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Characterization of Linuron Resistance in a Biotype of Common Purslane (Portulaca oleracea L.)

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CHARACTERIZATION OF LINURON RESISTANCE IN A BIOTYPE OF COMMON PURSLANE (*PORTULACA OLERACEA* L.)

By

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ABSTRACT

CHARACTERIZATION OF LINURON RESISTANCE IN A BIOTYPE OF COMMON PURSLANE (*PORTULACA OLERACEA* L.)

By

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A biotype of common purslane collected from a carrot (*Daucus carota* L.) field near Imlay City, Michigan was confirmed to be resistant to linuron using a flotation test kit. The resistant common purslane (R-POROL) was characterized and compared to linuron-susceptible common purslane (S-POROL). R-POROL and S-POROL were evaluated in field and greenhouse studies to determine their responses to preemergence (PRE) and postemergence (POST) applications of various photosynthetic inhibitors, their morphological and physiological characteristics, and the molecular basis for resistance.

S-POROL was killed by all PRE and POST applications of linuron or atrazine. R-POROL plants survived linuron or atrazine rates (2.24 and 4.48 kg ai/ha, respectively) after a transient slowing of growth. R-POROL was cross-resistant to cyanazine, diuron, and prometryn, but not to bentazon. R-POROL was negatively cross-resistant to bromoxynil. The resistance ratio for R-POROL was >300 for linuron and >400 for atrazine.

Morphological characterization of the two biotypes of common purslane indicated that susceptible plants had 30% heavier seeds, higher maximum seedling germination, higher

fresh and dry weights at all dates of measurements, and more numerous but smaller chloroplasts. In response to increasing CO_2 concentration, S-POROL exhibited a higher CO_2 compensation point and a lower stomatal limitation to CO_2 diffusion. Both R-POROL and S-POROL had similar light compensation points, total dark respiration, quantum yield, estimated maximum assimilation, and CO_2 photosynthetic efficiency. Increasing temperatures resulted in significantly higher CO_2 assimilation rates in R-POROL.

Measurements of photosynthesis and fluorescence at 3-day intervals after application with linuron or atrazine indicated that electron transfer from Q_A to Q_B was not inhibited in R-POROL. Therefore, any damage due to herbicide application was transitory. Linuron application at 3% to 12% the recommended field rate completely inhibited the electron transport in S-POROL and resulted in death by 14 days after treatment.

The levels of resistance and patterns of cross-resistance did not parallel those observed with the majority of triazine-resistant weeds. Sequence analysis of the *psb*A gene confirmed that R-POROL had a serine to threonine alteration at position 264 of the D1 protein. This novel mutation could explain the unique response of common purslane to the photosynthesis-inhibiting herbicides. This substitution was not observed previously in triazine-resistant plants at the whole-plant level.

To my parents and wife who believe in me

To my daughter whom I believe in

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KEY TO SYMBOLS AND ABBREVIATIONS

Φ = photochemical efficiency or quantum yield (μmol CO_2 fixed / mol PPFD)

Γ = CO_2 compensation point (μmol $CO_2 . mol^{-1}$)

A = CO_2 assimilation (μmol $CO_2 . m^{-2} . s^{-1}$)

A_{1200} = assimilation at full sunlight (1200 μmol $. m^{-2} . s^{-1}$)

A_{360} = CO_2 assimilation rate at 360 ppm

A_{360}/A_{max} = photosynthetic efficiency (%)

ALS = acetolactase synthase enzyme

A_{max} = estimated maximum assimilation (μmol $CO_2 . m^{-2} . s^{-1}$)

Atrazine = 2-chloro-4-ethylamino-6-isopropylamino-s-triazine

Bentazon = 3-(isopropyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide

Bromoxynil = 3,5-dibromo-4-hydroxybenzonitrile

c.p. = light compensation point (μmol $CO_2 . m^{-2} . s^{-1}$)

CHEAL = common lambsquarters, *Chenopodium album* L.

C_i = internal CO_2 concentration (μl$.liter^{-1}$)

C_{i360} = internal CO_2 concentration (μl$.liter^{-1}$) at atmospheric CO_2 concentration

Cyanazine = 2-{[4-chloro-6-(ethylamino)-s-triazin-2-yl] amino}-2-methylpropionitrile

DAS = days after seeding

DAT = days after treatment

DAUCA = carrot, *Daucus carota* L.

Diuron = 3-(3,4-dichlorophenyl)-1,1-dimethylurea

GR_{50} = herbicide dosage required to reduce fresh weight by 50% relative to the control

g_s = stomatal conductance ($mmol.m^{-1}.s^{-1}$)

Hexazinone = 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione

I_{50} = herbicide dosage (molar) that causes 50% inhibition of activity

k = estimated carboxylation efficiency (mol $CO_2.m^{-2}.s^{-1}$)

l_g = stomatal limitation to assimilation (%)

Linuron = *N*'-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea

OEC = oxygen-evolving complex

P680 = reaction center chlorophyll *a* dimer

PAR = photosynthetic active radiation

Pheo = pheophytin

POROL = common purslane, *Portulaca oleracea* L.

PPFD = Photosynthetic Photon Flux Density

PQ = plastoquinone

PQH_2 = plastohydroquinone

Prometryn = 2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine

PS II = photosystem II

Pyridate = O-(6-chloro-3-phenyl-4-pyridazinyl)-S-octyl-carbonothioate

Q_A = plastoquinone tightly bound to the D2 protein

Q_B = exchangeable plastoquinone bound to the D1 protein

R = resistant

R_d = total dark respiration ($\mu mol\ CO_2.m^{-2}.s^{-1}$)

RH = relative humidity

S = susceptible

Tyr_z = tyrosine 161 that acts as an electron donor to P680

Chapter 1

Literature Review

LITERATURE REVIEW

INTRODUCTION

Herbicides have changed the face of agricultural crop production. Herbicides are a reliable and inexpensive method of weed control. After the development of herbicides, growers no longer had to spend considerable time and effort removing weeds from fields. This tedious task continued throughout the growing season. Often, by the time a grower and his crew reached the end of a field and finished hand-weeding, it was time to start over again. Herbicide introduction also allowed farmers to put more land into production, since less time was spent on hand-weeding. Finally, herbicides simplified weed management so that a grower no longer has to depend completely on tillage, cover crops, fallow land, and crop rotation.

Since herbicides have become an integral part of agricultural production, herbicide resistance is an issue of major concern. There is great concern that herbicides will no longer be effective, with the ever-increasing number of resistant weeds. Since the first report of a simazine-resistant common groundsel (*Senecio vulgaris* L.) (Ryan 1970), herbicide resistance has received considerable attention from the scientific community, the growers, and the chemical industry.

In 1983, triazine-resistant weeds accounted for 67% of the documented cases of herbicide resistance (Heap 1998). Resistance to bipyridiliums accounted for 13%, synthetic

auxins 12%, and all other herbicide modes of action 8%. As new herbicides with new modes of action were being used, these proportions changed. In the period after 1984, triazine-resistant weeds accounted for only 15% of the newly reported cases. During that period, acetolactate synthase (ALS) inhibitors accounted for 28%, and bipyridiliums for 15% (Heap 1998). To date, 195 weed species worldwide are resistant to various herbicides in more than 42 countries. The current percentages of the resistant weed biotypes to each class of herbicide are as follows: 31% are resistant to triazine herbicides, 21% to ALS inhibitors, 14% to bipyridiliums, 8% to phenylureas/amides, 8% to synthetic auxins, 7% to ACCase inhibitors, 3% to dinitroanilines and the remaining 8% are resistant to other herbicide modes of action. It is estimated that about nine new resistant weed species have been recorded every year since 1978 (Heap 1998).

Triazine-resistance still constitutes the largest type of resistance in weeds. There are currently at least 60 triazine-resistant weed biotypes in 20 countries. Currently, triazine resistance is reported in nine *Amaranthus* species, five *Polygonum* species, and four *Chenopodium* species (Heap 1998). Following the triazines in numbers of resistant weed species are the ALS inhibitors and the bipyridiliums with 41 and 27 resistant biotypes, respectively.

Recently, more attention has been paid to weeds resistant to ALS inhibitors, due to the relatively short period between their introduction and the discovery of the first resistant weed.

A herbicide can be very effective on some weed species while having little or no effect on other weed species. This phenomenon is called herbicide selectivity and is not related to herbicide resistance. Factors such as field preparation and management, crop rotation, and intensity of herbicide use can determine which weed species will be controlled and which will become a serious pest in a field. Due to the ease of weed control with herbicides, farmers now grow one or a few crops every year. Although the farmer can achieve short-term financial profits, in the long term this practice leads to selection for resistant weeds. In the case of zero- or minimum-tillage systems, the reliance on and the frequent use of herbicides are two factors that encourage the development of herbicide resistance (Matthews 1991). In a crop rotation system, a weed has a lesser chance of becoming established compared to a system of continuous production with the same crop. In the latter case, the grower may be allowing weeds that were not controlled initially to reach serious levels. Thus, perennial weeds tend to become established in perennial crops or in no-till systems, and annual weeds tend to infest annual crops. In the case of onion (*Allium cepa* L.) and carrot (*Daucus carota* L.) fields, weeds such as prostrate

spurge (*Euphorbia humistrata* Engelm.) and common purslane (*Portulaca oleracea* L.) can reach serious infestation levels if not properly controlled. Prostrate spurge and common purslane have a prostrate spreading growth habit that makes their control difficult. These weeds are also prolific seed producers that shed seeds throughout the growing season. Common purslane also can produce adventitious roots when stems are broken by tillage or hoeing. Therefore, hand-weeding may not be of much benefit unless the weeds are removed from the field.

A carrot grower in Imlay City, Michigan, has been growing carrots continually for the last 25 years. Lack of crop rotation and the continuous use of the same herbicides have led to the establishment of common purslane that was no longer controlled by the application of linuron.

COMMON PURSLANE

Common purslane is a member of the Portulacaceae family that comprises 25 genera of mostly herbs and shrubs (Hyam and Pankhurst 1995). The Portulacaceae family is in the order Caryophyllales and is related to the Cactaceae and the Aizoaceae (Cronquist 1981). Common purslane has smooth, purplish-red, and fleshy cotyledons with fleshy and glabrous stems. The site of origin of common purslane is probably western Asia. It was introduced into the United States from southern Europe in mid 1800s (Anon. 1972). However, Holm et

al. (1977) proposed that common purslane may have originated in North Africa.

Salisbury (1961) believed that common purslane was introduced into many parts of the world as a vegetable food plant. Montgomery (1964) found evidence that common purslane was reported in Massachusetts as early as 1672. Holm et al. (1977) listed the sole use of common purslane as a food for pigs. However, it still is used widely as a salad vegetable in Europe, Latin America, Asia, and the Middle East (Hopen 1984).

Common purslane is one of 12 non-cultivated species that have been most successful in colonizing new areas with seeds spread by wind, water, and with crop seeds (Holm et al. 1977). The average life span of a plant is approximately 3 months, and the onset of low temperatures in autumn initiates senescence in most plants (Kiyoko and Cavers 1980).

Distinguishing characteristics of common purslane are:

1. Prostrate, reddish, and fleshy stems.
2. A watery sap.
3. Succulent leaves which are broad-rounded at the tips.
4. Small yellow flowers.
5. Conical capsules, bearing many seed each, that open around the middle (Holm et al. 1977).

Common purslane seeds can survive about 40 years in the soil (Darlington and Steinbauer 1961). Hopen (1972) found

that a higher population of common purslane plants developed on a smooth seedbed than on a rough seedbed. The highest germination was achieved when seeds were placed on the soil surface. High soil temperature is required for optimal germination. Growth was dependent on adequate phosphorus in the nutrient media.

Common purslane thrives in cultivated fields, gardens, and in other locations such as driveways and abandoned fields. The plant prefers an open habitat, and does well on many soil types. Common purslane is distributed throughout temperate and tropical areas of the world (Holm et al. 1977). In the United States, it is found in all states but is least common in the Pacific Northwest (Anon. 1970).

Common purslane is a serious weed problem in cultivated fields. It ranked as the second most important weed in onion and fourth in importance in tobacco (*Nicotiana tabacum* L.) (Vengris 1953). In potato (*Solanum tuberosum* L.) and corn (*Zea mays* L.), common purslane ranked eighth and ninth, respectively. Competitiveness is related to its succulent nature and to adventitious roots that readily form after plants are cut or broken (Connard and Zimmerman 1931). Plants have relatively small stomata on abaxial and adaxial surfaces of the leaf, specialized water storage tissues in the stem and leaves, thick cuticles, and many taproots with extensive secondary roots that spread close to the surface (Vengris et al. 1972).

Biological controls of common purslane do exist. Work by Cruttwell-McFadyen and Bennett (1995) showed that a number of polyphagous insects are potential biocontrol agents of common purslane. *Asphondylia portulacae* Möhn, a flower gall midge, and *Neolasioptera portulacae* Cook, a stem gall midge, have common purslane as their only hosts (Gagné 1994). The female *Asphondylia* inserts one egg into a flower bud, which will develop abnormally and will not produce seed. On the other hand, the female *Neolasioptera* lays several eggs in the stem galls of common purslane. The galls are elongate to globular up to 1.5 cm in diameter. The damage can retard or may completely prevent flower and seed formation (Cruttwell-McFadyen and Bennett 1995). Other insects have common purslane as one of their hosts, such as *Apion* sp. (D'Araújo et al. 1968), a flower gall weevil, and *Coleophora* sp., a case-bearer moth. Hopen (1984) indicated that purslane sawfly (*Schizocerella pilicornis*) can be an effective control agent of common purslane especially after the third generation of the season. By the fourth generation, most of common purslane plants were stripped of their leaves. Two types of purslane sawfly larvae were described by Hopen (1984). One is a leaf-miner larva that eats the leaves from within; the other feeds from the outside but can eat twice as much as the leaf-miner larva. If control of common purslane is desired, pesticides should be applied to the soil during the pupa stage of the leaf

miner so as not to kill the sawfly. Pesticide applications on common purslane will kill all feeding larvae.

Common purslane is one of many weeds and crops that have the C_4 pathway of photosynthesis. The C_4 pathway occurs in at least 13 families, 117 genera, and 485 species of the Angiospermae. Families with C_4 representatives include Aizoaceae, Amaranthaceae, Chenopodiaceae, Asteraceae, Portulacaceae, and Gramineae, among others (Downton 1975). Characteristics of C_4 plants are:

1. A four-carbon acid is the initial product of CO_2 fixation.
2. Kranz anatomy: a radial arrangement of chlorenchyma around vascular bundles. Chlorenchyma is differentiated into an inner layer of large and thick-walled cells surrounded by layers of palisade-like cells (Laetsch 1974).
3. Low O_2 compensation point.
4. No effect of low O_2 concentration on photosynthetic rate.

Laetsch and Kortschak (1972) found that senescent leaves of common purslane could simultaneously carry on C_3 and C_4 photosynthesis. This suggested no causal relation between the cellular structure or Kranz anatomy and CO_2 fixation pathways. Under water stress or short photoperiods, common purslane was capable of developing an acid metabolism

similar to plants having the Crassulacean acid metabolism (CAM) (Koch and Kennedy 1980).

Various aspects of the C_4 physiology of common purslane have been studied to understand its competitive ability. These include studies on C_4 photosynthetic pathway (Kennedy 1977; Kennedy and Laetsch 1973), enzyme activity (Kennedy 1976 and 1977), compensation point (Kennedy 1977; Treguna and Downton 1967), anatomy and cytology (Kennedy 1973), photorespiration (Kennedy 1976), photosynthesis rate, and response to salt and water stress (Kennedy 1977).

HERBICIDES

Photosystem II (PS II) Inhibitors

Although herbicide resistance was not a concern in the 1950s, predictions that herbicide resistance would eventually develop in weeds circulated in the scientific community (Abel 1954; Blackman 1950; Harper 1956). The fact that pesticide resistance had already appeared in insects and pathogens helped support predictions of herbicide resistance. Herbicide resistance was not a production issue because growers still did a considerable amount of hand-weeding and cultivation and did not rely completely on herbicides. It was not until 1968 that a biotype of common groundsel was confirmed to be highly resistant to simazine (Ryan 1970).

A worldwide survey completed in 1997 indicated that at least 195 weed biotypes are resistant to various classes of

herbicides (Heap 1998). Of those, 60 weed species (42 dicots, 18 monocots) had biotypes exhibiting triazine resistance. Worldwide, it is estimated that over three million hectares are infested with triazine-resistant weeds (Holt and LeBaron 1990), making this the most widespread resistance problem. Most of the resistant biotypes were found in monoculture maize fields in North America (LeBaron and McFarland 1990; Stephenson et al. 1990), or in orchards in Europe where simazine had been applied repeatedly for several years (LeBaron 1991).

Mode of Action of PS II Inhibitors. In the 1950s, the phenylurea monuron (Wessels and Van Der Veen 1956) and the *s*-triazine simazine (Moreland et al. 1959) were found to inhibit the Hill reaction in isolated chloroplasts. During the following decade, it was discovered that these chemicals block the Hill reaction by inhibiting the reducing end of electron transport of PS II (Duysens and Sweers 1963; Murata et al. 1966). A number of other chemicals were found to act in a similar manner. These include the uracils, triazinones, biscarbamates, nitriles, nitrophenols, substituted pyridazinones, phenylcarbamates, and cycloacrylates (Dodge 1991; Fuerst and Norman 1991; Phillips and Huppatz 1984). Some of these also have other modes of action. For example, the nitrophenols and nitriles act as uncouplers and the substituted pyridazinones inhibit fatty

acid desaturation and carotenoid biosynthesis (Fuerst and Norman 1991).

Target Site of PS II Inhibitors. Tischer and Strotman (1977) reported that the various PS II inhibitors compete for a common binding site on the thylakoid membranes of chloroplasts. Pfister et al. (1981), Oettmeier et al. (1984), and Boschetti et al. (1985), used photoaffinity labeling studies with the radioactive analogs (3-[^{14}C]azido derivatives) of atrazine and monuron. They demonstrated that the common binding site of these two herbicides was a 32-kDa protein, currently known as the D1 protein.

A schematic representation of the PS II reaction center is presented in Figure 1. According to this model, PS II-inhibiting herbicides act by displacing the plastoquinone at the Q_B binding site on the D1 protein, thereby blocking electron flow from Q_A to Q_B. On the basis of a stereochemical model, Gardner (1989) proposed that the triazines and ureas act as nonreducible analogs of plastoquinone, whereas the phenol-type herbicides (nitriles, dinitrophenols) act as non-reducible analogs of the semiquinone anion of plastoquinone.

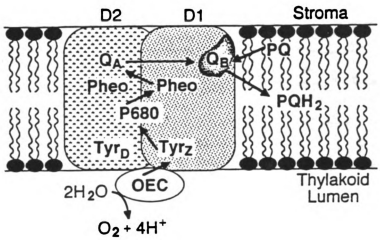

Figure 1. A schematic representation of the photosystem II reaction center complex and of the electron flow during normal photosynthesis (From Fuerst and Norman 1991). Arrows indicate the direction of electron flow. Abbreviations: OEC, oxygen-evolving complex; Tyr_z, tyrosine residue 161 that acts as electron donor to P680; P680, reaction center chlorophyll *a* dimer; Pheo, pheophytin; Q_A, plastoquinone tightly bound to the D2 protein; Q_B, exchangeable plastoquinone bound to the D1 protein; PQ, plastoquinone; PQH_2, plastohydroquinone.

According to current models describing the interactions of plastoquinone and PS II herbicides at the site of action, plastoquinone binds in the Q_B niche by hydrogen bonds between the two carbonyl oxygen molecules of plastoquinone and the amide backbone of histidine-215 and the hydroxyl of serine-264 of the D1 protein (Fuerst and Norman 1991; Tietjen et al. 1991; Trebst 1987). On the other hand, atrazine binds in the Q_B niche by hydrogen bonding with serine-264 and phenylalanine-265 as well as hydrophobic interactions with phenylalanine-255 (Figure 2).

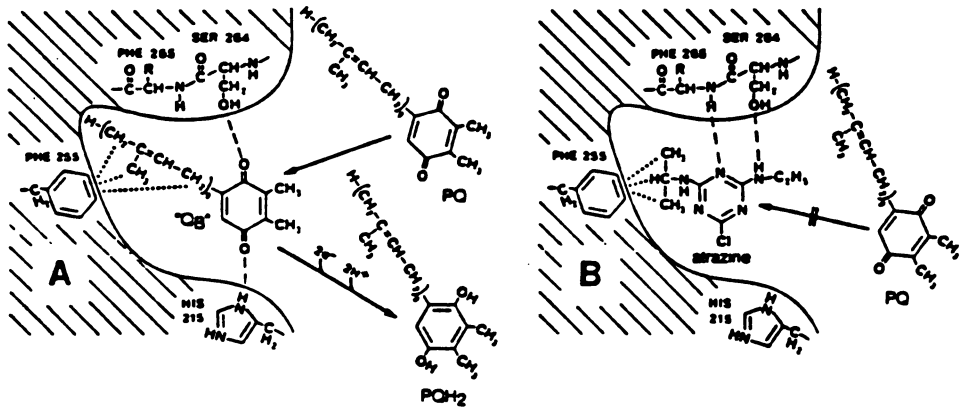

Figure 2. Schematic of the interaction of plastoquinone (A) and atrazine (B) in the Q_B-binding niche of the D1 protein (From Fuerst and Norman 1991). Hydrogen bonds and hydrophobic interactions are represented by dashed and dotted lines, respectively. Atrazine binding in the Q_B niche prevents the binding of plastoquinone. Abbreviations: PQ, plastoquinone: Q_B, bound plastoquinone; PQH_2, plastohydroquinone.

Trebst (1987) categorized herbicides that bind in the Q_B niche into two families based on their interaction with amino acids at this site: urea/triazine herbicides exhibiting a strong interaction with serine-264, and phenol herbicides interacting strongly with histidine-215.

Herbicidal Activity of PS II Inhibitors. It is generally accepted that PS II inhibitors block photosynthetic-electron transport and hence prevent the reduction of $NADP^+$ required for CO_2 fixation. However, this does not by itself result in death of susceptible plants. The ultimate death of plants is due to the oxidative stress generated when

photosynthetic-electron transport is blocked in the presence of PS II inhibitors. The net effect of blocking the electron transport is the destruction of the PS II reaction center and the photo-oxidation of lipid and chlorophyll molecules (Barry et al. 1990; Pallett and Dodge 1980).

Another herbicidal activity of PS II inhibitors was postulated by Gaba et al. (1987), Mattoo et al. (1989), Kuhn and Böger (1990), and by Gong and Ohad (1991). They suggested that the binding of diuron and atrazine at the Q_B site interferes with the degradation of the D1 protein, thus reducing the turnover rate of damaged D1 proteins. Herbicide-binding may block the access of a protease to the cleavage site near the Q_B niche, or it may cause a conformational change that restricts accessibility to this site. According to this hypothesis, the photo-damage to the D1 protein by the binding of the triazines and ureas at the Q_B niche prevents electron transfer from Q_A to Q_B, and is compounded by preventing the replacement or repair of the damaged D1 protein. Resistance to the PS II inhibitors is maternally inherited (Hirschberg and McIntosh 1983; Souza-Machado et al. 1978), which suggests that this trait is encoded by the chloroplast genome. Inheritance of the resistance trait is thus possible through seed only.

Mechanisms of Resistance to PS II Inhibitors in Weeds. To date, only two mechanisms of resistance to PS II-inhibiting herbicides have been identified in weeds, namely modified

target site and enhanced detoxification. No evidence exists for resistance to PS II inhibitors due to reduced absorption or translocation, sequestration or compartmentation, or due to repair of the toxic effects of herbicides.

Target-site based resistance. Resistance to PS II-inhibiting herbicides is due to a modification of the D1 protein of the PS II complex (LeBaron and McFarland 1990). This modification reduces the affinity of PS II herbicides at this site so that they no longer can compete effectively for the exchangeable plastoquinone Q_B. The most common form of modification at the target site is a substitution of glycine for serine at position 264 (Fuerst and Norman 1991; Mets and Thiel 1989; Trebst 1991). This modification was found to cause a 1000-fold reduction in atrazine affinity at the Q_B-binding site and greater than a 100-fold increase in atrazine resistance at the whole-plant level (Fuerst et al. 1986; Pfister and Arntzen 1979). Associated with the serine-264 to glycine mutation are a number of pleiotropic characteristics, such as a modified galactolipid composition and an increase in the degree of unsaturation of fatty acids (Lehoczki et al. 1985; Pillai and St. John 1981), reduced growth rate (Hobbs 1987), a high level of resistance to s-triazines, moderate level of resistance to triazinones, and little or no resistance to phenylureas such as diuron (Fuerst et al. 1986; Pfister and Arntzen 1979). Moreover, chloroplasts in triazine-resistant weeds were found to be similar to "shade chloroplasts", which develop under low

light intensities, with increased grana stacking and a reduced chlorophyll *a/b* ratio.

In tobacco and potato, a serine-264 to threonine mutation of the *psb*A gene has been selected in tissue culture (Sigematsu et al. 1989; Smeda 1990). In contrast to the more common serine-264 to glycine mutation, the serine-to-threonine mutation confers resistance to both atrazine and diuron. Other mutations conferring resistance to PS II inhibitors were selected in algae, in a cyanobacterium, and in higher plants. Depending on the altered residue, different levels and patterns of resistance are observed (Table 1).

Table 1. Amino acid alterations in the D1 protein of herbicide-resistant mutants and the type of resistance conferred (From Trebst 1991).

Amino Acid Alteration	Resistant to:	Organism	Refs.
Single Mutations:			
Phe 211 → Ser	atrazine (2.1X)/ DCMU (17X)	*Synechococcus*	(1)
Val 219 → Ile	metribuzin/DCMU/ ioxynil	*Chlamydomonas*	(2)
		Synechococcus	(1)
Ala 251 → Val	metribuzin/ atrazine (25X)/ diuron (5X)	*Chlamydomonas*	(8)

Table 1. (cont'd).

Amino Acid Alteration	Resistant to:	Organism	Refs.
Phe 255 → Tyr	atrazine (15X)/ Cyanoacrylate/ diuron (0.6X)	*Chlamydomonas* *Synechococcus*	(2) (4,12)
Gly 256 → Asp	atrazine/DCMU/ bromacil	*Chlamydomonas*	(2)
Ser 264 → Gly	atrazine (1000X)	*Amaranthus* *Anacystis*	(5) (6)
Ser 264 → Ala	metribuzin/ atrazine (84X)	*Anacystis* *Chlamydomonas* *Synechocystis*	(6) (2,3) (7)
Ser 264 → Thr	triazine (560X) diuron (40X)	*Nicotiana* *Euglena*	(9) (13)
Ser 264 → Asn	triazine	*Nicotiana*	(10)
Asn 266 → Thr	ioxynil	*Synechocystis*	(11)
Leu 275 → Phe	metribuzin/ bromacil/DCMU	*Chlamydomonas*	(2)
Double Mutations:			
Phe 255 → Tyr/ Ser 264 → Ala	urea/triazine	*Synechococcus*	(14)
Phe 255 → Leu/ Ser 264 → Ala	DCMU and reversal atrazine tolerance	*Synechocystis*	(15)
Phe 211 → Ser/ Ala 251 → Val	atrazine	*Synechocystis*	(15)

1 = Gingrich et al. 1988; 2 = Erickson et al. 1989;
3 = Pucheu et al. 1984; 4 = Ohad et al. 1987;
5 = Hirschberg and McIntosh 1983;
6 = Golden and Haselkorn 1985; 7 = Astier et al. 1986;
8 = Johanningmeier et al. 1987; 9 = Sato et al. 1988;
10 = Páy et al. 1988; 11 = Ajlani et al. 1989a;
12 = Hirschberg et al. 1987; 13 = Aiach et al. 1989;
14 = Horovitz et al. 1989; 15 = Ajlani et al. 1989b.

Enhanced metabolism. In the 1980s, a different type of resistance to PS II herbicides was found. In black-grass (*Alopecurus myosuroides* Huds.) biotypes found in England (Kemp and Caseley 1987; Moss and Cussans 1985) and Germany (Niemann and Pestemer 1984), in a rigid ryegrass (*Lolium rigidum* Gaud.) biotype found in Australia (Powles et al. 1990; Burnet et al. 1991), and in a velvetleaf (*Abutilon theophrasti* Medic.) biotype found in Maryland (Gronwald et al. 1989), resistance was determined to be due to enhanced herbicide detoxification. In these cases, resistant weeds "evolved" detoxification mechanisms similar to those commonly found in resistant crops such as corn. Although velvetleaf was resistant to simazine and atrazine, it was not cross-resistant to bentazon, cyanazine, linuron, or metribuzin.

Ecological Fitness of Resistant PS II Mutants. Most studies indicated that the serine-264 to glycine mutation of the *psb*A gene caused a significant reduction in relative ecological fitness of mutant plants. Triazine-resistant biotypes exhibited a reduction in CO_2 fixation, quantum yield, and seed and biomass production (Bowes et al. 1980; Conard and Radosevich 1979; Holt 1990; Holt et al. 1981; Jursinic and Pearcy 1988). In a few studies, there were no differences in fitness (Schönfeld et al. 1987). In others the resistant biotype was found more fit (Jansen et al. 1986). However, recent investigations conducted with near

isonuclear lines of canola (*Brassica napus* L.) (Gressel and Ben-Sinai 1985), common groundsel (van Oorschot and Van Leeuwen 1984), and black nightshade (*Solanum nigrum* L.) (Jacobs et al. 1988) clearly indicated that the *psb*A gene mutation reduced fitness. Studies conducted on common groundsel (McCloskey and Holt 1990 and 1991) also demonstrated that differences in the nuclear genome could compensate to some extent for reduced fitness conferred by the *psb*A mutation.

Although there is considerable evidence that the *psb*A mutation reduces plant fitness, there is a lack of agreement concerning the specific mechanism. The serine-264 to glycine mutation reduces the rate of electron transfer between Q_A and Q_B. However, it is still unclear how this is related to the reduction in photosynthetic capacity or relative fitness (Gronwald 1994). In addition, there are conflicting reports as to whether the serine-264 to glycine mutation reduces the whole-chain electron transport rate in isolated chloroplasts (Holt et al. 1981; Ort et al. 1983; Stowe and Holt 1988). However, a study with isonuclear lines of resistant and susceptible canola clearly showed that the slower rate of electron transfer in the resistant biotype was responsible for lower quantum yield and a lower maximum rate of photosynthesis. The lower quantum yield was attributed to the inefficient use of the separated charge in the PS II reaction center (Jursinic and Pearcy 1988).

Subsequent evidence suggested that the altered kinetics of Q_A to Q_B electron transfer associated with the serine-264 to glycine mutation increases susceptibility of PS II to photoinhibition. Kyle (1987) proposed that the slower rate of Q_A to Q_B electron transfer in triazine-resistant biotypes might increase the likelihood of radical formation in the Q_B-binding niche of the PS II reaction center. This was confirmed by Barber and Andersson (1992), who found that increased susceptibility to photoinhibition reduced fitness, particularly under high light environments, because of the high energy cost associated with the turnover of the D1 protein.

ALS Inhibitors

In 1982, chlorsulfuron was the first ALS inhibitor introduced for use in cereals. Due to the low use rates, sound environmental properties, low mammalian toxicity, wide crop selectivity, and high efficacy, the market share of all ALS inhibitors in 1991 was estimated at about $1.3 billion (Anon. 1991).

The mode of action of ALS-inhibiting herbicides is the inhibition of the acetolactate synthase enzyme present in the chloroplast. This is an important enzyme, because it is the first enzyme common to the biosynthesis of the branched-chain amino acids valine, leucine, and isoleucine (Saari et al. 1991). Sulfonylureas and other ALS inhibitors directly inhibit ALS activity.

The first reported case of resistance to an ALS inhibitor was that of chlorsulfuron resistance in prickly lettuce (*Lactuca serriola* L.) five years after its initial use (Mallory-Smith et al. 1990a). Forty-one weed species (32 dicots and 9 monocots) in 11 countries were documented to be resistant to ALS inhibitors (Heap 1998). Resistance in weeds is mainly due to an insensitive ALS enzyme (Saari et al. 1990 and 1992). The only example of resistance to ALS inhibitors due to enhanced metabolism in weeds was found in rigid ryegrass (Christopher et al. 1992). In contrast, crop tolerance to ALS inhibitors is due to the crops' ability to metabolize the herbicide rapidly, thus preventing it from reaching lethal levels (Brown and Nabers 1987; Sweetser et al. 1982; Takeda et al. 1986). Enhanced metabolism is found also in naturally occurring resistant weeds, such as black nightshade (Hutchison et al. 1984) and giant foxtail (*Setaria faberi* Herrm.)(Sweetser et al. 1982).

The high efficacy of ALS inhibitors, which originally made these herbicides very popular, was the main selection force for weed resistance. The long residual activity of many ALS inhibitors also increases the intensity and duration of the selection pressure, thus contributing to the rapid development of resistant weeds. In addition, continuous use of herbicides with the same mode of action such as the ALS inhibitors in monoculture or non-crop areas is a major factor contributing to the rapid development of

resistance in weeds (Mallory-Smith et al. 1991; Primiani et al. 1990).

What are the genetic factors behind the rapid development of weed resistance to ALS inhibitors? The initial frequency of resistant mutants in the field is an important variable. Mathematical models (Gressel and Segel 1990; Maxwell et al. 1990; Mortimer et al. 1992) suggested that an initial high frequency of resistant mutants will result in a more rapid concentration of resistant weeds in the field after only a few years of applications. Work on mouse-ear cress (*Arabidopsis thaliana* L.) (Haughn and Somerville 1987), alfalfa (*Medicago sativa* L.) (Stannard 1987), and tobacco (Mauvais 1989), suggested that the mutation frequency of ALS-inhibitor resistance is around 10^{-6} to 10^{-7}. However, Saari et al. (1991) proposed that the rapid appearance of resistance is not due to this initial high frequency of ALS mutations, but rather to the high selection pressure imposed by the ALS inhibitors on weeds.

The mode of inheritance for the resistance trait is another variable. Thompson and Thill (1992) found that resistance in Kochia (*Kochia scoparia* L.) is inherited as a dominant, nuclear trait. Mallory-Smith et al. (1990b) also found that sulfonylurea resistance in prickly lettuce was controlled by a single gene with incomplete dominance. Thus, immigration of resistant pollen or seed from infested fields could increase the proportion of resistant mutants, even in the absence of any selection pressure (Saari et al.

1991). The high selection pressure combined with weed characteristics such as high seed production, rapid and frequent seed germination, and open pollination, will accelerate the selection of resistant biotypes.

Resistance to ALS inhibitors in weeds developed more rapidly than resistance to other classes of herbicides (Rubin et al. 1992). About four to seven years elapsed between the initial use of an ALS inhibitor and the detection of a resistant weed biotype (Christopher et al. 1992; Pappas-Fader et al. 1993; Saari et al. 1992). In comparison, the first case of weed resistance to the photosynthetic inhibitors was about 10 years after their introduction. Differences do exist between the ALS inhibitors and the photosynthetic inhibitors in terms of mode of action and selection pressure. Still, resistance to both classes of herbicides is due to a mutation at the target site, making it less sensitive to the herbicide.

What are the causes for the time differential for resistance development between these two classes of herbicides? One plausible explanation is the difference in frequency of their use. Changes in management practices between the late 1950s, when photosynthetic inhibitors were first introduced, and the 1980s, when the ALS inhibitors were first introduced are also contributing factors. Tillage, hand-weeding, cover crops, fallow land, and crop rotation were still practiced, even after the advent of photosynthetic inhibitors. In the case of the ALS

inhibitors, monoculture and herbicide use were established already and commonly used by the majority of farmers.

Another possible explanation is the differential mode of inheritance between the ALS inhibitors and the photosynthetic inhibitors. ALS inhibitors are inherited by a single nuclear gene with incomplete dominance. This allows the resistance gene to be distributed by pollen and by seed. Dissemination of resistance via pollen could significantly hasten the development of resistance in previously non-infected fields, even before any ALS inhibitors were used. Resistance to the photosynthetic inhibitors, on the other hand, is maternally inherited and can only move through seed, thus limiting the time and range of dissemination of resistant mutants.

MANAGEMENT PRACTICES FOR RESISTANT WEEDS

Once resistant weeds are established, weed control strategies should shift more towards reducing the spread and severity of resistant-weed infestations and less towards comprehensive weed control.

The integration of simple, yet effective, management practices will help keep resistant weeds under control. Such practices include:

1. Rotating with herbicides having different modes of action.
2. Hand-weeding and cultivation in addition to herbicides.
3. Reducing the weed seed bank.

4. Preventing infestation of new fields by cleaning tillage and harvesting equipment between fields.
5. Planting weed-free certified seed.
6. Rotating crops.
7. Growing herbicide-resistant crops in the rotation, and applying broad-spectrum herbicides such as glyphosate.

Monitoring fields regularly for first signs of weed resistance could reduce establishment of resistant weeds. Additional integrated weed management practices were suggested by Matthews (1991), such as the planned use of non-selective herbicides and delayed or out of season planting.

All these practices have not yet eliminated the occurrence or spread of weed resistance. The major reasons behind that are the incompatibility between weed management practices and the desire by growers for short-term financial profit through complete weed control.

CONTROL PRACTICES FOR RESISTANT WEEDS

The resistance of common purslane to PS II inhibitors is an example of a mutation to a single class of herbicides. In such cases where resistance is due to a target-site mutation, control of resistant weeds should not be difficult. Matthews (1991) suggested using herbicides with alternative modes of action, or herbicides to which there is

no known resistance as a means to the successful control of resistant biotypes.

SUMMARY

Although resistance to ALS inhibitors appeared sooner than did the resistance to the PS II inhibitors, it can be argued that both classes of herbicides have the same potential for developing herbicide resistance in weeds. This is easily explained when considering the mode of action of the two herbicide classes or the genetic control of the resistance, as explained above. The only significant difference between the two classes of herbicides is that resistance to the ALS inhibitors is encoded by a nuclear gene, and thus can be transmitted by both seed and pollen, whereas the resistance to PS II inhibitors is encoded by the chloroplast and thus can be transmitted only maternally via the seed.

LITERATURE CITED

Abel, A. L. 1954. The rotation of weedkillers. *In*: Proceedings of the British weed control conference. Farnham, U.K.: The British Crop Protection Council. pp. 249-255.

Aiach, A., U. Johanningmeier, and E. Ohmann. 1989. A *psb*A mutation in diuron resistant *Euglena* leads to a change at ser 264 to thr in the D-1 protein. (In preparation).

Ajlani, G., D. Kirilovsky, M. Picaud, and C. Astier. 1989a. Molecular analysis of *psb*A mutations responsible for various herbicide resistance phenotypes in *Synechocystis* 6714. Plant Mol. Bio. 13:469-480.

Ajlani, G., I. Meyer, C. Vernotte, and A. Astier. 1989b. Mutation in phenol-type herbicide resistance maps within the *psb*A gene in *Synechococcus* 6714. FEBS Letters. 246: 207-210.

Anon. 1970. Selected weeds of the United States. Washington, DC: United States Department of Agriculture. 463 p.

Anon. 1972. Extent and cost of weed control with herbicides and an evaluation of important weeds. Washington, DC: United States Department of Agriculture. 277 p.

Anon. 1991. County NatWest WoodMac (Wood MacKenzie), The *NatWest Investment Bank Group*, London.

Astier, C., I. Meyer, C. Vernotte, and A. L. Etienne. 1986. Photosystem II electron transfer in highly herbicide resistant mutants of *Synechocystis* 6714. FEBS Letters. 207:234-238.

Barber, J. and B. Andersson. 1992. Too much of a good thing: Light can be bad for photosynthesis. TIBS 17:61-66.

Barry, P., A. J. Young, and G. Britton. 1990. Photodestruction of pigments in higher plants by herbicide action. I. The effect of DCMU (diuron) on isolated chloroplasts. J. Exp. Bot. 41:123-129.

Blackman, G. E. 1950. Selective toxicity and the development of selective weedkillers. J. R. Soc. Arts 98:499-517.

Boschetti, A., M. Tellenbach, and A. Gerber. 1985. Covalent binding of 3-azido-monuron to thylakoids of DCMU-sensitive and -resistant strains of *Chlamydomonas reinhardtii*. Biochim. Biophys. Acta 810:12-19.

Bowes, J., A. R. Crofts, and C. J. Arntzen. 1980. Redox reactions on the reducing side of photosystem II in chloroplasts with altered herbicide binding properties. Arch. Biochim. Biophys. 200:303-308.

Brown, H. M. and S. M. Nabers. 1987. Soybean metabolism of chlorymuron ethyl: physiological basis for soybean selectivity. Pestic. Biochem. Physiol. 29:112-120.

Burnet, M. W. M., O. B. Hildebrand. J. A. M. Holtum, and S. B. Powles. 1991. Amitrole, triazine, substituted urea, and metribuzin resistance in a biotype of rigid ryegrass (*Lolium rigidum*). Weed Sci. 39:317-323.

Christopher, J. T., S. B. Powles, and J. A. M. Holtum. 1992. Resistance to acetolactate synthase-inhibiting herbicides in annual ryegrass (*Lolium rigidum*) involves at least two mechanisms. Plant Physiol. 100:1909-1913.

Conard, S. G. and S. R. Radosevich. 1979. Ecological fitness of *Senecio vulgaris* and *Amaranthus retroflexus* biotypes susceptible or resistant to atrazine. J. Appl. Ecol. 16:171-177.

Connard, M. H. and P. W. Zimmerman. 1931. Origin of adventitious roots in cuttings of *Portulaca oleracea*. Contrib. Boyce Thomplson Inst. Plant Res. 3:337-346.

Cronquist, A. J. 1981. An integrated system of classification of flowering plants. Columbia Univ. Press, New York.

Cruttwell-McFadyen, R. E. and F. D. Bennett. 1995. Potential biocontrol agents of *Portulaca oleracea* L. from the Neotropics. Biological control. 5:189-195.

D'Araújo, S. A. G., C. R. Gonçalves, D. M. Galvão, A. J. L. Gonçalves, J. Gomes, M. N. Silva, and L. Simoni. 1968. Quarto Cátalogo dos insetos que vivem nas plantas do Brasil: Seus parasitos e predadores. Parte II. 1 Tomo, "Insetos, Hospedeiros e Inimigos Naturais", Min. da Agric., Depto. Do Def. E Inspeção Agropecuária. Rio de Janeiro. Brasil.

Darlington, H. T. and G. P. Steinbauer. 1961. The eighty-year period for Dr. Beal's seed viability experiment. Am. J. Bot. 48:321-325.

Dodge, A. D. 1991. Photosynthesis. *In*: Target Sites for Herbicide Action, R. C. Kirkwood, Ed. Plenum Press, New York. pp. 1-27.

Downton, W. J. S. 1975. The occurrence of C_4 photosynthesis among plants. Photosynthetica 9(1):96-105.

Duysens, L. N. M. and H. E. Sweers. 1963. Mechanism of two photochemical reactions in algae as studied by means of fluorescence. *In*: Studies on Microalgae and Photosynthetic Bacteria. Jpn. Soc. Plant Physiol. Tokyo: University of Tokyo Press. pp. 353-372.

Erickson, J. M., K. Pfister, M. Rahire, R. K. Togasaki, L. Mets, and J.-D. Rochais. 1989. Molecular and biophysical analysis of herbicide-resistant mutants of *Chlamydomonas reinhardtii*: Structure-function relationship of the photosystem II D1 polypeptide. The Plant Cell. 1:361-371.

Fuerst, E. P. and M. A. Norman. 1991. Interactions of herbicides with photosynthetic electron transport. Weed Sci. 39:458-464.

Fuerst, E. P., C. J. Arntzen, K. Pfister, and D. Penner. 1986. Herbicide cross-resistance in triazine-resistant biotypes of four species. Weed Sci. 34:344-353.

Gaba, V., J. B. Marder, B. M. Greenberg, A. K. Mattoo, and M. Edelman. 1987. Degradation of the 32 kD herbicide binding protein in far red light. Plant Physiol. 84:348-352.

Gagné, R. J. 1994. The gall midges of the neotropical region. Cornell Univ. Press, Ithaca, New York.

Gardner, G. 1989. A stereochemical model for the active site of photosystem II herbicides. Photochem. Photobiol. 49:331-336.

Gingrich, J. C., J. S. Buzby, V. L. Stirewalt, and D. A. Bryant. 1988. Genetic analysis of two new mutations resulting in herbicide resistance in the cyanobacterium *Synechococcus* sp. PCC 7002. Photosynthesis Research. 16:83-99.

Golden, S. S. and R. Haselkorn. 1985. Mutation to herbicide resistance maps within the *psb*A gene of *Anacystis nidulans* R2. Science. 229:1104-1197.

Gong, H. and I. Ohad. 1991. The PQ/PQH_2 ratio and occupancy of photosystem II-Q_B site by plastoquinone control the degradation of D1 protein during photoinhibition *in Vivo*. J. Biol. Chem. 266:21293-21299.

Gressel, J. and G. Ben-Sinai. 1985. Low intraspecific competitive fitness in a triazine-resistant, nearly nuclear-isogenic line of *Brassica napus*. Plant Sci. 38:29-32.

Gressel, J. and L. A. Segel. 1990. Herbicide rotations and mixtures: effective strategies to delay resistance. *In* Managing Resistance to Agrochemicals: From Fundamental Research to Practical Strategies. M. B. Green, H. M. LeBaron, and W. K. Moberg, Eds. (Washington , D.C.: American Chemical Society). pp. 430-458.

Gronwald, J. W. 1994. Resistance to Photosystem II inhibiting herbicides. *In*: Herbicide Resistance in Plants: Biology and Biochemistry, pp. 27-60. Lewis Publishers.

Gronwald, J. W., R. N. Andersen, and C. Yee. 1989. Atrazine resistance in velvetleaf (*Abutilon theophrasti*) due to enhanced atrazine detoxification. Pestic. Biochem. Physiol. 34:149-163.

Harper, J. L. 1956. The evolution of weeds in relation to resistance to herbicides. Proceedings of the British weed control conference. Farnham, U.K.: The British Crop Protection Council. pp. 179-188.

Haughn, G. and C. R. Somerville. 1987. Selection for herbicide resistance at the whole plant level. *In* Biotechnology in Agricultural Chemistry, Symposium series No. 334, H. M. LeBaron, R. O. Mumma, R. C. Honeycutt, and J. H. Duesing, Eds. (Washington, D.C.: American Chemical Society). pp. 98-107.

Heap, I. M. 1998. International survey of herbicide-resistant weeds. 1998 Herbicide-Resistance Action Committee Annual Report. WeedSmart, Corvallis, Oregon. 165 unnumbered pages.

Hirschberg, J. and L. McIntosh. 1983. Molecular basis of herbicide resistance in *Amaranthus hybridus*. Science. 222:1346-1348.

Hirschberg, J., N. Ohad, I. Pecker, and A. Rahat. 1987. Isolation and characterization of herbicide resistant mutants in the cyanobacterium *Synechococcus* R2. Z. Naturforsch. 39c:412-420.

Hobbs, S. L. A. 1987. Comparison of photosynthesis in normal and triazine-resistant *Brassica*. Can. J. Plant Sci. 67:457-466.

Holm, L. G., D. L. Plucknett, J. V. Pancho, and J. P. Herberger. 1977. *In*: The World's Worst Weeds: Distribution and Ecology. Honolulu, HI: University Press of Hawaii. 609 p.

Holt, J. S. 1990. Fitness and ecological adaptability of herbicide-resistant biotypes. *In*: Managing Resistance to Agrochemicals: From Fundamental Research to Practical Strategies. M. B. Green, H. M. LeBaron, and W. K. Moberg, Eds. Washington, D.C.: American Chemical Society Symposium Series. pp. 419-429.

Holt, J. S. and H. M. LeBaron. 1990. Significance and distribution of herbicide resistance. Weed Technol. 4:141-149.

Holt, J. S., A. J. Stemler, and S. R. Radosevich. 1981. Differential light responses of photosynthesis by triazine-resistant and triazine-susceptible *Senecio vulgaris* biotypes. Plant Physiol. 67:744-748.

Hopen, H. J. 1972. Growth of common purslane as influencing control and importance as a weed. Weed Science. 20:20-23.

Hopen, H. J. 1984. Common purslane, a problem weed in horticultural crops. Weeds Today. 15:5-6.

Horovitz, A., N. Ohad, and J. Hirschberg. 1989. Predicted effects on herbicide binding of amino acid substitutions in the D1 protein of photosystem II. FEBS Letters. 243:161-164.

Hutchison, J. M., R. Shapiro, and P. B. Sweetser. 1984. Metabolism of chlorsulfuron by tolerant broadleaves. Pestic. Biochem. Physiol. 22:243-247.

Hyam, R. and P. Pankhurst. 1995. Plants and their names, a concise dictionary. Oxford, Great Britain: Oxford University Press, 545 p.

Jacobs. B. F., J. H. Duesing, J. Antonovics, and D. T. Patterson. 1988. Growth performance of triazine-resistant and -susceptible biotypes of *Solanum nigrum* over a range of temperatures. Can. J. Bot. 66:847-850.

Jansen, M. A. K., H. Hobé, J. C. Wesselius, and J. J. S. van Rensen. 1986. Comparison of photosynthetic activity and growth performance in triazine-resistant and susceptible biotypes of *Chenopodium album*. Physiol. Veg. 24:475-484.

Johanningmeier, U., Bodner, and G. F. Wildner. 1987. A new mutation in the gene coding for the herbicide-binding protein in *Chlamydomonas*. FEBS Letters. 211:221-224.

Jursinic, P. A. and R. W. Pearcy. 1988. Determination of the rate limiting step for photosynthesis in a nearly isonuclear rapeseed (*Brassica napus* L.) biotype resistant to atrazine. Plant Physiol. 88:1195-1200.

Kemp, M. S. and J. C. Caseley. 1987. Synergistic effects of 1-aminobenzotriazole on the phytotoxicity of chlorotoluron and isoproturon in a resistant population of black-grass (*Alopecurus myosuroides*). Proceedings of the British crop protection conference-Weeds. Farnham, U.K.: The British Crop Protection Council. pp. 895-899.

Kennedy, R. A. 1973. Photosynthetic carbon metabolism in C_4 plants. Ph.D. Thesis. Univ. Calif., Berkley.

Kennedy, R. A. 1976. Relationship between leaf development, carboxylase enzyme activities and photorespiration in the C_4 plant *Portulaca oleracea* L. Planta 128:149-154.

Kennedy, R. A. 1977. The effects of NaCl-, polyethyleneglycol- and naturally-induced water stress on photosynthetic products, photosynthetic rates, and CO_2 compensation points in C_4 plants. Z. Pflanzenphysiol. 83:11-24.

Kennedy, R. A. and W. M. Laetsch. 1973. Relationship between leaf development and primary photosynthetic products in the C_4 plant *Portulaca oleracea* L. Planta 115:113-124.

Kiyoko, M. and P. B. Cavers. 1980. The biology of Canadian weeds. 40. *Portulaca oleracea* L. Can. J. Bot. 60:953-963.

Koch, K. and R. A. Kennedy. 1980. Characteristics of Crassulacean Acid Metabolism in the succulent C_4 dicot, *Portulaca oleracea* L. Plant Physiol. 65:193-197.

Kuhn, M. and P. Böger. 1990. Studies on the light-induced loss of the D1 protein in photosystem-II membrane fragments. Photosynth. Res. 23:291-296.

Kyle, D. J. 1987. The biochemical basis for photoinhibition of photosystem II. *In*: Topics in Photosynthesis, Vol. 9, Photoinhibition, D. J. Kyle, C. B. Osmond, and C. J. Arntzen, Eds. Amsterdam: Elsevier. pp. 197-226.

Laetsch, W. M. and H. P. Kortschak. 1972. Chloroplast structure and function in tissue cultures of a C_4 plant. Plant Physiol. 49:1021-1023.

Laetsch. W. M. 1974. The C_4 syndrome: a structural analysis. Ann. Rev. Plant Physiol. 25:27-52.

LeBaron, H. M. 1991. Distribution and seriousness of herbicide-resistant weed infestations worldwide. *In*: Herbicide Resistance in Weeds and Crops, J. C. Caseley, G. W. Cussans, and R. K. Atkin. Oxford: Butterworth-Heinemann. pp. 27-43.

LeBaron, H. M. and J. McFarland. 1990. Herbicide resistance in weeds and crops: An overview and prognosis. *In*: Managing Resistance to Agrochemicals: From Fundamental Research to Practical Strategies. M. B. Green, H. M. LeBaron, and W. K. Moberg, Eds. Washington, D.C.: American Chemical Society Symposium Series No. 421:336-352.

Lehoczki, E., E. Pölös, G. Laskay, and T. Farkas. 1985. Chemical composition and physical states of chloroplast lipids related to atrazine resistance in *Conyza canadensis* L. Plant Sci. 42:19-24.

Mallory-Smith, C. A., D. C. Thill, and L. L. Saari. 1991. Survey of sulfonylurea herbicide resistant agricultural sites in North America. *In* WSSA Abstracts, Vol. 31, Proceedings of the 1991 Meeting of the Weed Science Society of America (Champaign, IL: WSSA, 1991), p. 83.

Mallory-Smith, C. A., D. C. Thill, and M. J. Dial. 1990a. Identification of sulfonylurea herbicide-resistant prickly lettuce (*Lactuca serriola*). Weed Technol. 4:163-168.

Mallory-Smith, C. A., D. C. Thill, M. J. Dial, and R. S. Zemetra. 1990b. Inheritance of sulfonylurea herbicide resistance in *Lactuca* spp. Weed Technol. 4:787-790.

Matthews, J. M. 1991. Management of herbicide resistant weed populations. *In*: Herbicide Resistance in Plants: Biology and Biochemistry. Lewis Publishers. Boca Raton, FL. pp. 317-336.

Mattoo, A. K., J. B. Marder, and M. Edelman. 1989. Dynamics of the photosystem II reaction center. Cell 56:241-246.

Mauvais, C. 1989. DuPont Agricultural Products, Wilmington, DE. Unpublished results.

Maxwell, B. D., M. L. Roush, and S. R. Radosevich. 1990. Predicting the evolution and dynamics of herbicide resistance in weed populations. Weed Technol. 4:2-13.

McCloskey, W. B. and J. S. Holt. 1990. Triazine resistance in *Senecio vulgaris* parental and nearly isonuclear backcrossed biotypes is correlated with reduced productivity. Plant Physiol. 92:954-962.

McCloskey, W. B. and J. S. Holt. 1991. Effect of growth temperature on biomass production of nearly isonuclear triazine-resistant and -susceptible common groundsel (*Senecio vulgaris* L.). Plant Cell Environ. 14:699-705.

Mets, L. and A. Thiel. 1989. Biochemistry and genetic control of the photosystem II herbicide target site. *In*: Target Sites of Herbicide Action, P. Böger and G. Sandmann, Eds. CRC Press. Boca Raton, Fl. pp. 1-24.

Montgomery, F. H. 1964. Weeds of Canada and the northern United States. Toronto: Ryerson Press. 266 p.

Moreland, D. E., W. A. Gentner, J. L. Hilton, and K. L. Hill. 1959. Studies on the mechanism of herbicidal action of 2-chloro-4,6-bis(ethylamino)-*s*-triazine. Plant Physiol. 34:432-435.

Mortimer, A. M., P. F. Ulf-Hansen, and P. D. Putwain. 1992. Modelling herbicide resistance - a study of ecological fitness. *In* Resistance '91: Achievements and Developments in Combatting Pesticide Resistance, I. Denholm, A. L. Devonshire, and D. W. Hollomon, Eds. (London: Elsevier Applied Science). pp. 148-164.

Moss, S. R. and G. W. Cussans. 1985. Variability in the susceptibility of *Alopecurus myosuroides* (Black-grass) to chlortoluron and isoproturon. Aspects of Applied Biology 9, The Biology and Control of Weeds in Cereals. The Association of Applied Biologists, National Vegetable Research Station, Wellesbourne, Warwick, U.K. pp. 91-98.

Murata, N., M. Nishimura, and A. Takamiya. 1966. Fluorescence of chlorophyll in photosynthetic systems. II. Induction of fluorescence in isolated spinach chloroplasts. Biochim. Biophys. Acta. 120:23-33.

Nabers, S. and L. S. Privalle. 1990. Metabolism of primisulfuron by barnyardgrass. Pestic. Biochem. Physiol. 37:145-153.

Niemann, P. and W. Pestemer. 1984. Resistenz Verschiedener Herkunfte von Ackerfuchsschwans (*Alopecurus myosuroides*) Gegenuber Herbizidbehandlungen. Nachr. Dtsch. Pflanzenschutzdienst (Berlin) 36:113-118.

Oettmeier, W., K. Masson, H.-J. Soll, and W. Draber. 1984. Herbicide binding at photosystem II: A new azido-triazinone photoaffinity label. Biochim. Biophys. Acta 767:590-595.

Ohad, N. and J. Hirschberg. 1989. A similar structure of the herbicide binding site in photosystem II of plants and cyanobacteria demonstrated by site specific mutagenesis of the *psb*A gene. Photosynthesis Research.(in press).

Ohad, N., I. Pecker, and J. Hirschberg. 1987. Biochemical and molecular analysis of herbicide resistant mutants in cyanobacteria. *In*: Progress in Photosynthesis Research. Vol. III. (J. Biggins, ed.), pp. 807-810.

Ort, D. R., W. H. Ahrens, B. Martin, and E. W. Stoller. 1983. Comparison of photosynthetic performance in triazine-resistant and susceptible biotypes of *Amaranthus hybridus*. Plant Physiol. 72:925-930.

Pallett, K. E. and A. D. Dodge. 1980. Studies into the action of some photosynthetic inhibitor herbicides. J. Exp. Bot. 31:1051-1066.

Pappas-Fader, T., J. F. Cook, T. Butler, P. J. Lana, and J. Hare. 1993. Resistance of California arrowhead and smallflower umbrella plant to sulfonylurea herbicides. Proceedings of the Western Weed Science Society (Newark, CA: Western Society of Weed Science). p. 76.

Páy, A., M. A. Smith, F. Nagy, and L. Márton. 1988. Sequence of the *psb*A gene from wild type and triazine-resistant *Nicotiana plumbaginifolia*. Nucleic Acids Research. 16:8176.

Pfister, K. and C. J. Arntzen. 1979. The mode of action of photosynthetic II-specific inhibitors in herbicide-resistant weed biotypes. Z. Naturforsch. 34c:996-1009.

Pfister, K., K. E. Steinback, G. Gardner, and C. J. Arntzen. 1981. Photoaffinity labeling of an herbicide receptor protein in chloroplast membranes. Proc. Natl. Acad. Sci. U.S.A. 78:981-981.

Phillips, J. N. and J. L. Huppatz. 1984. Cyanoacrylate inhibitors of the Hill reaction. I. Nature of the inhibitor/receptor site interaction. Agric. Biol. Chem. 48:51-54.

Pillai, P. and J. B. St. John. 1981. Lipid composition of chloroplast membranes from weed biotypes differentially sensitive to triazine herbicides. Plant Physiol. 68:585-587.

Powles, S. B., J. A. M. Holtum, J. M. Matthews, and D. R. Liljegren. 1990. Herbicide cross-resistance in annual ryegrass (*Lolium rigidum* Gaud.): The search for a mechanism. *In*: Managing Resistance to Agrochemicals: From Fundamental Research to Practical Strategies. M. B. Green, H. M. LeBaron, and W. K. Moberg, Eds. Washington, D.C.: American Chemical Society Symposium Series No. 421:394-406.

Primiani, M. M., J. C. Cotterman, and L. L. Saari. 1990. Resistance of Kochia (*Kochia scoparia*) to sulfonylurea and imidazolinone herbicides. Weed Technol. 4:169-172.

Pucheu, N., W. Oettmeier, U. Heisterkamp, K. Masson, and G. F. Wildner. 1984. Metribuzin-resistant mutants of *Chlamydomonas reinhardtii*. Z. Naturforsch. 39c:437-439.

Rubin, B., M. Sibony, Y. Benyamini, and Y. Danino. 1992. Resistance to sulfonylurea herbicides in redroot pigweed (*Amaranthus retroflexus* L.). *In* WSSA Abstracts, Vol. 32, Proceedings of the 1992 Meeting of the Weed Science Society of America (Champaign, IL: WSSA, 1992), p. 66.

Ryan, G. F. 1970. Resistance of common groundsel to simazine and atrazine. Weed Sci. 18:614.616.

Saari, L. L., J. C. Cotterman, and D. C. Thill. 1991. Resistance to acetolactate synthase inhibiting herbicides. *In*: Herbicide Resistance in Plants: Biology and Biochemistry. Lewis Publishers. Boca Raton, FL. pp. 83-139.

Saari, L. L., J. C. Cotterman, and M. M. Primiani. 1990. Mechanism of sulfonylurea herbicide resistance in the broadleaf weed, *Kochia scoparia*. Plant Physiol. 93:55-61.

Saari, L. L., J. C. Cotterman, W. F. Smith, and M. M. Primiani. 1992. Sulfonylurea herbicide resistance in common chickweed, perennial ryegrass, and russian thistle. Pestic. Biochem. Physiol. 42:110-118.

Salisbury, E. 1961. Weeds and Aliens. London: St. James's Place. 384 p.

Sato, F., Y. Sigematsu, and Y. Yamada. 1988. Selection of an atrazine-resistant tobacco cell line having a mutant *psb*A gene. Molecular Genetics. 214:358-360.

Schönfeld, M., T. Yaacoby, O. Michael, and B. Rubin. 1987. Triazine resistance without reduced vigor in *Phalaris paradoxa*. Plant Physiol. 83:329-333.

Sigematsu, Y., F. Sato, and Y. Yamada. 1989. The mechanism of herbicide resistance in tobacco cells with a new mutation in the Q_B protein. Plant Physiol. 89:986-992.

Smeda, R. J. 1990. The physiological and molecular characteristics of atrazine resistance in photoautotrophic potato cells. Ph.D. Thesis, Purdue University, West Lafayette, IN.

Souza-Machado, V., J. D. Bandeen, G. R. Stephenson, and P. Lavigne. 1978. Uniparental inheritance of chloroplast atrazine tolerance in *Brassica campestris* Bird rape. Can. J. Plant Sci. 58:977-981.

Stannard, M. E. 1987. Weed control in alfalfa (*Medicago sativa* L.) grown for seed. M. S. Thesis, Montana State University, Bozeman, MT.

Stephenson, G. R., M. D. Dykstra, R. D. McLaren, and A. S. Hamill. 1990. Agronomic practices influencing triazine-resistant weed distribution in Ontario. Weed Technol. 4:199-207.

Stowe, A. E. and J. S. Holt. 1988. Comparison of triazine-resistant and -susceptible biotypes of *Senecio vulgaris* and their F1 hybrids. Plant Physiol. 87:183-189.

Sweetser, P. B., G. S. Schow, and J. M. Hutchison. 1982. Metabolism of chlorsulfuron by plants: biological basis for selectivity of a new herbicide for cereals. Pestic. Biochem. Physiol. 17:18-23.

Takeda, S., D. L. Erbes, P. B. Sweetser, J. V. Hay, and T. Yuyama. 1986. Mode of herbicidal and selective action of DPX-F5384 between rice and weeds. Weed Res. (Tokyo). 31:157-163.

Thompson, C. R. and D. C. Thill. 1992. University of Idaho, Moscow, ID. Unpublished results.

Tietjen, K. G., J. F. Kluth, R. Andree, M. Haug, M. Lindig, K. H. Müller, H. J. Wroblowsky, and A. Trebst. 1991. The herbicide binding niche of photosystem II - A model. Pestic. Sci. 31:65-72.

Tischer, W. and H. Strotmann. 1977. Relationship between inhibitor binding by chloroplasts and inhibition of photosynthetic electron transport. Biochim. Biophys. Acta. 460:113-125.

Trebst, A. 1987. The three-dimensional structure of the herbicide binding niche on the reaction center polypeptides of photosystem II. Z. Naturforsch. 42c:742-750.

Trebst, A. 1991. The molecular basis of resistance of photosystem II herbicides. *In*: Herbicide Resistance in Weeds and Crops. J. C. Caseley, G. W. Cussans, and R. K. Atkin, Eds. Oxford: Butterworth-Heinemann. pp. 145-164.

Treguna, E. B. and J. Downton. 1967. Carbon dioxide compensation in members of the Amaranthaceae and some related families. Can. J. Bot 45:2385-2387.

Van Oorschot, J. L. P., and P. H. Van Leeuwen. 1984. Comparison of photosynthetic capacity between intact leaves of triazine-resistant and susceptible biotypes of six weed species. Z. Naturforsch. 39c:440-442.

Vengris, J. 1953. Weed populations as related to certain cultivated crops in the Connecticut River Valley, Mass. Weeds 2:125-134.

Vengris, J., S. Dunn, and M. Stacewicz-Sapuncakis. 1972. Life history studies as related to weed control in the Northeast. 7. Common Purslane. Amherst, MA: University of Massachusetts, Agricultural Experiment Station, Research Bulletin No. 598, 46 p.

Wessels, J. S. C., and R. van der Veen. 1956. The action of some derivatives of phenylurethan and of 3-phenyl-1,1-dimethylurea on the Hill reaction. Biochim. Biophys. Acta 19:548-549.

Chapter 2

Discovery of a Common Purslane (*Portulaca oleracea* L.) Biotype Which is Resistant to Linuron

Discovery of a Common Purslane (*Portulaca oleracea* L.) Biotype Which is Resistant to Linuron

ABSTRACT

Greenhouse and field experiments were conducted to confirm and quantify linuron resistance in common purslane collected from a carrot field in Imlay City, Michigan. Preliminary evaluation was made using a flotation test kit to identify resistance to linuron and atrazine. Subsequent greenhouse experiments indicated that this common purslane biotype was resistant to 11.2 kg/ha linuron and 179 kg/ha atrazine. The resistance ratio (RR) was >300 for linuron and >400 for atrazine. The resistant common purslane was also highly resistant to diuron, cyanazine, and prometryn, but had a low level of negative cross-resistance to bromoxynil. Both resistant and susceptible biotypes of common purslane were equally sensitive to hexazinone and bentazon.

INTRODUCTION

Much of the early research on herbicide resistance involved the triazines. In 1990, Holt and LeBaron reported that triazine-resistant weeds accounted for 50% of the documented cases of weed resistance. In 1998, Heap determined that triazine resistance accounted for about 30% of reported resistance cases, since many cases of ALS resistance were reported in recent years. To date, 195 weed

species have been reported to be resistant to various herbicides (Heap 1998). To date, all triazine resistance in whole plants attributed to a target-site mutation is a result of a substitution of serine-to-glycine at position 264 in the D1 protein in photosystem II (PS II) (Hirschberg and McIntosh 1983). The triazine-resistant biotypes often have a slower growth rate than the susceptible biotypes and are usually ecologically less competitive (Ahrens and Stoller 1983; Holt and Radosevich 1983). Phenylurea and triazine herbicides affect the same site of action by inhibiting electron transfer from the thylakoid membrane-bound D1 protein of PS II to the plastoquinone pool in the chloroplast. Most triazine-resistant weeds also appear to have cross-resistance to the phenylurea herbicides such as diuron and linuron (Fuerst et al. 1986). However, whereas the resistance ratio ($GR_{50resistant}$ / $GR_{50susceptible}$) to the triazines ranges from 100 to 1000, it is only 1 to 4 to the ureas (Pfister and Arntzen 1979). In a few cases, triazine-resistant weeds were not found cross-resistant to phenylureas (Gray et al. 1995).

In 1991, a carrot (*Daucus carota* L.) grower in Imlay City, Michigan reported that linuron no longer controlled common purslane (*Portulaca oleracea* L.) in one field. The grower indicated that carrot was grown every year since 1965, except for 2 or 3 years with a small grain crop, and that a total of about 2.24 kg ai/ha linuron was applied every year carrot was grown. The objectives of the research

described here were to confirm and quantify the magnitude of resistance of common purslane to linuron, and the existence of any cross-resistance.

MATERIALS AND METHODS

Preliminary Evaluation with Flotation Test Kit. Common purslane plants that had survived linuron application were collected from a carrot field in Imlay City, Michigan in 1991. Plants also were collected from the Michigan State University (MSU) Muck Research Farm in Laingsburg, Michigan, where there was no evidence of resistance to linuron. Known atrazine-resistant (R-CHEAL) and susceptible (S-CHEAL) common lambsquarters (*Chenopodium album* L.) plants were used as controls. Common lambsquarters was used, because of its significance as a triazine-resistant weed. Heap (1998) found that triazine-resistance was reported in four *Chenopodium* species, and that common lambsquarters is the most frequently reported resistant weed in the world (16 countries). Both common purslane and common lambsquarters plants were tested for linuron resistance following procedures described by Mutch and Penner (1989) for the "Triazine-resistance test kit"[1]. The principle of the triazine test kit is leaf disc flotation after exposure to a herbicide and light. Leaf discs are placed in a solution containing a photosynthetic inhibitor herbicide and a vacuum is applied to force the solution into the intercellular

spaces of the discs, which subsequently sink to the bottom of the test tube. The test tube is then exposed to light. If the leaf discs are not affected by the herbicide in solution, they photosynthesize and the oxygen produced causes the discs to float. Thus, the plant is considered resistant to the herbicide in the solution if the leaf discs float, or susceptible if the leaf discs do not float after light exposure.

The test kit procedure was modified by adding linuron[2] instead of atrazine as the active ingredient. One-liter solutions of either linuron or a blank buffer[3] were mixed to contain the same ingredients as the triazine test kit. A 4-ml aliquot of the herbicide solution and three leaf discs (4 mm diameter) from a recently developed leaf were placed in each test tube. A tray containing 36 test tubes with the solution was placed inside a vacuum bell jar.

Preliminary tests during the day, using herbicide solutions or blank buffers, indicated that resistant common purslane leaf discs continued to photosynthesize and would not sink. Therefore, the bell jar was covered with a black cloth when applying the vacuum. A 500 mm-mercury vacuum was applied to the bell jar for 3 min, then released and reapplied for another 3 min, to force the solution into intercellular spaces and cause the leaf discs to sink. Tubes in which at least 2 of the 3 discs did not sink after

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[1] Neogen, Lansing, Michigan
[2] Lorox 50 DF was added to obtain 10^{-4} M linuron.
[3] Proprietary buffer, Neogen Triazine Resistance Test Kit.

the second vacuum application were discarded. The test tubes then were placed under a light source (2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 1 min to induce photosynthesis. Test tubes in which at least two leaf discs floated after light exposure were counted as resistant and those with no leaf discs floating were counted as susceptible. The test was conducted three times on common purslane plants collected from Imlay City for a total of 90 plants, and once for common purslane plants collected from the MSU Muck Research Station for a total of 36 plants. In addition, twenty plants each of atrazine-resistant and -susceptible common lambsquarters were tested using the linuron solution.

After testing the plants for resistance, the resistant (R) and susceptible (S) biotypes of common purslane were grown in 4-liter containers in separate greenhouses to obtain seed. Seeds were collected weekly for 3 weeks from 20 plants of each common purslane biotype. The seeds were sifted, cleaned, and stored at 3 C.

Greenhouse Studies. Separate preemergence (PRE) and postemergence (POST) herbicide application experiments were conducted on common purslane in the greenhouse to determine the effects of other photosynthesis-inhibiting herbicides on resistant common purslane. In the PRE experiments, seeds of resistant and susceptible common purslane were sown in 28 x 56-cm, 200-cell flats using BACCTO[4] soil mix, and a

[4] Michigan Peat Company, Houston, Texas

Blackmore[5] automatic seeder which places one seed per cell on the soil surface. Immediately after seeding, herbicide treatments were applied to the soil surface with a moving-belt bench sprayer equipped with an 8001E nozzle and calibrated at 1.6 kph, 187 l/ha, and 133 KPa. In one experiment, linuron (1X = 1.12 kg ai/ha) or atrazine (1X = 2.24 kg ai/ha) were both applied at 0X, 0.5X, 1X, 1.5X, and 2X rates to flats seeded with R-common purslane or S-common purslane. One flat of each biotype was sprayed with each rate of either linuron or atrazine. This experiment was conducted three times.

In another experiment, metribuzin (1X = 0.56 kg/ha) or terbacil (1X = 1.12 kg/ha) was applied at the same rates previously described for linuron and atrazine. This experiment was conducted twice. After spraying, clear plastic covers were placed over the flats for the duration of the studies to maintain high humidity and improve seed germination. Each experiment was arranged randomly on a greenhouse bench. The experimental design for both studies consisted of repeated measures with a three-way factorial arranged in a randomized complete block design with three replications. The three factors were: herbicide (linuron or atrazine for experiment 1, metribuzin or terbacil for experiment 2); common purslane biotype (resistant or susceptible); and herbicide rate (0X, 0.5X, 1X, 1.5X, and 2X). Counts of surviving seedlings were collected from each

[5] Blackmore Company Inc., Belleville, Michigan

experimental unit at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 days after treatment (DAT). All figures and discussion of results of the two PRE experiments are expressed as a percent of 200 seeds planted. Seedlings were irrigated using a mist nozzle to avoid disturbing the soil.

In the postemergence study, plants were established as described above. Three weeks after seeding, seedlings were transplanted into 946-ml plastic pots containing BACCTO mix, with one plant per pot. Plants were maintained in the greenhouse under natural and supplemental lighting (maximum of 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at midday) to provide 16 h day / 8 h night at 25 ± 5 C. Plants were watered and fertilized as needed with Peters 20-20-20 (20N-8.6P-16.6K) fertilizer solution[6]. In order to minimize variation due to plant size, common purslane plants were grouped by size prior to spraying. The experiment was designed to measure fresh weights of similar plants at five specific times after herbicide application. Each herbicide treatment was applied to plants of equal size two weeks after transplanting. Eight photosynthesis-inhibiting herbicides (linuron, 1X = 1.12 kg/ha; diuron, 1X = 2.24 kg/ha; atrazine, 1X = 2.24 kg/ha; cyanazine, 1X = 2.24 kg/ha; hexazinone, 1X = 2.24 kg/ha; prometryn, 1X = 2.24 kg/ha; bentazon, 1X = 1.12 kg/ha; and bromoxynil, 1X = 0.28 kg/ha) were applied at 0X, 0.5X, 1X, and 2X to resistant and susceptible common purslane, using the moving-belt bench sprayer as previously

described. After application, the plants were arranged in a randomized complete block design on a greenhouse bench. A total of 10 plants or replicates per biotype were sprayed with each herbicide treatment. Two plants per treatment were harvested at the soil surface, and fresh weights were recorded at 0, 4, 7, 10, and 14 DAT. Statistical analysis was conducted on fresh weight measurements for each herbicide within each biotype. Analysis of variance was conducted also on the combined data of the two biotypes for each date of measurement in order to determine the treatment effects across biotypes.

GR_{50} Calculation. An experiment was conducted to determine the dosage of linuron or atrazine required to reduce fresh weight to 50% of the control (GR_{50}). Both R and S-common purslane plants were established as described above. Linuron (0, 0.03, 0.07, 0.10, 0.13, and 0.17 kg ai/ha on S-common purslane, and 0, 6.7, 7.8, 8.9, 10, and 11.2 kg ai/ha on R-common purslane) or atrazine (0, 0.45, 0.89, 1.34, 1.79, and 2.24 kg ai/ha on S-common purslane, and 0, 44.8, 89.6, 134.4, and 179.2 kg ai/ha on R-common purslane) were applied three weeks after transplanting, and fresh weights of three plants for each treatment were determined at 0, 1, 4, 6, 9, 11, and 14 DAT. Non-linear regression analysis was used to extrapolate the herbicide dosage required to reach GR_{50}. Curve-fitting was performed using the Marquardt compromise method of successive approximations. The best

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[6] Scotts, Allentown, Pennsylvania

fit curve, evaluated by analysis of residuals and r^2, was the monomolecular asymptotic function (Hunt 1980) of the type:

$$Y = a * [1 - b * e^{(-c * X)}] \qquad [1]$$

Y represents fresh weight expressed as percent of the control, and X is the herbicide dosage. The constants a, b, and c are the asymptotic value, minimum value and rate constant, respectively. This polynomial was selected because it provided direct estimates of specific physiological processes and it exhibited curvilinear features that represented the data (Layne and Flore 1995). If a calculated GR_{50} value fell outside the lowest or highest herbicide dosage used, the respective (<) or (>) symbols were used to reduce prediction error. GR_{50} values are presented as the actual amount in kg ai/ha for each herbicide.

Field Study. A field experiment was conducted in 1993 in the carrot field near Imlay City, Michigan, where the putative resistance was first reported, to determine the response of common purslane to linuron and other photosynthesis-inhibiting herbicides under field conditions. A randomized complete block design was used with three replications. Field plots were 1.75 m wide by 12 m long, with 3 rows of carrots spaced 50 cm apart. Linuron was applied PRE or POST at 1.12, 2.24, 4.48, and 11.2 kg/ha. Atrazine was applied PRE or POST at 2.24 kg/ha. Terbacil

was applied PRE at 1.12 kg/ha. Metribuzin was applied POST at 0.28 kg/ha. Herbicides were applied with a CO_2 backpack sprayer equipped with a four nozzle (FF8002) boom at 180 L/ha and 201 KPa. PRE treatments were applied 9 days after planting. POST treatments were applied 50 days after common purslane emergence. At the time of POST application, common purslane plants were 8 to 15 cm in diameter and carrots were 30 to 36 cm in height. Visual injury ratings of common purslane were taken 28 and 70 days after application of PRE treatments, and 16 days after application of POST treatments. A scale of 1 to 10 was used in the visual injury ratings, in which 1 = no injury and 10 = complete kill.

RESULTS

Preliminary Evaluation with Flotation Test Kit. Leaf discs of common purslane collected from Imlay City, Michigan floated after soaking in linuron solution and exposure to light. Eighty-eight percent of the individual plants (80 plants out of 90 tested) were determined to be resistant to linuron. The R-common purslane biotype collected from Imlay City, Michigan will henceforth be referred to as R-POROL to indicate resistance to linuron. Leaf discs from 35 of 36 common purslane plants collected from the MSU Muck Research Farm did not float after exposure to light and appeared to be susceptible. In the case of the remaining plant, one of the three leaf discs floated while the other two, taken from

the same plant, did not. It was unclear why one leaf disc floated, but we speculate that it remained buoyant even after applying vacuum twice. The susceptible common purslane biotype is hereafter referred to as S-POROL. Atrazine-resistant common lambsquarters leaf discs did not float in the linuron solution, indicating that they were susceptible to linuron.

Greenhouse Studies. Results of the preemergence experiments are presented in Figures 1 and 2. R-POROL seedlings emerged two days after seeding, while S-POROL seedlings first emerged on the third day. Both biotypes reached maximum germination four to five days after seeding. Sixty to 80% of non-treated R-POROL and S-POROL seeds germinated, indicating no inherent differences in germination (Figures 1, 2). Only the 2X rate of linuron (2.24 kg ai/ha) reduced the stand of R-POROL below 20% by 12 DAT. On the other hand, all rates of linuron caused a significant reduction in stand of S-POROL by 8 DAT. Although percentage of surviving seedlings was lower at higher atrazine rates, no rate of atrazine caused significant reduction in stand of R-POROL. All rates of atrazine caused a lower stand of S-POROL by 8 DAT. It thus appears that R-POROL has a greater level of resistance to atrazine than to linuron. In this experiment, R-POROL seedlings demonstrated a low level of cross-resistance to metribuzin (Figure 2A, 2B). By 12 DAT, about 20% of R-POROL seedlings survived 0.5X metribuzin, whereas all S-POROL plants were killed by the same rate (Figure 2B).

R-POROL seedlings did not survive higher rates of metribuzin. On the other hand, terbacil was equally effective on R and S-POROL seedlings (Figure 2C, 2D). R-POROL does not appear to have cross-resistance to terbacil.

In the postemergence study, linuron at all rates produced lower fresh weights of R-POROL at all rating dates except for 7 DAT, compared with the control (Table 1). However, by 14 DAT the treated plants had recovered and started accumulating biomass. S-POROL plants showed significantly lower fresh weights at 4 DAT, and were dead by 10 DAT. With both biotypes of POROL, it took at least seven days for injury to be exhibited at any rate of linuron tested.

The response to diuron application was similar to linuron. Biomass production of R-POROL decreased until 10 DAT, then recovered, and began to increase. S-POROL plants were killed by all rates of diuron, and there were no surviving plants at 10 DAT.

Atrazine, cyanazine, and prometryn had little effect on R-POROL, indicating greater resistance to these herbicides than to linuron or diuron. Plants treated with atrazine were often larger than non-treated controls and thus had greater biomass accumulation than controls. Although biomass accumulation of R-POROL was reduced between 7 and 10 DAT with prometryn, plants recovered by 14 DAT and were similar to controls. On the other hand, S-POROL plants were killed by the lowest rate of each herbicide within 14 DAT. Hexazinone, another triazine, killed all S-POROL and

severely injured R-POROL. Although R-POROL plants survived 1.12 kg/ha (0.5X) and 2.24 kg/ha (1X) rates of hexazinone at 14 DAT, fresh weight was significantly reduced.

Bentazon killed all R-POROL and S-POROL plants at all rates. Bromoxynil was more toxic to R-POROL than to S-POROL. S-POROL appears to tolerate bromoxynil to some extent. S-POROL plants survived 0.14 and 0.28 kg/ha rates, but all were killed by the 2X rate (0.56 kg/ha) of bromoxynil. R-POROL plants survived the 0.5X (0.14 kg/ha) rate but all were killed by the 1X and 2X rates.

GR_{50} Calculation. The non-linear regression model (equation 1) indicated that very small amounts of linuron (<0.03X or 0.034 kg/ha) and atrazine (<0.2X or 0.5 kg/ha) were sufficient to reduce S-POROL fresh weight by 50% (Table 2). At 14 DAT, 50% reduction in fresh weight of R-POROL plants was calculated at >10X linuron and at >80X atrazine. The ratio of $GR_{50\ Resistant}$ to $GR_{50\ Susceptible}$, commonly referred to as the resistance ratio (RR), of common purslane to linuron was calculated at >300 at 14 DAT, meaning that R-POROL was at least 300 times more resistant to linuron than S-POROL. In addition, R-POROL is at least 400 times more resistant to atrazine than S-POROL.

Field study. At 28 DAT, injury on R-POROL treated PRE with linuron 1.12 kg/ha (1X) was similar to the control (Table 3). All other rates of linuron caused significant visual injury. Linuron at 2.24 kg/ha injured R-POROL similarly in field and greenhouse studies (30% injury and 30% reduction

in fresh weight, respectively). By 70 DAT, common purslane in all treated plots had recovered, except with linuron 11.2 kg/ha (10X). Injury on R-POROL due to atrazine and terbacil at 1X rates was significant at 28 DAT. However, plants recovered by 70 DAT and were not different from controls.

Sixteen days after POST treatment, common purslane treated with linuron 4.48 kg/ha (4X) or 11.2 kg/ha (10X) exhibited significant injury, but not complete death, and many common purslane plants survived (Table 3). All other treatments were not significantly different from the control. None of the herbicides at any application time or rate resulted in complete control of common purslane in the field.

DISCUSSION

The flotation test indicated resistance to linuron in the common purslane from Imlay City. Subsequent preemergence and postemergence bench spray experiments confirmed significant cross-resistance to linuron, atrazine, diuron, cyanazine, and prometryn in the resistant biotype. Field experiments indicated resistance of common purslane up to 11.2 kg/ha of linuron.

Greenhouse experiments, on the other hand, indicated that the growth rate of R-POROL plants was slowed for several days after linuron application. When treated with atrazine, however, the plants continued to develop at the same rate as non-treated controls. The response to diuron was similar to

that of linuron, and response to cyanazine was similar to that of atrazine. The R-POROL was obviously more resistant to atrazine than to the phenylureas. This is interesting since the field in which it was found had no known history of atrazine treatment.

The R-POROL demonstrated negative cross-resistance to bromoxynil, i.e., it was more susceptible to bromoxynil than S-POROL. Common purslane can tolerate bromoxynil up to 0.28 kg/ha (1X rates). Triazine-resistant weeds may have increased sensitivity to phenol-type herbicides such as bromoxynil (Durner et al. 1986; Oettmeier et al. 1982) and bentazon (De Prado et al. 1992; Van Oorschott et al. 1988). Much of the research dealing with negative cross-resistance was done in vitro on isolated thylakoids. Oettmeier et al. (1982) found that isolated chloroplasts of atrazine-resistant redroot pigweed (*Amaranthus retroflexus* L.) had higher sensitivity to bromoxynil than the susceptible biotype. Negative cross-resistance also has been observed at the whole-plant level. Van Oorschott et al. (1988) found that atrazine-resistant redroot pigweed, black nightshade (*Solanum nigrum* L.), and canola (*Brassica napus* L.) are more sensitive to photosynthesis reduction by bentazon injury. De Prado et al. (1992) found similar results where redshank (*Amaranthus cruentus* L.) and smooth pigweed (*Amaranthus hybridus* L.) survived atrazine and cyanazine applications, but were controlled by lower doses of bentazon. Fuerst et

al. (1986) also found that a triazine-resistant common lambsquarters had negative cross-resistance to bentazon.

Some triazine-resistant weeds have been found to exhibit little or no cross-resistance to phenylureas. Lehoczki et al. (1984) found that a triazine-resistant (1000X) horseweed (*Conyza canadensis* L.) was only ten times more resistant to diuron than the susceptible biotype. Fuerst et al. (1986) determined that a triazine-resistant smooth pigweed had slight cross-resistance to linuron and no resistance to diuron. Salhoff and Martin (1985) found that an atrazine-resistant kochia (*Kochia scoparia* L.) with cross-resistance to metribuzin and cyanazine was not cross-resistant to diuron. Similar results were found by van Oorschott et al. (1988) on redroot pigweed, black nightshade, and canola. Gray et al. (1995) determined in greenhouse experiments that two atrazine-resistant biotypes of velvetleaf (*Abutilon theophrasti* Medic.) were not cross-resistant to linuron, terbacil, or metribuzin. They attributed this lack of cross-resistance to the mechanism of atrazine resistance in velvetleaf. Whereas most triazine-resistance in weeds is due to an altered D1 protein (Hirschberg and McIntosh 1983), atrazine-resistance in velvetleaf is due to enhanced glutathione conjugation of the herbicide (Anderson and Gronwald 1991; Gronwald et al. 1989).

Of the few reports of triazine-resistant weeds having cross-resistance to linuron, the work by Fuerst et al. (1986) is typical; smooth pigweed was 830 times more

resistant to atrazine, but was only 3.4 and 1.4 times more resistant to linuron and diuron, respectively, compared to the susceptible biotype.

Our research has shown a much higher level of common purslane resistance to linuron and diuron. Although R-POROL exhibited a response typical of other triazine-resistant weeds with a high cross-resistance to cyanazine and prometryn, it was as sensitive as the susceptible biotype to bentazon.

The high level of resistance to linuron and diuron, the very high resistance to atrazine, the negative cross-resistance to bromoxynil, and sensitivity to bentazon indicate an interesting and unique example of resistance in this common purslane biotype, and may indicate a basis of resistance somewhat different than the usual serine-to-glycine mutation in the D1 protein of PS II.

Managing R-POROL in the field presents a challenge to carrot producers, due to the limited choices of herbicides labeled for use on carrot. In the past, Stoddard solvent was used to kill all emerged weeds in carrots. However, it is no longer labeled for use as a herbicide on carrot in Michigan. Crop rotation is another option for control of resistant weeds. Rotating to onions, for example, would allow growers to use oxyfluorfen, which is very effective on common purslane.

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LITERATURE CITED

Ahrens, W. H. and E. W. Stoller. 1983. Competition, growth rate, and CO_2 fixation in triazine-susceptible and -resistant smooth pigweed (*Amaranthus hybridus*). Weed Sci. 31:438-444.

Anderson, M. P. and J. W. Gronwald. 1991. Atrazine resistance in a velvetleaf (*Amaranthus hybridus*) biotype due to enhanced glutathione *S*-transferase activity. Plant Physiol. 96:104-109.

De Prado, R., M. Sanchez, J. Jorrin, and C. Dominguez. 1992. Negative cross-resistance to bentazon and pyridate in atrazine-resistant *Amaranthus cruentus* and *Amaranthus hybridus* biotypes. Pestic. Sci. 35:131-136.

Durner, J., A. Thiel, and P. Böger. 1986. Phenolic herbicides: correlation between lipophilicity and increased inhibitory sensitivity of thylakoids from higher plant mutants. Z. Naturforsch. 41c:881-884.

Fuerst, E. P., C. J. Arntzen, K. Pfister, and D. Penner. 1986. Herbicide cross-resistance in triazine-resistant biotypes of four species. *Weed Sci.* 34:344-353.

Gray, J. A., D. E. Stoltenberg, and N. E. Balke. 1995. Absence of herbicide cross-resistance in two atrazine-resistant velvetleaf (*Abutilon theophrasti*) biotypes. Weed Sci. 43:352-357.

Gronwald, J. W., R. N. Andersen, and C. Yee. 1989. Atrazine resistance in velvetleaf (*Abutilon theophrasti*) due to enhanced atrazine detoxification. Pestic. Biochem. Physiol. 34:149-163.

Heap, I. M. 1998. International survey of herbicide-resistant weeds. 1998 Herbicide-Resistance Action Committee Annual Report. WeedSmart, Corvallis, Oregon. 165 unnumbered pages.

Hirschberg, J. A. and L. McIntosh. 1983. Molecular basis of herbicide resistance in *Amaranthus hybridus*. Science 222:1346-1348.

Holt, J. S. and H. M. LeBaron. 1990. Significance and distribution of herbicide resistance. Weed Tech. 4:141-149.

Holt, J. S. and S. R. Radosevich. 1983. Differential growth of two common groundsel (*Senecio vulgaris*) biotypes. Weed Sci. 31:112-130.

Hunt, R. 1980. Asymptotic functions, p. 121-146. *In*: Plant growth curves. Univ. Park Press, Baltimore, MD.

Layne, D. R. and J. A. Flore. 1995. End-product Inhibition of photosynthesis in *Prunus cerasus* L. in response to whole-plant source-sink manipulation. J. Amer. Soc. Hort. Sci. 120(4):583-599.

Lehoczki, E., G. Laskay, E. Pölös, and J. Mikulás. 1984. Resistance of triazine herbicides in horseweed (*Conyza canadensis*). Weed Sci. 32:669-674.

Mutch, D. R. and D. Penner. 1989. Use of the triazine resistance kit for determination of triazine resistant weeds in the field. Proc. of NCWSS. 44:92.

Oettmeier, W., K. Mason, C. Fedtke, J. Konze, and R. R. Schmidt. 1982. Effect of different photosystem II inhibitors on chloroplasts isolated from species either susceptible or resistant toward s-triazine herbicides. Pestic. Biochem. Physiol. 18:357-367.

Pfister, K. and C. J. Arntzen. 1979. The mode of action of photosystem II-specific inhibitors in herbicide-resistant weed biotypes. Z. Naturforsch. 34c:996-1009.

Salhoff, C. G. and A. R. Martin. 1985. *Kochia scoparia* growth response to triazine herbicides. Weed Sci. 34:40-42.

Van Oorschott, J. L. P. and P. H. Van Leeuwen. 1988. Inhibition of photosynthesis in intact plants of biotypes resistant or susceptible to atrazine and cross-resistant to other herbicides. Weed Res. 28:223-230.

Table 1. Fresh weight response of resistant and susceptible common purslane after postemergence application of various photosynthesis-inhibiting herbicides

Table 1.

Herbicide	Rate	Rate (kg/ha)	R-Common Purslane Fresh Weight Days after treatment 4	7	10	14	S-Common Purslane Fresh Weight Days after treatment 4	7	10	14
			% of control							
Linuron	0.5X	0.56	55	87	29	63	32	7	---[a]	---
	1X	1.12	50	66	34	39	33	4	---	---
	2X	2.24	58	61	25	32	26	5	---	---
LSD[1] (0.05)			20	NS	23	33	32	55	---	---
LSD[2] (0.05)			21	40	NA[b]	NA				
Diuron	0.5X	1.12	65	38	33	56	57	9	---	---
	1X	2.24	84	43	20	28	28	5	---	---
	2X	4.48	103	31	19	18	29	9	---	---
LSD[1] (0.05)			25	6	29	53	42	28	---	---
LSD[2] (0.05)			40	17	NA	NA				
Atrazine	0.5X	1.12	80	103	112	105	54	13	13	---
	1X	2.24	97	97	91	90	70	10	---	---
	2X	4.48	79	87	119	109	57	12	---	---
LSD[1] (0.05)			NS	NS	NS	NS	NS	4	---	---
LSD[2] (0.05)			NS	26	NA	NA				
Cyanazine	0.5X	1.12	85	76	80	81	67	25	12	---
	1X	2.24	73	65	83	99	85	26	---	---
	2X	4.48	84	81	91	69	64	14	---	---
LSD[1] (0.05)			NS	NS	NS	NS	NS	34	---	---
LSD[2] (0.05)			NS	25	NA	NA				

LSD[1] is used to determine the significance of treatments within each biotype.
LSD[2] is used to determine the significance of similar treatments across biotypes.
[a] Treated plants are dead at this date of measurement.
[b] NA = LSD value is not applicable. At least one biotype is dead.

Table 1. (cont'd).

Herbicide	Rate	Rate (kg/ha)	R-Common Purslane Fresh Weight Days after treatment 4	7	10	14	S-Common Purslane Fresh Weight Days after treatment 4	7	10	14
			% of control							
Prometryn	0.5X	1.12	91	51	46	72	46	22	---	---
	1X	2.24	65	37	45	59	65	17	---	---
	2X	4.48	100	39	25	37	61	13	---	---
LSD[1] (0.05)			NS	NS	35	NS	18	24	---	---
LSD[2] (0.05)			NS	34	NA	NA				
Hexazinone	0.5X	1.12	33	7	9	5	20	4	---	---
	1X	2.24	19	7	8	3	29	7	---	---
	2X	4.48	30	9	---	---	21	5	---	---
LSD[1] (0.05)			NS	12	13	22	NS	4	---	---
LSD[2] (0.05)			48	7	NA	NA				
Bentazon	0.5X	0.56	28	9	---	---	42	13	9	---
	1X	1.12	21	8	---	---	35	10	---	---
	2X	2.24	20	5	---	---	25	11	---	---
LSD[1] (0.05)			18	45	NA	NA	27	10	---	---
LSD[2] (0.05)			17	24	NA	NA				
Bromoxynil	0.5X	0.14	14	8	3	4	32	10	35	69
	1X	0.28	9	2	---	---	17	6	18	37
	2X	0.56	4	1	---	---	11	3	---	---
LSD[1] (0.05)			23	6	NA	NA	15	12	54	55
LSD[2] (0.05)			16	8	NA	NA				

Table 2. Calculated GR_{50} values and resistance ratios (RR) of resistant and susceptible common purslane at various days after treatment (DAT) with linuron or atrazine.

		GR_{50}		
		Resistant	Susceptible	RR
	DAT	Kg ai/ha		
linuron	6	10.4	< 0.034 [c]	> 309
	9	7.4	< 0.034 [c]	> 221
	11	9.6	< 0.034 [c]	> 285
	14	> 11.2 [a]	< 0.034 [c]	> 333
atrazine	6	> 179 [b]	< 0.45 [c]	> 400
	9	> 179 [b]	< 0.45 [c]	> 400
	11	> 179 [b]	< 0.45 [c]	> 400
	14	> 179 [b]	< 0.45 [c]	> 400

[a] GR_{50} was reached at a calculated rate greater than the highest actual rate.

[b] GR_{50} was never reached even with the highest applied rate.

[c] GR_{50} was reached at a calculated rate lower than the lowest actual rate.

Table 3. Visual injury ratings of resistant common purslane after PRE[a] and POST[a] herbicide treatments. Imlay City, Michigan. 1993

				Visual Injury Rating[b]		
	Rate			28 DAT	70 DAT	16 DAT
Herbicide	(kg/ha)		Timing	PRE	PRE	POST
Linuron	1.12	1X	PRE	4	1	---
Linuron	2.24	2X	PRE	6	3	---
Linuron	4.48	4X	PRE	6	2	---
Linuron	11.2	10X	PRE	9	5	---
Atrazine	2.24	1X	PRE	8	4	---
Terbacil	1.12	1X	PRE	8	1	---
Linuron	1.12	1X	POST	---	---	3
Linuron	2.24	2X	POST	---	---	3
Linuron	4.48	4X	POST	---	---	5
Linuron	11.2	10X	POST	---	---	7
Atrazine	2.24	1X	POST	---	---	3
Metribuzin	0.28	0.5X	POST	---	---	2
Untreated Check				3	1	1
LSD (0.05)				2	3	2

[a] Timing = Herbicide application relative to common purslane growth stage

[b] Visual Injury Rating: 1 = no injury, 10 = complete kill

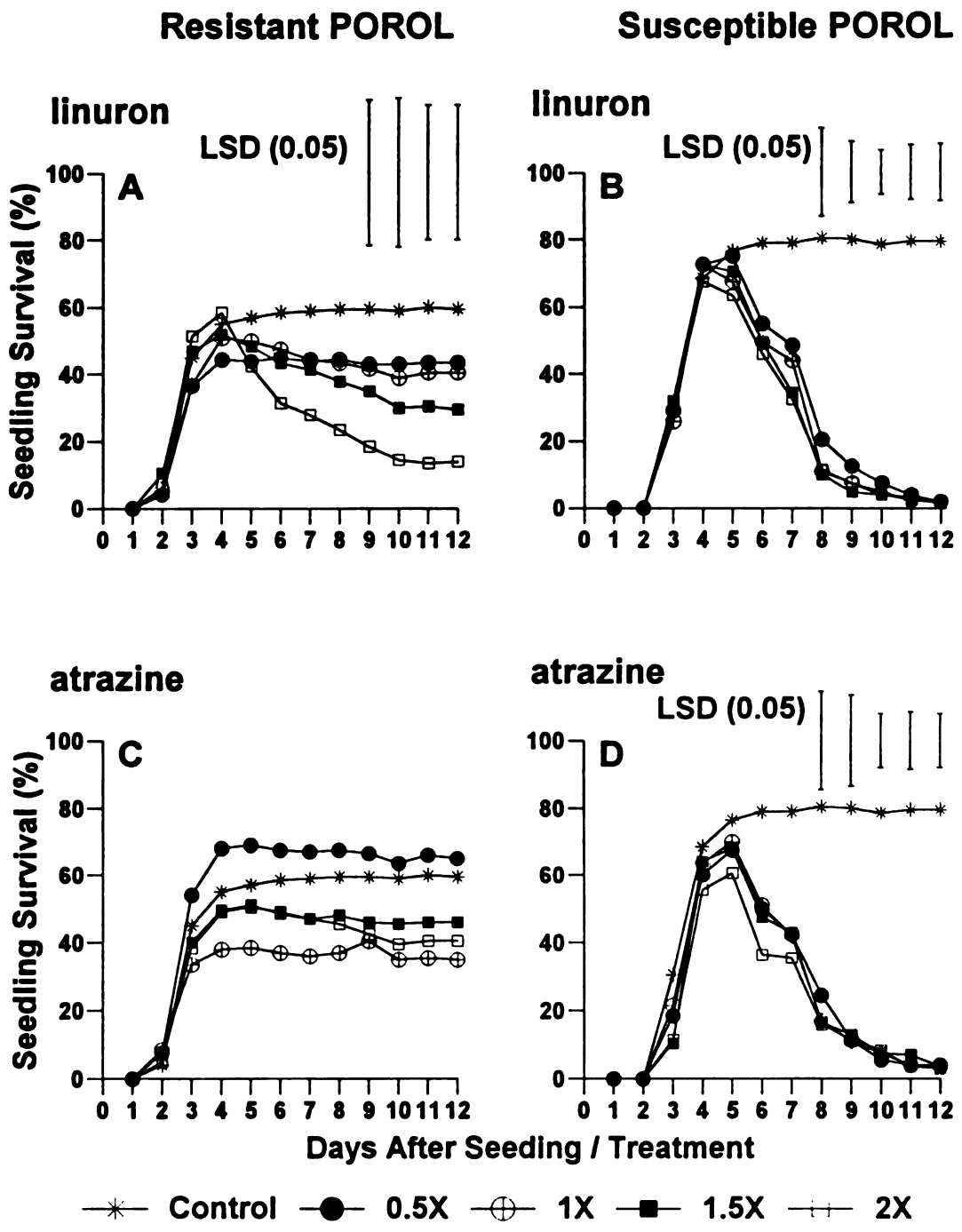

Figure 1. Seedling survival in resistant and susceptible common purslane treated preemergence with linuron or atrazine.

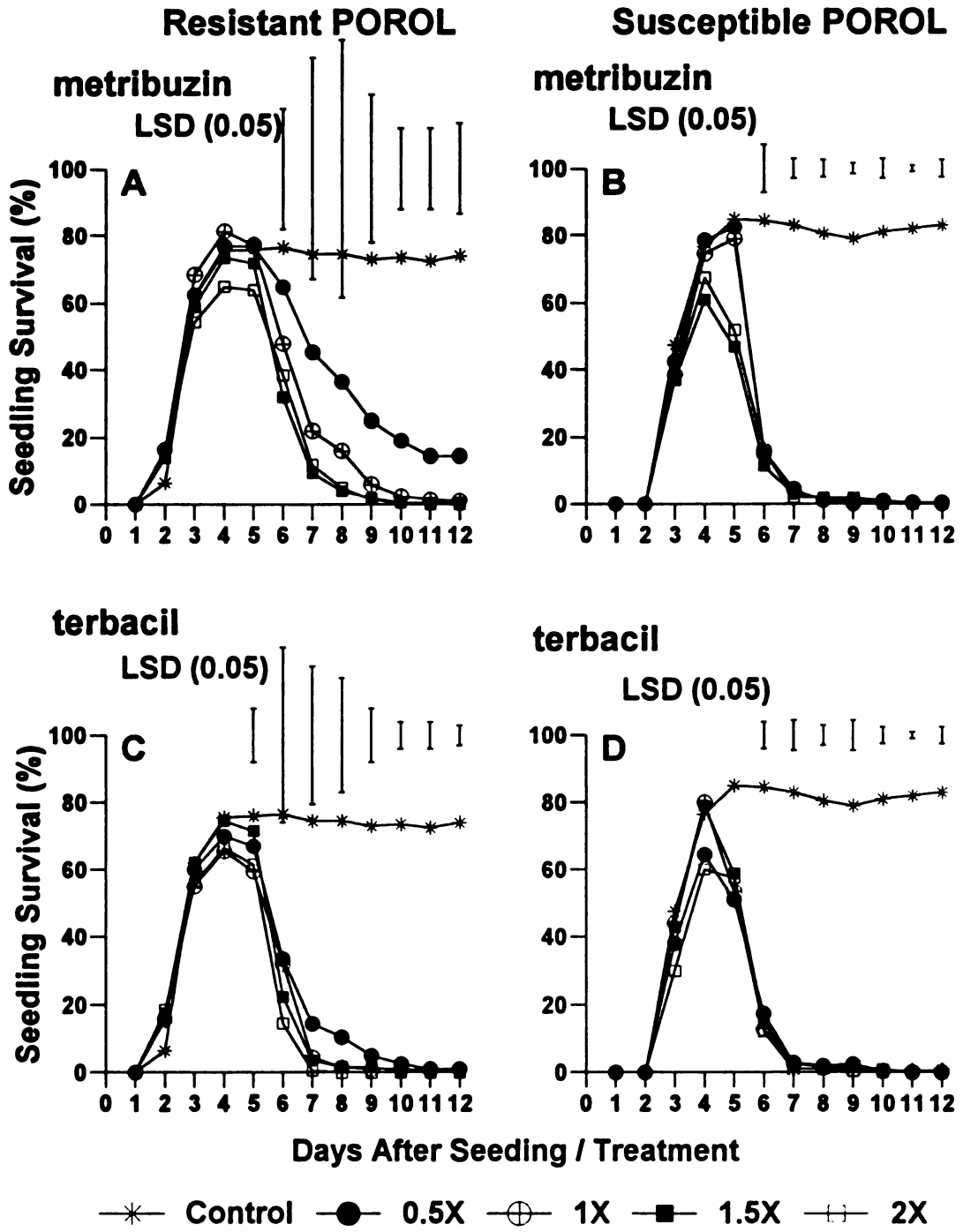

Figure 2. Seedling survival in resistant and susceptible common purslane treated preemergence with metribuzin or terbacil.

Chapter 3

Morphological and Physiological Characteristics of Linuron-Resistant Common Purslane (*Portulaca oleracea* L.)

Morphological and Physiological Characteristics of Linuron-Resistant Common Purslane (*Portulaca oleracea* L.)

ABSTRACT

Studies were conducted to compare morphological and physiological characteristics of linuron-susceptible and resistant common purslane. The susceptible biotype had heavier seed and germinated more rapidly than the resistant biotype. Two months after seeding, fresh and dry weights of susceptible common purslane plants were significantly greater than those of resistant plants. Chloroplasts and starch grains in the susceptible biotype were smaller, and fewer chloroplasts contained starch grains. Susceptible common purslane had a significantly higher CO_2 assimilation rate at temperatures ≥ 30 C. The susceptible biotype had a significantly lower CO_2 assimilation rate at CO_2 concentrations ≥ 600 $\mu l.liter^{-1}$, and at incident light levels ≥ 1300 $\mu mol.m^{-2}.s^{-1}$. The resistant and susceptible biotypes had similar carboxylation efficiencies and quantum yield.

INTRODUCTION

Since simazine resistance was reported in common groundsel (*Senecio vulgaris* L.) (Ryan 1970), 60 species (42 dicots and 18 monocots) were reported to be resistant to triazine herbicides worldwide (Heap 1998). Although

triazine-resistance has been the most common type of resistance reported (Pfister and Arntzen 1979; Radosevich and Appleby 1973), resistance has been reported to at least 14 other classes of herbicides (LeBaron 1991).

The mechanism of triazine resistance was first thought to be some property of the chloroplast associated with photosystem II (PS II) (Radosevich and DeVilliers 1976). Pillai and St. John (1981) noted changes in lipid composition in resistant biotypes of common groundsel, common lambsquarters (*Chenopodium album* L.) and redroot pigweed (*Amaranthus retroflexus* L.). Burke et al. (1982) observed differences in photosynthetic unit size and chlorophyll *a/b* ratio in triazine-resistant wild turnip (*Brassica campestris* L.). They proposed that any or all of these changes could account for the observed triazine resistance. Vaughn (1986) also reported differences in starch accumulation and chloroplast structure of atrazine-resistant canola (*Brassica napus* L.). In 1983, Hirschberg and McIntosh reported that the resistant biotype of smooth pigweed (*Amaranthus hybridus* L.) has a small alteration in the D1 protein of PS II so that triazine herbicides are no longer bound. This alteration (serine-to-glycine at position 264) in the D1 protein is accepted today as the usual cause for triazine resistance in weeds. In general, a common factor among all triazine-resistant weeds is their reduced competitive ability (Ahrens and Stoller 1983; Radosevich and Holt 1982).

Several researchers attempted to determine the physiological bases for competitive differences between the resistant and susceptible biotypes of triazine-resistant weeds. Holt et al. (1981) measured photosynthesis of resistant and susceptible biotypes of common groundsel (a C_3 plant) under various light intensities. Ort et al. (1983) used saturating CO_2 concentrations to measure photosynthesis of smooth pigweed (a C_4 plant) under various light intensities. Ahrens and Stoller (1983) used non-saturating CO_2 concentrations to measure photosynthesis of smooth pigweed under various light intensities. Hobbs (1987) measured photosynthesis of resistant and susceptible wild turnip and oilseed rape (*Brassica napus* L.) (C_3 plants) in response to various light and temperature regimes. Dekker and Sharkey (1992) measured the response of oilseed rape to different temperature regimes. In all cases, they found that CO_2 assimilation of the susceptible biotype exceeded that of the resistant biotype.

A linuron-resistant common purslane (*Portulaca oleracea* L.) biotype was discovered in a carrot field in 1991 in Imlay City, Michigan, and subsequent research confirmed cross-resistance to atrazine, cyanazine, prometryn, and diuron (Chapter 1). This resistant biotype has an unusually high resistance for linuron, compared to the typical triazine-resistant weeds as described by Fuerst et al. (1986).

The purpose of the research reported here was to determine the fitness of resistant (R-POROL) and susceptible (S-POROL) common purslane, to compare them to other known triazine-resistant weeds, and to determine differences in morphology and physiology of the two biotypes.

MATERIALS AND METHODS

Plant Material. R-POROL and S-POROL plants were grown in separate greenhouses to avoid cross-pollination. A photoperiod of 16 h light and 8 h dark was maintained with sunlight and with high-pressure sodium lighting, with an average intensity of 800 $\mu mol.m^{-2}.s^{-1}$. Mean daily temperatures of 25 ± 5 C were maintained during the study. The same light and temperature conditions were used in all greenhouse experiments. Plants were fertilized once weekly with Peters 20-20-20 fertilizer (20N-8.6P-16.6K) solution[1]. Seeds were collected for three weeks from 20 plants of each common purslane biotype. The seeds were sifted, cleaned, and stored at 3 C.

Seed Weight. Seeds of R-POROL and S-POROL were sampled 12 times with four sub-samples of 100 seeds each. The 100-seed samples were weighed with a Mettler AE200 balance. A t-test comparison was conducted on the combined means of each biotype.

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[1] Scotts, Allentown, Pennsylvania

Seedling Emergence. Seeds of R-POROL and S-POROL were planted individually in 200-cell (each cell 10 cc), 28 x 56-cm flats, with 10 flats as replicates per biotype. The flats were placed on a greenhouse bench under supplemental lighting of 800 $\mu mol.m^{-2}.s^{-1}$ with a photoperiod of 16 h light and 8 h dark. Mean daily temperatures were maintained at 25 ± 5 C. Clear plastic covers were placed over the flats to maintain humidity and protect young seedlings from desiccation. Seedlings were counted daily for two weeks. The experiment was repeated three times.

Fresh and Dry Weight Measurements. Two experiments were conducted to quantify differences in growth rates between R-POROL and S-POROL. In all experiments a replication consisted of one plant per pot. Common purslane was transplanted into 946-ml plastic pots containing BACCTO[2] high porosity professional planting mix (55%-65% horticultural sphagnum peat and 35%-45% perlite, with pH 5.5 - 6.5). In the first experiment, 100 plants each of R-POROL and S-POROL were transplanted 18 days after seeding (DAS). Three uniform plants of each biotype were harvested and weighed at 39, 42, 44, 47, 49, 54, and 59 DAS. In the second experiment, 250 plants each of R-POROL and S-POROL were transplanted 65 DAS and 16 plants per biotype were harvested and weighed at 77, 79, 84, 87, 89, and 94 DAS. The plants were dried in a forced hot air oven at 45 C for two days and then weighed. Fresh and dry weight

measurements were analyzed at each DAS using a t-test comparison between the two biotypes.

Ultrastructural Characteristics Using TEM. One leaf was removed from each of six 8-week-old plants per biotype. Several 1 mm^2 pieces were cut from each leaf, avoiding the midrib, and fixed under vacuum in 4% (w/v) glutaraldehyde - 1% (w/v) formaldehyde in 0.05M HEPES[3] buffer at room temperature. The residual fixative was removed by three washes of 2 min each with the same buffer. Tissue pieces were then post-fixed by a 2 to 4 h treatment with 1% (w/v) osmium tetroxide in water. Osmium tetroxide was removed by three washes of 20 min each in deionized water. The fixed leaf discs were dehydrated in a 25% graded series of ethanol solutions and then embedded in equivalent amounts of VCD resin (Spurr 1969) and Quetol 651 resin (Kushida 1974). Median cross-sections, 80-120 nm, were made from three samples per biotype using a RMC MTX ultramicrotome[4]. Sections with gold-silver reflectance were mounted on uncoated 300 mesh copper grids, stained with uranyl acetate and lead citrate (Hanaichi et al. 1986) for 5 min and observed with a Philips CM-10[5] transmission electron microscope operating at 100 kV. To assure randomness, mircrographs were taken of all the bundle sheath and Kranz mesophyll cell plastids (Fischer and Evert 1982). Prints at

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[2] Michigan Peat Company, Houston, Texas
[3] N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
[4] RMC, Tucson, Arizona
[5] Eindhoven, Netherlands

5,800X magnification were taken from at least three grids representing three different leaf samples. Contact prints (7.5 x 10 cm) of TEM negatives were prepared and overlapping prints were assembled to form a complete image of the area of interest. Total number of chloroplasts and starch grains of the two biotypes were counted from equal areas of measurement from the assembled TEM prints. Length and width measurements of chloroplasts and starch granules were taken to the nearest mm for both biotypes. The actual lengths and widths were obtained by dividing the measurements by the magnification level (5,800X), and are presented in μm units.

Photosynthetic Response Curves. Five uniform plants each of R-POROL or S-POROL and of triazine-resistant (R-CHEAL) or triazine-susceptible (S-CHEAL) common lambsquarters[6] were used to determine the rate of CO_2 assimilation (A) in response to increasing CO_2 concentrations, light intensities, and temperature levels. Triazine-resistant and -susceptible common lambsquarters (C_3 plant) were included to compare their photosynthetic performance to common purslane (C_4 plant). The common purslane and common lambsquarters were grown under the same conditions. Instantaneous measurements of photosynthetic carbon assimilation were obtained from attached leaves of 8-week-old plants using an "open" system, portable infrared gas

[6] Seeds of resistant and susceptible common lambsquarters were obtained from Dr. Donald Penner, Department of Crop and Soil Sciences, Michigan State University, E. Lansing, Michigan

analyzer and data logger (CIRAS-1[7]). Single leaves were equilibrated in the 2.5 cm^2 assimilation chamber for 60 sec before measurements were taken. For each measurement, the infrared gas analyzer recorded the following data: assimilation (A) in μmol $CO_2.m^{-2}.s^{-1}$, stomatal conductance (g_s) in $mmol.m^{-2}.s^{-1}$, and internal CO_2 concentration (C_i) in $\mu l.liter^{-1}$. Experiments were conducted in a walk-in growth chamber[8]. The growth chamber was programmed to the following settings: at 7 AM, air temperature = 28 C, relative humidity (RH) = 50%, photosynthetic active radiation (PAR) = 800 $\mu mol.m^{-2}.s^{-1}$; at 8 PM, air temperature = 20 C, RH = 50%, PAR = 0 $\mu mol.m^{-2}.s^{-1}$. Photosynthesis responses to changing CO_2 concentrations (CO_2 Response Curves) were determined by increasing the ambient CO_2 concentration stepwise to the following levels: 0, 200, 400, 600, 800, 1000, 1200, 1400, and 1600 ppm. Leaves were allowed to acclimate for 15 min at each CO_2 level before new measurements were taken, which allowed sufficient time for stomata to adjust. Temperature and light were kept constant at 28 ± 2 C and 1200 $\mu mol.m^{-2}.s^{-1}$, respectively. Photosynthesis responses to increasing light levels (Light Response Curves) were determined by increasing the incident light intensity in a stepwise manner with neutral density filters to the following levels: 10, 117, 278, 560, 740,

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[7] P.P. Systems, Haverville, Massachusetts
[8] Model PGV36, Controlled Environment Inc., Pembina, North Dakota

1250, and 2375 $\mu mol.m^{-2}.s^{-1}$. These levels were selected to allow for measurements at various light intensities from complete darkness to a light intensity beyond full sunlight. A 15 min period at each step allowed significant time for stomata to adjust. The chamber air temperature was kept constant at 28 ± 1 C and ambient CO_2 concentration at 359 ± 1 ppm. Measurements of CO_2 assimilation in response to increasing temperatures (Temperature Response Curves) were achieved by increasing the growth chamber air temperature from 10 C to 40 C at 10 C intervals, thus whole-plant temperature was changed, while maintaining incident light constant at 769 $\mu mol.m^{-2}.s^{-1}$ and CO_2 concentration at 355 ± 3 ppm. At each step, the temperature was allowed to equilibrate for 15 min before new measurements were taken.

Assimilation responses to increasing CO_2 levels, light, and temperatures were analyzed by non-linear regression for each biotype of the two plant species. Selecting the best-fit curve was performed using the Marquardt compromise method of successive approximations. The best-fit curve, evaluated by analysis of residuals and r^2, was the monomolecular model with position constants (Hunt 1980) of the type:

$$Y = a * [1 - b * e^{(-c * X)}] \qquad [1]$$

The constants a, b, and c are the asymptotic value, minimum value and rate constant, respectively. Y is the calculated CO_2 assimilation in $\mu mol.m^{-2}.s^{-1}$, and X is the independent

variable such as CO_2, light, or temperature level. This polynomial (equation 1) was selected because it provided direct estimates of specific physiological processes and it exhibited curvilinear features that represented the data. Individual leaf A vs. CO_2 concentration (AC_i Response Curves) were developed using this polynomial as per methods of Layne and Flore (1995). The AC_i response curve is the plot of calculated A vs. calculated C_i at each CO_2 concentration. The AC_i response curves are used in calculating the CO_2 compensation point (Γ) and the carboxylation efficiency (k). Figure 1 represents the AC_i response curves of R-POROL and S-POROL (Figure 1A), and of R-CHEAL and S-CHEAL (Figure 1B).

The CO_2 compensation point (Γ) is extrapolated from the AC_i plot as the C_i value when A is 0 $\mu mol.m^{-2}.s^{-1}$. Estimated carboxylation efficiency (k) is calculated as the slope in the linear portion between 0 and 400 $\mu l.liter^{-1}$.

Net CO_2 assimilation (A_{360}) and internal CO_2 concentration (C_{i360}) at atmospheric CO_2 level were determined from the non-linear regression equation by solving for Y when X = 360 ppm. Maximum net CO_2 assimilation rate (A_{max}) is determined as the asymptotic value (a) from the non-linear regression equation. Stomatal limitation to A (l_g) was calculated according to the differential method of Jones (1985). From the light response curves, light compensation point (c.p.) was extrapolated as the light level at which A was 0 $\mu mol.m^{-}$

$^{2}.s^{-1}$, i.e. by solving for X (light intensity in $\mu mol.m^{-2}.s^{-1}$) when Y (A in $\mu mol\ CO_2.m^{-2}.s^{-1}$) = 0. Total dark respiration rate (R_d) was extrapolated from the non-linear regression equation by solving for Y when X = 0 $\mu mol.m^{-2}.s^{-1}$. Photochemical efficiency or quantum yield (Φ) was calculated as the slope of A vs. PPFD in the linear portion between 10 and 278 $\mu mol.m^{-2}.s^{-1}$. Maximum net CO_2 assimilation rate (A_{max}) is determined as the asymptotic value (a) from the non-linear regression equation. Net CO_2 assimilation rate at full sunlight (A_{1200}) is determined from the equation by solving for Y when X = 1200 $\mu mol.m^{-2}.s^{-1}$.

RESULTS

Seed Weight. S-POROL seeds were approximately 30% heavier than R-POROL seeds (Table 1).

Seedling Emergence. R-POROL seedlings first emerged at 2 DAS. S-POROL seedlings first emerged at 3 DAS. Both biotypes achieved maximum emergence by 5 DAS. S-POROL achieved 80% emergence at 5 DAS, and R-POROL reached 65% by 4 DAS (Figure 2).

Fresh and Dry Weight Measurements. For all six harvest dates between 77 and 94 DAS, fresh and dry weight of S-POROL plants exceeded that of R-POROL (Figure 3).

Ultrastructural Characteristics Using TEM. The ultrastructure of the chloroplasts of R-POROL is markedly different from that of S-POROL (Figure 4). In equal areas

from TEM prints, there were 39 chloroplasts and 22 starch grains for R-POROL, compared to 82 chloroplasts and 29 starch grains for S-POROL. Thus, bundle sheath cells of the susceptible biotype contained more chloroplasts with fewer starch grains per chloroplast. Both S-POROL and R-POROL had a similar thylakoid network with few thylakoids per granum stack in the chloroplasts (Figure 4). Chloroplasts of R-POROL were significantly longer, 13.72 μm vs. 7.41 μm, but similar in width, 2.09 μm vs. 1.71 μm (Table 2). Starch grains of R-POROL are longer and wider than starch grains of S-POROL (Table 2, Figure 4).

Photosynthesis Response Curves.

CO_2 Response Curves. CO_2 assimilation (A) by R-POROL was significantly higher than by S-POROL at each CO_2 concentration ≥ 600 ppm (Figure 5A). CO_2 assimilation leveled off at a calculated maximum (A_{max}) of 23 μmol $CO_2.m^{-2}.s^{-1}$ for R-POROL and 14 μmol $CO_2.m^{-2}.s^{-1}$ for S-POROL (Table 3). Stomatal conductance (g_s) of R-POROL was significantly higher than that of S-POROL at CO_2 concentration ≥ 1000 ppm (Figure 5B). There were no differences in C_i (Figure 5C) for the two biotypes. At ambient CO_2 concentration (360 ppm), R-POROL and S-POROL had similar carboxylation efficiency rates (k), similar net CO_2 assimilation rates (A_{360}), and similar assimilation potential (A_{360}/A_{max}) (Table 3). The CO_2 compensation point (Γ) (Table 3), extrapolated

from the AC_i response curves (Figure 1), was significantly lower in R-POROL. Stomatal limitation to A (l_g) was significantly higher in R-POROL (Table 3).

Whereas A of the two biotypes of common purslane leveled off starting at CO_2 concentration of 600 ppm, A of R-CHEAL and S-CHEAL continued to increase, reaching a maximum at CO_2 concentration of 1400 ppm (Figure 5D). A (Figure 5D), g_s (Figure 5E), and C_i (Figure 5F) of R-CHEAL were significantly greater than those of S-CHEAL at CO_2 concentration $\geq$ 800 ppm. R-CHEAL and S-CHEAL had lower photosynthetic potentials (A_{360}/A_{max}) (19% and 13%) than R-POROL and S-POROL (60% and 70%) (Table 3). There was no difference in Γ between R-CHEAL and S-CHEAL, but k of R-CHEAL was significantly greater than that of S-CHEAL (Table 3).

Light Response Curves. A of R-POROL and S-POROL was similar at all light levels except 2375 $\mu mol.m^{-2}.s^{-1}$ (Figure 6A). There were no differences between the two biotypes for g_s (Figure 6B) and C_i (Figure 6C). At full sunlight (PPFD of 1200 $\mu mol.m^{-2}.s^{-1}$), both R-POROL and S-POROL were operating at similar photosynthetic potentials (A_{1200}/A_{max}) of about 88% of the maximum (Table 4). There were no differences in c.p., R_d, and Φ (Table 4).

In response to increasing light levels, A of R-CHEAL and S-CHEAL had a maximum rate at light intensity of 560 $\mu mol.m^{-2}.s^{-1}$ (Figure 6D). CO_2 assimilation of R-CHEAL was

significantly higher than S-CHEAL at light levels ≥ 740 $\mu mol.m^{-2}.s^{-1}$. g_s (Figure 6E) was significantly higher for R-CHEAL at all PPFD levels. There were no significant differences between C_i of R-CHEAL and S-CHEAL at all light levels (Figure 6F). The two common lambsquarters biotypes had similar c.p., R_d, Φ, A_{1200}, A_{max}, and A_{1200}/A_{max} (Table 4). Photosynthetic efficiency of R-CHEAL and S-CHEAL was at or near 100% compared to about 88% for R-POROL and S-POROL.

Temperature Response Curves. A (CO_2 assimilation) of S-POROL increased linearly with increasing chamber temperature between 10 C and 30 C but dropped at 40 C (Figure 7A). A of R-POROL leveled off at about 20 C. A of S-POROL was significantly greater than that of R-POROL at temperatures ≥ 30 C. A of R-CHEAL and S-CHEAL decreased with increasing temperatures (Figure 7D). The highest A level for S-CHEAL (16 $\mu mol\ CO_2.m^{-2}.s^{-1}$) was observed at 10 C. At 40 C, A dropped to 11 $\mu mol\ CO_2.m^{-2}.s^{-1}$ for R-CHEAL and 7 $\mu mol\ CO_2.m^{-2}.s^{-1}$ for S-CHEAL. A did not differ between R-CHEAL and S-CHEAL at any temperature, except at 40 C at which A of S-CHEAL was significantly lower.

g_s (stomatal conductance)of S-POROL was significantly greater than that of R-POROL at all temperatures ≥ 20 C (Figure 7B). There were no differences in g_s (Figure 7E) between the two biotypes of common lambsquarters.

C_i (internal CO_2 concentration) was statistically different between the two biotypes of common purslane (Figure 7C) at all temperatures except 30 C. C_i of S-CHEAL steadily increased with increasing temperatures, whereas C_i of R-CHEAL was similar at all temperatures (Figure 7F). At 40 C, C_i of S-CHEAL was significantly higher than C_i of R-CHEAL.

DISCUSSION

S-POROL was more vigorous, or fit, than R-POROL for most parameters examined. S-POROL had heavier and larger seeds, and greater fresh and dry matter accumulation. This is typical of the "fitness" model described by Hobbs (1987) for other triazine-resistant weeds in which the susceptible biotype is more vigorous. Holt and Radosevich (1983) showed that dry matter accumulation, height, number of leaves, and leaf area of the susceptible biotype of common groundsel were greater than those of the resistant biotype at all harvest dates under both low and high light regimes. They suggested that the slower growth in triazine-resistant plants was due to lowered photosynthetic capacity, which limited growth and reduced relative root production. Less vigorous growth of the resistant biotype has been shown in other triazine-resistant weed species also (Ahrens and Stoller 1983).

Resistant common purslane had more and slightly larger starch grains, and fewer and larger chloroplasts. However,

the question remains whether larger and more numerous starch grains in the resistant biotype are the cause for reduced growth rates due to competition with other processes of growth and development for available photosynthates, or are the result of an impaired electron transport system. Both resistant and susceptible common purslane had similar thylakoid networks with few thylakoids per granum.

Some triazine-resistant weeds that have been studied at the cellular level have had a micro-morphology quite different from this R-POROL. Triazine-resistant mustard (*Brassica campestris* L.)(Burke et al. 1982), canola (Vaughn 1986), smooth pigweed, and common lambsquarters (Vaughn and Duke 1984), had more thylakoids per granum, fewer starch grains, and thylakoid networks different from the susceptible biotypes and similar to plants grown under low light intensity. Vaughn and Duke (1984) suggested that the structural alterations observed in resistant plants are secondary effects of impaired photosynthetic electron transport, and are not the cause of triazine resistance.

In contrast to these results, Pölös et al. (1987) found that the chloroplast ultrastructure in triazine-resistant horseweed (*Conyza canadensis* L.) was not similar to plants grown under low light intensity. Chloroplasts of resistant plants lacked starch grains and contained fewer thylakoids per granum stack. They also observed no differences in rates of CO_2 fixation between resistant and susceptible horseweed. McCloskey and Holt (1990) regard such

observations as examples of traits controlled by the nuclear genome which compensated for the detrimental traits associated with triazine resistance, or as examples of a combination of nuclear genome effects and environmental conditions that favor the resistant biotype.

Both R-POROL and S-POROL reached saturating CO_2 assimilation rates at a CO_2 concentration of 600 ppm. R-POROL and S-POROL appeared to be light-limited. Thus, an increase in both light intensity and CO_2 concentration are required for an increase in CO_2 assimilation. In the case of common lambsquarters, the two biotypes were not light-limited and CO_2 assimilation continued to increase with increasing CO_2 concentration. The reasons behind these differences between common purslane and common lambsquarters are not evident, but appear to be related to their differential CO_2 assimilation pathway, i.e. C_3 pathway for common lambsquarters and C_4 for common purslane.

CO_2 assimilation of R-POROL was significantly higher at CO_2 concentration $\geq$ 600 ppm (Figure 5A), and at light intensities of 2375 $\mu mol.m^{-2}.s^{-1}$ (Figure 6A). Thus, R-POROL has the potential of outgrowing S-POROL under those growing conditions. However, a CO_2 concentration of 600 ppm and light intensity of 2375 $\mu mol.m^{-2}.s^{-1}$ are not possible under normal atmospheric conditions.

CO_2 assimilation of S-POROL increased more with increasing temperatures (Figure 7A), becoming significantly

different at temperatures ≥ 30 C. This is a plausible explanation for the observed higher growth rate of S-POROL at normal atmospheric conditions.

Under normal conditions of light (1200 $\mu mol.m^{-2}.s^{-1}$) and CO_2 (360 ppm), both R-POROL and S-POROL have similar assimilation rates. A cause of the lower growth rate in R-POROL could be the reduced efficiency of stomata. About 68% of the total resistance for CO_2 diffusion into the leaves is due to stomatal limitations in the resistant biotype compared to 28% in the susceptible biotype (Table 3). In addition, the difference between the stomatal limitations of R-POROL and S-POROL (68% - 28% = 40%) was three times the difference between the stomatal limitations of R-CHEAL and S-CHEAL (45% - 33% = 12%). With similar quantum yield and carboxylation efficiency, stomatal limitation to CO_2 diffusion must play a major role in the reduced growth rate of R-POROL.

Our results indicated that R-POROL and S-POROL had similar CO_2 assimilation rates at a non-saturating CO_2 concentration of 360 ppm. In addition, both biotypes had similar quantum yield. However, results with other resistant plants have been different. Ahrens and Stoller (1983) also used non-saturating CO_2 conditions in their measurements of CO_2 uptake in response to increasing light intensity and found that the susceptible biotype of smooth pigweed had higher rates of CO_2 assimilation than the resistant biotype. When they supplied leaves of both

biotypes with elevated CO_2 concentrations (up to 1000 ppm), they found no further increase in light-saturating CO_2 fixation rates. Since smooth pigweed is a C_4 plant, they concluded that the difference in CO_2 fixation rates was not due to differences in stomatal behavior or other characteristics of the leaf that affected CO_2 availability at the site of fixation in the chloroplast.

Ort et al. (1983) also worked on smooth pigweed, using saturating CO_2 concentrations up to 1500 ppm, and found that the rate of CO_2 assimilation in the susceptible biotype was always higher than in the resistant biotype and that the maximum rate of CO_2 assimilation was extremely high in both biotypes. The maximum rate of CO_2 assimilation in smooth pigweed was nearly equal to the highest rates for any species reported by Mooney et al. (1976). Ort et al. (1983) concluded that the differences couldn't be accounted for by a difference in stomatal aperture. The work of Holt et al. (1981) on common groundsel (C_3 plant) showed that at non-saturating CO_2 concentrations of 330 - 360 ppm, the rate of CO_2 assimilation and the quantum yield of the susceptible biotype in response to various light intensities was significantly higher than that of the resistant plants. They concluded that differences in photosynthesis were not due to differential leaf absorptance (the ratio of absorbed to incident radiation), but to differences in the photosynthetic system itself.

In our experiments, we compared common lambsquarters (a C_3 plant) to common purslane (a C_4 plant). We also used non-saturating CO_2 conditions similar to those reported by Ahrens and Stoller (1983). The CO_2 assimilation rate of R-POROL was significantly higher than that of S-POROL in response to CO_2 concentrations $\geq$ 600 ppm. In addition, the stomata played an important role in limiting CO_2 absorption in R-POROL.

The anatomical characteristics (chloroplast and starch grain size and numbers) are unique to common purslane and are not reminiscent of other triazine-resistant weeds such as mustard (Burke et al. 1982), canola (Vaughn 1986), smooth pigweed, and common lambsquarters (Vaughn and Duke 1984). Physiological characteristics such as the photosynthesis response of R-POROL to light, temperature, and CO_2 regimes is also different from other triazine-resistant weeds such as common lambsquarters (presented in this report), common groundsel (Holt et al. 1981), smooth pigweed (Ahrens and Stoller 1983; Ort et al. 1983), and canola (Dekker and Sharkey 1992; Hobbs 1987).

This linuron-resistant common purslane appears to be unique among triazine-resistant weeds.

LITERATURE CITED

Ahrens, W. H. and E. W. Stoller. 1983. Competition, growth rate, and CO_2 fixation in triazine-susceptible and resistant smooth pigweed (*Amaranthus hybridus*). Weed Sci. 31:438-444.

Burke, J. J., F. Wilson, and J. R. Swafford. 1982. Characterization of chloroplasts isolated from triazine-susceptible and triazine-resistant biotypes of *Brassica campestris* L. Plant Physiol. 70:24-29.

Dekker, J. H. and T. D. Sharkey. 1992. Regulation of photosynthesis in triazine-resistant and -susceptible *Brassica napus*. Plant Physiol. 98:1069-1073.

Fuerst, E. P., C. J. Arntzen, K. Pfister, and D. Penner. 1986. Herbicide cross-resistance in triazine-resistant biotypes of four species. Weed Sci. 34:344-353.

Fischer D. G. and R. F. Evert. 1982. Studies on the leaf of *Amaranthus retroflexus* (Amaranthaceae): Chloroplast polymorphism - Bot. Gaz. 143:146-155.

Hanaichi, T., T. Sato, T. Iwamoto, J. Malvasi-Yamashiro, M. Hoshino, and N. Mizuno. 1986. A stable lead by modification of Sato's method. J. Electron Microscopy (Japan) 35:304-305.

Heap, I. M. 1998. International survey of herbicide-resistant weeds. 1998 Herbicide-Resistance Action Committee Annual Report. WeedSmart, Corvallis, Oregon. 165 unnumbered pages.

Hirschberg, J. and L. McIntosh. 1983. Molecular basis of herbicide resistance in *Amaranthus hybridus* L. Science. 222:1346-1349.

Hobbs, S. L. A. 1987. Comparison of photosynthesis in normal and triazine-resistant *Brassica*. Can J. Plant Sci. 67:457-466.

Holt, J. S. and S. R. Radosevich. 1983. Differential growth of two common groundsel (*Senecio vulgaris*) biotypes. Weed Science. 31:112-120.

Holt, J. S., A. J. Stemler, and S. R. Radosevich. 1981. Differential light responses of photosynthesis by triazine-resistant and triazine-susceptible *Senecio vulgaris* biotypes. Plant Physiol. 67:744-748.

Hunt, R. 1980. Asymptotic functions, p. 121-146. In: Plant growth curves. Univ. Park Press, Baltimore.

Jones, H. G. 1985. Partitioning stomatal and non-stomatal limitations to photosynthesis. Plant, Cell and Environment. 8:95-104.

Kushida, H. 1974. A new method for embedding with a low viscosity epoxy resin "Quetol 651". J. Electron Microscopy. 23(3):197.

Layne, D. R. and J. A. Flore. 1995. End-product inhibition of photosynthesis in Prunus cerasus L. in response to whole-plant source-sink manipulation. J. Amer. Soc. Hort. Sci. 120(4):583-599.

LeBaron, H. M. 1991. Distribution and seriousness of herbicide-resistant weed infestations worldwide. p. 26-43 *in* J. C. Caseley, G. W. Cussans, and R. K. Atkin, eds. Herbicide Resistance in Weeds and Crops. Butterworth-Heinemann, Oxford, England.

Masabni, J. G. and B. H. Zandstra. 1998. Discovery of a common purslane (*Portulaca oleracea* L.) biotype which is resistant to linuron. Submitted to Weed Technology.

McCloskey, W. B. and J. S. Holt. 1990. Triazine resistance in *Senecio vulgaris* parental and nearly isonuclear backcrossed biotypes is correlated with reduced productivity. Plant Physiol. 92:954-962

Mooney, H. A., O. Björkman, F. Ehleringer, and J. Berry. 1976. Photosynthetic capacity of plants *in situ* Death Valley plants. Annu. Rep Carnegie Inst., pp. 410-413.

Ort, D. R., W. H. Ahrens, B. Martin, and E. W. Stoller. 1983. Comparison of photosynthetic performance in triazine-resistant and susceptible biotypes of *Amaranthus hybridus*. Plant Physiol. 72:925-930.

Pfister, K. and C. J. Arntzen. 1979. The mode of action of photosystem II-specific inhibitors in herbicide-resistant weed biotypes. Z. Naturforsch. 34c:996-1009.

Pillai, P. and J. B. St. John. 1981. Lipid composition of chloroplast membranes from weed biotypes differentially sensitive to triazine herbicides. Plant Physiol. 68:585-587.

Pölös, E., G. Laskay, Z. Szigeti, Sz. Pataki, and E. Lehoczki. 1987. Photosynthetic properties and cross-resistance to some urea herbicides of triazine-resistant *Conyza canadensis* Cronq. (L.). Z. Naturforsch. 42c:783-793.

Radosevich S. R. and A. P. Appleby. 1973. Studies on the mechanism of resistance to simazine in common groundsel. Weed Sci. 21:497-500.

Radosevich S. R. and O. T. DeVilliers. 1976. Studies on the mechanism of s-triazine resistance in common groundsel. Weed Sci. 24:229-232.

Radosevich, S. R. and J. S. Holt. 1982. Physiological responses and fitness of susceptible and resistant weed biotypes to triazine herbicides. Pages 163-183 *in* Herbicide Resistance in Plants. H. M. LeBaron, J. Gressel, eds.

Ryan, G. F. 1970. Resistance of common groundsel to simazine and atrazine. Weed Sci. 18:614.616.

Spurr, A. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31.

Vaughn, K. C. 1986. Characterization of triazine-resistant and -susceptible Isolines of Canola (*Brassica napus*). Plant Physiol. 82:859-863.

Vaughn, K. C. and S. O. Duke. 1984. Ultrastructural alterations to chloroplasts in triazine-resistant weed biotypes. Physiol. Plant. 62:510-520.

Table 1. Average seed weight per 100 seeds of resistant and susceptible common purslane.

	Weight of 100 seeds (mg)	Seeds / g
Resistant	8.1 ± 0.5	12,345
Susceptible	10.5 ± 0.6	9,524
Significance (0.05)	*	*

Means are averages of 4800 seeds per biotype.

Table 2. Chloroplast and starch grain size of resistant and susceptible common purslane measured from TEM prints taken at 5,800X magnification.

	Chloroplast				Starch Grain			
	Resistant		Susceptible		Resistant		Susceptible	
	Length	Width	Length	Width	Length	Width	Length	Width
	μm							
Mean	13.7	2.1	7.4	1.7	5.3	2.2	3.8	0.9
SD	8.9	0.8	2.8	0.4	3.4	1.3	1.5	0.3
CV	65%	60%	37%	24%	64%	58%	39%	31%

Table 3. CO_2 compensation point (Γ), estimated carboxylation efficiency (k), net CO_2 assimilation rate (A_{360}), estimated maximum assimilation (A_{max}), internal CO_2 concentration (C_{i360}) at atmospheric CO_2 concentration (360 ppm), photosynthetic efficiency (A_{360}/A_{max}), and stomatal limitation to A (l_g) of resistant and susceptible common purslane (C_4 plant) and common lambsquarters (C_3 plant). Figures are means of five leaf measurements.

Species	Γ	k	A_{360}	A_{max}	C_{i360}	A_{360}/A_{max}	l_g
	$\mu mol\ CO_2.mol^{-1}$	$mol\ CO_2.m^{-2}.s^{-1}$	$\mu mol\ CO_2.m^{-2}.s^{-1}$		$\mu l.liter^{-1}$	%	%
Common purslane							
Resistant	38	0.047	14	23	210	60	68
Susceptible	61	0.036	10	14	235	70	28
Significance (0.05)	*	NS	NS	*	NS	NS	*
Common lambsquarters							
Resistant	110	0.043	11	60	273	19	33
Susceptible	96	0.032	9	72	237	13	45
Significance (0.05)	NS	*	NS	*	*	NS	*

Table 4. Light compensation point (c.p.), total dark respiration (R_d), photochemical efficiency or quantum yield (Φ), assimilation at full sunlight 1200 $\mu mol.m^{-2}.s^{-1}$ (A_{1200}), estimated maximum assimilation (A_{max}), and CO_2 photosynthetic efficiency (A_{360}/A_{max}) of resistant and susceptible common purslane (C_4 plant) and common lambsquarters (C_3 plant). Figures are means of 5 leaf measurements.

			Φ			
			$\mu mol\ CO_2$ fixed			
Species	c.p.	R_d	/ mol PPFD	A_{1200}	A_{max}	A_{1200}/A_{max}
	$\mu mol\ CO_2.m^{-2}.s^{-1}$			$\mu mol\ CO_2.m^{-2}.s^{-1}$		%
Common purslane						
Resistant	69	-2.1	0.023	14	16	87
Susceptible	100	-2.7	0.019	11	12	88
Significance (0.05)	NS	NS	NS	NS	NS	NS
Common lambsquarters						
Resistant	81	-8.1	0.045	13	13	99
Susceptible	64	-5.6	0.037	9	8	100
Significance (0.05)	NS	NS	NS	NS	NS	NS

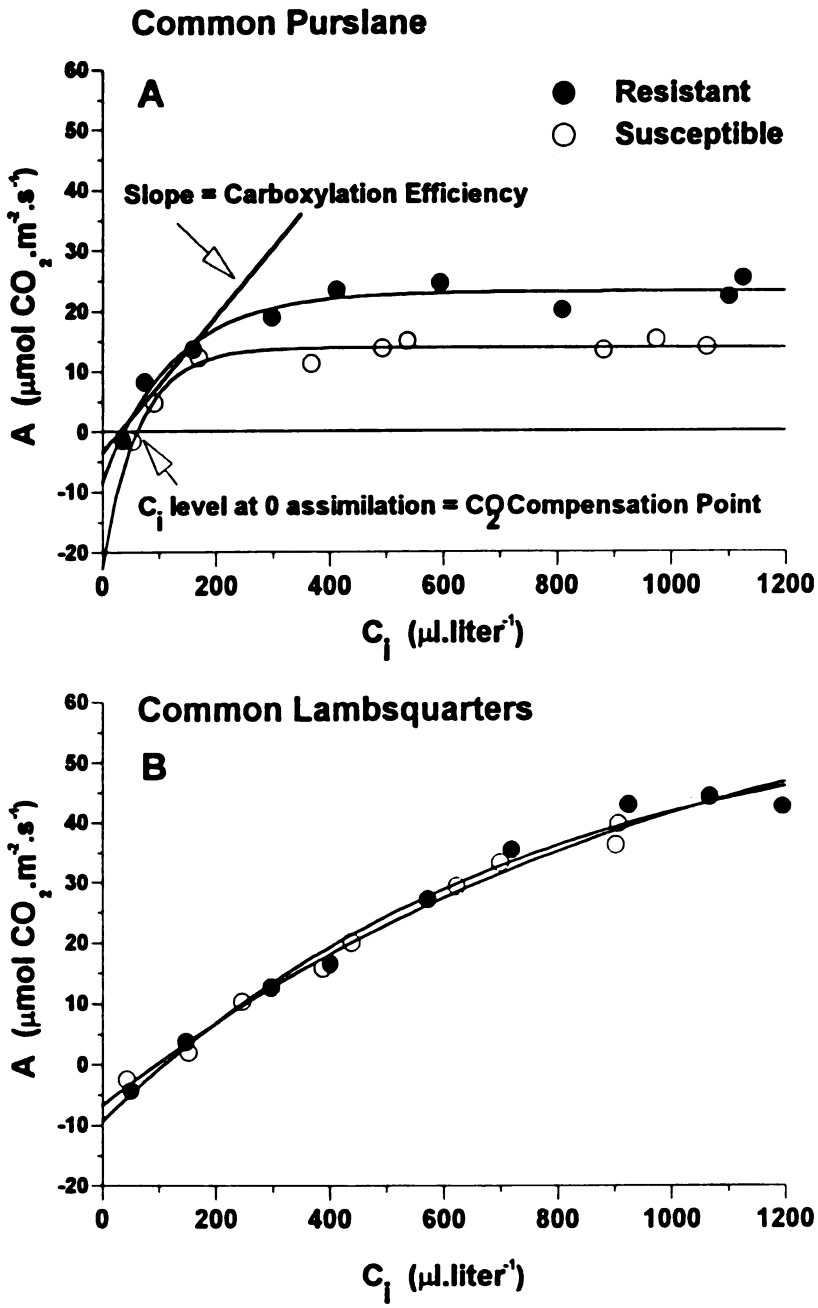

Figure 1. Assimilation vs. internal CO_2 concentration (AC_i curves) of resistant and susceptible common purslane and common lambsquarters.

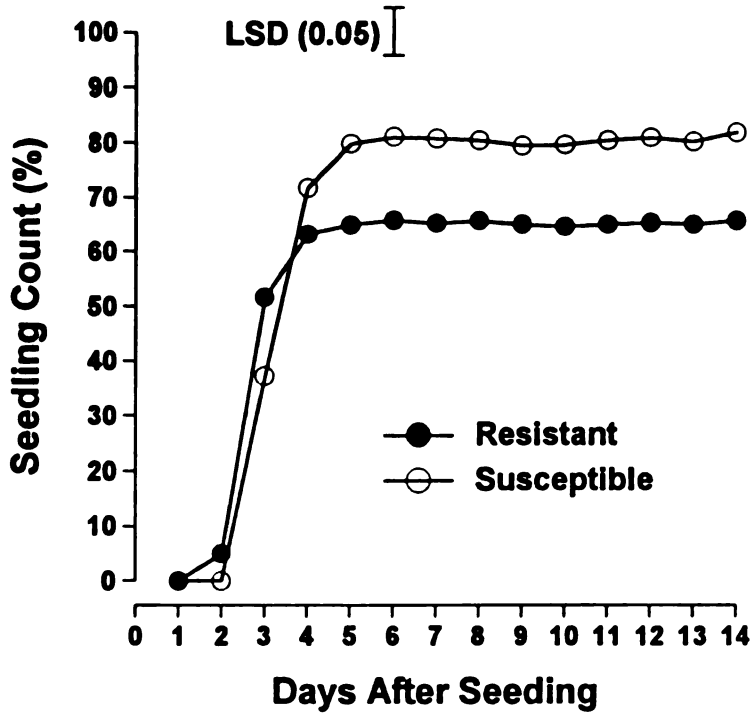

Figure 2. Daily counts of emerged seedlings of resistant and susceptible common purslane.

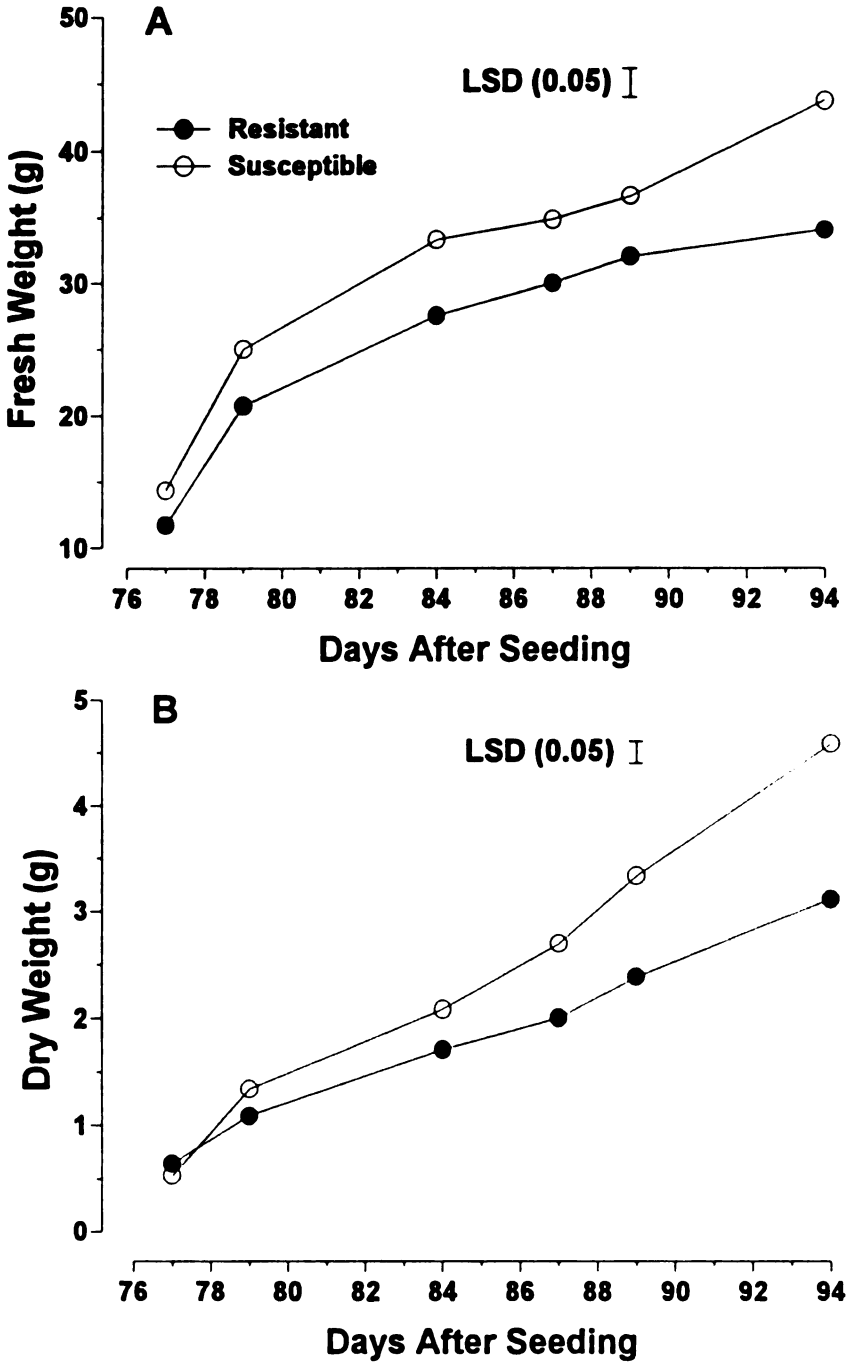

Figure 3. Fresh and dry weight measurements of resistant and susceptible common purslane taken at various dates between 77 and 94 days after seeding.

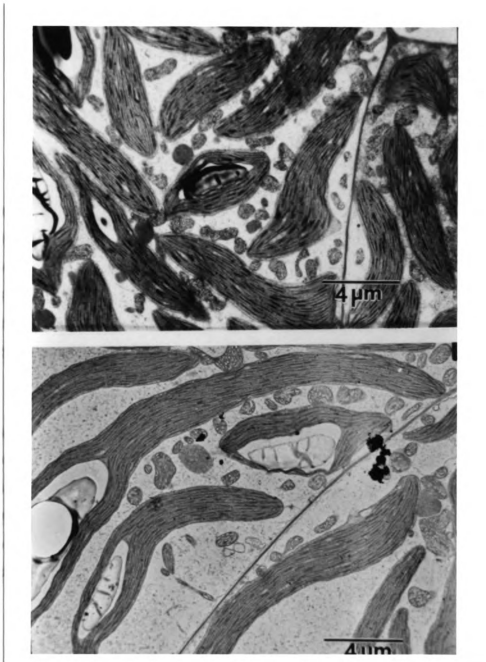

Figure 4. Ultrastructural characteristics of chloroplasts of susceptible (A) and resistant (B) biotypes of *Portulaca oleracea* L.

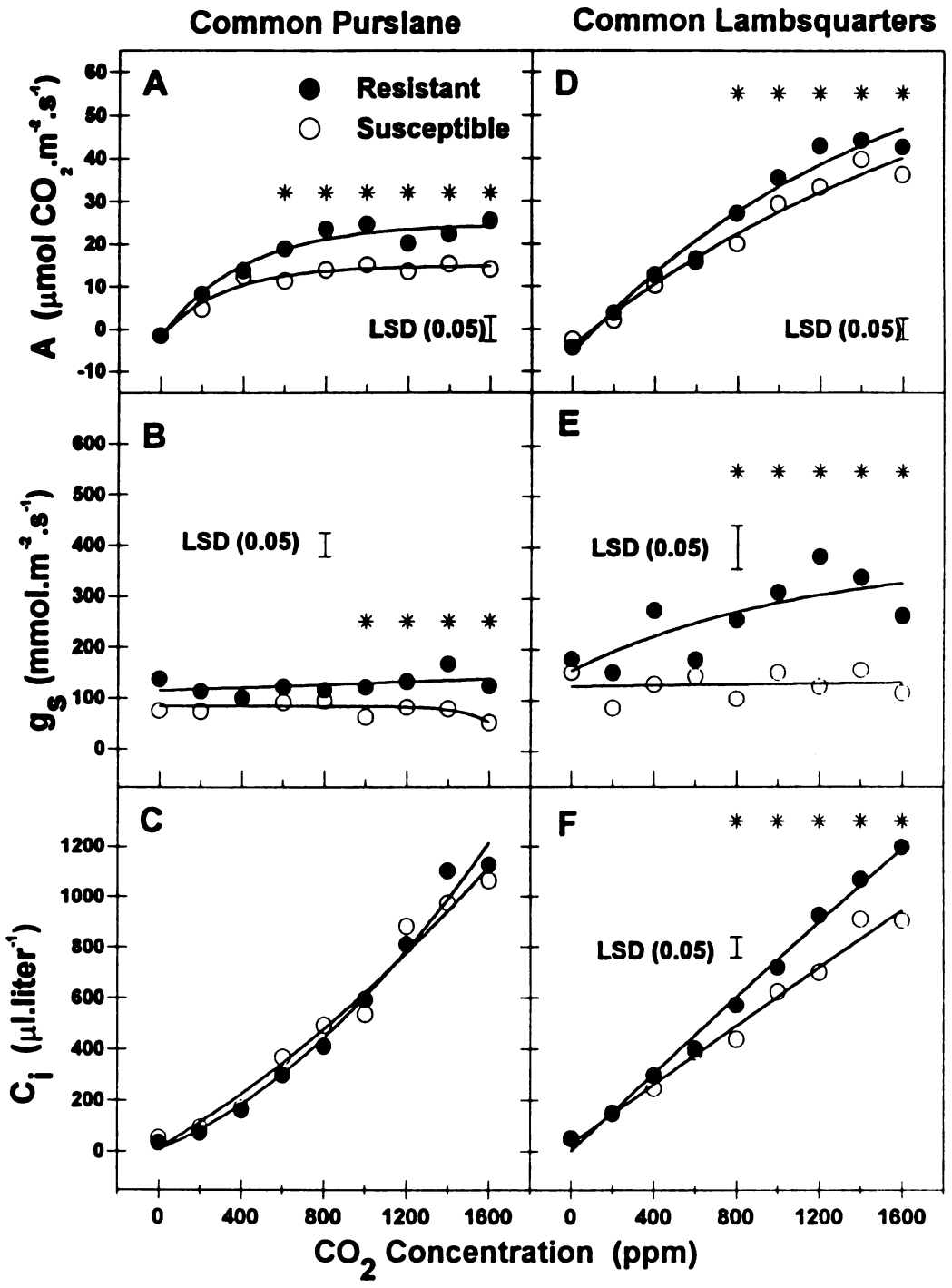

Figure 5. CO_2 response curves of resistant and susceptible common purslane and common lambsquarters.

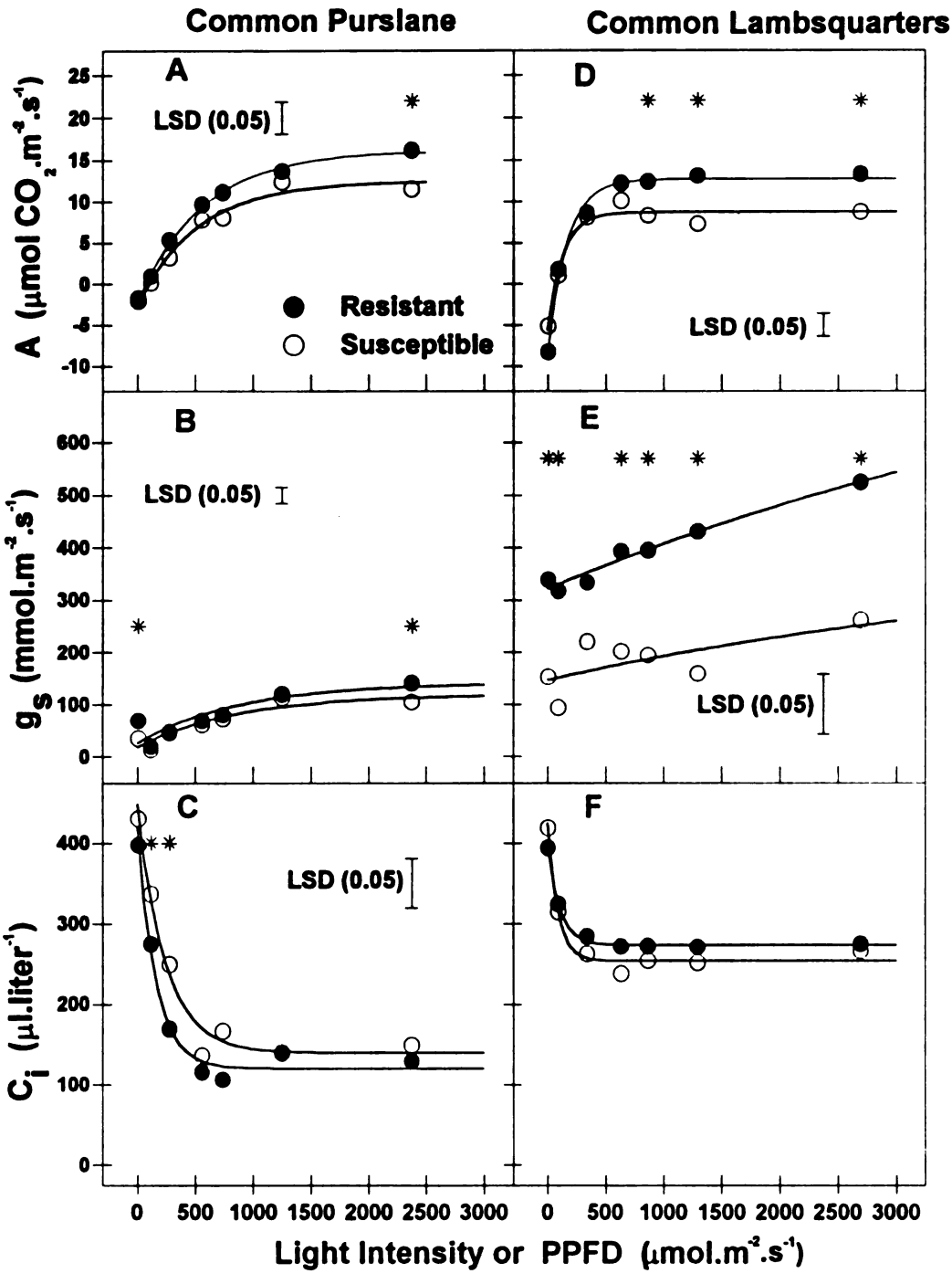

Figure 6. Light response curves of resistant and susceptible common purslane and common lambsquarters.

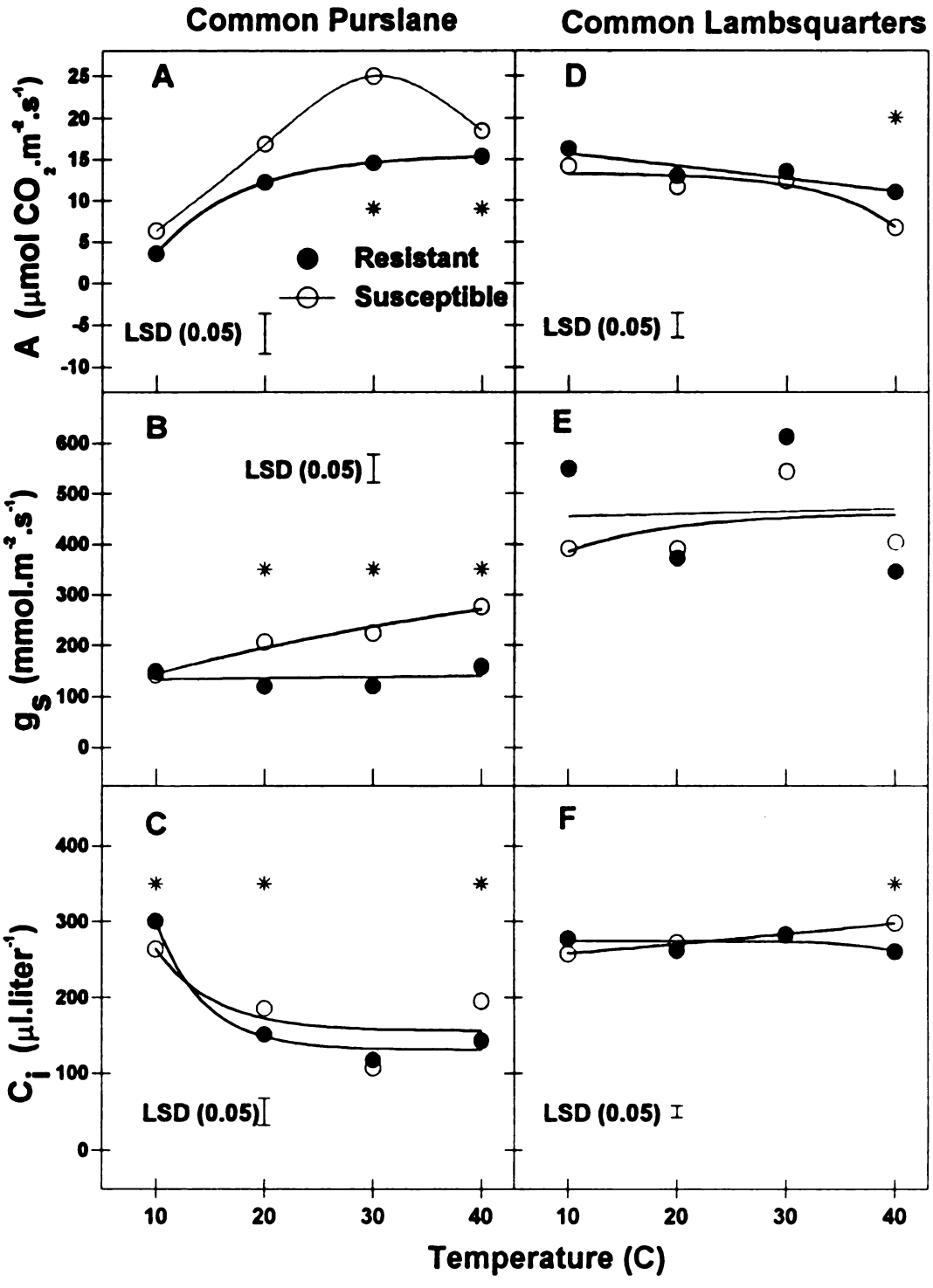

Figure 7. Temperature response curves of resistant and susceptible common purslane and common lambsquarters.

Chapter 4

A Novel Serine to Threonine Mutation in Linuron-Resistant Common Purslane (*Portulaca oleracea* L.)

A Novel Serine to Threonine Mutation in Linuron-Resistant Common Purslane (*Portulaca oleracea* L.)

ABSTRACT

Several photosynthesis-inhibiting herbicides were applied to linuron-resistant and susceptible common purslane and common lambsquarters, and O_2 evolution was measured by a Clark-type O_2 electrode. Resistance ratios (RR) based on O_2 evolution inhibition were 8 and > 6 for linuron and diuron, respectively, > 800 for atrazine, and > 20 for terbacil. Resistant common purslane was negatively cross-resistant to bentazon and pyridate (RR = 0.5 and 0.75, respectively). Time-course measurements of fresh weight, photosynthetic CO_2 assimilation, and photochemical efficiency indicated that linuron and atrazine inhibited electron transport in susceptible common purslane and common lambsquarters, resulting ultimately in death. Measurements of photochemical efficiency and CO_2 assimilation of resistant common purslane treated with linuron indicated transient injury from which plants recovered within 14 days of treatment. Recovery of resistant common purslane from atrazine injury was more rapid than from linuron injury for all measured variables. On the other hand, atrazine-resistant common lambsquarters had no cross-resistance to linuron and was equally injured at all rates as the atrazine-susceptible biotype. Sequence analysis of the D1 protein revealed that the resistance in common purslane is

due to a serine-to-threonine substitution at position 264. This is the first report of serine-to-threonine substitution observed at the whole plant level.

INTRODUCTION

Point mutations of the *psb*A gene have been correlated with resistance to triazine herbicides in several higher plants such as smooth pigweed (*Amaranthus hybridus* L.) (Hirschberg and McIntosh 1983) and black nightshade (*Solanum nigrum* L.)(Hirschberg et al. 1984) and in the green alga *Chlamydomonas reinhardtii* (Erickson et al. 1984). The *psb*A gene encodes the D1 protein which is essential in the electron transport pathway of photosystem II (PS II) and is the site of herbicide binding (Pfister et al. 1981).

Generally, triazine-resistance in weeds is associated with a reduction in relative ecological fitness. Triazine-resistant biotypes exhibit a reduction in CO_2 fixation, quantum yield, and seed and biomass production (Bowes et al. 1980; Conard and Radosevich 1979; Holt et al. 1981; Holt and LeBaron 1990; Jursinic and Pearcy 1988). However, some triazine-resistant biotypes have similar or greater biomass productivity (Warwick and Black 1981) or CO_2 assimilation rates (Schönfeld et al. 1987) compared to the susceptible biotypes. McCloskey and Holt (1990) regard these mutants as examples of traits controlled by the nuclear genome that have compensated for the detrimental traits associated with triazine resistance or as examples of a combination of

nuclear genome effects and environmental conditions that favor the resistant biotype of a non-isonuclear resistant and susceptible biotype pair.

Not all mutations in the D1 protein result in resistance to every triazine herbicide. A mutation of leucine-to-phenylanaline at position 275 of a *Chlamydomonas reinhardtii* mutant resulted in a 20-fold resistance to metribuzin and a 5-fold resistance to diuron, without any observed resistance to atrazine (Wildner et al. 1989).

In order to study the structure-activity relationship of the herbicide binding site in the D1 protein, various descriptive models have been made available. These models, based on the photosynthetic reaction center of the bacterium *Rhodopseudomonas viridis* and on the assignment of functional amino acid residues, describe the folding of the electron-carrier plastoquinone through the membrane and the topology of the binding niche for plastoquinone and herbicides (Egner et al. 1993; Sigematsu et al. 1989; Trebst 1987).

In addition to the descriptive models, point mutations in the *psb*A gene have been induced by site-specific mutagenesis in vitro to analyze the effects of the resulting modifications in the D1 protein on the electron transfer in PS II and on herbicide binding characteristics (Ohad and Hirschberg 1992).

A survey by Heap (1998) listed 42 dicots and 18 monocots that were resistant to triazines. A substitution of glycine for serine at position 264 in the D1 protein is the only

known naturally occurring mutation that causes triazine resistance in higher plants (Hirschberg et al. 1987). Associated with the serine-264 to glycine mutation are a number of pleiotropic characteristics such as modified galactolipid composition and an increase in the degree of unsaturation of fatty acids (Lehoczki et al. 1985; Pillai and St. John 1981), reduced growth rate (Hobbs 1987), high level of resistance to one or more triazine herbicides, and little or no resistance to phenylurea herbicides such as diuron (Fuerst et al. 1986; Pfister and Arntzen 1979). Moreover, chloroplasts in triazine-resistant weeds are similar to "shade chloroplasts", which develop under low light intensities and have increased grana stacking and a reduced chlorophyll *a/b* ratio (Gronwald 1994). Holt et al. (1983) observed altered patterns of O_2 evolution in chloroplasts of triazine-resistant common groundsel (*Senecio vulgaris* L.) and suggested that the altered kinetics may be due to an altered electron flow in the PS II complex of resistant chloroplasts. The reduction in photosynthetic efficiency is accompanied by a reduced capacity for CO_2 assimilation which reduces the growth rate and makes the resistant biotype less fit.

Our earlier research demonstrated that the triazine-resistant common purslane (R-POROL) responded differently to some conditions than most triazine-resistant weeds (Chapter 1). The research reported here was conducted to quantify the response kinetics of photosynthesis, fluorescence, and

growth rate of R-POROL and susceptible common purslane (S-POROL) treated with linuron or atrazine. Injury responses of common purslane were compared to those of a triazine-resistant (R-CHEAL) and susceptible (S-CHEAL) common lambsquarters. Finally, *psb*A gene sequencing of R-POROL and S-POROL was conducted to determine the genetic basis for the observed resistance.

MATERIALS AND METHODS

Time-Course Effects of Linuron and Atrazine.

General procedures. In order to quantify the injury response of common purslane over time, fresh weight, photosynthetic CO_2 assimilation, and chlorophyll fluorescence were measured at 4, 6, 9, 11, and 14 DAT. Fresh weight and chlorophyll fluorescence were measured at 4, 7, 11, and 14 DAT for common lambsquarters.

Plant Material. Seeds of R-POROL and S-POROL were planted in 28 x 56-cm, 200-cell flats using BACCTO[1] high porosity professional planting mix (55%-65% horticultural sphagnum peat and 35%-45% perlite, with pH 5.5 - 6.5) and a Blackmore automatic seeder[2]. Three weeks after seeding, seedlings were transplanted into 946-ml plastic pots containing BACCTO soil mix with one plant per pot. A photoperiod of 16 h light and 8 h dark was maintained with sunlight and with high-pressure sodium lighting yielding an average intensity

[1]Michigan Peat Company, Houston, Texas
[2]Blackmore Company Inc., Belleville, Michigan

of 800 $\mu mol.m^{-2}.s^{-1}$. The plants were watered and fertilized as needed with Peters 20-20-20 (20N-8.6P-16.6K) fertilizer solution[3]. In order to minimize variation due to plant size, plants were grouped by size prior to spraying. Herbicide treatments were applied to plants of nearly equal size 3 weeks after transplanting, using a moving-belt bench sprayer equipped with an 8001E nozzle and calibrated at 1.58 kph, 187 L/ha, and 133 KPa. Linuron was applied at rates of 0, 0.03, 0.07, 0.10, 0.13, and 0.17 kg ai/ha to S-POROL. Linuron at 0, 6.7, 7.8, 8.9, 10, and 11.2 kg ai/ha was applied to R-POROL. Atrazine was applied to S-POROL at 0, 0.45, 0.89, 1.34, 1.79, and 2.24 kg ai/ha, and to R-POROL at 0, 44.8, 89.6, 134.4, and 179.2 kg ai/ha.

Linuron was applied at rates of 0, 0.56, 1.12, 1.68, and 2.24 kg ai/ha to S-CHEAL. Linuron at 0, 1.12, 2.24, 4.48, and 6.72 kg ai/ha was applied to R-CHEAL. Atrazine was applied to S-CHEAL at 0, 1.12, 2.24, 3.36, and 4.48 kg ai/ha, and to R-CHEAL at 22.4, 44.8, 67.2, and 89.6 kg ai/ha. A total of 15 plants per biotype were sprayed with each herbicide treatment. Three plants or replicates per treatment were used in the measurement of the variables. After herbicide application, common purslane and common lambsquarters plants were arranged separately in a randomized complete block design on a greenhouse bench.

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[3] Scotts, Allentown, Pennsylvania

Analysis of variance was conducted and all means were compared by LSD within each biotype.

Fluorescence Measurements. A modulated fluorometer[4] was used to estimate the photochemical efficiency of R-POROL and S-POROL and of R-CHEAL and S-CHEAL after application with linuron or atrazine. The photochemical efficiency is a measure of the electron transport capacity from Q_A to Q_B in the PS II reaction center and is expressed as Fv/Fm or the ratio of variable to maximal fluorescence. Photochemical efficiency was recorded after a 0.4 sec burst of excitation light. Three readings were taken from each plant. Leaves were not dark-adapted before measuring Fv/Fm since we were interested in determining the herbicide effects relative to the control.

Photosynthetic CO_2 Assimilation. Instantaneous measurements of photosynthetic carbon assimilation were obtained from attached leaves using an "open" system, portable infrared gas analyzer and data logger[5]. Single leaves were equilibrated in the 2.5 cm^2 assimilation chamber for 60 sec before measurements were taken. Photosynthetic CO_2 assimilation (A) in $\mu mol\ CO_2.m^{-2}.s^{-1}$ was measured and recorded once for each plant.

Fresh Weight Measurements. Fresh weight of individual replications from each treatment was recorded using a Mettler AE200 balance.

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[4]Model OS-500, Opti-Sciences Inc., Tyngsboro, Massachusetts
[5]CIRAS-1, P.P. Systems, Haverville, Massachusetts

Electron-Transport Inhibition Measurements. In order to isolate viable chloroplasts, 30 g of leaves of each biotype was homogenized for 20 sec in the extraction medium of Radosevich and Devilliers (1976) and filtered through 12 layers of cheesecloth. The homogenate was centrifuged at 2400 rpm (Sorval SS-34 rotor) for 2 min at 4 C. The supernatant was collected and centrifuged at 4500 rpm for 10 min at 4 C. The resulting pellet was re-suspended gently in 2 ml of chloroplast suspension medium (Smeda et al. 1993). All procedures were completed at 4 C. Chloroplasts were maintained on ice and used immediately after isolation.

A Clark-type oxygen electrode[6] was used to measure the rate of electron transport in the presence of photosynthetic inhibitors. Aliquots of extracted chloroplasts containing the equivalent of 80 µg chlorophyll (Smeda et al. 1993) were placed in the sample cuvette. Sample temperatures were maintained at 29 C with a circulating water bath. Technical grade products of six photosynthesis-inhibiting herbicides (atrazine, linuron, diuron, terbacil, bentazon, and pyridate) were used in the preparation of concentrations ranging from 10^{-7} to 10^{-1} M. Dimethylsulfoxide (DMSO) was added at the equivalent of 1% on a volume basis. After the chloroplasts and the electron acceptor {3 mM $K_3Fe(CN)_6$} were added to the cuvette and sample temperatures had equilibrated, the photosynthesis-inhibiting herbicides were added under dim light and were allowed 1.5 to 3 min for

absorption. A light intensity of 225 $\mu mol.m^{-2}.s^{-1}$ was used to drive electron transport, and oxygen evolution was measured polarographically.

I_{50} concentrations (the herbicide concentrations required for 50% reduction in O_2 evolution) were calculated using non-linear regression. Curve-fitting was performed using the Marquardt compromise method of successive approximations. The best-fit curve, evaluated by analysis of residuals and r^2, was the exponential model (Hunt 1980) of the type :

$$Y = a * e^{(-b * X)} \qquad [1]$$

Y represents the O_2 concentration expressed as percent of the control, and X is the herbicide dosage. The constants a and b are predicted by the non-linear regression model. If a calculated I_{50} value fell outside the lowest or highest herbicide dosage used, the respective (<) or (>) symbols were used to reduce prediction error. I_{50} values are presented as the calculated molar concentration (M) for each herbicide. RR was determined by calculating the ratio of $I_{50\ Resistant}$ to $I_{50\ Susceptible}$. This experiment was conducted twice for each herbicide. The time interval between the replications was about 24 h.

Sequencing the D1 Protein. Total nucleic acids were isolated from S-POROL and R-POROL leaves according to Doyle and Doyle (1990). A 459-base-pair region of the *psb*A gene

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[6] DW2/2 Hansatech Instruments Ltd., Norfolk, England

encoding the herbicide-binding niche of D1 (Trebst 1991) was amplified by polymerase chain reaction (PCR) using the primers of Smeda et al. (1993) with total DNA as the template. The PCR cycle parameters were described by Yerkes and Weller (1995). PCR products were separated by gel electrophoresis, eluted, and cloned into a bacterial plasmid, pGEM-7Zf(+)[7]. Plasmids were integrated into a competent bacterium, *Escherichia coli* DH5α, and the transformed colonies were selected on Luria broth containing ampicillin (100 µg/ml) and Xgal (50 µg/ml). Plasmid DNA was isolated according to Maniatis et al. (1989). Both strands were sequenced by the Sanger chain termination method (Sanger et al. 1977) using vector primers, T7 and Sp6, and sequenase T7 DNA polymerase[8] to catalyze the reactions. Two sub-samples of two susceptible and two resistant plants were sequenced for a total of four replicates per biotype.

Strands of purified PCR products were sequenced also using the "dye dideoxy terminator" and the "dye primer" automated sequencing methods at the Michigan State University Sequencing Facility by using the ABI 373 DNA Sequencer[9]. Necessary primers were synthesized with a 308B DNA synthesizer[9] at the Macromolecular Facility, Department of Biochemistry, MSU.

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[7] Promega Corp., Madison, Wisconsin
[8] United States Biochemical Corp., Cleveland, Ohio
[9] Applied Biosystems, Foster City, California

RESULTS

Time-Course Effects of Linuron and Atrazine. Fresh weight of R-POROL treated with linuron started to decrease by 4 DAT, and ranged between 40 and 70% by 14 DAT (Figure 1A). Linuron had little or no effect on the photochemical efficiency (Fv/Fm) (Figure 1B) between 1 and 14 DAT. Photosynthetic CO_2 assimilation of R-POROL dropped at 1 DAT with linuron (Figure 1C). The trend of CO_2 assimilation showed a gradual recovery to about 70% with linuron rates of 6.7, 7.8, and 8.9 kg ai/ha. CO_2 assimilation for 10 and 11.2 kg ai/ha linuron remained at about 25% between 4 and 14 DAT. Although the photosynthetic rates of R-POROL treated with linuron dropped to about 25%, none of the plants died, and all recovered and continued to grow (Figure 1A).

Linuron application on S-POROL at rates much lower than those applied on R-POROL caused significant injury, resulting in the ultimate death of sprayed plants (Figure 1D). Photochemical efficiency of treated plants was reduced to zero by 14 DAT (Figure 1E). The most significant effect of linuron was on the photosynthetic CO_2 assimilation of S-POROL which shut down by 4 DAT (Figure 1F). Linuron injury in S-POROL was evident first in CO_2 assimilation, then in the photochemical efficiency, then in fresh weight.

Atrazine caused transitory reduction in fresh weight of R-POROL (Figure 2A). Some reduction was observed between 6 and 11 DAT, but fresh weights recovered to about 90% by 14 DAT. Atrazine had no observable effect on Fv/Fm (Figure 2B)

at all dates of measurements for R-POROL. At 14 DAT, CO_2 assimilation of R-POROL treated with 44.8 kg ai/ha atrazine was 55% of the control, compared to 35% at the highest rate of 179 kg ai/ha (Figure 2C). Average reductions of 40 to 50% were observed over all the measurement dates. This decrease in photosynthesis had no effect on the survivability of R-POROL, since all the treated plants continued to grow as shown in Figure 2A.

The response of S-POROL to low rates of atrazine was significant and resulted in death of all treated plants. Fresh weight of S-POROL started to decline by 4 DAT and continued to decrease to about 25% by 14 DAT (Figure 2D). At 11 DAT, few leaves were still present and the main components of fresh weight were the plant stems. Photochemical efficiency (Figure 2E) and CO_2 assimilation (Figure 2F) declined to 0% by 11 DAT and 4 DAT, respectively.

The responses of R-CHEAL and S-CHEAL to linuron are presented in Figure 3. Triazine-resistant and susceptible CHEAL were injured equally by linuron. Both R-CHEAL (Figure 3A) and S-CHEAL (Figure 3C) were dead by 14 DAT. By 11 DAT, electron transport was not detected in R-CHEAL (Figure 3B) or in S-CHEAL as indicated by the lack of measurable photochemical efficiency (Figure 3D).

Generally, atrazine application on R-CHEAL (up to 40X) had limited or no effect on Fv/Fm (Figure 4B). The only observable effect of atrazine on R-CHEAL was the 25 to 50%

reduction in fresh weight at 14 DAT (Figure 4A). Fresh weight reduction in R-CHEAL was due to stunting of growth instead of leaf drop. The effects of atrazine on fresh weight (Figure 4C) and Fv/Fm (Figure 4D) of S-CHEAL were similar to those of linuron on S-CHEAL (Figure 3C and 3D, respectively). By 14 DAT, all plants were dead except for those treated with 1.12 kg ai/ha atrazine, which resulted in a 50% reduction in fresh weight (Figure 4C).

Electron-Transport Inhibition Measurements. Results of the electron transport inhibition experiments are presented in Table 1. The RR value calculated for atrazine was the highest among the six herbicides used in this experiment. R-POROL was at least 800 times more resistant to atrazine, and 20 times more resistant to terbacil, than S-POROL. The RR of common purslane to linuron and diuron were similar and ranged between 6 and 8. A negative cross-resistance was determined for bentazon or pyridate with RR < 1.

Sequencing the D1 Protein. The partial sequence of the D1 protein obtained from R-POROL and S-POROL is presented in Table 2. The nucleotide sequences were compared to those of potato and black nightshade (previously sequenced by Dr. Stephen Weller, Purdue University, West Lafayette, Indiana). Of the 459 nucleotides sequenced, there was only one significant difference between R-POROL and S-POROL. At position 264, the AGT sequence in S-POROL encoding for serine is replaced with an ACT that encodes for threonine in

R-POROL. All other nucleotides and their resulting amino acids were similar for the two biotypes of common purslane.

Another type of nucleotide alteration is observed in Table 2. One nucleotide difference is observed in common purslane at positions 201, 202, 203, 246, 250, 267, 270, 275, 283, 286, 291, 294, 298, 304, 308, 310, 315, 316, and 321. None of these changes resulted in a different amino acid in common purslane. Except for the alteration at position 264, the homology between the D1 protein sequence of common purslane is highly conserved with that of potato and black nightshade.

DISCUSSION

The *psb*A gene sequences of R-POROL and S-POROL indicate a change at position 264, resulting in the substitution of the amino acid threonine for serine in the resistant biotype of common purslane. This is the first report of a serine-to-threonine mutation at the whole plant level of a linuron-resistant weed. However, a serine-to-threonine substitution has been reported in cell cultures. Sato et al. (1988) and Smeda et al. (1993) found a serine-to-threonine mutation in tissue-culture-selected strains of tobacco and potato, respectively.

While the serine-to-glycine mutation (Bettini et al. 1987; Blyden and Gray 1986; Mazur and Falco 1989) is the most common mutation at position 264 of the D1 protein, others have been discovered. For instance, a serine-to-

asparagine change was found in *Nicotiana plumbaginifolia* by Rey et al. (1990).

Additional evidence indicates that a mutation at position 264 is not the only mutation conferring triazine resistance in plants. Schwenger-Erger et al. (1993) determined that eight red goosefoot (*Chenopodium rubrum* L.) strains were resistant to metribuzin, atrazine, and diuron. Analysis of the *psb*A gene sequence showed the change to be in positions other than 264, including 219, 220, 229, 254, 266, 270, 272, and 273. Erickson et al. (1985) also found that strain Dr2 of *Chlamydomonas reinhardtii* has a valine-to-isoleucine mutation at position 219 and that strain Ar207 has a tyrosine-to-phenylalanine at position 255, resulting in 2-fold and 15-fold increase in resistance to atrazine, respectively. Neither strain had a mutation at position 264.

Another type of nucleotide mutation observed in triazine-resistant biotypes is a change in a nucleotide that doesn't result in a change in its encoded amino acid. This change was considered "silent" as described by Pay et al. (1988). They described a change that doesn't alter the amino acid in the TBR2 mutant strain of *Nicotiana plumbaginifolia*. Similar silent changes were observed in the *psb*A gene sequences of common purslane (Table 2).

The resistance of the threonine-264 mutant of common purslane to ureas and triazines was confirmed by measurements of chlorophyll fluorescence, fresh weight, and

of photosynthetic CO_2 assimilation. The thylakoid membranes of R-POROL can transport electrons even after treatment with 10 times the recommended rate of linuron (11.2 kg ai/ha) and with 80 times the recommended rate of atrazine (179.2 kg ai/ha). The resistant biotype appeared to be more resistant to atrazine than to linuron as evidenced by the full recovery of plants treated with much higher rates of atrazine, compared to the slower recovery with linuron.

The serine-to-glycine mutant of common lambsquarters survived 40 times the recommended rate of atrazine and showed only a transient injury to electron transport. However, this mutant had no tolerance to linuron and was dead by 14 DAT with 1.12 kg ai/ha. Triazine-resistant weeds with a serine-to-glycine mutation usually do not possess a high level of cross-resistance to ureas. Fuerst et al. (1986) calculated about a 4-fold increase in resistance to linuron in four weed species.

The increased levels of resistance to linuron and atrazine in R-POROL are atypical of serine-to-glycine mutant weeds, such as common lambsquarters. Thus, the influence of the altered D1 protein of R-POROL on herbicide resistance must be due to factors other than those known for the serine-to-glycine mutants. In an attempt to explain the possible reasons for higher levels of resistance in serine-to-threonine mutants, Sigematsu et al. (1989) used modeling techniques based on the similarities between PS II centers of higher plants and the reaction center of the purple

photosynthetic bacteria to predict the secondary structure of the mutant *psbA* gene with threonine in position 264. The predicted secondary structure indicated that a conformational change in the binding site conferred high levels of resistance to both triazine and urea herbicides. The usual mutation of serine-to-glycine provides resistance mainly to triazines, but not to ureas, due to the loss of the hydrogen bond between serine-264 and the amino alkyl side chain of the triazine ring (Fuerst and Norman 1991).

R-POROL has morphological and physiological differences from the susceptible biotype, such as reduced plant productivity and lower seed weight (Chapter 2). These characteristics, in addition to the physiological properties presented in this report, appear to be related to the serine-to-threonine mutation at position 264 of the D1 protein in R-POROL. The increased linuron-resistance in R-POROL appears to fit the model presented by Sigematsu et al. (1989) of a conformational change in the herbicide-binding niche of the D1 protein.

In conclusion, it appears that a serine-to-threonine change in the D1 protein results in mutants with increased levels of resistance to both triazines and ureas and with reduced fitness or competitive ability.

LITERATURE CITED

Bettini, P., S. McNally, M. Sevignac, H. Darmency, J. Gasquez, and M. Dron. 1987. Atrazine resistance in *Chenopodium album*. Plant Physiol 84:1442-1446.

Blyden, E. R. and J. C. Gray. 1986. The molecular basis of triazine herbicide resistance in *Senecio vulgaris* L. Biochem. Soc. Trans. 14:62.

Bowes, J., A. R. Crofts, and C. J. Arntzen. 1980. Redox reactions on the reducing side of photosystem II in chloroplasts with altered herbicide binding properties. Arch. Biochim. Biophys. 200:303-308.

Conard, S. G. and S. R. Radosevich. 1979. Ecological fitness of *Senecio vulgaris* and *Amaranthus retroflexus* biotypes susceptible or resistant to atrazine. J. Appl. Ecol. 16:171-177.

Doyle, J. J. and J. L. Doyle. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Egner, U., G-A. Hoyer, and W. Saenger. 1993. Modeling and energy minimization studies on the herbicide binding protein (D1) in photosystem II of plants. Biochim. Biophys. Acta. 1142:106-114.

Erickson, J. M., M. Rahire, P. Bennoun, P. Delepelaire, B. Diner, and J.D. Rochaix. 1984. Herbicide resistance in *Chlamydomonas reinhardtii* results from a mutation in the chloroplast gene for the 32-kilodalton protein of photosystem II. Proceedings of the National Academy of Sciences of the United States of America. 81(12): 3617-3621

Erickson, J. M., M. Rahire, J. D. Rochaix, and L. Mets. 1985. Herbicide resistance and cross-resistance: changes at three distinct sites in the herbicide-binding protein. Science 228:204-207.

Fuerst, E. P., C. J. Arntzen, K. Pfister, and D. Penner. 1986. Herbicide cross-resistance in triazine-resistant biotypes of four species. Weed Science. 34:344-353.

Fuerst, E. P. and M. A. Norman. 1991. Interactions of herbicides with photosynthetic electron transport. Weed Science. 39:458-464.

Gronwald, J. W. 1994. Resistance to Photosystem II inhibiting herbicides. *In*: Herbicide Resistance in Plants: Biology and Biochemistry, pp. 27-60. Lewis Publishers.

Heap, I. M. 1998. International survey of herbicide-resistant weeds. 1998 Herbicide-Resistance Action Committee Annual Report. WeedSmart, Corvallis, Oregon. 165 unnumbered pages.

Hirschberg, J. and L. McIntosh. 1983. Molecular basis of herbicide resistance in *Amaranthus hybridus*. Science. 222:1346-1348.

Hirschberg, J., A. Bleecker, D. J. Kyle, L. McIntosh, and C. J. Arntzen. 1984. The molecular basis of triazine-herbicide resistance in higher-plant chloroplasts. Z. Naturforsch. 39c:412-420.

Hirschberg, J., A. B. Yehuda, I. Pecker, and N. Ohad. 1987. Mutations resistant to photosystem II herbicides. Pages 336-352 in D. V. Wettstein and N. H. Chua, eds. Plant Molecular Biology. NATA ASI Ser. A: Life Sci. Vol. 140. Plenum Press, New York.

Hobbs, S. L. A. 1987. Comparison of photosynthesis in normal and triazine-resistant *Brassica*. Can J. Plant Sci. 67:457-466.

Holt, J. S., A. J. Stemler, and S. R. Radosevich. 1981. Differential light responses of photosynthesis by triazine-resistant and triazine-susceptible *Senecio vulgaris* biotypes. Plant Physiol. 67:744-748.

Holt, J. S., S. R. Radosevich, and A. J. Stemler. 1983. Differential efficiency of photosynthetic oxygen evolution in flashing light in triazine-resistant and triazine-susceptible biotypes of *Senecio vulgaris* L. Biochim. Biophys. Acta. 722:245-255.

Holt, J. S. and H. M. LeBaron. 1990. Significance and distribution of herbicide resistance. Weed Technol. 4:141-149.

Hunt, R. 1980. Asymptotic functions, p. 121-146. *In*: Plant growth curves. Univ. Park Press, Baltimore, MD.

Jursinic, P. A. and R. W. Pearcy. 1988. Determination of the rate limiting step for photosynthesis in a nearly isonuclear rapeseed (*Brassica napus* L.) biotype resistant to atrazine. Plant Physiol. 88:1195-1200.

Lehoczki, E., E. Pölös, G. Laskay, and T. Farkas. 1985. Chemical composition and physical states of chloroplast lipids related to atrazine resistance in *Conyza canadensis* L. Plant Sci. 42:19-24.

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1989. Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.

Masabni, J. G. and B. H. Zandstra. 1998a. Discovery of a common purslane (*Portulaca oleracea* L.) biotype which is resistant to linuron. Submitted to Weed Technology.

Masabni, J. and B. Zandstra. 1998b. Morphological and physiological characteristics of linuron-resistant common purslane (*Portulaca oleracea* L.). In preparation for submission to Weed Science.

Mazur, B. J. and S. C. Falco. 1989. The development of herbicide resistant crops. Annu. Rev. Plant Physiol. Mol. Bio.40:441-470.

McCloskey, W. B. and J. S. Holt. 1990. Triazine resistance in *Senecio vulgaris* parental and nearly isonuclear backcrossed biotypes is correlated with reduced productivity. Plant Physiol. 92:954-962.

Ohad, N. and J. Hirschberg. 1992. Mutations in the D1 subunit of photosystem II distinguish between quinone and herbicide binding sites. The Plant Cell. 4:273-282.

Páy, A., S. M. Smith, F. Nagy, and L. Márton. 1988. Sequence of the *psb*A gene from wild type and triazine-resistant *Nicotiana plumbaginifolia*. Nucleic Acids Res. 16: 8176.

Pfister, K. and C. J. Arntzen. 1979. The mode of action of photosystem II-specific inhibitors in herbicide-resistant weed biotypes. Z. Naturforsch. 34c:996-1009.

Pfister, K., K. E. Steinback, G. Gardner, and C. J. Arntzen. 1981. Photoaffinity labeling of an herbicide receptor protein in chloroplast membranes. Proceedings of the National Academy of Science of the United States of America. 78 (2):981-985.

Pillai, P. and J. B. St. John. 1981. Lipid composition of chloroplast membranes from weed biotypes differentially sensitive to triazine herbicides. Plant Physiol. 68:585-587.

Radosevich, S. R. and O. T. Devilliers. 1976. Studies on the mechanism of *s*-triazine resistance in common groundsel. Weed Sci. 24:229-232.

Rey, P., F. Eymery, and G. Peltier. 1990. Atrazine and diuron resistant plants from photoautotrophic protoplast-derived cultures of *Nicotiana plumbaginifolia*. Plant Cell Rep. 9:241-244.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Nat. Acad. Sci. 74:5463-5467.

Sato, F., Y. Sigematsu, and Y. Yamada. 1988. Selection of an atrazine-resistant tobacco cell line having a mutant *psb*A gene. Mol. Gen. Genet. 214:358-360.

Schönfeld, M., T. Yaacoby, O. Michael, and B. Rubin. 1987. Triazine resistance without reduced vigor in *Phalaris paradoxa*. Plant Physiol. 83:329-333.

Schwenger-Erger, C., J. Thiemann, W. Barz, U. Johanningmeier and D. Naber. 1993. Metribuzin resistance in photoautotrophic *Chenopodium rubrum* cell cultures: Characterization of double and triple mutations in the *psb*A gene. FEBS Letters 329:43-46

Sigematsu, Y., F. Sato, and Y. Yamada. 1989. A binding model for phenylurea herbicides based on analysis of a Thr264 mutation in the D-1 protein of tobacco. Pestic. Biochem. Physiol. 35:33-41.

Smeda, R. J., P. M. Hasegawa, P. B. Goldsbrough, N. K. Singh, and S. C. Weller. 1993. A serine-to-threonine substitution in the triazine herbicide-binding protein in potato cells results in atrazine resistance without impairing productivity. Plant Physiol. 103:911-917.

Trebst, A. 1987. The three-dimensional structure of the herbicide binding niche on the reaction center polypeptides of photosystem II. Z. Naturforsch. 42c:742-750.

Trebst, A. 1991. The molecular basis of resistance of photosystem II herbicides. *In*: Herbicide Resistance in Weeds and Crops, J. C. Caseley, G. W. Cussans, and R. K. Atkin (eds.), Butterworth-Heinemann Ltd., Oxford, UK, pp. 145-164.

Warwick, S. I., and L. Black. 1981. The relative competitiveness of atrazine susceptible and resistant populations of *Chenopodium album* and *C. strictum*. Can J. Bot. 59:689-693.

Wildner, G. F., U. Heisterkamp, U. Bodner, U. Johanningmeier. 1989. An amino acid substitution in the Q_B-Protein causes herbicide resistance without impairing electron transport from Q_A to Q_B. Z. Naturforsch. 44c:431-434.

Yerkes, C. N. and S. C. Weller. 1995. A photoautotrophic system for comparison of *psb*A mutants at the plant cell level. Submitted to Physiol. Plant.

Table 1. Herbicide rates required to reduce O_2 evolution from extracted chloroplasts by 50% (I_{50}), and the resistant ratio (RR), of resistant and susceptible common purslane.

	I_{50} (M)		
	Resistant	Susceptible	RR
atrazine	$> 10^{-3}$ [a]	$1.5 * 10^{-6}$	> 800
terbacil	$2.8 * 10^{-5}$	$< 1.4 * 10^{-6}$	> 20
linuron	$1.2 * 10^{-5}$	$1.4 * 10^{-6}$	8.1
diuron	$4.1 * 10^{-6}$	$< 6.5 * 10^{-7}$ [b]	> 6.3
pyridate	$4.9 * 10^{-5}$	$6.5 * 10^{-5}$	0.75
bentazon	$4.3 * 10^{-5}$	$8.6 * 10^{-5}$	0.5

[a] Calculated I_{50} is at a rate higher than the highest rate applied.

[b] Calculated I_{50} is at a rate lower than the lowest rate applied.

Table 2. Partial D1 protein sequence and predicted amino acids of resistant (R-POROL) and susceptible (S-POROL) common purslane and of potato and black nightshade.

Key to abbreviations:

A = Codon numbering per Naber et al. (1990)
B = R-POROL
C = S-POROL
D = Potato and black nightshade sequences obtained from Dr. Stephen Weller (Dept. of Horticulture, Purdue, West Lafayette, Indiana)
E = Amimo acid equivalent to the nucleotide sequence

--- = indicates same codon as R-POROL

Table 2.

A	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	220	221
B	CTT	ATG	CAC	CCA	TTT	CAC	ATG	TTA	GGT	GTC	GCG	GGT	GTA	TTC	GGC	GGC	TCC	CTA	TTT	AGT	GCT	ATG	CAT	GGT	ACT	TCT
C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
D	---	---	---	---	---	---	---	---	GGC	GTG	GCT	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
E	Leu	Met	His	Pro	Phe	His	Met	Leu	Gly	Val	Ala	Gly	Val	Phe	Gly	Gly	Ser	Leu	Phe	Ser	Ala	Met	His	Gly	Thr	Ser

A	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247
B	AGT	TTG	ATC	AGG	GAA	ACC	ACA	GAA	AAT	GAA	TCT	GCT	AAT	GAA	GGT	TAC	AGA	TTC	GGT	CAA	GAG	GAA	GAA	ACT	TAC	AAT
C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
D	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	TAT	---
E	Ser	Leu	Ile	Arg	Glu	Thr	Thr	Glu	Asn	Glu	Ser	Ala	Asn	Glu	Gly	Tyr	Arg	Phe	Gly	Gln	Glu	Glu	Glu	Thr	Tyr	Asn

A	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	**264**	265	266	267	268	269	270	271	272	273
B	ATC	GTA	GCT	GCT	CAT	GGT	TAT	TTT	GGC	CGA	TTG	ATC	TTC	CAA	TAT	GCT	**ACT**	TTC	AAC	AAT	TCT	CGT	TCT	TTA	CAC	TTC
C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	**AGT**	---	---	---	---	---	---	---	---	---
D	---	---	GCC	---	---	---	---	---	---	---	---	---	---	---	---	---	**AGT**	---	---	AAC	---	---	TCG	---	---	---
E	Ile	Val	Ala	Ala	His	Gly	Tyr	Phe	Gly	Arg	Leu	Ile	Phe	Gln	Tyr	Ala	**Thr Ser**	Phe	Asn	Asn	Ser	Arg	Ser	Leu	His	Phe

A	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299
B	TTC	TTA	GCT	GCT	TGG	CCT	GTA	GTG	GGT	ATT	TGG	TTT	ACT	GCT	TTA	GGT	ATT	AGT	ACT	ATG	GCG	TTC	AAC	CTA	AAC	GGT
C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
D	---	CTA	---	---	---	---	---	---	---	ATC	---	---	ACC	---	---	---	---	AGC	---	---	GCT	---	---	---	AAT	---
E	Phe	Leu	Ala	Ala	Trp	Pro	Val	Val	Gly	Ile	Trp	Phe	Thr	Ala	Leu	Gly	Ile	Ser	Thr	Met	Ala	Phe	Asn	Leu	Asn	Gly

A	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325
B	TTC	AAT	TTC	AAC	CAG	TCT	GTA	GTT	GAT	AGT	CAA	GGT	CGT	GTA	ATT	AAT	ACC	TGG	GCT	GAT	ATC	ATT	AAC	CGT	GCT	AAC
C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
D	---	---	---	---	CAA	---	---	---	GAC	---	CAG	---	---	---	---	AAC	ACT	---	---	---	---	ATC	---	---	---	---
E	Phe	Asn	Phe	Asn	Gln	Ser	Val	Val	Asp	Ser	Gln	Gly	Arg	Val	Ile	Asn	Thr	Trp	Ala	Asp	Ile	Ile	Asn	Arg	Ala	Asn

A	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345
B	CTT	GGT	ATG	GAA	GTT	ATG	CAT	GAA	CGT	AAT	GCT	CAT	AAC	TTC	CCT	CTA	GAC	CTA	GCT	GCT
C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
D	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
E	Leu	Gly	Met	Glu	Val	Met	His	Glu	Arg	Asn	Ala	His	Asn	Phe	Pro	Leu	Asp	Leu	Ala	Ala

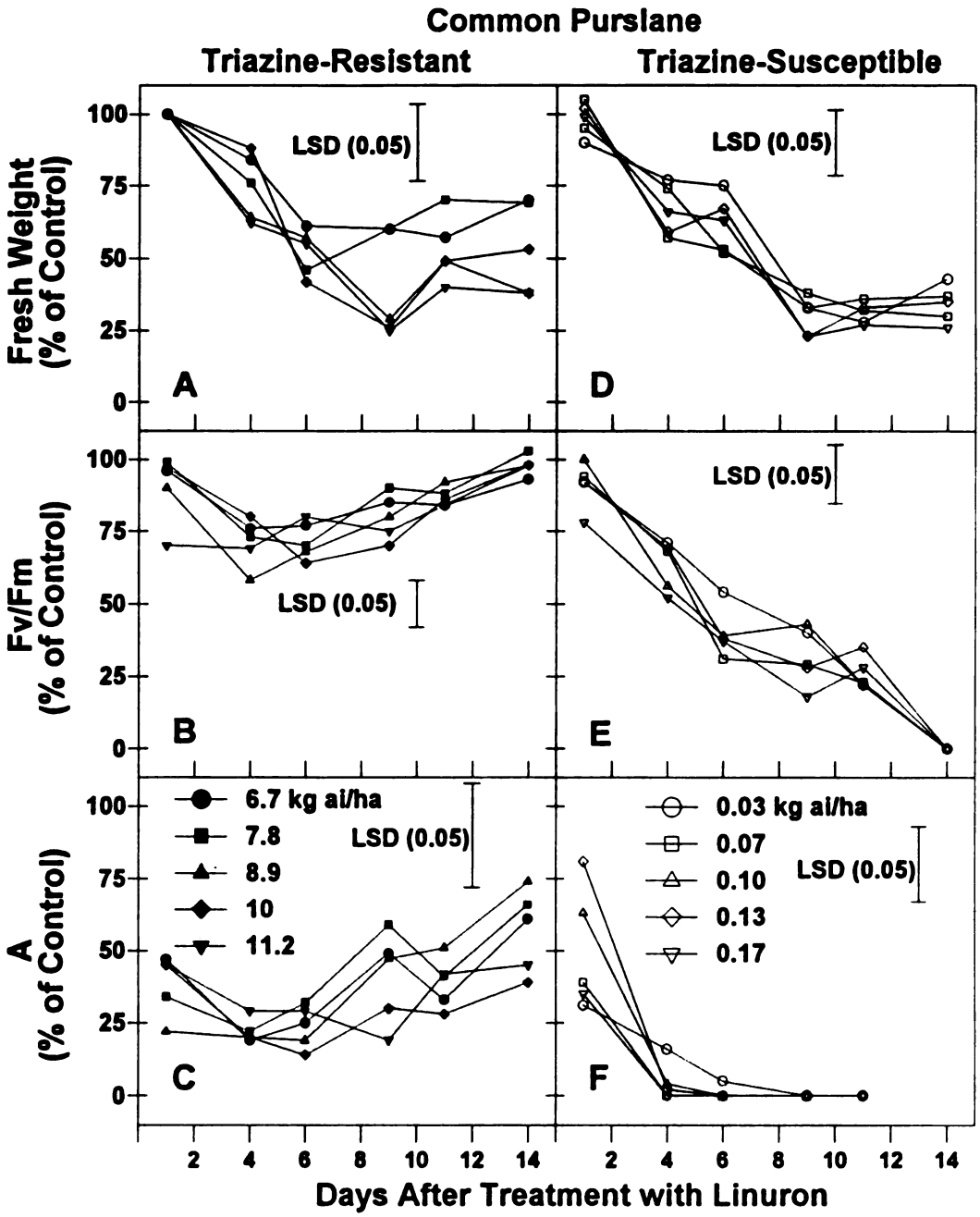

Figure 1. Fresh weight, photochemical efficiency (Fv/Fm), and photosynthetic CO_2 assimilation (A) expressed as percent of controls, for resistant (A, B, C) and susceptible (D, E, F) common purslane at various dates after treatment with linuron.

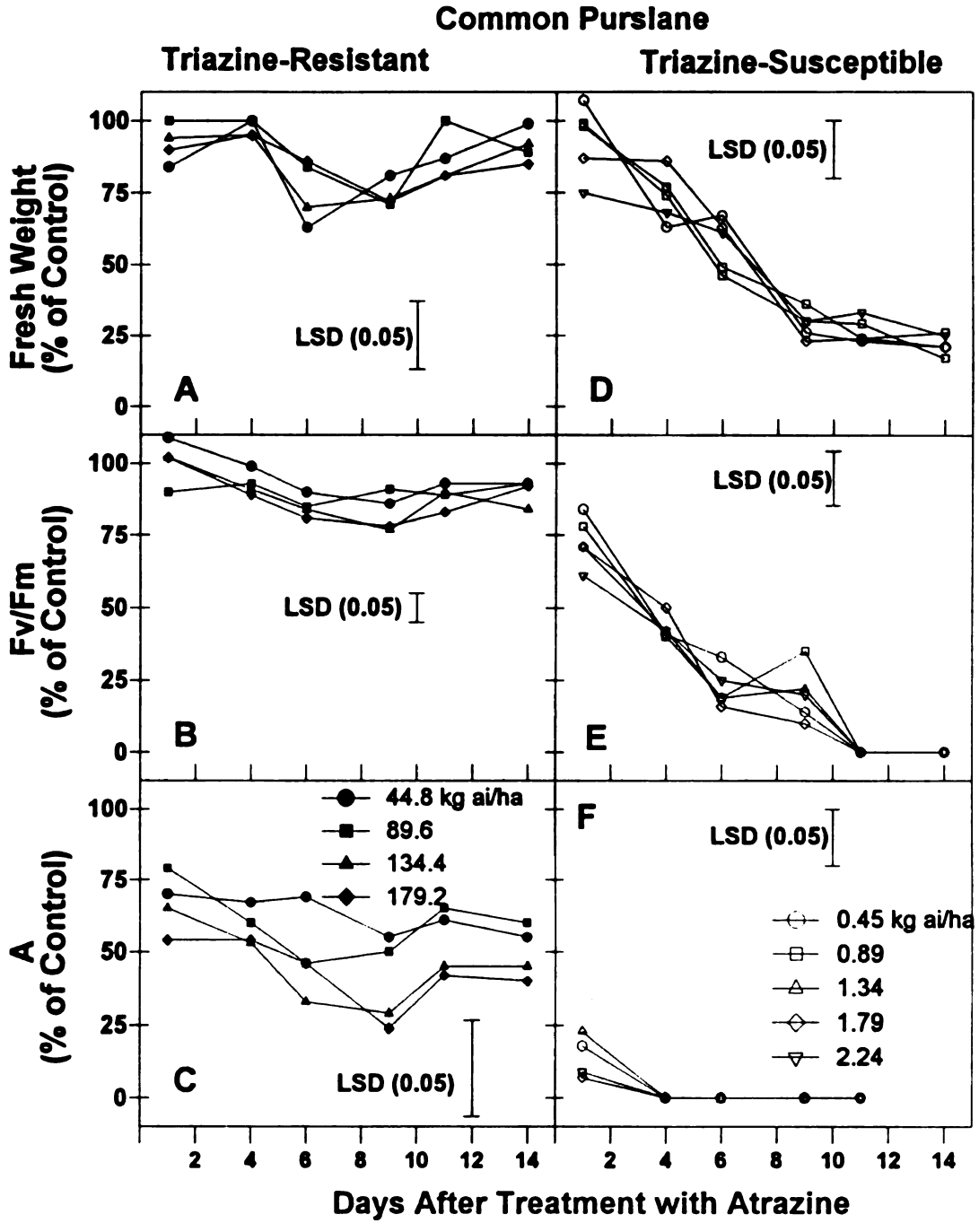

Figure 2. Fresh weight, photochemical efficiency (Fv/Fm), and photosynthetic CO_2 assimilation (A) expressed as percent of controls, for resistant (A, B, C) and susceptible (D, E, F) common purslane at various dates after treatment with atrazine.

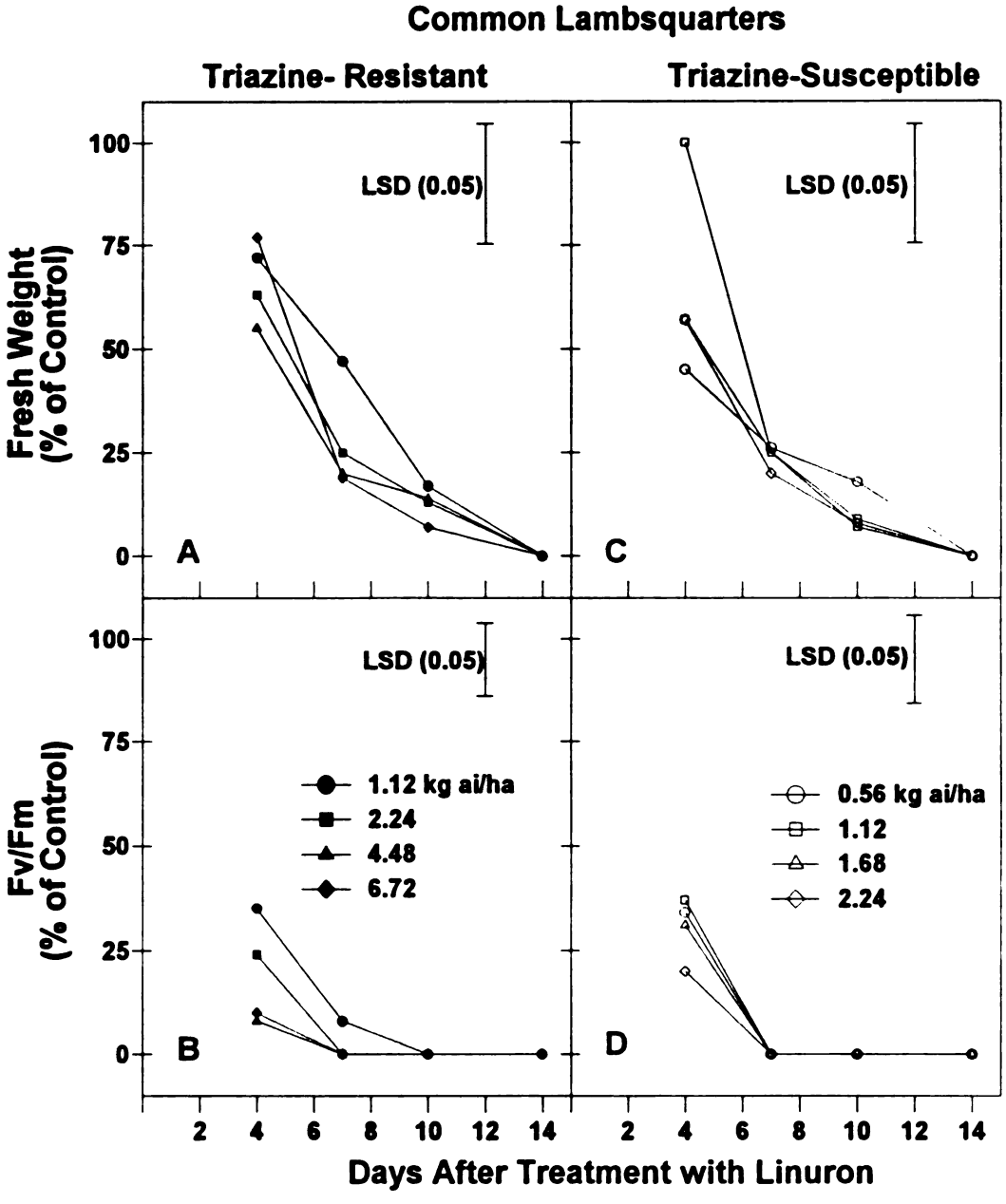

Figure 3. Fresh weight and photochemical efficiency (Fv/Fm) expressed as percent of controls, for resistant (A, B) and susceptible (C, D) common lambsquarters at various dates after treatment with linuron.

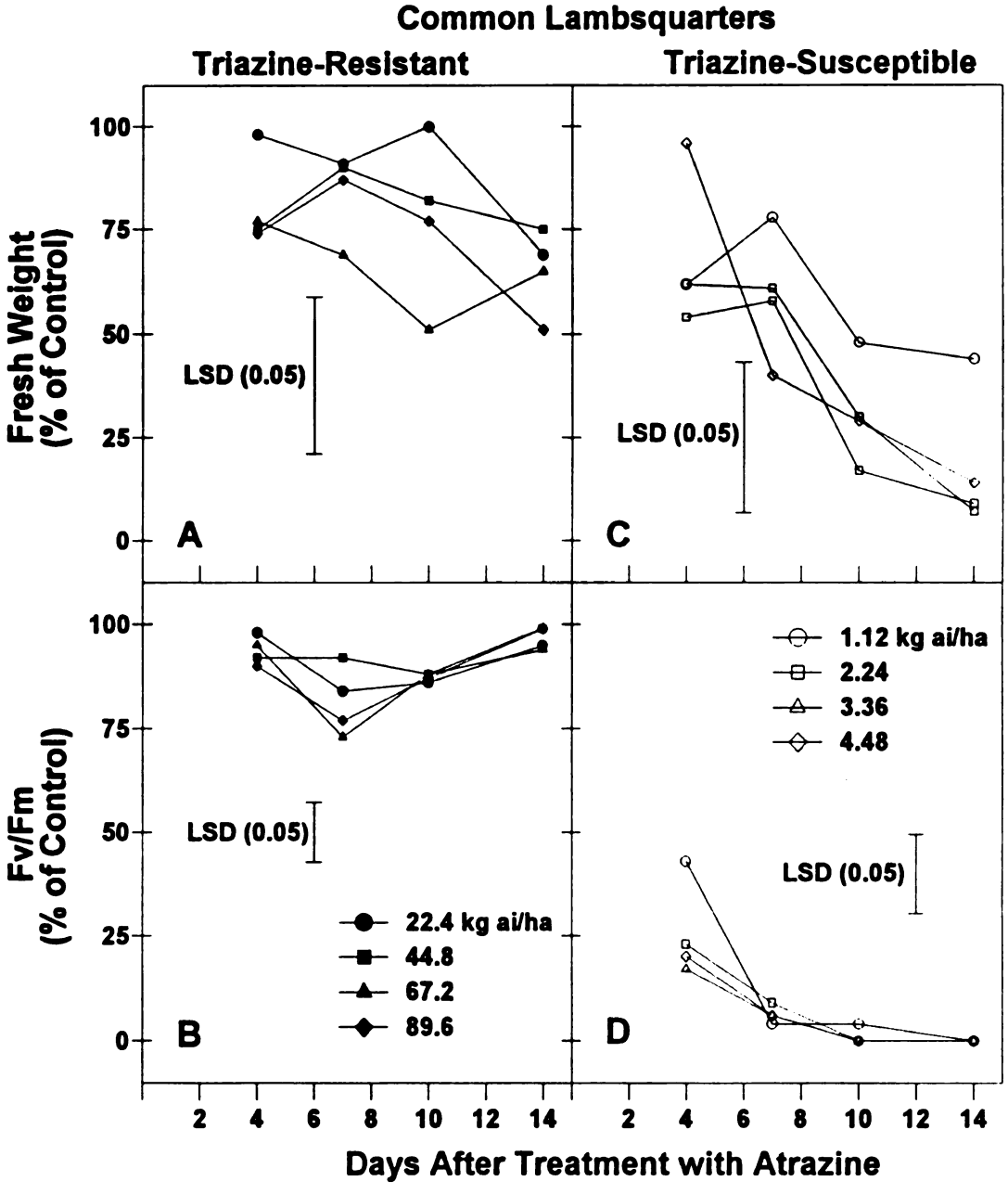

Figure 4. Fresh weight and photochemical efficiency (Fv/Fm) expressed as percent of controls, for resistant (A, B) and susceptible (C, D) common lambsquarters at various dates after treatment with atrazine.