Myclobutanil as a Curative Agent for Chrysanthemum White Rust

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ABSTRACT

Bonde, M. R., Peterson, G. L., Rizvi, S. A., and Smilanick, J. L. 1995. Myclobutanil as a curative agent for chrysanthemum white rust. Plant Dis. 79:500-505.

In response to a recent outbreak of chrysanthemum white rust, caused by *Puccinia horiana,* in California, research was conducted to test the fungicide myclobutanil for its effectiveness as a foliar chemical control for the disease. Myclobutanil applied 5 days after exposure of susceptible plants to sporidial inoculum had extremely strong curative properties and usually prevented disease development in either inoculated plants or cuttings obtained from presymptomatic infected mother plants. Even though lesions with pustules developed on inoculated plants when they were sprayed with the fungicide at 10, 15, or 20 days after sporidial inoculation, the pathogen produced few sporidia in an otherwise conducive environment. Myclobutanil at 100 mg a.i./liter, however, was not highly effective for preventing infection when sprayed onto plants 5 days before inoculation, although it did reduce disease incidence. The data indicate that myclobutanil (100 mg a.i./liter) used as a dip treatment for chrysanthemum cuttings prior to planting is suitable as a regulatory treatment for exclusion and eradication of chrysanthemum white rust.

Puccinia horiana Henn., the causal agent of chrysanthemum white rust, is a serious chrysanthemum pathogen indigenous to eastern Asia (14). It was first discovered in Japan in 1895 (13) and described by Hennings (13) in 1901. Since 1963, white rust has been reported in several European countries, the United Kingdom (2), New Zealand, South Africa (11), Australia (10), and South America (7).

Contrary to specific reports, *P. horiana* was never found on U.S. chrysanthemum plants until 1977 (see Kahn and Wheeler [16]). Isolated introductions of the pathogen and disease were reported in the states of New Jersey (20) and Pennsylvania (7) in 1977, and in Washington (Dan Williams, Washington Department of Agriculture, *personal communication*) and Oregon (12) in 1990. In each case, the disease was present only in hobbyist plantings and not in commercial nurseries, and the pathogen was eradicated by destruction of the diseased plants. White rust was found in Santa Barbara County, California (4), beginning in December 1991, and in 1992 in Santa Clara (5) and Santa Cruz (6) counties, California. These outbreaks were of considerable concern, both to the California Department of Food and Agriculture (CDFA) and to the U.S. Animal and Plant Health Inspection Service (APHIS), because they occurred in commercial nurseries and therefore posed a significant threat for extensive spread and economic losses.

Upon recognition of the disease in California and discussions among CDFA, APHIS, Agricultural Research Service (ARS), and industry personnel, a joint ARS/APHIS research project was initiated to obtain research data to support or reject an emergency strategy recently implemented to prevent spread and new introductions of the pathogen. This information was required in order to make critical decisions as to the best way to proceed in eradicating chrysanthemum white rust with as little disruption to the industry as possible.

Dickens (8,9) tested several fungicides to control chrysanthemum white rust in Great Britain. One of these, myclobutanil, was highly effective as a curative treatment when sprayed onto plants at 100 mg a.i./liter (the only concentration reported). Other fungicides were tested by Dickens (8,9) and others (10,18); however, only hexaconazole, propiconazole, and myclobutanil gave "complete control" (9). Myclobutanil is known to have systemic, protective, and curative activity against a wide range of fungal plant pathogens (17,19,21) and is a sterol demethylation inhibitor (DMI) of ergosterol biosynthesis (17) sold under the trade names Eagle, Nova, Rally, Prothane, and Systhane. It is the only highly effective fungicide for chrysanthemum white rust that is registered for use in California on horticultural crops, although it is not registered for chrysanthemums.

Our main goal was to determine the efficacy of myclobutanil as a dip treatment for cuttings from U.S. chrysanthemum cultivars for controlling the spread of the pathogen into disease-free greenhouses or nurseries. Currently, myclobutanil is not registered for use as a dip treatment for any crop in the United States; however, under an emergency label it is being used as such for chrysanthemum cuttings in California. Our study was initiated to determine the adequacy of this exclusion protocol recently implemented in California by CDFA and APHIS. The study was conducted at their request. Our second objective was to determine if myclobutanil could be used to eradicate the disease in a planting without total destruction of the plants.

MATERIALS AND METHODS

Pathogen maintenance. Bare-rooted chrysanthemum cuttings (cultivar unknown) infected with *P. horiana* were sent to the Foreign Disease-Weed Science Research (FDWSR), Frederick, Maryland, from Santa Cruz County, California. Three to 10 pustules were present on each cutting. The pathogen was transferred to healthy chrysanthemum plants every 2 weeks. Initially, *P. horiana* was propagated on cultivar Red Delano, but after a host susceptibility study, Splendor (provided by Yoder Brothers, Inc., Alva, Florida) was used.

Chrysanthemum plants. Splendor was used as a disease-maintenance/inoculum-donor plant because the pathogen produced a large number of sporulating pustules on inoculated plants. After consultation with industry representatives, five test cultivars (Detroit News, Coral Pomona, Cherry Pomona, Super White, and Super Yellow) were selected for inclusion in our studies. They were provided by Yoder Brothers as rooted cuttings shipped overnight to Frederick, Maryland, from Alva, Florida. After arriving in Frederick, they were transplanted to 10.2-cm or 15.2-cm clay pots (depending on the

Product names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Accepted for publication 3 February 1995.

purpose of the plants) containing a greenhouse soil mix in addition to lime, fertilizer, and a wetting agent. Inoculum donor plants (Splendor) were grown in 10.2-cm clay pots, as were all test plants except mother plants, which were to be used as a source of cuttings, which were planted in 15.2-cm clay pots. All plants were fertilized one to two times per week with 20:20:20 general purpose fertilizer.

Inoculation (mist) chamber. Inoculations were performed in an inoculation (mist) chamber consisting of a 1.83-m-long by 0.91-m-wide by 1.22-m-high frame constructed from 5.7-cm-diameter PVC pipe covered on all sides with 3-mil clear plastic. A ridged plastic drainpipe with a row of 1.9-cm-diameter holes on each of the two opposite sides of the pipe was suspended inside at the top center of the chamber. The plastic drainpipe was connected to a cold mist humidifier. When the chamber was in use, a fine mist was forced into the pipe, which acted as a manifold to equally distribute moisture and a small amount of air turbulence throughout the chamber. The turbulent moist air was required for uniform effective distribution of inoculum. While the chamber was in operation, relative humidity was maintained at 97 to 100%.

Inoculation of plants. Chrysanthemum plants were inoculated by incubating healthy plants next to infected (20 to 24 days postinoculation) plants for 18 hr at 16 to 18°C in the inoculation chamber in a 4:1 ratio, respectively. Infected plants were distributed evenly throughout the chambers. Each was on an inverted 10.2-cm clay pot to raise it above the canopy of the healthy plants. Following the inoculation/incubation period, inoculated plants were placed in the greenhouse at 22 to 28°C for disease development.

Application of myclobutanil. Two different methods were used for treatment of plant material with the fungicide: i) Rooted plants were sprayed with myclobutanil until runoff at a concentration of 100 mg a.i./liter (250 mg Eagle, 40% WP per liter), unless stated otherwise, by means of a 3.8-liter (1-gal) garden sprayer. Control plants were sprayed until runoff with water; and ii) Cuttings from mother plants, following a 24-hr storage period in a 4°C refrigerator, were dipped in a suspension of myclobutanil at 100 mg a.i./liter prior to treating cut ends with Rootone F (Dragon Corp., Roanoke, VA) and planting in 10.2-cm clay pots.

Application 5 days prior to inoculation to determine prophylactic effects. Eight plants of each of the five cultivars were sprayed with myclobutanil as previously described at 100 mg a.i./liter. Six additional plants per cultivar were sprayed until runoff with water as untreated controls. Five days after treatment, five treated and three untreated plants per cultivar were inoculated with *P. horiana* by placement in

Table 1. Level of infection[x] of plants inoculated with *Puccinia horiana* 5 days after a foliar protectant application of myclobutanil (100 mg a.i./liter)

Treatment	Number of plants infected in each experiment											
	No pustules			1–4 pustules			5–100 pustules			>100 pustules		
Cultivar	1	2	3	1	2	3	1	2	3	1	2	3
Fungicide treatment[y]												
Detroit News	0	1	1	5	4	1	0	0	3	0	0	0
Coral Pomona	4	3	1	1	0	1	0	2	3	0	0	0
Cherry Pomona	2	3	2	2	1	0	1	1	3	0	0	0
Super White	5	4	2	0	0	0	0	1	2	0	0	1
Super Yellow	3	3	1	2	1	1	0	1	3	0	0	0
Control[z]												
Detroit News	0	0	0	0	0	0	0	0	0	3	3	3
Coral Pomona	0	0	0	0	0	0	1	0	0	2	3	3
Cherry Pomona	0	0	0	0	0	0	1	0	0	2	3	3
Super White	0	0	0	0	0	0	0	0	0	3	3	3
Super Yellow	0	0	0	0	0	0	0	0	0	3	3	3

[x] Data presented are for numbers of pustules on test plants at 30 days after incubation of plants in a mist chamber with infected donor plants acting as a source of inoculum.

[y] Five plants were used per cultivar per experiment for the pathogen/fungicide treatment. Noninoculated/fungicide-treated plants did not develop disease and data are not presented in table.

[z] Three plants were used per cultivar per experiment for the pathogen/nonfungicide (water) treatment. Noninoculated/nonfungicide-treated plants did not develop disease and are not presented in the table.

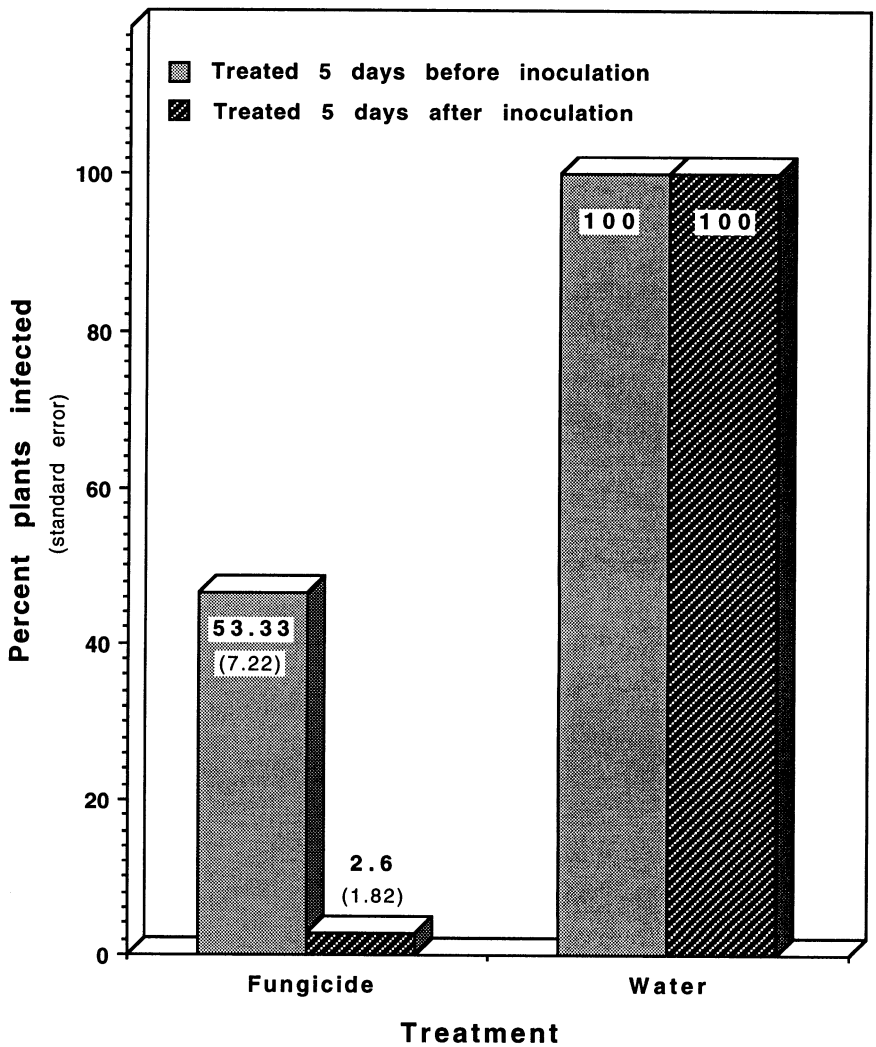

Fig. 1. Comparison of percentage of plants infected by *Puccinia horiana* when sprayed with myclobutanil at 100 mg a.i./liter or with water either 5 days before or 5 days after inoculation with the pathogen. Data were collected 30 days after inoculation and were pooled for the five cultivars. The 2.6% infection for plants treated with the fungicide 5 days after inoculation represents two plants with one or two pustules per plant, respectively, and demonstrates the high curative properties of the fungicide.

the mist chamber with infected plants as previously described. Three fungicide-treated plants and three water-sprayed plants per cultivar were placed in a second chamber without infected plants to serve as uninoculated controls. The experiment was repeated on two additional dates.

Application 5 days after inoculation to determine curative effects. Eight plants of each of the five cultivars were inoculated with *P. horiana* as described. Six additional plants per cultivar were placed in a second mist chamber as uninoculated controls. Five days after inoculation, five inoculated and three uninoculated plants per cultivar were sprayed (treated) with myclobutanil at a concentration of 100 mg a.i./liter as described. Three inoculated and three uninoculated plants per cultivar served as untreated controls. This experiment was repeated on two additional dates.

Efficacy of myclobutanil at various concentrations applied 5 days after inoculation. Thirty plants each of Detroit News, Cherry Pomona, and Super White were inoculated with *P. horiana,* and five plants of each cultivar served as uninoculated controls. Five days after inoculation, six sets of five inoculated plants of each cultivar were sprayed with myclobutanil at 100, 50, 25, 10, 5, or 0 mg a.i./liter. Five uninoculated plants of each cultivar were sprayed with water to serve as uninoculated non-fungicide-treated controls. The experiment was conducted three times, each on a different date.

Dip treatment of cuttings from infected plants. Fifteen plants of each of the five cultivars were inoculated with *P. horiana* in the mist chamber. Ten plants of each cultivar were used as uninoculated controls. Five days after inoculation, five cuttings were taken from each plant, placed in a plastic bag, and refrigerated for 24 hr. The next day, 30 cuttings from inoculated plants of each cultivar and 10 from uninoculated plants were dipped (treated) for 3 s in myclobutanil (100 mg a.i./liter). The lower ends of the cuttings were dipped in Rootone F and planted in 10.2-cm clay pots. Twenty cuttings originating from inoculated and 10 from uninoculated plants per cultivar were dipped in water, treated with Rootone F as untreated controls, and planted. From 2 to 6 hr after planting, plants were placed under a misting system in a greenhouse cubicle for 4 days, then transferred to a greenhouse at 22 to 28°C. This experiment was conducted three times, each on a different date. Using a new set of mother plants, the experiments were repeated an additional three times exactly the same as the first set of three experiments, except that three cultivars instead of five were used.

Efficacy of myclobutanil at various stages of disease development. Twenty-four plants of each of five cultivars were inoculated with *P. horiana.* Eight plants of each cultivar were placed in a mist chamber without infected plants to serve as uninoculated controls. Five days after inoculation, a set consisting of four inoculated and one uninoculated plant per cultivar was treated with myclobutanil (100 mg a.i./liter) by spraying until runoff. Additionally, two inoculated and one uninoculated plant per cultivar were sprayed with

Table 2. Level of infection[x] on plants inoculated with *Puccinia horiana* 5 days before a foliar spray application of myclobutanil (100 mg a.i./liter)

Treatment	Number of plants infected in each experiment											
	No pustules			1–4 pustules			5–100 pustules			>100 pustules		
Cultivar	1	2	3	1	2	3	1	2	3	1	2	3
Fungicide treatment[y]												
Detroit News	5	5	5	0	0	0	0	0	0	0	0	0
Coral Pomona	5	5	5	0	0	0	0	0	0	0	0	0
Cherry Pomona	5	5	4	0	0	1	0	0	0	0	0	0
Super White	5	5	5	0	0	0	0	0	0	0	0	0
Super Yellow	4	5	5	1	0	0	0	0	0	0	0	0
Control[z]												
Detroit News	0	0	0	0	0	0	0	0	0	3	3	3
Coral Pomona	0	0	0	0	0	0	0	0	0	3	3	3
Cherry Pomona	0	0	0	0	0	0	0	1	0	3	2	3
Super White	0	0	0	0	0	0	0	0	0	3	3	3
Super Yellow	0	0	0	0	0	0	0	1	0	3	2	2

[x] Data presented are for numbers of pustules on test plants at 30 days after incubation of plants in a mist chamber with infected donor plants acting as a source of inoculum.
[y] Five plants were used per cultivar per experiment for the pathogen/fungicide treatment. Noninoculated/fungicide-treated plants did not develop disease and data are not presented in table.
[z] Three plants were used per cultivar per experiment for the pathogen/nonfungicide (water) treatment. Noninoculated/nonfungicide-treated plants did not develop disease and are not presented in the table.

Table 3. Three-way analysis of variance[y] for infection levels of *Puccinia horiana* on three chrysanthemum cultivars sprayed with specific concentrations of myclobutanil followed by 30 days incubation

Source	df	Sum of squares	Mean square	*F* value	*P* value[z]
Cultivar	2	7.678	3.839	15.022	0.0001
Experiment	2	4.844	2.422	9.478	0.0001
Fungicide rate	3	237.661	79.220	309.993	0.0001
Cultivar × Experiment	4	3.122	0.781	3.054	0.0188
Cultivar × Fungicide rate	6	5.789	0.965	3.775	0.0016
Experiment × Fungicide rate	6	7.556	1.259	4.928	0.0001
Cultivar × Experiment × Fungicide rate	12	7.544	0.629	2.460	0.0060
Residual	144	36.800	0.256		

[y] Dependent variable was infection level, and independent variables were cultivar, experiment, and fungicide rate.
[z] Significant differences exist among all sources ($P \geq 0.05$).

Table 4. Chrysanthemum rust infection levels on three cultivars after foliar spray application of different concentrations of myclobutanil 5 days after inoculation with *P. horiana* and 25 days incubation

Myclobutanil (mg a.i./liter)	Cultivar and infection level		
	Detroit News[w]	Cherry Pomona[x]	Super White[y]
0	3.00 a[z]	3.00 a	3.00 a
5	3.00 a	3.00 a	3.00 a
10	2.87 b	3.00 a	3.00 a
25	1.60 c	2.33 b	2.80 a
50	0.47 d	0.87 c	0.93 b
100	0.00 e	0.00 d	0.20 c

[w] Infection level = -0.548 (log myclobutanil concentration)^2 – 1.080 (log myclobutanil concentration) + 4.189; $R^2 = 0.968$.
[x] Infection level = -1.954 (log myclobutanil concentration)^2 – 2.809 (log myclobutanil concentration) + 2.060; $R^2 = 0.978$.
[y] Infection level = -2.391 (log myclobutanil concentration)^2 – 4.139 (log myclobutanil concentration) + 1.301; $R^2 = 0.938$.
[z] n = 15: Infection levels within each column with the same letter are not significantly different using Fisher's Protected Least Significant Difference ($P = 0.05$). 0 = no infection; 1 = 1–4 pustules/plant; 2 = 5–100 pustules/plant; 3 = more than 100 pustules/plant with coalesced lesions.

water as fungicide-untreated controls. This procedure was conducted with a new set of plants at 10, 15, and 20 days after inoculation, and the experiment was repeated twice.

Data collection and analysis. Plants in all studies were examined for symptoms 10, 20, and 30 days after inoculation. A disease rating scale was developed where 0 = no infection, 1 = fewer than five pustules per plant, 2 = five to 100 pustules per plant, 3 = more than 100 pustules per plant, and 4 = more than 100 pustules per plant and two or more leaves with coalesced lesions over at least 75% of the leaf area. The scale was designed to emphasize differences at the lower numbers of pustules, since we were interested in complete control (eradication). Because disease category 3 was not common, and 100 or more pustules usually resulted in coalesced lesions, categories 3 and 4 were combined in the analyses and given the designation 3.

Data for effects of fungicide rates on pustule numbers for three chrysanthemum cultivars sprayed with myclobutanil 5 days after inoculation were analyzed by ANOVA, and treatment means were separated by Fisher's Protected Least Significant Difference. Infection levels were assigned numbers, where 0 = no pustules, 1 = one to four pustules, 2 = five to 100 pustules, and 3 = greater than 100 pustules per plant. Data were initially analyzed by a three-way analysis with infection level the dependent variable and cultivar, experiment, and fungicide rate the independent variables. Because of a significant difference among cultivars in numbers of pustules, the cultivars were then analyzed separately, with infection level the dependent variable and fungicide rate and experiment the independent variables.

RESULTS

Application of myclobutanil 5 days prior to inoculation. Ten days following inoculation with *P. horiana*, no symptoms were observed on any of the plants in three experiments. At 20 days, one inoculated/fungicide-treated plant of Coral Pomona, rated 2 in the first experiment, and one plant of Detroit News, rated 1 in the second repeat of the experiment, had pustules. In the third repeat of the experiment, 11 inoculated/fungicide-treated plants, rated 1 to 3, had developed pustules. All untreated inoculated control plants were infected (ratings 2 to 3) in all three experiments. No symptoms were noted on any of the uninoculated controls. Results of the 30-day readings are presented in Table 1 and Figure 1. The application of myclobutanil 5 days prior to inoculation, although decreasing the amount of disease, was not satisfactory, at least with a single application, as a protective treatment.

Application of myclobutanil 5 days after inoculation. Results of the 30-day readings are presented in Table 2 and Figure 1. Only two plants in the inoculated/fungicide-treated plants in a total of three experiments (Table 2) developed pustules, and each had only two pustules on a single leaf. None of the uninoculated plants developed pustules.

Curative ability of myclobutanil at different concentrations. Ten days after inoculation, no pustules were observed on any of the plants in three experiments at 100, 50, 25, 10, 5, or 0 mg a.i./liter, although most of the inoculated plants showed chlorotic symptoms, indicating a successful inoculation and initiation of infection.

After 20 days, no pustules were observed on any of the plants inoculated/fungicide-treated at 100 mg a.i./liter. Light infection was observed (0 to less than 100 pustules per plant) on plants inoculated/fungicide-treated at 50 mg a.i./liter. Heavy infection (greater than 100 pustules per plant) was observed on plants inoculated/fungicide-treated at 25, 10, 5, or 0 mg a.i./liter. No symptoms were observed on uninoculated control plants.

The results of the observations at 30 days were similar to those at 20 days; the infection level decreased with the concentration of myclobutanil. Although there were significant differences among cultivars and experiments (Table 3), myclobutanil consistently controlled white rust in all experiments (Table 4, Fig. 2).

Application of myclobutanil as a dip treatment for cuttings from infected plants. Ten days after inoculation, no pustules were observed on any fungicide-treated plants, although most of the inoculated plants showed chlorotic symptoms, indicating successful inoculation and initiation of infection. After 20 days, no pustules were observed in any of the six experiments of the inoculated/fungicide-treated plants. Over 90% of the inoculated/fungicide-untreated plants were infected. None of the uninoculated (fungicide-treated or untreated) controls was infected. At 30 days, only two of 720 inoculated/fungicide-treated plants (0.3%) were infected, with two to three pustules each. Pustules developed on 94% of the inoculated/fungicide-untreated plants, and

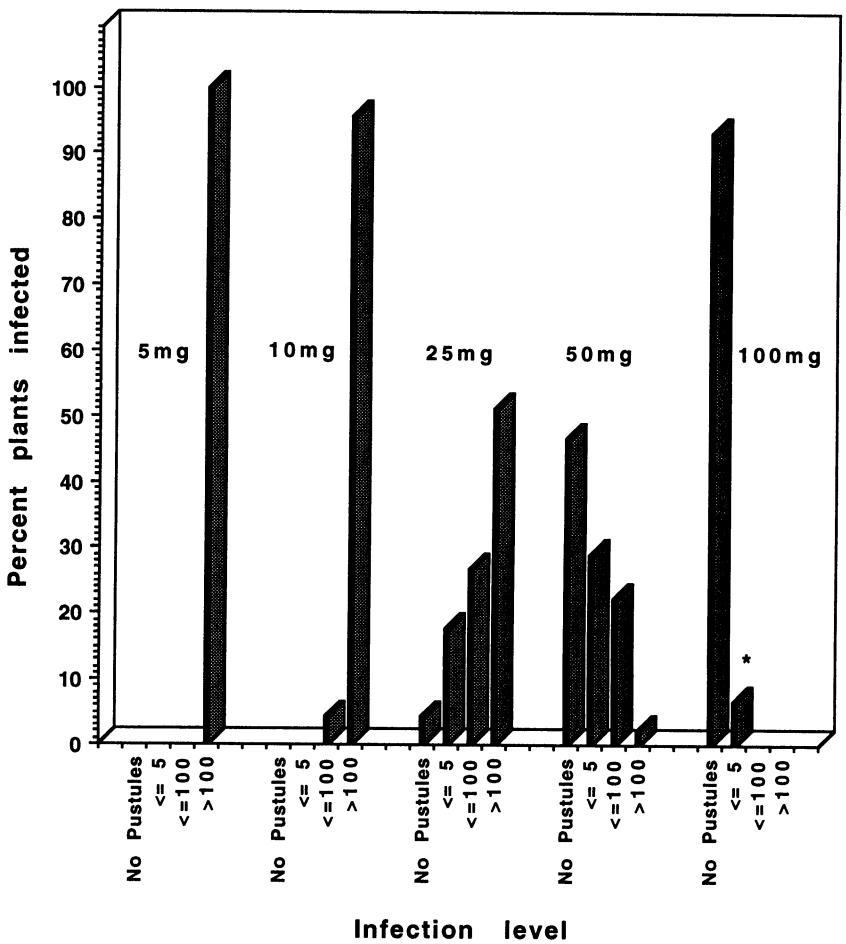

Fig. 2. Percentage of plants (combined data for five cultivars) in each of four infection categories (based on numbers of pustules) after foliar spray treatment with myclobutanil at 5, 10, 25, 50, or 100 mg a.i./liter 5 days after inoculation with *Puccinia horiana*. Note that at 25 and 50 mg a.i./liter, many plants were in the intermediate categories for numbers of pustules, and control was unsatisfactory. At the highest fungicide concentration, two plants (designated with a star) each had one to two pustules. Data are for plants examined 30 days after inoculation. All control plants (sprayed with water instead of fungicide solution) had more than 100 pustules per plant.

Table 5. Levels of infection on plants foliar spray treated with myclobutanil (100 mg a.i./liter) at 5, 10, 15, or 20 days after inoculation with *Puccinia horiana*

Time / Cultivar	Number of plants infected in each experiment											
	No pustules			1–4 pustules			5–100 pustules			>100 pustules		
	1	2	3	1	2	3	1	2	3	1	2	3
5 Days[z]												
Detroit News	4	4	4	0	0	0	0	0	0	0	0	0
Coral Pomona	4	4	4	0	0	0	0	0	0	0	0	0
Cherry Pomona	4	4	4	0	0	0	0	0	0	0	0	0
Super White	2	4	4	1	0	0	0	0	0	0	0	0
Super Yellow	4	4	4	0	0	0	0	0	0	0	0	0
10 Days[z]												
Detroit News	0	0	1	0	0	0	0	0	0	4	4	3
Coral Pomona	0	0	0	0	0	0	0	0	0	4	4	4
Cherry Pomona	0	0	0	0	0	0	0	0	0	4	4	4
Super White	0	0	1	0	0	0	0	0	0	4	4	3
Super Yellow	0	0	0	0	0	0	0	0	0	4	4	4
15 Days[z]												
Detroit News	0	0	0	0	0	0	0	0	0	4	4	4
Coral Pomona	0	0	0	0	0	0	0	0	0	4	4	4
Cherry Pomona	0	0	0	0	0	0	0	0	0	4	4	4
Super White	0	0	0	0	0	0	0	0	0	4	4	4
Super Yellow	0	0	0	0	0	0	0	0	0	4	4	4
20 Days[z]												
Detroit News	0	0	0	0	0	0	0	0	0	4	4	4
Coral Pomona	0	0	0	0	0	0	0	0	0	4	4	4
Cherry Pomona	0	0	0	0	0	0	0	0	0	4	4	4
Super White	0	0	1	0	0	0	0	0	0	4	4	3
Super Yellow	0	0	0	0	0	0	0	0	0	4	4	4

[z] Four inoculated plants per cultivar per experiment were sprayed with myclobutanil at 100 mg a.i./liter 5, 10, 15, or 20 days after inoculation, and plants were examined for pustules 30 days after inoculation. Two additional inoculated and one uninoculated plant per cultivar per time treatment per experiment were sprayed with water as fungicide-untreated controls and also examined 30 days after inoculation. All inoculated fungicide-untreated plants developed heavy infection (data not included in table) and all uninoculated fungicide-untreated plants developed no disease (data not included in table).

most had more than 100 per plant. No uninoculated controls (fungicide-treated or untreated) had developed pustules.

Efficacy of myclobutanil foliar sprays at various stages of disease development. At 10 days after inoculation, all plants inoculated and then spray-treated 5 days later showed chlorotic symptoms, indicating successful infection, but no pustule formation. All uninoculated (fungicide-treated or untreated) plants were free of symptoms.

Twenty days after inoculation, plants inoculated and treated 5 days afterward, except for two of four plants of one cultivar (Super White in one experiment), were free of pustules. Plants treated with myclobutanil 10 days after inoculation showed a reduced amount of disease compared to controls. All other inoculated plants were highly infected. No symptoms were observed in the uninoculated (fungicide-treated or untreated) control plants.

Thirty days after inoculation, plants inoculated and fungicide-treated 5 days afterward, with the exception of Super White in one experiment (two out of four), remained pustule free (Table 5). All other inoculated plants, including those treated at 10, 15, or 20 days after inoculation, were heavily infected, with 100 or more pustules per plant. No symptoms were observed on any of the uninoculated fungicide-treated or untreated control plants.

DISCUSSION

Based on the results of this study, it is apparent that myclobutanil applied in a spray treatment at the rate of 100 mg a.i./liter had significant activity against *P. horiana*. Myclobutanil applied in a single protectant application was not highly effective as a protective fungicide, although it significantly reduced disease. In a situation where complete control is not required or repeated applications are feasible, myclobutanil may be satisfactory as a protectant.

In contrast, the application of the fungicide 5 days after inoculation with *P. horiana* was strongly curative and almost totally prevented disease development in both inoculated plants and cuttings from infected plants. (Although not discussed in this paper, the curative effects of the fungicide also were tested on a second isolate of the pathogen from California and an isolate from Mexico, with results nearly identical to those presented here.) Triazole fungicides, of which myclobutanil is a representative, characteristically have strong curative properties (1).

The curative effects of myclobutanil also were observed by Dickens (9); however, he did not test myclobutanil as a dip treatment for cuttings. The amount of infection we observed on plants or cuttings treated with myclobutanil 5 days after inoculation was extremely low (only a few rare pustules) and possibly could have been eliminated by increasing the fungicide concentration or making an additional application. We note, however, that the quantity of inoculum used in our study was extremely high, and the environment was highly conducive to infection. Consequently, conditions were considerably more favorable for disease than in normal commercial production.

Although lesions with pustules developed on inoculated plants when they were sprayed with myclobutanil at 10, 15, or 20 days after inoculation, pustules produced few sporidia when tested by placing excised leaves over agar in a humid environment (data not presented). Hence, these pustules would contribute little toward disease spread. In agreement with Dickens (9), we observed no obvious plant growth regulator effects with the use of myclobutanil.

Myclobutanil is registered for use on several crops, including peaches, cherries, nectarines, ornamentals, and grapes (22). On apples, myclobutanil is used to control apple scab (*Venturia inaequalis* (Cooke) G. Wint.); however, a spray program based solely on myclobutanil resulted in inadequate control (15). The near total control of chrysanthemum white rust in our study using a myclobutanil dip can be attributed partly to the complete coverage of the host surface by submersion of the cuttings into the fungicide solution.

Development of fungicide resistance is a concern with the demethylation inhibiting fungicides such as myclobutanil, which share a common, site-specific mode of action. Fungicides with a single inhibition site have a higher risk of selecting fungicide-resistant pathogen populations than do multiple-site inhibitors (17). There is evidence of resistance having developed to myclobutanil in the apple scab pathogen (3). Therefore, it is necessary that additional fungicides be tested and registered for control of white rust.

The results of our study indicate that myclobutanil used as a cutting dip at the rate of 100 mg a.i./liter would effectively exclude chrysanthemum white rust from commercial greenhouses or nurseries. Although myclobutanil currently is not registered for use as a dip on any crop, we believe that it should be registered for that purpose for chrysanthemums.

ACKNOWLEDGMENTS

We sincerely thank Andrew Bishop and Jane Trolinger, Yoder Brothers, Inc., for providing the chrysanthemum plant materials; John Long, Rohm and Haas Co., for providing the myclobutanil during the course of the study; and Ted Boratynski, APHIS, Plant Protection and Quarantine, El Centro, California, for the pathogen cultures. We thank Conrad Krass, California Department of Food and Agriculture, for providing especially valuable guidance during this study. We also thank Eddie Rippeon, Steve Flook, and Jennifer Haines, USDA-ARS, Frederick, for valuable technical assistance that made the study possible.

LITERATURE CITED

1. Agrios, G. N. 1988. Plant Pathology. 3rd ed. Academic Press, New York.
2. Baker, J. J. 1967. Chrysanthemum white rust in England and Wales 1963-66. Plant Pathol. 16:162-166.
3. Braun, P. G., and McRae, K. B. 1992. Composition of a population of *Venturia inaequalis* resistant to myclobutanil. Can. J. Plant Pathol. 14:215-220.
4. California Department of Food and Agriculture. 1991. Plant pathology highlights. Calif. Plant Pest Dis. Rep. (Oct.-Dec.) 10:87-89.
5. California Department of Food and Agriculture. 1992. Plant pathology highlights. Calif. Plant Pest Dis. Rep. (Jan.-May) 11:18-19.
6. California Department of Food and Agriculture. 1992. Plant pathology highlights. Calif. Plant Pest Dis. Rep. (June-Sept.) 11:36.
7. CMI Distribution Maps of Plant Diseases. 1989. *Puccinia horiana* map no. 403. CABI.
8. Dickens, J. S. W. 1990. Studies on the chemical control of chrysanthemum white rust caused by *Puccinia horiana*. Plant Pathol. 39:434-442.
9. Dickens, J. S. W. 1991. Evaluation of some newer fungicides, in comparison with propiconazole, against chrysanthemum white rust (*Puccinia horiana*). Tests of agrochemicals and cultivars 12 (1991) Ann. Appl. Biol. (Suppl.) 118:32-33.
10. Exley, P. J., Giles, R. J., Pascoe, I. G., and Guy, G. L. 1993. The impact and control of white rust of chrysanthemums in Australia. Abstr. Int. Congr. Plant Pathol., 6th.
11. Firman, I. D., and Martin, P. H. 1968. White rust of chrysanthemums. Ann. Appl. Biol. 62:429-442.
12. Griesbach, J. A., Milbrath, G. M., and Thomson, T. W. 1991. First occurrence of chrysanthemum white rust caused by *Puccinia horiana* on florists' chrysanthemum in Oregon. Plant Dis. 75:431.
13. Hennings, P. 1901. Einige neue japanische Uredineen. Hedwigia 40:25-26.
14. Hiratsuka, N. 1956. Three species of chrysanthemum-rusts in Japan and its neighboring districts. Sydowia, Ser. 2. Suppl. 1:34-44.
15. Jones, A. L., Ehret, G. R., El-Hadidi, M. F., Zabik, M. J., and Cash, J. N. 1993. Potential for zero residue disease control programs for fresh and processed apples using sulfur, fenarimol, and myclobutanil. Plant Dis. 77:1114-1118.
16. Kahn, R. P., and Wheeler, W. H. 1969. *Puccinia horiana* - not known to occur in the United States. Plant Dis. Rep. 53:420.
17. Köller, W. 1988. Sterol demethylation inhibitors: Mechanisms of action and resistance. Pages 79-88 in: Fungicide Resistance in North America. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
18. Orlikowski, L. B., and Wojdyla, A. 1981. Chemical control of chrysanthemum white rust. Acta Hortic. 125:201-206.
19. Orpin, C., Bauer, A., Bieri, R., Faugeron, J. M., and Siddi, G. 1986. Myclobutanil, a broad-spectrum systemic fungicide for use on fruit, vines, and a wide range of other crops. Pages 55-62 in: Br. Crop Prot. Conf. - Pests Dis., 1986.
20. Peterson, J. L., Davis, S. H., Jr., and Weber, P. V. V. 1978. The occurrence of *Puccinia horiana* on chrysanthemum in New Jersey. Plant Dis. Rep. 62:357-360.
21. Rohm and Haas Co. 1992. Eagle WSP: Turf and ornamental fungicide (Pending EPA Registration). Rohm and Haas Co., Independence Mall West, Philadelphia, PA.
22. Thomson, W. T. 1994. Agricultural Chemicals. Book IV. Fungicides. 1993-94 Rev. Thomson Publications, Fresno, CA. p. 144.