IR-4 Bio-Pesticide Report

Grant Title: Evaluation of KeyPlex 350 DP for the Control of Citrus Greasy Spot and as a Resistance Management Tool

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Bio-Pesticide Common Name: KeyPlex 350 DP

Registrant: Morse Enterprises Limited, Inc. Brickell East Floor Ten 151 South East 15 Road Miami, FL 33129

Petition: To be sent to EPA 1st quarter 2003 or after December 7, 2002.

<u>Proposed Label Use Directions:</u> Apply 2 to 3 quarts per acre as a foliar spray. Apply in early summer on expanded flush and repeat on subsequent flushes.

Ingredients: Yeast hydrolysate (exemption pending at EPA) and other ingredients.

<u>Plan of Work – Objective:</u> To evaluate KeyPlex 350 DP alone and in rotation with Abound for the control of Greasy Spot. Abound is not recommended to be applied sequentially, because of the possibility of resistance developing. Various spray protocols will be evaluated and compared with University of Florida recommended chemical controls for Greasy Spot on citrus intended for both processed (juice) and fresh fruit markets.

<u>Potential Benefits:</u> Reduce the chance of the Greasy Spot fungus developing resistance to Abound and offer an alternative control to heavy metal fungicides based on copper. Provide efficacy data to support the registration of the Bio-Pesticide, KeyPlex 350 DP.

Introduction

There are approximately 621,000 acres of commercial citrus groves currently under cultivation in Florida of which over 80% is processed for juice. One of the primary concerns of citrus growers is defoliation which causes reductions in yield. The fungal disease Greasy Spot, *Mycosphaerella citri*, is the major causative agent of citrus tree defoliation in Florida. Greasy Spot is a slow developing disease. The initial infection occurs during the summer on young tender flush. The disease gradually progresses during the summer and fall culminating in leaf drop during the winter and early spring. The current controls are mainly copper fungicides and petroleum oil. Abound (azoxystrobin) has been recently labeled for use on citrus to control Greasy Spot but has a potential resistance problem characteristic of strobilurin class fungicides. According to L. W. Timmer (U. of Fla. IFAS, Lake Alfred, FL), "A strobilurin fungicide should not be applied more than once a year for greasy spot control" due to likely resistance. Fungicides are currently applied once or twice during the summer months depending on disease severity.

Materials and Methods

The study site was a 10-acre citrus grove planted in 1982 with 'Redblush' grapefruit trees (*Citrus paradisi* Macf.) on 'Swingle' citrumelo [*Citrus paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] rootstock located 0.4 mile east of the Florida Research Center in Indian River County, Florida. Typical horticultural practices were provided to the grove site with the exception that no chemical sprays were applied to the foliage seven months prior to the study period. Trees were irrigated via a microirrigation system as necessary. Trees were fertilized via the same irrigation system. Size matched trees were used for the study. A complete randomized block design with 1 tree per replicate and 10 replicates for a total of 10 trees per treatment were used (Figure 1). A minimum of four young branches with healthy, spring flush leaves were selected in each tree and tagged on 06/20/03 prior to the first treatment. The selected shoots were spread around the tree in order to minimize position and coverage variations.

Test materials, Abound Flowable (Syngenta Crop Protection, Inc.; Greensboro, NC); Kocide 101 (Griffin Corporation, Valdosta, GA); KeyPlex 350 DP (Morse Enterprises Limited, Inc., Miami, FL); Petroleum Oil FC 435-66 (Diamond R Fertilizer Co., Inc., Ft. Pierce, FL); Latron CS-7 (Rohm and Hass Co., Philadelphia, PA) were prepared as indicated in Table 1 and were applied by means of a tractor-powered hydraulic sprayer equipped with a handgun calibrated to deliver 1.4 gal per tree. This rate per tree was based on an assumed spray volume of 167 gal per acre applied to 120 trees per acre and was deemed to provide good coverage to the foliage.

First Application (07/10/03)		Second Application (08/26/03)	
Treatment	Rate / Acre	Treatment	Rate / Acre
Untreated Control	-	Untreated Control	-
Abound + Latron CS-7*	12.4 oz	None	-
Abound + Latron CS-7*	12.4 oz	Petroleum Oil FC 435-66	7.0 gal
Abound + Latron CS-7*	12.4 oz	Kocide 101 +	4.0 lb +
		Petroleum Oil FC 435-66	5.0 gal
Abound + Latron CS-7*	12.4 oz	KeyPlex 350 DP	3.0 qt
Abound + Latron CS-7*	12.4 oz	KeyPlex 350 DP +	3.0 qt +
		Petroleum Oil FC 435-66	5.0 gal
KeyPlex 350 DP	3.0 qt	KeyPlex 350 DP	3.0 qt
KeyPlex 350 DP +	3.0 qt +	KeyPlex 350 DP	3.0 qt
Petroleum Oil FC 435-66	5.0 gal		_

Table 1

*Abound was applied with Latron CS-7 surfactant at 1 pint/100 gal

Trees were first sprayed on 07/10/03 and a second time on 08/26/03. Leaves on the previously tagged branches were examined and scored for percent defoliation and Greasy Spot infection on 01/22/04. The treated trees did not receive any additional foliar treatments of any kind for the duration of the experiment.

Disease ratings were carried out on 01/22/04, in a blind experimental design where the investigators were not aware of the treatments. Percent defoliation was determined by counting the number of leaves remaining on the previously tagged shoot as well as those that had dropped off. Greasy spot infection severity was determined visually from 0 to 5 via images from a Greasy Spot Rating Scale developed by T.R. Gottwald, USDA, Ft. Pierce, FL. The area affected by greasy spot on the remaining leaves was rated on a scale of 0 = no greasy spot, 1 = 1-5% affected leaf area, 2 = 6-10%, 3 = 11-15%, 4 = 16-20%, and 5 = >20%. The scale can be seen at the following webpage: http://www.lal.ufl.edu/timmer/greasyspotratingscale.htm.

Statistical analyses of the data sets were performed using the Systat 10 statistical analysis package for Windows (SPSS, Inc., Chicago, IL),. Significance for data was at Alpha = 0.05.

Results

Very little defoliation was noted for all treatments irrespective of treatment and no significant differences were observed. However, trees treated with KeyPlex 350DP alone or in combination with oil showed no defoliation at all (Table 2).

No. of	Treatment Description	Percent	Greasy Spot
Sprays		Defoliation *	Severity Rating
0	Untreated Control	1.2% a	1.88 a
1	Abound 1X;	4.5% a	0.85 b
2	Abound 1X; Oil 1X	4.6% a	0.74 bc
2	Abound 1X; Kocide 101 + Oil 1X	0.8% a	0.38 bc
2	Abound 1X; KeyPlex 350 DP 1X	3.7% a	0.65 bc
2	Abound 1X; KeyPlex 350 DP + Oil 1X	2.4% a	0.64 bc
2	KeyPlex 350 DP 1X; KeyPlex 350 DP 1X	0.0% a	0.40 bc
2	KeyPlex 350 DP + Oil 1X; KeyPlex 350 DP 1X	0.0% a	0.25 c

Table 2.

* Means (\pm SD) followed by the same letter in columns are not significantly different based on Kruskal-Wallis All-Pairwise Comparison Test at the 5% level.

Disease severity ratings varied depending on the treatment with all treatments significantly different from the untreated control with the single application of Abound offering the least control. The KeyPlex 350 DP + Oil 1X; KeyPlex 350 DP 1X two spray treatment demonstrated the lowest visible foliar infection and was followed by the Abound 1X; Kocide 101 + Oil 1X treatment. No significant differences between any of the two spray treatment regimes were observed (Table 2).

Discussion

This study was designed to evaluate the effectiveness of KeyPlex 350 DP, a candidate Bio-Pesticide, first as a viable treatment for Greasy Spot control in citrus and secondly as a rotation option for use with strobilurin class fungicides (i.e. Abound) for resistance management. Unfortunately there were no significant differences between treatments for defoliation, but only the two spray KeyPlex 350 DP treatments and the Abound 1X; Kocide 101 + Oil 1X treatment were less than the untreated control. It is likely that if the rating took place two months later there would have been greater time for disease progression to occur and thereby revealing significant differences in treatments based on percent defoliation.

All treatments provided significant reduction of greasy spot based on greasy spot severity rating. Interestingly, the KeyPlex 350 DP + Oil 1X; KeyPlex 350 DP 1X treatment provided greater reduction of greasy spot severity than one application of Abound, but was not significantly better than any of the other two spray treatments. This finding supports the use of two applications of KeyPlex 350 DP, one with and one without Petroleum Spray Oil, as an alternative to Abound for greasy spot control on citrus foliage. Additionally, our data suggests that a single application of Abound is not as effective as two applications of any of the treatment regimes tested.

Summary

Our study suggests that KeyPlex 350 DP is a viable treatment for Greasy Spot control on citrus foliage. In addition KeyPlex 350 DP represents a rotation option for use with strobilurin class fungicides (i.e. Abound) for resistance management. Additional testing of KeyPlex 350 DP is required to determine its use to control of rind blotch on Grapefruit also caused by *Mycosphaerella citri*.