AN ABSTRACT OF THE THESIS OF

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Title : Resistance to Atrazine and Diuron in California Brome

Abstract approved:

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Four greenhouse bioassays and two chlorophyll fluorescence tests were conducted to characterize atrazine (6-chloro- N-ethyl- N'- (1 methyl-1,3,5-triazine,-2,4-diamine) and diuron (N'-(3,4- dichlorophenyl) - N, N' - dimethyl urea) resistance in California brome(*Bromus carinatus* H & A). In greenhouse bioassays, differences in atrazine resistance among accessions of California brome existed in a range from 0.3 to 1.0 ppm. Resistance ratios for the two accessions with the greatest atrazine resistance were 2 to 9, 2 to 4, and 10 to 20-fold greater than the most susceptible accession for plant height, dry weight per plant, and mortality, respectively. One of the atrazine-resistant accessions was also more tolerant to diuron than the other accessions, suggesting cross resistance.

Chlorophyll fluorescence of the same 10 accessions of California brome was measured 12 hours and 1 week after application of atrazine and diuron. The terminal fluorescence (T) of all accessions 12 hours after treatment was significantly higher than the untreated controls for both atrazine and diuron. Because increased T fluorescence results from the binding of herbicides to the D1 protein of PS II and the inhibition of electron transport, we concluded that the mechanism of resistance in these accessions of California brome was not alteration of the chloroplastic binding site. Fluorescence measurements 1 week after treatment showed differences in rate of recovery among accessions. The greatest reduction in T between the two dates occurred for the two accessions identified as most resistant to atrazine in the bioassays. This differential recovery further suggests that resistance to atrazine and diuron in California brome results from other biochemical or biophysical processes such as metabolism of the herbicide or absorption of photochemical energy by other pigments such as the xanthophylls.

Resistance to Atrazine and Diuron in California Brome

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INTRODUCTION

Herbicide technology has played a vital role in increasing food production to feed the ever increasing world population. Herbicide usage is often cheaper than cultivation, is less damaging to soil structure, and greatly reduces the competition for scarce plant resources by controlling weeds. As a result of its cost effectiveness and simplicity of use, the farming community of the developed and developing world has used herbicides extensively over the last 20 years. In the process, many cultural weed control practices have been reduced and rotations of crops nearly abandoned, resulting in the selection of resistant weed biotypes that no longer can be controlled by once effective herbicides.

The incidence of herbicide resistance in weeds was first reported in the 1970's, and has risen dramatically over the past 10 years. At present, at least 57 weed species including 40 dicots and 17 monocots have evolved resistance to triazine herbicides (Table 1) and at least 60 species have resistance to one or more herbicides from 14 other classes (Holt et al., 1993). Most cases of herbicide resistance have occurred in situations where herbicides with similar modes of action were used repeatedly in the same field, thus selecting for increased resistance within species that had formerly been susceptible.

Weed management strategies to control resistant weeds require better understanding of herbicides and weeds, including mechanisms, evolution, and inheritance of resistance. A review by Holt (1992) failed to discover any cases where triazine resistance resulted in unmanageable weed problems, apparently because

Table 1. Occurrence and distribution of herbicide resistant weed biotypes selected under agricultural conditions.

Herbicide class	Year first detected	Number of species with resistant biotypes	Number of known sites
ACCase inhibitors	1982	8	>1000
ALS inhibitors	1986	8	>1000
Amides	1986	2	2
Aminitriazoles	1986	2	2
Arsenicals	1984	1	2
Benzonitriles	1988	1	1
Bipyridiliums	1976	18	>50
Carbamates	1988	2	70
Dinitroanilines	1973	5	>20
Phenoxyacetic acids	1962	6	5
Picloram	1988	1	1
Pyridazinones	1978	1	3
Substituted ureas	1983	7	>50
Triazines	1968	57	>1000
Uracils	1988	2	1

Adapted from Holt et al., 1993

of the availability of other weed management methods. Herbicide rotation has been looked upon as one alternative to delay the occurrence of herbicide resistance in weeds based on the initial assumption that all herbicide-resistant biotypes were less fit than the unselected types in the absence of the herbicide. A new development in herbicide resistance is the recent discovery of multiple and cross-resistance in weeds (Holt et al., 1993) These multiple-resistant weeds cannot be managed simply by changing herbicides since any new herbicide used would have a high probability of selecting for resistance to itself. The suggestion has also been made that herbicide rotations or mixtures might not be helpful in delaying the rate of appearance of resistance in cases where fitness of resistant biotypes is nearly the same as susceptible biotypes.

Determination of the variability in response within a herbicide-treated population is important in developing management strategies, understanding resistance evolution, and responding effectively. Our ability to do this would be enhanced by developing rapid methods for detecting, verifying, and reporting the incidence of resistance, at the regional, national, and international levels.

California brome (*Bromus carinatus* H & A) is native to the Rocky Mountains and Pacific Coast regions of the western U.S. (Hughes et al., 1962). California brome has become a troublesome perennial weed in grasses grown for seed in the Willamette Valley of Oregon, reducing crop yields and complicating the seed cleaning process (Mueller-Warrant et al., 1991). The authors reported that grass seed growers in Oregon have failed to control California brome with diuron (N'-(3,4- dichlorophenyl) - N, N' - dimethyl urea), atrazine (6-chloro- N-ethyl- N'- (1 methyl-1,3,5-triazine,-2,4-diamine), and simazine (6 chloro-N , N'-diethyl-1,3,5-triazine-2-4-diamine), even at rates injuring established crops. It was also noted that from1960 to 1987 atrazine and simazine have been widely used for seedling grass control in orchardgrass grown for seed. Diuron was

even less effective than atrazine and simazine on this weed, but is currently the only long-residual herbicide registered in grass seed crops .

Known mechanisms of resistance to photosynthetic-inhibiting herbicides involve either an altered site of action, which reduces binding of the herbicide, or enhanced metabolism of the herbicide. Since California brome has proliferated in fields that were repeatedly treated by triazine and diuron, it is probable that this species has developed resistance to one or both of these herbicides. There is a need to verify such events so that appropriate management strategies can be developed. The objectives of these experiments are: (1) to quantify the resistance to atrazine and diuron among California brome accessions, and (2) to identify mechanisms of resistance.

Various protocols have been designed to detect triazine resistance. Among the many methods used to detect PS II resistance in weeds are field and greenhouse tests on whole plants, floating leaf disc, measurements of electron transport inhibition in herbicide-treated isolated chloroplasts, herbicide binding studies, chloroplast DNA analysis, and fluorescence of chlorophyll in isolated chloroplasts and intact plants. In our studies, we used greenhouse bioassays and chlorophyll fluorescence of intact plants to detect levels of atrazine and diuron resistance among accessions of California brome. Laboratory analysis of chloroplast DNA is underway but only preliminary results are presented.

LITERATURE REVIEW

2 .1. Botanical chracteristic and importance of California Brome as a weed

California brome (*Bromus carinatus* H.& A.) is native to the Rocky Mountains and Pacific Coast regions of the western US (Hughes et al.,1962). This native species is widely distributed in the Pacific northwest, is highly variable in morphology, and is generally viewed as a short -lived perennial (Hitchcock, Cronquist, and Ownbey,1984). Rojas (1990) studied dormancy and vernalization of California brome seed and reported that a quantitative vernalization period of about 20 days is required. He reported that seeds left on the soil surface remain dormant up to 7 months, while most buried seeds germinated readily, implying a generally short-lived seed bank. In Oregon, this weed is more frequently found on slightly higher elevations, relatively well-drained soils, such as those used for orchardgrass (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.) seed production, than on the lower, more poorly drained soils used for perennial ryegrass (*Lolium perenne* L.) (Mueller-Warrant and Mellbye, 1991).

California brome has become a troublesome perennial weed in orchard grass grown for seed in Oregon because of the absence of effective control measures for established plants and decreasing availability of seedling control treatments (Mueller-Warrant and Mellbye, 1991). Mueller-Warrant (1987) reported that California brome has been increasing in tall fescue and orchardgrass seed production fields by tolerating normal use rates of diuron, simazine, and atrazine. There was inconsistent control of this weed between seasons and locations, with diuron being even less effective than atrazine or simazine. It was speculated that differences between tests in susceptibility of

California brome seedlings to diuron and atrazine might be due to genetic differences in tolerance among ecotypes present at various locations (Mueller-Warrant and Mellbye,1991).

2. 2. The evolution of herbicide resistance in weeds

The fact that not all members of a weed population are equally sensitive to a herbicide is a major factor in the evolution of herbicide resistance (Cobb, 1992). The earliest prediction of the evolution of herbicide resistance in weeds was made by Harper in 1956 based on the understanding of resistance to drugs in bacteria and insecticides in arthropods. This prediction was realized in 1970 when Ryan reported the failure of simazine and atrazine to control groundsel (*Senecio vulgaris* L.) in a nursery. Since then, numerous cases of herbicide resistance have been reported (LeBaron and Gressel, 1982). In almost all reported cases, the evolution of herbicide resistance resulted from strong and constant selective pressure in monocultures treated with the same herbicide. This process is understood to involve small numbers of individual plants that are resistant to the herbicide before it is ever applied and build up to large populations when the herbicide selection pressure is imposed. Holt (1992) and Holt et al.,(1993) reviewed worldwide reports of herbicide resistant weeds, finding that there was no evidence that any cases of resistance occurred from mutations caused by herbicides. Thus, the occurrence of a resistant population is understood to result from natural gene mutations that are artificially selected for by the herbicides (LeBaron and Gressel, 1982).

Two key biological processes help to understand the dynamics of resistance and suggest ways to develop management strategies:(1) processes related to fitness of

resistant biotypes relative to the susceptible biotypes and crop species, and (2) processes that contribute to gene flow in space and time (Radosevich et al.,1991). According to the authors, the major factors related to fitness and gene flow which influence the development of herbicide resistance are immigration, demography, inheritance, and competition. Furthermore, most of the plant groups that have developed resistance are reported to be wholly or partially self-pollinated, herbaceous annuals, autogamous, and associated with agriculture (LeBaron and Gressel, 1982). Herbicide resistance in general is most likely to occur if some or all of the following are present: (1) the herbicide has a high degree of control of the target species, (2) the weed's seed has a short life in the soil seed bank, (3) the herbicide has a long soil persistence, (4) the herbicide is used frequently, (5) the herbicide has a single site of action, (6) the use rate is high, (7) annual herbicide rotation is not practiced, and (8) herbicides are not mixed on the crop (Zimdahl, 1993). However, relatively little is known concerning the levels of genetic variation in herbicide resistance in natural populations, occurrence and spread of resistant plants before any selection due to herbicides, field mutation rates, and the establishment and spread of resistance within populations (Warwick 1991).

The genetics of resistance, as reviewed by Gressel and Segel (1982), suggests the modes of inheritance for herbicides resistance include all classical modes of inheritance from monogenic through polygenic, maternal inheritance, and dominant and recessive genes. A recent review on inheritance of herbicide resistance by Gressel (1985) revealed that resistance to triazines in corn, to picloram, phenmedipham, and bentazone in tobacco, and to metribuzin in soybeans was generally inherited on one or at most two major nuclear genes. However, triazine resistance in weeds has proven to be a special case, and is maternally inherited because the D1 protein is a chloroplast

gene product. Andersen et al. (1987) disclosed the existence of a different form of triazine-resistance inheritance in weed biotypes of velvetleaf *(Abutilon theophrasti* Medik.*)*. Resistance in this weed was shown to be controlled by a single, partially dominant nuclear gene that was not cytoplasmically inherited. In algae in the laboratory, diuron resistance was found to be maternally inherited. There is at best only a small degree of cross tolerance between atrazine, metribuzin, and diuron in the resistant biotypes (Gressel, 1985). Fuerst et al., (1986) found resistant biotypes of smooth pigweed, common lambsquarters and common groundsel, with strong resistance to atrazine and bromacil, a slight resistance to buthidozole, and little or no resistance to diuron. The crop canola showed a similar pattern of cross resistance.

2. 3. The mechanisms of atrazine and diuron resistance

The earliest studies on the mechanism of resistance indicated that differential uptake, translocation, and metabolism of atrazine and simazine were not responsible for differences in herbicide sensitivity between resistant and susceptible biotypes of common groundsel (Radosevich and Appleby, 1973) and on other weed species (Jensen et al., 1977; Radosevich, 1977). Tolerance to triazine herbicides has been attributed either to a rapid metabolic detoxification of the herbicide (Shimabukuro, 1968) or to a modified site of action that prevents herbicide binding within the chloroplasts (Arntzen et al., 1982). Susceptible as well as resistant biotypes of some weed species like wild turnip rape (*Brassica campestris* L.) can rapidly metabolize atrazine (Ali and Machado, 1984). According to the authors, 90% or more of the metabolized atrazine in shoots and roots of both the susceptible and resistant biotypes was in the form of 2-hydroxyatrazine, suggesting that hydroxylation was the major

pathway for atrazine metabolism in these plants. The mechanism of detoxification of triazines by resistant weeds was not the same mechanism of detoxification found in corn. Triazine-resistant weeds degraded atrazine at a much lower rate than corn (Gressel, 1985). Jensen et al.(1977) made extensive studies on atrazine metabolism by 53 grass species of sub-families Festucoideae, Panicoideae, and Eragrostoideae. According to their study, members of the sub family Panicoideae showed greater tolerance to atrazine because of their ability to detoxify it. *Bromus inermis*, a Festucoideae, was reported to be slower in detoxification of atrazine. However, LeBaron (1991) reported that there was an absence of clear relationships between plant families and genera in their tendency to evolve resistance, with resistance usually developing in one, or at most, a few species in a weed community exposed to a herbicide.

Several classes of herbicides, including the ureas, triazines, and phenols, are now known to inhibit photosystem II (PS II) activity by displacing plastoquinone from the QB binding site, and in doing so preventing electron flow from QA to the rest of the electron transport chain (Ashton and Crafts, 1981; Cobb, 1992). Inhibition arises from the reversible binding of one herbicide molecule per electron-transport chain to a high affinity site on the 32 kDa protein, also called the D1 protein of PS II, which is the secondary quinone acceptor QB (Tischer et al., 1977; Pfister and Arntzen, 1979). Binding of triazines to this high affinity site on the chloroplast thylakoid membrane inhibits electron transport (Tischer et al., 1977). There are no published findings of binding without inhibition except for quinone binding (Vermas et al., 1983), and loss of the 32-kDa protein results in loss of the binding site (Herrmann et al., 1984). Hirschberg et al. (1984) cloned the psbA gene from atrazine resistant and susceptible biotypes of black nightshade (*Solanum nigrum* L.) and redroot pigweed *(Amaranthus*

retroflexus L.), and detected a single base substitution from serine to glycine at amino acid 264 as the basis of resistance to this herbicide. This amino acid substitution causes a change in the redox equilibrium of PS II electron acceptors, resulting in a ten-fold decrease in the rate of electron transfer between QA and QB in atrazine-resistant chloroplasts in the absence of the herbicide (Bowes et al., 1980). This mutation reduces the susceptibility of the photosynthetic electron transport system to symmetrical triazines by roughly 1000 fold (Hirschberg et al., 1984). In addition to resistance to the herbicide, the resistant biotypes possess other alterations in chloroplast properties. These include a greatly reduced rate of QA to QB electron transfer and a reduced level of saturation of thylakoid membrane lipids (Arntzen et al. 1982 and Chapman et al. 1985).

By further examination of the QB binding site, Trebst (1987) suggested that inhibitors with a carbonyl or equivalent group (ureas, triazines, triazinones) oriented towards the peptide bond close to serine 264 could form a hydrogen bridge to this peptide bond. Substitution at position 264 from serine to glycine yields atrazine resistance, while serine to alanine or threonine produces resistance to diuron. This observation led to the proposal that a serine hydroxyl group may be essential for atrazine binding (Cobb, 1992). The binding of herbicides to PS II as reported by Gressel (1985) is clearly not covalent, and it was described as a very loose binding, not even much stronger than the diffusive force. It was also noted that the binding is competitive. Atrazine or metribuzin can easily be driven off the thylakoid by diuron, lenacil, propanil, pyramin, and to a lesser extent by other PS II inhibitors, indicating that they have a common target or binding site.

2. 4. Methods frequently used to determine atrazine and diuron resistance in weeds

A variety of laboratory, greenhouse, and field techniques have been devised for recognizing herbicide resistant plants (Truelove and Hensley, 1982; Clay and Underwood, 1989). However, not all of them enable the different mechanisms of resistance to be distinguished (Van Oorschot, 1991). Because different herbicides affect different processes, selection of procedures to be used will depend on the primary metabolic processes known to be affected in susceptible plants (Truelove and Hensley, 1982). Van Oorschot (1991) reviewed the methods available for identification of resistant biotypes with regard to triazine resistant weeds, and summarized them as field treatments, whole-plant studies, leaf photosynthesis and leaf disc floating, electron transport properties of isolated chloroplasts, and chlorophyll fluorescence in intact leaves. Binding studies and a rapid detection of chloroplast DNA involvement in atrazine resistance in plants (McNally et al., 1987) have been used to rapidly detect the reaction of plants to the herbicides.

For whole plant assays, seeds are collected from resistant plants in continuously treated fields and from susceptible plants in areas where the herbicide has not been used. Treatments are then applied in greenhouse as pre- or post emergence, and the response is evaluated. Greenhouse trials are more effective than field trials because the herbicide treatment can be made more uniformly and variability due to other environmental factors can also be reduced. In field trials, failure to achieve control is not always caused by resistance. In general, field trials and whole plant studies in the greenhouse do not reveal the mechanism of resistance that is involved (Van Oorschot

The floating disk method was first described by Truelove et al. (1974). In this method, leaf disks that remain floating on a phosphate buffer containing the herbicide are considered to be resistant. In that case, photosynthesis is relatively undisturbed and the buoyancy results from the production of oxygen in the intercellular air spaces. This method doesn't discriminate between rapid detoxification and chloroplastic resistance (Van Oorschot 1991).

Chlorophyll fluorescence, measurable with commercially available fluorometers, has been suggested as a tool for investigation of photosynthesis mechanisms and herbicide resistance in plants (Shaw et al., 1986). Bowes et al. (1980) and Ahrens et al. (1981) investigated the use of chlorophyll fluorescence as a potential screening system for triazine resistance in various crop and weed species. Measurements of chlorophyll fluorescence emission kinetics from leaves have also been extensively employed in studies of photosynthetically active herbicides (Cadahia et al.; 1982; and Richard et al., 1983) because they offer a rapid, specific and non-destructive technique to evaluate herbicide activity *in vivo*. The chlorophyll fluorescence is a product of light energy absorbed by plants (and in chloroplasts *in vitro*) that is not used in photosynthesis or given off as heat. When photosynthesis is operating at maximum efficiency, there is little energy loss to fluorescence (Lichtenthaler, 1988).

The principle of fluorescence measurement and assessment of the capabilities of the photosynthetic apparatus is made by exposing a dark-adapted plant to light and measuring the resultant fluorescence emissions (inductions). The amount of light fluoresced is normally reported by arbitrary units. This phenomenon was observed and detailed by Kautsky and Hirsch (1931) and the resulting fluorescence induction curve has since been termed the Kautsky effect. The now widely accepted interpretation of the basic fluorescence transient features were formulated by Papageorgiou (1975). The

Kautsky curve (Figure 1), exhibits fluorescence levels usually termed O , I , D , P , M, S , T (Papageorgiou, 1975). The "O" level or the ground fluorescence (Fo) is the minimum fluorescence that occurs when all the electron acceptors in the electron transport system are open and is considered to be insensitive to inhibitors. However, damage to PS II after prolonged exposure to herbicides may increased Fo. The "I" and "D" are intermediate levels that correspond to an increase and slight dip in the rise to the "P" level. The "P" level, usually termed the fluorescence peak (Fp) or the fluorescence maximum (Fm) occurs when the electron acceptors are maximally reduced. The rise from Fo to Fm is the time it takes to fully reduce all the primary electron acceptors (Gressel, 1985). This rise to Fm is very rapid in PS II inhibited plastids of triazine-treated sensitive plants or in diuron-treated atrazine resistant biotypes (Gressel, 1985). "S" and "M" are the intermediate declines and rises in fluorescence levels as the photosynthetic apparatus fully adjusts to the available light and fluorescence approaches the 'T" or terminal level. Triazine-treated susceptible biotypes do not return to this lower steady-state level, while resistant biotypes do (Gressel, 1985). Variable fluorescence (Fv) corresponds to the fluorescence increase from Fo to Fm. Stress decreases the Fv of dark-adapted leaves (Lichtenthaler, 1988). The rise from O to P is a fast phase kinetics rise. The decline from P to T is a slower phase decline to steady state fluorescence level. The ratio of variable fluorescence to maximum fluorescence (Fv/Fm) is a measure of photochemical efficiency (Oquist and Ogren, 1985; Adams et al., 1990; Ottander and Oquist, 1991). A decline in Fv/Fm can serve as a convenient parameter to assess herbicide effectiveness in inhibiting electron transport (Lichtenthaler, 1988).

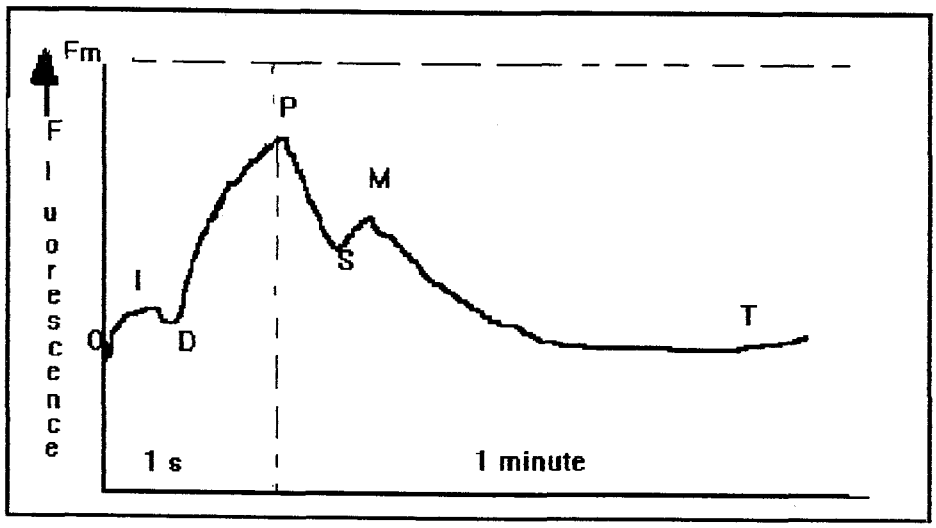

Figure 1. Schematic representation of the Kautsky phenomenon in leaves and interpretation of fluorescence variations.

" O , initial fluorescence (Fo) ; all PS II reaction centers are open. (the initial electron acceptor of PS II, QA, is fully oxidized.) O-I, partial photoreduction of QA- by the plastoquinone pool lowers fluorescence to D (" dip "). D-P, accumulation of NADPH and reduced plastoquinone (due to low rate of carbon assimilation) leads to reduction of QA, fluorescence rises to P ("peak"). Fm, stationary fluorescent level in the presence of diuron. It represents the maximum fluorescence yield, when all reaction centers of PS II are closed. Fm may be higher than P. P-S, enhanced reoxidation of QA- and buildup of a pH difference across the thylakoid membranes causes fluorescence decline to S ("steady state"). S-M, Increased reduction of QA and decrease in pH results in the fluorescence rise to M ("maximum") since a lag in CO2 assimilation retards electron flow. M-T, further decline of fluorescence to T ("terminal" level) is caused mainly by increase in pH and /or by reoxidation of QA-, as non-cyclic electron flow is enhanced due to activation of the carbon reduction cycle".

Adapted from Briantais, et al., 1986.

Fluorescence induction curves change as a result of many factors. Changes in fluorescence induction curves can be compared between herbicide treated and control plants, between species, or between biotypes after treatment by herbicides. These changes in fluorescence induction curves have already been used as the basis of intact plant bioassays for evaluating the penetration and effectiveness of urea and atrazine herbicides in different species (Ahrens et al., 1981; Richard et al., 1983, Habash et al., 1985; Shaw et al., 1986).

Selection of specific chlorophyll fluorescence induction parameters to measure plant injury from herbicides may depend on the type of equipment used and the objectives of the measurements. Lichtenthaler (1988) listed several fluorescence parameters to be used for detecting stress in predarkened intact plants, including a fast fluorescence rise signal and a slow fluorescence decrease from maximum to steady state fluorescence. Malkin and Michael (1971), Cahen et al. (1976), and Habash et al. (1985) used the area above the induction curve between "O" and "P", and the reduction in this area was suggested to be the most sensitive monitor of diuron inhibition. According to the authors, in a diuron-poisoned system the area above the curve is directly related to the concentration of QA, but without treatment this area is considerably larger and proportional to plastoquinone. Measurement of this area has been reported to be complex . However, a simple indicator is given by t1/2, one-half of the time required for the rise in fluorescence from "O "to "P" (or from Fo to Fm) (Bolhar-Nordenkampf et al., 1989). Fisher (1983) used the percent decay from "P" to "T" 30 s after the onset of illumination to evaluate hard red winter wheat cultivars for metribuzin tolerance. Ahrens et al.(1981), Melcarek et al. (1977), and Richard et al. (1983) used terminal steady state level of fluorescence (T) to detect injury by chilling

or herbicide. The decline in Fv/Fm resulting from atrazine treatment of wheat leaves has also been suggested to serve as a convenient parameter to assess herbicide effectiveness.

Greenhouse bioassays and fluorescence induction tests have also been compared for better interpretation of induction parameters. For example, Shaw et al. (1985) made a comparison between various chlorophyll induction curves and metribuzin, diuron, and atrazine concentrations in the laboratory studies. These authors reported that the height of I ,P, and fluorescence level 30 s after the onset of illumination (T) were useful at 4 to 8h after transplanting but poorly correlated with herbicide concentration at later times, and even negatively correlated after 24h. The authors explained that after extended exposure to a photosynthetic inhibitor, chloroplasts within the plant are destroyed and begin to lose their ability to emit fluorescence due to the production of a permanent quenching state. The experiment also revealed that eight hours after transplanting into varying diuron concentrations, the heights of I , P, and T were significantly lower at 8 mg kg^{-1} compared to 4 mg kg^{-1}, indicating a reduction in the overall height of the chlorophyll fluorescence curve. Ahrens et al. (1981) found greater fluorescence in untreated resistant biotypes than in susceptible biotypes in six weed species, including *Bromus* species. The authors concluded this high native fluorescence by resistant biotypes results from an altered functional PS II since fluorescence is a measure of the loss of energy previously absorbed by the PS II complex.

Bahler et al. (1987) used CRF (change in relative fluorescence) between two readings, i.e., before and after treatment with atrazine, to evaluate atrazine tolerance of three warm season grasses, side oats grama (*Bouteloua curtipendula* (michx) Torr.), Indian grass (*Sorghastrum nutans* (L.) Nash), and switchgrass *(Panicum virgatum* L.).

Different procedures vary the length of time between exposure to herbicides and measurements of fluorescence. For example, in cereals both tolerant and susceptible plants are initially affected by the herbicide. Tolerance in cereals is attributed to the ability of some plants to metabolize the herbicide to non-toxic products more rapidly than other plants can (Shimabukuro and Swason, 1969; Ahrens et al., 1981; Cadahia et al., 1982). As a consequence, differences in fluorescence induction curves between tolerant and susceptible cereal cultivars tend to be small, and take a relatively long time to become apparent (Harris and Camlin 1988). According to these authors, the day after application of chlorotoluron, metoxuron, and atrazine, both tolerant and sensitive cultivars of winter wheat showed a distinct change in their fluorescence induction curves, exhibiting an apparently instantaneous rise to P. Quenching after P to T was also negligible. This pattern has been described as typical of that produced by photosystem II blockage of the electron transfer chain by binding to the 32 kDa herbicide binding proteins of the photosystem II complexes.

Harris and Camlin (1988) have classified winter wheat varieties as tolerant and susceptible by using the chlorophyll fluorescence induction parameters P/T (or quenching after P) and the area above the curve 7 days after chlorotouluron, metoxuron, and atrazine applications. They defined susceptible cultivars as those where after 7 days, the curves for the herbicide-treated and untreated differed significantly for P/T and area above the curve. Tolerant cultivars were also defined as those where the curves for the herbicide treated and control plants showed no difference after 7 days. The chlorophyll fluorescence induction curves of herbicide-treated resistant and susceptible cultivars of wheat were not different 24 hours after herbicide application.

MATERIALS AND METHODS

Four sets of greenhouse bioassays and two sets of chlorophyll fluorescence measurements were made to detect the reaction of California brome accessions to atrazine and diuron. The greenhouse experiments were conducted at Oregon State University USDA-ARS Forage Seeds Production Research greenhouse and the fluorescence measurements were made at the Faculty of Forestry and Wild Life laboratory of OSU.

3.1. Source and the background of the seeds of California brome used in the studies

All the California brome seeds used in these experiments were kindly provided by George Mueller-Warrant. A total of 93 accessions of California brome with varying rates of tolerance to atrazine had been growing in a nursery at Hyslop farm since 1987. Seven suspected resistant and three presumed susceptible accessions of California brome were selected for these experiments. The variability in their reaction to herbicides and chracteristic features of the ten accessions(Table 2) were obtained together with the seeds. According to Mueller-Warrant (1993 personal communication) the California brome accessions were collected from two locations; the Shedd area and Mary's Peak. The Shedd area represented fields continuously treated with atrazine and diuron for more than 20 years while Mary's Peak represented an area of no known herbicide use. The brome seeds used in all experiments were from the 1991 harvest of the nursery at Hyslop. The presumed susceptible types came from the Shedd area rather than Mary's Peak, despite the known use of herbicides in the Shedd area fields.

Table.2. Characteristics of California brome accessions used in the experiments

Test number	Accession number	50% heading date in May 3 years average	Source	Previous reaction to atrazine	Morphology
R1	12	11.27	Shedd	resistant	high vigor
R2	91	24.05	Shedd	resistant	fuzzy, low vigor
S3	56	30.15	Mary's Peak	susceptible	
R4	65	29.94	Mary's Peak	resistant	
S5	75	18.81	Shedd	susceptible	
R6	31	10.41	Shedd	resistant	
R7	11	6.99	Shedd	resistant	
R8	20	9.44	Shedd	resistant	
R9	38	7.35	Shedd	resistant	hairy
S10	54	27.97	Mary's Peak	susceptible	

3. 2. Greenhouse experiments

3.2.1. Determination of atrazine rates for control of California brome (Greenhouse experiment 1)

The objective of this experiment was to find the range of atrazine rates to be used in the greenhouse for assessment of atrazine resistance in California brome.

The experiment consisted of five accessions of California brome designated as R1,R2,S3,R4 and S5 of which accessions S3 and S5 were presumed susceptible while accessions R1,R2,and R4 were suspected resistant. Eight rates of atrazine (0.0 ppm, 0.01 ppm, 0.03 ppm , 0.1 ppm, 0.3 ppm, 1.0 ppm, 3.0 ppm, and 10 ppm) were used pre-plant soil incorporated. Each rate was applied separately in 10 kg of soil, and mixed in an electric cement mixer. The herbicide atrazine (480 g/L) was diluted for each rate to give a total of 0.5 L of solution. Before adding this herbicide solution to dry soil, 0.5L of water was added to 10 kg soil and mixed well in the mixer. The herbicide solution was then injected into the soil while the mixer was in motion to uniformly mix the herbicide with the soil. The treated soil was then transferred into pots holding 385 g of soil. Seeds of each accession were then planted in the pots. A randomized complete block design was used with uniform distribution of light in three replications. Supplementary light was utilized from 5 a.m. to 6 p.m. Average day/night temperature in the greenhouse during the growing period was 21.4/14.5C.

Plant height was recorded weekly and dry weight per plant was recorded 30 days after planting. Differences in plant height and dry weight per plant for different accessions and atrazine rates were determined by analysis of variance (ANOVA) and multiple range tests, when appropriate. All statistical analysis were conducted using

Statgraphics version 5 software. The dose response for the five accessions across the rates of atrazine was calculated to determine the GR 50 (growth reduction 50%) with the help of a BASIC program.

3.2.2. Effect of pre-emergence atrazine rates on growth, dry weight per plant, and percent mortality of 7 suspected resistant and 3 susceptible accessions of California brome (Greenhouse experiment 2 and 3)

Two greenhouse experiments (experiment 2 and 3) were conducted in spring 1993 and in fall 1994, respectively. The objective of the two experiments was to determine the relative resistance / susceptibility to pre-emergent atrazine by 7 suspected resistant and 3 presumed susceptible accessions of California brome in the greenhouse. Accessions S3, S5 and S10 represent susceptible while the remaining accessions, R1,R2,R4,R6,R7,R8, and R9, are the suspected resistant as described earlier. In experiment 2, six rates of atrazine (0.0 ppm,0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm and 0.6 ppm) were applied pre-emergence. In experiment 3, rates were modified; consisting of 0.0 ppm, 0.3 ppm, 0.6 ppm, 0.9 ppm, 1.2 ppm and 1.5 ppm.

In both experiments, plant population was thinned to 6 plants per pot immediately after seedling emergence. The plants were watered daily and a liquid fertilizer was applied once per week uniformly to all treatments. The pots were arranged in a randomized complete block design of factorial experiment consisting of 6 replications. Supplementary light was provided by fluorescence bulbs from 5 a.m. to 6 p.m and the day/night average temperature for experiment 2 was 30/17.1C,and for experiment 3, was 24.4/18.3C. Growth rates were recorded every ten days by measuring plant height. Dry weight per plant was measured by cutting the seedlings 2 cm above soil and drying them in the oven 30 days and 40 days after planting for experiment 2 and 3 respectively. Percent mortality was also determined by counting the

number of plants per pot alive 3 weeks after planting. Differences in plant height, dry weight per plant, and percent mortality among accessions and among rates of atrazine application were determined by using analysis of variance and multiple range tests. A GR-50 for height and dry weight and LD-50 for mortality were calculated from the means. Resistant ratios were then obtained by dividing the GR-50 or LD-50 of all accessions by the GR-50 or LD-50 of the most susceptible accession.

3. 2 .3. Effect of pre-emergent diuron rates on growth, dry weight per plant and percent mortality of 7 suspected resistant and 3 presumed susceptible accessions of California brome (Greenhouse experiment 4)

The objectives of this experiment were

1. To determine the reactions of the same ten accessions of California brome to varying levels of diuron, and identify accessions resistant or susceptible to diuron.
2. To determine whether atrazine-resistant California brome accessions have developed multiple or cross resistance to diuron.

This experiment was conducted in winter 1994 side by side with pre-emergent experiment 3 of atrazine. The treatments include, the same 10 accessions with eight rates of diuron (0.0 ppm, 0.9 ppm, 1.8 ppm, 2.7 ppm, 3.6 ppm, 4.5 ppm, 5.4 ppm, and 6.4 ppm) applied pre-emergent, after planting the weed seeds in pots. Similar design and growing conditions used in experiment 3 were provided. Evaluations similar to those used in experiment 3 were utilized but the analysis was made by using MSTAT and Statgraphics softwares.

3. 3. Determination of atrazine and diuron resistance in California brome by using whole plant chlorophyll fluorescence

3.3.1. Chlorophyll fluorescence 12 hours after atrazine and diuron applications and before exposure to light

The objectives of this experiment were:

1. To detect resistant and susceptible accessions and to determine the mechanisms of resistance of California brome by induction of chlorophyll fluorescence before the occurrence of photochemical damage to PS II by the herbicides.
2. To determine the mechanism of resistance to atrazine and diuron in California brome by using the chlorophyll induction parameters.

The same ten accessions of California brome were used to detect resistance and susceptibility by intact plant chlorophyll fluorescence method. Seedlings transplanted from the control plants of experiment 2 were used for intact plant chlorophyll fluorescence. Young seedlings were obtained from regrowth of transplanted and cut plants. When seedling height was about 30 cm, plants from each accession were assigned to diuron, atrazine and control treatments with 4 replications. All aerial parts of the plants were dipped in clean water for 5 minutes before dipping into herbicide solutions in order to insure uniform retention of herbicides by the surfaces. Atrazine and diuron 100 micromolar concentrations were prepared, each in 8 L of water . The plants were dipped either in atrazine or diuron for 15 minutes in the dark, and kept in the dark for 12 hours until fluorescence was measured on each plant. The control plants were dipped in water for 15 minutes and kept in the dark similarly. Chlorophyll fluorescence measurements were made between 12 and 16 hours after herbicide application. The chlorophyll fluorescence was measured on the same day for all treatments with an integrating fluorometer (Pacific, Fluorotec, Burnaby, British

treatments with an integrating fluorometer (Pacific, Fluorotec, Burnaby, British Colombia, Canada) attached to a 286 micro computer (Fluoroview software package versions 0.5e and 0.8 Pacific Fluorotec) in the Faculty of Forestry laboratory of Oregon State University. The integrating fluorometer used in the experiments as described by Vidaver et al.(1991) measures the chlorophyll fluorescence emissions of an entire dark-adapted seedling in a non-destructive and non-invasive manner, rapidly and accurately. The protocol outlined by Vidaver et al.(1991) for chlorophyll fluorescence measurement, and seedling management before measurements, was followed while conducting these experiments. This procedure includes transferring seedlings to the laboratory environment at late afternoon of the day before chlorophyll fluorescence measurements, watering them, and keeping them in a well ventilated environment. Adjustments of light levels, duration of scans, and preparing the fluorometer was done before putting the seedlings into the sphere. Transferring seedlings into the sphere of fluorometer was also done in complete darkness. The fluorometer was adjusted to scan for 180 seconds and the output of the fluorescence curve and kinetic parameters were printed. Induction parameters Fo, Fm, Fv, Fv/Fm, time to reach Fo and Fm, and T or terminal level (steady state fluorescence) were examined. Analyses were made only on the normalized fluorescence parameters Fv, relative Fm, Fv/Fm, and T. Induction curves were used to interpret the results.

3.3 2. Chlorophyll fluorescence of California brome accessions 1 week after atrazine and diuron applications

The objective of this measurement was to determine resistance and susceptibility to herbicides based on the recovery potential of accessions, 1 week after treatments with the herbicides.

These fluorescence measurements were made by following the same general procedures on the same plants that had been scanned the previous week. One week after exposure to the respective herbicides and light in the greenhouse, chlorophyll fluorescence induction parameters were recorded and analyzed, and relative resistance or susceptibility of each accession determined. The recovery rate from inhibition of electron transport by herbicides was determined by examining changes in the chlorophyll fluorescence induction parameters 1 week after the first measurement and by comparisons with inductions of the untreated plants.

RESULTS

4.1. Greenhouse experiments

4.1. 1. Determination of Atrazine rates for control of California brome (Greenhouse experiment 1)

Differences in plant height and dry weight per plant were significant among accessions of California brome with atrazine rates between 0.3 and 1.0 ppm. As indicated in Tables 3 and 4, the GR-50 value for plant height reduction was the highest for the suspected resistant accession R1. For dry weight per plant reduction, the two suspected resistant accessions R1 and R2 showed higher values than the others. These GR-50 values tentatively separated the two accessions of California brome R1 and R2 as more resistant than the rest of the accessions.

Rates of atrazine below 0.3 ppm were equally tolerated by all accessions while rates above 1.0 ppm were not tolerated by any accessions (Table 5). These range finding studies revealed that accessions of California brome can be compared for their relative resistance to atrazine by using atrazine rates between 0.3 and 1.0 ppm in the greenhouse.

Table 3 Effect of pre-plant atrazine rates on plant height of 5 accessions of California brome (Greenhouse experiment 1)

Rate ppm	Plant height 20 days after planting (cm) Accession R1	R2	S3	R4	S5	mean	S.E	P
	----------cm----------							
0	25.7 a	26.7 b	24.0 a	29.7 a	26.3 a	26.5	1.02	0.5
0.01	29.3 a	35.3 a	30.0 a	28.7 a	29.3 a	30.5	0.9	0.25
0.03	27.0 a	28.0 b	28.0 a	30.0 a	28.0 a	28.3	0.6	0.6
0.1	26.0 a	29.3 b	27.0 a	30.3 a	27.3 a	28.1	0.6	0.3
0.3	24.3 a	30.7 ab	26.0 a	25.0 a	25.0 a	26.2	1.5	0.7
1	13.3 b	10.7 c	5.3 b	8.7 b	14.0 b	10.5	1.38	0.3
3	6.0 bc	0.7 d	2.0 b	4.0 bc	2.0 c	2.9	0.9	0.3
10	2.0 c	0.5 d	0.0 c	0.0 c	0.0 c	0.5	0.41	0.5
Mean	19.3	20.2	17.9	19.5	19			
S.E	0.087	0.57	0.8	0.94	0.6			
P	0.00	0.00	0.00	0.00	0.00			
GR-50	1.64	0.91	0.74	0.73	1.05	1.23		
R^2	94	63	89	95	98	88		

Within columns, means followed by the same letter are not significantly different according to 5% LSD test.

Table 4 Effect of pre-plant atrazine rates on dry weight per plant of 5 California brome accessions (Greenhouse experiment 1)

Rate ppm	Dry weight per plant at 30 days after planting Accession R1	R2	S3	R4	S5	mean	S.E	P
	.....g / plant.....							
0	0.033 bc	0.035 a	0.043 a	0.023 c	0.034 bc	0.034	0.0009	0.0009
0.01	0.041 ab	0.042 a	0.044 a	0.042 a	0.063 a	0.047	0.003	0.16
0.03	0.042 ab	0.044 a	0.043 a	0.032 b	0.049 ab	0.042	0.003	0.47
0.1	0.049 a	0.034 a	0.046 a	0.027 bc	0.047 b	0.04	0.002	0.07
0.3	0.022 c	0.026 ab	0.007 b	0.014 d	0.027 c	0.019	0.002	0.1
1	0.006 d	0.005 c	0.003 b	0.002 e	0.004 d	0.004	0.0004	0.12
3	0.002 d	0.011 bc	0.001 b	0.002 e	0.003 d	0.004	0.001	0.5
10	0.002 d	0.002 c	0.002 b	0.002 e	0.002 d	0.002	0.007	0.38
Mean	0.025	0.025	0.024	0.018	0.029	0.024		
S.E	0.0013	0.002	0.0015	0.0008	0.0019			
P	0.00	0.00	0.00	0.00	0.00			
GR-50	0.41	0.5	0.23	0.3	0.34	0.57		
R^2	96	98	55	66	70	79		

Within columns, means followed by the same letter are not significantly different according to 5% LSD test.

Table 5 Effect of pre-plant atrazine rates on dry weight per plant as percent of untreated control on 5 accessions of California brome (Greenhouse experiment 1)

Rate	Accession					
ppm	R1	R2	S3	R4	S5	Mean
	Dry weight per plant as % of untreated control					
0.01	124.2	120	102.3	182.6	185.2	138.2
0.03	127.8	125.7	106.9	139.1	144.1	123.5
0.1	148.5	97.1	106.9	117.4	138.2	117.6
0.3	66.6	74.3	16.3	60.8	79.4	55.8
1	18.2	14.3	6.9	8.6	11.7	11.8
3	6.1	31.4	2.3	8.6	8.8	11.8
10	6.1	5.7	4.6	8.6	5.8	5.8

4.1.2. Effect of pre-emergent atrazine rates on growth, dry weight per plant, and percent mortality of 10 accessions of California brome (Greenhouse experiment 2)

The objective of this experiment was to quantify the presumed resistance to atrazine among California brome accessions The relative resistance and susceptibility among the 10 accessions of California brome was determined on the basis of plant height, dry weight per plant and mortality percent of seedlings treated with pre-emergent atrazine at rates between 0.1 ppm and 0.6 ppm. Our experiment discriminated two suspected resistant accessions of California brome, R2 and R9, from the other 8 accessions tested as being more resistant to atrazine, based on significantly greater height, greater dry weight per plant, and lesser percent mortality than the other accessions (ANOVA and 5 % LSD tests) (Tables 6,7,8,10).

As indicated on Table 9, the two most resistant accessions required two to four fold higher atrazine rates than the most susceptible accession 10 to cause similar 50 % reductions in height, three to five fold higher rates for dry weight per plant, and nearly 20 fold higher rates for 50% mortality. Our study has confirmed the previously reported cases of field tolerance to atrazine in California brome accessions by Mueller-Warrant (1987), where the absence of complete control with atrazine was noted even at a rate of 4.5 kg ha^{-1}. In addition, we have distinguished the two accessions R2 and R9 to be the most tolerant among the accessions of California brome tested. This type of experiment, however, has limitations on determining the mechanisms involved in the observed resistance by the two accessions of California brome.

In the process of quantifying the resistance ratios of California brome accessions to atrazine, we observed greater differences for percent mortality and dry weight per plant than for plant height. Since the height of a single escaped plant in a

susceptible accession could be the same as the height of all other plants in a resistant population, differences in plant height as we measured are probably not the best indicators of herbicide resistance.

Table 6 Effect of pre-emergent atrazine rates on plant heights of 10 accessions of California brome (Greenhouse experiment 2)

Accession	Atrazine application rates ppm								
	0	0.1	0.2	0.3	0.4	0.6	Mean	S.E	P
	Plant height 20 days after planting								
	.....cm.....								
R1	22 abc	19.6 ab	14.3 ab	18.5 a	6.8 bc	3.7 ab	14.1	0.9	0.000
R2	19.0 c	17.3 b	14.3 ab	9.5 b	13.8 ab	4.2 ab	13.0	0.9	0.001
S3	25.6 a	17.5 b	12.5 b	12.8 ab	4.5 c	1.0 b	12.3	0.8	0.000
R4	22.2 abc	25.0 a	12.0 b	12.3 ab	6.3 c	1.0 b	13.1	0.7	0.000
S5	22.3 abc	21.0 ab	17.0 ab	8.0 b	7.8 bc	0.9 b	12.9	0.9	0.000
R6	23.1 ab	21.3 ab	15.0 ab	7.6 b	8.8 bc	5.1 ab	13.5	0.8	0.000
R7	21.8 bc	20.6 ab	17.0 ab	7.3 b	14.0 ab	4.0 ab	14.2	1.1	0.000
R8	22.8 ab	20 ab	12.6 b	11.5 b	4.6 c	2.4 ab	12.4	0.9	0.000
R9	20.8 bc	22.8 ab	20.5 a	18.8 a	17.8 a	6.0 a	17.8	0.8	0.000
S10	23.6 ab	12.7 b	12.7 b	13.0 ab	1.5 c	2.5 ab	12.2	0.9	0.000
Mean	22.3	20.5	14.8	11.9	8.6	3.12			
S.E	0.4	0.7	0.8	0.7	0.8	0.5			
P	0.11	0.5	0.35	0.005	0.001	0.2			

Means followed by same letters within columns are not significantly different according to 5 % LSD test.

Table 7 Effect of pre-emergent atrazine rates on dry weight per plant of 10 accessions of California brome (Greenhouse experiment 2)

Accession	Atrazine rates ppm								
	0	0.1	0.2	0.3	0.4	0.6	mean	S.E	P
	Dry weight per plant 30 days after planting								
	.....g /plant.....								
R1	0.033 ab	0.018 ab	0.011 a	0.016 a	0.003 b	0.002 abc	0.01	0.002	0.02
R2	0.025 b	0.016 ab	0.008 ab	0.003 b	0.009 a	0.002 abc	0.01	0.002	0.004
S3	0.044 a	0.011 b	0.006 ab	0.006 b	0.002 b	0.001 c	0.01	0.001	0.001
R4	0.035 ab	0.015 ab	0.004 b	0.004 b	0.002 b	0.002 bc	0.01	0.001	0.001
S5	0.040 ab	0.012 b	0.008 ab	0.003 b	0.003 b	0.002 bc	0.01	0.001	0.001
R6	0.041 ab	0.013 b	0.005 ab	0.003 b	0.003 b	0.002 ab	0.01	0.008	0.001
R7	0.041 ab	0.013 b	0.004 b	0.003 b	0.005 b	0.002 abc	0.01	0.001	0.001
R8	0.040 ab	0.014 b	0.011 a	0.003 b	0.005 b	0.002 abc	0.01	0.001	0.001
R9	0.028 ab	0.024 a	0.011 a	0.006 b	0.005 b	0.002 a	0.01	0.001	0.001
S10	0.040 ab	0.009 b	0.006 ab	0.004 b	0.002 b	0.002 abc	0.01	0.001	0.001
Mean	0.036	0.014	0.007	0.005	0.004	0.002			
S.E	0.002	0.001	0.001	0.007	0.005	0.0001			
P	0.04	0.13	0.15	0.004	0.02	0.29			

Means followed by same letters within columns are not significantly different according to 5 % LSD test.

Table 8 Effect of pre-emergent atrazine rates on percent mortality of 10 California brome accessions (Greenhouse experiment 2)

Accession	Atrazine application rates ppm							
	0.1	0.2	0.3	0.4	0.6	mean	S.E	P
	.....	.....	.....	Mortality %.....	.....	.....	.....	.....
R1	36.1 cd	86.1 ab	50.0 b	100.0 a	100.0 a	74.0	4.4	0.00
R2	19.4 d	61.1 bc	80.5 a	69.4 b	72.2 b	60.4	5.0	0.01
S3	63.8 abc	97.2 a	80.5 a	100.0 a	100.0 a	88.0	4.3	0.00
R4	61.0 abc	83.3 a	100.0 a	100.0 a	100.0 a	89.0	2.7	0.00
S5	80.0 ab	61.0 bc	100.0 a	100.0 a	100.0 a	88.0	4.1	0.00
R6	50.0 bcd	100.0 a	100.0 a	97.0 a	97.0 a	89.0	3.6	0.00
R7	44.0 bcd	89.0 a	100.0 a	92.0 a	100.0 a	85.0	2.8	0.00
R8	61.0 abc	97.0 a	97.0 a	96.0 ab	100.0 a	88.0	4.7	0.00
R9	25.0 d	50.0 c	80.0 a	69.0 b	94.0 a	64.5	2.9	0.00
S10	91.6 a	97.0 a	97.2 a	100.0 a	100.0 a	97.0	0.9	0.00
Mean	53.3	82.2	88.6	91.3	96.4			
S.E	3.9	2.9	2.5	2.1	1.1			
P	0.00	0.00	0.00	0.00	0.00			

Means followed by same letters within columns are not significantly different according to 5 % LSD test.

Table 9. The resistance ratios of accessions of California brome to atrazine as determined by 50 % mortality, height, and dry weight per plant (Greenhouse experiment 2)

Accession	Rates giving 50% reduction, R-square, and ratio of R/S (atrazine rates ppm)								
	Plant height			Dry weight			% mortality		
	ppm			ppm			ppm		
	GR-50	R^2	R/S	GR-50	R^2	R/S	LD-50	R^2	R/S
R1	0.32	79.0	2.2	0.14	87.7	3.9	0.12	54.6	13.0
R2	0.38	80.0	2.5	0.13	88.5	3.5	0.18	73.2	20.0
S3	0.21	95.0	1.4	0.06	89.6	1.5	0.06	77.5	6.6
R4	0.27	85.0	1.8	0.05	91.6	1.4	0.07	99.9	8.0
S5	0.27	92.0	1.8	0.05	90.1	1.4	0.08	56.6	9.2
R6	0.25	92.0	1.7	0.04	86.4	1.1	0.10	99.0	11.1
R7	0.31	76.0	2.1	0.04	81.8	1.0	0.10	99.0	11.5
R8	0.24	95.0	1.6	0.07	93.6	2.0	0.05	82.7	5.1
R9	0.55	71.0	3.7	0.16	92.8	4.4	0.18	95.0	20.2
S10	0.15	78.0	1.0	0.04	85.6	1.0	0.01	76.7	1.0
All	0.31	99.0		0.07	94.8		0.08	97.7	

Table 10. Effect of atrazine rates on dry weight per plant as percent of the untreated control of 10 California brome accessions (Greenhouse experiment 2)

Accession	Atrazine rates in ppm				
	0.1	0.2	0.3	0.4	0.6
	Dry weight per plant as % of untreated control				
R1	54.5	33.3	48.4	9.1	6.1
R2	64.0	32.0	12.0	36.0	8.0
S3	25.0	13.6	13.6	4.5	2.2
R4	42.8	11.4	11.4	5.7	5.7
S5	30.0	20.0	7.5	7.5	5.0
R6	31.7	12.2	7.3	7.3	4.9
R7	31.7	9.8	7.3	12.2	4.9
R8	35.0	27.5	7.5	12.5	0.1
R9	85.7	39.3	21.4	17.9	7.1
S10	22.5	15.0	10.0	5.0	5.0
Mean	38.9	19.4	13.9	11.1	5.6

4.1.3. Effect of pre-emergent atrazine rates on growth and mortality of 10 accessions of California brome (Greenhouse experiment 3)

This experiment was a continuation of experiment 2. Higher atrazine rates than those used in the experiment 2 were included to determine the relative resistance and susceptibility of the 10 accessions of California brome to increasing rates of atrazine. As indicated in Tables 11-15, the previously identified resistant accession R9 showed greatest resistance to increasing rates of atrazine. This accession possessed a nine fold greater GR-50 for height reduction, four fold greater GR-50 for dry weight per plant reduction, and eleven fold greater LD-50 value for percent mortality as compared to the most susceptible accessions S3 (for height) and S10 (for dry weight and mortality). Relatively moderate resistances were seen in other suspected resistant accessions, and resistance ratios as determined by their GR-50 and LD-50 values were not as high as accession R9. Accessions R2, R7,and R8 showed about two fold greater LD-50 values as compared to the most susceptible accession S10 (Table 14).

Accessions differed in dry weight per plant in the untreated controls, where the presumed susceptible accession S3 weighed significantly more than the other accessions, and the suspected resistant accession R9 weighed significantly less than the other accessions (Table 12). This indicates that in absence of herbicides at least some of the resistant types were inferior to some of the susceptible types.

Table 11. Effect of atrazine rates on heights of 10 California brome accessions (Greenhouse experiment 3)

Accession	Plant height 40 days after planting					
	Atrazine application rates ppm					
	0	0.3	0.6	0.9	1.2	1.5
	.....cm.....					
R1	37.8 ab	41.5 ab	27.3 abc	16.7 bdc	3.8 de	4.7 bc
R2	37.7 ab	30.8 e	33.2 ab	26.0 ab	22.0 b	4.7 bc
S3	42 a	45.2 a	15.2 e	27.2 ab	3.3 de	5.3 bc
R4	39.3 ab	40.7 b	36.0 a	11.7 cd	3.3 de	0.8 c
S5	36 b	33.8 de	25.5 bc	23.3 abc	1.7 de	0.0 c
R6	38.7 ab	39.2 bc	34.5 ab	13.8 bcd	10.5 cd	3.8 c
R7	39.7 ab	37.5 bcd	32.8 abc	14.5 bcd	8.3 cde	2.2 c
R8	38.2 ab	38.5 bc	27.5 abc	20.5 abc	17.0 bc	8.8 bc
R9	37.2 b	35.3 cd	37.2 a	33.2 a	33.5 a	27.5 a
S10	40.7 ab	40 b	22.8 cd	6.2 d	0.0 e	14.7 b
Mean	38.7	38.3	29.2	19.3	10.35	7.25
S.E	0.5	0.5	1.2	1.5	1.04	1.14
P	0.4	0.001	0.001	0.009	0.0001	0.0001

Means followed by same letters within columns are not significantly different according to 5 % LSD test.

Table 12. Effect of atrazine rates on dry weight per plant of 10 California brome accessions (Greenhouse experiment 3)

Accession	Dry weight 40 days after planting					
	Atrazine rates ppm					
	0	0.3	0.6	0.9	1.2	1.5
	.....g / plant					
R1	0.190 ab	0.160 ab	0.060 b	0.013 cd	0.005 c	0.008 bcd
R2	0.180 ab	0.100 cd	0.100 ab	0.050 ab	0.030 a	0.01 bcd
S3	0.200 a	0.180 a	0.020 b	0.030 bcd	0.007 bc	0.007 bcd
R4	0.170 abc	0.130 bcd	0.196 a	0.020 cd	0.005 bc	0.004 d
S5	0.133 bc	0.095 cd	0.040 b	0.040 abc	0.004 c	0.005 cd
R6	0.190 ab	0.140 abc	0.090 ab	0.012 cd	0.009 bc	0.006 bcd
R7	0.130 bc	0.140 abc	0.060 ab	0.012 cd	0.007 bc	0.006 bcd
R8	0.170 abc	0.130 abcd	0.050 b	0.030 abcd	0.020 b	0.014 b
R9	0.120 c	0.090d	0.090 ab	0.060 a	0.040 a	0.03 a
S10	0.180 ab	0.150 ab	0.030 b	0.009 d	0.005 c	0.013 b
Mean	0.17	0.13	0.07	0.03	0.013	0.01
S.E	0.007	0.005	0.015	0.003	0.001	0.001
P	0.4	0.006	0.34	0.003	0.001	0.001

Means followed by same letters within columns are not significantly different according to 5 % LSD test.

Table 13. Effect of atrazine rates on percent mortality of 10 California brome accessions (Greenhouse experiment 3)

Accession	Percent mortality 40 days after planting				
	Atrazine application rates ppm				
	0.3	0.6	0.9	1.2	1.5
	..... %				
R1	8.3 a	27.8 bc	44.4 bcd	91.7 ab	91.7 ab
R2	0.0 a	02.8 c	27.8 cd	30.5 de	83.3 ab
S3	8.3 a	58.3 a	41.7 bcd	97.2 a	83.3 ab
R4	8.3 a	25.0 bc	58.3 abc	94.4 a	97.2 ab
S5	8.3 a	36.1 ab	52.7 bc	88.8 ab	100.0 a
R6	0.0 a	13.8 bc	72.2 ab	75.0 ab	88.8 ab
R7	0.0 a	11.1 bc	47.2 bc	66.7 bc	97.2 ab
R8	2.7 a	25.0 bc	44.4 bcd	39.4 cd	75.0 ab
R9	2.8 a	05.6 c	11.1 d	05.6 e	25.0 c
S10	0.0 a	22.2 bc	88.8 a	100.0 a	72.2 b
Mean	3.88	22.8	48.8	68.9	81.3
S.E	1.03	2.9	3.8	3.03	2.9
P	0.18	0.004	0.004	0.0001	0.001

Means followed by same letters across the column are not significantly different according to 5 % LSD test.

Table 14. The resistance ratios of accessions of California brome to atrazine as determined by 50 % mortality, height , and dry weight per plant (Greenhouse experiment 3)

Accession	Rates giving 50% reduction, R-squqre, and ratios of R/S (atrazine rates in ppm)								
	Plant height			Dry weight			% mortality		
	GR-50	R^2	R/S	GR-50	R^2	R/S	LD-50	R^2	R/S
	ppm			ppm			ppm		
R1	0.74	85.5	1.2	0.31	78.9	1.3	0.76	89.7	1.4
R2	0.82	77.0	1.4	0.52	95.0	2.3	1.20	72.0	2.1
S3	0.60	70.5	1.0	0.26	78.0	1.1	0.57	78.6	1.0
R4	0.72	81.5	1.2	0.57	56.0	2.5	0.70	91.0	1.3
S5	0.77	79.0	1.3	0.43	95.0	1.9	0.71	92.0	1.3
R6	0.80	84.0	1.3	0.37	89.8	1.6	0.74	81.2	1.3
R7	0.76	89.0	1.3	0.44	75.0	1.9	0.88	87.0	1.6
R8	0.97	93.0	1.6	0.38	94.0	1.7	1.08	87.0	1.9
R9	5.36	68.3	8.9	0.87	94.0	3.8	6.03	65.0	10.7
S10	0.87	65.0	1.5	0.23	80.0	1.0	0.56	64.0	1.0
All	0.95	94.0		0.45	95.0		0.89	94.0	

Table 15. Effect of atrazine rates on dry weight per plant as percent of the untreated control of 10 California brome accessions (Greenhouse experiment 3)

Accession	Dry weight per plant				
	Atrazine rates in ppm				
	0.3	0.6	0.9	1.2	1.5
	..... % of untreated control				
R1	84.2	31.6	6.8	2.6	4.2
R2	55.5	55.5	27.7	16.7	5.6
S3	90.0	10.0	15.0	3.5	3.5
R4	76.5	115.2	11.8	2.9	2.4
S5	71.4	30.0	30.0	3.0	3.8
R6	73.7	47.4	6.3	4.7	3.2
R7	107.6	46.2	17.6	5.4	4.6
R8	76.5	29.4	17.6	11.8	8.2
R9	75.0	75.0	50.0	33.3	25.0
S10	83.3	16.7	5.0	2.8	7.2
Mean	76.5	41.2	17.6	7.6	5.9

4. 1. 4 . Effect of diuron rates on growth, dry weight per plant and mortality percent of 10 accessions of California brome (Greenhouse experiment 4)

The objective of this experiment was to determine the relative resistance to diuron of the 10 accessions previously characterized for their resistance to atrazine. Response of the accessions to diuron was evaluated by plant height, dry weight per plant, and percent mortality at 40 days after planting (Tables 16-20). We found that the atrazine resistant accession R9 was also extremely tolerant of diuron, showing greater height and dry weight per plant and lower mortality from diuron treatment than all other accessions. On the other hand, the moderately atrazine-resistant accession R4 was the most susceptible to diuron. The atrazine-susceptible accession S3 showed relatively high tolerance to diuron, second only to the most resistant accession R9.

The over all effects of diuron on plant height was not as great as atrazine (Table16.). However, multifactor ANOVA for 10 accessions and 8 rates of diuron on plants height 40 days after planting revealed significant differences among accessions, among rates of diuron, and their interaction. Single factor ANOVA on plant height of the 10 accessions at each diuron rate showed significant differences among accessions at all rates except 4.5 ppm. Maximum tolerable rates for all accessions of California brome were not determined in this experiment because even 50% plant height reductions were not obtained for accessions R1, S3, R6, R7, R8, and R9 at the rates used. This resulted in extrapolated GR-50 values for these accessions greater than the highest rate of diuron tested, 6.3 ppm. Accessions R9, S3, and R1 had resistance ratios of 6.8x, 2.2x, and 2.0x for 50% reduction in plant height from diuron compared to accession R4 (Table 19). Accession R2 was fairly tolerant to diuron, but was not as resistant to diuron as it was to atrazine.

The dry weight per plant for the 10 accessions and 8 rates of diuron showed significant differences among accessions, among diuron rates, and their interaction (Table 17). Accessions R9, S3, R2, and R8 showed greater GR- 50 values as compared to the others with ratios 2.3x, 1.8x, 1.8x, and 1.7x, respectively.

The percent mortality of the 10 accessions 40 days after planting differed significantly among accessions and among rates. However, the interaction of accession with diuron rate was not significant, probably because rates were too low to kill many seedlings in any accession. Differences in percent mortality among accessions was only found at diuron rates of 4.5 ppm and greater. At 4.5 ppm, the highest mortality was for accession R4, while at 5.4 ppm, mortality of both accessions R4 and R2 was significantly higher than the other accessions. At the highest diuron rate nearly all accessions showed similar mortality, with only accession R9 significantly lower than the others, indicating its extreme tolerance to diuron (Table 18).

According to our results, California brome accessions generally showed greater tolerance to diuron than to atrazine. Accession R4, however, was relatively more susceptible to diuron than the other accessions, but even it would only be partially controlled at normal field rates of 2-3 kg ha^{-1}. Accession S3, which was relatively susceptible to atrazine, tolerated high rates of diuron in this experiment. Of the two most atrazine-tolerant accessions, R2 and R9, accession R9 was also extremely resistant to diuron, indicating the development of cross resistance to both herbicides in California brome.

Table 16. Effect of diuron rates on heights of 10 California brome accessions (Greenhouse experiment 4)

Accession	Plant height 40 days after planting								
	Diuron rates ppm								
	0	0.9	1.8	2.7	3.6	4.5	5.4	6.3	Mean
	..... cm								
R1	40.7 b	44.0 abcd	42.2 b	41.8 ab	28.8 bcd	25.5 ab	37.2 a	17.5 bc	34.7
R2	34.8 c	37.3 e	36.0 c	28.0 bcd	25.8 d	15.2 ab	7.0 d	12.5 bc	24.6
S3	43.3 ab	47.8 a	48.3 a	45.2 a	39.5 abc	30.8 a	28 ab	24.0 ab	38.4
R4	46.8 a	47.0 ab	43.0 ab	36.8 abcd	32.3 abcd	11.0 b	14.2 cd	11.2 bc	30.3
S5	38.8 bc	41.0 cde	43.5 ab	25.5 cd	28.2 cd	22.8 ab	30.7 ab	11.2 bc	30.2
R6	41.5 ab	42.0 bcde	44.5 ab	41.5 ab	32.2 abcd	32.0 a	22.2 bc	17.0 bc	34.1
R7	39.5 bc	38.8 de	43.0 ab	23.7 d	26.3 d	16.7 ab	24.0 bc	8.5 c	27.6
R8	41 b	37.5 e	43.2 ab	39.2 abc	43.3 a	29.8 a	29.3 ab	12.5 bc	34.5
R9	41.5 ab	37.8 e	41.3 bc	40.0 a	40.8 ab	30.3 a	33.3 ab	35.2 a	37.5
S10	41.8 ab	45.2 abc	44.5 ab	45.7 a	39.8 abc	17.5 ab	23.8 bc	9.2 c	33.4
Mean	40.9	41.9	42.9	36.7	33.7	23.2	24.9	15.8	
S.E	0.6	0.6	0.6	1.6	1.4	1.9	1.4	1.6	
P	0.01	0.001	0.03	0.01	0.04	0.1	0.001	0.009	

Means followed by same letters within columns are not significantly different according to 5 % LSD test.

Table 17. Effect of diuron rates on dry weight per plant of 10 California brome accessions (Greenhouse experiment 4)

Accession	Dry weight per plant 40 days after planting								
	Diuron rates ppm								
	0	0.9	1.8	2.7	3.6	4.5	5.4	6.3	Mean
	..... g / plant								
R1	0.266 a	0.201 bc	0.163 a	0.140 a	0.066 b	0.045 abc	0.078 a	0.035 ab	0.124
R2	0.149 e	0.180 bcd	0.142 a	0.119 ab	0.076 b	0.057 abc	0.024 cd	0.045 ab	0.099
S3	0.216 abcd	0.231 ab	0.211 a	0.128 a	0.176 a	0.052 abc	0.044 bcd	0.078 a	0.142
R4	0.252 ab	0.265 a	0.171 a	0.090 abc	0.086 b	0.015 abc	0.017 d	0.026 b	0.115
S5	0.184 cde	0.136 d	0.195 a	0.055 bc	0.071 b	0.057 abc	0.05 abcd	0.017 b	0.096
R6	0.227 abc	0.199 bc	0.160 a	0.116 ab	0.062 b	0.078 a	0.038 bcd	0.028 ab	0.113
R7	0.193 bcde	0.212 abc	0.153 a	0.049 c	0.060 b	0.030 c	0.042 bcd	0.019 b	0.095
R8	0.189 bcde	0.175 bcd	0.170 a	0.104 a	0.112 ab	0.057 abc	0.055 abc	0.02 b	0.11
R9	0.157 de	0.165 cd	0.160 a	0.111 abc	0.109 ab	0.075 ab	0.056 ab	0.059 ab	0.112
S10	0.229 abc	0.224 abc	0.168 a	0.122 a	0.086 b	0.034 bc	0.038 bcd	0.033 ab	0.117
Mean	0.206	0.199	0.169	0.103	0.09	0.05	0.044	0.036	
S.E	0.007	0.006	0.009	0.007	0.008	0.005	0.004	0.006	
P	0.005	0.009	0.84	0.01	0.08	0.11	0.03	0.31	

Means followed by same letters within columns are not significantly different according to 5 % LSD test.

Table 18. Effect of diuron rates on percent mortality of 10 California brome accessions (Greenhouse experiment 4)

Accession	Mortality 40 days after planting								
	Diuron rates ppm								
	0.9	1.8	2.7	3.6	4.5	5.4	6.3	Mean	S.E
	..... %								
R1	0.0 b	5.5 a	6.2 bc	41.5 a	36.0 cd	25.0 bc	72.0 a	23.3	2.9
R2	0.0 b	11.2 a	33.3 ab	50.2 a	47.2 abcd	72.2 a	77.7 a	36.5	4.7
S3	0.0 b	5.5 a	12.83 abc	33.5 ab	38.8 bcd	52.8 ab	55.7 a	24.9	2.5
R4	0.0 b	19.5 a	19.5 abc	39.0 a	77.8 a	69.3 a	66.7 a	36.5	3.6
S5	5.7 ab	8.5 a	41.7 ab	30.3 ab	54.2 abcd	39.0 bc	69.5 a	31.1	3.7
R6	11.2 a	11.2 a	25 abc	36.2 a	41.7 bcd	45.8 abc	61.0 a	29	3.12
R7	8.3 ab	5.5 a	47.2 a	47.2 a	68.3 abc	45.8 abc	83.3 a	38.2	3.8
R8	0.0 b	2.8 a	13.8 abc	22.2 ab	44.3 abcd	47.2 ab	75.0 a	25.7	2.6
R9	0.0 b	2.8 a	0.0 c	5.7 b	22.3 d	16.7 c	15.3 b	7.8	2.4
S10	0.0 b	8.5 a	8.8 bc	30.7 ab	58.8 ab	48.5 ab	80.5 a	29.4	2.2
Mean	2.5	8.1	20.8	33.6	48.9	46.2	65.7		
S.E	1.2	1.9	4	3.2	3.9	3.2	3.3		
P	0.3	0.7	0.1	0.1	0.05	0.01	0.001		

Means followed by same letters within columns are not significantly different according to 5 % LSD test.

Table 19. Resistance ratios of accessions of California brome to diuron as determined by 50 % mortality, height, and dry weight per plant reductions (Greenhouse experiment 4)

Accession	Rates giving 50% reduction, R-squqre, and ratios of R/S (diuron rates in ppm)							
	Plant height			Dry weight				% mortality
	GR-50	R^2	R/S	GR-50	R^2	R/S	LD-50	R^2
	ppm			ppm			ppm	
R1	7.50	55.5	2.0x	2.12	93.9	1.1x	5.59	69.0
R2	4.26	80.3	1.2x	3.65	81.3	1.8x	3.83	92.9
S3	8.08	72.4	2.2x	3.53	70.9	1.8x	5.63	90.9
R4	3.70	84.0	1.0x	1.99	86.2	1.0x	3.45	80.5
S5	5.58	62.6	1.5x	2.48	71.2	1.2x	4.61	80.8
R6	6.36	76.0	1.7x	2.6	96.0	1.3x	5.39	95.6
R7	4.33	73.4	1.2x	2.11	81.7	1.1x	3.51	80.4
R8	6.75	52.6	1.8x	3.36	91.6	1.7x	5.06	85.6
R9	25.22	46.5	6.8x	4.56	86.0	2.3x	26.67	68.0
S10	5.13	65.0	1.4x	2.55	93.0	1.3x	4.5	83.3
All	5.96	81.3		2.97	94.6		5.06	92.7

Table 20. Effect of diuron rates on dry weight per plant as percent of the untreated control of 10 accessions of California brome(Greenhouse experiment 4)

Accession	Dry weight per plant						
	Diuron rates in ppm						
	0.9	1.8	2.7	3.6	4.5	5.4	6.3
	% of untreated control						
R1	75.6	61.3	52.6	24.8	16.9	29.3	13.2
R2	120.8	95.3	79.9	51.0	38.3	16.1	30.2
S3	106.9	97.7	59.3	81.5	20.6	20.4	36.1
R4	105.2	67.9	35.7	34.1	5.9	6.7	10.3
S5	73.9	105.9	29.9	38.6	30.9	27.2	9.2
R6	87.7	70.5	51.1	27.3	34.4	16.7	12.3
R7	109.8	79.3	25.4	31.1	15.5	21.8	9.8
R8	92.6	89.9	55.0	59.3	30.2	29.1	10.6
R9	105.1	101.9	70.7	69.4	47.8	35.7	37.6
S10	97.8	73.4	53.3	37.6	14.8	16.6	14.4
Mean	96.6	82.0	50.0	43.7	24.3	21.4	17.5

4. 2. Characterization of atrazine and diuron resistance in California brome with whole plant chlorophyll fluorescence

Two chlorophyll fluorescence measurements were made on ten accessions of California brome in order to further characterize the resistance to atrazine and diuron, and to possibly detect mechanisms involved in the resistance. Chlorophyll fluorescence was first measured 12 hours after exposure to atrazine, diuron, and control treatments but before exposure to light. The second measurement was made on the same treated plants after keeping them in the greenhouse for one week so that the recovery from inhibition of electron transport could be monitored. The chlorophyll fluorescence induction parameters obtained 12 hours after exposure to atrazine and diuron revealed the type of resistance in California brome to be not the D1 protein binding-site mutation reported in species such as *Senecio vulgaris* by Arntzen et al.(1982) because of the evidence of inhibition of electron transfer in all accessions tested. Increased fluorescence (in the presence of PS II inhibitors) is indicative of altered electron transport properties and implies binding of the herbicide to the D1 protein. The second measurement discriminated accessions of California brome into relatively resistant and susceptible types on the basis of their relative recovery from inhibition of electron transport caused by atrazine and diuron.

For all accessions of California brome, T level fluorescence 12 hours after treatment was increased by both atrazine and diuron compared to control plants (Table 21). Accessions R2 and R9 that consistently showed greatest resistance to atrazine and diuron in the greenhouse bioassays showed the greatest increase in their T level fluorescence for both atrazine and diuron. These increases in T reflect blockage of electron transfer chain, implying binding to the 32 kDa D1 protein of photosystem II complexes occurred in all accessions of California brome tested in this experiment.

Increased terminal fluorescence was described by Richard et al., 1983 as a reliable, consistent, and sensitive parameter not subject to a large degree of variability with different plants, leaf position, age, or periods of dark adaptations for assessing photosynthetic electron transport inhibition by atrazine and diuron. According to the authors, an increase in T level would occur if excitation energy was prevented from being utilized for photochemistry. This basic principle of inhibition of electron transport as evidenced by increased levels of T fluorescence in all resistant and susceptible accessions of California brome was used to conclude that the mechanism of resistance to atrazine or diuron by California brome was not the classic mutation of the chloroplastic D1 protein but by rather some other biochemical or biophysical mechanism.

In addition to increased T level fluorescence, reduction in Fm/T ratios for all accessions of California brome 12 hours after atrazine or diuron treatments relative to their respective controls is also a good indicator of inhibition of electron transport. The Fm/T ratios were significantly reduced by atrazine and diuron treatments from their respective controls for all accessions (Table.22.). Decrease in the ratio of Fm/T 12 hours after atrazine or diuron treatment is typical evidence for the blockage of electron transfer chain by binding of those herbicides to 32 kDa D1 protein. Our results agree well with the work of Harris and Camlin (1988) where they reported that resistant as well as susceptible cultivars of winter wheat showed a similar decrease in the ratio of peak fluorescence to terminal fluorescence from their respective controls at one day after chlorotoluron, metoxuron, and metoxuron-simazine applications, but differing ratios at one week after herbicide treatment. According to the authors, this ratio was only useful in discriminating cultivars of wheat into resistant and susceptible groups only at one week after herbicide application rather than sooner because of the time

Table 21. The fluorescence parameter T(terminal fluorescence) of ten accessions of california brome 12 hours and 1 week after atrazine and diuron application

Accession	T (relative units)						% reduction in T by 1 week versus 12 hours after treatment	
	Control		Atrazine		Diuron			
	12 h	1 week	12 h	1 week	12 h	1 week	Atrazine	Diuron
R1	0.3290 ab	0.2252 b	0.8140 bc	0.4066 b	0.8699 b	0.5037 a	50.10	41.30
R2	0.4258 a	0.2915 ab	1.4458 a	0.5775 ab	1.5906 a	0.6081 a	60.10	61.60
S3	0.3265 ab	0.2605 ab	0.5986 bc	0.5578 b	0.6369 b	0.4168 a	6.60	34.50
R4	0.3676 ab	0.2387 ab	0.5688 bc	0.4966 b	0.4997 b	0.4814 a	12.30	4.10
S5	0.3651 ab	0.3058 ab	0.6928 bc	0.5229 b	0.6820 b	0.4786 a	24.50	29.30
R6	0.3432 ab	0.2957 ab	0.6372 bc	0.4774 b	0.5866 b	0.5025 a	25.10	13.60
R7	0.2615 b	0.2752 ab	0.8013 bc	0.5517 b	0.6073 b	0.4828 a	31.20	19.70
R8	0.3202 ab	0.2547 ab	0.7186 bc	0.5369 b	0.5048 b	0.5400 a	26.40	-7.90
R9	0.2970 b	0.3370 a	1.1566 ab	0.8066 a	1.3454 ab	0.5155 a	30.26	61.60
S10	0.2878 b	0.2512 ab	0.3369 c	0.4573 b	0.5047 c	0.5164 a	-35.60	-1.90
Mean	0.3324	0.2736	0.777	0.5391	0.7828	0.5045		
S.E	0.048	0.022	0.048	0.022	0.048	0.022		
P	0.2	0.54	0.04	0.16	0.0002	0.9		

Means followed by same letters within columns are not significantly different according to 5 % LSD test.

Table 22. The ratios of fluorescence parameters Fm/T of ten accessions of california brome 12 hours and 1 week after atrazine and diuron application

Accession	Fm/T					
	Control		Atrazine		Diuron	
	12 h	1 week	12 h	1 week	12 h	1 week
R1	4.93 b	8.06 ab	2.48 ab	3.88 ab	2.23 abc	3.35 a
R2	8.78 ab	9.4 ab	2.02 b	5.74 a	1.96 abc	5.13 a
S3	4.93 b	12.3 a	3.03 ab	3.05 b	2.97 abc	4.14 a
R4	4.25 b	7.38 ab	3.12 ab	3.47 b	3.54 a	3.47 a
S5	5.0 b	5.96 ab	2.66 ab	3.61 b	2.82 abc	4.40 a
R6	5.95 ab	6.36 b	2.91 ab	3.63 b	2.74 abc	3.90 a
R7	8.87 ab	6.94 b	3.53 ab	3.66 b	3.77 a	4.32 a
R8	5.11 ab	7.38 ab	2.84 ab	3.39 b	3.29 ab	3.28 a
R9	9.94 a	7.01 b	3.54 ab	2.84 b	2.03 abc	4.81 a
S10	6.03 ab	6.93 b	4.23 a	3.69 ab	3.05 abc	3.26 a
Mean	6.38	7.79	3.04	3.7	2.84	4.01
S.E	0.53	0.59	0.2	0.22	0.13	0.22
P	0.2	0.4	0.5	0.3	0.08	0.58

Within columns, means followed by same letter are not significantly different according to 5 % LSD test.

requirement for biochemical processes involved in inactivating the herbicides in the resistant cultivars.

Our second fluorescence measurement made on the same plants 1 week after the previous treatments generally showed a decrease in T and an increase in Fm/T ratios for both atrazine and diuron treatments as compared to the 12 hours results (Table 22). This reduction in T was not observable in the susceptible accession 10 in both atrazine and diuron treatments and on suspected resistant accession 8 with diuron treatment. Differences in levels of T measured 1 week after diuron treatment were not significant among accessions, although differences were significant for atrazine treatment, where accessions R2 and R9 still possessed greater T level fluorescence. Reductions in the T level fluorescence from 12 hours to 1 week interacted with accessions. T level fluorescence of suspected resistant accessions R1, R 2, and R9 declined by 50%, 60% and 30% in atrazine treatment (Figure 2) and by 41% , 61%, and 61% in diuron treatment (Figure 3) respectively. This decline is a good indicator of greater recovery from electron transport inhibition caused by the two herbicides for those accessions. Although there were significant reductions in T level fluorescence by 1 week in the resistant types, the levels of T fluorescence were still not as low as the controls. The higher level of T fluorescence in atrazine or diuron treatments than in their respective controls even after 1 week for suspected resistant plants implies that complete inactivation of the herbicides probably takes longer than this time.

The Fm/T ratio at the second fluorescence measurement increased over the first measurement in both atrazine and diuron (Table 22). This change is associated with recovery from inhibition of electron transport, with greater quenching of fluorescence after the peak level of fluorescence. Differences in this ratio after 1 week were not significant among accessions for diuron. The Fm/T ratios for atrazine and diuron

treatments were lower than their respective controls, indicating incomplete inactivation of the herbicides by any of the accessions of California brome in one week's time (Table 22). After 12 hours of the herbicides treatment, the chlorophyll fluorescence induction curves of the resistant and the susceptible accessions were different from their respective untreated control induction curves (Figures 4,6,8). After 1 week of herbicide exposure, the chlorophyll fluorescence induction curves of the resistant accessions were almost similar to their untreated control induction curves (Figures 5,7,9).

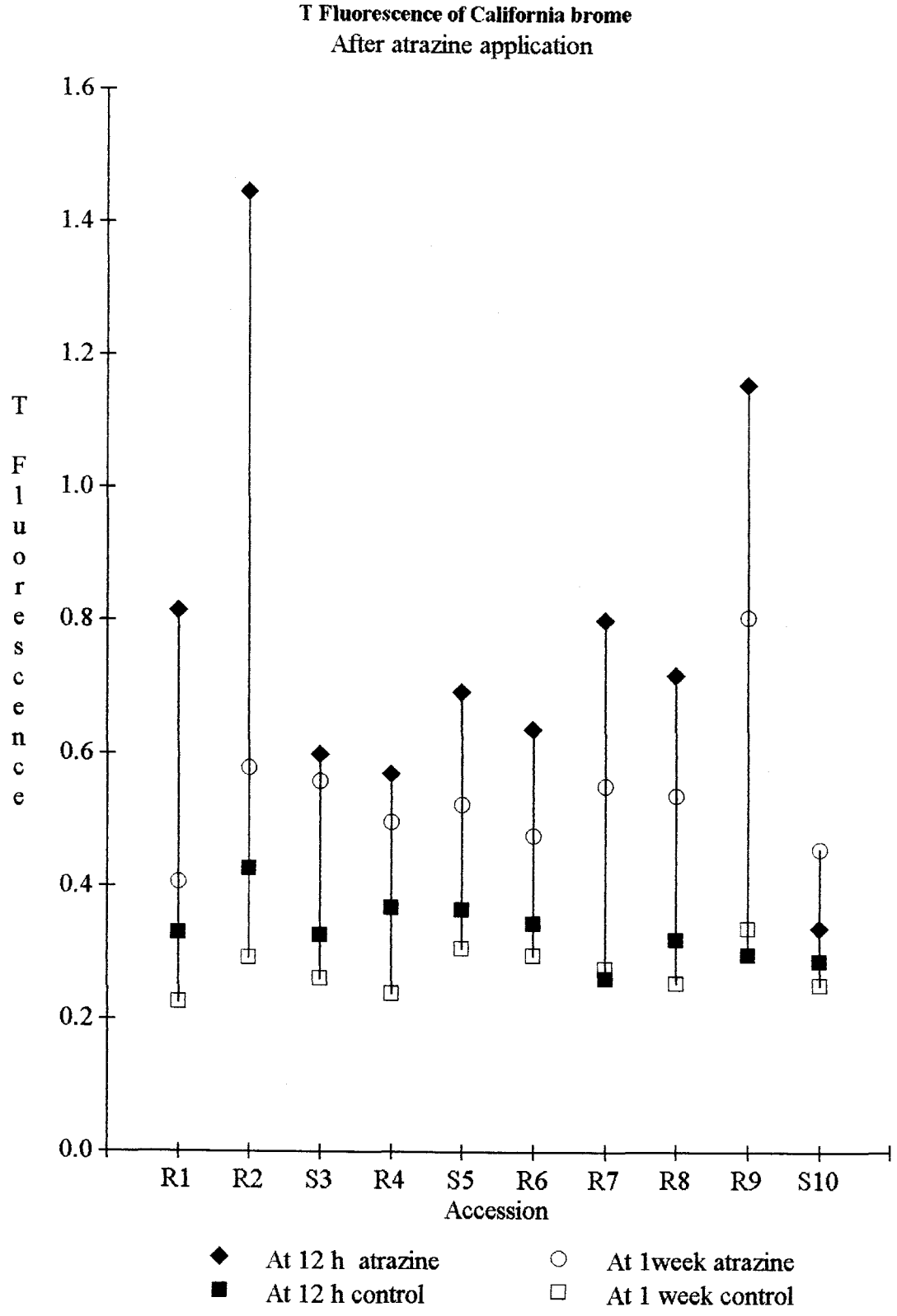

Figure 2. Change in T fluorescence of R and S from atrazine treatment.

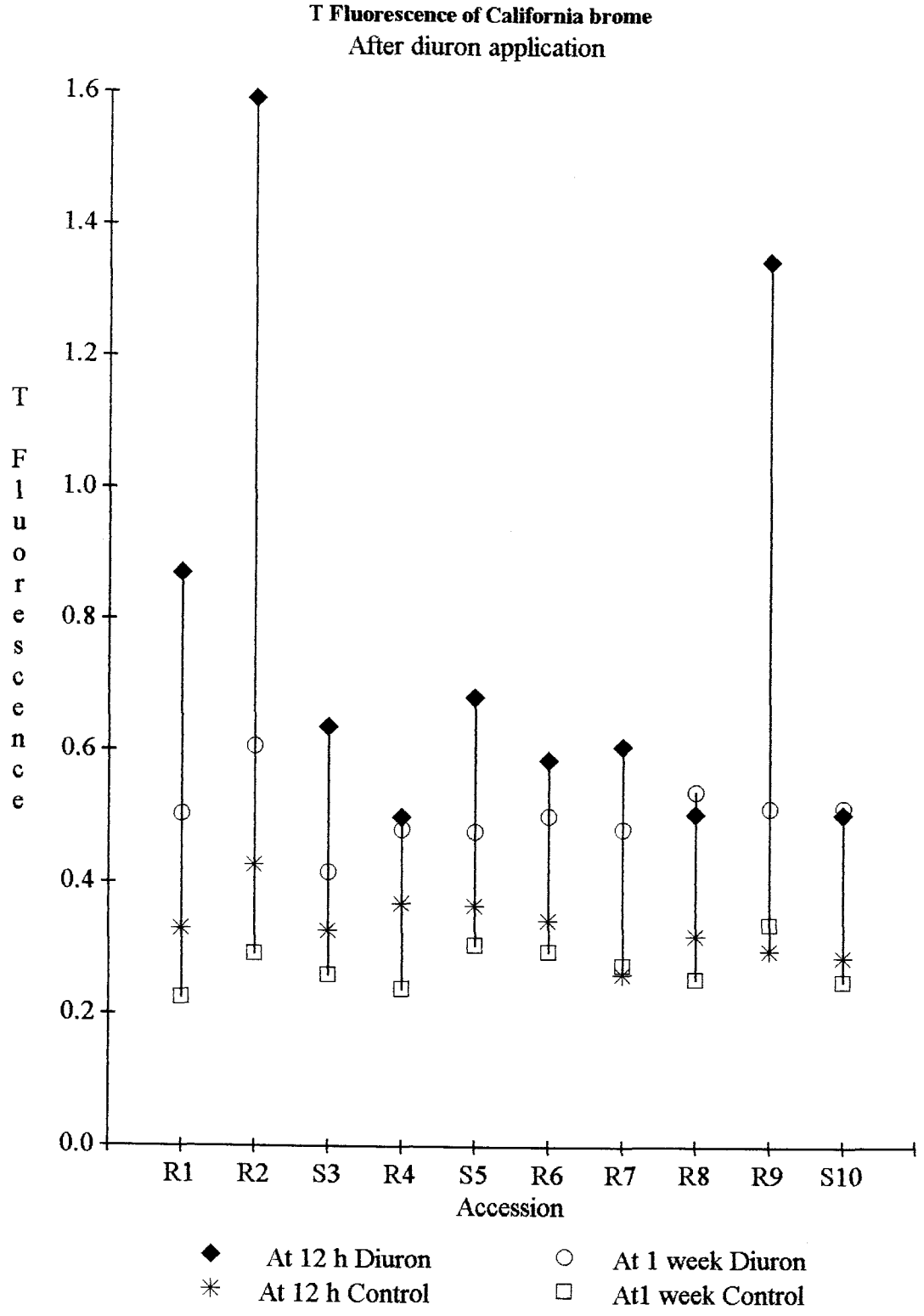

Figure 3. Chgange in T fluorescence of R and S from diuron treatment.

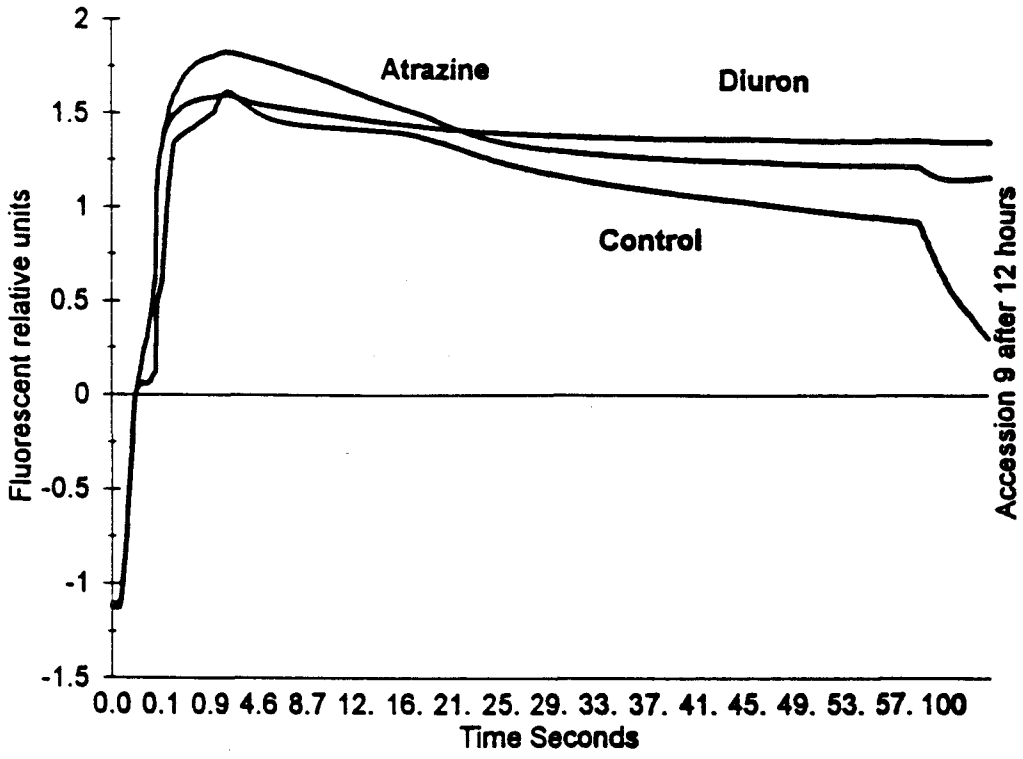

Figure 4. Chlorophyll fluorescence induction curves of the identified resistant accession R9 showing electron transport inhibition by atrazine and diuron 12 hours after treatment.

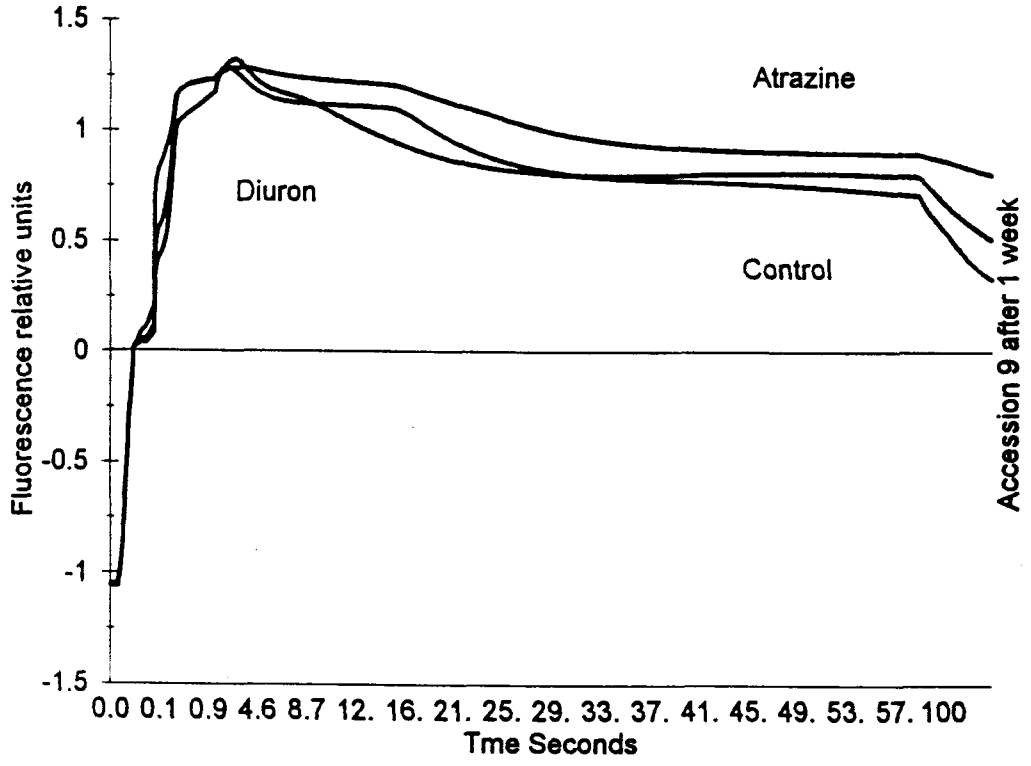

Figure 5. Chlorophyll fluorescence induction curves of the identified resistant accession R9 showing similar inductions for atrazine, diuron and the control 1 week after herbicides treatment.

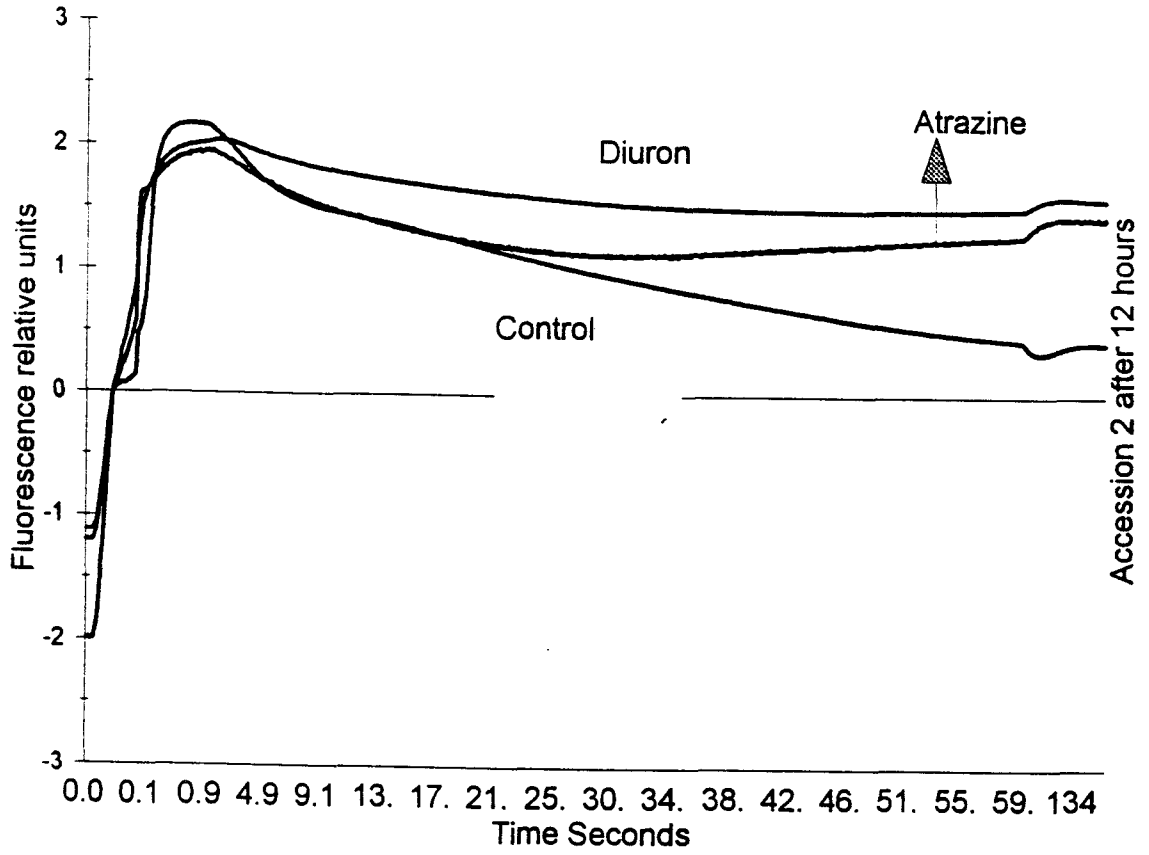

Figure 6. Chlorophyll fluorescence induction curves of the identified resistant accession R2 showing electron transport inhibition by atrazine and diuron12 hoursafter treatment.

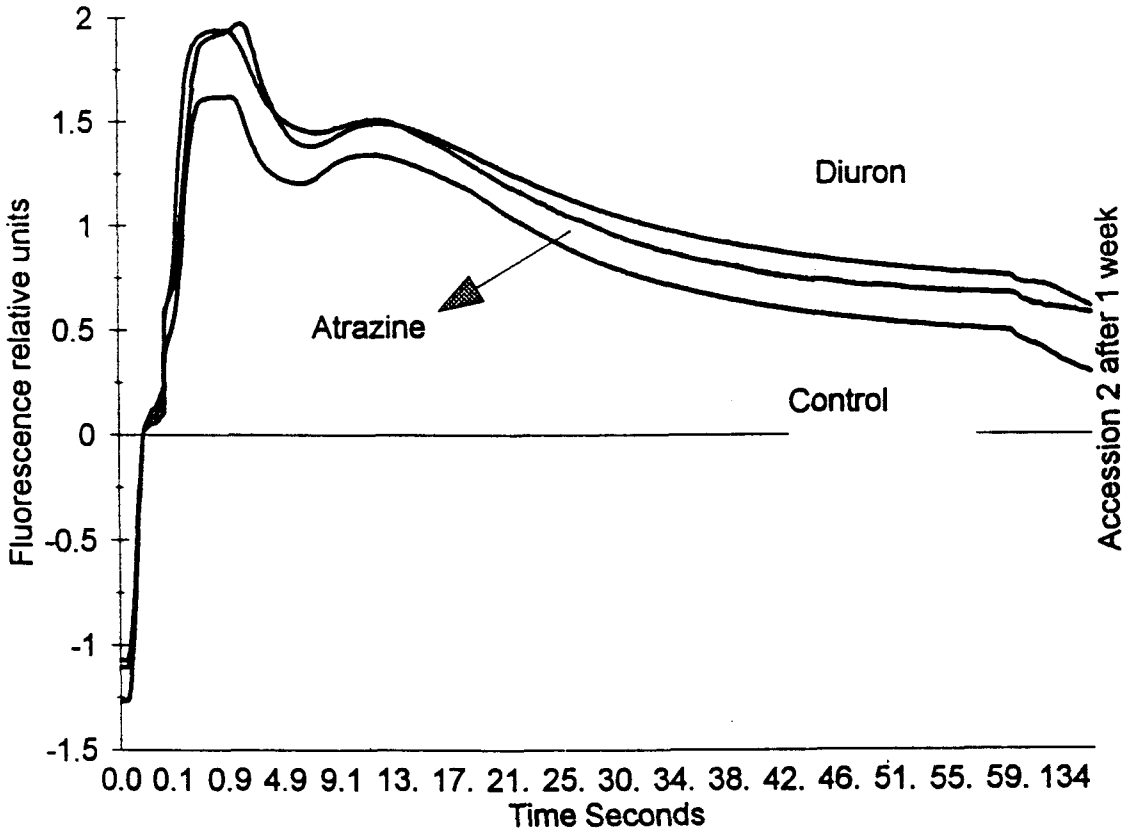

Figure 7. Chlorophyll fluorescence induction curves of the identified resistant accession R2 showing similar inductions of atrazine and diuron treatments with the control 1 week after herbicides treatment.

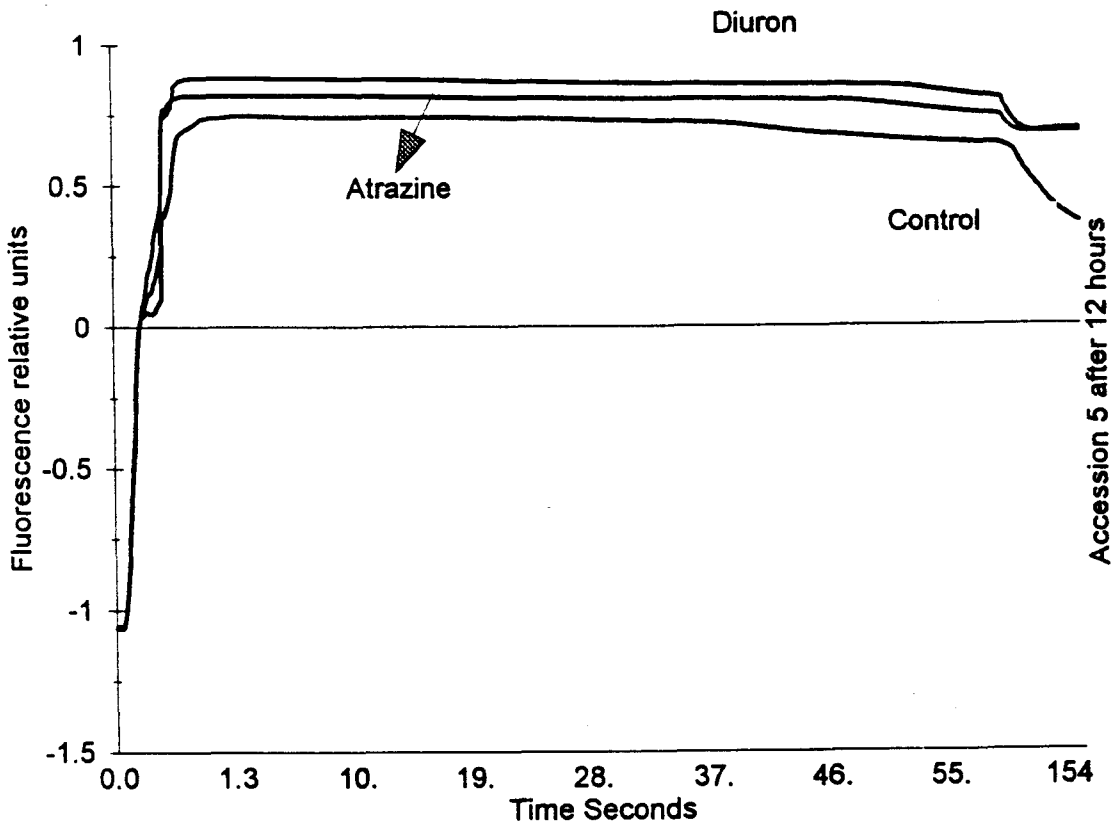

Figure 8. Chlorophyll fluorescence induction curves of the presumed susceptible accession S5 showing electron transport inhibition by atrazine and diuron 12 hours after treatment.

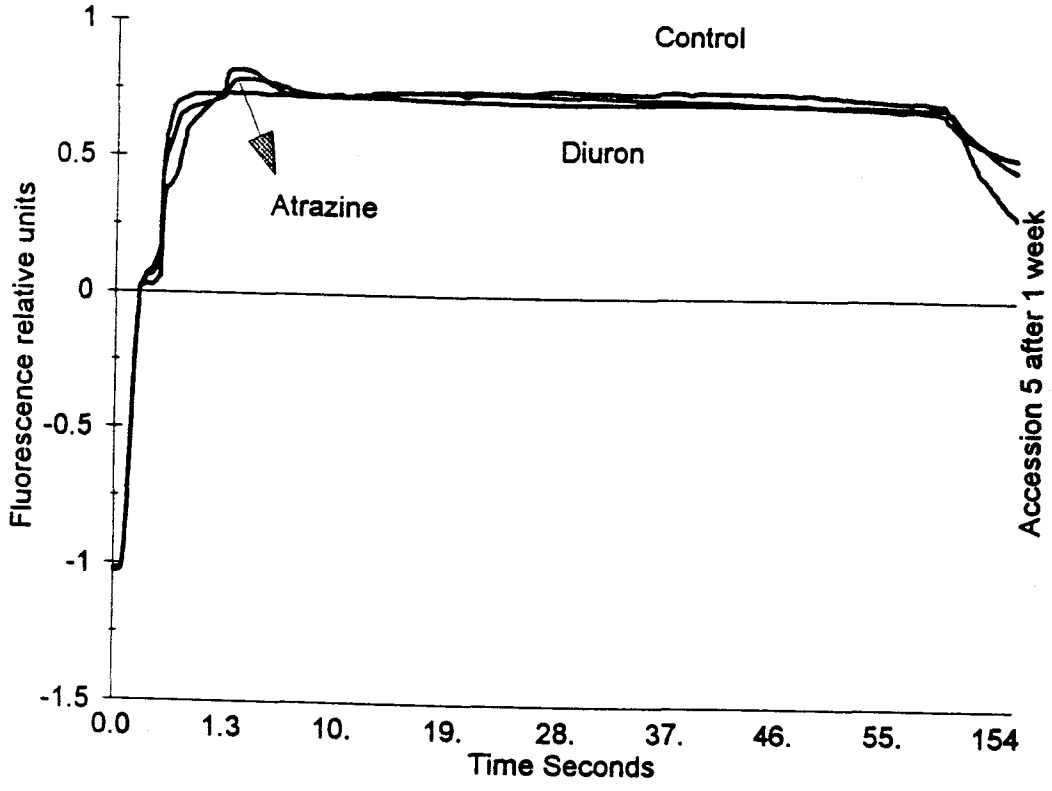

Figure 9. Chlorophyll fluorescence induction curves of the presumed susceptible accession S5 showing verey little recovery from electron transport inhibition by atrazine and diuron 1 week after treatment.

DISCUSSION

The greenhouse bioassays we used have distinguished two accessions of California brome as the most resistant to atrazine as compared to other accessions tested in the greenhouse. One of the two atrazine resistant accessions also showed extreme resistance to diuron, indicating the occurrence of cross resistance in California brome. The discrimination into resistant and susceptible types was based on their resistant ratios of the calculated GR-50 and LD-50 values. The calculated GR-50 for reductions in plant height and in dry weight per plant and the LD-50 for percent mortality of R/S clearly showed differences in the resistance ratios among biotypes and was considered as a resistant factor. Accordingly, the two most resistant accessions showed resistance factors reaching 2 to 9 times for plant height reductions, 2 to 4 times for dry weight per plant reductions and 10 to 20 times for the percent mortality caused by atrazine. A diuron resistant accession also showed higher resistance ratio reaching 2 and 7 times for plant height reduction and for dry weight reductions respectively and 7 times LD-50 value for mortality as compared to the most susceptible type. These resistance factors are far lower than the resistance factors of mutant resistant biotypes of *Chenopodium album* or *Senecio vulgaris* L. where their resistant ratios to atrazine reached 1000 fold (Hirschberg et al., 1984 Fuerest et al., 1986). The resistance ratios we obtained for diuron on the other hand, were greater than the same author"s findings, which were only 0.8 to 1.0. We do not know what resistant ratios we would get from isolated chloroplasts of California brome since we did not make any measurements.

Although there are no reports on multiple resistance by atrazine resistant biotypes to diuron reaching the ratios we observed, our bioassays have clearly indicated its existence in California brome. The occurrence of diuron resistance in the field has

repeatedly been reported by Mueller-Warrant (1987) and Mueller-Warrant and Melbye (1991) where diuron-tolerant bromes proliferated in response to wide use of diuron in perennial ryegrasses grown for seed and in winter wheat. Accordingly, increased field rates of 2.2 kg ha^{-1} have shown control of bromes reaching only 62%. The greenhouse bioassays we used have also indicated the greater tolerance of bromes to diuron than to atrazine. The occurrence of differences in resistance to the same rate of diuron among accessions according to our studies can explain the existence of diuron resistant accessions in California bromes.

The chlorophyll fluorescence of intact plants has been used by many researchers as a probe to detect herbicide resistant biotypes in crops or weeds. Our first chlorophyll fluorescence measurement 12 hours after atrazine and diuron treatment showed increased T fluorescence and reduced Fm/T ratios of all accessions subjected to atrazine and to diuron. Since these parameters are known as typical indicators for electron transport inhibition by herbicides that bind at the 32kDa D1 protein of PS II, we conclude the mechanism of resistance seen in the greenhouse bioassays to be biochemical or biophysical. This first chlorophyll measurement was more important in detecting the mechanism of resistance in California brome to atrazine and diuron than to discriminating accessions into resistant and susceptible types. Because a greater increase in T was observed on the two most resistant accessions and because T of nearly all accessions was increased by both atrazine and diuron compared to their respective untreated controls, we were not able to detect any kind of target-site-based resistance. These kinds of results have also been reported by Harris et al.(1988) where all resistant and susceptible cultivars of wheat had inductions of fluorescence showing signs of susceptibility when measured 1 day after herbicide treatment. But we suspect some other form of alteration that reduces the flow of electrons from QA to QB of the

two most resistant accessions because of the existence of higher native fluorescence or higher T even on untreated controls of these accessions as compared to the other accessions.

Reduced T levels and increased Fm/T were observed on two resistant accessions 1 week after first measurement compared to other accessions. We considered these as good indicators of recovery from electron transport inhibition resulting from the herbicides. Since the levels of this reduced T of the resistant accessions were still higher than their respective untreated controls, we suggest the requirement of more than 1 week time for inactivation of the herbicides by resistant accessions. However, we do not know how long it would take if the herbicides would be applied to the roots in the soil instead of the foliar drench.

Since we used the previously chracterized suspected resistant and susceptible accessions seeds in our experiments, and their progeny generally showed similar reactions to herbicides, we can assume that the resistance is inherited. However, further studies would be required to characterize the inheritance patterns.

As has been indicated earlier, we are also investigating whether the chloroplast DNA is involved in the resistance of California brome to atrazine. By following the protocol used by McNally et al. (1987), we tried to investigate a point mutation in the chloroplast psbA gene at codon 264 resulting in amino acid substitution of serine to glysine by using Mae1 restriction enzyme. This enzyme has a restriction site which is present in susceptible but not in the resistant plants. According to the authors, this Mae1 enzyme with a restriction site CTAG, allows discrimination between susceptible and resistant at the level of 264 codon. Following this method, we tested the ten accessions of California brome but we were unable to see polymorphism at the

specified site indicating the absence of the mutation known to cause chloroplastic resistance in species such as *Senecio vulgaris* (data not presented).

Our studies have determined the existence of atrazine and diuron resistant accessions of California brome and a cross resistant accession for atrazine and diuron. The mechanism of resistance was presumed to be metabolic although the two most resistant accessions possessed unusual fluorescence patterns.

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