Effect of Norflurazon (Zorial Rapid 80®) Mixed With Pendimethalin (Prowl®) and Prometryn (Caparol®) on Cotton Stand Establishment and Yield

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Abstract

The effect on cotton stand establishment and seed cotton yield of various rates of norflurazon applied in combination with pendimethalin or both pendimethalin and prometryn was determined in field studies conducted at the Maricopa Agricultural Center in 1994 and 1995 in a sandy loam soil. Cotton stand counts were highest when only pendimethalin was applied or when no herbicide was used. Tank mixing prometryn with pendimethalin did not significantly reduce plant populations. Tank mixing increasing amounts of norflurazon with pendimethalin resulted in decreasing plant populations in both the wet and the dry plant experiments. Tank mixing increasing rates of norflurazon with both pendimethalin and prometryn caused a similar decline in plant populations in both the wet and the dry plant experiments. The symptoms of dying cotton seedlings and the stand count data indicated that norflurazon was the component of the tank mixtures that caused seedling mortality. The effect of the herbicide treatments on seed cotton yields was much less than on stand counts, but the same trends discussed above were evident. However, at the label rate for norflurazon in coarse textured soils, 0.5 lb a.i./A, seed cotton yields were not significantly reduced. The smaller effect of the herbicide treatments on seed cotton yields was due to the bush type nature of DPL 5415 and increased growth of surviving plants when plant populations were reduced. The data indicates that yield losses were not significant unless plant populations were reduced below about 20,000 to 25,000 plants/A.

Introduction

In recent years, low rates of norflurazon (0.5 to 0.75 lb a.i./A) have been tank mixed with traditionally used herbicides, primarily pendimethalin or trifluralin, for suppression of purple and yellow nutsedge (*Cyperus rotundus* and *Cyperus esculentus*, respectively) and for control of annual morningglory (*Ipomoea*) species. Occasionally a three component herbicide tank mix including a dinitroaniline herbicide (pendimethalin or trifluralin), prometryn, and norflurazon has been applied preplant-incorporated for control of annual morningglory species. Norflurazon has long been registered for cotton in the Southeastern U.S., but was not registered for use in Arizona prior to the 1993 cotton season primarily because of concerns about crop injury on coarse textured soils. Coarse textured soils containing 0.5 to 1 percent organic matter and with low adsorptive capacity for herbicide molecules are common in Arizona cotton production areas. Although cotton has some tolerance to norflurazon, preplant-incorporated applications of norflurazon to these coarse textured soils at rates used in Southeastern U.S. cotton production areas (1 to 2 lb a.i./A) cause unacceptable injury and stand loss. The injury occurs because norflurazon is readily available for uptake by cotton seedlings from the soil water solution due to the low adsorptive capacity of coarse textured soils in Arizona.

The objective of this study was to investigate the effects of various rates of norflurazon (0.5 to 1.25 lb a.i./A) on cotton stand establishment and seed cotton yields when mixed with pendimethalin or both pendimethalin and prometryn.

Materials and Methods

Field studies were conducted at the University of Arizona, Maricopa Agricultural Center (MAC) in 1994 and 1995. The coarse textured soil in the study field was a Casa Grande sandy loam, which contained 0.7 percent organic matter, 65 percent sand, 20 percent silt, and 15 percent clay. The experimental design was a randomized complete block design with 6 blocks in which individual plots were four 40 inch rows by either 90 (1994) or 40 (1995) feet long. Herbicide treatments were applied to the same plots each year of the study. The effects of the herbicide combinations were examined using both a plant to moisture strategy (wet planting), and a plant dry and irrigate up strategy (dry planting). Preemergence herbicide applications [see data tables for combinations and rates in lb. of active ingredient (a.i.) per acre] were made to flat ground on April 6, 1994 and March 24, 1995, and incorporated using a tandem finishing disk to a depth of about two inches. The wet plant experiments were established by listing to form a large peaked bed and pre-irrigating the field to fill the soil profile with moisture. The field was then mulched and planted to moisture with Delta and Pine Land (DPL) cotton variety 5415, an upland cotton variety, on April 20, 1994 and April 10, 1995. The dry soil mulch placed over the seed row by the planting operation to conserve soil moisture was removed 4 or 5 days after planting to facilitate seedling emergence. The dry plant experiments were established by listing, mulching with a bed shaper, and planting DPL 5415 about 0.5 inches deep. The dry plant experiments were planted on the same day as the wet plant experiments, and irrigated on the day of planting or the following day. In 1995, the dry plant experiment was irrigated on April 11, 1995. The maximum, minimum, and average air temperatures, as well as, the average soil temperature at the 2 inch depth for the 14 day period after planting in 1995 were as listed in Table 1. A period of cold weather following planting in 1995 interacted with the herbicide treatments to increase seedling mortality compared to the 1994 dry plant experiment. Due to the extensive stand loss, the dry plant experiment was destroyed by disking, the beds were reshaped, replanted, and irrigated on May 31, 1995. No herbicides were applied to the plots following the initial preplant-incorporated herbicide applications each year. The experiments were hand weeded several times during the season. Early season stand counts were made in late May, June or early July depending on the experiment and year. In 1994, the percent canopy present in the center two rows of each plot was measured on July 11, 1994 with 100 percent representing a crop canopy with no gaps and 0 percent indicating no crop canopy. Each percentage point equaled 1.8 feet of crop row so that a rating of 99 percent indicated that a canopy gap of 1.8 feet was present in the center 2 rows of a 90 foot plot. The cotton plants were about 24 inches tall at the time of the canopy measurements. The center two rows of the four row plots were machine harvested on October 3, 1994 and November 14, 1995. Analysis of variance in combination orthogonal contrasts were used to determine if linear trends existed in the data and to elucidate differences between treatments. In addition, where appropriate, differences between treatments were evaluated using Duncan's multiple range test.

Results and Discussion

Cotton stand counts were highest when pendimethalin was used alone (treatment 1 in 1994 and 1995) or when no preplant-incorporated herbicide was applied (treatment 11 in 1995) in both the wet plant and the dry plant experiments in both years of the study (Table 2 and 3). These two treatments were the standards against which other treatments were compared. Tank mixing prometryn with pendimethalin (treatment 6) did not significantly reduce plant populations compared to the standard treatments in either the wet plant or the dry plant experiments in either year of the study (Table 2 and 3). In the 1994 wet plant experiment, tank mixing increasing amounts of norflurazon with pendimethalin (treatments 1 to 5) or with both pendimethalin and prometryn (treatments 6 to 10) resulted in decreasing plant populations (Table 2). The decline in plant populations with increasing rates of norflurazon was a significant linear trend in both the two component herbicide mixture (plant popln. = 33,201 - 13,229 x norflurazon lb a.i./A, $r = -0.738$, $p<0.001$), and in the three component herbicide mixture (plant popln. = 34,193 - 13,339 x norflurazon lb a.i./A, $r = -0.770$, $p<0.001$). There was no significant difference in the level (i.e., intercept) or slope of the two regression equations. Similar significant linear declines in plant populations with increasing amounts of norflurazon also occurred in the 1994 dry plant experiments (Table 2). In the dry plant experiment, the regression equation for the two component mixture (plant popln. = 35,327 - 12,380 x norflurazon lb a.i./A, r = -0.676, p<0.001) had a significantly higher level or intercept (p=0.026) than the regression equation for the three component mixture (plant popln. = 34044 - 14025 x norflurazon lb a.i./A, r = -0.739, p<0.001). However, given the overall similarity of the regression equations, the 1994 experiments can be generalized by calculating the average slope of the regression equations, -13243 plants/lb a.i., and determining that an increase in norflurazon rate of 0.10 lb a.i./A resulted in an average decrease of 1,324 plants/A. The 1994 plant population data and the symptomatology of the cotton seedlings indicated that norflurazon was the component of the herbicide mixes that caused cotton seedling mortality.

The decrease in plant populations in 1994 caused by increasing norflurazon rates resulted in reduced canopy development in the cotton seed row (Table 2). In the wet plant experiment, the decline in canopy development with increasing rates of norflurazon was a significant linear trend in both the two component mixture (% canopy = 98.0 - 19.8 x norflurazon lb a.i./A, r = -0.576, p=0.001), and in the three component mixture (% canopy = 98.3 - 17.8 x norflurazon lb a.i./A, r = -0.672, p<0.001). There was no significant difference in the level (i.e., intercept) or slope of the two regression equations. Similar significant linear declines in canopy development with increasing amounts of norflurazon also occurred in the 1994 dry plant experiment where the regression equation for the two component mixture (% canopy = 97.6 - 15.5 x norflurazon lb a.i./A, r = -0.514, p=0.004) had a significantly smaller slope (p=0.021) than the regression equation for the three component mixture (% canopy = 104.5 - 33.1 x norflurazon lb a.i./A, r = -0.756, p<0.001). The reductions in canopy development as increasing amounts of norflurazon were applied were correlated with the previously discussed declines in plant populations. The correlation coefficients between plant populations and percent canopy development (Table 2) for the wet plant-two component mixes (treatments 1 to 5), wet plant-three component mixes (treatments 6 to 10), dry plant two-component mixes (treatments 1 to 5), and dry plant three-component mixes (treatments 6 to 10) were r=0.781 (p<0.001), r=0.796 (p<0.001), r=0.900 (p<0.001), and r=0.880 (p<0.001), respectively.

Seed cotton yields were also affected by the amount of norflurazon applied preplant incorporated in 1994 (Table 4). In the wet plant experiment, orthogonal contrasts indicated that there were no significant differences in seed cotton yields between treatments that included 0.5 lb a.i./A of norflurazon and treatments that did not include norflurazon (Table 4). Note that the label rate for norflurazon in coarse textured soils is 0.5 lb/A. Similar results were obtained in the dry plant study in 1994. However, there was a significant linear decrease in seed cotton yield as the amount of norflurazon applied increased above 0.5 lb a.i./A in the wet plant-two component mixtures (yield = 3893 - 689 x norflurazon lb a.i./A, r = -0.439, p=0.015), in the dry plant-two component mixtures (yield = 3788 - 652 x norflurazon lb a.i./A, r = -0.557, p=0.001), and in the dry plant-three component mixtures (yield = 4052 - 1101 x norflurazon lb a.i./A, r = -0.730, p<0.001) (Table 4). The trend of declining yield with increasing amounts of norflurazon was not significant at p=0.05 in the wet plant-three component mixtures (yield = 3876 - 418 x norflurazon lb a.i./A, r = -0.349, p=0.059). The changes in seed cotton yields as increasing amounts of norflurazon were applied (Table 4) were correlated with reductions in canopy development (Table 2). The correlation coefficients between percent canopy development and seed cotton yields for the wet plant-two component mixtures (treatments 1 to 5), wet plant-three component mixtures (treatments 6 to 10), dry plant two-component mixtures (treatments 1 to 5), and dry plant three-component mixtures (treatments 6 to 10) were r=0.828 (p<0.001), r=0.614 (p<0.001), r=0.894 (p<0.001), and r=0.789 (p<0.001), respectively.

Increasing amounts of norflurazon applied preplant-incorporated resulted in decreases in plant populations in the 1994 experiments. The planting densities in 1994 were relatively low and the reductions in plant populations resulted in gaps in the cotton canopy as indicated by the correlation between plant populations and percentage of canopy. These gaps tended to vary in size (i.e., length of seed row without cotton plants), with the gaps becoming more numerous and larger as the rate of norflurazon increased. Due to the bush-type growth habit of DPL 5415, small canopy gaps were closed early in the season by the growth of plants adjacent to the gaps. However, larger gaps in the canopy that filled in later in the season or remained open until the end of the season resulted in the seed cotton yield reductions described above. In biological systems, increasing amounts of a chemical may cause a gradual increase or decrease in a measured response, or the chemical may have no effect on a measured response until a threshold amount is exceeded after which greater amounts of chemical cause a change in the response being measured. Increasing amounts of norflurazon appeared to cause the former type of response with respect to plant populations in that plant populations declined linearly as increasing amounts of norflurazon were applied. In contrast, increasing amounts of norflurazon caused a threshold type of response with respect to yield in that seed cotton yields did not begin to decline until the rate of norflurazon exceeded 0.5 lb a.i./A. Cotton plants exhibit a plastic response to plant density. Plants in areas of low density (i.e., low numbers of plants) grow larger than in areas of high plant densities. Thus, over a wide range of plant populations, 25,000 to 45,000 plants/A, yield changes relatively little with changes in plant populations because this plastic growth response to density results in the effective use of available space and resources (Norton et. al., 1995). The data collected in this study suggest that yield losses begin to occur when plant populations decline below about 20,000 to 25,000 plants/A (Table 2) because the gaps in the canopy become too large and exceed the plastic growth response of cotton. The data collected in this study is consistent with previous upland cotton plant population studies (Norton et. al., 1995; Silverthooth et. al., 1994).

The plant population data collected in the wet plant experiment and after the first planting of the dry plant experiment (Dry I) in 1995 were similar to the data collected in 1994 in that increasing amounts of norflurazon mixed with pendimethalin (treatments 1 to 5) or with both pendimethalin and prometryn (treatments 6 to 10) resulted in decreasing plant populations (Table 3). The reductions in plant populations were significant linear trends in the wet plant-two component mixtures (plant popln. = 52,151 - 23,813 x norflurazon lb a.i./A, $r = -0.745$, $p<0.001$), and in the three component mixtures (plant popln. = 46,702 - 13,088 x norflurazon lb a.i./A, $r = -0.445$, $p<0.029$). Significant linear declines in plant populations with increasing amounts of norflurazon also occurred in the dry plant two component mixture (plant popln. = 30,688 - 21,562 x norflurazon lb a.i./A, $r = -0.904$, $p<0.001$), and in the three component mixture (plant popln. = 27,773 - 18,759 x norflurazon lb a.i./A, $r = -0.740$, $p<0.001$). Because it was obvious there would be significant yield reductions due to the severe stand losses in the 1995 dry plant experiment, the experiment was replanted. There were no significant differences in stand counts between any of the treatments in the replanted dry plant experiment (Dry II) in 1995 (Table 1). This was probably because replanting occurred when environmental conditions were more favorable for cotton germination and establishment, and because the 68 day interval between replanting and application of the preplant-incorporated herbicides allowed some dissipation of the herbicides.

The weather conditions following planting in 1994 were generally favorable for cotton germination and seedling growth. The 24 h average soil temperatures at the 2 inch depth, indicative of temperatures in the zone of the germinating cotton seedlings in the wet plant experiment, were 81, 77, 71 F in the three days following planting. The irrigation water applied to dry planted cotton to germinate the seed cools the soil down considerably and increases the heat capacity of the soil so that the soil is slow to warm up in response to radiant energy. The irrigated beds typically cool to within a few degrees of the 24 h minimum air temperature. The 24 h minimum air temperatures following planting in 1994 were 59, 60, 60 F in the three days following planting. Thus, the soil temperatures in the dry planted beds for the critical first few days after planting were in a range that facilitates good cotton seed germination and seedling growth. In contrast to the weather conditions in 1994, conditions in 1995 were suboptimal during germination and early seedling growth of the cotton. The period of cold weather that occurred immediately following planting in 1995 (Table 1) caused much greater stand loss than occurred in the 1994 dry plant experiment. For example, in the dry plant experiment of 1995, the plant populations in the treatments that included 1.0 lb norflurazon/A were about 30 percent of the plant populations in the treatments that did not include norflurazon. In contrast, in the 1994 dry plant experiment, plant populations in the treatments that included 1.0 lb norflurazon/A were about 66 percent of the plant populations in the treatments that did not include norflurazon. The soil temperatures at level of the seed in the dry planted beds following irrigation, as judged by being within a few degrees of the 24 h minimum air temperature, were well below the optimum for cotton germination and seedling emergence (Table 1). The plant population reductions in the wet plant experiments were about the same in the 1994 and 1995 experiments probably because the soil temperatures at the depth of the cotton seeds remained considerably above the night time air temperatures due to the thermal insulating properties of soil.

Similar to the results obtained in 1994, orthogonal contrasts indicated that there were no significant differences in seed cotton yields between treatments that included 0.5 lb a.i./A of norflurazon and treatments that did not include norflurazon in the 1995 wet plant and dry plant experiments (Table 4). There was a significant linear decrease in seed cotton yield as the amount of norflurazon applied increased above 0.5 lb a.i./A in the wet plant-two component mixtures (yield = 3600 - 642 x norflurazon lb a.i./A, $r = -0.3721$, $p=0.043$), but the trend of decreasing seed cotton yield with increasing amounts of norflurazon was not significant in the wet plant-three component mixtures (yield = 3411 - 116 x norflurazon lb a.i./A, $r = -0.0751$, $p>0.5$). There was considerably more variation in the 1995 wet plant data compared to the 1994 data because some of the variation in stand counts, canopy gaps, and yield was due to the cool weather conditions in 1995 (Table 1) and was not related to differences between treatments in rates of norflurazon. In the 1995 replanted dry plant experiment, there were no seed cotton yield differences between treatments related to norflurazon rates except that treatments 1 (pendimethalin alone) and 11 (no herbicide) had lower yields than most of the other treatments (Table 4). Due to the dissipation of herbicide concentrations in the soil during the 68 day interval between the application of the preplant-incorporated herbicides and replanting or lack of herbicide, poor weed control was obtained in these treatments. Although all treatments were hand weeded several times, it was still difficult to eliminate yield losses due to weed competition in treatments 1 and 11 because of the late planting and vigor of the weeds. The dissipation of the herbicides also reduced the effect of the norflurazon on stand counts (Table 3, Dry II data) and thus yield (Table 4). The replant data indicates that fields treated with norflurazon that suffer severe stand loss from chilling injury or other factors can be replanted (when weather conditions are favorable) and irrigated every other row to obtain a stand. In this scenario, the degree of stand loss in replanted fields caused by norflurazon will vary from none as in this experiment, up to the levels documented in this study, with the degree of stand loss depending primarily on the length of the time interval between application of preplant-incorporated herbicides and replanting, the rate of norflurazon used, and weather conditions at the time of replanting.

Conclusions

Several implications of the data collected in this study should be emphasized. The risk of cotton injury due to the use of norflurazon or other herbicides should be evaluated against the well documented yield benefits that result from good weed control. Once the decision is made to use norflurazon, growers should use production practices that optimize cotton germination and seedling growth. These practices include planting into a favorable weather forecast when soil temperatures will be adequate. The data collected in this study clearly indicate that the risk of herbicide injury from norflurazon is greatly increased by cold weather especially in fields that are dry planted. This cold weather effect also occurs with the injury of cotton roots by dinitroaniline herbicides such as pendimethalin (Prowl) and trifluralin (Treflan). In many situations, root injury caused by dinitroaniline herbicides is combined with seedling diseases that severely stunt the growth of cotton seedlings. The roots of these injured seedlings do not rapidly grow out of the zone of herbicide treated soil resulting in increased absorption of norflurazon and expression of norflurazon symptoms on the foliage. Norflurazon does not directly affect root growth of developing cotton seedlings. Thus, although norflurazon symptoms are present in the foliage, other important causes of cotton injury in these situations include cold temperature, seedling diseases, and dinitroaniline herbicide injury. Other factors under the control of growers are the selection of cotton variety and the amount of seed planted per acre. Varieties with good seedling vigor will be more resistant to injury and planting higher numbers of seeds per acre will compensate to some extend for the stand loss caused by norflurazon and other factors. Seeding rates should also be increased as environmental factors affecting cotton germination and seedling growth become less favorable. Lastly, if norflurazon is being used in a field with a severe weed problem, such as purple nutsedge, where the competitive effect of the weed will increase the stress imposed on developing cotton seedlings, it becomes even more important to plant during favorable weather conditions and to increase seeding rates.

References

Norton, E.R., J.C. Silvertooth, and S.W. Stedman. 1995. Plant population evaluation for upland cotton. Cotton, A College of Agriculture Report. University of Arizona, Series P-99: 25-28.

Silvertooth J.C., E.R. Norton, and S.W. Stedman. 1994. Plant population evaluation for upland cotton. Cotton, A College of Agriculture Report. University of Arizona, Series P-96: 24-25.

Table 1. Air and soil temperatures at the Maricopa Agricultural Center following planting on April 11, 1995.

Date	Air Temperature Maximum	Air Temperature Minimum	Air Temperature Average	Soil Temperature 2" Depth Average
	(°F)	(°F)	(°F)	(°F)
4/11	79	38	60	67
4/12	87	40	64	69
4/13	89	45	68	68
4/14	79	53	66	64
4/15	72	51	61	62
4/16	73	39	54	61
4/17	67	41	53	59
4/18	68	44	54	59
4/19	61	44	51	56
4/20	73	38	56	61
4/21	70	46	59	62
4/22	77	42	60	62
4/23	83	47	65	67
4/24	87	45	68	70

Table 2. Effect of preplant incorporated norflurazon, pendimethalin, and prometryn on cotton stand establishment and canopy development in a sandy loam soil in 1994.

Treatment	Rate	Wet Plant Stand Count	Wet Plant Canopy[a]	Dry Plant Stand Count	Dry Plant Canopy
	lb a.i./A	*plants/A*	%	*plants/A*	%
1. pendimethalin	0.62	34086	97	34545	93
2. pendimethalin norflurazon	0.62 0.5	25785	90	32900	95
3. pendimethalin norflurazon	0.62 0.75	22034	82	27382	89
4. pendimethalin norflurazon	0.62 1.0	20449	80	23886	85
5. pendimethalin norflurazon	0.62 1.25	17351	72	19590	72
6. pendimethalin prometryn	0.62 1.2	34195	96	32125	95
7. pendimethalin norflurazon prometryn	0.62 0.5 1.2	29173	92	29706	93
8. pendimethalin norflurazon prometryn	0.62 0.75 1.2	21090	83	24176	84
9. pendimethalin norflurazon prometryn	0.62 1.0 1.2	22094	85	20292	73
10. pendimethalin norflurazon prometryn	0.62 1.25 1.2	17727	72	14835	55

[a]Percentage of canopy with 100% representing a crop canopy with no gaps and 0% indicating no crop canopy. One percentage point equals 1.8 feet of canopy (or a gap of 1.8 feet) in the center 2 rows of a plot.

Table 3. Effect of preplant incorporated norflurazon, pendimethalin, and prometryn on cotton stand establishment in a sandy loam soil in 1995.

Treatment	Rate	1995		
		Wet	Dry I	Dry II[a]
	lb a.i./A		*plants/A*	
1. pendimethalin	0.62	50179 a[b]	28871 a	32416 ab
2. pendimethalin norflurazon	0.62 0.5	46718	24055	34146 ab
3. pendimethalin norflurazon	0.62 0.75	30117	13371	35852 ab
4. pendimethalin norflurazon	0.62 1.0	27128	8059	30952 b
5. pendimethalin norflurazon	0.62 1.25	23268	3618	36453 a
6. pendimethalin prometryn	0.62 1.2	49078 a	28677 a	31738 ab
7. pendimethalin norflurazon prometryn	0.62 0.5 1.2	40256	15924	36506 a
8. pendimethalin norflurazon prometryn	0.62 0.75 1.2	27189	15028	34981 ab
9. pendimethalin norflurazon prometryn	0.62 1.0 1.2	40837	9257	29693 b
11. no herbicide	-	49937 a	27237 a	31146 ab

[a]Dry I are data for the first planting whereas Dry II are data for the second planting (i.e. replanting).
[b]Means within columns followed by the same letter do not significantly differ (P=0.05, Duncan's multiple range test).

Table 4. Effect of preplant incorporated norflurazon, pendimethalin, and prometryn on seed cotton yield in a sandy loam soil.

Treatment	Rate	1994		1995	
		Wet	Dry	Wet	Dry II[a]
	lb a.i./A	*lb of seed+lint/A*			
1. pendimethalin	0.62	3818 a[c]	3592 a	3557 a	2227 de
2. pendimethalin norflurazon	0.62 0.5	3750 a	3663 a	3463 ab	2523 bcd
3. pendimethalin norflurazon	0.62 0.75	3200	3433	2965	2710 ab
4. pendimethalin norflurazon	0.62 1.0	3327	3241	2931	2691 ab
5. pendimethalin norflurazon	0.62 1.25	2959	2727	2837	2766 ab
6. pendimethalin prometryn	0.62 1.2	3911 a	3244 ab	3500 ab	2502 bcd
7. pendimethalin norflurazon prometryn	0.62 0.5 1.2	3782 a	3566 a	3430 ab	2674 ab
8. pendimethalin norflurazon prometryn	0.62 0.75 1.2	3215 a	3304	2818 ab	2612 abc
9. pendimethalin norflurazon prometryn	0.62 1.0 1.2	3636 a	3128	3637 a	2694 ab
10. pendimethalin norflurazon prometryn	0.62 1.25 1.2	3375 a	2465	-	-
11. no herbicide	-	-	-	3581 a	1925 e

[a]Dry II are data for the second planting (i.e., replanting).
[b]Means within columns followed by the same letter do not significantly differ (P=0.05, Duncan's multiple range test).