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Neutralizers and Pesticides and their Sequence of Application to Acid-delinted Cottonseed: Effects on Germination, Stand, and Verticillium Wilt of Cotton[1]

Earl B. Minton[2]

ABSTRACT

Neutralizing acid residues on cottonseed after HCl delinting contributed to seedling emergence and survival in field plantings and emergence in greenhouse plantings, but not necessarily to survival in the greenhouse where seedling diseases were severe or to germination in the laboratory. Similar results were obtained with an experimental neutralizer (EN) and the standard neutralizer (NH_3) used alone or in combination with fungicides and the insecticide, disulfoton. The percentage of plants with foliar symptoms of Verticillium wilt was reduced by the neutralizers and pesticides. Decrease in wilt symptoms was related to increased plant populations. Percentage germination, emergence and survival of seedlings and Verticillium wilt were similar when EN and pesticides were applied simultaneously or sequentially. Use of EN can eliminate ammonia and ammonium chloride pollutants currently associated with acid delinting of cottonseed.

***Additional index words:* *Gossypium hirsutum* L., Pollution, Ammonia, Ammonium chloride, Acid delinting, Fungicide, Insecticide.**

BARRE (2) found that delinting seed of cotton with sulfuric acid reduced seedling losses from external infestation with *Glomerella gossypii* Edg. Today, commercial seed processing plants use either sulfuric acid or gaseous, anhydrous hydrochloric acid (3, 4, 5, 6, 9) to delint cottonseed. After delinting, the acid residues on seed must be neutralized to obtain consistent performance from fungicides subsequently applied to the seed and to prevent deterioration of bags during storage. Anhydrous ammonia (NH_3) is applied in a closed chamber as a separate operation to neutralize acid residues on delinted cottonseed, but this process produces the pollutants ammonia and ammonium chloride. A neutralizer that avoids the production of pollutants is urgently needed in order to improve the environment of seed processing plant. Also, simultaneous application of neutralizer and pesticides should reduce processing time and eliminate some equipment. A saving for the processors and the farmers could thus be realized. The objective of these investigations was to compare the performance of HCl-delinted cottonseed treated with two neutralizers, three fungicides, and one insecticide with two sequences of application of one neutralizer and the pesticides.

MATERIAL AND METHODS

Processing of Seed. Seed of *Gossypium hirsutum* L., 'Stripper 32' and 'Tamcot SP37', were delinted with gaseous, anhydrous HCl and then subjected to various cleaning, neutralization, and pesticide treatments in two separate commercial seed processing facilities as shown in Fig. 1. The experimental neutralizer (EN), which was developed by Cargill, Inc.,[3] contains organic and inorganic buffers and was applied at 1.5 g/kg of seed. The

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[2] Plant pathologist, Cotton Research Laboratory, FR-SEA-USDA in cooperation with the Texas Agric. Exp. Stn., Lubbock.

[3] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable. The chemical composition of EN has not been released because of pending patent rights.

standard neutralizer, anhydrous ammonia (NH_3), was applied at 2.5 g/kg of seed.

The following pesticides and rates were used:

Seed protectant fungicide: Captan, 75% wettable powder, 29.5% flowable: *N*-{(trichloromethyl)thio}-4-cyclohexene-1,2-dicarboximide, 1.25 g/kg of seed.

Thiram, 29.5% flowable: bis(dimethylthiocarbamoyl) disulfide, 1.25 g/kg of seed.

Systemic fungicide: Carboxin, 29.5% flowable: 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide, 2.5 g/kg of seed.

Systemic insecticide: Disulfoton, 95% oil base: *0,0*-diethyl *S*-{2-(ethylthio)ethyl} phosphorodithioate, 5.2 ml/kg of seed.

Seed from each treatment were placed in plastic bags for laboratory, greenhouse, and field evaluations.

Germination in the Laboratory. Standard procedures were used to germinate the seed at alternating 20 to 30 C and constant 18 C regimes (1). The seeds were germinated within 2 months after they were treated. About 3 weeks after the first germination test, the test was repeated. Treatments were randomized in complete blocks with 12 replications.

Greenhouse Evaluation of Seed. Seed were planted in Amarillo loam soil in the greenhouse 4 months after treatment. The soil was naturally infested with seedling disease organisms, primarily *Rhizoctonia solani* Kühn and *Fusarium* spp. The soil had been used for seedling disease evaluation during the last 3 years. The inoculum density was maintained by adding autoclaved, acid-delinted cottonseed (not treated with pesticides) to the soil every 6 months.

Treatments were randomized in complete blocks with six replications. Forty seeds were planted per plot and covered with 3.8 cm of soil. Healthy seedlings (those with no apparent aboveground disease symptoms) and diseased seedlings were counted at 2 to 3-day intervals during the period of emergence for about 30 days after planting. Diseased specimens were washed in running tap water for 20 min and then plated on 1.5% water agar to identify the organisms present. Greenhouse temperature ranged from 16 to 33 C.

Field Evaluation of Seed. Seed were planted in Amarillo loam soil 8 months after treatment. Herbicide had been applied to the soil for 6 years, but cotton had been grown for 3 consecutive years. A four-row cone planter equipped with seed-press wheels was used to space the seeds uniformly and cover them with 5 cm of soil. A plot was one row, on 1 m centers and 5.5 m long, and 100 seed were planted to each. Treatments were randomized in complete blocks with six replications. Stand counts were made 14 and 30 days after planting. Plants with foliar symptoms of Verticillium wilt were counted 15 and 31 August, and 1 and 15 September.

Analysis of Data. All data were subjected to analysis of variance and Duncan's multiple range test.

RESULTS AND DISCUSSION

Laboratory Germination

Stripper 32. Percentage of germination at 20 to 30 C and 18 C differed among the treatments at 5 and 7 days, respectively, but not at 12 days (Table 1). Germination was similar for comparable pesticide treatments applied after the use of each neutralizer and between the two sequences of application of EN and the pesticides. The percentage of nub-root seedlings under the two temperature regimes indicated that seed quality was medium. The percentage of nub-root seedlings is directly related to reduced quality of the seed, and values from 4 to 8% are obtained with medium quality seed. Germination and seedling survival may be reduced by coating seed with disulfoton (7, 8, 10, 11), but this insecticide did not affect germination of Stripper 32 seed.

Percentage of seed germination was about 2% lower under each temperature regime for the second test in comparison with the first test conducted about 3 weeks earlier. These small but significant ($P < 0.05$) differences in germination probably were not caused by the treatments or by deterioration of the seed, since a high percentage of the seed germinated and the seedlings subsequently emerged in greenhouse and field plantings several months later, regardless of treatment.

Tamcot SP37. Percentage of germination at 5, 7, and 12 days differed among the treatments at both temperature regimes (Table 1). The highest germination was obtained with the nonneutralized seed. Germination was similar for comparable pesticide

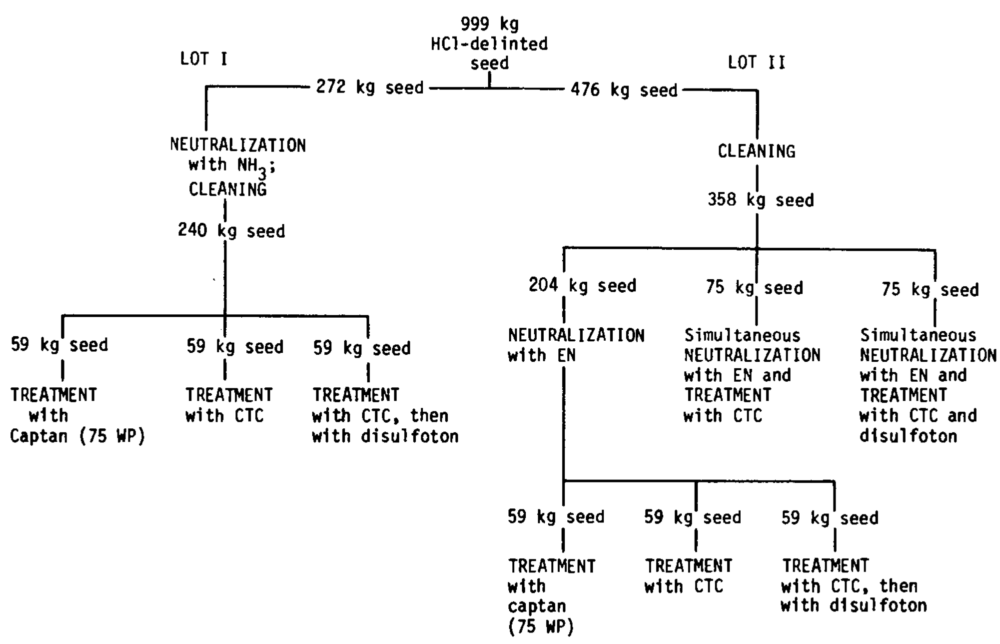

Fig. 1. Flow chart for cleaning, neutralization, and pesticide treatment of cottonseed after acid delinting. NH_3 = anhydrous ammonia at 2.5 g/kg; EN = experimental neutralizer at 1.5 g/kg; CTC = Captan 29.5% F at 1.25 g/kg; Thiram 29.5% F at 1.25 g/kg; Carboxin 29.5% F at 2.5 g/kg; Captan 75 WP at 1.25 g/kg; Disulfoton 96% oil base at 5.2 ml/kg.

treatments applied after use of each neutralizer and between the two sequences of application of the EN and the pesticides. The low percentage of nub-root seedlings indicated that seed quality was in the acceptable range.

Both Cultivars. The average percentage of germination within each temperature regime for seed of cultivars with different levels of seed quality indicated that neutralization of residual HCl after delinting of cottonseed is not necessary. However, it is known that acid residues must be neutralized to prevent deterioration of bags during storage of acid-delinted seed. The slightly higher percentage of nub-root seedlings obtained with each treatment within cultivars germinated at 18 C than at 20 to 30 C was expected. This small difference did not indicate any adverse effects of the neutralizers or pesticides on seed quality.

Greenhouse Evaluation

Stripper 32. Emergence and survival of seedlings differed significantly among the treatments, but were similar for comparable pesticide treatments applied after use of each neutralizer (Table 1). Combination of fungicides gave higher seedling emergence, but not necessarily survival than Captan 75 WP used alone. Seedling emergence was similar after simultaneous application of the pesticides and the EN and after split applications of the same chemicals.

Tamcot SP37. Emergence and survival of seedlings differed significantly among the treatments (Table 1). Results were similar for comparable pesticide treatments applied after use of each neutralizer, except for higher survival for NH_3 + Captan-Thiram-Carboxin than for EN + Captan-Thiram-Carboxin. Seedling emergence was higher for the treatment Captan-Thiram-Carboxin used alone or in combination with Disulfoton than for Captan 75 WP, neutralized only, or none (check). However, this relationship was not always evident for seedling survival following severe postemergence damping-off.

Both Cultivars. Contrary to germination performance, the greenhouse data indicated best survival was obtained when neutralization was accompanied or followed by treatment with fungicides or fungicides and an insecticide. The low emergence and survival for seedlings of both cultivars were caused by seedling diseases. *R. solani* was the organism most frequently cultured from diseased seedlings. *Fusarium* spp. were found less frequently. The rates of fungicides used including the combinations were too low to protect the seedlings from the severe seedling disease.

Field Evaluation

Stripper 32. Although seedling survival was similarly high among all fungicide treatments, stands were higher for seed coated with fungicides than for seed only neutralized or nonneutralized seed (Table 2). Most of the benefit from the fungicide treatments was in the control of pre-emergence damping-off, since only a few seedlings even from seed that were not coated with fungicides were killed by postemergence damping-off.

Table 1. Effects of acid neutralizers, pesticides, and sequence of application on germination of cottonseed and emergence and survival of cotton seedlings in the greenhouse soil beds, 1976.

	Germination in the laboratory						Greenhouse tests	
	Constant 18 C			Alternating 20–30 C			Seedlings‡	
		After 12 days			After 12 days			
Treatment†	After 5 days	Total	Nub-root	After 7 days	Total	Nub-root	Emergence	Survival
	%							
				Stripper 32				
None (check)	73 a*	73 a	6 a	59 abc	80 a	4 a	38 d	9 bc
NH_3 (neutralizer)	66 c	70 a	6 a	52 d	75 a	5 a	32 d	13 abc
NH_3 + captan 75 WP	70 abc	73 a	6 a	59 abc	75 a	6 a	48 bcd	15 abc
NH_3 + captan-thiram-carboxin	66 c	74 a	7 a	58 abc	77 a	5 a	64 ab	24 a
NH_3 + captan-thiram-carboxin + disulfoton	68 abc	74 a	6 a	60 abc	78 a	6 a	61 ab	15 abc
Experimental neutralizer (EN)	71 ab	73 a	6 a	58 abcd	78 a	4 a	39 cd	6 c
EN + captan 75 WP	66 c	73 a	6 a	57 bcd	77 a	5 a	41 cd	11 bc
EN + captan-thiram-carboxin	70 abc	72 a	7 a	55 cd	80 a	5 a	48 bcd	16 abc
EN + captan-thiram-carboxin + disulfoton	66 c	76 a	6 a	64 a	74 a	6 a	48 bcd	10 bc
EN-captan-thiram-carboxin	66 c	76 a	6 a	60 abc	76 a	6 a	56 abc	22 ab
EN-captan-thiram-carboxin-disulfoton	67 bc	75 a	7 a	63 ab	77 a	5 a	67 a	25 a
				Tamcot SP37				
None (check)	87 a	91 a	2 c	87 a	91 a	1 a	44 d	29 ab
NH_3 (neutralizer)	78 b	85 b	3 b	83 abc	88 ab	1 a	37 de	16 cde
NH_3 + captan 75 WP	72 cde	79 cd	3 b	84 ab	88 ab	2 a	52 cd	14 e
NH_3 + captan-thiram-carboxin	72 cde	80 bcd	3 b	80 bcd	84 bc	1 a	71 ab	40 a
NH_3 + captan-thiram-carboxin + disulfoton	78 b	84 b	2 c	79 bcd	85 bc	2 a	75 a	26 bcde
EN	74 bcd	82 bcd	3 b	74 e	80 c	2 a	33 e	15 de
EN + captan 75 WP	69 de	77 d	3 b	75 de	83 bc	2 a	49 cd	14 e
EN + captan-thiram-carboxin	67 e	78 cd	4 a	77 de	83 bc	2 a	66 ab	24 bcde
EN + captan-thiram-carboxin + disulfoton	72 cde	81 bcd	2 c	77 de	84 bc	2 a	76 a	27 bcd
EN-captan-thiram-carboxin	71 de	79 cd	3 b	76 de	84 bc	2 a	61 bc	28 abc
EN-captan-thiram-carboxin-disulfoton	76 bc	83 bc	3 b	78 cde	84 bc	2 a	73 a	26 bcde

* For each cultivar and within each column, values followed by a letter in common are not significantly different at the 0.05 level of probability, as determined by Duncan's multiple range test. † A dash (-) between chemicals indicates that they were mixed and applied together; a plus (+) between chemicals indicates that they were applied sequentially. Further details of treatments are shown in the text and Fig. 1.

‡ Emergence and survival at 15 and 30 days, respectively, after planting.

Lower percentage of Verticillium wilt occurred 31 August for some fungicide treatments than for others or no fungicides (Table 2). These trends occurred on the other dates, but the differences were not significant.

Tamcot SP37. Seedling survival was higher for neutralized than for nonneutralized seed (Table 2). Stands were further increased by the use of pesticides, but results were similar for comparable treatments applied after use of each neutralizer except for higher stands for captan when applied after NH_3 than when applied after EN. The sequence of application of the EN and the pesticides did not significantly affect seedling survival.

Leaf symptoms of Verticillium wilt were delayed and reduced in plants from neutralized seed in comparison with plants from nonneutralized seed (Table 2). The prevalence of wilt was further reduced, although not always significantly, by the application of pesticides. Wilt percentages were similar for comparable pesticide treatments applied after use of each neutralizer.

Both Cultivars. Hydrochloric acid-delinted cottonseed should be neutralized and subsequently treated with fungicides for high percentage of emergence and survival of seedlings in field plantings. Stands were similar for pesticide treatments when applied after use of each neutralizer, but were generally higher for combination of fungicides than for only Captan 75 WP.

The percentage of plants with foliar symptoms of Verticillium wilt was lower for neutralized than for nonneutralized seed. Further reduction in disease expression was generally obtained by coating the seeds with fungicides. Decrease in Verticillium wilt was related to increased plant populations, which is well known, and perhaps by the fungicides per se as we have found in other studies (unpublished data).

CONCLUSIONS

Control of pre-emergence and postemergence damping-off and Verticillium wilt is important in cotton production. Reducing losses from these diseases can be accomplished by planting properly processed and treated seed. Residues on cottonseed after delinting with gaseous anhydrous HCl should be neutralized to enhance maximum survival of seedlings and minimum prevalence of Verticillium wilt in field plantings. The EN and NH_3 neutralizers effectively increased seedling survival and performed similarly with comparable pesticide treatments. Simultaneous application of EN and pesticides resulted in seedling survival equal to or higher than, and wilt prevalence equal to or lower than those from split applications of the same chemicals. Thus, EN and pesticides may be mixed and applied together. This procedure would eliminate one step and some equipment from the procedure currently used for treating acid-delinted cottonseed.

Use of EN can eliminate the pollutants (ammonia and ammonium chloride) associated with acid delinting of cottonseed. The EN remains on the seed indefinitely and inactivates residual acid on the seed coat. This residual action should improve the performance of seed in field plantings and reduce the breakdown of the bags during storage. There are no known adverse effects or pollutants produced from use of EN.

Table 2. Effects of acid neutralizers, pesticides, and sequence of application on survival of cotton seedlings and prevalence of Verticillium wilt in the field, 1977.

	Seedling survival‡		Plants with Verticillium wilt		
Treatment†	16 May	31 May	15 Aug.	31 Aug.	15 Sept.
			Stripper 32		
None (check)	45 e*	46 c	26 a	36 a	43 a
NH_3 (neutralizer)	57 bcd	54 bc	15 a	28 abcd	36 a
NH_3 + captan 75 WP	62 abc	61 ab	20 a	28 abcd	40 a
NH_3 + captan-thiram-carboxin	65 ab	60 ab	22 a	27 bcd	32 a
NH_3 + captan-thiram-carboxin + disulfoton	68 a	68 a	16 a	25 cd	31 a
Experimental neutralizer (EN)	51 de	47 c	25 a	34 ab	37 a
EN + captan 75 WP	62 abc	62 ab	18 a	25 cd	32 a
EN + captan-thiram-carboxin	71 a	68 a	16 a	26 bcd	34 a
EN + captan-thiram-carboxin + disulfoton	56 cd	54 bc	21 a	33 abc	42 a
EN-captan-thiram-carboxin	65 ab	68 a	18 a	24 d	32 a
EN-captan-thiram-carboxin-disulfoton	62 abc	59 ab	16 a	27 bcd	33 a
			Tamcot SP37		
None (check)	41 e	40 e	41 a	64 a	73 a
NH_3 (neutralizer)	60 cd	59 bc	33 abc	45 bc	47 cd
NH_3 + captan 75 WP	70 a	70 a	25 bc	41 bc	48 bcd
NH_3 + captan-thiram-carboxin	66 abc	67 a	22 c	38 c	42 d
NH_3 + captan-thiram-carboxin + disulfoton	69 ab	67 a	32 abc	46 bc	52 bcd
EN	54 d	51 d	32 abc	49 b	61 b
EN + captan 75 WP	59 cd	57 cd	34 ab	49 b	59 bc
EN + captan-thiram-carboxin	62 bc	64 ab	28 bc	44 bc	52 bcd
EN + captan-thiram-carboxin + disulfoton	71 a	68 a	28 bc	44 bc	50 bcd
EN-captan-thiram-carboxin	64 abc	64 ab	30 abc	41 bc	54 bcd
EN-captan-thiram-carboxin-disulfoton	65 abc	68 a	27 bc	39 c	46 cd

* For each cultivar and within each column, values followed by a letter in common are not significantly different at the 0.05 level of probability, as determined by Duncan's multiple range test. † A dash (-) between chemicals indicates that they were mixed and applied together; a plus (+) between chemicals indicates that they were applied sequentially. Further details of treatments are shown in the text and Fig. 1.

‡ Based on number of seeds planted 28 April.

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