Growth performance of triazine-resistant and -susceptible biotypes of *Solanum nigrum* over a range of temperatures

BRIAN F. JACOBS
Department of Botany, Duke University, Durham, NC 27706, U.S.A.
JOHN H. DUESING
Ciba–Geigy Corporation, Research Triangle Park, NC 27709-2257, U.S.A.
JANIS ANTONOVICS
Department of Botany, Duke University, Durham, NC 27706, U.S.A.
AND
DAVID T. PATTERSON
United States Department of Agriculture, Duke University Phytotron, Duke University, Durham, NC 27706, U.S.A.

Received March 3, 1987

JACOBS, B. F., DUESING, J. H., ANTONOVICS, J., and PATTERSON, D. T. 1988. Growth performance of triazine-resistant and -susceptible biotypes of *Solanum nigrum* over a range of temperatures. Can. J. Bot. **66**: 847–850.

Growth of triazine-resistant and -susceptible biotypes of *Solanum nigrum* was studied over a range of temperatures to test whether resistant biotypes show reduced performance under high temperatures. Each biotype was represented by parental lines and reciprocal F_1 crosses. Resistant biotypes were inferior in growth relative to susceptible biotypes. Temperature had no differential effect on the relative performance of resistant and susceptible biotypes. Our results with *Solanum nigrum* do not support the hypothesis that the infrequent occurrence of the resistant biotypes in southern regions is explained by their decreased growth at high temperatures.

JACOBS, B. F., DUESING, J. H., ANTONOVICS, J., et PATTERSON, D. T. 1988. Growth performance of triazine-resistant and -susceptible biotypes of *Solanum nigrum* over a range of temperatures. Can. J. Bot. **66** : 847–850.

La croissance de biotypes de *Solanum nigrum* résistants et sensibles à la triazine fut étudiée à plusieurs températures afin de vérifier si les biotypes résistants ont une performance réduite à hautes températures. Chaque biotype était représenté par des lignées parentales et des croisements F_1 réciproques. La croissance des biotypes résistants était inférieure à celle des biotypes sensibles. La température n'a pas eu d'effet sur la performance relative des biotypes résistants et sensibles. Les résultats obtenus avec *Solanum nigrum* n'appuient pas l'hypothèse selon laquelle la présence rare des biotypes résistants dans les régions du sud s'expliquerait par leur diminution de croissance aux températures élevées.

[Traduit par la revue]

Introduction

Resistance to the *s*-triazines is now known for over 40 weed species representing 26 genera in 7 families (H. M. LeBaron, personal communication). Most of these species are annuals with high fecundities, short generation times, and often with cosmopolitan distributions. Although the *s*-triazines are effective against a broad spectrum of weeds and have been used over a wide latitudinal range, it is noteworthy that many of the resistant biotypes first appeared in the cooler regions of the temperate zone (H. M. LeBaron, personal communication). In the United States, it is notable that there have been no reports of resistant biotypes from the southeastern states or the midwestern corn belt (LeBaron and Gressel 1982). The rarity of resistant biotypes in more southern climates may reflect either the effect of prevailing agricultural practices in those regions or physiological effects of the resistance genes such that resistant plants are less able to survive at high temperatures. Support for the latter hypothesis comes from a number of observations. Donnelly and Hume (1983) showed that when a commercial *Brassica napus* cultivar with *s*-triazine resistance derived from a resistant biotype of *Brassica campestris* was compared with the same cultivar without resistance, the *s*-triazine-resistant cultivar showed a 21% decrease in dry-matter production at 25:20°C (light:dark) but no difference at 15:10°C.

Gasquez *et al.* (1981) found that resistant biotypes of *Polygonum lapathifolium* showed 51% reduction in total dry-matter yield when compared with normal biotypes in a growth chamber at 27°C but only a 22% reduction at 20°C. A similar trend (55 vs. 4% reduction) was seen in dry-matter yields of plants grown in a warm versus cool greenhouse. Gasquez *et al.* (1981) also demonstrated enhanced low-temperature germination in resistant biotypes of *Polygonum lapathifolium* and *Amaranthus retroflexus* but not in those of *Solanum nigrum* or *Chenopodium album*. Pillai and St. John (1981) reported that chloroplast membrane lipids from triazine-resistant biotypes of three weeds were richer in unsaturated fatty acids than were lipids from susceptible biotypes. The presence of such unsaturated fatty acids has also been implicated in enhanced resistance to low-temperature stress. Gressel (1985) drew attention to similar changes in the lipid composition of chloroplast membranes of triazine-resistant and chilling-resistant plants.

The present study was designed to investigate the temperature response of *s*-triazine-resistant and -susceptible biotypes on a uniform genetic background. Previous studies had compared either biotypes from different localities (Gasquez *et al.* 1981) or biotypes only partially homogenized by backcrossing (Donnelly and Hume 1983). We used resistant and susceptible biotypes of *Solanum nigrum* represented by parental types and F_1s derived from reciprocal crosses. Because atrazine resistance is encoded by a chloroplast gene and therefore cytoplasmically inherited, F_1 reciprocal crosses differ in their susceptibility but have a common nuclear background. We assessed the relative growth of resistant and susceptible bio-

Printed in Canada / Imprimé au Canada

types under a range of temperatures in growth chambers and tested the hypothesis that resistant-biotype performance is diminished at high temperatures.

Materials and methods

Solanum nigrum biotypes resistant to *s*-triazine were first reported from France and West Germany in 1976 (H. M. LeBaron, personal communication). Seed material of atrazine-resistant and -susceptible biotypes of *Solanum nigrum* was obtained from G. Gasquez, Dijon, France, and F_1 progency were generated by reciprocal crosses. Cytoplasmic type was verified by fluorescence excitation and decay measurements taken on atrazine-treated leaf disk samples of five individuals of each of the four genotypes, using a fluorometer (Plant Productivity Meter, Richard Brancker Research Ltd., Canada) (Ducrest and Gasquez 1978). Verification of the hybrid status of the F_1 plant material was provided by observations of intermediate characters (stem pubescence and mature leaf size) in the F_1 individuals. Two related experiments were conducted.

Experiment 1

To assess the effects of a wide range of temperatures on growth, replicate individuals of each of four biotypes, represented by resistant and susceptible parents and their reciprocal F_1 progeny, were grown at each of three temperature regimes: 12:6, 19:13, and 26:20°C (light:dark). Plants were grown in controlled-environment chambers in the Duke University Phytotron for 4 weeks. Plants were grown in a completely randomized design and were rotated twice weekly over the course of the experiment. Plants were maintained under a 16-h photoperiod (400 μmol m^{-2} s^{-1} PAR), rooted in a 1:1:1 mix of vermiculite–Turface–gravel, and watered with half-strength Hoagland's solution in the morning and with distilled water in the afternoon. Plants were started in flats in the greenhouse, transplanted as seedlings to pots, and placed in the appropriate growth chambers. Plants that died during the first 2 days of the experiment because of transplant shock were replaced with plants that had been maintained under the same experimental conditions. Plants were harvested after 30 days and the following measurements were obtained per individual: survival; leaf area; and biomass of leaves, stem, and reproductive parts. Biomass measurements were taken on material that was oven-dried at 40°C for 10 days. Reproductive biomass was taken as a single, composite measurement per biotype. The biomass data for the 12:6°C (light:dark) treatment was also taken as a single measurement per biotype. In both cases this was made necessary by the small amount of material being weighed.

Experiment 2

To investigate the effect of large temperature changes on growth rate, 10 individuals of each of the four biotypes from the 12:6 and the 26:20°C regimes of experiment 1 were transferred to 35:29 and 12:6°C, respectively, and grown for an additional 30 days. The growth performance of each individual was assessed as in experiment 1.

Results

The resistant biotypes of *Solanum nigrum* were inferior in growth performance relative to the susceptible biotypes (Table 1). The results were consistent across all temperature regimes, with no significant biotype × temperature interaction (Table 2) even though the temperature regimes produced about a 10-fold difference in total aboveground biomass (Table 1). Similar trends were obtained for leaf area (Table 1), although biotype differences were not significant (Table 2). Reciprocal F_1 crosses resembled the female parent in growth response, indicating that differences among the parents due to nuclear effects were small and that cytoplasmic type was primarily responsible for the decreased growth performance of resistant biotypes. After the large temperature reversals imposed in experiment 2, resistant biotypes again yielded less than susceptible biotypes (Table 3), although the difference was not significant (Table 4). There was no differential effect of a switch to high or to low temperature, as shown by the absence of a significant biotype × temperature interaction (Table 4). In terms of total dry weight, the resistant parent tended to outperform the resistant F_1s, while the reverse was true for susceptible types, but the differences were not significant (Table 1). A similar trend has been observed in rapeseed (D. J. Hume, personal communication).

Only the resistant biotypes experienced any mortality and then only during the first 12 days of the first experiment. Mortality was almost equally divided between the parental (four deaths) and F_1 (five deaths) resistant biotypes, with most deaths occurring at the intermediate temperature. This suggests the resistant biotypes may be also less hardy at the seedling stage.

Discussion

The present study comparing resistant and susceptible biotypes of *Solanum nigrum* under noncompetitive conditions found the susceptible biotypes to be superior in both total biomass and reproductive output. Thus the present study supports the idea of a "cost" of resistance, with the resistant biotypes being somewhat less fit, or photosynthetically less efficient, than the susceptible in a herbicide-free environment (Burke *et al.* 1982; Ort *et al.* 1983; Paterson and Arntzen 1982; Kyle *et al.* 1983; Warwick and Marriage 1982; Warwick 1980; Holt and Radosevich 1983; Holt 1983; Holliday and Putwain 1980). In many earlier studies resistant and susceptible biotypes were sampled from widely separated populations and sometimes even from different latitudes. One may expect that nuclear genes would vary with geographical origin and could account for the observed differences. Weaver and Warwick (1982) and Warwick and Marriage (1982) have shown a large effect of geographical origin on total biomass and flowering time of *Chenopodium* and *Amaranthus*. To be valid, comparisons between resistant and susceptible biotypes must minimize differences in nuclear background not necessarily correlated with cytoplasmic type. This can be achieved by reciprocal crosses as in the present study or by a backcrossing program in the manner of Kihara (1982). The results of the present study, therefore, confirm a cytoplasmic effect that is independent of any nuclear differences.

The observation that a large number of the triazine-resistant biotypes were first reported from the more northern latitudes led to our speculation that this might be due in part to a decreased fitness of the resistant biotypes, relative to the susceptible, when grown under high temperatures (Donnelly and Hume 1983; Gasquez *et al.* 1981). The present study, however, found no such pattern in *Solanum nigrum*. Thus, the geographic pattern suggested by the first reports of resistant biotypes, combined with our results, indicates that other factors, such as the persistence of triazine in the soil and regional agronomic practices, are likely to be important where the resistant weeds first appeared. Triazine is degraded more rapidly in warm climates (LeBaron and Gressel 1982), while crop rotation and (or) herbicide rotation reduce selective pressures favoring herbicide resistance. Donnelly and Hume (1983) used a backcrossing scheme to homogenize the nuclear background of resistant and susceptible strains. However, it is not clear how many backcross generations were involved and the degree

TABLE 1. Growth of *s*-triazine-resistant and -susceptible biotypes after 30 days

Temp. regime, °C (light:dark)	Biotype	Biomass (mg) Stem	Leaf	Total (stem + leaf)	Reproductive parts	Leaf area (cm²)
12:6	Susceptible parent	—	—	0.27 (—)	0	5.47 (0.81)
	Susceptible F_1	—	—	0.24 (—)	0	6.48 (1.47)
	Resistant F_1	—	—	0.17 (—)	0	6.09 (0.89)
	Resistant parent	—	—	0.19 (—)	0	4.36 (0.90)
19:13	Susceptible parent	0.20	0.75	0.95 (0.04) *a*	0	177.10 (4.17) *ab*
	Susceptible F_1	0.20	0.85	1.05 (0.03) *a*	0.02	192.90 (5.84) *a*
	Resistant F_1	0.11	0.44	0.55 (0.07) *b*	0	164.30 (10.36) *b*
	Resistant parent	0.12	0.55	0.67 (0.06) *b*	0	187.20 (8.83) *ab*
26:20	Susceptible parent	0.80	1.01	1.85 (0.09) *ab*	0.09	344.40 (20.28) *a*
	Susceptible F_1	0.79	1.15	1.94 (0.06) *a*	0.08	370.70 (19.41) *a*
	Resistant F_1	0.73	0.95	1.64 (0.08) *bc*	0.07	389.40 (22.75) *a*
	Resistant parent	0.63	0.87	1.49 (0.07) *c*	0.02	362.10 (21.80) *a*

NOTE: Standard errors are given in parentheses. Values followed by the same letter are not significantly different at $P < 0.05$, using Duncan's multiple-range test within each temperature treatment.

TABLE 2. Analyses of variance on biomass and leaf-area measurement from experiment 1

	df	SS	MS	*F*	Probability (>*F*)
		Biomass			
Biotype	3	2.684	0.895	19.1	0.001
Temperature	1	17.177	17.177	367.5	0.001
Biotype × temperature	3	0.208	0.069	1.5	0.227
Error	72	3.365	0.047		
		Leaf area			
Biotype	3	4 869.6	1 623.2	0.6	0.631
Temperature	1	693 960.0	693 960.1	247.1	0.001
Biotype × temperature	3	10 353.7	3 451.2	1.2	0.306
Error	72	202 237.4	2 808.9		

NOTE: Data were untransformed. Log transformation did not improve normality and gave essentially similar results.

TABLE 3. Growth of *s*-triazine-resistant and -susceptible biotypes after 60 days

Temp. regime, °C (light:dark)	Biotype	Biomass (mg) Stem	Leaf	Total (stem + leaf)	Reproductive parts	Leaf area (cm²)
26:20 to 12:6	Susceptible parent	2.69	3.11	5.80 (0.37)	1.12	640.92 (26.13)
	Susceptible F_1	2.95	3.84	6.79 (0.51)	0.85	696.07 (22.93)
	Resistant F_1	2.62	3.10	5.72 (0.20)	0.57	656.83 (26.52)
	Resistant parent	2.84	3.23	6.07 (0.32)	0.19	710.27 (27.29)
12.6 to 35:29	Susceptible parent	2.33	2.52	4.85 (0.29)	0.05	1287.16 (55.16)
	Susceptible F_1	2.34	2.63	4.97 (0.33)	0.07	1331.13 (53.92)
	Resistant F_1	2.15	2.40	4.55 (0.28)	0.05	1281.94 (65.14)
	Resistant parent	2.06	2.59	4.65 (0.20)	0.03	1218.78 (44.89)

NOTE: Standard errors are given in parentheses. No differences in total weight or leaf area are significant, using Duncan's multiple range test within each temperature treatment.

to which the nuclear backgrounds were similar. Temperature-related effects need to be assessed in a number of other *s*-triazine-resistant species before any general conclusions can be drawn.

In this regard it is prudent to consider that comparisons of plant performance are often made under a limited range of environments. Life-history differences such as delayed germination, late flowering, and a slower rate of development, which have been attributed to resistant biotypes (Mapplebeck *et al.* 1982), may not always be disadvantageous under field conditions. There is also the possibility that relative biotype fitness may change under different environmental conditions

TABLE 4. Analyses of variance on biomass and leaf area measurements from experiment 2

	df	SS	MS	F	Probability ($>F$)
		Biomass			
Biotype	3	3.088	1.029	0.9	0.463
Temperature	1	31.250	31.250	26.3	0.001
Biotype × temperature	3	0.847	0.282	0.2	0.870
Error	72	85.545	1.188		
Total	79	120.730			
		Leaf area			
Biotype	3	3 455.4	11 451.8	0.6	0.649
Temperature	1	7 290 033.7	7 290 033.7	351.9	0.001
Biotype × temperature	3	61 577.7	20 525.9	1.0	0.402
Error	72	1 491 404.5	20 714.0		
Total	79	8 877 371.3			

NOTE: Data were untransformed. Log-transformed data did not improve normality and gave essentially similar results.

and with different nuclear backgrounds. Relative biotype growth performance under low temperatures may differ for *Solanum nigrum* and *Brassica napus* in part because of life-history characteristics intrinsic to each. *Brassica napus* is a spring-planted annual, which flowers in early summer. *Solanum nigrum* is an annual that begins germination later, after the soil is warm, and upon reaching reproductive maturity in a month or so, flowers until frost. Thus *Solanum nigrum*, a plant of tropical origin, may be less adversely affected by high temperatures than *Brassica napus*, a plant whose relatives are well known for their low-temperature hardiness. Clearly, additional studies are needed on a wide range of species of contrasting growth form and geographic origin to test if the lack of temperature effects found with *Solanum nigrum* are typical of other species with *s*-triazine-resistant biotypes.

Acknowledgements

Dr. Homer LeBaron of the Ciba–Geigy Corporation provided information on the distribution of *s*-triazine-resistant weeds. Tom Peeler assisted with fluorescence measurements. Damon Smith and Elaine Perkins provided assistance in making Phytotron harvests. Funding was provided by a grant from the Duke University Research Council and the Department of Botany, Duke University. Partial support for the controlled-environment studies was provided by National Science Foundation Grant BSR 83-14925 to the Duke University Phytotron and by the U.S. Department of Agriculture.

BURKE, J. J., WILSON, R. F., and SWAFFORD, J. R. 1982. Characterization of chloroplasts isolated from triazine susceptible and triazine resistant biotypes of *Brassica campestris* L. Plant Cell Physiol. **70**: 24–29.

DONNELLY, M. J., and HUME, D. J. 1983. Photosynthetic rates and growth analysis of triazine resistant and triazine susceptible rapeseed. North Cent. Weed Control Conf. Proc. **38**: 51.

DUCREST, J. M., and GASQUEZ, J. 1978. Fluorescence as a means of detecting and chloroplastic resistance of *Chenopodium album* and *Poa annua* to atrazine. Chemosphere, **7**: 691–696.

GASQUEZ, J., DARMENCY, H., and COMPOINT, J. P. 1981. Comparaison de la germination et de la croissance de biotypes sensibles et résistants aux triazines chez quatre espèces de mauvaises herbes. Weed Res. **21**: 219–225.

GRESSEL, J. 1985. Herbicide tolerance and resistance: alteration of site of activity. *In* Weed physiology. Vol. II. Herbicide physiology. Chap. 7. *Edited by* S. O. Duke. CRC Press, Boca Raton, FL.

HOLLIDAY, R. J., and PUTWAIN, P. D. 1980. Evolution of herbicide resistance in *Senecio vulgaris*: variation in susceptibility to simazine between and within populations. J. Appl. Ecol. **17**: 779–791.

HOLT, J. S. 1983. Growth and ecological fitness of two common groundsel (*Senecio vulgaris* L.) biotypes. Weed Sci. Soc. Am. Abstr. **1983**: 78.

HOLT, J. S., and RADOSEVICH, S. R. 1983. Differential growth of two common groundsel biotypes (*Senecio vulgaris*). Weed Sci. **31**: 112–120.

KIHARA, H. 1982. Importance of cytoplasm in plant genetics. Cytologia, **47**: 435–450.

KYLE, D. J., ARNTZEN, C. J., and ROBINSON, H. R. 1983. Similarities in photosynthetic biochemistry from independently arising atrazine resistant biotypes from four species suggest a common biochemical/genetic modification. Michigan State University, Department of Energy, Plant Research Laboratory. 18th Annu. Rep. pp. 58–61.

LEBARON, H. M., and GRESSEL, J. 1982. Herbicide resistance in plants. J. Wiley and Sons, New York.

MAPPLEBECK, L. R., MACHADO, V. S., and GRODZINSKI, B. 1982. Seed germination and seedling growth characteristics of atrazine susceptible and resistant biotypes of *Brassica campestris*. Can. J. Plant Sci. **62**: 733–739.

ORT, D. R., AHRENS, W. H., MARTIN, B., and STOLLER, E. W. 1983. Comparison of photosynthetic performance in triazine resistant and susceptible biotypes of *Amaranthus hybridus*. Plant Physiol. **72**: 925–930.

PATERSON, D. R., and ARNTZEN, C. J. 1982. Detection of altered inhibition of photosystem II reactions in herbicide resistant plants. *In* Methods in chloroplast biology. *Edited by* M. Edelman, R. B. Hallick, and N. H. Chua. Elsevier Biomedical Press, New York. pp. 109–118.

PILLAI, P., and ST. JOHN, J. B. 1981. Lipid composition of chloroplast membranes from weed biotypes differentially sensitive to triazine herbicides. Plant Physiol. **68**: 585–587.

WEAVER, S. E., and WARWICK, S. I. 1982. Competitive relationships between atrazine resistant and susceptible populations of *Amaranthus retroflexus* and *A. powellii* from southern Ontario. New Phytol. **92**: 131–139.

WARWICK, S. I. 1980. Differential growth between and within triazine-resistant and triazine-susceptible biotypes of *Senecio vulgaris* L. Weed Res. **20**: 299–303.

WARWICK, S. I., and MARRIAGE, P. B. 1982. Geographical variation in populations of *Chenopodium album* resistant and susceptible to atrazine. I. Between- and within-population variation in growth and response to atrazine. Can. J. Bot. **60**: 483–493.