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FUSARIUM STEM ROT OF CHRYSANTHEMUMS (CHRYSANTHEMUM X MORIFOLIUM RAMAT.) CAUSED BY FUSARIUM SOLANI (MART.) APPEL & WR: IN VITRO FUNGICIDE EFFICACY AND DISEASE CONTROL STUDIES

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Abstract. In vitro **studies were conducted using fungicide amended potato dextrose agar (PDA) (Difco) plates to determine the efficacy of benomyl (Benlate 50W), ectaconazol (Vangard 10W), mancozeb (Manzate 200 80 W), captan (Captan 50W), propiconazol (Tilt 3.6EC), pyroxyfur (Grandstand 7EC), and chlorothalonil (Daconil 75W). Benomyl and ectaconazol completely inhibited mycelial growth at 10 mg/liter followed by mancozeb at 100, chlorothalonil at 1000 and propiconazol at 5000 mg/liter. Captan and pyroxyfur, at rates of 1000 and 10,000 mg/liter, respectively, did not completely inhibit mycelial growth. Eradicative control studies with potted chrysanthemums resulted in no significant disease control with any of these fungicides compared to the untreated controls. Daminozide (B-Nine) amended PDA at 1000 mg/liter caused a 27% mycelial growth reduction compared to the controls. Daminozide amended with benomyl did not affect the fungicidal activity** in vitro. **In greenhouse studies with inoculated potted chrysanthemums, daminozide plus benomyl was less effective than benomyl alone for disease control.**

Fusarium stem rot of *Chrysanthemum* X *morifolium* caused by *Fusarium solani* was first observed and described by Engelhard et al. (3, 4, 5). In this paper, we review the symptomology and discuss disease control relating to chrysanthemum stock plants.

Disease development in stock plants grown in ground beds of Myakka fine sand can start at any wound site during the growing period. Fusarium stem rot can be divided into 3 stages of development (Figs. 1-4). Initial stage (Fig. 1): dieback of the wound site with the stub becoming necrotic and turning black. The necrotic lesion on the stem enlarges and progresses downward. A red to brown discoloration of the pith and vascular tissue can extend down from the infected wound site (5). Wilt stage (Fig. 2): progression of the dieback down the stem affecting the lateral branches. The leaves of these branches wilt from the lack of water caused by the breakdown of the vascular tissue and then become necrotic and withered. Perithecia (reddish-orange) of the perfect stage (*Nectria haematococca* Berk. & Br.) may be present on severely affected stem bases with warm, moist conditions needed for perithecial development. Final stage (Fig. 3): complete dieback of all branches (blight) of plant. Perithecia can be seen further up the stem and in greater abundance than in the wilt stage. Fig. 4 illustrates healthy wound sites.

Symptom development of fusarium stem rot is frequently confused with symptoms of fusarium wilt cause by *Fusarium oxysporum* amend. Snyd. at Hans. f. sp. *chrysanthemi* (1, 2). Early symptom identification will aid in distinguishing between the 2 diseases. *F. solani* can cause a reddish-brown discoloration of the pith whereas *F. oxysporum* causes a dark discoloration which is limited to the vascular strands (5). Further, *F. oxysporum* causes a reduction in leaf size, chlorosis of younger leaves, and leaf curvature which is not found with stem rot infections (2). The wilt disease is usually more destructive than stem rot. Stem rot is prevalent when the ambient temperature is above 27°C and the canopy humidity approaches 100% relative humidity.

Materials and Methods

In vitro study. Sterilized potato dextrose agar (PDA-Difco) was prepared per manufacturer's direction and autoclaved at 121°C for 25 min. Benomyl (Benlate 50W), mancozeb (Manzate 200 80W), ectaconazol (Vangard 10W), captan (Captan 50W), pyroxyfur (Grandstand 7EC), propiconazol (Tilt 3.6EC) and neem leaf extract were each added to autoclaved PDA when the media had cooled to 60°C. PDA plates were prepared with benomyl and ectaconazol at 1, 10, 100 and 500 mg/liter; mancozeb and captan were prepared at 10, 100, 500 and 1000 mg/liter; propiconazol at 10, 100, 1000, and 5000 mg/liter; pyroxyfur at 10, 100, 5000 and 10,000 mg/liter; chlorothalonil at 10, 100, 1000 mg/liter; and neem leaf extract at 1000, 5000, 10,000 and 100,000 mg/liter. All PDA amended plates were allowed to dry for 48 hr prior to inoculation with a 4-mm diameter mycelial plug of 7-day-old *F. solani*. All plates were incubated at 29°C in the dark. Radial growth measurements were taken after 10 days with treatments replicated 5 times

Fig. 1-4. Clockwise: Stem rot of chrysanthemums ('Cirbronze') experimentally inoculated with a *Fusarium solani* propagule suspension. 1) initial stage; 2) wilt stage; 3) final stage; 4) healthy harvest site.

per concentration. Growth measurements were taken at 2 points (diameter) on each plate and then averaged. Neem extract was prepared by taking 50 g of fresh leaves, washing them in tap water, and then blending with 100 ml of sterile water. The mixture was then filtered through a Whatman #4 filter and centrifuged for 15 min at 10,000 g. The supernatant was collected and filtered through a 0.45 μm membrane filter. Specific quantities of the purified neem leaf extract were then added to autoclaved PDA medium and poured into petri dishes.

Eradicative study. Twelve-wk-old 'Dark Yellow Paragon' stock plants displaying typical fusarium stem rot were used in these studies. Plants were sprayed twice a week with the fungicides. Benomyl, mancozeb, and chlorothalonil were used on a rotational basis and an ammonium nitrate fertilizer was applied twice weekly. Ninety infected plants (initial stage) were randomly selected from a previous inoculation study in experimental plots, transplanted into 15-cm pots using Metro-Mix 500 (W. R. Grace and Co., Cambridge, MA 02140) and placed in the greenhouse. A single application of benomyl at 120 and 240 mg, captan at 240 and 480 mg and mancozeb 200 at 240 and 480 mg per liter of water was applied as a foliar spray to runoff using a hand-held pump sprayer. Seventy-two hr after the fungicide applications, stem sections (discs) and perithecia were taken from each treatment and cultured on PDA to determine the percent inhibition of *F. solani*. The control treatments were sprayed with sterile water and then cultured. Each treatment consisted of 10 plants.

Daminozide interaction study. Daminozide (B-Nine 85SP) was incorporated with PDA plates per the procedure used in the in vitro chemical screening study at 100, 200, 1000, 5000, and 10,000 mg/liter concentrations. Daminozide was also incorporated with benomyl in PDA at the same concentrations. A 4-mm mycelial plug of 7-day-old *F. solani* was placed in the center of each petri plate and then incubated for 11 days at 29°C in the dark. Radial growth measurements were taken at 2 points on each plate and then averaged.

Rooted 'Cirbronze' and 'Fiesta' cuttings were planted 3 per 15-cm pot using Metro-Mix 500 soil media and placed on benches in the greenhouse. After first flush, these plants were inoculated (sprayed to runoff) at the wound sites with a *F. solani* propagule suspension (1 x 10^6). Twenty-four hr after inoculation, a single chemical application of either benomyl (120 mg/liter, daminozide (23 mg/liter), benomyl plus daminozide (120 mg + 23 mg/liter) or a water control were applied as a foliar spray to the point of runoff. Disease evaluations were made 12 wk after inoculation. Cuttings were harvested on a weekly basis using a 2-inch cutting guide. Ammonium nitrate fertilizer was applied twice weekly and no additional fungicides were used during these studies.

Results and Discussion

Complete (100%) inhibition of mycelial growth of *F. solani* occurred with benomyl and ectaconazol at 10 mg/liter, mancozeb 200 at 100 mg/liter, chlorothalonil at 1000 mg/liter, and propiconazol at 5000 mg/liter. Pyroxyfur at 10,000 mg/liter and captan at 1000 mg/liter did not provide 100% inhibition (Table 1). Neem leaf extract was ineffective in inhibiting the growth of *F. solani* at concentrations up to 100,000 mg/liter (Table 1).

Benomyl plus captan (120 mg + 240 mg/liter) was the most effective eradicant tested (Table 2) but was not significantly different than any of the other treatments. However, all the fungicides tested were ineffective in inhibiting peritherical activity, the germination of ascospores and sporulation.

Table 2. The percent recovery of *Fusarium solani* from fungicide treated plants.

Treatments	Rate (mg/liter)	*F. solani* isolated (%)
Benomyl	120	95 a[z]
Benomyl	240	90 a
Mancozeb	240	85 a
Mancozeb	480	80 a
Captan	240	90 a
Captan	480	80 a
Benomyl + captan 50W	240	75 a
Benomyl + captan 50W	480	90 a
Control	—	100 a

[z]Mean separation by Duncan's multiple range test, 5% level. Each data point represents the mean for 10 plants per treatment.

Daminozide at 1000 mg/liter and above caused reduced growth rates of *F. solani* (Table 3) in our *in vitro* studies. Further, when daminozide was added to benomyl amended agar at 100 mg/liter and higher, no change in the efficacy of benomyl was observed.

There was no significant difference in the mean number of lesions per plant in the daminozide treatment (Table 4) compared with the other treatments. The dominozide plus benomyl treatment had larger lesions than any of the other treatments and a general trend toward high disease incidence than the other treatments.

The most effective fungicides tested *in vitro* for control of *F. solani* were benomyl and ectaconazol at 10 mg/liter. Radial growth was reduced by captan and pyroxyfur at the

Table 1. The effect of various fungicides on the *in vitro* growth of *Fusarium solani*.[z]

Treatments	Rate (mg/liter)							
	1	10	100	500	1000	5000	10,000	100,000
	Mycelial growth (cm)							
Benomyl	5.8	NG[y]	NG	NG	—	—	—	—
Mancozeb	—[x]	8.5	NG	NG	NG	—	—	—
Ectacomazol	2.0	NG	NG	NG	—	—	—	—
Propiconazol	—	2.0	1.6	—	1.1	NG	—	—
Pyroxyfor	—	4.3	3.3	—	—	2.6	2.5	—
Captan	—	6.4	4.3	2.0	1.7	—	—	—
Chlorothalonil	—	4.7	4.4	—	NG	—	—	—
Neem leaf extract[w]	—	—	—	—	8.0	8.0	8.0	5.5

[z]The control treatment after 10 days growth at 29°C was 8.5 cm. Each data point represents the mean of 5 replicates.
[y]NG = No mycelial growth.
[x]Concentrations that were not evaluated have a hyphen (—).
[w]The neem leaf extract study growth measurements represents 6 days growth response. The control mycelial growth for this treatment was 8.0 cm.

Table 3. The effects of daminozide and benomyl on the *in vitro* growth of *Fusarium solani*.[z]

Treatments[y]	Rate (mg/liter)				
	100	200	1000	5000	10,000
	Mycelial growth (cm)				
Daminozide	8.3	8.2	6.1	5.0	3.8
Benomyl	NG[w]	NG	NG	NG	NG
Daminozide + benomyl	NG	NG	NG	NG	NG

[z]All treatments were replicated 5 times, with each data point representing the mean of all replicates/treatment.
[y]The control treatments mycelial growth at the 10-day period at 29° was 8.3 cm.
[w]NG = No mycelial growth.

Table 4. The effects of daminozide and/or benomyl foliar spray applications on fusarium stem rot.[z]

Treatment	Rate (mg/liter)	Cirbronze		Fiesta	
		Lesions/ plant	Lesion length (cm)	Lesions/ plant	Lesion length (cm)
Daminozide	23	6.9 a	1.3 c	1.5 ab	3.0 a
Benomyl	120	4.2 a		0.9 b	
Daminozide + benomyl	23 + 120	7.0 a	1.4 c	2.9 a	2.5 a
Inoculated control	—	7.5 a	4.8 a 1.8 b	1.7 ab	2.2 a 2.7 a

[z]Mean separation within columns by Duncan's multiple range test, 5% level. The data represents the mean of two replicates each with a sample population of 3 plants. Plants were harvested and then spray-inoculated with 1 x 10^6 propagules/ml of *Fusarium solani* to runoff.

highest rates used. The use of *in vitro* testing methods used in this study may not be the most effective means of measuring the basic activity of pyroxyfur, therefore future *in vivo* studies will be evaluated to determine this compound's full potential.

The use of chlorothalonil, mancozeb, and/or benomyl in inoculated plot studies has not resulted in acceptable control of fusarium stem rot. Our *in vitro* studies have established that these compounds are fungicidal to *F. solani* at rates lower than used in our inoculated plot studies. Possible reasons why these compounds are ineffective in these situations could be due to poor spray penetration, very high inoculum used, or the large number of wound sites. Our eradicative study results confirm that the above compounds plus captan, and a benomyl plus captan combination were all ineffective in controlling stem rot after the infection has become established.

Singh et al. (6) reported neem leaf extract inhibited *F. oxysporum* at 25,000 mg/liter. At 100,000 mg/liter complete inhibition of *F. solani* was not obtained. At this concentration, radial growth reduction of 31% was observed. At the concentrations used in these studies, a field equivalent rate would be approximately 84 lb. of leaf material per 100 gal of water. Possible variations in activity may be due to the age of the leaf tissue used and processing procedures. Our leaf sources were seedlings less than 1-yr-old.

The *in vitro* results indicate that daminozide does not affect the efficacy of benomyl at 100 mg/liter and above and that daminozide at 100 mg/liter and above reduced mycelial growth of *F. solani* by 27%. A possible explanation of the daminozide effect could be due to its growth regulator (retardant) effects. In greenhouse studies with daminozide plus benomyl, a consistent but not significant ($P=0.05$) increase in both the leasions per plant and the lesion length compared to the benomyl treatment alone was observed. The rate of benomyl used in the greenhouse study was 600 mg/liter and at that rate no antagonistic effect to the compounds efficacy or disease incidence should have been observed. The exact nature of the daminozide plus benomyl interaction is not known at this time. Further studies with benomyl, daminozide and other fungicides will be evaluated to determine their interaction.

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