INFLUENCE OF GLYPHOSATE (ROUNDUP ULTRA®) RATE AND TIME OF APPLICATION ON WEED CONTROL AND PERFORMANCE OF DP5415RR COTTON IN ISRAEL: FIELD AND LABORATORY EXPERIMENTS

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Abstract

Field and laboratory trials were conducted at four locations in Israel in 1998 and 1999 to evaluate the performance of glyphosate-resistant cotton (Roundup Ready, cv. DP5415RR). Cotton plants treated postemergency (POST) with glyphosate at various rates and growth stages did not show any visual injury. Late application of glyphosate (8- to 10- leaf stage) resulted in deformed cotton flowers particularly in the first blooming flush. Anthers were degenerated and released very little pollen. Final plant mappings have shown that the late application of glyphosate (1.44 kg/ha) resulted in a significant increase in the number of bolls per plant and a concomitant decrease in boll weight of the bottom fruiting branches. In spite of the observed detrimental effects, no yield reduction was recorded. Excellent weed control was observed in all experiments at all herbicide rates and timings tested. However, control of troublesome weeds like purple nutsedge (*Cyperus rotundus*) was improved when late and high application rates were used. Cross-pollination between 'DP5415RR' and a neighboring local cultivar 'Sivon' was evaluated by testing the response to glyphosate of the progeny collected from 'Sivon' plants grown at different distances. Less than 10% gene transfer was detected at 1 m distance and less than 0.5% at 35 m from the RR plot.

Introduction

Traditionally, weed control programs in cotton have been dependent on soil-applied and post-directed herbicides. With the adoption of glyphosate-resistant (Roundup Ready™) cotton varieties, glyphosate has become a major component of the weed management program. This herbicide provides control of a broad-spectrum grass and broadleaf weeds. Being "environmentally friendly" further facilitates its acceptance by the farmers (Askew and Wilcut 1999).

The transgenic cotton contains a CP4 gene construct that exhibits excellent vegetative tolerance to glyphosate (Jones and Snipes, 1999). However, over the top application after the 4th leaf stage was reported to result in lower pollination, fruit abortion, deformed bolls and yield reduction (Kerby and Voth, 1998; Vargas et al., 1998). Recent experiments indicated possible negative interactions between environmental factors and response of RR cotton to glyphosate (Jones and Snipes 1999). The aims of our study were to evaluate the performance of DP5415RR cotton under Israeli management and its response to various herbicides applied PPI, PRE and POST. We also examined the risk of gene transfer by cross-pollination to other cotton cultivars.

Material and Methods

DP5415RR cotton was examined in four locations throughout Israel during the 1998 and 1999 cotton seasons. RR and non-transgenic cotton cultivars were planted in May 1998 and April 1999 in commercial cotton fields prepared for planting according to the local practices. Plots were 4 cotton rows (96cm apart) wide and 15 to 20 m long replicated 4 to 6 times in a randomized complete block design. Herbicides were applied PPI, PRE or POST (over the top) as needed using a motorized knapsack sprayer equipped with a 4 m boom with 10 T-jet nozzles delivering 100 L/ha. Glyphosate (Roundup Ultra®) was applied POST, either early (2 to 3 leaves), medium (4 to 5 leaves) or late (8 to 10 leaves) growth stages at rates of 0, 0.72 and 1.44 kg ae/ha. Weed control efficacy and crop response to the treatments was visually assessed as needed. Plant mapping was carried out just before the harvest and included number of bolls and their position on the sympodium, boll weight and number of seeds/boll. Total cotton-seed yield/plot was recorded following mechanical picking.

Flowers and bolls were sampled from each plot throughout the season and their morphology was examined. When needed, scanning electron microscope (SEM) observations were made on fresh flowers without fixation, using a Jeol scanning microscope. (Model JSM-5410LV, Japan).

Seed cotton was collected by hand from adjacent non-transgenic cotton ('Sivon') border rows for analysis of pollen dispersal from transgenic plants, up to 50 m eastward (wind direction) and westward. At each distance ca. 100 bolls were hand-picked at random from the top, middle and bottom of the plants. Harvest was done mechanically in 2 rows of each plot. Seed cotton was ginned in a laboratory gin, and a sub-sample of 200 seeds was planted in a sand:peat mixture and

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transferred to the greenhouse. Cotton seedlings at the fully expanded cotyledons were sprayed with glyphosate (1.44kg ae/ha) using a chain belt-driven laboratory sprayer. Surviving plants were counted and considered resistant.

The response of DP5415 and DP5415RR cultivars to various cotton herbicides was also examined under field conditions. Herbicides (see Table 3) were applied PPI, PRE or Post as described above on plots 2 rows wide and 12m long and replicated 8 times in a split blocks design. DP5415RR cotton seedlings grown in the greenhouse in pots were sprayed with glyphosate at 2, 4, 6 and 8 leaves stage with glyphosate up to 4.32 kg ae/ha (12 L of Roundup Ultra/ha). Shoot fresh and dry weight were determined 14 days after treatment.

Results and Discussion

Response of Weeds and RR Cotton to Glyphosate

No visual damage or growth retardation was observed on the cotton plants treated with glyphosate at all rates and times of application tested (Table 1). Similarly, no significant effects were found in seed cotton yield or any quality traits tested. Greenhouse experiments confirmed these results (data not shown).

Table 1. Effect of PRE and POST treatments on DP5415RR seed cotton yield at three experimental sites in Israel, 1999. All plots including the control were PPI treated with trifluralin + fluometuron (1.0+1.0 kg/ha); EP = Early Post - 2-3 leaves; MP = Medium Post – 4-5 leaves; LP = Late Post – 8-10 leaves. Data presented as means±sd.

Glyphosate (kg ae/ha)	Applic' time	Seed cotton yield (kg/ha)		
		M'-Gilboa	Nahhal-Oz	Negba
0.00	PPI	5672±150	5559±140	5962±302
0.72	EP	5317±170	5913±205	5851±466
1.44	EP	5169±465	5856±342	5788±164
0.72	MP	5614±210	5828±211	5476±584
1.44	MP	5467±113	5650±377	5740±492
0.72	LP	5482±258	5872±456	5813±258
1.44	LP	5567±153	5546±253	6274±443
0.72+0.72	EP+LP	5369±326	5747± 81	5630±455
1.44+1.44	EP+LP	4961±263	5822±137	5476 ±514

No significant differences (Student's p=0.05)

Weed Control

All glyphosate treatments provided effective and acceptable weed-control until harvest. In 1998 experiments, all the weeds that emerged before the crop were controlled with glyphosate applied pre-planting, hence no much weed infestation was observed even at the untreated plots. However, toward the end of the season, some infestation with common cocklebur (*Xanthium strumarium*) and Palmer amaranth (*Amaranthus palmeri*) was observed in the untreated control plots.

In the experiments conducted during 1999, glyphosate at all rates provided excellent control of the following annual weeds: heliotrop (*Heliotropium arbainense*), prostrate pigweed (*Amaranthus blitoides*), redroot pigweed (*Amaranthus. retroflexus*), *Moluccella laevis, Chrozophora tinctoria, Datura ferox,* puncturevine (*Tribulus terrestris*), and black nightshade (*Solanum nigrum*). Good control of perennial weeds such as johnsongrass (*Sorghum halepense*), bermudagrass (*Cynodon dactylon*) and field bindweed (*Convolvulus arvensis*) were observed, particularly at the high rates. Subsequent flushes of black nightshade and prostrate pigweed seedlings appeared following the early application of glyphosate at the 2- to 4- leaf stage. This phenomenon was more significant after the drip irrigation was started (early June, 1999). Higher rates and late application of glyphosate resulted in excellent control of purple nutsedge (*Cyperus rotundus*), indicating the potential of using the RR trait for combating this troublesome weed in cotton.

Effect of Late Application of Glyphosate

The first flowers developed on plants treated 'late Post' at high rates of glyphosate, appeared abnormal with partially developed anthers that contained little pollen and a pistil with a stigma protruding much above the stamens (Fig. 1). These symptoms were less frequent and weaker at early application and low glyphosate rates. No such effects were observed in control plants or in flowers developed later in the season.

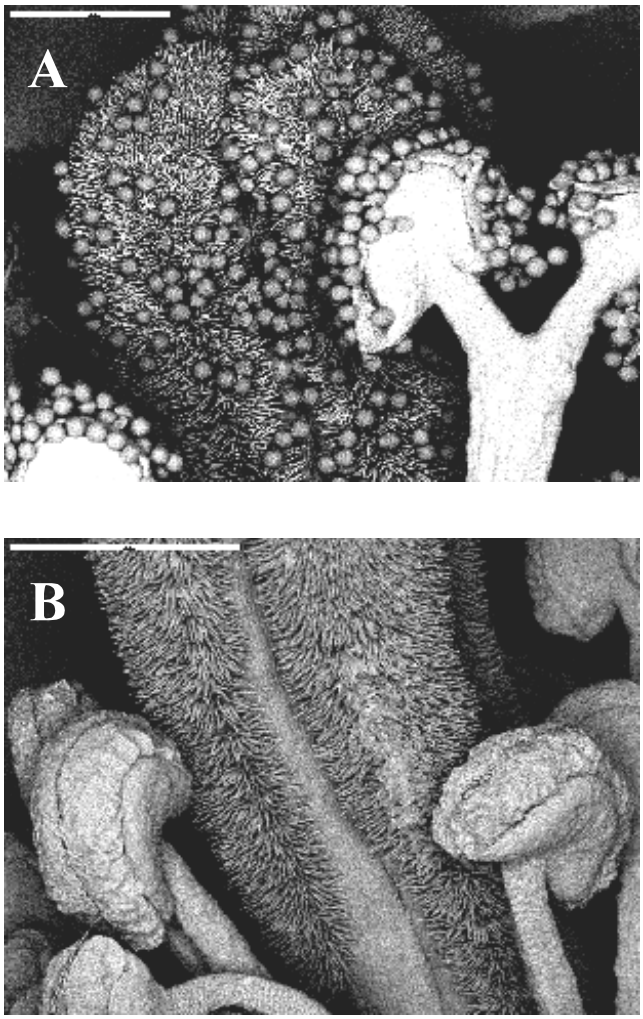

Figure 1. Stigma and anthers of cotton flower untreated (A) and treated (B) with glyphosate 1.44kg ae/ha at late application (8 to 10 leaves).

Some of the early bolls developed on plants treated 'late' and at the high rate were deformed mostly with one degenerated compartment resulting in a 'moon shaped' bolls. In addition, the number of bolls per plant was significantly higher in this treatment, with a parallel decrease in the boll weight and number of seeds per boll. This was particularly apparent in bolls developed on the first and second position sympodia of the 5th to 10th fruiting branches (Table 2). It seems that the plant attempts "to compensate" in response to the initial injury caused by the herbicide by increasing the rate of fruit set.

Response of RR Cotton to Other Herbicides

No difference were observed in the response of the two varieties DP5415 and DP5415RR to various cotton herbicides commonly used in Israel (Table 3).

Table 2. Effect of herbicides applied PRE and POST on DP5415RR cotton fruiting pattern: Nahhal-Oz and Negba, 1999. PPI = preplant incorporation of fluometuron+trifluralin; EP = Early Post - 2-3 leaves; MP = Medium Post – 4-5 leaves; LP = Late Post – 8-10 leaves*.

Glyphosate (kg ae/ha)	Applic' time	Average Boll wt. (g/boll)**		Number of bolls/plant	
		Nahhal-Oz	Negba	Nahhal-Oz	Negba
0.00	PPI	5.4a	4.1cd	18.7a	26.1b
0.72	EP	4.9a	3.9bc	22.0ab	24.5b
1.44	EP	5.1a	3.6bcd	25.8b	28.3ab
0.72	MP	5.0a	3.7b	22.7ab	26.9ab
1.44	MP	5.3a	2.9a	22.2ab	28.8ab
0.72	LP	4.9a	3.5b	25.9b	27.7ab
1.44	LP	4.9a	4.1cd	25.3b	29.3ab
0.72+0.72	EP+LP	4.8ab	4.2d	21.4ab	29.3ab
1.44+1.44	EP+LP	4.4b	4.2d	27.1b	33.9a

*Means followed by the same letter within a column are not significantly different using Student's p=0.05 analysis.
**Bolls on 1st and 2nd positions of branches developed on nodes 5 to 10.

Table 3. Effect of different cotton herbicides on seed cotton yield of DP5415 and DP5415RR– Negba, 1999. Pyrithiobac was applied at 9 to 10 leaves. Data presented as means±sd.

Herbicide	Rate (kg ai/ha)	Applic' time	Seed cotton (kg/ha)	
			DP5415	DP5415RR
Trifl.+Fluom.	1.25+1.25	PPI	5758±490	5539±576
Pendimethalin	2.50	PRE	5553±579	5931±371
Prometryn	2.00	PRE	5914±442	5887±457
Pyrithiobac	0.13	POST	5618±356	5615±509

Gene Transfer from RR Cotton to Other Cotton Cultivars

Cross-pollination between DP5415RR and the local cotton cultivar 'Sivon' (Acala type) was examined within the range of 50 m from the RR plot. The proportion of cross-pollination was up to 10% at 1 m distance and none further than 35 m (Fig. 2). Interestingly, the proportion of cross-pollination at plots located westward of the RR plot (against the wind direction) was larger then that observed in the eastern side, indicating that pollen was moved mostly by insects (especially bees) rather than wind.

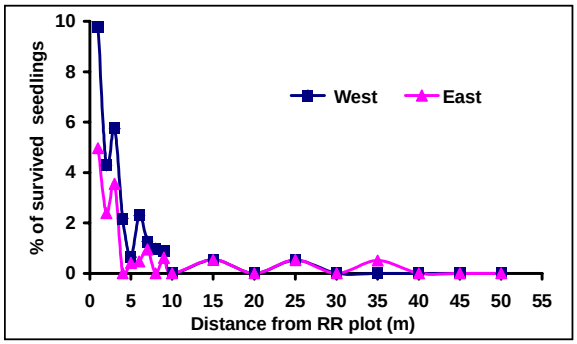

Figure 2. Frequency of transgenic pollen dissemination into border rows located east and west to the test plot - Negba 1998.

Summary

Over the top application of glyphosate at the recommended rate (0.72 kg ae/ha) and at the double rate (1.44 kg ae/ha) applied at up to 4 to 5 leaves stage in DP5415RR provides good and season-long annual weed control. Crop safety is excellent without any visible damage to the vegetative parts or reduction in seed cotton yield and quality.

Late application of glyphosate (8- to 10- leaves) may result in reduction in boll size and number of seeds per boll with a parallel increase in boll number per plant. No significant reduction yield was observed.

The risk of gene transfer from the RR cotton to other non-transgenic cotton cultivars is limited to 10 m.

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