

## **Pymetrozine Interferes with Transmission of *Tomato Yellow Leaf Curl Virus* by the Whitefly *Bemisia tabaci***

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Pymetrozine, a novel compound belonging to the class pyridine-azomethines, is a feeding inhibitor labeled for use against plant pests in the order Hemiptera. Pymetrozine was evaluated for its ability to interfere with whitefly transmission of the begomovirus *Tomato yellow leaf curl virus* (TYLCV). Pymetrozine was applied as Fulfill<sup>TM</sup> 50 WG at two rates (0.291 and 0.582 g formulation l<sup>-1</sup>) to tomato seedlings with four to six true leaves. Viruliferous whiteflies (three to five per plant) were added 1, 4, 7 and 11 d after a single application of pymetrozine, and transmission rates were determined 4 wk after the addition of whiteflies. Pymetrozine provided protection against transmission of TYLCV by viruliferous whiteflies for up to 1 wk after a single application. No phytotoxicity was observed on tomato transplants. These results indicate that pymetrozine could be an effective tool for tomato transplant producers to protect susceptible transplants from infection by begomoviruses, such as TYLCV. Pymetrozine might also work well as part of an integrated approach to begomovirus management in greenhouse tomato fruit production.

KEY WORDS: TYLCV; begomovirus; Fulfill<sup>TM</sup>; whitefly transmission; virus management.

### INTRODUCTION

The whitefly species complex, *Bemisia tabaci* Genn., has a worldwide distribution and is recognized as a pest on over 500 plant species, including many important agricultural crops (4,7,10,24). *B. tabaci* has been shown to transmit more than 100 viruses belonging to the genus *Begomovirus* (family *Geminiviridae*) (3,5,12). Begomoviruses are transmitted in a persistent manner by *B. tabaci* and can be acquired by immature or adult whiteflies. These viruses can be acquired during an access period as short as 10 min by adults, followed by a latent period of 4 to 21 h, and can then be transmitted in as little as 10 min (11). Begomoviruses are important pathogens in the subtropics and tropics, and have been shown to reduce yields significantly in a number of crops (21). Begomoviruses are spread over short and medium length distances (a few meters to a few kilometers) by their highly mobile vector and over greater distances through the movement of infected plant material (22,26).

The begomovirus *Tomato yellow leaf curl virus* (TYLCV) (reviewed in ref. 8) has spread beyond its origins in the eastern Mediterranean, and has become a major concern for tomato production in the Mediterranean, the Caribbean, southeastern United States and Japan (17,19,21,23,25,27). It appears that some of this spread in geographic distribution has been due to the movement of TYLCV-infected transplants or viruliferous whiteflies on

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commercial plant material (21,22). This is a very difficult virus to manage and improved strategies are needed to reduce crop losses and to prevent introduction of the virus to new areas through the movement of infected vegetable and ornamental transplants (22).

TYLCV is managed in the field primarily through the use of insecticides that reduce whitefly populations. Nicotinoid insecticides are very important in begomovirus management. These insecticides are systemic in plants, fast-acting and can be highly effective in minimizing begomovirus spread in production fields. However, their use in greenhouses has been limited. Nicotinoids are not used on plants for fruit production in planthouses due to detrimental effects of these chemicals on insect pollinators. Their use in transplant production is restricted in order to limit the development of insecticide resistance in target insect species (6). Therefore, begomoviruses are managed in greenhouses in the USA through exclusion and/or repeated applications of contact insecticides, although often with little success.

Pymetrozine, marketed by Syngenta Inc. (Greensboro, NC, USA) under the names Fulfill<sup>TM</sup>, Chess<sup>TM</sup>, Plenum<sup>TM</sup>, and Sterling<sup>TM</sup>, is a compound with specific activity against Hemiptera (aphids, planthoppers and whiteflies) (1,13,14,20). It is the only member of a relatively new class of insecticides, the pyridine-azomethines, and has been demonstrated to be a feeding inhibitor (15). Pymetrozine was shown to cause immediate and irreversible cessation of feeding in aphids which results in death within days due to starvation (18). Laboratory studies measuring electrical penetration indicated that pymetrozine blocked stylet penetration (15,16). Pymetrozine is highly mobile in plants. It can be taken up from the soil as well as through the leaves (13). Pymetrozine is translocated in both a systemic (acropetal and basipetal directions *via* the phloem and the xylem) and translaminar manner (29,30). In tomato, approximately one-third of the extractable pymetrozine was found in the intercellular fluids (30).

Numerous greenhouse and field studies with both vegetable and cotton crops have demonstrated that pymetrozine reduced whitefly populations (1,13,20). Pymetrozine was shown to be as effective as a nicotinoid in reducing whitefly adults and nymph populations on sweet pepper in Spain (20).

Studies have shown that pymetrozine can interfere with aphid transmission of plant viruses. This compound significantly reduced the acquisition and subsequent transmission of *Cauliflower mosaic virus* (CaMV), a plant virus transmitted in a semi-persistent manner by several species of aphids (2). Pymetrozine reduced significantly the acquisition of CaMV from treated plants, but did not affect the transmission rate of already viruliferous aphids. It had no effect on transmission rates of *Potato virus Y* (PVY) by aphids that had already acquired the virus; however, acquisition rates of PVY from treated plants were reduced (16). Pymetrozine was demonstrated to reduce by 97% the transmission of *Potato leaf roll virus* (PLRV), a plant virus persistently transmitted by the aphid *Myzus persicae* (Sulzer) (16).

The objective of the present study was to evaluate the ability of pymetrozine to interfere with the transmission of the begomovirus TYLCV by whiteflies.

## MATERIALS AND METHODS

**Whiteflies** Whiteflies (*Bemisia tabaci*, biotype B) (9) used in this study were adult populations of mixed age and gender. They were reared on tomatoes (cv. 'Florida Lanai') infected with TYLCV in a growth chamber (23–25°C, 14 h light). Whiteflies were obtained

from infected plants by gently tapping whitefly-infested leaves over a yellow, non-sticky plastic card and collecting the adults on the card with an aspirator.

**Virus culture** The *Tomato yellow leaf curl virus* isolate used in this study was obtained from an infected tomato plant in a commercial planthouse in 1997 (22). The virus was maintained in tomato Florida Lanai by whitefly transmission. This virus has been sequenced and identified as an isolate of TYLCV (severe strain) first recognized in Israel (22).

**Test plants** used for this study were two cultivars of tomato (*Lycopersicon esculentum* Mill.), Florida Lanai and 'Solar Set', which are equally susceptible to TYLCV. All test plants were planted and evaluated in 128- or 24-cell Todd Planter Flats (Speedling, Inc., Sun City, FL, USA). The studies were conducted at the Gulf Coast Research and Education Center in Bradenton, Florida (USA) in a glass greenhouse under natural lighting with an average daytime temperature of 30°C. Each experiment was conducted three times.

**Pymetrozine** was used in the form of Fulfill 50 WG (Syngenta). Two rates were tested, 0.582 g formulation  $l^{-1}$ , and 0.291 g formulation  $l^{-1}$ , in combination with a non-ionic surfactant (NIS), Dyne-amic®, 2.5 ml  $l^{-1}$  (Helena Chemical Co., Memphis, TN, USA). The lower rate of the formulation is that currently labeled for use in the USA and the higher rate is labeled for use in some locations outside the USA.

**Inoculation of TYLCV to tomato seedlings by whiteflies** Viruliferous adult whiteflies reared on TYLCV-infected Florida Lanai tomato plants were used to transmit the virus to test plants to determine the effect of pymetrozine on transmission of TYLCV. An average of three to five whiteflies per test plant were introduced into cages constructed of white nylon organdy cloth bags with internal supports made of PVC plastic pipes fitted together to form a three-dimensional frame. The Todd planter flats were placed on shallow trays inside the cages for watering. Flats were watered daily by filling the tray with water poured through the sides of the bags, thereby preventing the escape of any whiteflies. Whiteflies were introduced onto test plants by tapping aspirators containing the whiteflies over test plants at randomly selected sites above the flat through Velcro-lined openings in the organdy cage.

**Evaluating the effect of pymetrozine on transmission of TYLCV** A study was conducted to determine if pymetrozine would have any effect on the transmission of TYLCV by whiteflies to tomato seedlings. The two rates of pymetrozine were each applied to 128 Florida Lanai tomato plants. Pymetrozine-treated tomato plants were compared with an equal number of tomato plants sprayed with water or with water plus 2.5 ml  $l^{-1}$  NIS (control). Pymetrozine was applied 4 wk after test plants germinated (plants had four to six true leaves). Whiteflies were added 24 h after the application of pymetrozine and allowed to remain on test plants until the end of the experiment. The severity of symptoms observed was recorded weekly for each plant showing symptoms, for 3 wk beginning 2 wk after the addition of whiteflies. At the same time, plants were inspected for the presence of live adult whiteflies. Each rate of pymetrozine was tested three times.

The mean of the percent of TYLCV-infected plants (three replications over time) was compared between plants treated with pymetrozine plus NIS and those treated with water plus NIS. The means were transformed using an arc-sine square root transformation and compared using Analysis of Variance (SAS 8e, SAS Institute, Cary, NC, USA).

**Residual activity of pymetrozine on transmission of TYLCV** The effectiveness of pymetrozine in interfering with the transmission of TYLCV at different days after a single application (residual activity) to tomato transplants, was estimated by applying pymetrozine once followed by the addition of viruliferous whiteflies at one of four different times afterward. Test plants, either Florida Lanai or Solar Set, were grown in 24-cell Todd Planter Flats, and pymetrozine was applied one week after test plants emerged. Twenty plants were used in each treatment and an equal number of untreated control plants were sprayed with water alone or water plus  $2.5 \text{ ml l}^{-1}$  NIS. Residual activities for the two rates of pymetrozine were estimated by the addition of viruliferous whiteflies to pymetrozine-treated plants 1, 4, 7 or 11 days after treatment. Whiteflies were allowed to remain on test plants until the end of the experiment. Experiments were replicated three times.

The mean incidence of TYLCV-infected plants was calculated for each replication and treatment. Means were transformed using an arc-sine square root transformation and were separated using Fisher's Protected LSD (SAS 8e).

**Incidence of TYLCV in test plants** The incidence of TYLCV-infected plants was monitored weekly to determine the effect of pymetrozine on transmission of TYLCV. Test plants were inspected for symptom expression at weekly intervals for 3 wk beginning 2 wk after the addition of whiteflies. The severity of symptom expression on each infected plant was recorded on a 0 to 4 scale: 0 = no symptoms; 1 = downward leaf curling/cupping (DLC/C); 2 = DLC/C + reduced leaf size (RLS), and yellowing at base of leaflet; 3 = DLC/C, RLS, and mild chlorosis; 4 = all TYLCV symptoms: DLC/C, RLS, severe chlorotic margins, and stunting of plant. An index of the symptom severity (mean symptom severity) was calculated by multiplying the number of plants in each rating category (above 0) by the number of the symptom rating (1, 2, 3 or 4), summing across all rates, and dividing the sum by the number of plants showing symptoms.

A nucleic acid spot hybridization assay was used to confirm the presence of virus in plants showing mild symptoms and in any plants that were questionable. Young leaves from control and test plants were collected and extracted (22). A  $20\text{-}\mu\text{l}$  sample was taken from the expressed sap and added to  $140 \mu\text{l}$  of TE buffer (10 mM Tris-acetate, 1 mM EDTA, pH 8.0). Twenty  $\mu\text{l}$  of 1 M NaOH was added and the contents were mixed and incubated at room temperature for 10 min. Extracts were then treated with  $20 \mu\text{l}$  of 3 M sodium acetate, pH 5.2. The contents were mixed and incubated at room temperature for 10 min. Supernatants of the extracted samples were spotted in  $20\text{-}\mu\text{l}$  aliquots onto TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0)-wetted Nytran membranes (Schleicher and Schuell, Inc., Keene, NH, USA) using a blotting manifold (Hybrid-Dot Manifold, Life Technologies, Inc., Gaithersburg, MD, USA). Membranes were air-dried and then baked at  $65^\circ\text{C}$  for 30 min. TYLCV DNA was detected using a 312 bp probe from the intergenic region of TYLCV DNA (GenBank Acc. No. L12219) that was labeled with  $^{32}\text{P}$  using an Amersham High Prime labeling system (Amersham, Arlington Heights, IL, USA). This probe was hybridized to blots at  $65^\circ\text{C}$  overnight and rinsed under high stringency conditions. Blots were exposed to X-ray film for 4 to 24 h at  $-70^\circ\text{C}$ .

**Phytotoxicity** Test plants were treated with pymetrozine at the two rates, but were not inoculated with TYLCV. They were visually evaluated at weekly intervals for 3 wk after a single application of pymetrozine for indications of phytotoxicity such as stunting, foliar necrosis, and/or chlorosis.

## RESULTS

**Effect of pymetrozine on TYLCV incidence and symptom expression** Both the 0.291 g  $l^{-1}$  and the 0.582 g  $l^{-1}$  rate of pymetrozine as Fulfill 50 WG significantly reduced the transmission frequency based on the incidence of TYLCV-infected tomato plants ( $P \leq 0.01$ ) when whiteflies were exposed to plants 24 h after treatment (Fig. 1, Table 1). The two rates of pymetrozine were equally effective. Mean symptom severity indexes of pymetrozine-treated plants at 4 wk after inoculation were 1.3 and 1.5 (0.291 g formulation  $l^{-1}$  and 0.582 g formulation  $l^{-1}$ , respectively), whereas the mean symptom severity indexes of the water-treated plants were 3.0 to 2.8, respectively. Significant differences in the severity of the symptoms expressed in TYLCV-infected plants were observed at  $P \leq 0.1$  but not at  $P \leq 0.05$ , between pymetrozine- and water-treated plants.



Fig. 1. Tomato plants cv. 'Florida Lanai' 4 weeks after inoculation with *Tomato yellow leaf curl virus* (TYLCV). Plants on the left were treated with pymetrozine (0.582 g  $l^{-1}$  Fulfill 50 WG plus 2.5 ml  $l^{-1}$  Dyne-amic<sup>®</sup>). The plants on the right were controls, treated with water (plus 2.5 ml  $l^{-1}$  Dyne-amic<sup>®</sup>). An average of five whiteflies per plant were introduced transferred from TYLCV-infected tomato plants to test plants 24 h after application of pymetrozine or water.

No live whiteflies were observed on any pymetrozine-treated plants during the weekly inspections, whereas live adult whiteflies were readily observed during the weekly inspections of water-treated control plants (data not shown). These observations are consistent with previous reports (13,14,20). Pymetrozine appeared to act on whiteflies

TABLE 1. Effect of single applications of pymetrozine at two rates on the transmission of *Tomato yellow leaf curl virus* (TYLCV) to tomato seedlings by whiteflies introduced 24 h after treatment

Treatment <sup>z</sup>	Incidence (%) of TYLCV-infected plants
0.291 g pymetrozine $l^{-1}$	1.7 a <sup>y</sup>
Water control	74.8 b
0.582 g pymetrozine $l^{-1}$	4.5 a
Water control	61.5 b

<sup>z</sup>Pymetrozine was formulated as Fulfill 50 WG plus 2.5 ml  $l^{-1}$  Dyne-amic®.

<sup>y</sup>Each value represents the mean of three replications (128 plants per treatment per replication), as established 4 wk after inoculation. Arc-sine square root transformation was used on percentages where appropriate, prior to data analysis; non-transformed data are presented. Means followed by different letters indicate significant differences by ANOVA ( $P \leq 0.01$ ) for either rate of pymetrozine.

TABLE 2. Residual effect of single applications of pymetrozine at two rates on the mean incidence of *Tomato yellow leaf curl virus* (TYLCV) in tomato seedlings

Treatment <sup>z</sup>	No. of days between application of pymetrozine and addition of whiteflies			
	1	4	7	11
0.291 g a.i. $l^{-1}$	1.7 <sup>y</sup> a	25.0 a	51.7 a	68.3 a
Water control	93.3 b	93.3 b	91.7 b	90.0 a
<i>P</i> value	$P \leq 0.0019$	$P \leq 0.0078$	$P \leq 0.0005$	ns <sup>x</sup>
0.582 g a.i. $l^{-1}$	0.0 a	6.7 a	33.3 a	65.0 a
Water control	85.0 b	68.3 b	83.3 b	83.3 a
<i>P</i> value	$P \leq 0.0009$	$P \leq 0.0425$	$P \leq 0.0310$	ns

<sup>z</sup>Pymetrozine was formulated as Fulfill 50 WG plus 2.5 ml  $l^{-1}$  Dyne-amic®.

<sup>y</sup>Each value represents the mean of three replications (20 plants per replication), as established 4 wk after inoculation. Arc-sine square root transformation was used on percentages where appropriate, prior to data analysis; non-transformed data are presented. Means followed by different letters indicate significant differences by ANOVA.

<sup>x</sup>ns = not significant at  $P \leq 0.05$ .

very quickly, since inspections 48 h after whiteflies were transferred to treated plants did not reveal any live whiteflies, whereas many live whiteflies were readily observed 48 h after transfer to water-treated plants. There was no evidence of whitefly reproduction on pymetrozine-treated plants – no eggs were observed and no adults emerged from treated plants. Whitefly eggs were observed on water-treated control plants and adults were seen to emerge at the time of the third symptom evaluation.

No evidence of phytotoxicity was ever observed on any plants treated with either rate of pymetrozine.

**Residual activity of pymetrozine** Both rates of pymetrozine reduced the incidence of plants infected with TYLCV for up to 7 days after a single application (Table 2). The means of the incidence of TYLCV-infected plants were statistically lower ( $P \leq 0.05$ ) in pymetrozine-treated plants compared with water-treated plants when whiteflies were transferred 1, 4 or 7 days following treatment at either rate of pymetrozine. There were no significant differences between the mean incidence of TYLCV-infected plants when viruliferous whiteflies were transferred to plants 11 days after application of either rate of pymetrozine. At the 0.291 g  $l^{-1}$  rate, pymetrozine reduced the incidence of TYLCV-infected plants an average of 98.0%, 73.2%, 43.2%, and 24.1% when whiteflies were transferred 1, 4, 7 and 11 days, respectively, after a single application of pymetrozine.

At the  $0.582 \text{ g l}^{-1}$  rate, pymetrozine reduced the incidence of TYLCV-infected plants an average of 100%, 90.2%, 60.0% and 22.0%, when whiteflies were added 1, 4, 7 and 11 days, respectively, after a single application of pymetrozine.

## DISCUSSION

Pymetrozine-treated plants had significantly lower incidences of TYLCV-infected plants than water-treated control plants when exposed to viruliferous whiteflies. The mode of action of pymetrozine, which is an immediate and irreversible cessation of stylet penetration (15,18), appears to be sufficiently rapid to reduce significantly the transmission of TYLCV by adult whiteflies to treated tomato seedlings. In order to interfere with TYLCV transmission, whiteflies would have to be affected by pymetrozine early in the feeding process. This may be the case, since whiteflies begin feeding by probing followed by intercellular penetration of leaf tissue (28). Approximately one-third of the extractable pymetrozine in a plant is found in the intercellular fluids of tomato leaves (30), and therefore it is likely that whiteflies would encounter pymetrozine very early in the feeding process. Studies with aphids on potato plants treated with pymetrozine showed that initial stylet penetration was not affected; however, aphid feeding time was significantly reduced and time to subsequent penetrations was significantly increased (16). Pymetrozine may have a similar effect on whitefly feeding behavior (30).

Transmission rates of TYLCV were significantly reduced and symptoms were milder in the pymetrozine-treated plants than in the water-treated plants. It is not clear why milder symptoms were observed during the period of the evaluation. Perhaps this reflects a lower initial inoculum level due to the interference of pymetrozine with whitefly feeding. It is also possible that pymetrozine may have some effect on plant defense mechanisms.

One application of pymetrozine was found to interfere significantly with the transmission of TYLCV for 7 days but not for 11 days. The residual activity of pymetrozine is not surprising, considering it can be absorbed by the plant through the leaves, shoots, and roots and can be translocated through both the xylem and phloem (29,30). In our studies, it would be expected that some of the pymetrozine applied to the leaves would be absorbed through the leaves and shoots, but that some would drip onto the soil, and could be taken up by the plant from the soil, thus extending the residual activity.

The data indicate that pymetrozine may provide a useful new tool for producers to protect transplants from infestation by whiteflies, infection by TYLCV and other begomoviruses, and infection by those viruses transmitted in a persistent manner by whiteflies. It is expected that pymetrozine would work well as part of an integrated approach to virus management. Its unique chemistry would interface well with the nicotinoid insecticides that are the most important means of control at this time. Pymetrozine would also provide producers with an added protection from aphids and viruses semi- and persistently transmitted by aphids (16). The use of pymetrozine in the planthouse could reduce the incidence of TYLCV in transplants and help in the production of virus-free transplants, an important factor in the successful management of this economically significant virus. Based on these data, transplant producers in Florida have used pymetrozine on tomato transplants with good results. Growers report a noticeable decrease in the number of complaints from clients regarding TYLCV-infected transplants when pymetrozine has been incorporated into transplant production practices.

In addition, pymetrozine could be useful to control whiteflies and whitefly-transmitted viruses in tomatoes grown for fruit production in planthouses. This compound has been shown to have activity against Homoptera and to have little to no impact on beneficial insect species (20). Pymetrozine could be very useful in planthouses where most insecticides employed against whiteflies are not compatible with the use of pollinators, predators and parasitoids. In addition, it would be expected that the spread of TYLCV within tomato fruit production houses would be reduced by the use of pymetrozine.

Pymetrozine has been proposed as a means of control for whitefly populations on a wide range of crops including cotton and several vegetables (1,19). Many of these crops suffer significant losses due to begomoviruses and other viruses persistently transmitted by species of Homoptera. These data suggest that pymetrozine could be used in various crops as an effective tool to manage both whiteflies and begomoviruses.

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