Potential benefits and risks associated with the introduction of herbicide-resistant transgenic cotton in Israel

Hagai Yasuor, Gil Tsuk and Baruch Rubin
Department of Field Crops, Vegetable and Genetics, Institute of Plant Sciences and Genetics, Faculty of Agriculture, Food and Environmental Science, The Hebrew University of Jerusalem, Rehovot 76100, Israel

Summary Glyphosate-resistant cotton (RR) varieties are commercially grown worldwide. Field and laboratory studies were conducted during the last four years to examine the possible introduction of RR cotton into the Israeli cropping system. The aims of the study were to elucidate the effect of glyphosate rate and time of application on weed control, crop development and yield of RR cotton and to estimate the risk of trait introgression to other cotton cultivars. Glyphosate applied at various cotton growth stages and at various rates provided excellent control of most annual and perennial weeds without visual injury to the crop. Glyphosate improved the control of troublesome weeds such as *Euphorbia geniculata* Ortega and *Cyperus rotundus* L. Early treatments (up to 4 to 5 leaf stage) resulted in late flashes of weeds that emerged after the start of irrigation that caused significant yield loss, indicating the crucial need for split glyphosate applications or amendment with a residual herbicide. Late (over 5-leaf stage) and split treatments resulted in abnormal cotton flowers with partially developed anthers and pollen grains. The stigma, although protruded above the stamens in the treated plants, functions normally. A 'moon' shape bolls were developed from damaged flowers. Generally, no significant yield reduction was recorded. Less than 10% of gene transfer from the RR cotton to other non-transgenic 'Acala' type cotton cultivars was detected at 1 m distance and less than 0.5% at 35 m. No cross-pollination between cotton and its weedy relatives occurs in Israel. The results indicate that transgenic RR cotton can be introduced safely into the Israeli cotton management with the potential of reducing herbicide load in the environment, improving weed management, and improving the farmer's income.

Keywords Glyphosate resistance, stigma, anther, cross-pollination.

INTRODUCTION

Cotton is the major irrigated summer crop in Israel, heavily infested with annual and perennial weeds. Weed control relies mostly on pre-emergence applied herbicides. Traditional weed control programs in cotton are mostly dependent on soil-applied and post-directed herbicides. With the adoption of glyphosate-resistant (RR, Roundup Ready™) cotton varieties, glyphosate has become a major component of the weed management programs. RR cotton varieties are now grown commercially in the US and experimentally in several other locations in the world. Glyphosate provides a broad-spectrum control of annual and perennial grass and broadleaf weeds. Being cheap and 'environmentally friendly' further facilitate its acceptance by the farmers. (Askew and Wilcut 1999, Nida *et al*. 1996, Yasuor *et al*. 2000). However, over reliance on a single herbicide or transgenic crop may increase the risk of resistance evolution or a shift in the weed population toward those weeds which confer natural tolerance (Rubin 1996).

Glyphosate is a competitive inhibitor of the 5-enol -pyruvylshikmate-3-phosphate synthase (EPSPS) in the shikimic acid pathway, leading to the biosynthesis of the aromatic amino acids tryptophan, tyrosine, and phenylalanine (Ray 1989). The transgenic cotton that contains a CP4 EPSPS gene from *Agrobacterium* spp. exhibits excellent vegetative tolerance to glyphosate (Yasuor *et al*. 2000). However, over the top (OTT) application after the fourth leaf stage was reported to result in lower pollination, boll 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 (Jones and Snipes 1999). No such negative response of RR-soybean was reported.

Our previous studies have pointed out that late OTT application of glyphosate resulted in male sterile flowers. The anthers did not open and the pollen grain seems to collapse during their development. (Yasuor and Rubin 2001, Pline *et al.* 2002). Chen and Hubmeier (2001) reported that the male sterility caused by glyphosate is due to a low expression of the CP4 EPSPS in male reproductive tissues. Reports from USA (Umbeck *et al.* 1991) and Australia (Llewellyn and Fitt 1996) have shown a limited transfer of the resistance gene from transgenic cotton to non-transgenic cultivars. The aims of this study were to elucidate the effect of glyphosate rate and time of application on weed control, crop development and yield of RR cotton and to estimate the risk of trait introgression to other cotton cultivars.

MATERIALS AND METHODS

DP5415RR Acala type cotton was examined in commercial cotton fields prepared for planting according to the local practices. Plots were four cotton rows wide (96 cm apart), 15 to 20 m long, and replicated 4 to 6 times in a randomised complete block design. Herbicides were applied pre-plant incorporated (PPI), pre-emergence (PRE), OTT or as a directed spray (only the lower leaves intercepting the spray), using a motorised knapsack sprayer equipped with a 4 m boom delivering 100 L ha^{-1}. Glyphosate (Roundup Ultra®) was applied OTT, either early at 2–3 leaf stage (E), medium at 5–6 leaf stage (M) or late at 8–10 leaf stage (L) at rates of 0, 0.7, 1.1 and 1.4 kg a.e. ha^{-1}. Weed control efficacy and crop response to the treatments was visually assessed as needed. 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 Jeol scanning microscope. (5410LV, Japan).

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 per boll. Total cotton-seed yield per plot was recorded following mechanical picking. Hundred bolls were hand-picked at random from the top, middle and bottom of 10 plants. from adjacent non-transgenic Acala type cotton ('Sivon') rows (up to 50 m), for analysis of cross-pollination. Seeds (200 per sample) were planted in Rehovot soil and seedlings were treated OTT with glyphosate (1.4 kg a.e. ha^{-1}) using a chain belt-driven laboratory sprayer. Surviving plants were counted and considered resistant.

RESULTS AND DISCUSSION

All glyphosate treatments provided effective and acceptable weed-control until harvest. Excellent control (>95%) of the following annual weeds was recorded: heliotrope (*Heliotropium arbainense* Fresen.), prostrate pigweed (*Amaranthus blitoides* S.Watson), redroot pigweed (*A. retroflexus* L.), *Moluccella laevis* L., *Chrozophora tinctoria* L., jimsonweed (*Datura ferox* L.), puncturevine (*Tribulus terrestris* L.), and black nightshade (*Solanum nigrum* L.). Good control (80 to 90%) of perennial weeds such as johnsongrass (*Sorghum halepense* L.), bermudagrass (*Cynodon dactylon* L.) and field bindweed (*Convolvulus arvensis* L.) were observed particularly at high rates.

Weeds in plots treated with glyphosate at an early stage were temporally weed free, but a new flash of common cocklebur (*Xanthium strumarium* L.), Palmer amaranth (*Amaranthus palmeri* S.Watson), and prostrate pigweed was observed after the drip irrigation has started (8 to 10 weeks after emergence).

Glyphosate applied twice, very early (first leaf stage) and at the 4 to 5 leaf stage, resulted in an effective control of purple nutsedge as compared to the recommended treatment with MSMA (Table 1). We assume that the early treatment combined with the competition provided by the crop plants prevented further development of the weed. Additional late directed application (67 days after planting, DAP) of glyphosate did not improve the nutsedge control (Table 1). These data indicate the potential of using the RR trait for combating this troublesome weed in cotton and the importance of the application timing on the control of purple nutsedge.

Trials conducted in the 1998–2001 seasons at five locations demonstrated that glyphosate caused no visual damage to the vegetative part of the crop at all rates and times of application tested. These results were further confirmed in greenhouse experiments. RR plants grown in pots were treated with a range of glyphosate (0 to 4.3 kg ha^{-1}) applied OTT at various growth stages (cotyledons to 8-leaf stage).

Normal (0.7 kg ha^{-1}) and high rates (1.4 kg ha^{-1}) of glyphosate applied OTT at the 8–10 leaf stage when flower buds appear (square initiation) resulted in abnormal flowers. The injured flowers were characterised by partially developed anthers containing undeveloped pollen grains, and a stigma protruding much above the stamens (Figure 1). Cross sections and SEM conducted in anthers two days before anthesis have shown that the pollen development has ceased at earlier stages with an empty and collapsed structure (Figure 2). The pistil however, did not exhibit any deformations, apart from some elongation of the style that resulted in a stigma extruding above the anthers. The pistil remains functional, and when hand-pollinated with normal pollen grains, produced normal boll and seed cotton yield (data not shown). SEM observations have shown that in non-pollinated bolls the ovules did not develop within

Table 1. Response of purple nutsedge to herbicides applied OTT or as directed spray.

Herbicide	Application time (DAP)	Rate (kg ha^{-1})	Nutsedge infestation (%)[a]
Control	–	–	80 ± 4
MSMA	18 + 40	1.0 + 1.0	40 ± 7
Glyphosate	18 + 40	0.7 + 1.4	5 ± 1
Glyphosate	30 + 40	1.1 + 1.4	10 ± 1
Glyphosate	18 + 40 + 67	0.7 + 0.7 + 1.1	5 ± 1
Glyphosate	30	1.1	10 ± 1

[a] Infestation level was visually estimated 100 DAP.

the lock (the compartment within the ovary) beyond their embryonic stage, whereas in the hand-pollinated bolls the embryo was further developed to form the seed with its primordial fibres. Damaged flowers that were bagged at the early morning of anthesis to prevent cross-pollination by insects were aborted within two weeks (data not shown).

These symptoms were barely detected in plots where glyphosate was applied early (up to 5-leaf stage) or at low rates. The negative effect of the applied herbicide was visible from three weeks after application and gradually disappeared within five to six weeks after application. This phenomenon well coincides with the initiation of the premordia of the fruiting branches and squares (Mauney 1986). Hence, the damage occurs at the very early stages of the flower development. This 'critical period' of the herbicide effect may appear again if the herbicide is applied again during the reproductive phase.

Plant mapping conducted before harvest revealed that many of the bolls developed on lower fruiting branches (6 to 16 nodes) were deformed ('moon shaped'). These bolls were significantly smaller and produced less seed/boll as compared to the untreated control (Table 2). The effects were much more pronounced in boll positioned first on each branch, indicating less efficient pollination in certain growth stages. Treated plants compensate for the early damage by producing more bolls on higher branches (above the 20th branch) (data not shown).

Further experiments have shown that glyphosate applied as a directed spray at late and/or very late (12–13 leaves) growth stages caused no damage to the vegetative parts of cotton plants.

Table 2. Effect of herbicides applied PPI and POST on DP5415RR cotton boll weight and seed cotton yield.

Glyphosate (kg ha^{-1})	Appl. time	Bolls $plant^{-1}$	Boll wt. (g)	Yield (t ha^{-1})[c]
–[a]	PPI	18.7 b[b]	5.4 a[b]	5.56
0.7	E	22.0 ab	4.9 a	5.91
1.4	E	25.8 a	5.1 a	5.86
0.7	M	22.7 ab	5.0 a	5.83
1.4	M	22.2 ab	5.3 a	5.65
0.7	L	25.9 a	4.9 a	5.87
1.4	L	25.3 a	4.9 a	5.55
0.7+0.7	E+L	21.4 ab	4.8 ab	5.75
1.4+1.4	E+L	27.1 a	4.4 b	5.82

[a] Standard treatment – trifluralin and fluometuron (1.0+1.0 kg ha^{-1}); [b] Means followed by the same letter are not significantly different at P=0.05; [c] No significant differences were detected.

Figure 1. Flowers taken from untreated (right) and treated (left) cotton plants at 8-leaf stage with glyphosate 1.44 kg ha^{-1}.

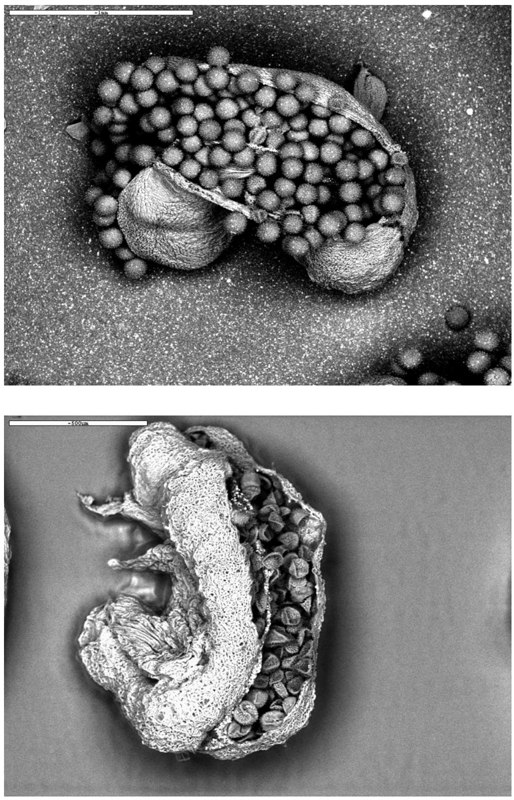

Figure 2. SEM micrograph of anthers and pollen grains of untreated (above) and treated (below) cotton plants at 8 leaf stage with glyphosate 1.44 kg ha^{-1}.

However, abnormal flowers and fruit setting were observed followed by an excessive elongation of the main cotton stem by more than 40 cm above the untreated plants and a significant yield reduction. We speculate that this is the response of the barren plant caused by the very late application of glyphosate.

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 (Figure 3). 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. In Israel, cotton can not cross-pollinate with weeds, thus demonstrating the low risk associated with dispersal of the transgenic trait to the environment.

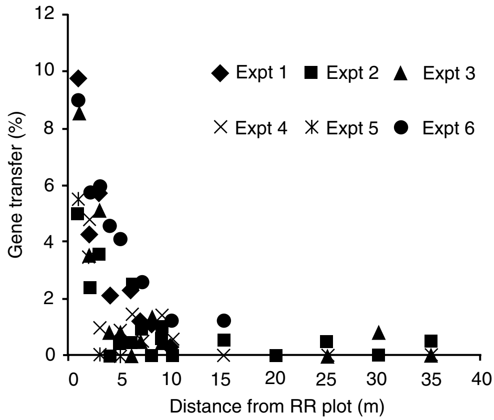

Figure 3. Frequency of transgenic pollen dissemination from RR into non-transgenic cotton grown nearby. Data are means of six different experiments conducted in three years and three locations.

ACKNOWLEDGMENTS

The authors wish to express their thanks to The Israel Cotton Board, E. Abiri and Monsanto Company for their support of the project. The help of M. Litvak, Kibbutz Negba and Kibbutz Hasolelim is highly appreciated.

REFERENCES

Askew, S.D. and Wilcut, J.W. (1999). Cost and weed management with herbicide programs in glyphosate-resistant cotton (*Gossypium hirsutum*). *Weed Technology* 13, 308-313.

Chen, Y.S. and Hubmeier, C. (2001). Histochemical analyses of male reproductive development in glyphosate-tolerant cotton (*Gossypium hirsutum*). Proceedings of the 18th Asian-Pacific Weed Science Society Conference, pp. 437-441.

Jones, M.A. and Snipes, C.E. (1999). Tolerance of transgenic cotton to topical application of glyphosate. *Journal of Cotton Science* 3, 19-26.

Kerby, T. and Voth, R. (1998). Roundup Ready® – introduction experiences in 1997. Proceedings Beltwide Cotton Conferences, pp. 26-29.

Llewellyn, D. and Fitt, G. (1996). Pollen dispersal from two field trials of transgenic cotton in Namoi Valley, *Australia. Mol. Breed*. 2, 157-166.

Mauney, J.R. (1986). Vegetative growth and development of fruiting sites. *In* 'Cotton physiology', eds J.R. Mauney, and J.McD. Stewart, pp. 11-28. (The Cotton Foundation, Publisher, Tennessee).

Nida, D.L., Kolacz, K.H., Buehler, R.E., Deaton, W.R., Schuler, W.R., Armstrong, T.A., Taylor, M.L., Ebert, C.C., Rogan, G.J., Padgette, S.R. and Fuchs, R.L. (1996). Glyphosate-tolerant cotton: Genetic characterization and protein expression. *Journal of Agricultural and Food Chemistry* 44, 1960-1966.

Pline, W.A., Viator, R., Wilcut, J.W., Edmisten, K.L., Thomas, J.F. and Wells, R. (2002). Reproductive abnormalities in glyphosate-resistant cotton may be due to lower CP4-EPSPS levels in male reproductive tissue. *WSSA Abstracts* 42, 254.

Ray, T.B. (1989). Herbicide as inhibitors of amino acid biosynthesis. *In* 'Target sites of herbicide action', eds P. Böger and G. Sandmann, pp. 105-126. (CRC Press, Boca Raton, Florida, USA).

Rubin, B. (1996). Herbicide-resistant weeds – the inevitable phenomenon: mechanisms, distribution and significance. *Zeitschrift fur Pflanzenkrankheiten und Pflantzenschutz* XV, 17-32.

Umbeck, P.F., Barton, K.A., Nordheim, E.V., Mccarty, J.C., Parrott, W.L and Jenkins J.N. (1991). Degree of pollen dispersal by insects from a field test of genetically engineered cotton. *J. Econ. Entoml*. 84, 1943-1950.

Vargas, R.N., Wright, S. and Martin-Duvall, T.M. (1998). Tolerance of Roundup Ready cotton to roundup ultra applied at various growth stages. Proceedings Beltwide Cotton Conferences, pp. 847-848.

Yasuor H., Sibony, M., Rubin, B., Litvak, M., Flash, I. and Gat. E. (2000). Influence of glyphosate (Roundup Ultra®) rate and time of application on weed control and performance of DP5415RR cotton in Israel: field and laboratory experiments. Proceedings Beltwide Cotton Conferences, pp. 1480-1483.