Sublethal Doses of Mefenoxam Enhance Pythium Damping-off of Geranium

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Abstract

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The effect of sublethal doses of fungicides on fungicide-resistant *Pythium* isolates is unknown but potentially relevant to disease management. Occasional grower reports of Pythium disease increases after fungicide applications and our observations of greater radial growth in vitro on fungicide-amended media than on nonamended media suggests that *Pythium* isolates may be stimulated by sublethal doses of fungicides. The objectives of this study were to determine whether *Pythium* isolates were stimulated by sublethal doses of mefenoxam in vitro and whether this stimulation had any influence on Pythium damping-off of geranium seedlings. A mefenoxam-resistant isolate of *Pythium aphanidermatum* displayed 10% mean radial growth increase in vitro with mefenoxam at 1×10^{-10} µg/ml compared with growth on nonamended agar (nonsignificant). Geranium seedlings treated with one of eight mefenoxam concentrations were inoculated with 5-mm-diameter colonized agar plugs and evaluated for disease severity every 24 h. The area under the disease progress curve and the survival curve were estimated for each treatment and compared. Significant increases in damping-off were observed with mefenoxam at 1×10^{-6} and 1×10^{-10} µg/ml. Our data indicate that a *Pythium* isolate with resistance to mefenoxam can be stimulated by sublethal doses of the fungicide, and that this stimulation can result in significantly higher rates of Pythium damping-off of geranium seedlings.

Pythium diseases have devastating consequences if not managed properly (21). In ornamental production facilities, *Pythium* spp.-caused damping-off, black leg, and root rots can hamper the productivity of an operation by reducing seed germination, destroying seedlings, killing cuttings, and reducing crop quality. Because most *Pythium* spp. are not host specific, management practices such as cultivar resistance and crop rotation are not employed (21,35).

Prevention, by sanitation and chemical control, and eradication, by scouting and discarding plants displaying disease symptoms and by applying fungicide treatments as needed, are the most commonly used management practices. Due to the high cost of fungicides and the small number of products registered for ornamental use against *Pythium* spp., chemical control is restricted to a few different active ingredients. Previous reports have demonstrated resistance to mefenoxam and propamocarb in *Pythium* spp. in ornamentals as well as turfgrass and field crops (1,20,38,39,43–46,56). Of general concern is the lack of control of Pythium diseases once a resistant strain is present in a greenhouse operation. Applying the fungicide to which resistance was developed would provide resistant strains a favorable environment where they can thrive. The motile zoospores and other propagules produced by *Pythium* spp. can be easily dispersed throughout ornamental operations by recirculating watering systems (28,47). Therefore, if a fungicide-resistant strain was responsible for disease in a facility where that chemical was the only treatment for *Pythium* infections, a progressive increase of disease levels could be expected as the pathogen spreads through production areas. Occasionally, growers have reported sudden increases in Pythium disease levels following the application of fungicides, raising the concern that a resistant isolate is present (G. W. Moorman and C. D. Garzón, *unpublished*).

Under experimental conditions, it has been observed that some fungicide-resistant oomycete and fungal isolates grow faster in agar media amended with fungicide than in nonamended media (G. W. Moorman and C. D. Garzón, *unpublished*). Although these observations are common, they are usually disregarded as experimental error or artifact, even if they are consistently reproducible. Our observations on fungicide-resistant *Pythium* isolates suggested the possibility of stimulating effects of fungicides on these isolates at sublethal doses, and that similar physiological responses might occur both in vitro and in planta.

Metabolic stimulation by toxic compounds at low doses has been previously reported in diverse scientific literature (5–9,12,16–18,21,22,24–26,29,30,36,37,48,49,52,53,55). In 2003, Calabrese and Baldwin (14) brought to general awareness the concept of "hormesis". Hormesis is defined as an adaptive response that is either directly induced or the result of compensatory biological processes following homeostasis disruption (14). This adaptive response is characterized by biphasic dose responses of similar quantitative features with respect to amplitude and range to the stimulatory response. Hormesis is a physiological process where factors that trigger homeostatic disruptions at high doses produce adaptive responses at low doses that can result in metabolic stimulation (13). This adaptive response appears to be a general biological phenomenon, and evidence shows that it may be independent of environmental stressor, biological end point, and experimental model system (11). Some examples of systems that display hormetic behavior include plant growth stimulation by herbicides (17,18) and radiation (31,36), human cancer cells stimulation by chemicals (7,23), and fungal and oomycete stimulation by fungicides and other chemicals (5,25,26,48,49,52).

The dose-effect phenomenon has been intensively studied in recent years because of its important implications in multiple biological systems (16,27). However, little is known about the effect of low doses of fungicides on fungi and oomycetes (22,25,26). Mefenoxam and the closely related fungicide metalaxyl are the active ingredients of several fungicide products extensively used to prevent and control plant diseases caused by Oomycetes (*Pythium, Phytophthora*, and *Albugo* spp., among others) on diverse crops, including ornamentals and nursery crops. This chemical is systemic and is highly effective, placing a high selective pressure on populations of oomycetes. Frequent use results in the selection of resistant isolates (32,39,56). Usually considered negligible, the actual effects of fungicides at sublethal doses on fungi and oomycetes are unknown.

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The objectives of this study were to examine the dose effect of mefenoxam on *Pythium* isolates in vitro and to determine whether sublethal doses of mefenoxam increased damping-off of geranium seedlings.

Materials and Methods

Mefenoxam-sensitivity determination. The mefenoxam sensitivity of six *Pythium* isolates (two *Pythium aphanidermatum* and four *P. cryptoirregulare*) was determined as previously described (38). Isolates were rated as resistant if their growth was not reduced by 50% by mefenoxam at 100 µg/ml (Syngenta Crop Protection Inc., Wilmington, DE).

Effect of sublethal mefenoxam doses on *Pythium* spp. in vitro. Each *Pythium* isolate was evaluated individually. *Pythium* mycelia grown on corn meal agar (CMA; BBL, Voigt Global Distribution Inc., Lawrence, KS) at 20°C for 3 days was transferred to water agar (WA) and allowed to grow for an additional 3 days before transferring 5-mm-diameter plugs to CMA amended with mefenoxam at 11 concentrations. Eleven fungicide treatments were prepared by doing serial dilutions two orders of magnitude apart ranging from a reference concentration (RC = 100 µg/ml or 1 µg/ml for resistant and sensitive isolates, respectively) to RC × 10^{-20}. Cultures on nonamended CMA were used as controls. The average radial growth of each isolate at 20°C was calculated by measuring two perpendicular radii per isolate, three replicates per isolate, and compared with the average growth of the control. The isolate most stimulated by sublethal doses of fungicide (P18, *P. aphanidermatum*) was selected for further experiments in vitro and in planta. The experiment described above was repeated three additional times with isolate P18.

Effect of low-dose fungicide treatments on damping-off of seedlings. An in planta assay was designed in order to determine the effect of exposure to low doses of mefenoxam on the ability of a mefenoxam-resistant *P. aphanidermatum* isolate (P18) to cause damping-off of geranium seedlings. Geranium seed (*Pelargonium* × *hortorum* 'Orbit White'; Park Seed Co., Greenwood, SC) were germinated on sterile filter paper circles (9 cm in diameter, Grade 410; VWR, West Chester, PA) soaked with a fertilizer solution (20% N, 20% P_2O_5, 20% K_2O, and 200 ppm N) inside sterile disposable petri plates (10 cm in diameter; VWR) and kept in growth chambers at 23°C in the dark for 2 days, followed by full-spectrum light for 16 h/day at 23°C for the remainder of the experiment (Fig. 1). Each germination plate contained five seeds. Seedlings were allowed to grow for 5 days before replacing the fertilizer solution with a fungicide-amended fertilizer or with fungicide-free fertilizer solution. After 2 days of exposure to the fungicide, seedlings were inoculated with 5-mm-diameter plugs of 3-day-old P18 mycelium on CMA. One plug was placed on the main root of each seedling, 5 mm away from the stem. Seven fungicide treatments, plus a control (fertilizer alone) were applied to the geranium seedlings. Eight treatments (100 µg/ml, 1 µg/ml, 1 × 10^{-4} µg/ml, 1 × 10^{-6} µg/ml, 1 × 10^{-10} µg/ml, 1 × 10^{-12} µg/ml, 1 × 10^{-18} µg/ml, and 0 µg/ml as a control) were selected for seedling experiments due to growth chamber space restrictions. Eight plates per treatment were distributed in eight trays in a randomized complete block design inside the growth chamber and incubated at 23°C. Every 24 h after inoculation (HAI), the disease incidence was evaluated by counting the number of seedlings infected (n = 1 to 5), and disease severity was quantified using a disease scale (0% = no infection, 25% = root infected, 50% = root and up to half of the stem infected, 75%= root and stem infected, and 100% = seedling completely infected or dead). Disease progress was evaluated every 24 h for 7 days, and the area under the disease progress curve (AUDPC) of each treatment was calculated for the first 96 h. The AUDPC at each time was estimated per treatment. This experiment was repeated three times.

Data analysis. Statistical analyses were conducted with SAS (SAS System for PC, v. 9.2; SAS Institute Inc., Cary, NC) and graphs were created with R v. 2.10.1 (42).

In vitro. Each petri dish was defined as one experimental unit, with the arithmetic mean of two perpendicular measures of radial growth as response. A generalized linear model (GLM) was used to infer differences between and across treatments, replicates, and trials. Student's t tests for small samples were performed to identify treatments that produced significant stimulation compared with the control within each trial repetition.

Seedling assay. The experimental unit for the seedling assay was defined as each petri plate containing five geranium seedlings. Mean disease severity over 168 h at 24-h intervals was obtained by calculating the arithmetic mean of all five seedlings. After 96 h, more than 50% of the seedlings were dead and all the seedlings were dead in most treatments; therefore, analyses were limited to the first 96 HAI. A GLM was used to infer differences in mean plate severity between and across trays, repetitions, and treatments. Autocorrelation between the observations was controlled by including time in the model. The AUDPC was estimated for each treatment and a GLM was used to compare treatments over AUDPCs. A Wilcoxon rank sum test with continuity correction (nonparametric) was performed to assess for significant differences between treatment AUDPCs at 72 and 96 HAI without making distributional assumptions. Finally, a Cox-proportional hazards function was used to estimate the differences in the mean estimated time to death of the seedlings per plate and tray.

Results

Mefenoxam-sensitivity determination. Radial growth of four of the six isolates tested was not inhibited by mefenoxam at 100 ppm (Table 1); hence, they were considered resistant to that fungicide (P18, P25, P50, and P80). The other two isolates (P16 and P27) were mefenoxam sensitive.

Effect of sublethal doses of mefenoxam on *Pythium* spp. in vitro. All the *Pythium* isolates tested grew faster than the control when treated with one or more of the sublethal mefenoxam concentrations (Table 2). Growth increases ranged between 1 and 22%. The largest growth increases of resistant isolates P18, P25, P50, and P80 were obtained at mefenoxam concentrations of 1 µg/ml, 1 × 10^{-18} µg/ml, 1 × 10^{-14} µg/ml, and 1 × 10^{-4} µg/ml, respectively. The largest growth increases of sensitive isolates P16 and P27 were obtained at mefenoxam concentrations of 1 × 10^{-6} µg/ml and 1 × 10^{-20} µg/ml, respectively. The isolate most stimulated by low doses of mefenoxam was P18. Therefore, P18 was selected for the in vitro and seedling assays that followed. Differences among treatment replicates within each trial repetition were not significant. Differences between treatments and between trial repetitions were significant. Interactions between trial repetitions and treatments were significant. Comparisons of the normalized in

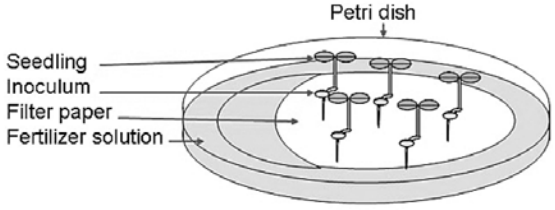

Fig. 1. Germination plate set up.

Table 1. *Pythium* isolate sensitivity to mefenoxam

Isolate ID	Species	Mefenoxam sensitivity[z]
P16	*Pythium aphanidermatum*	S
P18	*P. aphanidermatum*	R
P25	*P. cryptoirregulare*	R
P27	*P. cryptoirregulare*	S
P50	*P. cryptoirregulare*	R
P80	*P. cryptoirregulare*	R

[z] R = resistant, 50% effective concentration > 100 µg/ml and S = sensitive, 50% effective concentration < 100 µg/ml.

vitro radial growth for P18 (four trial repetitions) showed a maximum growth increase of 10% at 1×10^{-14} µg/ml, which was not statistically significant. Student's *t* test of the mean radial growth at each fungicide dose compared with the mean of the control showed significant stimulation at one or more doses within trial repetitions (Table 3; Fig. 2).

Effect of low-dose fungicide treatments on damping-off. No significant differences were found between seedling assay repetitions; therefore, results of the three trials were pooled together for the analysis. Increased damping-off severity on geranium seedlings caused by the resistant *Pythium aphanidermatum* isolate P18 in the presence of low doses of mefenoxam was observed at 24, 48, and 72 HAI (Table 4). At 24 h, doses of mefenoxam between 1 and 1×10^{-18} µg/ml caused increases ranging from 13 to 61%. At 48 and 72 h, all the treatments caused increases ranging from 0.01 to 54.7% and from 0.1 to 33%, respectively. The highest increases in severity at 24, 48, and 72 h were obtained at a mefenoxam rate of 1×10^{-10} µg/ml. After 72 h, significant differences between the AUDPC (increased disease severity) of fungicide treatments and the control were identified with mefenoxam at 1×10^{-4} µg/ml, 1×10^{-6} µg/ml, and 1×10^{-10} µg/ml by GLM (P = 0.0456, 0.0366, and 0.0114, respectively) and the Wilcoxon rank sum test (P = 0.0229, 0.0155, and 0.0049, respectively) (Fig. 3; Tables 4 and 5). The Wilcoxon rank sum test also identified significant differences between treatments of 100 and 1×10^{-4} µg/ml, 1×10^{-6} and 1×10^{-10} µg/ml, and between 1×10^{-10} µg/ml and 1×10^{-12} µg/ml and 1×10^{-18} µg/ml; after both 72 and 96 h of observations (Table 5).

Survival analysis. Large differences in survival curves were found between the control (0 µg/ml) and mefenoxam at 1×10^{-6} and 1×10^{-10} µg/ml after 96 h (Fig. 4). On average, *P. aphanidermatum* isolate P18 killed all the seedlings 99 HAI in the nonchemical control and after 86 and 79 h when exposed to mefenoxam at 1×10^{-6} and 1×10^{-10} µg/ml, which was 16 and 26% faster than the control, respectively. However, these differences were not statistically significant.

Discussion

Sublethal doses of mefenoxam stimulated a mefenoxam-resistant *P. aphanidermatum* isolate, causing a significant increase of damping-off on geranium seedlings, as reflected by significant differences in AUDPC between the nonchemical control and low-dose mefenoxam treatments. The fungicide concentrations involved in stimulation were very low (1×10^{-4} to 1×10^{-10} µg/ml). Although in vitro radial growth was consistently stimulated by sublethal doses of mefenoxam by as much as 24% in individual trials, there was large variation between trials. Therefore, it was determined that the maximum mean stimulation in vitro (10%) was not significantly different from the nontreated control. Significant interactions between the variance of trials and treatments indicate that results could not be directly compared across trials, which affected our ability to identify treatments that produced consistent radial growth stimulation. Instead, the range of fungicide concentrations at which radial growth stimulation was observed was used to design the seedling assay. As expected, sublethal fungicide treatments resulted in increased disease severity of damping-off of geranium seedlings. Disease severity in the seedling assay results was easier to reproduce than in in vitro assay results. The observed variation was probably a result of the difficulty of preparing extremely low fungicide concentrations and homogeneously mixing them with growing media. Different times between fungicide stock solution preparation and application from trial to trial may also have affected the effective doses used as treatments. Also, other factors may have affected the physiology of the pathogen (i.e., developmental stage of the mycelium used as inoculum according to a plug's location within a plate). Calabrese and Baldwin (14) have stated that hormesis is not easy to study because strict experimental design requirements must be fulfilled in order to detect hormetic effects with statistical significance, in addition to selection of adequate end points. It is possible that, in spite of our ef-

Table 3. Probability (*P*) values generated by Student's *t* test of mycelial radial growth of resistant *Pythium aphanidermatum* isolate P18 in vitro, comparing mefenoxam treatments versus control within trial repetitions[z]

	Student's *t* test			
Dose (µg/ml)	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
1×10^{-18}	0.209	0.038*	0.151	0.523
1×10^{-16}	0.841	0.061	0.001*	0.035
1×10^{-14}	0.016*	0.797	0.034*	0.905
1×10^{-12}	0.014*	0.513	0.002	0.000
1×10^{-10}	0.001*	0.049	0.103	0.154
1×10^{-8}	0.031*	0.359	0.237	0.017*
1×10^{-6}	0.089	0.113	0.133	0.107
1×10^{-4}	0.037*	0.706	0.005	0.016
1×10^{-2}	0.005*	0.032*	0.010	0.005
1	0.000*	0.350	0.802	0.000
100	0.029	0.292	0.000	0.000

[z] Significant differences between treatments and the controls were found within trials. Several treatments resulted in radial growth significantly larger or smaller than the controls ($P < 0.05$). Treatments with radial growth larger than the controls are highlighted in bold fonts; * indicates significant stimulation, *P* value of treatments at which mycelial growth was significantly larger than the control.

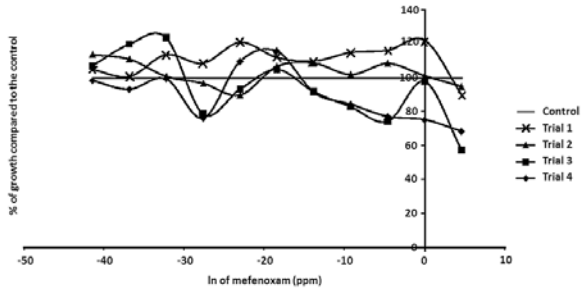

Fig. 2. Comparison of mean radial growth of *Pythium aphanidermatum* isolate P18 exposed for 24 h to sublethal doses of mefenoxam (µg/ml) among repetitions. Although stimulation was observed in all repetitions, no statistically significant differences were found.

Table 2. Percentages of in vitro radial growth increase or decrease of *Pythium* isolates on corn meal agar amended with mefenoxam relative to the control[z]

Concentration	**P16**	**P18**	**P25**	**P27**	**P50**	**P80**
RC	–5	–1	2	8	–9	3
RC × 10^{-2}	0	22	8	7	–5	2
RC × 10^{-4}	–5	16	5	7	–8	5
RC × 10^{-6}	7	15	6	3	–9	6
RC × 10^{-8}	7	10	0	5	5	4
RC × 10^{-10}	6	12	6	5	2	6
RC × 10^{-12}	–1	21	0	5	2	3
RC × 10^{-14}	–5	8	9	10	–3	4
RC × 10^{-16}	1	13	5	6	6	6
RC × 10^{-18}	6	1	3	8	3	4
RC × 10^{-20}	2	5	10	11	–2	4

[z] Reference concentration (RC) for sensitive and resistant isolates was 1 and 100 µg/ml, respectively.

forts, improvements to the experimental design of the in vitro assay are needed to increase the reproducibility of our results (i.e., more treatments, doses within the hormetic curve, and replicates or smaller dose spacing; 11). Also, other end points may reflect in vitro stimulation better than mean radial growth (i.e., respiration rate and enzyme production).

Low-dose stimulation has been reported in bacteria, fungi, plants, and animals, including humans, in numerous publications (6,8–10,15). For example, low doses of titanium chloride, magnesium chloride, and sodium chloride, and low doses of penicillin, had hormetic effects on the in vitro growth of *Escherichia coli* and *Staphylococcus* spp., respectively (29,37). Jensen (30) observed nonmonotonic responses in wheat to several herbicides, with stimulation at low doses and inhibition at higher doses. Gabliks et al. (24) reported growth increases in cultured mouse liver cells exposed to organophosphate insecticides at concentrations below the toxic threshold. Blumber and Loefer (4) reported a stimulant effect of neomycin on the growth of the protozoan *Tetrahymena gelii* in vitro. Calabrese et al. (16) gathered hundreds of scientific publications with results that fit hormetic models in diverse systems, providing strong evidence for biphasic response as a general phenomenon.

The hormetic effect is believed to be caused by an overcompensation of the cell to an irritant stimulus or by direct stimulation (6). Based on his studies on the hydroid *Laomedea flexuosa* (53) and the marine yeast *Rhodotorula rubra* (54,55), Stebbing proposed that hormesis is a consequence of overcorrections to low levels of inhibitory challenge (i.e., overcompensation due to a disruption of homeostasis), and that it may be linked to the acquisition of tolerance to higher loads of an agent. Direct stimulatory response has been proposed as another possible cause of hormesis (12). Schumacher (50) proposed the activation of cellular stress resistance mechanisms as a model that might explain the hormetic effects of low levels of damage. However, an increasing number of studies suggest that several metabolic pathways may be involved in responses to different stressors (14).

The first observations of high-dose inhibition and low-dose stimulation by toxic compounds in fungi were reported by Schulz (48,49), who observed that various agents detrimental to yeast metabolism at high doses had stimulatory effects at low doses. He postulated that, "for every substance, small doses stimulate, moderate doses inhibit and large doses kill." Additional studies focused on yeast metabolism reported similar findings. Branham (5) reported that CO_2 production increases using several doses of different chemicals that affect yeast metabolism. Cook et al. (19) reported higher proliferation in yeast suspensions with 1,2,5,6-

Table 4. Effect of sublethal doses of mefenoxam on geranium seedling damping-off 24, 48, and 72 h after inoculation[x]

	Disease severity (%)[y]			
Conc. (µg/ml)[z]	24 h	48 h	72 h	AUDPC
100	11.1	46.5	64.3	1,565.3 b
1	14.1	63.3	75.0	1,930.3 ab
1×10^{-4}	15.4	66.3	82.6	**2,078.9 a**
1×10^{-6}	16.1	65.0	78.9	**2,097.5 a**
1×10^{-10}	18.5	71.3	83.0	**2,152.8 a**
1×10^{-12}	15.7	53.3	72.6	1,818.8 ab
1×10^{-18}	13.0	53.0	78.3	1,818.8 ab
0	11.5	46.1	62.4	1,569.5 b

[x] Area under the disease progress curves (AUDPC) was compared among treatments and against the fungicide-free control using a generalized linear model (GLM). Least squares means (LSMEAN) AUDPC values significantly larger than that of the control are highlighted in bold.

[y] Disease severity scale: 0% = no infection, 25%= root infected, 50% = root and up to half of the stem infected, 75% = root and stem infected, and 100% = seedling completely infected or dead. Disease severity was measured per experimental unit every 24 h and averaged per treatment. Treatments with the same letter were not significantly different according to the Tukey-Kramer LSMEANS test (GLM).

[z] Mefenoxam concentration.

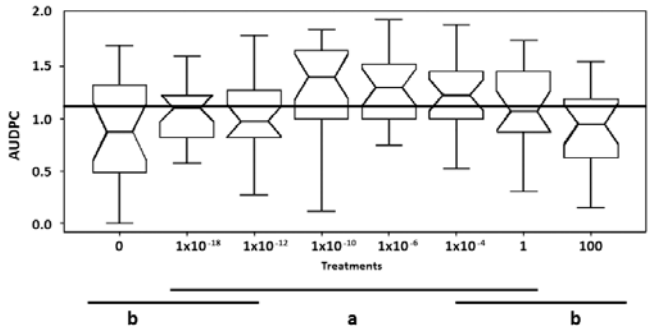

Fig. 3. Comparison of the area under the disease curves (AUDPC) of damping-off caused by *Pythium aphanidermatum* isolate P18 on geranium seedlings (*Pelargonium* × *hortorum* 'Orbit White') exposed to sublethal doses of mefenoxam (µg/ml) or fertilizer alone for 72 h. Nonoverlapping notches provide strong evidence of difference between the medians.

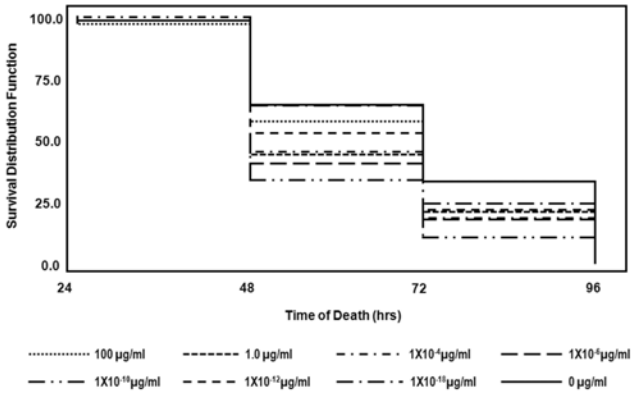

Fig. 4. Seedling survival curves per treatment. A larger number of seedlings were killed by *Pythium aphanidermatum* isolate P18 after 72 and 96 h when exposed to mefenoxam at 1×10^{-10} µg/ml than to fertilizer alone. Differences with the control were not statistically significant.

Table 5. Probability (*P*) values generated by nonparametric comparisons (Wilcoxon rank sum test) of disease severity between treatments at 72 and 96 h after inoculation (upper right and lower left, respectively)[z]

Dose (µg/ml)	100	1	1×10^{-4}	1×10^{-6}	1×10^{-10}	1×10^{-12}	1×10^{-18}	Control
100	…	0.0664	0.0089*	0.0044*	0.0023*	0.3500	0.1693	0.9562
1	0.0845	…	0.598	0.433	0.093	0.373	0.531	0.0865
1×10^{-4}	0.0054*	0.3615	…	0.7764	0.3557	0.1661	0.0767	**0.0229***
1×10^{-6}	0.0028*	0.3695	0.8915	…	0.3455	0.0884	0.0821	**0.0155***
1×10^{-10}	0.0019*	0.1211	0.4095	0.4533	…	0.0339*	0.0156*	**0.0049***
1×10^{-12}	0.2143	0.4550	0.1160	0.1067	0.0432*	…	0.8690	0.2576
1×10^{-18}	0.0647	0.7086	0.1594	0.1336	0.0378*	0.7169	…	0.2529
Control	0.843	0.130	**0.0303***	**0.0217***	**0.0062***	0.258	0.227	…

[z] Treatments with area under the disease progress curve values significantly higher than the control are highlighted in bold; * indicates treatments significantly different.

dibenzanthracene 9×10^{-4} molar than at any other concentration of this chemical, and total inhibition when four times this amount was used. Southam and Ehrlich (52) observed that western red-cedar heartwood extracts at low doses were stimulatory for *Fomes officinalis*, a wood-decaying fungus, in vitro but inhibitory at high doses. In that study, Southam and Ehrlich used the term hormesis to describe the low-dose stimulation phenomenon for the first time. They were also the first to report hormesis in a plant-pathogenic fungus. Although metabolic stimulation by low doses of toxic compounds has been previously reported for fungi and other plant pathogens, this is the first study to demonstrate that exposure of an oomycete to sublethal doses of a fungicide results in increased disease incidence and severity.

Accidental exposure of *Pythium* spp. and other pathogens to sublethal fungicide concentrations can occur in ornamental facilities that use recirculating watering systems. Although it is not recommended that fungicides be applied through recirculating watering systems, they leach out of pots after watering and enter holding tanks, where they are diluted progressively. Resistant isolates may be exposed to sublethal doses of fungicides when growers apply reduced doses of fungicides in order to reduce disease control expenses, a common but not recommended practice. Reduced doses of fungicides in combination with other control methods, such as host resistance (40,41), fungicide mixes (3), host resistance enhancers (2,33), biological control agents (34), and silicon amendments (51), have been reported as promising alternative methods for disease management. Rotation of fungicides with different modes of action is fundamental to prevent the selection of fungicide-resistant strains, which could be stimulated by fungicides even at recommended doses.

Exposure to sublethal doses of mefenoxam can result in the infection and loss of a much larger proportion of seedlings than in the absence of the fungicide in a short period of time. Hence, the financial impact of the pathogen-stimulatory effects of sublethal doses may be significant for growers. It remains to be determined whether sublethal doses of mefenoxam can also increase Pythium disease levels in other crops, established plants, and cuttings.

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