Effect of Foliar-Applied Salicylic Acid on Cotton Flowering, Boll Retention, and Yield

J.J. Heitholt[1,2], J.H. Schmidt[2], and J.E. Mulrooney[3]
[2]USDA-ARS, P.O. Box 345, Stoneville, MS 38776 and [3]USDA-Forest Service, Starkville, MS 39759

Salicylic acid (2-hydroxybenzoic acid) may help regulate several plant functions, including systemic acquired resistance to pathogens and the formation of flowers. The objective of this study was to characterize the effects of foliar-applied salicylic acid on cotton (*Gossypium hirsutum* L.) flowering, boll retention, and yield. Field experiments were conducted at two Mississippi locations in 1995 and at one location in 1997. In 1995, a single application of sodium salicylate (0, 17.1, 51.3, or 171 g ha^{-1}) was made two to three weeks prior to flowering. In 1997, nine sequential applications of sodium salicylate (51.3 g ha^{-1}) or salicylic acid (44.3 g ha^{-1}) or a check solution (Tween 20, polyoxyethylene sorbitan monolaurate only) were made beginning when the first floral buds were present and ending at first flower. In one of the 1995 tests and in the 1997 test, the cotton was not treated with insecticides after planting. Although physiological responses to exogenously-applied salicylic acid on cotton have been reported elsewhere, in the present study, flower production, boll retention, and yield were not significantly affected.

Foliar applications of salicylic acid have been shown to affect flower induction and flower numbers in several species. In minute duckweed (*Lemna paucicostata*), salicylic acid increased the percent of plants flowering under both long- and short-day lengths (Watanabe and Takimoto, 1979). Kharana and Cleland (1992) showed that 10 μM salicylic acid or benzoic acid induced flowering of *L. paucicostata* LP6. Both exogenous salicylic acid and the salicylic acid fraction of brown ambrosia aphid (*Dactynotus ambrosiae*) honeydew that had been feeding on cocklebur, *Xanthium strumarium* L. induced flowering in inflated duckweed, *L. gibba* (Cleland and Ajami, 1974). However, in Japanese morningglory {[*Ipomoea nil* (L.) Roth]=[*Pharbitis nil* (L.) Choisy]}, salicylic acid reduced flowering (Groenewald and Visser, 1978).

In mung bean (*Vigna radiata* L.), three foliar sprays of 7.2 and 72 μM salicylic acid increased seed yield per plant by 19 and 46%, respectively (Singh and Kaur, 1980). In cheena millet (*Panicum miliaceum* L.), salicylic acid increased plant height and grain number (Datta and Nanda, 1985).

In addition to possible involvement in flowering, the knowledge of the role of salicylic acid in systemic acquired resistance and pest resistance has recently been advanced (Raskin, 1992; Delaney et al., 1994; Ryals et al., 1996; Sticher et al., 1997). Plant drenches of salicylic acid at 61 and 123 μM also increased cotton root gossypol concentration by 35 and 47%, respectively (Khoshkhoo et al., 1993). Soaking cotton seedling root systems for 30 s in 0.1% salicylic acid reduced shoot fresh weight and root-knot nematode (*Meloidogyne incognita* race 3) egg numbers per root biomass (Hedin et al., 1995). Herbivory by *Helicoverpa zea* (Boddie) increased cotton leaf salicylic acid and H_2O_2 concentration (Bi et al., 1997), a response frequently observed following pathogenesis (Klessig and Malamy, 1994). However, foliar applications of salicylic acid did not affect cotton foliar resistance to *H. zea*. Hypersensitivity response to bacterial blight (*Xanthomonas campestris* pv *malvacearum*) in cotton was reported to be related to the accumulation of salicylic acid in cotyledons (Martinez et al., 2000).

Since the agronomic effects of salicylic acid on cotton in the mid-south USA have not been reported, the objective of this study was to determine whether foliar applications of sodium salicylate or salicylic acid would increase yield, flowering, and boll retention in field-grown cotton.

[1]Author for correspondence. Current address: Texas Agricultural Research and Extension Center, 17360 Coit Road, Dallas, TX 75252, j-heitholt@tamu.edu

MATERIALS AND METHODS

Cotton (cv. Deltapine 50) was grown in the field in two locations (3 km apart) near Stoneville, MS in 1995. Seed were planted on 18 April 1995 (Test 1) at one location on a mixed soil type (a Bosket very fine sandy loam and a Dundee very fine sandy loam) and on 10 May 1995 for the second location on Dundee silty clay (Test 2). No attempt was made to match planting dates between Test 1 and Test 2. In 1997, Test 3 was planted on 23 May at the same site where Test 1 was two years earlier. Conventional fungicide seed-treatment and planting procedures were used. Row spacing was 102-cm and plots were four rows wide and 5.2 m long. Final plant stand density averaged 11 plants m^{-2}.

In Tests 1 and 2, foliar treatments were applied on only one date during the floral bud stage, approximately two to three weeks before first bloom. The treatments consisted of one application of 0, 0.625, 1.87, or 6.25 mM Na salicylate (Sigma Chemical Co., St. Louis, MO). All solutions contained 0.05% (v/v) Tween 20 (polyoxyethylene sorbitan monolaurate, Sigma Chemical Co., St. Louis, MO) and were applied using a backpack sprayer with flat-fan nozzles. The rate of solution applied was 171 L ha^{-1} (17.1 mL m^{-2}). Thus, rates of sodium salicylate were 0, 17.1, 51.3, and 171 g ha^{-1}. Application dates were 14 June 1995 for Test 1 and 15 June 1995 for Test 2. Initial flowering for Test 2 occurred approximately one week later than flowering for Test 1. An additional unsprayed check treatment, was included in both tests.

Because the results in Tests 1 and 2 indicated that a one-time salicylic acid spray had little effect, we changed to multiple treatments in Test 3 to consist of a check (Tween 20 only), foliar sprays of 1.87 mM sodium salicylate (pH 5.4) with 0.05% Tween 20, and foliar sprays of 1.87 mM salicylic acid (pH 3.1) also with 0.05% Tween 20. Application dates were 16 June, 18 June, 20 June, 23 June, 25 June, 27 June, 1 July, 8 July, and 15 July 1997. The spray rate for each application was 17.1 mL m^{-2} (171 L ha^{-1}). For sodium salicylate and salicylic acid, respectively, seasonal totals of 462 g and 395 g were applied per ha.

In Tests 1 and 3, plots were not treated with insecticide. In Test 2, plots were treated with insecticide on 27 May, 10 June, 22 June, 1 July, 15 July, 3 Aug., and 12 Aug. when insect pests typical of mid-South USA cotton reached threshold levels.

On selected dates during flowering (approximately twice weekly), white flowers were counted on one of the inner rows of each plot (5.28 m^2 per plot). In upland cotton, petals of blooms are white or cream colored on the day of anthesis. Petals are inconspicuous the day prior to anthesis and are pink or red the day following anthesis. Thus, it is possible to find and count all flowers at anthesis on a given day without concern for counting flowers that reached anthesis on an earlier or later date. An estimate of flowers reaching anthesis, on days when counts were not made, was made by interpolation. Subsequently, total seasonal flower production was calculated (Heitholt, 1993).

As the crop matured, open bolls were counted and hand-harvested from 4.6 m^2 of one inner row per plot (same row as that used for the flower count). Hand-harvest dates were 25 Aug., 6 Sept., 18 Sept., 27 Sept., and 6 Oct. 1995 for Test 1. Harvest dates were 7 Sept., 27 Sept., and 9 Oct. 1995 for Test 2. For Test 3, harvest dates were 9 Sept., 16 Sept., 30 Sept., 6 Oct., 16 Oct., 23 Oct., and 3 Nov. 1997. Boll retention (seasonal boll number divided by seasonal flower number), yield, boll numbers, and boll size (lint per boll) were determined.

The treatments in all three experiments were arranged in a randomized complete block design with six replications. An analysis of variance (ANOVA) was performed on all data. For Tests 1 and 2, sources of variation were replicate, control, rate (control), and error. For Test 3, only three treatments were used, so the sources of variation were replicate, treatment, and error.

RESULTS AND DISCUSSION

Tests 1 and 2 indicated that none of the treatments significantly affected yield (Table 1). It is possible that treatment of plants with salicylic acid can induce defenses against microbes, insects, and herbivores (Metraux and Raskin, 1992). Therefore, we originally hypothesized that a positive effect from salicylate was more likely in Test 1, which did not receive insecticide, than in Test 2.

Other yield-related factors, such as flower production, boll numbers, boll retention percentage, and boll size were likewise unaffected by foliar-applied salicylate (Table 1). Flower production, boll numbers, and boll retention in this study were typical of previous studies with Deltapine 50 (Heitholt, 1993).

In Test 3, a severe insect infestation greatly reduced flowering and yield. Yield was not significantly (P=0.08) affected by either salicylic acid or sodium salicylate (Table 2). Flower numbers, boll retention, and boll size were also unaffected. Others have reported that 10^{-4} to 10^{-10} M salicylic acid decreased abscisic acid-induced leaf abscission (as measured by petiole breakage) in kidney bean (*Phaseolus vulgaris* L.) (Apte and Laloraya, 1982). In Tests 1, 2, and 3, differences in leaf damage due to treatments were not observed. However, a high incidence of boll rot was generally observed in Test 3 regardless of treatment (no data collected).

Table 1. Effect of sodium salicylate on flower production, boll characteristics, and lint yield of Deltapine 50 cotton in 1995 at two locations.

Na Salicylate Treatment	Seasonal Flowers	Bolls	Boll Retention	Lint Yield	Boll Size
Test 1 No Insecticide	number m^{-2}		%	kg ha^{-1}	g (lint)
Unsprayed	104	65.7	63	970	1.47
Tween 20 only	107	63.9	60	973	1.53
17.1 g ha^{-1}	110	66.2	60	1010	1.52
51.3 g ha^{-1}	110	63.3	57	938	1.48
171.0 g ha^{-1}	106	70.2	66	1040	1.48
LSD (0.05)[a]	ns	ns	ns	ns	ns
Test 2 Conventional Insecticide					
Unsprayed	114	55.7	49	764	1.37
Tween 20 only	117	54.6	47	739	1.37
17.1 g ha^{-1}	114	53.2	47	725	1.36
51.3 g ha^{-1}	110	54.0	50	751	1.40
171.0 g ha^{-1}	114	49.5	43	708	1.44
LSD (0.05)[a]	ns	ns	ns	ns	ns

[a]LSD values for Test 1 were 11, 5.3, 9, 81, and 0.07 and for Test 2 were 13, 6.3, 14, 70, and 0.12.

In contrast to Patil and Wele (1992) factors other than salicylic acid obviously controlled the reproductive growth in field studies reported here. In the study by Patil and Wele (1992), salicylic acid may have increased yield by decreasing transpiration in a water-limited environment.

The finding that Test 1 (no insecticides) exhibited a greater yield than Test 2 (insecticides applied) needs an explanation. In Tests 1 and 2 (1995), extremely warm August temperatures in the mid-Delta impeded reproductive growth. Reproductive development of the earlier planted cotton (i.e., Test 1) was about one week further advanced than Test 2. Although one week is not extremely long, fruit growth in Test 1 apparently escaped much the warm temperatures that occurred in August 1995. This hypothesis is supported by the observation that earlier maturing cultivars tended to outyield later maturing cultivars at sites adjacent to Tests 1 and 2 that year (Heitholt and Meredith, 1998). In Test 3 (1997), the low yields (395 to 487 kg ha^{-1} vs. 708 to 1040 kg ha^{-1} in 1995) were likely due to a late planting date combined with high insect pressure.

Despite salicylic acid's lack of effect here, the results should not be interpreted to preclude the importance of salicylic acid in systemic acquired resistance or its role for protecting cotton against insects and pathogens. Future studies need to characterize genotypic differences in response to salicylic acid concentrations and determine whether those differences can affect pathogen resistance or yield.

Table 2. Effect of nine sequential pre-bloom foliar sprays of Tween 20, 1.87 mM salicylic acid, or 1.87 mM sodium salicylate on flower production, boll characteristics, and lint yield of Deltapine 50 cotton in Test 3 (1997). The field was not treated with insecticide.

Salicyclic Acid Treatment	Seasonal Flowers	Bolls	Boll Retention	Lint Yield	Boll Size
	number m^{-2}		%	kg ha^{-1}	g (lint)
Tween 20 only	37.2	31.4	85.7	432	1.39
Salicyclic acid	40.1	33.1	83.2	487	1.48
Na salicylate	34.9	27.3	80.3	395	1.46
LSD (0.05)[a]	ns	ns	ns	ns	ns

[a]LSD values for Test 3 were 7.9, 5.3, 14.2, 75, and 0.09.

ACKNOWLEDGMENT

The authors thank D. Boykin for statistical advice.

LITERATURE CITED

Apte, P.V., and M.M. Laloraya. 1982. Inhibitory action of phenolic compounds on abscisic acid-induced abscission. J. Exp. Bot. 33:826–830.

Bi, J.L., J.B. Murphy, and G.W. Felton. 1997. Does salicylic acid act as a signal in cotton for induced resistance to *Heliocoverpa zea*? J. Chem. Ecol. 23:1805-1818.

Cleland, C.F., and A. Ajami. 1974. Identification of the flower-inducing factor isolated from aphid honeydew as being salicylic acid. Plant Physiol. 54:904–906.

Datta, K.S., and K.K. Nanda. 1985. Effect of some phenolic compounds and gibberellic acid on growth and development of cheena millet (*Panicum miliaceum* L.). Indian J. Plant Physiol. 28:298–302.

Delaney, T.P., S. Uknes, B. Vernooij, L. Friedrich, K. Weymann, D. Negrotto, T. Gaffney, M. Gut-Rella, H. Kessmann, E. Ward, and J. Ryals. 1994. A central role of salicylic acid in plant disease resistance. Science 266:1247–1250.

Groenewald, E.G., and J.H. Visser. 1978. The effect of arachidonic acid, prostaglandin and inhibitors of prostaglandin synthetase, on the flowering of excised *Pharbitis nil* shoot apices under different photoperiods. Z. Pflanzenphysiol. 88:423–429.

Hedin, P.A., B. Tang, and R.G. Creech. 1995. Effect of bioregulators on development and reproduction of root-knot nematode in cotton plant roots. Miss. Agric. Forest. Exp. Stn. Bulletin 1028, 6 p.

Heitholt, J.J. 1993. Cotton boll retention and its relationship to lint yield. Crop Sci. 33:486–490.

Heitholt, J.J., and W.R. Meredith, Jr. 1998. Yield, flowering, and leaf area index of okra-leaf and normal-leaf cotton isolines. Crop Sci. 38:643–648.

Kharana, J.P., and C.F. Cleland. 1992. Role of salicylic acid and benzoic acid in flowering of a photoperiod-insensitive strain of *Lemna paucicostata* LP6. Plant Physiol. 100:1541–1546.

Khoshkoo, N., P.A. Hedin, and J.C. McCarty, Jr. 1993. Effects of bioregulators on the terpenoid aldehydes in root-knot nematode infected cotton plants. J. Agric. Food Chem. 41:2442–2446.

Klessig, D.F., and J. Malamy. 1994. The salicylic acid signal in plants. Plant Mol. Biol. 26:1439–1458.

Martinez, C., J.C. Baccou, E. Bresson, Y. Baissac, J.F. Daniel, A. Jalloul, J.L. Montillet, J.P. Geiger, K. Assigbetse, and M. Nicole. 2000. Salicylic acid mediated by the oxidative burst is a key molecule in local and systemic responses of cotton challenged by an avirulent race of *Xanthomonas campestris* pv *malvacearum*. Plant Physiol. 122:757–766.

Metraux, J.P., and I. Raskin. 1993. Role of phenolics in plant disease resistance. Pages 191–209 *in* I. Chet ed. Biotechnology in plant disease control. Wiley-Liss, Inc.

Patil, S.M., and A.D. Wele. 1992. Yield of cotton as influenced by antitranspirants and land surface modification. Punjabrao Krishi Vidyapecth Res. J. 16:265–266.

Raskin, I. 1992. Role of salicylic acid in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:439–463.

Ryals, J.A., U.H. Neuenschwander, M.G. Willits, A. Molina, H.-Y. Steiner, and M.D. Hunt. 1996. Systemic acquired resistance. Plant Cell 8:1809–1819.

Singh, G., and M. Kaur. 1980. Effect of growth regulators on podding and yield of mung bean (*Vigna radiata* L. Wilczek). Indian J. Plant Physiol. 23:366–370.

Sticher, L, B. Mauch-Mani, and J.P. Metraux. 1997. Systemic acquired resistance. Annu. Rev. Phytopathol. 35:235-270.

Wantanabe, K., and A. Takimoto. 1979. Flower-inducing effects of benzoic acid and some related compounds in *Lemna paucicostata* 151. Plant Cell Physiol. 20:847–850.