Fusarium Wilt of Chrysanthemum: Complete Control of Symptoms With an Integrated Fungicide-Lime-Nitrate Regime

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Florida Agricultural Experiment Stations Journal Series No. 4541.

Accepted for publication 2 April 1973.

ABSTRACT

Benomyl 50W, and BAS 3201-F 50W [methyl 1-(methylthioethylcarbamoyl)-2-benzimidazolecarbamate], drenched on potted chrysanthemums grown on a high-lime, all-nitrate-nitrogen cultural regime, provided complete control of the symptoms of Fusarium wilt (*Fusarium oxysporum* f. sp. *chrysanthemi*) on the highly susceptible cultivar 'Yellow Delaware'. Rates as low as 15 g active ingredient/100 liters (0.125 lb/100 gal) drenched two times at 200 ml per 15-cm pot (6-inch) per application were effective and were not phytotoxic. Calcium hydroxide added to the soil and $NaNO_3$ as the only nitrogen source, contributed additively to the control of the disease.

Lack of foliage and stem symptoms, excellent development of the flowers, and normal top weights and heights of the plants confirmed the control of Fusarium wilt. This fungicide, high-lime, all-nitrate-nitrogen regime appears to be the first report of the complete control of the symptoms of Fusarium wilt of chrysanthemum.

Phytopathology 63:1256-1259

Additional key word: chemotherapy.

Certain chemical and nutritional factors reduce the development of Fusarium wilt in the host (1, 3, 5, 6, 7, 8, 9, 10, 11, 12). Edgerton & Moreland (3), Fisher (6), and Jones & Overman (8) reported lime reduced Fusarium wilt in tomato, and Woltz & Engelhard (12) had the same result with chrysanthemum. Using nitrate nitrogen rather than ammoniacal nitrogen reduced the development of Fusarium wilt in chrysanthemum according to Woltz & Engelhard (12), and in cotton according to Albert (1). The additive effect of lime and nitrate in reducing Fusarium wilt was shown in cotton by Albert (1), and in chrysanthemum by Woltz & Engelhard (12). Biehn & Dimond (2) reduced the severity of the symptoms of Fusarium wilt of tomato with benomyl. According to the literature, no nutritional or fungicidal treatments, or combinations thereof, have provided complete control of the symptoms of Fusarium wilt.

The objective of this research was to determine the feasibility of obtaining complete control of Fusarium wilt [*Fusarium oxysporum* (Schlecht.) f. sp. *chrysanthemi* Litt., Armst. & Armst.] of chrysanthemum in an integrated system in which the partially effective factors of fungicidal chemicals, lime, and nitrate nitrogen were combined.

MATERIALS AND METHODS.–In Experiment 1, rooted cuttings of *Chrysanthemum morifolium* Ramat 'Yellow Delaware' were planted 10 August 1971 in 15-cm plastic pots containing methyl bromide treated Myakka fine sand containing 30% (v/v) of peat. The treatments were replicated four times with four plants per replication. The plants were inoculated on 24 August using 70 ml/pot of spore suspension containing 200,000 conidia/ml of *F. oxysporum* f. sp. *chrysanthemi*. The pathogen was grown in petri plates on potato-dextrose agar. The conidia were harvested by flooding the plates with deionized water, and filtering the suspension through cheesecloth. The culture was a single-spore isolate obtained from G. M. Armstrong (University of Georgia, Griffin). The cultivar Yellow Delaware was selected because of its severe disease reaction to the above forma specialis (4).

Roots of the plants were injured just prior to inoculation by making a cut in the soil 2.5-cm from the stem and 8-cm deep on opposite sides of each plant. The 70 ml of spore suspension were poured into the slits in the soil in each pot.

Fungicides were evaluated in a high-lime, $NaNO_3$ cultural regime which was previously shown by Woltz & Engelhard (12) to provide a significant degree of control of Fusarium wilt. Dry $Ca(OH)_2$ at 2 g/kg of soil was thoroughly incorporated in the soil and allowed to equilibrate for one week prior to potting except that no $Ca(OH)_2$ was added to treatment No. 18 (Table 1).

The four chemicals used in the soil drenches included benomyl 50W, which was selected for its chemotherapeutic potency against Fusarium fungi, its systemic movement in a number of plants, and its commercial availability. BAS 3201-F 50W [methyl 1-(methylthioethylcarbamoyl)-2-benzimidazole carbamate] and thiabendazole 160 60W [2-(4-thiazolyl)benzimidazole] were selected because of their similarity in chemical structure and fungicidal activity to benomyl, and AN 2639 6E (n-decyl alcohol, 54%; and n-octyl alcohol, 45%) because the manufacturer reported the material to have systemic fungicidal activity against *Fusarium* sp. The fungicides, except AN 2639, were used at rates of 15 and 60 g of active ingredient per 100 liters (0.125 and 0.5 lb/100 gal) of water. AN 2639 was used at the rates of 30 and 60 g active ingredient/100 liters (0.25 and 0.5 lb/100 gal). Pots in Schedule A were drenched with fungicides at the rate of 200 ml/pot 3 and 13 days after planting, respectively. Pots in Schedule B received additional drenches with fungicides at 100 ml/pot 31 and 53 days after potting, respectively. The plants were grown with four hours supplementary lighting at night until 21 September, when they were maintained under natural short days and allowed to flower.

Plants were fertilized twice weekly with 100 ml/pot of nutrient solution. All treatments except No. 18 received a nutrient solution formulated from 3.2 g $NaNO_3$, 1.0 g $NaH_2PO_4 \cdot H_2O$, 0.85 g KCl, and 0.2 g $MgSO_4 \cdot 7H_2O$ dissolved in deionized water and diluted to 1 liter. In No. 18, 1.5 g NH_4NO_3 replaced the 3.2 g $NaNO_3$ as the nitrogen source. The solution containing $NaNO_3$ was shown to suppress Fusarium wilt development, whereas the solution containing NH_4NO_3 was shown to enhance the degree of wilt development in chrysanthemum (12).

Micronutrients were applied as needed to control foliage deficiency symptoms. The foliage of all plants was sprayed until thoroughly wet with ferric oxalate at the rate of 120 g/100 liters (1 lb/100 gal) on 21 and 28 August 1971. Ferric oxalate, manganese sulfate, and zinc sulfate, each at the rate of 120 g/100 liters (1 lb/100 gal), were sprayed on the foliage of all plants on 31 August and 5 October. Chelated iron (NaFe diethylenetriamine pentaacetate) at 0.09 g/l was added to the fertilizer drench on 3 September.

Pots were held in a greenhouse at 38 ± 2 C daily maxima and 25 ± 2 C minima for development of disease.

The plants were harvested 113 days after planting when they were rated for (i) foliage and stem symptoms of Fusarium wilt, (ii) desirability as commercially salable pots, and (iii) flower development. The tops of the plants were also weighed (Table 1).

A second experiment was conducted under similar conditions except the rooted cuttings were potted on 7 March 1972, 200 ml/pot of benomyl was drenched at the rate of 15 g/100 liters 3 and again 10 days after planting, the plants were inoculated 14 days after planting using 70 ml/pot of spore suspension containing 2,000,000 conidia/ml of *F. oxysporum* f. sp. *chrysanthemi*, and final disease ratings and height measurements were made 86 days after planting. The plants were exposed to natural long days and did not flower. The plants were sprayed with ferric oxalate at the rate of 120 g/100 liters (1 lb/100 gal) on 24 March and with ferric oxalate, manganese sulfate, and zinc sulfate, each at the rate of 120 g/100 liters (1 lb/100 gal) on 31 March, 7 and 14 April, to prevent minor element deficiencies.

The rating system used for Fusarium wilt was: 0 = no visible symptoms; 1 = leaf chlorosis and/or vascular discoloration in leaf; 2 = symptoms for 1 plus curvature of leaf and/or stem; 3 = symptoms for 2 plus wilting; 4 = symptoms for 3 plus stunting; and 5 = dead plant.

The rating system used to determine desirability as a commercially salable pot of plants was: 1 = free of visible symptoms of Fusarium wilt on the foliage and stems, plants uniform in height, and flowers uniformly developed; 2 = free of visible Fusarium wilt

TABLE 1. Effect of chemical drenches on Fusarium wilt control and plant development in 'Yellow Delaware' chrysanthemums

No.	Treatment	Rate active ingredient (g/100 liters)	Schedule[a]	Inoculated	Fusarium[b] wilt (0-5)	Commerc.[c] plants (1-3)	Flower[d] develop. (0-5)	Avg top wt per rep. (g)
1	Benomyl 50W	60	A	Yes	0	1.2	0	1,008
2	Benomyl 50W	60	B	Yes	0	1.0	0	832
3	Benomyl 50W	15	A	Yes	0	1.0	0	911
4	Benomyl 50W	15	B	Yes	0	1.0	0	821
9	BAS 3201-F 50W	60	A	Yes	0	1.0	.1	847
10	BAS 3201-F 50W	60	B	Yes	0	1.2	.2	843
11	BAS 3201-F 50W	15	A	Yes	0	1.2	.2	912
12	BAS 3201-F 50W	15	B	Yes	0	1.7	.2	920
13	AN 2639 6E	60	A	Yes	0.4	2.2	1.0	763
14	AN 2639 6E	60	B	Yes	1.3	2.7	1.5	701
15	AN 2639 6E	30	A	Yes	1.0	2.5	1.3	782
16	AN 2639 6E	30	B	Yes	1.7	3.0	1.4	717
17	Control-high lime			Yes	1.6	2.7	1.8	648
18	Control-low lime			Yes	4.0	3.0	3.5	364
19	Control-high lime			No	0	1.2	1.1	805
20	Benomyl 50W	60	A	No	0	1.2	0.4	861
21	Benomyl 50W	60	B	No	0	1.5	0.1	866
24	BAS 3201-F 50W	60	B	No	0	1.7	0.8	812
25	AN 2639 6E	60	B	No	0	1.2	0.5	732
$LSD_{.05}$					0.5	0.6	0.5	27

[a] A = pots drenched two times at 200 ml/pot. B = pots drenched as in A, plus they received two additional drenches with fungicides at 100 ml/pot.

[b] Graded system in which 0 = no visible symptoms to 5 = dead plant at harvest.

[c] One = plants with no disease symptoms, all plants and flowers uniformly developed at harvest; two = no disease symptoms but plants and/or flowers not uniformly developed; three = one or more plants with Fusarium symptoms.

[d] Graded system in which 0 = all flowers open (petals extended) to 5 = plants dead.

TABLE 2. Effect of benomyl fungicide, nitrogen source, and pH on Fusarium wilt development in 'Yellow Delaware' chrysanthemums

No.	Inoculated	Nitrogen source	Fungicide (Benomyl)	Initial soil acidity (pH)	Fusarium[a] wilt (0-5)	Average[b] height (cm)	Average[b] weight (g)
1	No	$NaNO_3$	Yes	7.4	0	107	211
2	No	$NaNO_3$	Yes	6.2	0	110	207
3	No	$NaNO_3$	No	7.4	0	104	187
4	No	$NaNO_3$	No	6.2	0	110	195
5	No	NH_4NO_3	Yes	7.4	0	122	226
6	No	NH_4NO_3	Yes	6.2	0	123	221
7	No	NH_4NO_3	No	7.4	0	120	213
8	No	NH_4NO_3	No	6.2	0	121	213
9	Yes	$NaNO_3$	Yes	7.4	0	109	212
10	Yes	$NaNO_3$	Yes	6.2	0.1	113	216
11	Yes	$NaNO_3$	No	7.4	3.4	94	155
12	Yes	$NaNO_3$	No	6.2	4.4	89	112
13	Yes	NH_4NO_3	Yes	7.4	0.3	115	218
14	Yes	NH_4NO_3	Yes	6.2	1.4	119	214
15	Yes	NH_4NO_3	No	7.4	4.8	87	60
16	Yes	NH_4NO_3	No	6.2	5.0	76	12
$LSD_{.05}$					0.5	9	26

[a] Graded system in which 0 = no visible wilt symptoms to 5 = dead plant.
[b] Each replication had four plants.

symptoms, plants uneven in height, and/or all flowers not uniformly developed; and 3 = Fusarium wilt symptoms present on one or more plants in a pot.

The rating system used for the development of individual flowers was: 0 = flowers open (petals were extended); 1 = petals opening but were not extended to a horizontal plane; 2 = flower buds showing color; 3 = buds "breaking" open to expose color; 4 = buds small and not showing color; and 5 = plants dead.

The pH of the soil was determined with a standard pH meter. In Experiment 1, determinations were made 30 days after planting and again at harvest, or 114 days after planting. The pH determinations for Experiment 2 were made at planting time. The soil samples for determining pH were prepared with 50-50 (v/v) soil-deionized water which had equilibrated 30 minutes.

RESULTS.—The most significant result of this research was the demonstration that benomyl 50W and BAS 3201-F 50W drenched on potted plants grown under the high-lime, $NaNO_3$ regime produced plants that were completely free of foliage and stem symptoms of Fusarium wilt (Table 1, 2). Plants treated with AN 2639 had visible symptoms of wilt, whereas almost all plants treated with thiabendazole were killed by the chemical (Nos. 5-8 inclusive).

Comparing ratings or top weights in Experiment 1 for "Fusarium wilt", "Flower development", "Average weight per rep", and "Commercial plants" between treatments 17 and 18 (Table 1) demonstrated the significant reduction in disease effects by the use of the high level of lime and $NaNO_3$-fertilization as compared to the low level of lime and NH_4NO_3-fertilization previously demonstrated by Woltz & Engelhard (12). Similar comparison of the plants receiving the benomyl or BAS 3201-F treatments superimposed on the high-lime, $NaNO_3$-fertilizer regime, demonstrated the additional wilt control provided by the fungicides (Treatments 1 through 12 versus 17, Table 1).

Treatment with benomyl 50W and BAS 3201-F 50W resulted in commercially salable pots of plants; i.e., the plants had green foliage, the leaves, stems, and flowers were free of disease symptoms, and were of uniform size and general appearance (Table 1). An arbitrary commercial plant rating of 1 to 1.8 was judged to be in the top quality bracket. Both inoculated and noninoculated plants treated with benomyl and BAS 3201-F fell within the desirable range, indicating no serious phytotoxicity from the integrated chemical-cultural approach to Fusarium wilt control. Similar results with benomyl were obtained in Experiment 2 (Table 2). Inoculated plants treated with AN 2639 and plants grown under the high-lime, $NaNO_3$ and low-lime, NH_4NO_3 cultural regimes were of poor quality because of Fusarium wilt. The average heights and average weights of the tops of the plants free of wilt symptoms varied somewhat, although visually there were no major differences among the plants (Table 1, 2).

Wilted plants were present in all the inoculated treatments receiving AN 2639 and in the high- and low-lime control plants. None of the noninoculated plants had foliage or stem symptoms.

The initial pH of the soil in Experiment 1 in the high-lime treatments was 7.9 and at harvest was 8.6, whereas the low-lime treatment was 6.5 and 7.0 initially and at harvest, respectively. In Experiment 2, the initial pH of the high-lime treatments was 7.4 and of the low-lime treatment 6.2 (Table 2).

Among the three substituted benzimidazole compounds used in Experiment 1, the aliphatically substituted benomyl and BAS 3201-F were effective in disease control and lacked phytotoxicity, whereas the ring-substituted thiabendazole was so phytotoxic that 11 of 16 plants were killed when treated two times at the low rate of application.

In Experiment 2, the chemotherapeutic effect of benomyl in controlling Fusarium wilt was clearly demonstrated in Treatment 9 vs. 11, 10 vs. 12, 13 vs. 15, and 14 vs. 16 (Table 2). In addition, control provided by using $NaNO_3$ rather than NH_4NO_3 as a nitrogen source (Treatments 10 vs. 14, 11 vs. 15, and 12 vs. 16) and the higher pH level versus the lower (Treatments 11 vs. 12, 13 vs. 14, and 15 vs. 16) was shown. The additive effect of chemotherapeutant, all NO_3 nitrogen, and pH was demonstrated in treatment 9, which was the only treatment in which all of 16 plants in the four replications were free of Fusarium wilt symptoms (Table 2). The average height of the plants in Treatment 9 was not different from the heights of noninoculated plants, indicating no phytotoxic effects from the treatments.

DISCUSSION.–Complete control of symptoms of Fusarium wilt in chrysanthemums was accomplished by simultaneously using factors or conditions which, separately, each provide a significant reduction in disease development in the host. The effect of using a chemotherapeutant, lime, and all-nitrate-nitrogen fertilization was shown to be additive and resulted in the control of Fusarium wilt of chrysanthemum. This additive principle for the control of plant diseases requires that all factors that provide some increment of disease control be used simultaneously to give the greatest over-all degree of integrated plant disease control possible. It could be applicable to all diseases and offers hope for the control of difficult-to-control vascular wilt diseases. This principle provides for a more balanced approach to disease control and lessens the pressure and dependence on disease control solely with fungicidal chemicals.

A producer of potted chrysanthemums who has a history of Fusarium wilt should be able to use this chemical-cultural disease control procedure with relatively little additional cost. Benomyl, at 15 g active ingredient/100 liters, applied two times at 200 ml per 15-cm (6-inch) diam pot per application, would cost less than $0.01 for four pots at current USA prices. The cost of hand labor or automatic application would be extra. The costs for liming and nutrient materials should not differ significantly from his regular program. This program is, therefore, feasible on both a biological and an economical basis.

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