Proc. Fla. State Hort. Soc. 116:294-297. 2003.

A REFEREED PAPER

AZAFENIDIN EFFICACY ON WEEDS AND SEEDLING SAFETY IN CITRUS

SHIV D. SHARMA
CCS Haryana Agriculture University
Regional Research Station
Kamal, Haryana, India

MEGH SINGH[1]
University of Florida, IFAS
Horticultural Sciences Department
Citrus Research and Education Center
700 Experiment Station Road
Lake Alfred, FL 33850

Additional index words. herbicide, phytotoxicity, rootstocks, weeds

Abstract. **The effects of different rates of the herbicide azafenidin alone and in the tank mix with other preemergence herbicides were examined on some important weed species of citrus groves. Azafenidin alone had immediate damaging effects on emerging seedlings of test weeds, which became bleached and died 1 week after treatment (WAT) even at the lowest rate (0.28 kg a.i./ha). No significant difference in the control of test weeds was obtained with tank mix applications of azafenidin with bromacil or diuron, or with azafenidin alone. One week after treatment, ≤23% control was obtained in tall morningglory, milkweed vine, and hairy beggarticks, 26% to 73% control in Brazil pusley, and 100% control in redroot pigweed when norflurazon, bromacil, diuron, or simazine herbicides were applied alone. Two weeks after treatment, all test herbicides, including azafenidin at the lowest rate of 0.28 kg a.i./ha, achieved 100% control of the test weed species. Symptoms of phytotoxicity of azafenidin were obvious on the upper leaves of citrus rootstock seedlings. The order of symptom severity was Volkamer > Carrizo = Swingle, and injury increased with the rate of azafenidin. Carrizo seedling height was significantly reduced at higher rates (1.68 and 2.2 kg a.i./ha) of azafenidin 4 WAT. Upper leaves of Volkamer were bleached by 2.2 kg a.i./ha. Beyond 4 weeks, all three rootstocks resumed normal growth in all azafenidin treatments. Therefore, azafenidin could be used as an effective and potential broad-spectrum herbicide in citrus groves at lower rates (0.28 kg a.i./ha).**

Predicting herbicide efficacy is challenging. Herbicide activity is influenced by environmental conditions at application time (Doran and Anderson, 1976), herbicide rate (King and Oliver, 1992), weed emergence, weed size (Kells et al., 1984), and interactions with other herbicides (Hatzios and Penner, 1985). Soil-applied preemergence herbicides (PRE) prevent seed germination and reduce early weed growth to a certain extent. However, the application of post-emergence herbicides (POST) like Roundup is essential (Singh and Tucker, 1983). Application of PRE and POST herbicides reduces weed competition with citrus trees, but at a high cost because of the need for repeated use of herbicides at very high rates. Bromacil at 8.9 $kg \cdot ha^{-1}$, simazine at 10.6 to 20.7 $kg \cdot ha^{-1}$, norflurazon at 11.1 $kg \cdot ha^{-1}$, diuron at 8.9 $kg \cdot ha^{-1}$ as PRE and glyphosate at 14.7 kg a.i./ha^{-1} as POST are being applied annually to achieve optimum control of the broad spectrum of weeds in citrus groves (Futch and Singh, 2000). Furthermore, repeated high rates of application increase the risks of tree phytotoxicity and environmental contamination. Repeat applications of the same active compound could increase the chance of developing resistance against the chemical (Bradshaw et al., 1997). Glyphosate has been used worldwide for more than 20 yr, and its intensive use in agriculture has led to the appearance of resistant weed populations (Holt et al., 1993; Powles and Holtum, 1994). Powles et al. (1998) reported the development of resistance to glyphosate in rigid rye grass. Similarly, Lior et al. (2001) reported that the plantain (*Plantago lagopus* L.) population was found to be diuron resistant.

In recent years, low rate technology herbicides have become increasingly popular for weed control because of improved weed control efficacy and reduced environmental loading (Turner et al., 2000). Use of alternative herbicides like azafenidin that have broad-spectrum and longer duration of weed control have potential benefits of reducing the high costs associated with repeated applications of high rates of other soil applied herbicides. Azafenidin is absorbed through roots and shoots of susceptible plants (Amuti et al., 1997).

This research was supported by the Florida Agricultural Experiment Station, and approved for publication as Journal Series No. R-08326. Thanks to Gary Test for his valuable help during experiment.

[1]Corresponding author; e-mail: msingh@crec.ifas.ufl.edu.

Azafenidin can be tank mixed with bromacil, diuron, glyphosate, and acts by inhibiting the porphyrin biosynthetic pathway at a site that causes the accumulation of a photodynamic porphyrin intermediate, protoporphyrin IX. Protoporphyrin IX absorbs light and transfers this energy to thylakoid, creating a highly reactive singlet oxygen species that indiscriminately reacts with cellular components, resulting in cell membrane disruption. Its use may minimize phytotoxicity and the environmental contamination risk, and also could minimize the risk of resistance development caused by continuous and repeated use of other herbicides. Azafenidin is temporarily withdrawn by DuPont, but may change their mind in the future to exploit the positive aspects of improved weed control by azafenidin.

The objective of this study was to examine the bio-efficacy of azafenidin on some important weeds of citrus groves and its phytotoxicity to three citrus rootstocks.

Materials and Methods

Plant material. Two experiments were conducted under controlled environment greenhouse conditions at the University of Florida, Citrus Research and Education Center at Lake Alfred, Fla. In both experiments, tall morningglory (*Ipomoea purpurea* (L.) Roth.), hairy beggarticks (*Bidens frondosa* L.), redroot pigweed (*Amaranthus retroflexus* L.), and milkweed vine (*Morrenia odorata* (H&A) Lindl.) were tested. The second experiment additionally contained Brazil pusley (*Richardia brasiliensis* (Moq.). Weeds were grown in soil collected from the top profile (0 to 15 cm) of a well-drained Candler sand (Hyperthermic, unbolted Typic Quartzipsamments) from Davenport, Fla. with a pH of 6.5 and 0.9% organic matter. This site has not been used for any agricultural crops for over 8 yr and is presumed to be free of herbicide residues. The soil was sieved and filled to a depth of 7.5 cm in metal trays (48 × 33 × 10 cm) with holes at the base which were covered by placing paper towels; the soil was soaked completely with water overnight to bring its moisture level to field capacity. The next day, seeds of test weed species were planted in single rows in these trays (one row of each weed seed in each tray and more than 100 seeds in a row). There were four replicated trays.

Herbicides and bioefficacy study. In the first experiment, the effect of azafenidin (2-[2,4-dichloro-5-(2-propynyloxy)phenyl]-5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-a]pyridin-3[2H]-one) (Milstone™ WG 80%) alone or in tank mix with bromacil (Hyvar X 80% a.i.) or diuron (Karmax® DF 80% a.i.) was evaluated. Azafenidin was used at 0.56, 0.84, 1.1, 1.4 kg a.i./ha alone and at all rates in tank mix with bromacil or diuron at 2.2 kg a.i./ha. In the second experiment, the bioefficacy of various PRE herbicides [azafenidin, norflurazon (Solicam® WG 80%), bromacil, diuron, simazine (Princep® 4L 48% a.i.)] was examined as individual treatments. Azafenidin at 0.3, 0.56, 0.84, 1.1, and 1.4 kg a.i./ha and all other herbicides at 2.2 kg a.i./ha were applied as separate treatments.

Freshly prepared aqueous solutions of test herbicides were applied one day after sowing of weed seeds. Spray was accomplished using a chamber track sprayer (Allen Machine Works, MI, USA). The sprayer was fitted with a Teejet 8003 flat fan spray nozzle (Spraying System Co., Wheaton, IL, USA) delivering a carrier volume of 189 $L \cdot ha^{-1}$ at 138 kPa pressure. After spraying, the trays were returned to a greenhouse maintained at 25 °C (±2 °C) temperature and 70% (±5%) relative humidity under normal daylight conditions. The experiments were conducted during November and December 2000. Uniform amounts of water were applied to the soil surface after herbicide application to infiltrate the herbicide into soil.

Phytotoxic injury to germinating seedlings was rated for each weed species until the phytotoxicity symptoms were established up to a maximum of 4 weeks after treatment (WAT). Data are presented for 1 WAT and 2 WAT because the injury to the germinating seedlings reached a maximum and was fully established within this period. A scale of 0 to 100 was used; 0 indicating no injury and 100 indicating complete damage or death (Frans et al., 1986).

Citrus rootstock phytotoxicity. Three-month-old rootstock seedlings of Volkameriana lemon (*Citrus volkameriana* Ten. & Pasq.), Carrizo citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.], and Swingle citrumelo [*Poncirus trifoliata* (L.) Raf. × *Citrus paradisi* Macf.], were purchased from a local nursery. Seedlings are generally more sensitive to herbicides than saplings or older trees, making them excellent test plants for phytotoxicity study. Therefore, these rootstock seedlings were used to generate information about their susceptibility to azafenidin (Singh and Achhireddy, 1984). One rootstock per pot was transplanted into pots of 20 cm height and 18 cm top diameter (5 kg soil) in the top profile of soil (0 to 15 cm) as mentioned above. Rootstocks were allowed to establish themselves for 3 weeks before azafenidin treatments were applied. Azafenidin effect was assessed at 0.56, 1.1, 1.68, and 2.2 kg a.i./ha. Volume of herbicide solutions for pots were calculated on the basis of the weight of field soil, considering a 15 cm furrow slice per hectare. The soil moisture in pots was brought to field capacity before herbicide application. The amount of herbicide for each individual pot was mixed in 50 ml of water and applied uniformly to the top of the soil in the pots. There were four replications per treatment and the experiment was repeated once. Rootstock height before herbicide application and at 4 WAT was measured to determine any effect of azafenidin on seedling growth. Foliar symptoms of phytotoxicity of azafenidin to citrus rootstocks weres visually rated 4 WAT on a 0 to 100 scale based on visible foliar phytotoxic symptoms (0 - no phytotoxicity; 100 - 100% damage or plant died due to herbicide phytotoxicity). Rootstocks were maintained until 12 weeks after treatment application and any change in growth and/or symptoms was recorded.

Statistical analysis. Experiments were analyzed as randomized complete blocks with four replications and both experiments were repeated once. Each individual weed species was analyzed separately. The data were combined to present the mean values after performing a test of homogeneity of variance. The data were subjected to ANOVA after performing an arc-sine transformation but are presented in the original form for clarity; the means were separated using Fisher's Protected Least Significant Difference test (LSD $P \leq 0.05$). In citrus phytotoxicity experiment with rootstock, observation scale of 0-10 was converted into percent data and ANOVA was performed as mentioned.

Results and Discussion

Experiment I. All weed species in experiment 1 germinated. Azafenidin treatment had an immediate damaging (bleached) effect on these emerging seedlings, which became chlorotic

Table 1. Effect of azafenidin alone and in tank mix with Bromacil and Diuron on weed growth.

Treatment	Rate kg a.i.·ha^{-1}	Percent injury 1 WAT			
		Tall morningglory	Hairy beggarticks	Redroot pigweed	Milkweed vine
Azafenidin	0.56	91	89	100	90
+ Bromacil	2.2	94	94	100	90
+ Diuron	2.2	83	89	100	90
Azafenidin	0.84	88	90	100	91
+ Bromacil	2.2	94	95	100	93
+ Diuron	2.2	88	94	100	90
Azafenidin	1.1	95	94	100	94
+ Bromacil	2.2	91	95	100	93
+ Diuron	2.2	90	94	100	91
Azafenidin	1.4	95	95	100	94
+ Bromacil	2.2	95	95	100	91
+ Diuron	2.2	90	95	100	93
Control		0	0	0	0
LSD ($P = 0.05$)		15	6	0	4

and died within 1 week even with the lowest rate (0.56 kg a.i./ha) of azafenidin (Table 1). Even lower rates of 0.28 kg a.i./ha in the second study provided complete mortality after 2 weeks of spraying (Table 2). Similarly, Amuti et al. (1997) reported that azafenidin produces necrotic symptoms quickly in weed species, which died within a few days of emergence. Zandstra and Masabni (2000) also reported that susceptible seedlings die soon after emergence. In the present study, tank mix application of azafenidin with bromacil or diuron achieved similar control of weed species as were obtained with azafenidin alone. Zandstra and Masabni (2000) reported that application of azafenidin at 0.56 kg a.i./ha or higher plus glyphosate 1.12 kg a.i./ha gave acceptable control of broadleaf and grass weeds for 90 d and that a second application of azafenidin gave season-long weed control.

Experiment II. From the first study, it was not possible to differentiate the effect of azafenidin alone or its effect of tank mix with bromacil or diuron on any of the test weed species. Therefore, a second study was conducted using lower rates of azafenidin (0.28-1.4 kg a.i./ha), bromacil, simazine, norflurazon, and diuron at 2.2 kg a.i./ha. These herbicides were included in this experiment to directly compare azafenidin to currently available PRE herbicides. All the test weed seeds germinated within 3 to 5 d of sowing. Similar to the first experiment, the emerging seedlings were chlorotic and died within one day of emergence when azafenidin was applied at any rate (Table 2). Weed control was lower with norflurazon, bromacil, diuron, and simazine at one WAT for all the weed species except redroot pigweed, but increased significantly at 2 WAT (Table 2). Except for milkweed vine, total control was achieved with each of these herbicides 2 WAT (Table 2). Based on these results, application of low rates of azafenidin (0.28 kg a.i./ha) could be used as an alternative to other PRE herbicides which have to be used repeatedly and at higher rates to control a broad spectrum of weeds in citrus (Futch and Singh, 2000). Lior et al. (2001) reported that the plantain (*Plantago lagopus* L.) population at one site was found to be diuron resistant. They demonstrated that the polymorphic nature of plantain induced resistance to different herbicides, due to selection pressure imposed by the repeated herbicide application. Examples of glyphosate resistance have also been observed (Powles and Holtum, 1994).

Rootstock phytotoxicity study. There was no significant difference in the increased height of Volkamer and Swingle rootstocks between the untreated control and any of the azafenidin treatments 4 WAT (Table 3). In Carrizo, however, height was significantly reduced by higher rates (1.68 and 2.2 kg a.i./ha) of azafenidin. The phytotoxicity symptoms of

Table 2. Effect of preemergence herbicides on selected weed species.

Treatment	Rate kg a.i.·ha^{-1}	Percent control 2 WAT				
		Tall morningglory	Milkweed vine	Hairy beggarticks	Brazil pusley	Redroot pigweed
Azafenidin	0.28	100	100	100	100	100
Azafenidin	0.56	100	100	100	100	100
Azafenidin	0.84	100	100	100	100	100
Azafenidin	1.1	100	100	100	100	100
Azafenidin	1.4	100	100	100	100	100
Norflurazon	2.2	100	100	100	100	100
Bromacil	2.2	100	81	100	100	100
Diuron	2.2	100	83	100	100	100
Simazine	2.2	100	83	100	100	100
Control		0	0	0	0	0
LSD ($P = 0.05$)		0	3	0	0	0

Table 3. Effect of different rates of azafenidin on three citrus rootstocks.

		Increase in height (cm)[z]			Phytotoxicity[z]		
Treatment	Rate kg a.i.·ha^{-1}	Volkamer	Carrizo	Swingle	Volkamer	Carrizo	Swingle
Azafenidin	0.56	9.2	4.9	3.3	0	0	0
Azafenidin	1.1	6.6	6.1	5.6	10	5	5
Azafenidin	1.68	8.4	3.6	2.7	10	5	5
Azafenidin	2.2	4.3	3.1	4.1	18	5	5
Control		8.3	5.7	3.6	0	0	0
LSD (P = 0.05)		4.7	1.4	2.5			

[z]Increase in tree height (cm) and phytotoxicity (scale of 0-100) were observed 4 WAT.

azafenidin appeared on the upper leaves of the rootstocks tested. The order of symptom severity was Volkamer > Carrizo = Swingle. Symptoms included intraveinal chlorosis/bleaching or yellowish discoloration of leaves, and increased on Volkamer lemon with the rates of azafenidin (0.56 to 2.2 kg a.i./ha) applied to the soil. At 2.2 kg a.i./ha rate, the upper leaves appeared chlorotic in Volkamer. Beyond 4 WAT, the symptoms in Volkamer appeared on older leaves as well, and persisted even after 3 months following treatment (data not included). All rootstocks treated with azafenidin at 0.56 to 1.68 kg a.i./ha showed no phytotoxic symptoms beyond 4 WAT and were growing similar to the control treatments. Seedling height was almost equal under all azafenidin treatments and similar to the control beyond 4 WAT (data not presented). Azafenidin has also generally been reported to be safe on tree crops (Turner et al., 2000). Zandstra and Masabni (2000) applied azafenidin to apple, asparagus, blueberry, cherry, peach, strawberry, and spearmint at rates of 0.14 to 1.68 kg a.i./ha alone or in combination with foliar active herbicides. Azafenidin at 1.68 a.i./ha caused no phytotoxicity to any of the tree crops. Asparagus tolerated 1.68 kg·ha^{-1}, strawberry was sensitive to 0.56 kg·ha^{-1}, and spearmint was injured by 0.14 kg·ha^{-1} azafenidin. In a crop safety test, Turner et al. (2000) reported that with application of azafenidin at 1.68 kg·ha^{-1} and higher, growth and quality parameters such as trunk diameter, yield, and percent sucrose, have been equal to or greater than in untreated controls or standard treatments. These workers have also reported that azafenidin provided broad-spectrum weed control for 4 to >6 months depending upon rate and environmental conditions.

In the present study, negligible phytotoxic effects on citrus rootstocks and effective weed control at low application rates confirm the potential of azafenidin for citrus groves. Further experiments on the degradation and leaching aspect of azafenidin are required to understand the environmental fate and impact of azafenidin in typically sandy soils of Florida Citrus groves.

Literature Cited

Amuti, K., A. Trombini, L. Giammarrusti, C. Sbriscia, H. Harder, and J. Gabard. 1997. Azafenidin: A new, low use rate herbicide for weed control in perennial crops, industrial weed control, and forestry. Proc. British Crop Protection Council-Weeds. pp. 59-66.

Bradshaw, L. D., S. R. Padgette, S. L. Kimball, and B. H. Wells. 1997. Perspectives on glyphosate resistance. Weed Tech. 11:189-198.

Doran, D. L. and R. N. Anderson. 1976. Effectiveness of bentazon applied at various times of day. Weed Sci. 24:567-570.

Frans, R., R. Talbert, and D. Marx. 1986. Experimental design and techniques for measuring and analyzing plant responses to weed control practices, pp. 29-46. In N. D. Camper (ed.). Research Methods in Weed Science. Southern Weed Sci. Soc., Champaign, IL.

Futch, S. H. and M. Singh. 2000. 2000 Florida Citrus Pest management guide: Weeds. University of Fla. Coop. Ext. Ser. (HS 107) p. 33.1.1-33.1.11.

Hatzios, K. K. and D. Penner. 1985. Interactions of herbicides with other agrochemicals in higher plants. Rev. Weed Sci. 1:1-63.

Holt, J. S., S. B. Powles, and J. A. M. Holtum. 1993. Mechanisms and agronomic aspects of herbicide resistance. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44:203-229.

Kells, J. J., W. F. Meggitt, and D. Penner. 1984. Absorption, translocation, and activity of fluazifop-butyl as influenced by plant growth stage and environment. Weed Sci. 32:143-149.

King, C. A. and L. R. Oliver. 1992. Application rate and timing of acifluorfen, bentazon, chlorimuron, and imazaquin. Weed Tech. 6:526-534.

Lior, E., M. Sbony, T. Hassar-Nadler, J. Kigel, and B. Rubin. 2001. Multi-herbicide resistance in plantain (*Plantago lagopus* L.) populations. Proc. Weed Sci. Soc. Amer. 41:96.

Powels, S. B. and J. A. M. Holtum. 1994. Herbicide resistance in plants, pp. 353. In S. B. Powels and J. A. M. Holtum (eds.). Biol. Biochem., Lewis Publishers, Boca-Raton, Fla.

Powles, S. B., D. F. L. Colwill, J. J. Dellow, and C. Preston. 1998. Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. Weed Sci. 46:604-607.

Singh, M. and D. P. H. Tucker. 1983. Preemergence herbicides for container-grown citrus. HortScience 18:950-952.

Singh, M. and N. R. Achhireddy. 1984. Tolerance of citrus rootstocks to preemergence herbicides. J. Environ. Hort. 2:73-76.

Turner, R. G., K. S. Amuti, J. R. C. Leavitt, M. V. Naidu, E. Bollin, Jr., A. S. Klemens, R. H. N. Park, and L. B. Gillham. 2000. Performance, soil and plant metabolism, and dissipation of azafenidin in citrus, grape, sugarcane, and pome fruit in the United States. Proc. Weed Sci. Soc. Amer. 40:45.

Zandstra, B. H. and J. G. Masabni. 2000. Azafenidin for preemergence weed control in perennial crops. Proc. Weed Sci. Soc. Amer. 40:93.