The ecological fitness of *Amaranthus retroflexus* and *A. blitoides* resistant to acetolactate synthase (ALS) inhibitors and atrazine

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Summary The ecological fitness in *Amaranthus retroflexus* and *A. blitoides* was studied in the field in the absence of herbicide in monoculture and under competitive conditions. The competition studies were conducted at the following combinations: ALS-resistant *Amaranthus retroflexus* (SuR) *vs.* the wild type (SuS) and the multiple-resistant *A. blitoides*: ALS- and triazine-resistant (SuR/TR) *vs*. wild type (SuS/TS) and SuR/TR *vs.* ALS-sensitive and triazine-resistant (SuS/TR) biotypes. The experiments were conducted in the field on a sandy loam soil during the summer of 1996 and 1997, using different seedlings proportions: 100%S; 75%S/25%R; 50%S/50%R; 25%S/75%R; 100%R at a constant stand of 400 plants m^{-2}. The SuR and SuS *A. retroflexus* biotypes attained similar shoot biomass and the relative yield total (RYT) =1, indicating that the ALS-resistance trait in *A. retroflexus* is not associated with reduced ecological fitness. Under monoculture conditions, the growth of the three *A. blitoides* biotypes SuR/TR, SuS/TR, and SuS/TS was similar. In replacement series experiments when SuS/TS biotype was grown in a mixture with SuR/TR at different proportions, the individual SuS/TS plant was much superior in its growth as compared to the SuR/TR. A negative effect of association was observed – 'amensalism', where the total biomass of the SuR/TR plants per plot was dramatically reduced whereas that of the SuS/TS was virtually unchanged. Under competition, the biomass produced by each individual plant of SuR/TR and SuS/TR was similar. However, the relative yield (RY) of the different biotypes at the pair combinations was less than the combine potential and the RYT was lower than one, indicating that there is an antagonism effect between the biotypes. We concluded that the trait conferring resistance to ALS inhibitor herbicides in *A. retroflexus* and *A. blitoides* is not associated with a growth penalty and did not incur ecological penalty in the field. The data clearly demonstrate that the triazine resistance trait is the cause of the observed reduction in growth rendering the SuS/TR and SuR/TR less fit than the wild type.

Keywords ALS, *Amaranthus blitoides*, *Amaranthus retroflexus*, competition, ecological fitness, herbicide resistance, sulfonylurea, triazine.

INTRODUCTION

Amaranthus is a common annual broadleaf genus of tropical origin, widely distributed all over the world (Kigel 1994). In Israel, *A. retroflexus* L. (redroot pigweed), and *A. blitoides* S. Watson (prostrate pigweed) are major agricultural weeds infesting all irrigated summer crops. Resistance to almost all herbicide groups has been reported *Amaranthus* spp. (Rubin 2000, Sibony 2001, Sibony *et al*. 2001). Moreover, we recently reported on a multiple resistance to triazines (TR) and acetolactate synthase (ALS) inhibiting herbicides in *A. blitoides* (SuR) (Sibony and Rubin 2002 this proceedings).

In weeds, the ability to establish, survive, and reproduce successfully (i.e. weed fitness), is one of the most important factors influencing the appearance and persistence of herbicide-resistant biotypes (Maxwell *et al*. 1990). The assumption that resistant weed biotypes are *a-priori* less fit than the susceptible ones in the absence of herbicide application is based on early studies of triazine resistance. These studies indicated a marked reduction in the reproductive capacity and low competitiveness of the TR biotypes relative to the triazine-susceptible (TS) of most species in the absence of the herbicide, e.g. *A. hybridus* L. (smooth pigweed) (Ahrens and Stoller 1983), *A. retroflexus* L. (redroot pigweed) (Conard and Radosevich 1979), and *A. rudis* (common waterhemp) (Anderson *et al*. 1996). Weaver and Warwick (1982) have shown that TR *A. retroflexus* and *A. powellii* produced less biomass and seeds than the TS biotype. However, we have shown that under non-competitive conditions the TR biotype of *Phalaris paradoxa* was superior to the corresponding TS biotype in seed germinability and seedling emergence, and equal or superior in CO_2 uptake and biomass accumulation (Schonfeld *et al*. 1987).

Resistance to ALS inhibiting herbicides was reported in 72 weed species worldwide (Heap 2002). Numerous studies have shown that resistance to ALS inhibitors in *Lactuca serriola* (Alcocer-Ruthling *et al*. 1992a, 1992b) and *Kochia scoparia* (Thompson *et al*. 1994, Christoffoleti and Westra 1994), is not associated with reduced fitness.

Lolium rigidum from Australia have evolved cross and multiple resistance to a wide range of herbicides

(Holtum and Powles 1991). The multiple-resistant biotype (SLR 31) grown in isolation or in mixture had a similar phenology, biomass production, and fecundity as the susceptible biotype, (Holt and Thill, 1994). This indicates that the mechanisms conferring multiple resistance may not carry a substantial fitness penalty.

The aims of this research were to evaluate the competitiveness of a multiple-resistant *A. blitoides* (SuR/TR) biotype as compared to ALS-susceptible and triazine-resistant (SuS/TR) biotype and to the wild type (SuS/TS), and to evaluate the ecological fitness of SuR and SuS *A. retroflexus* biotypes.

MATERIALS AND METHODS

Plant material Seeds of resistant *A. retroflexus* (SuR) and multiple-resistant *A. blitoides* (SuR/TR) were collected from plants which survived sulfometuron and simazine field treatments at Ganot, in the Coastal Plain of Israel. Seeds from the corresponding wild types (SuS) and (SuS/TS) were collected from an untreated field at Kfar Shmuel. Seeds from the ALS-susceptible and TR *A. blitoides* (SuS/TR) were collected from a corn field at Yavne. Two weeks old seedlings were transplanted in the field at the University Research Farm (Rehovot).

Replacement series competitive studies The experiments were conducted at the following combination: SuS *vs*. SuR biotypes of *A. retroflexus*; SuS/TS *vs.* SuR/TR and SuS/TR *vs.* SuR/TR biotypes of *A. blitoides*. Seedlings of each biotype were planted in the field, at a density of 400 plants m^{-2} (121 plants per plot). Five varying proportions were chosen: 100%S; 75%S/25%R; 50%S/50%R; 25%S/75%R; 100%R for the different biotype mixtures (de Wit 1960). The plots were irrigated as needed. *A. blitoides* and *A. retroflexus* plants were harvested before seed ripening, in order to prevent seed-shed and diminish the chances of infestation with herbicide-resistant weeds in the field. Plants were uprooted, their shoot cut at soil level, oven dried at 70°C for 3 days and dry weight was recorded. The total plot shoot biomass of each biotype and the relative yield (RY = shoot biomass of the biotype grown in mixture/shoot biomass of the same biotype grown in a pure stand) were calculated. In addition, the sum of the RY of the competing biotypes was determined as the relative yield total (RYT) (de Wit and van den Bergh 1965).

Data analysis All experiments were arranged in randomised complete block design, with four replicates for each biotype and for each pair of biotypes. The experiments were conducted twice, during the summer of 1996 and 1997. Analysis of variance (ANOVA) was run on all data. Since there was no significant interaction of year by harvest date, data from the 1996 and 1997 were pooled for statistical analysis. Regression coefficients that describe the effects of the different mixtures were tested for similarity by *t*-test (P = 0.05), using joint regression analysis (JMP, SAS Institute Inc, USA).

RESULTS AND DISCUSSION

All transplanted plants were present at the time of harvest, indicating that seedling survival was not affected by competition at a density of 400 plants m^{-2}.

Amaranthus retroflexus The *A. retroflexus* SuR and SuS biotypes had a similar development pattern when grown under monoculture conditions, with a similar shoot biomass, leaf area, shoot height and seed yield per plant (data not shown). When grown under competitive conditions, the SuR and SuS biotypes attained similar shoot biomass with a similar regression line and a comparable plant biomass in the 50%/50% mixture. The relative yield total (RYT) was approximately one in all the mixtures of the replacement series, implying a similar competitiveness in the two biotypes (Figure 1). These data indicate that the ALS-resistance trait in *A. retroflexus* is not associated with reduced ecological fitness.

Amaranthus blitoides Under monoculture conditions, the growth of the three *A. blitoides* biotypes SuR/TR, SuS/TR, and SuS/TS was similar (data not shown).

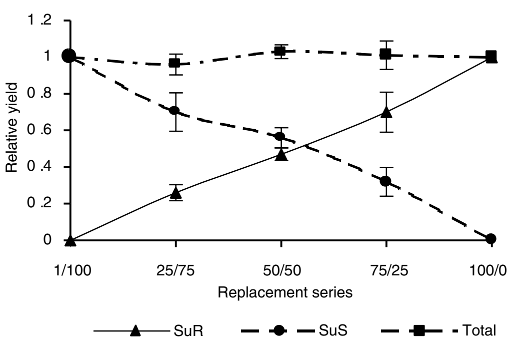

Figure 1. Relative shoot dry biomass and relative shoot total biomass of *A. retroflexus* biotypes grown under competition in a replacement series at various proportions and constant density (400 plants m^{-2}). Vertical bars indicate standard error.

Competition between SuR/TR and SuS/TS In a pure stand – 100% SuR/TR and 100% SuS/TS, the shoot biomass of the biotypes was similar. However, when grown in mixtures (25:75; 50:50; 75:25), the individual SuS/TS plants were much superior in their growth as compared to SuR/TR plants. The total biomass of the SuR/TR plants per plot was dramatically reduced whereas that of the SuS/TS was virtually unchanged. The lack of mutual effect under competition suggests that in spite of the inhibited growth of the SuR/TR plants, the SuS/TS plants were not able to exploit the extra resources available. This phenomenon defined as 'amensalism' (Barbour *et al.* 1999), was evident as the relative yield was less than the combined potential of the two biotypes. These data indicate that neither biotype reached its growth potential and contributed its expected share to the total yield (Figure 2a). The RYT was lower than one indicating that there is an antagonistic effect between the biotypes. The basis for the observed amensalism is probably due to a severe competition for light, as the TR plants confer low photosynthetic capacity caused by the mutation in the *psbA* gene.

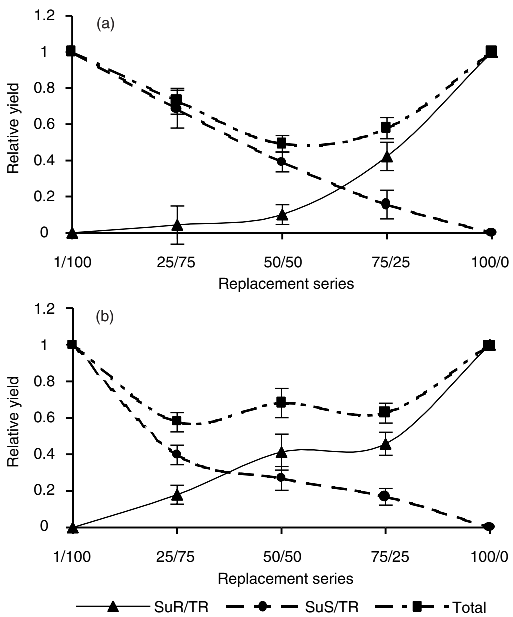

Figure 2. Relative shoot dry biomass and relative shoot total biomass of *A. blitoides* biotypes grown in a replacement series at various proportions and constant density (400 plants m^{-2}) under competition of SuR/TR *vs*. SuS/TS (a) and of SuR/TR *vs*. SuS/TR (b). Vertical bars indicate standard error.

Competition between SuR/TR and SuS/TR The biomass production of both TR *A. blitoides* biotypes, SuR/TR and SuS/TR that differ only in their sensitivity to ALS inhibitors in a pure stand was similar. The biomass produced by each individual plant was also similar and was not significantly influenced by the presence of the competitors. However, when the sum biomass per plot was calculated for each biotype in the three mixture proportions tested, their shoot biomass was significantly lower than that predicted. The RY of each biotype in any mixture did not reach its own potential. Hence, the calculated RYT of the two biotypes was lower than one, indicating a mutual antagonism between the two biotypes (Figure 2b). Furthermore, the average shoot biomass in the 50%/50% mixture was similar for both biotypes and the competition judged to be nil. This indicates that the two biotypes compete for the same resources, but there is a mutual antagonism.

The mutual antagonism observed in both replacement series experiments in *A. blitoides* supports our assumption that it was caused by the TR trait rather than the resistance to ALS inhibitors. Hence, we assume that these two prostrate pigweed biotypes SuS/TR and SuR/TR were less robust than the wild type SuS/TS due to the mutation in the *psbA* gene (Sibony and Rubin, 2002 this proceedings).

Our data indicate that the trait conferring resistance to ALS inhibitor herbicides in *A. retroflexus* and *A. blitoides* is not associated with a growth penalty and did not result in an ecological cost in the field. Similar trends were reported for sulfonylurea-resistant *Kochia scoparia* biotypes (Thompson *et al*. 1994, Christoffoleti and Westra 1994). Lior (2001) also demonstrated that *Plantago lagopus* resistant to sulfonylurea was not less competitive than the susceptible biotype. However, the lower ecological fitness observed in the multiple-resistant *A. blitoides* SuR/TR is probably due to a negative canopy interactions, caused by a less efficient light use in the TR plants. It can be argued that when the application of ALS inhibitor herbicides is terminated, *A. retroflexus* SuR biotypes will continue to be present in the field, since no reduced fitness is expected.

In contrast, when a mixture of *A. blitoides* SuR/TR and SuS/TS biotypes grow in the absence of the selector, the SuR/TR biotype should slowly disappear and the entire population will become SuS/TS. This phenomenon may explain the low spread and limited distribution of the multiple resistant biotype SuR/TR in Israel.

ACKNOWLEDGMENTS

This study was supported by the Deutsche Forschungsgemeinschaft, as a part of the Trilateral German-Israeli-Palestinian Research Program and by the Chief Scientist of the Ministry of Agriculture, Israel.

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