Effects of the Plant Growth Regulator Mepiquat Chloride on Silverleaf Whitefly (Homoptera: Aleyrodidae) Infestations on Cotton[1,2]

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ABSTRACT Cotton, *Gossypium hirsutum* L., plants were treated with the plant growth regulator mepiquat chloride (Pix™) in greenhouse and field plot tests to determine its effect on infestations of the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring. In the greenhouse, Pix-treated plants were significantly shorter and had thicker leaves than untreated plants by the end of the second week following application. Most of the Pix treatments caused significant reductions in adults, eggs and nymphs on leaves by fourth and final week of the test. In a separate test, leaf water potentials were equal for Pix-treated and well-watered plants which were significantly lower than for water-stressed and control plants. Pix-treated plants in two field plot tests showed the same changes in plant morphology observed in Pix-treated plants in the greenhouse. However, leaf water potentials were the same as in untreated plants and no reductions in whitefly infestations were found during the 6 to 7 wk test periods. Pix-treated plants in a third field plot test showed no changes in plant morphology or in whitefly infestations. The reduced whitefly infestations of Pix-treated plants in the greenhouse, but not in field plots, may have been due to reduced leaf water potentials in Pix-treated greenhouse plants but not in field plants.

KEY WORDS Pix, mepiquat chloride, silverleaf whitefly, *Bemisia* spp., cotton

During preliminary greenhouse studies, fewer adult silverleaf whiteflies, *Bemisia argentifolii* Bellows and Perring (formerly called strain "B" of *Bemisia tabaci* Gennadius) were observed on potted cotton plants, *Gossypium hirsutum* L., treated with Pix™, (mepiquat chloride, N,N-dimethylpiperidinium chloride, BASF Corp., Research Triangle Park, NC) than on untreated plants (Unpubl. data, L. E. and E. W. D.). Plants were treated with Pix in irrigation water to reduce their size and growth rate for caged plant studies with whiteflies. Mepiquat chloride controls plant growth by reducing the synthesis of the plant

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hormone, gibberellic acid. Most of the effects observed are caused by the suppression of cell enlargement. Smaller cells in treated cotton plants result in reduced leaf area (smaller leaves), shorter branches and main stem, and thickening of leaves caused by an increased layer of cells that develops (Hake et al. 1991). Allelochemicals (gossypol, tannins, flavonoids) are also found in increased quantities in treated cotton plants (McCarty and Hedin 1994). Cotton plants treated with an appropriate dose of mepiquat chloride devote fewer resources to growth and more to fruit retention and boll development with the potential of greater lint yield (Briggs 1980). Effects of mepiquat chloride are greatest on plants with rank growth; cotton plants growing normally show minimal effects (Boman and Westerman 1994).

Plant growth regulators such as ethephon (2-chloroethyl phosphonic acid) that remove late-season cotton fruiting forms have reduced overwintering infestations of several insect pests (Henneberry et al. 1988). However, little information is available on the effects to insects of treating cotton plants with mepiquat chloride. Chlormetquat chloride, a compound similar in function to mepiquat chloride, reduced aphid, *Hyperomyzus lactucae* (L.), densities on black currant, *Ribes nigrum* L. (Singer and Smith 1976). Zummo et al. (1983) reported that cotton bollworm *Helicoverpa zea* (Boddie) larvae, caused less damage and had lower survival rates on cotton plants treated with mepiquat chloride because of increased concentrations of allelochemicals. However, Graham et al. (1987) reported no increased plant resistance to artificial infestations of tobacco budworm, *Heliothis virescens* F., on cotton treated with mepiquat chloride. Schroeder and Vega (1994) found that treating cucumber, *Cucumis sativus* L., with mepiquat chloride had no effect on populations of silverleaf whitefly, but the addition of a commercial surfactant did cause subsequent reductions in whitefly oviposition. We know of no studies on the effects of treating cotton plants with mepiquat chloride on subsequent infestations of the silverleaf whitefly.

Our objectives were to confirm greenhouse observations of reduced numbers of silverleaf whiteflies on cotton plants treated with mepiquat chloride and to determine whether the reductions were sustained under field conditions.

Materials and Methods

General methods. Greenhouse tests were conducted at the Western Cotton Research Laboratory, Phoenix, AZ. Greenhouses were 2.7 × 3.7 m and were temperature controlled to 25 to 32°C by evaporative cooling and electric heating. Field tests were conducted during 1994 at the University of Arizona, Maricopa Agricultural Center (MAC) farm, Maricopa, AZ where soil type is a sandy loam and the growing season is approximately 245 d. Deltapine 50 (DPL-50) was the cultivar planted for both greenhouse and field tests. Fields at the MAC farm are grown under the supervision of a farm manager who controls fertilization, irrigation, and insect pests needed to achieve maximum production. Spraying for insect pests usually limits testing to early through mid-season.

Populations of adult silverleaf whiteflies were determined by counting the numbers of resting adults on the undersides of leaves at the fifth main stem node from the plant terminal. Populations of immature whiteflies were determined from counts of eggs and nymphs (including the pupal stage) on 3.88 cm^2 leaf disks removed from sector two of fifth main stem node leaves (Naranjo and Flint 1994). Sample leaf disks were taken from plants centrally located in field plots. Whiteflies collected from the greenhouse in September 1994, and from fields at MAC in September and October 1994, were tested at our laboratory using RAPD-PCR analysis (Gawel and Bartlett 1993) and determined to be the "B" strain of *B. tabaci*. Subsequently, this strain was described as a new species, *B. argentifolii* (Bellows et al. 1994).

Data sets with four or fewer means were evaluated by analysis of variance (ANOVA) using a randomized block design. A least significant difference (LSD) was calculated where significant *F* values were obtained (Gomez and Gomez 1984). Data sets with more than four means were tested using Ryan-Einot-Gabriel-Welsch-F test (SAS Institute 1988). Data for two treatments were tested by t-tests. The 0.05 level of significance was used for all statistical tests. Untested means are presented with their standard errors (SE).

Greenhouse tests. Cotton plants used in greenhouse tests were grown in 3-liter pots containing commercial potting soil. The greenhouse was fumigated routinely with Vapon 2(2,2-dichlorovinyl dimethyl phosphate, Prentiss Drug and Chemical Co., Floral Park, NY) to control silverleaf whitefly (uninfested greenhouse). Plants used in the tests were approximately 15 to 20 cm tall and were at the first-flower-bud to first-bloom stage of development. Maximum response to mepiquat chloride occurs in cotton plants that are at first bud through 2 to 3 wk after first-flower (Landivar et al. 1992). Both soil and foliar treatments were made, each at three levels of active ingredient, plus water only and untreated controls. The soil treatments included the original dose of Pix (Pix contains 4.3% mepiquat chloride) that led to these tests, 0.28 ml per plant, and lower doses of 0.15 and 0.07 ml per plant. These doses were administered in 100 ml water to plants in pots held in saucers to retain excess water for reabsorption. Foliar sprays were made at the label rate of 1.12 liter Pix per hectare and lower doses of 0.58 and 0.29 liter per hectare. Foliar doses of Pix were based on the label rate per hectare divided by 98,760 plants per hectare: 0.0113, 0.0058 and 0.0029 ml per plant. This amount of Pix was applied in sufficient water to obtain mist coverage (not to runoff) of the upper leaf surfaces using a hand sprayer operated at a pressure of 2.2 kg per cm^2. Three uninfested plants for each of eight treatments were arranged in a randomized block design in a greenhouse containing silverleaf whitefly infested cotton plants (infested greenhouse). Data for plant height, leaf thickness, and whitefly eggs, nymphs, and resting adults were collected weekly for 4 wk following the initial treatments. At the end of this test the infested greenhouse was fumigated to reduce the numbers of whiteflies infesting the source plants and the test was repeated with fresh plants from the uninfested greenhouse.

We also compared the effects of treating cotton plants with Pix with the effects of induced water stress, a condition known to increase whitefly infestations (Flint et al. 1994). Five uninfested cotton plants at approximately first bloom were used for each of four treatments: 1) Pix applied at 0.28 ml in 100 ml water initially and 2 wk later, 2) irrigation every day to maintain well-watered condition, 3) irrigation only when plants showed wilting to maintain water-stressed condition, and 4) normal irrigation every 2 d, our usual procedure. Measurements of leaf water potential were made at 21, 23 and 27 d following the initiation of the test. Leaf water potential is the pressure (-bars) required to cause interstitial leaf sap to be exuded from the leaf petiole. Fifth main stem node leaves below the plant terminal were collected for the first sample and the next main stem node leaf closest to the terminal was collected for succeeding samples. Leaf samples were collected by the methods of Meron et al. (1987) and tested in the pressure chamber by the methods of Turner (1987). All readings were made within 0.5 h of leaf excision. Three micrometer measurements of leaf thickness were made per leaf nearest the fifth main stem node.

Small plot field tests. Five plots for each of six treatments were arranged in a randomized block design in a larger field of cotton planted 15 April. Each plot consisted of one row of cotton 12 m in length with six contiguous plots per each of five interior rows of cotton. The five interior rows of cotton were separated from each other by 28 m. The six treatments were 1) Pix applied once at 1.12 liter per hectare, 2) Pix at 1.12 liter per hectare initially and 4 wk later, 3) Pix at 2.24 liter per hectare, 4) Pix at 2.24 liter per hectare initially and 4 wk later, 5) water only and 6) untreated control. Plants were approximately 30 cm tall and were at the first bloom stage of development when the initial spray applications were made on 6 June (second applications were made 5 July). Plots were sprayed with an amount of spray proportional to 280 liter spray per hectare using a hand sprayer at a pressure of 2.2 kg per cm^2. This amount of spray allowed full coverage of the upper leaf surfaces of the plants but not to dripping. Data for plant height, leaf thickness, leaf water potential and numbers of eggs and nymphs of the silverleaf whitefly were collected the 13 and 27 June and the 11, and 25 July. The entire field was sprayed with insecticide 27 July and on a schedule, thereafter, to control whiteflies, ending the test.

Large plot field tests. Four plots for each of two treatments were arranged in each of two cotton fields. The two fields, numbered 2 and 116 at the MAC farm, were planted 15 and 16 April, respectively, and were separated by 1 km. Plot sizes were 12 rows × 20 m in field 2 and 12 rows × 12 m in field 116. Plots were continuous for the lengths of the fields to facilitate ground rig application of Pix. The two treatments in each field were: 1) Pix at 1.12 liter per hectare initially and 4 wk later, and 2) untreated. Plants in field 2 were approximately 45 cm tall and were just beginning to bloom. Plants in field 116 were approximately 30 cm tall and had initiated blooming 1 wk before treatment. The shorter but earlier blooming plants in field 116 reflected planting on historically less productive soil. Initial spray application was made to both fields 15 June and a second application was made 13 July. Plots were sprayed using a 12-row bloom with two nozzles per row directed at the top and each side of the row. Applications were made at a rate of 187 liter spray per hectare at a pressure of 22 kg per cm^2 for full plant coverage. Data for plant height, leaf thickness, and numbers of eggs and nymphs of the silverleaf whitefly were collected 17, 30 June and 15, 28 July. Field 116 was sprayed for lygus bugs, *Lygus hesperus* Knight, 6 July (acephate, 0.56 kg AI per hectare) and both fields were sprayed with insecticide 28 July (after sample collection) and on schedule, thereafter, for control of whiteflies, ending the tests.

Results

Greenhouse tests. Cotton plants treated with foliar or soil applications of Pix in the greenhouse generally were significantly shorter and had significantly thicker leaves at the end of the second week and thereafter following the initial application. However, in both tests, the numbers of resting adults and eggs and nymphs per cm^2 did not show significant reductions until the fourth week following the initial application (Table 1). By the end of the fourth week, foliar and soil applications of Pix (average of all Pix treatments as a percent of the control ± SE) had reduced plant height (Test 1 = 77 ± 2%, Test 2 = 85 ± 1%), numbers of resting adults (Test 1 = 36 ± 7%, Test 2 = 54 ± 3%), eggs on leaves

Table 1. Effects of Pix on plant growth and silverleaf whitefly populations on cotton plants in the greenhouse four weeks after applications.*

				Silverleaf whitefly		
					Number/cm^2 leaf disk	
Applic.	Pix ml/plant*	Plant height cm	Leaf thickness mm	Resting adults/leaf	Eggs	Nymphs
Test 1 5 April - 5 May**						
Foliar	0.0029	28.7 bc	0.36	52 b	49 b	9 b
Foliar	0.0058	28.4 bc	0.43	84 ab	52 b	13 b
Foliar	0.0113	24.9 c	0.46	27 b	35 b	8 b
Soil	0.07	27.7 bc	0.38	48 b	42 ab	8 b
Soil	0.15	26.7 bc	0.43	68 b	55 ab	14 b
Soil	0.28	25.4 bc	0.43	24 b	58 ab	17 b
Foliar	water	32.0 a	0.33	83 ab	73 ab	33 ab
Control	–	35.1 a	0.30	139 a	114 a	37 a
Test 2 5 May - 2 June**						
Foliar	0.0029	35.1 b	0.40 ab	4 b	3	7 b
Foliar	0.0058	37.5 b	0.41 ab	5 b	4	7 b
Foliar	0.0113	36.4 b	0.47 a	5 b	4	8 b
Soil	0.07	35.4 b	0.42 a	4 b	3	4 b
Soil	0.15	36.6 b	0.41 ab	8 b	7	11 ab
Soil	0.28	33.8 b	0.43 a	4 b	3	6 b
Foliar	water	46.6 a	0.35 b	14 a	5	10 ab
Control	–	41.9 ab	0.33 b	11 ab	11	19 a

* Greatest foliar dose equals 1.12 liter Pix per hectare. Soil doses applied in 100 ml water per plant.
** Means not followed by a common letter, within a test and column, are significantly different, ANOVA, Ryan-Einot-Gabriel-Welsch multiple F test, $P = 0.05$.

(Test 1 = 43 ± 3%, Test 2 = 36 ± 6%), nymphs on leaves (Test 1 = 31 ± 4%, Test 2 = 38 ± 5%) and increased leaf thickness (Test 1 = 138 ± 5%, Test 2 = 141 ± 3%). There was little difference in the results of foliar or soil application or between doses by the end of the fourth and final week following the initial application. However, the greatest dose applied to the soil, 0.28 ml, was approximately 25-fold greater than the greatest foliar dose of 0.0113 ml Pix per plant.

Pix-treated plants and plants watered daily (well-watered) generally had significantly lower leaf water potentials (less pressure required to exude interstitial sap) than water-stressed and control plants (Table 2). The increases in leaf water potential over the three sample dates reflect possible differences in the watering cycles, sunlight conditions or leaf position. The results indicated that Pix-treated plants had leaf water potentials comparable to well-watered plants. Plant height was significantly reduced and leaf thickness significantly increased in Pix-treated or water-stressed plants in comparison to well-watered and control plants in this greenhouse test.

Small plot field test. At 5 and 7 weeks after initial applications of Pix to single row plots, plants were significantly shorter and had thicker leaves compared to water-sprayed and control plants (Table 3, 11 and 25 July). However, there were no significant reductions in leaf water potential or in the numbers of whitefly eggs and nymphs on leaves. There appeared to be little difference in the results of the two dose levels or whether they were applied once or twice. The data indicate that effects of the Pix treatments on plant height and leaf thickness were generally apparent 3 wk following treatment (27 June). The results of this test differ from previous results in the greenhouse in

Table 2. Effects of Pix and well-watered, water-stressed or normal irrigation conditions on leaf water potential, plant height and leaf thickness on cotton plants in the greenhouse.*

Treatment	Leaf water potential -bars**			Plant height** cm	Leaf thickness** mm
	25 May	27 May	2 June		
Pix 0.28 ml	10.6 b	11.2 b	14.6 b	40.6 c	0.59 a
Well-watered	10.7 b	11.5 b	15.0 b	55.8 b	0.43 c
Water-stressed	12.4 a	14.2 a	18.3 a	43.2 c	0.51 b
Control	12.4 a	13.8 a	15.1 b	63.5 a	0.46 c
LSD	1.4	1.8	1.9	5.1	0.04

* Means of five plants per treatment, treatments initiated 4 May. Pix applied 0.28 ml per plant in 100 ml water on 4 and 20 May, otherwise control irrigation. Well-watered = excess water daily, water-stressed = minimum water at leaf wilt, control = normal watering every other day.

** Plant height an leaf thickness measured 3 June. Means within a column not followed by a common letter are significantly different, ANOVA, LSD tests, $P = 0.05$.

Table 3. Effects of Pix on plant growth and silverleaf whitefly populations in cotton plants in single-row plots, MAC farm, 1994.*

Pix l/ha (no. applic.)	Plant height** cm	Leaf thickness** mm	Leaf water potential** -bars	Silverleaf whitefly Avg./3.88 cm² leaf disk** Eggs	Nymphs
			13 June		
1.12	40.6	0.38	14.4	0.04	0.04
1.12 (2)	40.3	0.40	13.8	0.12	0.00
2.24	40.3	0.38	13.3	0.04	0.00
2.24 (2)	40.8	0.39	13.9	0.04	0.08
Water	39.6	0.40	14.0	0.04	0.04
Control	38.9	0.39	14.2	0.04	0.08
			27 June		
1.12	51.7 ab	0.35 ab	13.4	0.08	0.04
1.12 (2)	52.0 ab	0.34 b	12.8	0.23	0.12
2.24	50.6 ab	0.36 a	13.7	0.12	0.12
2.24 (2)	48.2 b	0.35 ab	13.5	0.19	0.23
Water	55.4 a	0.32 c	14.3	0.16	0.16
Control	55.1 a	0.31 c	13.0	0.19	0.08
			11 July		
1.12	56.9 bc	0.39 a	13.6	0.62	0.50
1.12 (2)	57.2 abc	0.39 ab	13.1	0.43	0.50
2.24	54.6 c	0.40 a	13.2	0.39	0.39
2.24 (2)	55.2 bc	0.40 a	14.1	0.58	0.50
Water	62.4 ab	0.36 bc	13.8	0.70	0.43
Control	63.7 a	0.35 c	13.2	0.58	0.43
			25 July		
1.12	60.7 b	0.35 b	12.8	18.24	8.92
1.12 (2)	61.5 b	0.39 a	13.2	16.30	7.37
2.24	59.4 b	0.38 a	12.7	13.19	5.43
2.24 (2)	57.5 b	0.38 a	13.0	15.52	8.15
Water	68.0 a	0.31 c	13.2	14.74	6.21
Control	69.0 a	0.31 c	13.1	17.07	5.82

* Five single-row plots each 10 m long per treatment on 6 June. Averages of 5 plants for height, 10 leaves for thickness, 3 plants for leaf water potential and 10 leaf disks for eggs and nymphs.

** Means within a column and date not followed by a common letter are significantly different, ANOVA, Ryan-Einot-Gabriel-Welsch multiple F test, $P = 0.05$.

that reductions in leaf water potential were not obtained in Pix-treated plants (although the morphological changes were obtained) and whitefly infestations were not reduced.

Large plot field tests. Ground rig application of Pix to 12-row field plots in Field 2 caused significant reductions in plant height and significant increases in leaf thickness from 30 June (2 wk following application) to the end of the test, 28 July (Table 4). However, the numbers of eggs and nymphs on leaves were not significantly reduced by Pix treatment (except for nymphs on 30 June). In field 2, the anticipated morphological effects of Pix on plant growth were obtained, but whitefly infestations were not reduced.

Plant height, leaf thickness, and whitefly infestations were not significantly affected (with minor exceptions) in Pix-treated plots in field 116 (Table 4). The lack of effects on Pix on plant growth, in contrast to our other experiments, may be explained by the generally lower plant growth rate (nonrank growth) of cotton in field 116. The failure of Pix treatments to affect whitefly infestations was consistent with our other field plot tests.

Discussion

Leaves of cotton plants treated with Pix in the greenhouse generally had fewer adults, eggs, and nymphs of the silverleaf whitefly 4 wk following treatment than untreated plants, while leaves of plants treated with similar doses in field plots did not. Reduced infestations in the greenhouse appeared to be caused by reduced numbers of adults on leaves. Similar counts of adults were not made in the field. However, the lack of effect of Pix treatment on the numbers of eggs and nymphs in the field suggests that adults were not disturbed by the treatment. Zummo et al. (1984), working with Pix-treated cotton plants in field cages, infested bagged plants with neonatal bollworm larvae, *H. zea*, and determined larval survival at 7 d. They showed that reduced survival was associated with measured increases in tannins and terpenoids (which began to increase 2 d after application) in Pix-treated plants. They concluded that the observed mortality was caused by induced host plant resistance. However, increased resistance to the tobacco budworm, *H. virescens,* was not found in artificially (Graham et al. 1987) or naturally (Pfrimmer 1984) infested cotton plants treated with Pix (although typically shorter plants were obtained). Our measurements of plant height and leaf thickness confirm the usual morphological effects of Pix and we presume that allelochemicals also increased in treated plants. However, we doubt that these chemicals affected silverleaf whitefly densities.

The lower leaf water potentials (-bars) of Pix-treated and well-watered plants than of water-stressed and (usually) control plants in the greenhouse were not observed in plants in the field. Cotton plants in the field usually have lower leaf water potentials in response to increased irrigation frequency (Flint et al. 1995). Water-stressed cotton plants (with concomitantly higher leaf water potentials) are significantly more attractive to silverleaf whiteflies than are well-watered plants (Flint et al. 1994). We suggest that greenhouse-grown

Table 4. Effects of Pix applied by ground rig on cotton plant growth and silverleaf whitefly infestations in two field plot tests, MAC farm, 1994.†

Date	Treatment	Plant height‡ cm	Leaf thickness** mm	Silverleaf whitefly Avg./3.88 cm^2 leaf disk‡ Eggs	Nymphs
			Field 2		
17 June	Pix	45	0.38	0.19	0.16
	Control	44	0.38	0.08	0.08
30 June	Pix	58	0.36**	0.35	0.16
	Control	65**	0.30	0.47	0.39*
15 July	Pix	64	0.43**	5.90	1.90
	Control	76**	0.33	9.93	2.10
28 July	Pix	70	0.36**	118.73	20.95
	Control	84**	0.30	95.06	19.01
			Field 116		
17 June	Pix	32	0.41	0.16	0.12
	Control	32	0.41	0.19	0.04
30 June	Pix	40	0.38	1.16	0.62
	Control	44*	0.36	1.32	0.58
15 July	Pix	54	0.33	14.28	2.10
	Control	57	0.33	14.90	4.97**
28 July	Pix	66	0.30	42.68	8.92
	Control	69	0.28	43.84	6.98

†Plot size field 2 = 12 rows × 20 m, field 116 12 rows × 12 m. Four replicate plots per treatment, both fields. Plots sprayed on 15 June and on 13 July with 1.12 liter Pix per hectare.

‡Pairs of means compared by *t*-tests, * = significant at $P = 0.05$, ** = significant at $P = 0.01$.

cotton plants treated with Pix had lower infestations of whiteflies than untreated plants because they had lower leaf water potentials. Conversely, we suggest that treated and untreated plants in the field did not differ in whitefly infestations because they did not differ in leaf water potentials. The effect of Pix on leaf water potential of plants in the greenhouse may have been caused by differences in the soil and holding conditions. The original observation of reduced numbers of silverleaf whiteflies on greenhouse cotton plants treated with Pix was verified but the effect was not obtained under field conditions.

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