NUTSEDGE (*CYPERUS* SPP) CONTROL IN ALFALFA

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ABSTRACT

The competitive advantage of purple and yellow nutsedge in low desert alfalfa production areas of Southern California and Arizona is due to a combination of factors. These include frequent harvest and irrigation, high incident solar radiation, high summer temperatures that are supra-optimal for alfalfa growth, and the presence of C_4 photosynthesis and vegetative reproduction (e.g., tubers) in nutsedge species. Research in Arizona has shown that only three herbicides have the ability to suppress the growth of purple nutsedge in alfalfa in the low desert: Eptam (EPTC), Zorial (norflurazon) and glyphosate (Roundup, Touchdown and other trade names). Multiple Eptam granule applications can reduce the amount of nutsedge foliage in alfalfa hay and the density of purple nutsedge shoots (stem/ft^2) compared to untreated areas but nutsedge density in both treated and untreated areas increased during the first two years of the alfalfa stand. Zorial granules applied at 1 to 3 lb ai/A in a heavily infested alfalfa field (60 to 80% nutsedge cover) resulted in poor control (30 to 40% control) following a single spring application and 76 to 94% control in treatments receiving two applications per year (i.e., spring and summer) for 2 years. Zorial was not applied in the third year of the study but residual soil concentrations of norflurazon continued to provide substantial suppression of purple nutsedge. By August of the third year, the 2 lb ai/A per year and lower rate treatments did not keep purple nutsedge densities from rebounding to pretreatment levels while the 3 and 4 lb ai/A per year rate provided 51 and 75% suppression, respectively. Similar purple nutsedge suppression was observed in a second study where treatments were initiated in the spring following a fall alfalfa planting in a field with a lower initial density. In another study, glyphosate was applied in the summer and fall in a fallow field infested with purple nutsedge that mimicked the appearance of heavily infested, recently cut and irrigated fields in Parker Valley, AZ. Two to 4 glyphosate applications at 1.5 lb ae/A greatly reduced nutsedge tuber sprouting and shoot emergence in the following spring. Experiments to date suggest that reducing purple nutsedge tuber populations in alfalfa fields may be greatly facilitated by the commercial introduction of Roundup Ready alfalfa.

Key words: weed control, purple nutsedge, alfalfa, EPTC, Eptam, norflurazon, Zorial, glyphosate, Roundup, Touchdown

INTRODUCTION

Purple (*Cyperus rotundus*) and yellow nutsedge (*Cyperus esculentus*) are economically important perennial weeds with world-wide distributions that thrive in hot, moist conditions (Holm *et al.*, 1977) such as those found in southwestern alfalfa production areas. They are similar in their morphology, physiology and biology (Holm *et al.*, 1977; Wills, 1987) and both species utilize C_4 photosynthesis (Holt and Orcutt, 1991). In addition to utilizing CO_2 more

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efficiently, C_4 plants have higher temperature optimums for photosynthesis than C_3 plants such as alfalfa or cotton and photosynthesis becomes light saturated at much higher light intensities in C_4 plants than in C_3 plants (Radosevich et al., 1997; Wills, 1987). Purple nutsedge is more common than yellow nutsedge in low desert agricultural fields in Arizona (McCloskey et al., 1997). Purple nutsedge rarely reproduces by seed but reproduces extensively by rhizomes and tubers (Wills, 1987). Rhizomes that extend upward produce new shoots which in turn produce new rhizomes. Rhizomes that extend horizontally or downward produce tubers that can form other tubers resulting in chains of tubers or the formation of new shoots (Wills, 1987). Purple and yellow nutsedge tubers contain a large store of carbohydrates that can facilitate rapid growth following shoot emergence (Holt and Orcutt, 1991). The extensive underground biomass and vegetative reproduction of purple and yellow nutsedge make these species difficult to control in a frequently harvested perennial crop such as alfalfa. The lack of alfalfa vigor during hot summer months in the low desert caused by supra-optimal temperatures for alfalfa growth (Feltner and Massengale, 1965; Robison and Massengale, 1968) and resulting lack of crop competition compounds the difficulty in controlling these species.

Although many herbicides are registered for use in alfalfa, most of them (e.g., Balan, Buctril, Poast, Select, Trifluralin, or 2,4-DB) do not suppress purple or yellow nutsedge. Other herbicides such as Pursuit and Raptor do suppress nutsedges but are generally used in the fall after planting for weed control during alfalfa establishment. These imidazolinone herbicides are also limited to a single application per year in growing alfalfa and Pursuit may not be used during the last year of the stand life because of its long soil residual and potential to injure following crops. Herbicides with significant effect on purple and yellow nutsedge include Eptam (EPTC), Zorial (norflurazon) and glyphosate. Zorial has a long soil residual like Pursuit and the potential to injure following crops and is best used where cotton or alfalfa can be planted following a treated alfalfa crop. Eptam has less restrictive crop rotation limitations compared to Zorial due to its short soil residual and great volatility. Eptam can be applied to alfalfa multiple times in a growing season for nutsedge suppression. Tickes (1991) initiated an experiment in a one year old alfalfa field infested with purple nutsedge where Eptam 10G granules were applied five times per year after successive harvests during February to July in 1990 and 1991 at rates of 2, 3 and 4 lb ai/A per application. The treatments reduced the amount nutsedge foliage in the hay by 60 to 95% and there was little difference between the various rates. The Eptam treatments suppressed the density (stems/ft^2) of purple nutsedge 30 to 50% compared to untreated areas but the density of shoots increased steadily during the two years of repeated Eptam applications. Glyphosate has no soil residual effect but is toxic to alfalfa and can only be used on a limited basis as a spot treatment. The development of glyphosate tolerant alfalfa (i.e., Roundup Ready alfalfa) will allow repeated glyphosate applications in a growing season. Because of Eptam results discussed above and the development of glyphosate tolerant alfalfa, additional experiments were initiated to evaluate the efficacy of Zorial 5G granules and postemergence glyphosate applications for nutsedge suppression in alfalfa.

PROCEDURES

Zorial 1 Experiment. A field experiment with 4 replications and 20 x 60 ft plots was established during 1996 in Parker Valley (located in southwestern La Paz County, Arizona) in a 2 year old alfalfa stand on a Gilman loam soil with 1.8, 44, 40, and 16% organic matter, sand, silt, and clay, respectively (see data tables for treatments). In 1996, the first and second applications were made on 30 April 1996 and 7 August 1996, respectively. The granule formulation of Zorial was applied with a Valmar small plot granule applicator (model 1255 PT).

Zorial 2 Experiment. The field was planted on 29 October 1997 using grower collected 'CUF 101' alfalfa seed in a sandy loam soil similar to that described above. The initial herbicide treatments were applied on 30 April 1998 following harvest (i.e., cutting and hay removal). All of the granule herbicides were applied using a ground driven Valmar granule applicator (model 1255 PT). Individual plots were 32 feet wide by 300 feet long and were arranged in a randomized complete block design with three blocks. There were no emerged purple nutsedge shoots or summer annual grasses present at the time of the initial applications. The second protion of the split application treatments were applied on 8 September 1998. In 1999, the spring herbicide treatments were applied on 4 March 1999 and the summer portion of the split applications were applied on 22 June 1999. In general, the granule herbicides were incorporated within 2 to 4 days of application by flood irrigation. Visual estimates of percent weed control compared to the untreated plots were made following hay harvest at various times as indicated in the data tables.

Glyphosate Experiments. Two experiments were conducted at the University of Arizona Campus Agricultural Center in Tucson, Arizona using a natural field population of purple nutsedge from 31 August 1999 through 21 March 2000 and from 7 July 2001 through 11 April 2002. During 2000 between experiments, the field was disked to spread tubers and irrigated frequently to reestablish a dense purple nutsedge infestation. The experiments were arranged in a randomized complete block design with 4 replications and the individual plots were 12 x 24 ft in 1999 and 12 x 20 ft in 2001. The field was irrigated every 10 to 12 days, with regular irrigation ceasing at the end of October in 1999 or the end of September in 2001. Glyphosate was applied at 1.5 lb ae/A and the formulations used were Roundup Ultra (1999, Monsanto), Roundup UltraMax (2001, Monsanto) and Touchdown IQ (2001, Syngenta). Roundup Ultra was sprayed sequentially on 31 August and 28 September in 1999. In 2001, the glyphosate formulations were sprayed either 2 or 4 times sequentially on 7 July, 4 August, 20 August, and 20 September. The herbicides were applied with ammonium sulfate (8.5 lb/100 gal H_2O) using a CO_2 pressurized backpack sprayer equipped with four TeeJet flat-fan, XR8003VS nozzles in a carrier volume of 16.7 gal/A at 3 mph. Phytotoxicity was evaluated approximately every 7 days until 2 Nov 1999 and 18 Oct 2001. Phytotoxicity was visually estimated on a whole-plot basis using a scale of 0 to 10 with 0 = no injury, 4 to 5 = severe stunting, 6 to 7 = chlorosis and 9 to 10 = necrosis. In addition, after 2 irrigations the following spring, regrowth was estimated by counting the number of shoots within 2 x 24 ft strip down the middle of the plots in March 2000 or by counting the number of shoots within a 0.25 m^2 or a 0.5 m^2 subplot in high and low density plots, respectively, in April 2002.

RESULTS AND DISCUSSION

Zorial 1 Experiment. A study involving a two year Zorial 5G application program was initiated in the spring of 1996 in two year old 'CUF 101' alfalfa that had about 61 to 74% purple nutsedge groundcover within the alfalfa stand. Two annual Zorial granule applications during April and August of 1996, and March and June of 1997 at total rates of 3 to 4 lb ai/A per year provided fair to good purple nutsedge control in established alfalfa in 1997 (Table 1 and Knowles et al., 1997). The residual soil concentrations of norflurazon from these treatments continued to provide some suppression of purple nutsedge in 1998 but at labeled rates of 1.5 and 2 lb ai/A per year purple nutsedge control was low, 15 to 30% (Table 1). In August 1998, the above label rate of 3 lb ai/A per year only provided fair nutsedge suppression (51%), while 4 lb ai/A per year still provided satisfactory suppression (75%).

Zorial 2 Experiment. Zorial 5G (1, 1.5, 2, 2.5, and 3 lb ai/A), Treflan TR10 (2 lb ai/A), and Visor 2.5G (0.25 and 0.5 lb ai/A) were first applied on 30 April 1998 about 22 weeks after planting before purple nutsedge and summer annual grasses emerged. As of 4 August 1998, purple nutsedge was the predominate weed in this experiment. Purple nutsedge distribution in the plots was highly variable with many plots in the second block having fairly dense infestations as shown by the percent ground cover data (Table 2). Purple nutsedge suppression was estimated based on the degree of chlorosis, and amount of bleached leaves and necrotic plants in 1998. Little thinning or reduction of purple nutsedge populations was observed until 1999 and then this parameter was also used in evaluating nutsedge suppression. Purple nutsedge suppression on 27 May 1998 increased linearly as the rate of Zorial 5G applied increased from 1.5 to 3.0 lb ai/A (Table 2). The degree of nutsedge suppression resulting from the initial spring Zorial 5G applications increased with time, reached a maximum on 4 August 1998 and declined later in the fall (Table 2). The slow increase in efficacy was probably due to increased herbicide soil incorporation and plant uptake caused by the cumulative increase in the amount of applied irrigation water. Zorial rates of 1.5 to 3.0 ai/A provided moderate purple nutsedge suppression (57 to 72%) on 4 August 1998 (Table 2).

Purple nutsedge was still the predominant weed in the study when the second part of the split Zorial 5G and Treflan TR10 treatments were applied on 8 September 1998. Single spring applications of Zorial 5G at rates ranging from 1 to 3 lb ai/A still provided moderate (42 to 68%) purple nutsedge suppression on 8 October 1998 (Table 2). A previous experiment found that superior nutsedge control required a second of application of Zorial 5G during the summer (Knowles et al., 1998). Similarly, split spring and summer applications of Zorial 5G at annual rates of 2 or 3 lb ai/A per year provided good (80 to 88%) purple nutsedge suppression in the fall of the first year of this study (Table 2). On 8 October 1998, the split 2 (1+1) lb ai/A Zorial treatment and the two split 3 lb (1.5+1.5 and 2+1) ai/A Zorial treatments resulted in greater purple nutsedge suppression in the fall than the 2 lb ai/A and 3 lb ai/A single spring application treatments, respectively, although the latter treatments provided greater suppression in the spring and summer (Table 2). The two annual split 3 lb ai/A Zorial 5G treatments did not result in significantly better purple nutsedge control than the split 2 lb ai/A Zorial 5G treatment when evaluated on 8 October 1998 (Table 2).

In the spring 1999, the Zorial 5G (1, 1.5, 2, 2.5, and 3 lb ai/A), Treflan TR10 (2 lb ai/A), and Visor 2.5G (0.5 lb ai/A) treatments were reapplied to the same plots in one year, 5 month old ‘CUF 101' alfalfa on 4 March 1999 before significant amounts of purple nutsedge emerged. The second part of the split applications were reapplied on 22 June 1999. During the summer of 1999, purple nutsedge was still the predominate weed in this test. Similar to 1998, there was a slow increase in Zorial 5G efficacy on purple nutsedge during the spring of 1999 with the single spring 1.5 to 3 lb ai/A Zorial 5G applications resulting in very good control (89 to 96%) of this weed on 22 June 1999 (Table 3). The annual application rate of 1 lb ai/A Zorial 5G resulted in only fair purple nutsedge control (at best 75%) and was significantly worse than the other Zorial treatments (Table 2). The Treflan TR10 and Visor 2.5G treatments resulted in poor control (0 to 5%) of purple nutsedge similar to the results obtained in 1998. Interestingly, during June, July and August of 1999, there was little difference between treatments that received 1.5, 2, 2.5 or 3 lb ai/A of Zorial 5G in a single spring application (Table 3). In addition, there were no significant differences in purple nutsedge control between treatments that received 1.5 lb ai/A of Zorial 5G or more in a single spring application versus treatments that received the same annual Zorial rate in split applications (Table 2). Thus, the split 2 lb ai/A Zorial 5G treatment and the two split 3 lb ai/A Zorial 5G treatments were not significantly different from the single spring applications of 2 and 3 lb ai/A, respectively, during the second year of this study (Table 3). On two occasions, once in early summer of each year of the study, a few alfalfa plants with white leaves were observed in a plot that received a single spring application of 3 lb ai/A of Zorial 5G.

In conclusion, in a newly planted alfalfa field, there does not appear to be any benefit in applying more than 1.5 to 2 lb ai/A of Zorial if a Zorial 5G treatment program is initiated in the spring following a fall planting. This is in contrast to the conclusion of Knowles et al. (1997) that 3 or more lb ai/A per year were needed to provide adequate purple nutsedge control in heavily infested fields when a Zorial treatment program was not started until the second or third year of the alfalfa stand. However, similar to previous results (Knowles et al., 1997), two or more years of Zorial treatments were required to provide adequate purple nutsedge control and to substantially reduce nutsedge population densities. In the Zorial 2 Experiment, there was no difference in purple nutsedge control between split application (spring and summer) treatments versus single spring application treatments in the second year of the alfalfa stand when the same amount of Zorial was applied annually. However, during the first year, a summer application of Zorial did substantially increase the degree of purple nutsedge suppression observed in the fall.

The Zorial 5G formulation of norflurazon used in the alfalfa experiments conducted in 1996 to 1999 discussed above is no longer available but an alfalfa use section was added to the Zorial Rapid 80 label. Although the sprayed formulation results in less deposition of herbicide on the ground than the granule formulation, the loss of herbicide on crop and weed leaf surfaces can be minimized by spraying Zorial Rapid 80 immediately after the dried hay is baled and removed from the field before substantial regrowth occurs and by using a high carrier volume. Our data suggest that the best results will be obtained by planting alfalfa in the fall (October) so that it can get established during cooler conditions without competition from purple nutsedge. A Zorial treatment regime for purple nutsedge should be initiated during the first summer of an alfalfa planting to avoid developing dense infestations. The current Zorial Rapid 80 label allows the application of 1 lb ai/A in the spring 5 months after planting and allows a second application of 1 lb ai/A latter in the year. The maximum label rate of 2 lb ai/A per year should be used because some Zorial Rapid 80 will be inactivated on plant surfaces. Our results suggest that in subsequent years an additional 2 lb ai/A application can be made each spring to early summer to avoid the extra application costs of split applications. In view of the long soil residual of Zorial and the rotational restrictions on the Zorial label, established alfalfa fields should not be treated during the last year of the stand in order to reduce the risk of injury to rotational crops.

Glyphosate Experiments. The development of glyphosate tolerant alfalfa will allow purple nutsedge control strategies that use multiple glyphosate applications. Following cutting, harvest and baling of alfalfa in Parker Valley, purple nutsedge shoots rapidly grow in the absence of an alfalfa canopy and shading. These patches often have the appearance of monospecific nutsedge stands because the alfalfa crowns are below the nutsedge foliage. We applied two or four sequential glyphosate applications to a similar dense infestation of purple nutsedge in a fallow field (i.e., no crop competition) to assess the efficacy of glyphosate for purple nutsedge control. Roundup Ultra was sprayed sequentially on 31 August and 28 September in 1999. Fourteen days after the initial treatment (DAT) at 1.5 lb ae/A, the plots appeared slightly chlorotic and the plants were not growing (phytotoxicity rating of 2.6 on a 0 [no injury] to 10 [necrosis] rating scale) but by 30 DAT the plants were starting to grow and the plots appeared green again (phytotoxicity rating of 1.0). After the second glyphosate application, the phytotoxicity ratings increased dramatically and by 41 and 63 days after the initial treatment the phytotoxicity ratings were 6.1 and 8.4, respectively. On 22 March 2000, the amount of spring shoot emergence from tubers was reduced from 305 shoots/m^2 in the untreated control to 5.7 shoots /m^2 in the glyphosate treated plots indicating that two sequential fall applications at 1.5 lb ae/A in Roundup Ready alfalfa would have the potential to dramatically reduce purple nutsedge infestations.

In the second glyphosate experiment, treatments were initiated earlier in the year beginning on 7 July with sequential applications made on 4 August, 20 August, and 20 September 2000. Sixteen days after the initial treatments purple nutsedge was chlorotic with a phytotoxicity rating of 4.2 but by 33 DAT the purple nutsedge population was recovering and the phytotoxicity rating was 2.4. After the second glyphosate application, nutsedge phytotoxicity increased to 5.2 and 7.1 at 40 and 61 DAT, respectively. There was no significant difference between the plots treated with the Roundup Ultramax or the Touchdown IQ glyphosate formulations. Spring purple nutsedge emergence was measured on 11 April 2001 and was 384 shoots/m^2 averaged across the two glyphosate formulations compared to 628 shoots/m^2 in the untreated plots. After three sequential 1.5 lb ae/A glyphosate applications, these treatments had a phytotoxicity rating of 9.5 averaged across the two glyphosate formulations 61 DAT on 6 September 2000. After 4 sequential applications, purple nutsedge shoot emergence in the spring was 33 shoots/m^2 averaged across both glyphosate formulations which was a 95% reduction in spring shoot emergence.

The research reported here indicates that summer and fall applications of glyphosate in Roundup Ready alfalfa will be a good tool for purple and yellow nutsedge control in low desert alfalfa production areas. Future research will be conducted in low desert alfalfa production areas in farmer fields to determine the number of applications and the appropriate rates of glyphosate needed for nutsedge control. The efficacy glyphosate alone versus combinations of Zorial and glyphosate or combinations of Eptam and glyphosate also need to be investigated. In the presence of crop competition and shading from alfalfa, postemergence glyphosate applications alone may be sufficient to control troublesome nutsedge species along with many other weed species in alfalfa.

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TABLES

Table 1. Effect of residual norflurazon on purple nutsedge control in 1997-98 in a Parker Valley alfalfa field. The first 1997 Zorial 5G treatments were applied on 6 March 1997 and the second part of the split application treatments were applied on 17 June 1997.

		Purple Nutsedge Control[1]				
Treatment	Rate	9-11-97	10-13-97	5-30-98	7-6-98	8-7-98
	(lb ai/A)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Untreated	-	0 d	0 f	0 e	0 d	0 e
Zorial 5G	1 + 1	61 bcd	50 cde	38 bc	34 cd	24 cd
Zorial 5G	1.5	46 d	36 e	19 d	16 e	15 de
Zorial 5G	1.5+ 1.5	81 ab	74 b	68 a	63 b	51 b
Zorial 5G	2	49 d	45 de	23 cd	25 de	24 cd
Zorial 5G	2 + 2	92 a	89 a	83 a	83 a	75 a
Zorial 5G	3	71 abc	64bc	46 b	43 c	36 bc
Zorial 5G[2]	2 + 1	59 cd	53 cd	39 bc	38 cd	30 cd

[1]Data are means of 4 replications; means within a column followed by the same letter are not significantly different at P=0.05 according to Duncan's Multiple Range Test.
[2]The initial norflurazon application in this treatment was 2 lb ai/A of Zorial Rapid 80 applied on 30 April 1996. Most of the Zorial Rapid 80 was intercepted by purple nutsedge foliage and not the soil. Thus, the norflurazon was not incorporated by the irrigation following application and poor control was initially obtained. Therefore, the granular Zorial formulation was used for all subsequent applications.

Table 2. Purple nutsedge weed control and percent ground cover in 1998 in a Parker Valley alfalfa field. The first applications of norflurazon (Zorial 5G), thiazopyr (Visor 5G), and trifluralin (Treflan TR10) were made on 30 April 1998 and the second portion of the split treatments were applied on 8 September 1998.

		Nutsedge Cover[1]	Purple Nutsedge Control in 1998[2]				
Treatment	Rate	9-8-98	5-27	7-6	8-4	9-8	10-8
	(lb ai/A)	*(% plot)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Untreated	-	10, 90, 40	0 g	0 f	0 g	0 d	0 f
Zorial 5G	1	10, 5, 7	20 de	28 cde	52 cd	35 c	42 e
Zorial 5G	1.5	10, 25, 3	23 de	32 cd	57 bcd	38 bc	45 de
Zorial 5G	2	5, 8, 4	32 bc	42 ab	65 ab	55 a	60 cd
Zorial 5G	2.5	50, 10, 10	32 b	42 ab	72 a	52 a	68 bc
Zorial 5G	3	5, 65, 2	46 a	50 a	68 ab	55 a	55 cde
Zorial 5G	1 + 1	10, 80, 5	25 cde	28 cde	48 d	32 c	80 ab
Zorial 5G	1.5 + 1.5	8, 10, 5	27 bcd	35 bc	63 abc	48 ab	82 ab
Zorial 5G	2 + 1	5, 3, 20	25 cde	32 cd	68 ab	40 bc	88 a
Visor 2.5G	0.25	20, 75, 10	12 f	20 e	23 ef	0 d	0 f
Visor 2.5G	0.5	10, 75, 10	18 ef	25 de	33 e	7 d	0 f
Treflan TR10	2 + 2	35, 90, 10	12 f	20 e	17 f	3 d	0 f

[1]Purple nutsedge percent ground cover is listed in replication order (i.e., rep. 1, 2 and rep. 3).
[2]Data are means of 3 replications; means within a column followed by the same letter are not significantly different at P=0.05 according to Duncan's Multiple Range Test.

Table 3. Purple nutsedge weed control in 1999 in a Parker Valley alfalfa field. Applications of norflurazon (Zorial 5G), thiazopyr (Visor 5G), and trifluralin (Treflan TR10) were made on 4 March 1999 and the second portion of the split treatments were applied on 22 June 1999.

Treatment	Rate	Purple Nutsedge Control[1]				
		4-15-99	5-20-99	6-22-99	7-21-99	8-30-99
	(lb ai/A)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Untreated	-	0 d	0 e	0 c	0 d	0 d
Zorial 5G	1	47 c	57 c	75 b	70 c	63 c
Zorial 5G	1.5	58 b	68 b	89 a	85 ab	80 b
Zorial 5G	2	62 ab	68 b	92 a	87 ab	83 b
Zorial 5G	2.5	60 cb	73 ab	90 a	83 b	82 b
Zorial 5G	3	68 ab	78 a	96 a	88 ab	89 ab
Zorial 5G	1 + 1	47 c	58 c	88 a	83 b	83 b
Zorial 5G	1.5 + 1.5	70 a	70 b	96 a	93 a	97 a
Zorial 5G	2 + 1	65 ab	73 ab	89 a	90 ab	96 a
Visor 2.5G	0.5	0 d	13 d	5 c	5 d	0 d
Treflan TR10	2 + 2	0 d	0 e	3 c	0 d	0 d

[1]Data are means of 3 replications; means within a column followed by the same letter are not significantly different at P=0.05 according to Duncan's Multiple Range Test.