reduced zoospore infection. These results have several significant practical implications.

Propamocarb remains an effective option for *Phytophthora* disease in a fungicide resistance management program, but it must be used preventively and with extra caution. Contaminated irrigation water is a primary source of inoculum for *Phytophthora* diseases in nursery production (20,21) where zoospores are the principal propagules (34,40,44). The fact that propamocarb effectively reduced seedling mortality by zoospore infection (Table 2) suggests that this fungicide can provide

good protection of nursery crops if applied to plants prior to arrival of zoospore inoculum. This compound also can slow the disease progression by inhibiting sporangium production and zoospore motility (Table 1). Sporulation, zoospore motility, zoospore germination, and infection all are important steps in the disease cycle. They are particularly significant for disease development of polycyclic pathogens like *P. nicotianae* by fueling secondary inoculum buildup. It must be pointed out that propamocarb had little effect on mycelial growth and infection (Tables 1 and 2). We performed additional in vitro assays with 10 other isolates and demonstrated that their mycelial growth had similar levels of propamocarb sensitivity (data not shown). For example, isolates 23A8 and 23A9 had EC₅₀ values of 4.7 and 5.2 mg/ml, respectively. These observations suggest that once infection takes place, this fungicide may not prevent local disease spreading by mycelium. They also may be indicative that fungicide resistance to this compound in *P. nicotianae* is on the rise. Thus, repeated use of propamocarb should be avoided and resistance to this compound should be closely monitored.

Our results suggest that the inconsistency between in vitro and in vivo propamocarb efficacy data in the literature is a reflection of different growth stages differentially responding to this compound. Most previous in vitro assays were done with mycelial growth, suggesting insensitivity to this compound (29,31,48). In contrast, the vast majority of documented in vivo data were obtained from field trials (1,10,36), where zoospores were likely the major type of primary and secondary inoculum. As a result, this fungicide provided good protection of crops in field trials. Our contention is supported by the data of Reiter et al. on the propamocarb sensitivity of mycelial growth versus sporangium production, oospore formation, and infection of tomato by *P. infestans* (37).

In vitro mycelium growth is a standard measurement for resistance screening to various fungicides including propamocarb (2,9,14,16,25,28,31,43,45). The contrasting efficacy data between mycelium versus other growth stages in this study (Table 1, Figs. 1 to 3) along with those in the literature suggest that this "standard" measurement is not the best one for screening of propamocarb resistance in *Phytophthora* species. An in vitro assay with mycelial growth to determine the status of propamocarb resistance is easy and fast, but solely relying on this measurement may overestimate the threat of resistance. In this case, additional growth stages must be tested in order to reliably determine whether isolates have developed resistance to this compound. An alternative approach would be to use a more sensitive growth stage such as zoospore germination (Table

Table 2. Efficacy of propamocarb in controlling mortality of *Pelargonium × hortorum* cv. White Orbit seedlings caused by *Phytophthora nicotianae*

Isolate	No. of infected plants ^x					
	Mycelial plug		<i>P</i> ^z	Zoospore		<i>P</i>
	Nontreated control	Propamocarb-treated ^y		Nontreated control	Propamocarb-treated	
26E8	4.5	4	0.50	3	0.2	0.001
16C8	5	5	1.0	4.7	0.5	0.001
18C6	5	4.5	0.5	4	1.7	0.001
25J9	5	5	1.0	4.5	1.8	0.001
23C7	4.5	4	1.0	5.5	1.8	0.001
23C3	5	4.5	0.17	4.8	1.8	0.001
18C4	5	5	1.0	4	1.5	0.001
3A4	5	5	1.0	2.8	0.7	0.001
31E2	4.5	5	0.5	4.5	1.2	0.001
31A5	5	5	1.0	6.8	2.8	0.001
23A9	5	5	1.0	3.5	1.5	0.001
30J7	4.5	4.5	0.83	2.8	0.2	0.001
30J5	5	4	0.17	2.5	0.0	0.001
31D7	4	3	0.17	5.7	1.2	0.001
31A8	4	3.5	0.5	4.2	1.7	0.04
18C8	5	4.5	0.5	3.3	0.7	0.001
1E3	5	5	1.0	6.3	1.2	0.001

^x Symptoms began at the inoculation points with necrosis, then spread down to cause blackened stems and seedling collapsing or up to cause leaf curling and plant wilting. The number of infected plants is mean value of six replicates from two repeating tests. The total number of test plants per replicate was five for inoculation tests with mycelial plugs and 10 for those with zoospores.

^y Seedlings treated with propamocarb at 3.6 mg/ml (equivalent to 6 fl. oz./10 gal, double the labeled rate) in water-soluble fertilizer

^z Mann-Whitney *U* test was performed to determine differences in mortality of geranium seedlings between propamocarb treatment and nontreated control.

1, Fig. 3) in propamocarb resistance screening programs. This is because the objective of disease control is to break the disease cycle rather than to control every step in the cycle. A long-term solution would be to identify DNA markers tied to *in vivo* propamocarb resistance.

Propamocarb sensitivity is a multigenic trait in *P. nicotianae*. This hypothesis is supported by both distinct sensitivities among the growth stages (Tables 1 and 2) and a continuous distribution of mycelial growth among isolates assessed in the present study (Fig. 1). It was not clear whether propamocarb insensitivity of mycelial growth and infection is a result of the natural variation or of selection pressure due to use of the fungicide (31). The fact that the EC₅₀ values of isolates 23A8 and 23A9, recovered back in 1968, were nearly three times the label rate points to the first possibility. However, there were variations in propamocarb sensitivity among isolates from different nurseries and even among those from the same nurseries. For example, three irrigation water isolates from one nursery in southwestern Virginia had higher EC₅₀ values (ranging from 13.7 to 90.1 mg/ml) than those from other nurseries in central and eastern Virginia. The originating nursery of these three isolates did not appear to have used propamocarb more frequently than other locations; but we cannot be certain whether the three isolates had more exposure to this compound, as resistant isolates could have been brought into this production facility in contaminated soil or on infected plants. Propamocarb sensitivity had no clear association with mating type (data not shown). The genetic details of propamocarb ineffectiveness against mycelial growth and infection process are yet to be determined. Identification of linked molecular markers and quantitative trait loci is warranted to determine the number of genes responsible for propamocarb sensitivity as well as the degree of contribution of individual genes.

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