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Mutant Weeds of Iowa: s-Triazine-Resistant Plastids Chenopodium album L.

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Mutant Weeds of Iowa: s-Triazine-Resistant Plastids in *Chenopodium album* L.[1]

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S-triazine resistance in common lambsquarters (*Chenopodium album* L.) has been reported previously but not in Iowa. A study was conducted using a chlorophyll *a* fluorescence assay to confirm the presence of resistance in *C. album* populations in Iowa. Variable chlorophyll *a* fluorescence assays confirmed that mutations conferring resistance exist in five (5) geographically separated populations of *C. album* in Iowa: relatively greater fluorescence yields were measured in untreated resistant tissue compared with untreated susceptible plants, relatively greater fluorescence yield in treated compared with untreated susceptible leaf disks; and similar fluorescence yields of treated and untreated resistant tissue. This is the first report of an s-triazine resistant plant found in an Iowa agroecosystem.
INDEX DESCRIPTORS: chlorophyll fluorescence, atrazine, chloroplasts, herbicide resistance.

S-triazine resistance in higher plants was first discovered in 1968 in *Senecio vulgaris* L. by Ryan (1970). Subsequent studies revealed that this type of herbicide resistance was not a consequence of differential herbicide accumulation, membrane permeability, metabolism, translocation or uptake (Radosevich, 1970). Photo-affinity label studies indicated that an analogue of atrazine [6-chloro-*N*-ethyl-*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine] exhibited reduced binding to the 32kD chloroplast protein, D-1, in the resistant (R) biotype (Pfister et al. 1981; Steinback et al. 1981). This protein is encoded on the psbA gene in the chloroplast genome (Hirschberg and McIntosh 1983). The R variant has a single nucleotide base pair substition of adenine for guanine. This mutation results in substitution of a serine codon (AGT) for a glycine codon (GGT) at position 264 in the polypeptide product. This single base pair mutation results in a profound decrease in the ability of the protein to bind s-triazines which reduces toxicity.

S-triazine herbicides act by binding to the D-1 protein in the chloroplast thylakoid membranes and thereby inhibit photosynthetic electron transport. Chlorophyll fluorometry is an intrinsic probe of photosystem II function (Krause and Weis, 1984). When s-triazine herbicides bind to the D-1 protein and block electron transport, light energy absorbed by chloroplasts is quickly reemitted as fluorescence. The relative strength of the fluorescence signal is an ideal indicator of s-triazine susceptibility and resistance.

S-triazine resistance has been reported previously in *C. album* (Bandeen et al. 1982, Bandeen and McLaren 1976) at several locations, but not in Iowa. This type of herbicide resistance has been confirmed in one other plant species in Iowa (*Kochia scoparia* L. Schrader) (Dekker et al. 1987) found in a railroad yard. To date, no species with triazine-resistance has been reported to exist in an Iowa agroecosystem. The objective of this study is to determine whether if several populations of *C. album* discovered in Iowa agricultural fields possess s-triazine resistance.

METHODS AND MATERIALS

The putative triazine-resistant (R) *C. album* plants evaluated in this study came from seed gathered in five geographically separated agricultural fields (Table 1). The fields were farmed by five different growers in Northeastern Iowa. Susceptible (S) *C. album* plants were grown from seed gathered from two different fields on the Iowa State University Agrticultural Experiment Station. Plants from all locations were grown in a glasshouse at Iowa State University. Leaf disks were taken from all plants just prior to analysis from young, fully expanded leaves showing no evidence of senescence or injury. Leaf disks were analyzed with variable chlorophyll *a* leaf disk fluorescence methodologies reported previously (Ali and Souza Machado 1981, Ahrens et al. 1981; Truelove and Hensley, 1982), and as modified by Dekker et al., 1987. Briefly, leaves were taken from the plants immediately before treatment with 1×10^{-4} M technical grade atrazine in phosphate buffer. Two 8-mm disks were taken, one from each side of the midvein, from each of two leaves of each plant of each type (R,S) evaluated. This sampling procedure was repeated on a second plant from each location. One leaf disk from one side of a leaf's midvein was placed in a dish with buffer solution, the disk from the other side of the vein was placed in a dish with atrazine plus buffer. Fluorescence measurements were made after the disks in the dishes were placed in the dark for at least 5 min. The disks were then placed on paper towels. The fluorometer probe was placed directly on the disk and measurements were made immediately. After this, the disks were placed in their respective solutions and placed in the light until further measurements were made. Fluorescence evaluations were made just prior to treatment, and again at 105 or 120 minutes after treatment. During this interval, the disks in the dishes were maintained under metal halide lights providing light of ca. 200 μ moles quanta/m^2/s. Due to the consistent fluorescence yield of similar tissue under similar experimental conditions, data presented are from one representative disk of each type (R,S) from each location (Dekker et al. 1987).

RESULTS AND DISCUSSIONS

Several consistent changes in variable chlorophyll fluorescence (VCF) occurred due to genotype and herbicide treatment. VCF yields observed in the two Story County samples were similar in the control tissue at the beginning and end of the experiment, and in the treated disks before treatment (Figures 1,2). The disks exposed to atrazine treatment from these two locations showed a dramatic rise in the maximum VCF (F_{max}) (Krause and Weis 1984). This change in VCF yield in response to atrazine is consistent with blocked electron transport in the chloroplast and is definitive evidence of traizine susceptibility. VCF yields observed in disks taken from plants from all the other locations were similar in both the control and atrazine treated disks, before and after treatment (Figures 3,4,5,6,7). This lack of effect in the presence of atrazine is evidence in support of an altered herbicide binding site associated with R (Pfister et al. 1981, Steinback et al. 1981). The peak VCF (F_p) (Krause and Weis 1984) associated with untreated R leaf disks (Figures 3-7) was relatively greater than that associated with the untreated susceptible disks (Figures 1,2). This differential response was consistent with previous observations of a less efficient electron transport apparatus in R

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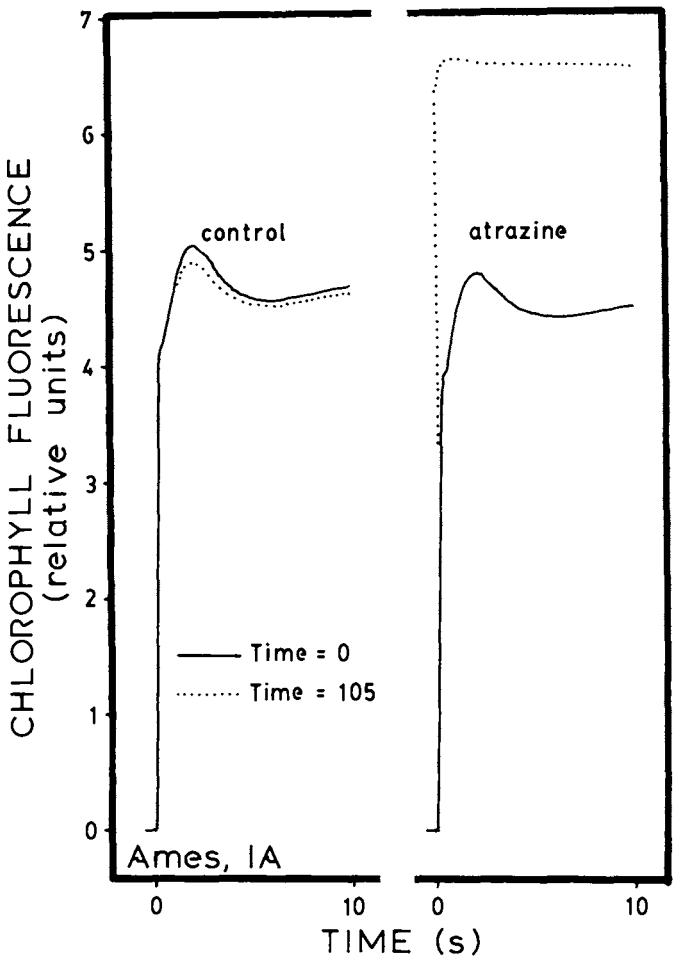

Fig. 1. Variable chlorophyll *a* fluorescence (relative units) following a 10s illumination period of dark-adapted leaf disks of triazine-susceptible *Chenopodium album* before, and after 105 min, treatment in buffer solution only (control) or in 1×10^{-4} M atrazine plus buffer solution (atrazine); plants were grown from seed collected near Ames, Iowa.

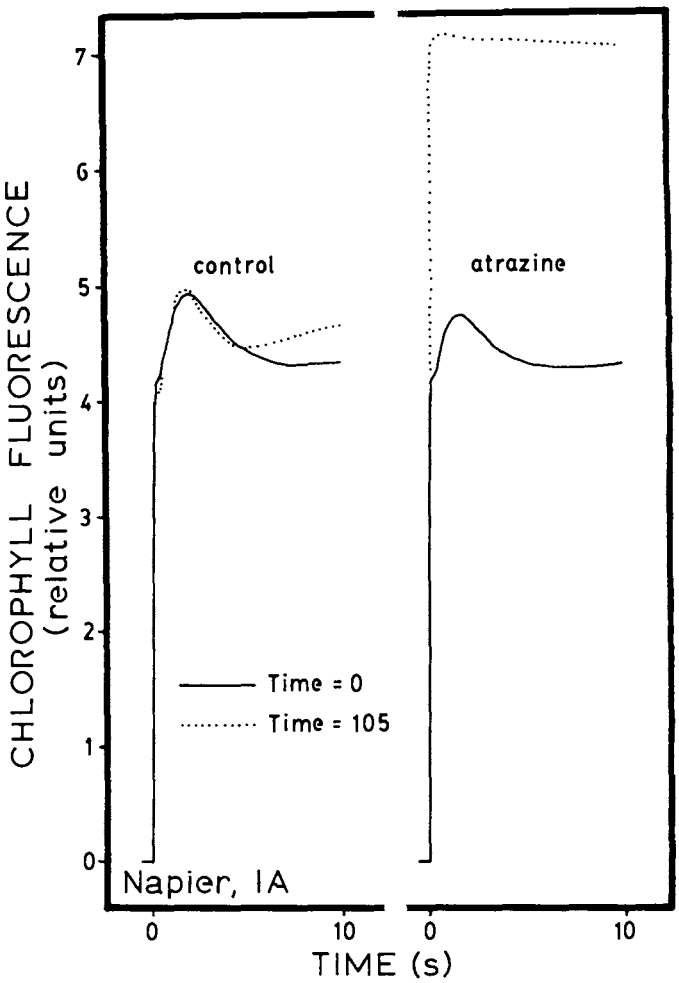

Fig. 2. Variable chlorophyll *a* fluorescence (relative units) following a 10s illumination period of dark-adapted leaf disks of trizaine-susceptible *Chenopodium album* before, and after 105 min, treatment in buffer solution only (control) or in 1×10^{-4} M atrazine plus buffer solution (atrazine); plants were grown from seed collected near Napier, Iowa.

(Arntzen et al. 1979, Burke et al. 1982, Dekker and Westfall 1987a,b).

Of those samples that were shown to be R, most occurred in agricultural fields with a minimum of four years of continuous corn (*Zea mays* L.) production and the use of atrazine or cyanazine [2-[4-chloro-6-[ethylamino]-1,3,5-triazine-2-yl)amino]-2-methylpropanenitrile] for weed control (Table 1; Figures 3-7). This is consistent with previous reports of R appearing in fields in which the weed flora were subjected to continuous s-triazine selection pressure. A different cropping and herbicide usage situation occurred at the Waukon, Iowa site (Table 1; Figure 7). Resistant *C. album* appeared in this field with three years continuous corn production and atrazine or cyanazine usage followed by a 5 year oats-alfalfa cropping hiatus in which no herbicides were used. Apparently, once established, R weeds were competitive enough with other plants to persist for some time after the use of the selective herbicide is discontinued.

This is the first evidence of triazine-resistance in *C. album* in Iowa. These are also the first cases of R found in a species in an agricultural situation in Iowa. This report is the third in a series (Dekker and Dekker 1987, Dekker et al. 1987) on mutant weeds in the natural flora of Iowa.

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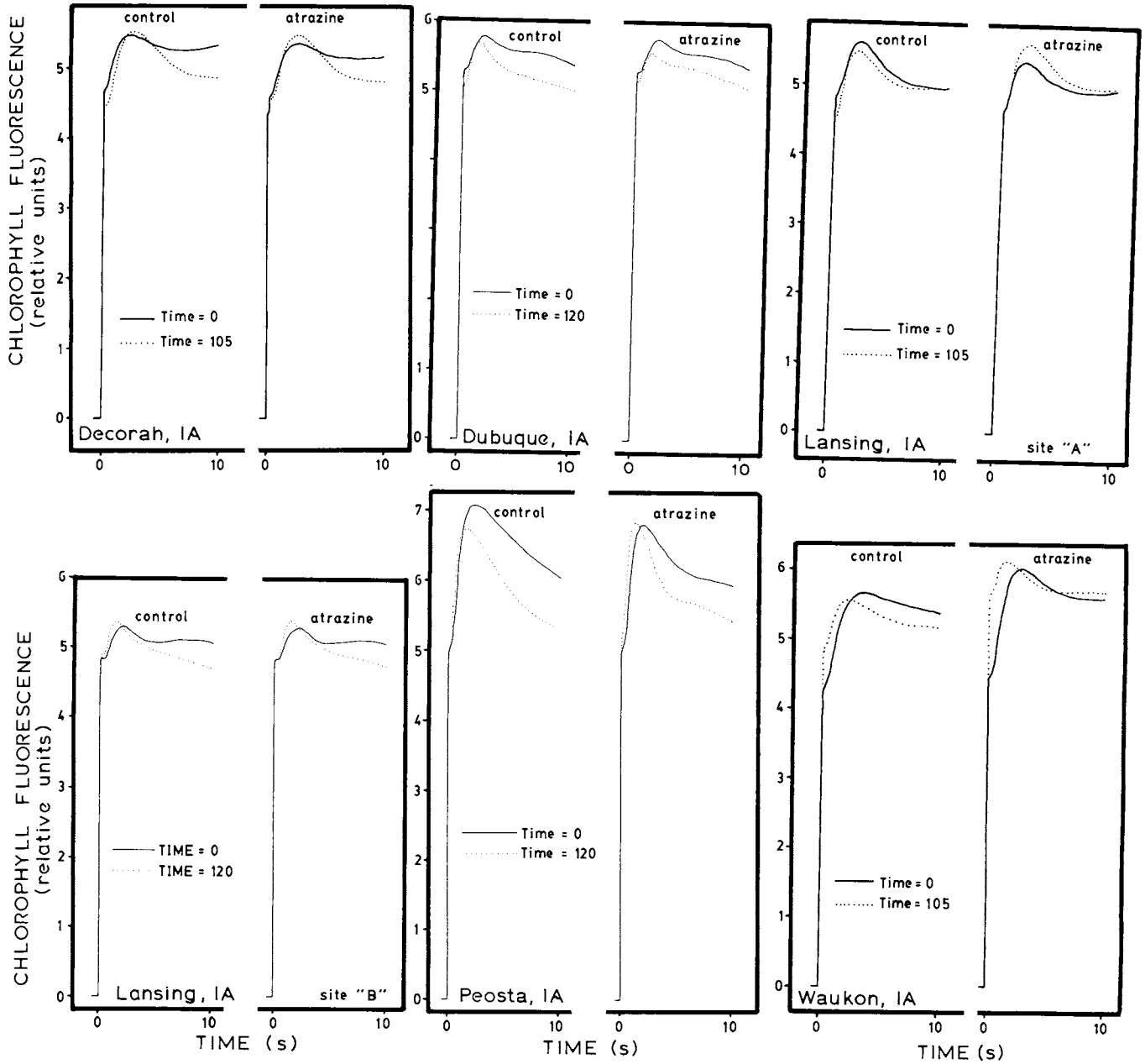

Fig. 3. Variable chlorophyll *a* fluorescence (relative units) following a 10s illumination period of dark-adapted leaf disks of triazine-resistant *Chenopodium album* before, and after 105 or 120 min, treatment in buffer solution only (control) or in 1×10^{-4} M atrazine plus buffer solution (atrazine); plants were grown from seed collected near Decorah, Dubuque, Lansing (2 sites: A,B), Peosta, and Waukon, Iowa.

Table 1. Sites from which *Chenopodium album* seed was obtained for the evaluations reported: Location (nearest city, county to field), Crop History (crops grown and years), Herbicide History (Herbicide types applied for weed control and years), the Year the Problem was First Observed and the Year the Seed was Collected for Testing for resistance.

Location	Crop History	Herbicide History	Year problem first observed	Year seed collected for testing
Ames, Story Co.	Corn-soybean rotation	Several	—	Fall, 1984
Napier, Story Co.	Corn-soybean rotation	Several	—	Fall, 1982
Decorah, Winneshiek Co.	Continuous corn (1972-1986)	Agrazine plus alachlor (1972-1986)	Spring, 1986	Fall, 1986
Dubuque, Dubuque Co.	Continuos corn (1979-1988)	Atrazine plus metolachlor (1988); cyanazine plus metolachlor (1987), atrzine (1985-86), atrazine plus alachlor (1979-1984)	1985	Summer, 1987
Peosta, Dubuque Co.	Continuous corn (1979-1988)	Atrazine plus alachlor or atrazine plus metolachlor (1979-1988)	1987	Summer, 1987
Lansing, Allamakee Co.	Continuous corn (1982-1987)	Atrazine plus alachlor or atrazine plus cyanazine plus alachlor (1982-1987)	Spring, 1985	Fall, 1986
Waukon, Allamakee Co.	Corn-oats-hay rotation: Corn (1978-1980; 1985-1988); oats (1981); alfalfa (1982-1984)	Atrazine plus alachlor or cyanazine plus alachlor (1978-1980; 1985-1988)	1986	Fall, 1986

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