

Diseases

Fungicide Evaluations For The Control of *Phymatotrichum* Root Rot

Dick Hine, Plant Pathologist; Roy Whitson, former Research Assistant; Jim Armstrong, Pima County Agricultural Agent; Don Howell, Yuma County Extension Director; Ron Cluff, Graham County Extension Director

Summary

Eight sterol-inhibiting fungicides including propiconazol, etaconazol (Ciba-Geigy), bitertanol, triadimefon, triadimenol (Bayer), imazalil (Janssen), fenarimol (Elanco), and XE-770 (Chevron) were evaluated in the laboratory for activity against *Phymatotrichum omnivorum*, the causal fungus of *Phymatotrichum* root rot of cotton. The fungicides inhibited mycelial growth of *P. omnivorum* at concentrations as low as 0.1 parts per billion. Significant stunting of cotton seedlings occurred following simulated pre-plant applications of propiconazol (Tilt) at rates equivalent to 0.56-2.24 kg/ha. Using this stunting of cotton seedlings as a bioassay, it was determined that propiconazol persisted in field soil for 3-5 mo. Results of field evaluations of propiconazol in 1983 and 1984 determined that foliar applications at 0.56 kg/ha, or side-dress applications of granules at 1.12 to 2.24 kg/ha, applied 4-8 wk after planting, provided statistically significant control of root rot in cotton.

In 1984, leaves of 5-wk-old container-grown cotton plants were treated with C-14-labeled propiconazol (obtained from Ciba-Geigy) to determine whether control of root rot from foliar sprays was the result of downward translocation of the fungicide. Plant tissues were completely oxidized to carbon dioxide, and the C-14 analyzed by liquid scintillation. Over an 8-wk period, 0.07-0.23% of the applied radioactivity was translocated to the roots. Using these percentages, the concentration of propiconazol in the roots from a foliar application of 0.56 kg/ha was calculated to be 1.52-4.31 ug/g, or more than 250X the concentration necessary to inhibit mycelial growth of *Phymatotrichum omnivorum*.

Bioassay techniques also demonstrated downward translocation of Tilt into cotton tap roots from foliage applications. These findings indicate that the control achieved from foliar applications resulted from the translocation of propiconazol to the roots. This is the first report of controlling *Phymatotrichum* root rot of cotton with application of a fungicide applied to foliage.

The authors would like to thank the following individuals for time and help in establishing the field plots: Tom Clark, Jr., Art Pacheco, Barkley Company of Arizona (Gabe Piceno, Jesus Tovar), Dr. Curtis Engle (Mobay), and Dr. Scott Ferguson (Ciba-Geigy).

Since 1981, we have been looking at the potential of a new Ciba-Geigy fungicide (propiconazol or TILT) for control of *Phymatotrichum* root rot of cotton. Laboratory studies demonstrated that *Phymatotrichum omnivorum* was sensitive to TILT at concentrations as low as 1.0 ppb, and that foliar and soil applications of TILT reduced disease and increased yield of cotton in root rot infested areas. In 1983 and 1984 we evaluated 5 additional fungicides which have the same mode of action (inhibition of sterol synthesis) as TILT for toxicity against *P. omnivorum* and control of root rot in the field. Evaluations of these chemicals were made in small replicated plots at the University of Arizona Experimental Farm at Marana. Large, replicated field plots were also made in Yuma, Graham and Pima County. Because of the floods of 1983 we did not collect any data from our large plots (3 locations in Marana or in Graham County). During 1984, plots were established in Yuma County and at 3 locations in Marana. In addition, because foliar applications of TILT reduced root rot in field trials in 1982 and 1983, it seemed likely that the fungicide had moved downward from the foliage to the root system in sufficient concentration to prevent infection by *P. omnivorum*. Studies were initiated during 1983 and 1984 to prove this downward translocation.

Materials and Methods

Evaluation of Toxicity:

Besides TILT, the other fungicides evaluated (and their manufacturers) were: Vangard (Ciba-Geigy); Bayleton and Baytan (Mobay); Imazalil (Janssen); and XE-779 (Chevron). The fungicides were obtained as commercial formulations of either emulsifiable concentrates, or wettable powders. Potato dextrose agar (PDA) was amended with the fungicides to produce concentrations of 100, 10, 1.0, or 0.1 ppb.

An 8mm diameter plug of mycelium, from a 2-wk-old culture of *P. omnivorum*, was transferred to a control or fungicide-amended plate. Five replicates were prepared for each chemical and concentration, and the entire evaluation was replicated a minimum of three times, on successive weeks. Measurements of the greatest radial growth of the mycelium were made at 24 hour intervals, up to 96 hours. The data are presented as the percent reduction in growth at 96 hours, relative to the control.

Field evaluations for control of root rot:

During the summer of 1984 one large, replicated field plots was established in San Luis (Yuma County, Barkeley Farms), and three plots in Marana (2 plots with Art Pacheco and 1 plot with Tom Clark). At each of the four locations, granules (2.5% a.i.) of a slow release and regular release formulation of Tilt injected with a tractor mounted Gandy apparatus approximately 6" deep and 8" from the seed row at the rates of 1.0 and 0.5 lbs a.i./A. Four row plots, approximately 600 ft in length were replicated four times. The applications were made when the plants were approximately 2 months in age. The fields were irrigated within 24 hrs of the application in order to activate the granules.

Also, small plots were established at the University of Arizona Experimental Farm at Marana to evaluate 2 new, highly active chemicals, Baytan (Mobay) and XE-779 (Chevron) in comparison with Tilt in controlling root rot.

In the small replicated plots, plant counts were made weekly from July through September.

Systemic Translocation of TILT in Cotton Plants:

Carbon-14 labeled TILT was obtained from the Ciba-Geigy Corporation. Cotton plants (Deltapine 55) were grown in a sterilized mixture of field soil and peat moss (2:1), contained in 3 ft sections of 4 inch diameter PVC sewer pipe, in order to allow for the development of a tap root similar to that occurring in a field situation.

Treatments were made on groups of 5 and 8 week old plants. Each plant was treated with 1.0 ml (5-wk-old plants) and 1.5 ml (8-wk-old plants) of an aqueous solution of C-14 TILT (0.25 microcuries/ml), by placing drops of the solution along the leaf veins (maximum of 0.25 ml/leaf) of the upper 4-6 leaves, and allowing the drops to evaporate and/or be absorbed by the leaf. Control plants were treated in a similar manner with a solution of the emulsifier used in preparing the C-14 TILT solution. Plants from both groups (3 treated and 1 control) were removed from the containers at 1, 2, 3, 4 and 8 weeks after the treatment, and prepared by oxidizing the tissues to carbon dioxide using a PACKARD TRI-CARB, model B306 tissue oxidizer. The amount of radioactivity in the tissues was determined on a BECKMAN LS 7000 liquid scintillation counter.

During the summer of 1984, the foliage of DP 55 plants growing in the field at Marana were sprayed with Tilt at varying rates and at different times. The plants were collected at 9 different occasions from September through October and brought to the laboratory for bioassay of the fungicide. A technique utilizing Phymatotrichum omnivorum was developed to determine if propiconazol had been translocated into the tap roots of the sprayed plants. Sections of root tissue were challenged with actively growing mycelium of P. omnivorum in an agar system.

Results and Discussion

The laboratory studies (Table 1) indicated that all of the sterole-inhibitors were highly active against P. omnivorum. Tilt, Vanguard, XE-779, Imazalil, Baytan and Bayleton significantly reduced mycelial growth at concentrations ranging from 1.0 to 0.1 parts per billion. These are the most active fungicides ever tested against P. omnivorum. From these studies several of the most active were selected for evaluation in the field during 1983 and 1984.

Unfortunately, excessive flood damage occurred in all of our Marana plots during 1983. A plot in the Safford area was also flooded out. We were unable to collect any meaningful data from these plots.

The 1984 situation in the large plots at Marana was also a disappointment. One large test with Tom Clark was destroyed by hail. The 2 plots with Art Pacheco were established in kill patterns that had occurred every year for many years. Disease incidence in these 2 areas was too low to measure any effect of the fungicide treatments. Excessive rainfall at periodic intervals may have been the reason for lack of disease.

The small plots at the Experimental farm yielded good information on the activity of Tilt and Baytan. The data is presented in Tables 3 and 4. Foliar and side-dress applications of Baytan will be compared with Tilt in large trials during 1985.

Systemic Translocation in TILT in Cotton Plants:

The results of the translocation experiments are present in Table 2. The data are present as the percentage of radioactivity recovered in various tissues of the cotton plant, relative to the original dose. Over the 8 weeks from the original treatment, most of the applied dose (entire plant) had been lost by evaporation, metabolism, and/or defoliation. The majority of the remaining activity was concentrated in the leaves (dosed, non-dosed, and new growth), with approximately 0.25% of the applied dose showing up in the root. These results show that TILT does move systemically within the cotton plant, and although the amount is small, basipetal translocation does occur.

Based upon the amount of TILT which would be applied to a cotton plant in the field at a rate of 0.5 lb a.i./acre (ca. 5 mg), the weight of the roots sampled in these studies, and the percentage of the radioactivity from the applied dose detected in these roots, some theoretical estimates of the concentration of TILT in the root tissues can be made. These estimates assume that the radioactivity detected in the roots represent TILT and/or its metabolites, and that these metabolites may also be toxic to P. omnivorum. These calculations estimate that TILT and its metabolites, despite the low percentage of the applied dose reaching the roots, could reach and maintain a concentration within the roots in excess of 50 ppb for 8 weeks following a foliar treatment. Based upon the sensitivity of the fungus to TILT, as determined in the previous experiment, the 50 ppb concentration within the roots should be sufficient to inhibit the growth of mycelium, and greatly reduce the ability of the fungus to kill the plant. This study also indicates that an application to younger, more actively growing plants may result in more of the fungicide reaching the roots than if applied to plants prior to squaring.

These studies have determined that the 'sterol-inhibiting' fungicides show great promise for the control of Phymatotrichum root rot. The results of translocation studies have determined that a small percentage of radioactivity from a foliar application of radio-labeled TILT was translocated to the roots of cotton plants, suggesting that this fungicide has the unique ability to translocate basipetally.

Table 1. Percent Reduction in Mycelial Growth of An Isolate of Phymatotrichum omnivorum from Rillito, Arizona after 96 hour Exposure to Fungicide Amended Potato Dextrose Agar

Fungicide (Manufacturer)	Concentration (parts per billion)			
	100	10	1.0	0.1
XE-779 (Chevron)	86%	69%	34%	19%
TILT (Ciba-Geigy)	95%	58%	24%	17%
VANGARD (Ciba-Geigy)	82%	62%	23%	---

IMAZALIL (Janssen)	77%	39%	*11%	---
BAYTAN (Mobay)	80%	23%	* 0%	---
BAYLETON (Mobay)	81%	20%	* 0%	---
BENLATE (DuPont)	55%	*11%	---	---

* Indicates that these are the only values not significantly different from the control (LSD-0.05).

Table 2. Percentage of the Applied Dose of Radioactivity Remaining in Various Tissues of Cotton Plants Treated at 5 and 8 Weeks after Emergence with C-14 labeled TILT

Sampling Interval After Treatment	Tissue				
	Apical Meristem	Leaves & Petioles	Stem	Roots	Entire Plant
1					
5th Week Treatment					
1 week	0.34	73.21	1.76	0.07	75.45
2 weeks	0.38	64.47	0.47	0.22	65.56
3 weeks	0.44	19.61	0.42	0.23	20.57
4 weeks	0.10	7.74	0.29	0.13	8.34
8 weeks	0.10	1.63	0.28	0.11	2.25
1					
8th Week Treatment					
1 week	0.10	61.11	0.31	0.11	62.00
2 weeks	0.20	38.90	0.24	0.12	39.71
3 weeks	0.10	32.22	0.23	0.06	32.86
4 weeks	0.06	18.45	0.16	0.04	18.84
8 weeks	0.05	5.01	0.12	0.05	5.27

1. An aqueous solution of C-14 labeled TILT (0.25 uCi/ml) was applied to the leaves (0.25 ml/leaf) of 5 week (1.0 ml/plant) and 8 week (1.5 ml/plant) old cotton plants.

Table 3. Effect of Baytan, Tilt and X-779 on incidence of *Phymatotrichum* Root Rot of cotton in tests at Marana, Arizona (1984)

Number of dead plants per 50 feet of row at 3 different sampling dates		
<u>Replication 1</u>	<u>Replication 2</u>	<u>Replication 3</u>

	<u>8/28</u>	<u>9/18</u>	<u>10/1</u>	<u>8/28</u>	<u>9/18</u>	<u>10/1</u>	<u>8/28</u>	<u>9/18</u>	<u>10/1</u>
Baytan Foliar (6/8/84)									
16 oz a.i./A	3	10	10	4	17	17	3	3	3
8 oz a.i./A	15	19	20	0	1	2	0	3	3
Baytan Foliar (6/28/84)									
16 oz a.i./A	3	4	8	4	4	4	1	1	1
8 oz a.i./A	3	5	5	17	36	36	34	48	48
Baytan DF (trench)									
16 oz a.i./A	2	7	7	3	3	3	7	8	8
8 oz a.i./A	4	6	7	10	11	11	2	2	3
Tilt Foliar									
16 oz a.i./A	1	2	2	2	4	5	4	17	19
8 oz a.i./A	6	12	12	19	47	47	3	10	10
XE-779									
1 lb a.i./A (trench)	18								
1 lb a.i./A (foliar)	6	9	9	5	12	12	12	2	2
Check	3	15	15	6	15	16	17	21	31

a
All plots were established on 6/18/84 except 2 Baytan Foliar treatments applied on 6/28/84. Baytan DF and one X-779 treatment were side-dressed (3" deep) in the plant bed. Delta Pine 55 was planted on April 20, 1984. The foliar applications were applied in approximately 50 gallons of water per acre.

Table 4. A comparison of foliar applications of Tilt with a side-dress application of Chevron XE-779 for control of Phymatotrichum Root Rot of Cotton at Marana, AZ (1984)

Treatments	<u>Number of dead plants per 50 ft of row at 2 sampling dates</u>					
	<u>Replication 1</u>		<u>Replication 2</u>		<u>Replication 3</u>	
	<u>9/18</u>	<u>10/1</u>	<u>9/18</u>	<u>10/1</u>	<u>9/18</u>	<u>10/1</u>
Tilt (foliar)						
1 lb a.i./A	3 (42)	4 (43)	2 (25)	2 (25)	3 (52)	27 (52)
0.5 lb a.i./A	58 (116)	58 (91)	8 (43)	16 (43)	28 (50)	29 (49)
Chevron XE-779 (Side-dress)						
2 lb a.i./A	16 (36)	16 (59)	26 (42)	27 (44)	18 (29)	26 (31)
1 lb a.i./A	2 (30)	22 (32)	30 (48)	31 (51)	52 (29)	52 (40)
0.5 lb a.i./A	50 (51)	52 (49)	29 (47)	29 (49)	65 (85)	66 (96)

a
All treatments were applied on 6/28/84. Delta Pine 55 was planted on April 20, 1984. The foliar applications were applied in approximately 50 gal/A of water. The XE-779 was side-dressed approximately 3 inches deep in the bed.

b
Alternate rows were treated. The adjacent untreated row was assumed to be a check. 3(42) means that there were 3 dead plants in the 50 foot row on 9/18. The (42) means that the untreated adjacent row had 42 dead plants.