AN ABSTRACT OF THE THESIS OF

Modesto T. Madrid, Jr. for the degree of Doctor of Philosophy in Horticulture presented on July 31, 1990.

Title: Onion (*Allium cepa* L.) Cultivar Tolerance to Bromoxynil

Abstract approved: _____
Garvin D. Crabtree

In onions, sensitive cultivars diminish the usefulness of the selective herbicide bromoxynil. Tolerant cultivars can overcome this limitation. A better understanding of the biochemistry, physiology and genetics of bromoxynil tolerance could lead to improved weed control programs in onions.

Onion cultivar response to bromoxynil was studied under greenhouse conditions. Tolerant cultivars were identified using survival and fresh weight of surviving plants as indicator variables. Cultivars 'Utah Yellow Sweet Spanish' (YSS) and 'White Bermuda' were designated as tolerant and sensitive, respectively. Bromoxynil rates eliciting minimum and maximum responses in percent kill and fresh weight of the surviving plants were determined. Cultivar 'Winner' was identified as tolerant from the screening of cultivars with 5.0 kg ae/ha bromoxynil.

Mechanisms that confer cultivar tolerance to bromoxynil in onions were studied by examining the effects of ethofumesate, piperonyl butoxide and hydrolysis of cyano ^{14}C bromoxynil in Utah YSS and White Bermuda. Ethofumesate did not enhance bromoxynil phytotoxicity to Utah YSS. Piperonyl butoxide did not modify bromoxynil phytotoxicity to either Utah YSS or White Bermuda. Hydrolysis of cyano ^{14}C bromoxynil in Utah YSS and White Bermuda yielded no significant differences in the amount of unextractable radioactivity from the plant residues or in the amount of 3,5-dibromo-4-hydroxybenzonitrile formed. Hydrolysis of the cyano moiety of bromoxynil occurs in both Utah YSS and White Bermuda. It is not the mechanism that confers tolerance to bromoxynil in the onion cultivar Utah YSS.

Onion (*Allium cepa* L.) Cultivar Tolerance to Bromoxynil

by

Modesto T. Madrid, Jr.

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed July 31, 1990

Commencement June 1991

APPROVED:

Professor of Horticulture in charge of major

Chairman of Department of Horticulture

Dean of Graduate School

Date thesis is presented July 31, 1990

Typed by Modesto T. Madrid, Jr.

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INTRODUCTION

The improvement of plants for the benefit of man came with the dawn of plant domestication. Starting from the exclusion of undesirable plants from a population, the breeding of desirable plants followed. Recently, cloning, transfer and expression of desirable genes have been achieved with enormous gains in the understanding of plant biology. Genes that enable plants to withstand phytotoxic rates of herbicides have been isolated and expressed in a number of crops, (Gasser and Fraley, 1989) inspite of concerns about possible (1) harmful effects of a foreign gene, (2) gene transfer to non-target organisms and (3) increased herbicide residue levels on crops and the environment.

To reap the full benefits from the development of herbicide-tolerant crop cultivars without unreasonable adverse effects, these questions need to be addressed: (1) With which herbicide will tolerance be feasible and desirable? (2) With what crops will tolerance be most useful? (3) What weed species are to be controlled?

The implications of these questions are apparent with the use of bromoxynil for weed control in onions. They focus on the growing importance of herbicide tolerant crops as a component in the development of integrated weed management systems.

Onions (*Allium cepa* L.) are an important vegetable in Oregon. Production of 312,000 tons of dry bulbs valued at 47 million dollars from 13,000 acres (Miles, 1990) reflects the yield levels, dollar per acre and the historical acreage planted to the crop. Control of weeds is an essential part of onion production, since crop yields are drastically reduced by the presence of weeds. Slow growing and poor competitors, onions easily get smothered by weeds. Onion growers plant, raise and harvest the crop in essentially weed-free fields. During fallow periods, weeds are not allowed to grow in fields where onions are to be planted. Massive off-season weed growth could result in the stockpiling of allelochemicals which can inhibit onions (Menges, 1987; Connick, et al 1989). Weeds are not allowed to mature seed so as to break their reproductive cycle. The control of weeds necessarily involves the integration of techniques including crop rotation, timely tillage and seeding, proper spacing, cultivation, handweeding and herbicides to optimize their effectiveness and control weeds ecologically and economically. Two major cultivar types are planted in Oregon. Sweet Spanish types are grown in eastern Oregon and storage type onions are grown in western Oregon. Herbicides registered for weed control in onions are grouped according to the broad edaphic and environmental features of the major growing areas.

In the mineral soils of eastern Oregon, bensulide, DCPA, glyphosate, oxyfluorfen, monocarbamide dihydrogen sulfate, bromoxynil, trifluralin and fluazifop are registered. The soil-applied herbicides bensulide, DCPA, and trifluralin are largely ineffective in the organic soils of western Oregon. This

results in fewer herbicide options for the western Oregon grower and leaves them with glyphosate, oxyfluorfen, monocarbamide dihydrogen sulfate and fluazifop. Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) is registered for use with Sweet Spanish types but not for storage type cultivars because crop tolerance is unacceptable. Occasional crop injury may be encountered in the Sweet Spanish types but the herbicide provides excellent control of weed species belonging to the Cruciferae, Solanaceae, Compositae, Convolvulaceae, Polygonaceae and Boraginaceae families.

It was of interest to overcome the limitations imposed by sensitive cultivars on the usefulness of bromoxynil for weed control in onions. The main objectives of this research were (1) to develop a screening methodology to identify bromoxynil-tolerant onion cultivars, and (2) to study the mechanisms that may confer tolerance to bromoxynil in onion cultivars.

REVIEW OF LITERATURE

As food, onions are relatively poor in nutritional value. Medicinal properties of the plant are recorded throughout history and evidence to support assertions on the therapeutic value of onions are reviewed by Augusti (1990). Attraction to onions and their allies arise from the delightful flavor and odor of the sulfur compounds they generate. Important products of cysteine metabolism in onions are S-alk(en)ylcysteine sulfoxides (alliins) which are essentially inactive and compartmentalized in the plant cell. When the cells are bruised or crushed, they come in contact with enzymes, specifically S-alk(en)ylcysteine sulfoxide lyase (alliinase) which brings about the reactions shown in Figure 2.1.

The products of interest include mono, di and trisulfides which are the major flavor components, thiopropanal-s-oxide, the lachrymatory factor, pyruvic acid and ammonia. Qualitative and quantitative differences in the amounts of disulfides have been used as bases for classifying odors in *Allium spp.* as well as in grading onions as mild or pungent. Saghir, et al, (1966) attributed the onion-like odor to the predominance of propenyl disulfides, the garlic-like odor to allyl disulfides, and the cabbage-like odor to methyl disulfides. Pungency, on the other hand, is based on the qualitative differences in the amounts of dipropyl disulfides, methyl-n-propyl disulfides and n-propylallyl disulfides (Bernhard, 1968).

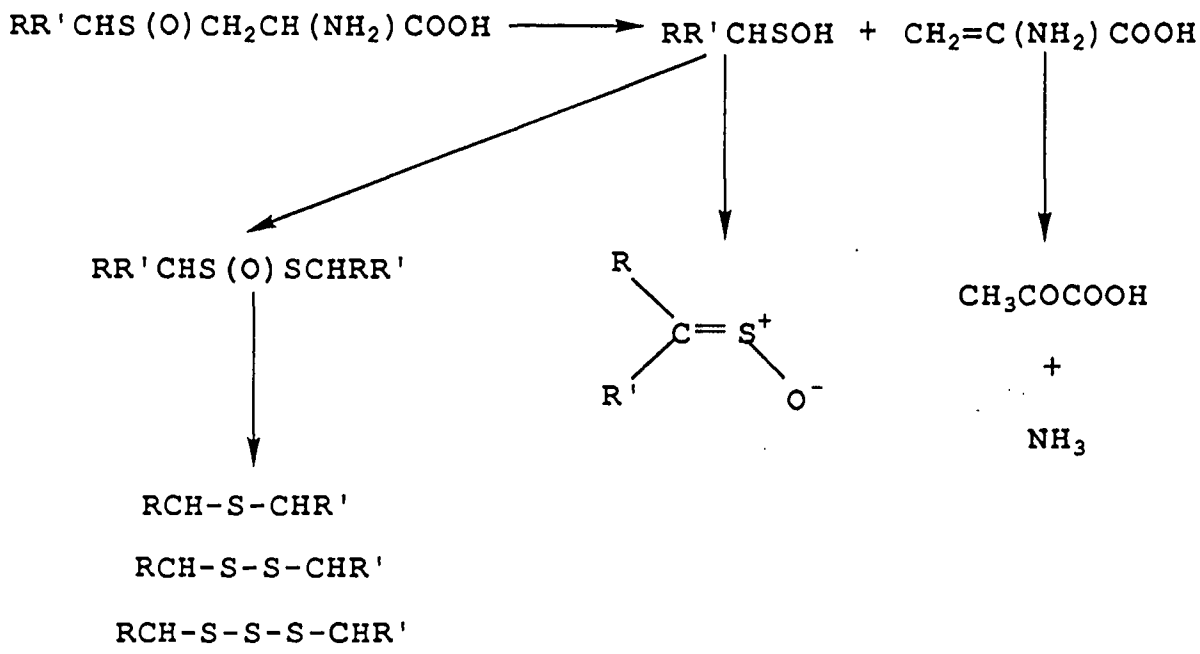

Figure 2.1 S-alk(en)yl cysteine sulfoxides and alliinase.

The biological activity of onions to other organisms can be profound. Crude onion juice inhibits the growth of *Escherichia coli*, *Pseudomonas pseudocyaneus*, *Salmonella typhi*, and *Bacillus subtilis* (Abdou, et al, 1972). The glutamyl peptides of s-alk(en)ylcysteine sulfoxides are known to possess antifungal, anti-viral, tumor-inhibiting activity. Di-n-propyl disulfide is an attractant to the onion maggot (Matsumoto, 1970).

The formation of thiocyanates in onions from unknown glucosides as suggested by Robinson (1980) has not been fully studied. In the Cruciferae family, cysteine metabolism leads to the formation of glucosinolates which are acted upon by the enzyme thioglucosidase. Thiocyanates, isothiocyanates, cyano-epithioalkanes, nitriles, amines and oxazolidine thiones are formed from the action of thioglucosidase on glucosinolates as outlined in Figure 2.2.

The number of natural products with diverse chemical properties need not be alarming. So far, only the oxazolidine-thiones (OZT), specifically goitrin (5-vinyl OZT) has attracted serious attention as being aggravating to people with thyroid condition, otherwise peoples right to pungency, another of life's finest pleasures need not be curtailed. The presence of chemically active constituents which are biosynthetically traceable to cysteine metabolism have not been ignored. Reduction in the amount of glucosinolates are a major achievement in the crop improvement efforts that made rapeseed oil prominent in the human

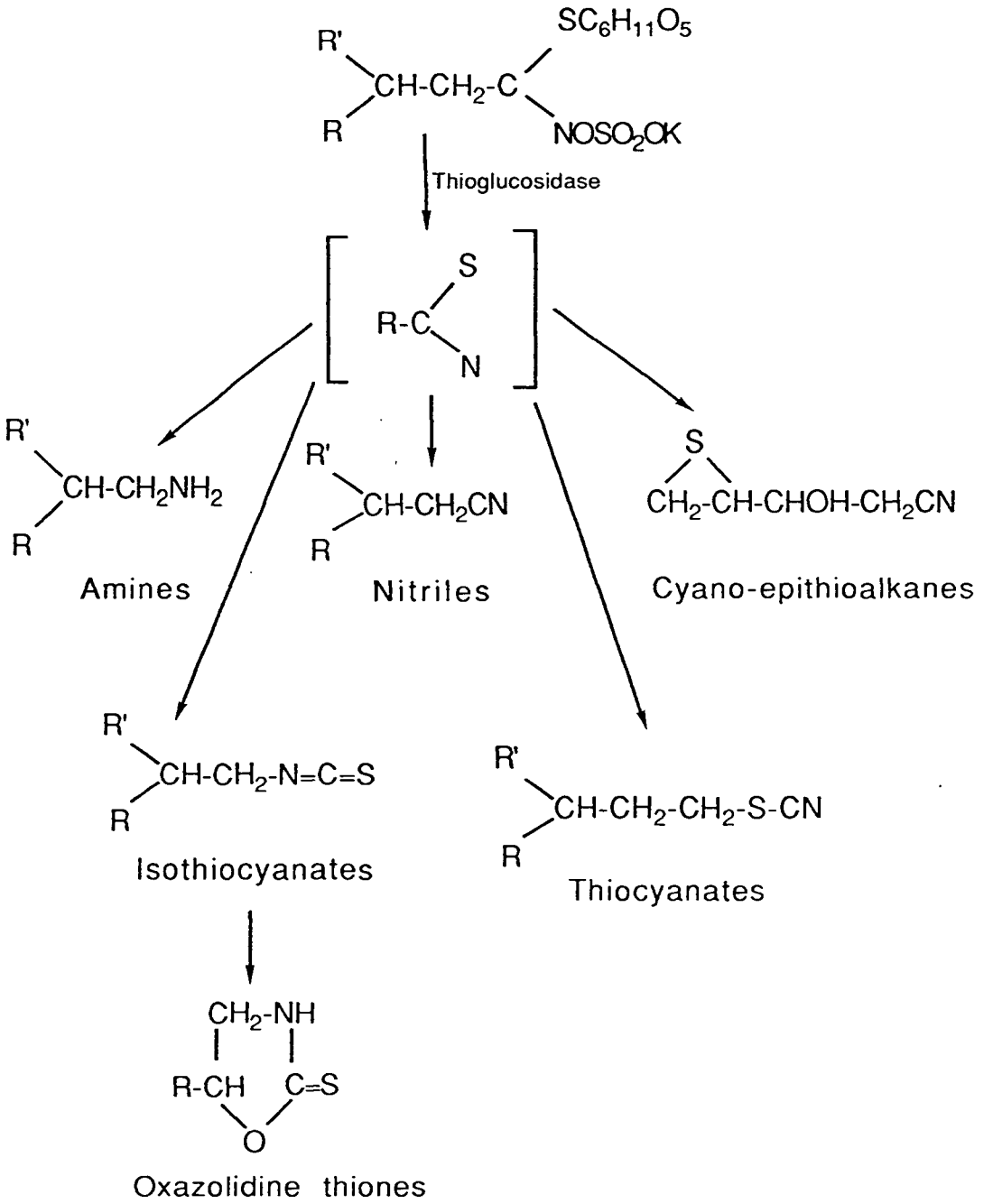

Figure 2.2 Glucosinolate decomposition by thioglucosidase.

diet. The presence of cyanates and nitriles in Chinese cabbage, cabbage and cole slaw is under scrutiny (West, et al, 1977; Daxenbichler, et al, 1977, 1979).

The chemical nature of disease resistance in plants was first reported in onions (Stahman and Jones, 1955). Resistance to smudge, *Colletotrichum circinans* was traced to two water soluble phenols, protocatechuic acid and catechol, which were associated with bulb scale color. Colored bulbs were resistant; while white bulbs were susceptible.

Resistance to the black mold, *Aspergillus nigricans* goes the opposite direction with the white bulbs being resistant and the colored bulbs susceptible (Stevenson and Walker, 1953). Resistance to thrips is conditioned by the glossiness of onion leaves. Onion cultivars that produce waxy rods and toothed platelets are not preferred by thrips (Molenaar, 1984). In the white rot disease of onion caused by *Sclerotium cepivorum*, cultivars that do not release S-alk(en)ylcysteine sulfoxides through their roots are tolerant. Root exudates from sensitive cultivars are acted upon by soil microbes producing diallyl disulfides which in turn stimulate the germination of *Sclerotium cepivorum* (Coley-Smith and Parfitt, 1986).

Bromoxynil is a herbicide for the control of certain broadleaf weeds in small grains, corn, sorghum, flax, garlic, onions, mint, turf and non-crop areas.

Synthesized in 1896 (Auwers and Ries) starting with p-hydroxybenzaldehyde, the final product came about by oximation, dehydration and bromination. More recent synthetic processes involve the single step bromination of p-cyanophenol or the reaction of 3,5-dibromo-4-hydroxyben-zaldehyde with hydroxylamine. Discovery of the herbicidal properties of bromoxynil was reported by three research groups working independently. Wain, (1963) found bromoxynil by replacing the nitro group with a nitrile in the herbicide dinitrocresol. Carpenter and Heywood (1963) synthesized nitrile compounds based on the known activity of indole-3-acetonitrile. Herbicidal activity was reported from p-cyanophenols with I, Br or Cl at the 2 and 6 positions. Hart, Bishop and Cook (1964) discovered the activity of 4-hydroxybenzonitrile in conjunction with compounds related to halogen substituted thyroxines.

Bromoxynil is a restricted herbicide and the butyl ester formulation has been withdrawn after it has been found to cause birth defects in rats. Toxicology of bromoxynil (WSSA, 1989) suggests no unreasonable adverse effects to the environment, non-target organisms and consumers of treated produce when used as indicated on the label. Clinical evidence of problems with bromoxynil exposure was reported in France (Conso, et al, 1977) where plant workers experienced symptoms ranging from fever, headache, dizziness, vomiting, asthenia, weight loss and or myalgia of the legs. Prompt recovery from these symptoms occurred when the workers were separated from exposure.

The mechanism of action of bromoxynil in plants has been traced to its ability to elicit two types of cellular action. Moreland (1980) described bromoxynil as an inhibitory uncoupler, that is, it inhibits photosynthetic electron transport at the photosystem II site and uncouples oxidative phosphorylation and respiration. It is unique in its ability to affect the processes of photosynthesis and respiration, both of which are vital cellular processes.

Sanders, et al, (1984) observed the changes in *Matricaria inodora* following treatment with bromoxynil and concluded that the greater susceptibility of the species cannot be explained by the in-vitro data on photosynthetic interference. Further studies with *M. inodora* and *Viola arvensis* suggested that the differential responses between the species were not due to differences in chloroplast binding, electron transport inhibition and uncoupling activity.

Metabolism of bromoxynil and its role in higher plants was investigated earlier by Schafer and Chilcote (1970) who demonstrated that $^{14}CO_2$ evolution from cyano labeled bromoxynil by tolerant wheat plants exceeded that of the sensitive coast fiddleneck. Buckland, et al, (1973) demonstrated in greater detail, the complex metabolic pathways of bromoxynil transformation in tolerant wheat plants. Starting with hydrolysis of the octanoic ester to free bromoxynil, three consecutive and/or concurrent steps follow, which includes (a) hydrolysis of the cyano group to an amide and carboxylic acid followed by decarboxylation, (b)

replacement of one or both bromines by hydroxy groups and their hydrolytic products, and (3) replacement of one or both bromines by hydrogen and their hydrolytic products. Sanders and Pallet (1987) working on the metabolism of bromoxynil in *M. inodora* and *V. arvensis* demonstrated that bromoxynil remained essentially unchanged in the susceptible species. Breakdown of bromoxynil in the resistant *V. arvensis* was documented.

Early studies on the possible effects of bromoxynil on microbial populations found the herbicide inhibitory to fungi and bacteria in vitro (Smith and Fletcher, 1964). Inhibited microorganisms, however,resumed growth after the herbicide was broken down. Under field conditions, Smith (1971) found the degradation of bromoxynil to be fairly rapid. At the high application rate of 14 kg ae/ha, over 50% of the applied bromoxynil was degraded in 2 weeks. In-vitro degradation by *Flexibacterium* BR4 produced 3,5-dibromo-4-hydroxybenzamide and 3,5-dibromo-4-hydroxybenzoic acid as degradation products (Smith and Cullimore, 1974). Cullimore and Kuhout (1974) demonstrated in-vitro as well as in-vivo degradation of bromoxynil by *Hormidium barlowi*. Hsu and Camper (1975) reported slow degradation of bromoxynil in 4 bacterial and 2 fungal species. Harper (1977) isolated *Nocardia sp.* of the rhodochrous group utilizing benzonitriles as sole carbon and nitrogen sources.

Stalker, Kenny and McBride (1986) reported the metabolism of bromoxynil by *Klebsiella pneumonia* subsp. *ozaenae* with nitrilase activity specific for the herbicide. Stalker and McBride (1987) cloned the bromoxynil degrading gene and expressed it in *Escherichia coli.* The gene was later expressed in transgenic tobacco (Stalker, et al, 1988).

Kristufek, et al (1987) demonstrated that *Streptomyces felleus* took up 95% of the bromoxynil in the culture media, degraded 50% and deposited 45% in the cell. Neuzil, et al, (1988) showed enzymic degradation of bromoxynil in cell-free extracts of *S. felleus* by cleavage of the aromatic ring catalyzed by an Fe dependent dioxygenase.

Bromoxynil phytotoxicity to onions is conditioned by the stage of growth of the crop, rate of herbicide application and the environmental conditions before, during and after application time. At the very early stages, onions are generally sensitive and gradually become tolerant with age. Low temperatures, low light intensity and high relative humidity at application time and up to 3 days after enhance bromoxynil phytotoxicity to onions (Menges and Tamez, 1981). Agamalian (1967) reported injury to Southport White Globe with 0.56 kg/ha at the flag leaf stage. Greater tolerance was observed with the same rate applied after the one and one half leaf stage. Split applications of 0.28 kg/ha plus 0.28 kg/ha 14 days apart did not cause injury to Colorado #6 Yellow Sweet Spanish (Dunster, 1968).

At the 1-2 leaf stage, 0.28 to 0.74 kg/ha bromoxynil did not cause injury to the cultivars Australian Brown or White Globe. At the flag and one half leaf stage, 0.28 to 0.56 kg/ha was phytotoxic to Oregon Danvers (Crabtree, et al, 1981). Stanger (1982) working with breeding lines used as parents for hybrid onion seed production observed severe injury to certain lines while other lines appeared unaffected.

DEVELOPMENT OF TOLERANCE SCREENING METHODOLOGY

ABSTRACT

Greenhouse screening studies were undertaken to detect and assess cultivar tolerance to bromoxynil in onions. At the 2 leaf stage, seedlings were sprayed with bromoxynil at rates ranging from 0.5 to 4.0 kg ae/ha. Response to the treatments was quantified in terms of survival and fresh weight of the surviving plants two weeks after spraying. Two cultivars, 'Utah Yellow Sweet Spanish' and 'White Bermuda' showed markedly contrasting responses and were designated as tolerant and sensitive, respectively.

Dose-response relationships in Utah Yellow Sweet Spanish and White Bermuda were different in terms of the rates that elicit the minimum and the maximum responses. At 5.0 kg ae/ha, survival ranging from 72 to 90% and fresh weights of the surviving plants ranging from 65 to 98% of the untreated control were observed in Utah YSS. Cultivars 'Winner' and 'Utah YSS' withstood up to 10 times the normal field rates of application under greenhouse conditions.

INTRODUCTION

Differential response between crops and weeds to a herbicide is the basis for selective weed control. Differences in tolerance within weed species can hasten the evolution of a tolerant population within a weedy species, or in herbicide-sensitive crops, tolerant cultivars may provide adequate margins of safety.

Differential tolerance to bromoxynil based on assessments of phytotoxicity is detected by assigning scores on a graded scale of arbitrary values (Wax, et al, 1974; Pantone, et al, 1988). By visually comparing the effect of a treatment with an untreated control, reasonably objective phytotoxicity ratings can be obtained. Since visual evaluations are non-destructive, periodic ratings provide a means of monitoring the rate of recovery from herbicide injury. Visual assessments may follow the Weber-Fechner law which states that the response of the eye to a stimulus is a linear function of the log of the stimulus (Lindow and Andersen, 1986). To what extent this is true in using visual ratings to assess herbicide phytotoxicity is unknown. With the difficulties inherent in visual comparisons it is desirable that alternative methods of assessing herbicide tolerance be developed. The method of assessment, should be able to identify cultivars that can survive the treatment and assess recovery from herbicide injury. A combination of quantal and quantitative assessments are employed in the succeeding experiments to illustrate the potential usefulness of the method in herbicide tolerance detection in onions.

REVIEW OF LITERATURE

Differential tolerance to bromoxynil has been reported in both crops and weeds. Wax, et al, (1974) found many soybean cultivars developed in the U. S. and Canada tolerant while Japanese cultivars are sensitive to 0.3 kg/ha. Stanger (1982) observed certain breeding lines of onions to be tolerant of bromoxynil. Crabtree, et al, (1981) found Oregon Danvers, the major cultivar grown in western Oregon sensitive to 0.56 kg/ha sprayed at the cotyledonary and 1 1/2 leaf stage.

In weeds, Price, et al, (1983) found genetic variability in response to 0.13 kg/ha in *Clarkia Williamsonii*. The genes for tolerance to bromoxynil in the species were found to occur at frequencies higher than expected from mutation rates. Pantone, et al, (1988) reported differential tolerance to bromoxynil at 0.12 to 0.50 kg/ha in *Amsinckia intermedia* and *Amsinckia gloriosa*.

The major objective of this study was to develop a screening methodology to identify onion cultivars tolerant of bromoxynil. A step-wise approach to the development of the methodology included (1) assessment of the natural variability in survival and fresh weight of the surviving cultivars, (2) examination of the dose-response relationships in onion cultivars that exhibited markedly contrasting responses, and (3) screening onion cultivars with a discriminating rate of application and using previously identified tolerant and sensitive check cultivars.

MATERIALS AND METHODS

Assessment of variation in sensitivity to bromoxynil between onion cultivars

Seeds of onion cultivars were planted separately in rows spaced 3 cm apart in trays measuring 33 X 27 X 12 cm filled with greenhouse mix. Seeds were covered 1 cm deep and the trays were sub-irrigated overnight. The trays were placed on a greenhouse bench under artificially supplemented 14 hr photoperiod at 26/18°C day/night temperatures and watered with half strength Hoagland solution twice a week starting 14 days after seeding. When onions were at the 2 leaf stage, the trays were sprayed with bromoxynil at 4, 2, 1, 0.5 and 0 kg acid equivalent/ha using a chain-driven greenhouse sprayer delivering 365 liters/ha. Treated trays were arranged in a randomized complete block design.

Factorial experiments involving the five herbicide rates and varying numbers of cultivars were undertaken. Experiment 1 which included only two cultivars, namely Sweet Spanish and White Bermuda was replicated three times. Experiment 2 consisting of three Yellow Sweet Spanish (YSS) cultivars namely Currier, Utah and Valencia; three Globes (G) namely Downing Yellow, Early Yellow and Golden; Stockton Early Red and Walla Walla Sweet giving a total of eight cultivars was replicated four times. In experiment 3, Utah YSS, Early Yellow Globe, Walla Walla, Stockton Early Red, White Bermuda and two strains of Oregon Danvers namely, Beirley and Leedy were included in four replications. Assessment of the effects of the treatments were made 2 weeks after spraying.

The total number of treated plants, number of survivors, and fresh weights of the surviving plants were recorded. Plants with >1 cm green tissue above the ground surface at the time of assessment were considered to have survived. Recovery from herbicide injury as reflected by fresh weight of the surviving plants expressed as percent of the untreated control and arcsin transformed survival ratio (survivors/treated) were regressed on bromoxynil rates of application.

Dose-response relationships in tolerant and sensitive cultivars

To characterize the contrasting responses between Utah YSS and White Bermuda, seeds of the 2 cultivars were separately planted in trays and established as described earlier. Varying rates of bromoxynil up to 32 kg/ha were applied. Two factorial experiments with herbicide rates X 2 cultivars replicated 5 and 4 times respectively were undertaken. The highest rate used in experiment 1 was 8 kg/ha while in experiment 2, the rate was raised to 32 kg/ha. The total number of plants treated, number of plants killed and fresh weight of the surviving plants were recorded. Plants with <1 cm green tissue above the ground surface 2 weeks after treatment were considered to have been killed.

Arcsin transformed kill ratio (killed/treated) and fresh weight of surviving plants expressed as percent of the untreated control, were regressed on bromoxynil rates.

Screening of onion cultivars for tolerance to bromoxynil

Onion cultivars were screened for tolerance to bromoxynil at a discriminating rate of 5 kg ae/ha which is equivalent to 10 times the normal field rates of application. In each tray, seven or eight cultivars were seeded separately including Utah YSS and White Bermuda. Two trays were used to accomodate the number of entries included in a treatment. Experimental design was a randomized complete block replicated 4 times. Untreated controls were provided. Fresh weight of the surviving plants expressed as percent of the untreated control and angular transformed percent survival were subjected to analysis of variance. Means were separated by Tukey's test.

RESULTS AND DISCUSSION

Assessment of variation in sensitivity to bromoxynil between onion cultivars

The increasing rate of bromoxynil brought a concomitant decline in the survival ratio. At the highest rate of 4 kg/ha (Figure 3.1) Sweet Spanish had 0.2 survival ratio while none survived in White Bermuda. Bromoxynil accounted for 69% of the variability in survival ratio but survival of the 2 cultivars was not significantly different.

Fresh weight of the surviving plants expressed as percent of the untreated controls are shown in Figure 3.2. Increasing bromoxynil rates brought decreasing fresh weights and the difference between the cultivars was statistically significant. Bromoxynil accounted for 73% of the variation in fresh weight of the surviving cultivars.

The findings strongly suggest that survival and fresh weight of the surviving plants are complementary response variables that should reinforce assessments of herbicide phytotoxicity. Two cultivars that are equally sensitive to bromoxynil may not show differences in survival but still have significant differences in the ability to recover from herbicide injury as shown by the differences in fresh weights of the surviving plants.

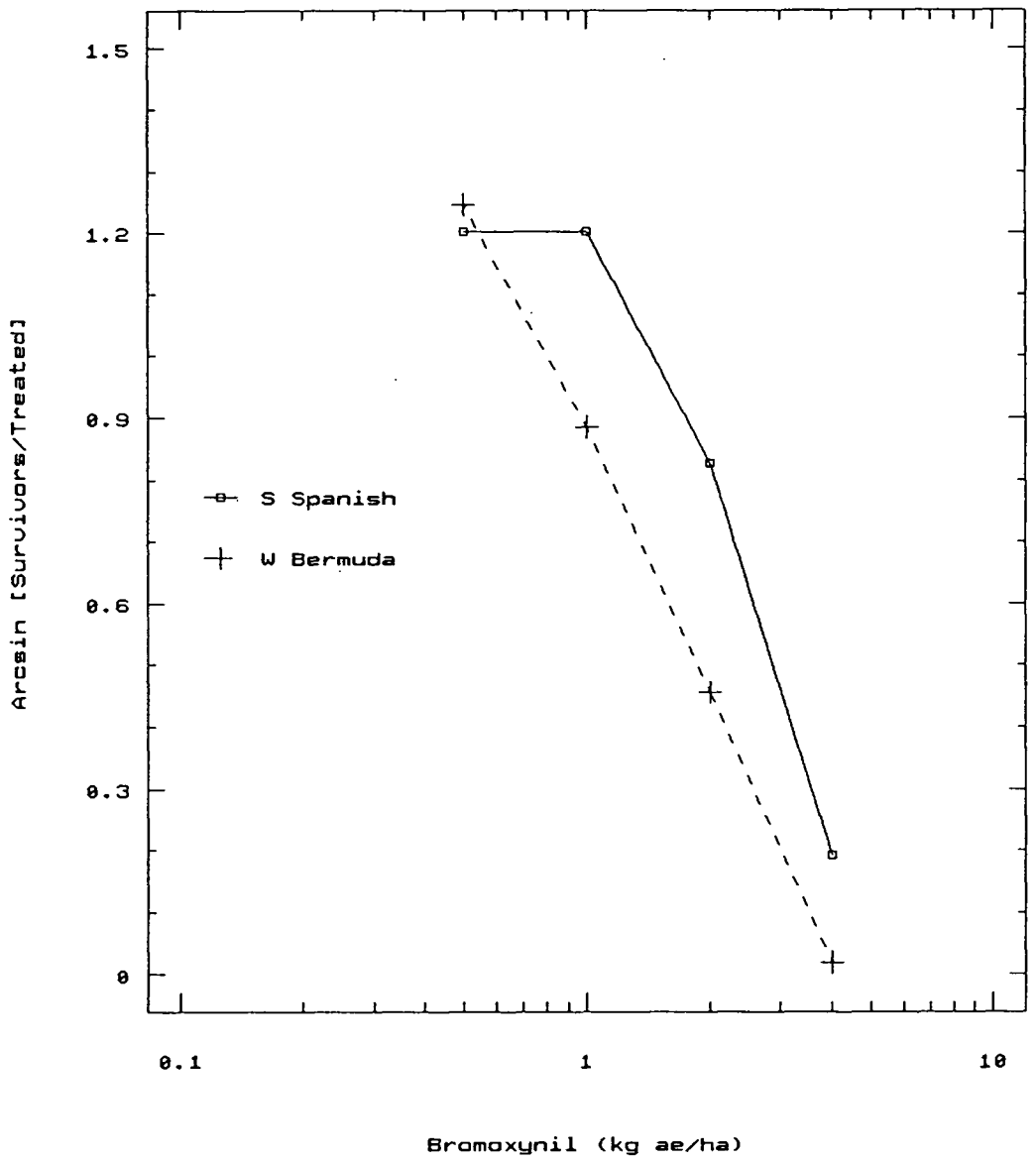

Figure 3.1 Arcsin transformed survival ratio up to 4 kg ae/ha.

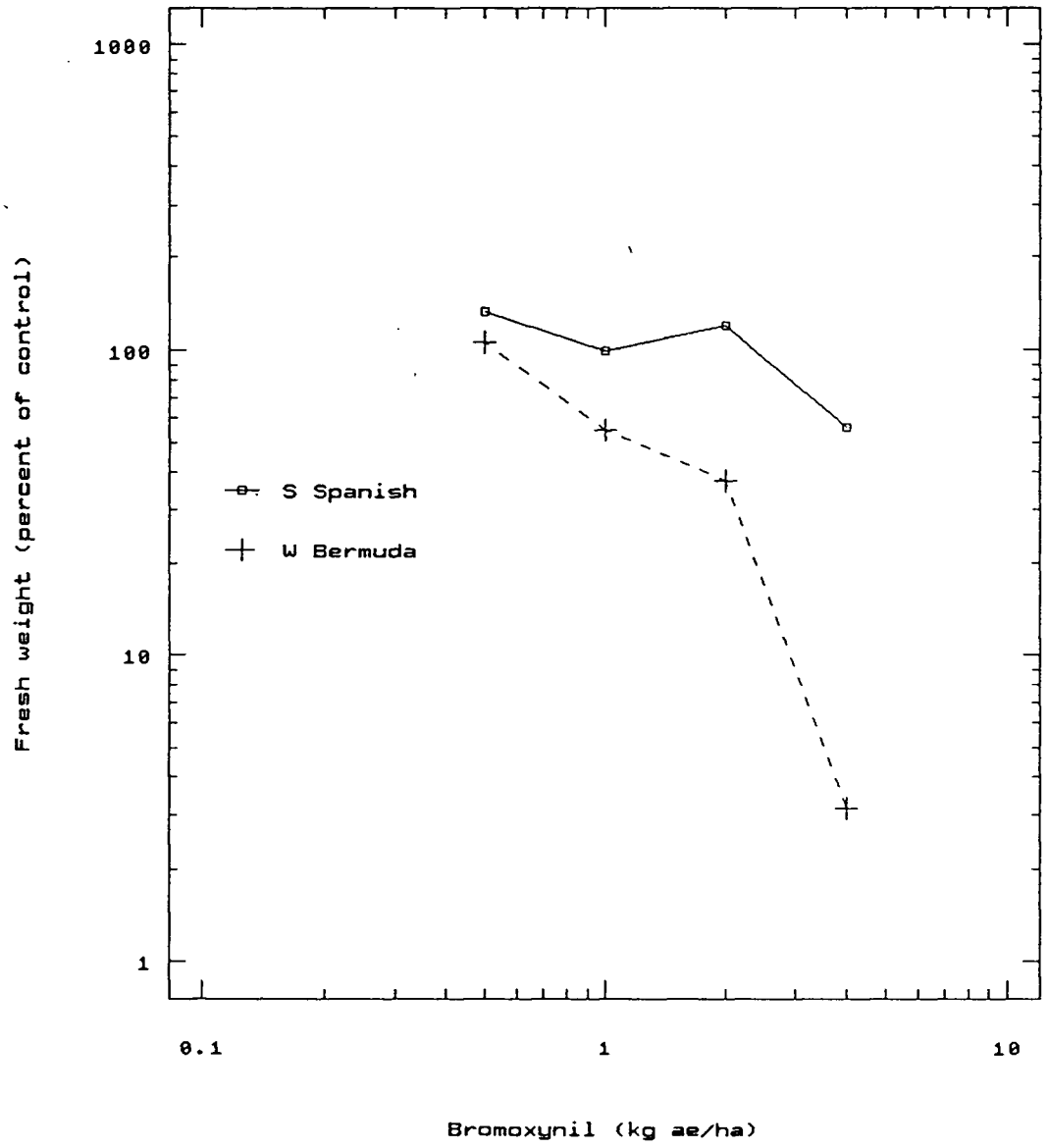

Figure 3.2 Fresh weight (percent of control) up to 4 kg ae/ha.

Results of experiment 2 are presented in Figure 3.3. Bromoxynil accounted for only 19% of the variability in the survival of the 8 cultivars included. Arcsin transformed survival ratio at the highest rate of 4 kg/ha were between 0.5 and 0.9. Fresh weights of the surviving plants expressed as percent of the untreated control are shown in Figure 3.4. Most of the cultivars included in this experiment exhibited a modest decline in fresh weight with increasing bromoxynil rates. Utah YSS was unusually different in producing fresh weights that were greater than the untreated control at 4 kg/ha. Bromoxynil accounted for only 23% of the variation in fresh weight of the surviving cultivars.

Experiment 3 was designed such that the sensitive cultivar White Bermuda from experiment 1, the tolerant cultivar Utah YSS from experiment 2, two strains of Oregon Danvers, Beirley and Leedy, Early Yellow Globe, Stockton Early Red and Walla Walla were included. The cultivars were selected to confirm the earlier findings from experiments 1 and 2 with White Bermuda and Utah YSS and at the same time find out how the 2 strains of Oregon Danvers and other cultivars compared with the tolerant and sensitive cultivars.

Results in Figure 3.5 show the decline in survival of the cultivars with increasing rates of bromoxynil. The herbicide accounted for 49% of the variability in survival of the cultivars. More importantly, the earlier findings on the differences between Utah YSS and White Bermuda were confirmed. Survival

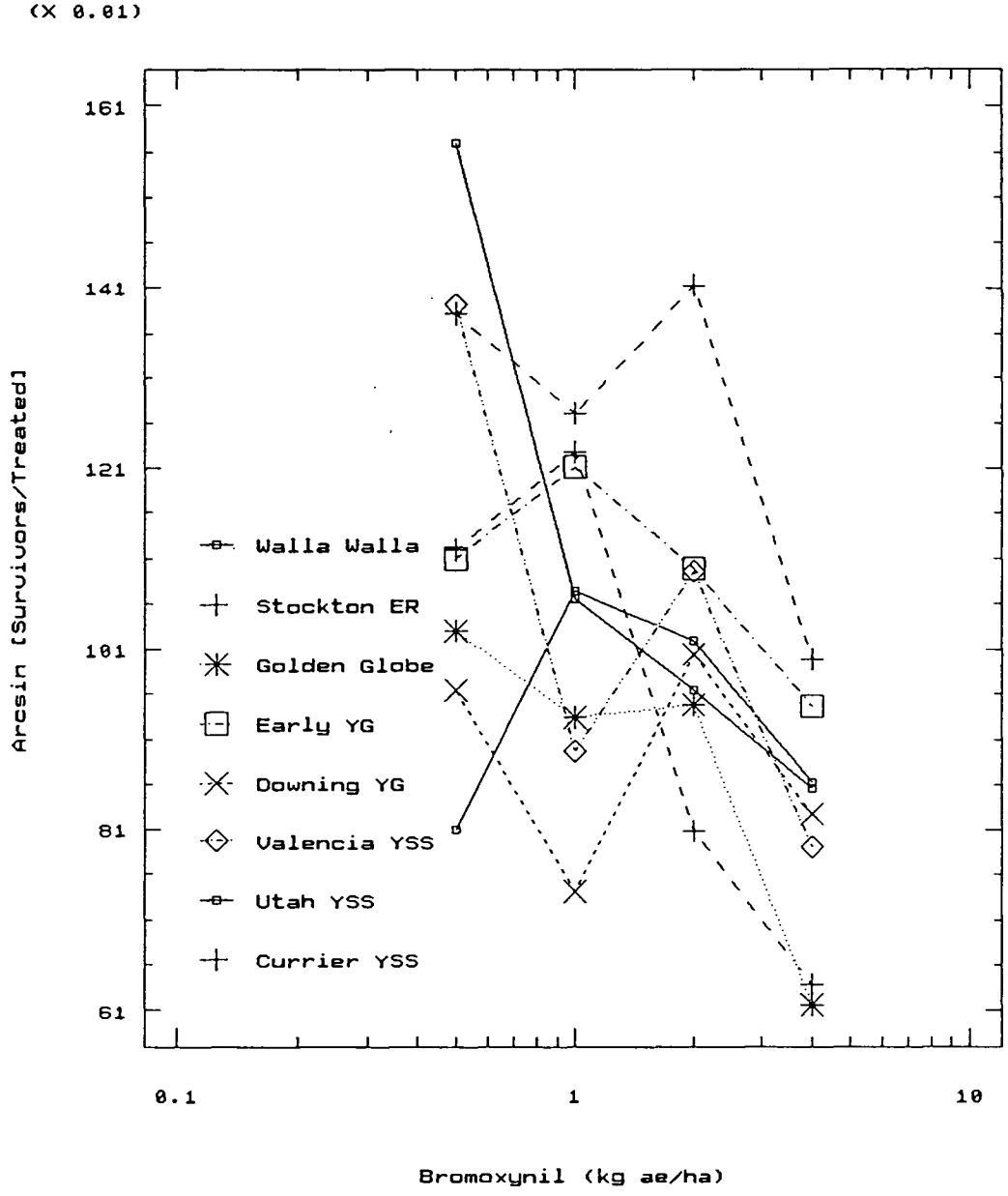

Figure 3.3 Arcsin transformed survival ratio of eight cultivars.

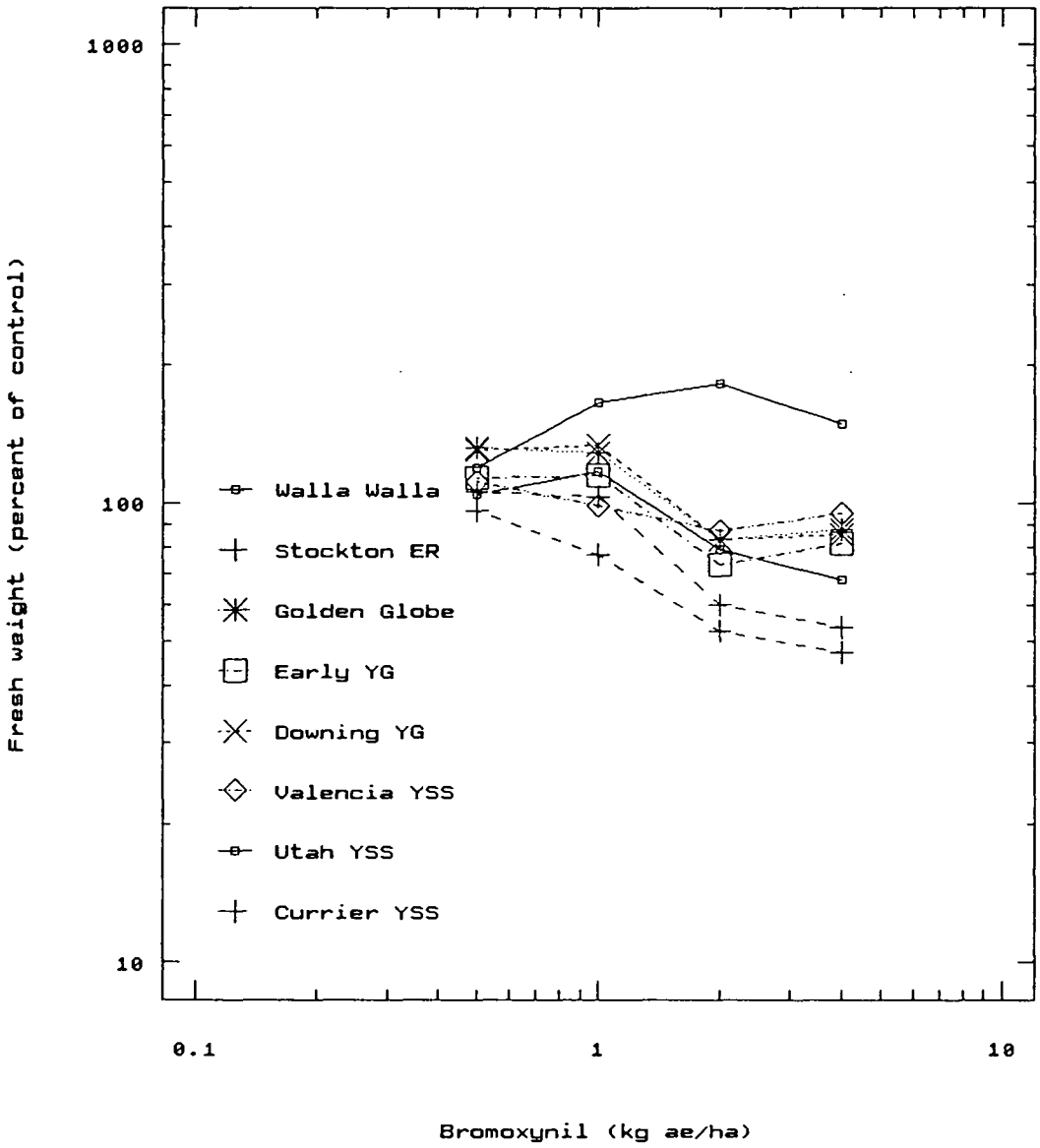

Figure 3.4 Fresh weight (percent of control) of eight cultivars.

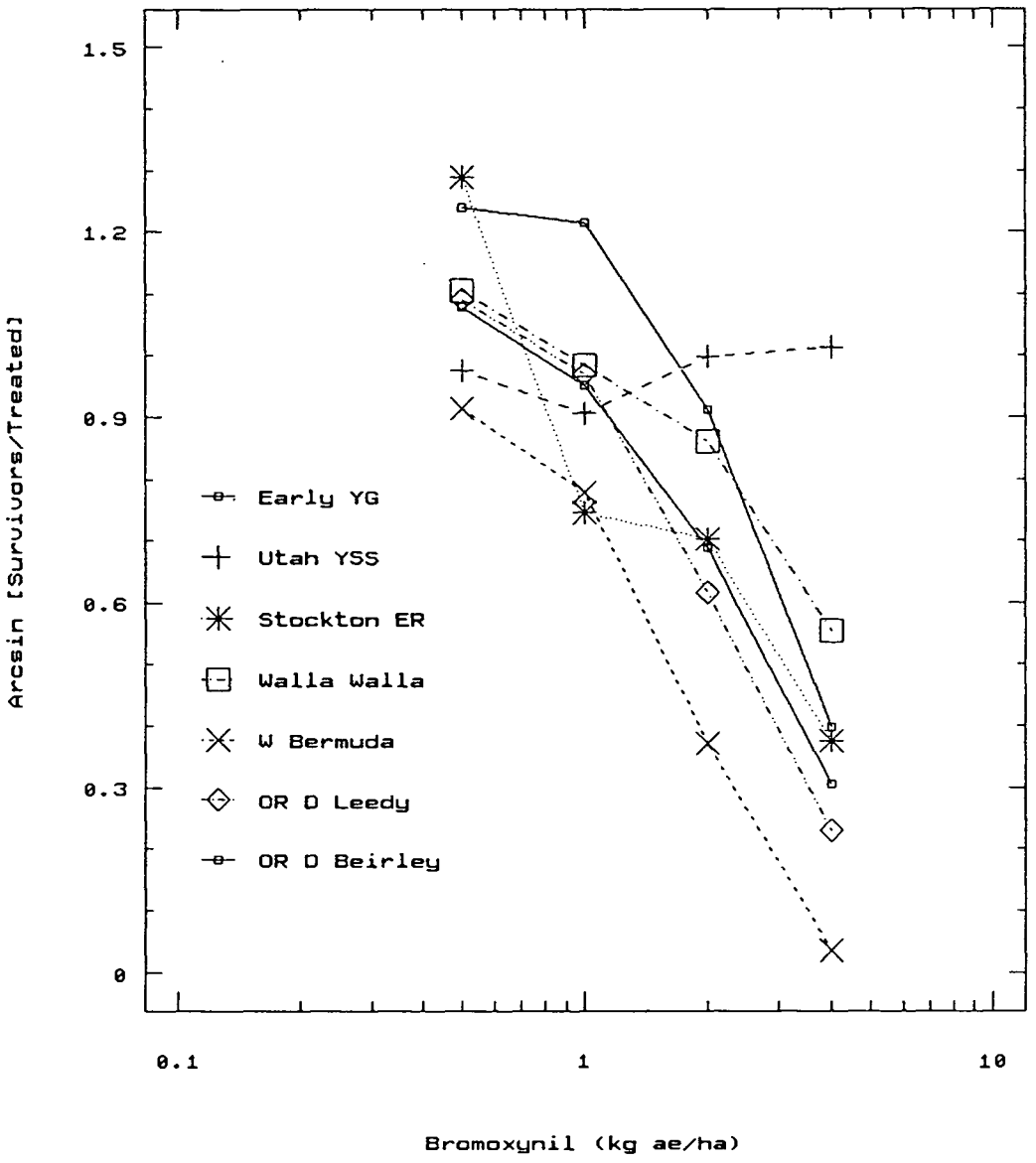

Figure 3.5 Arcsin transformed survival ratio of seven cultivars.

of the 2 strains of Oregon Danvers were in the 0.30's compared with Utah YSS which was in the 0.80's.

Fresh weight of the surviving plants (Figure 3.6) with bromoxynil accounting for 42% of the variability indicates that there is considerable variability in the response to bromoxynil. More importantly, the markedly consistent contrasting differences between Utah YSS and White Bermuda is demonstrated not only in terms of survival but also in the fresh weight of plants.

Regression of the arcsin transformed survival ratio and fresh weight of the survivors expressed as percent of the untreated control on bromoxynil rate of application gave r^2 values from 0.19 to 0.73. This wide range in the values derived draw attention to the limitations of regression in quantifying differences in a comparative assay.

Dose-response relationships in tolerant and sensitive cultivars

Bromoxynil accounted for 57% of the variability in kill ratio of the two cultivars, and the difference between Utah YSS and White Bermuda were statistically significant. With application rates of up to 8 kg/ha (Figure 3.7) less than 10% kill was observed in Utah YSS while up to 90% kill was registered with White Bermuda.

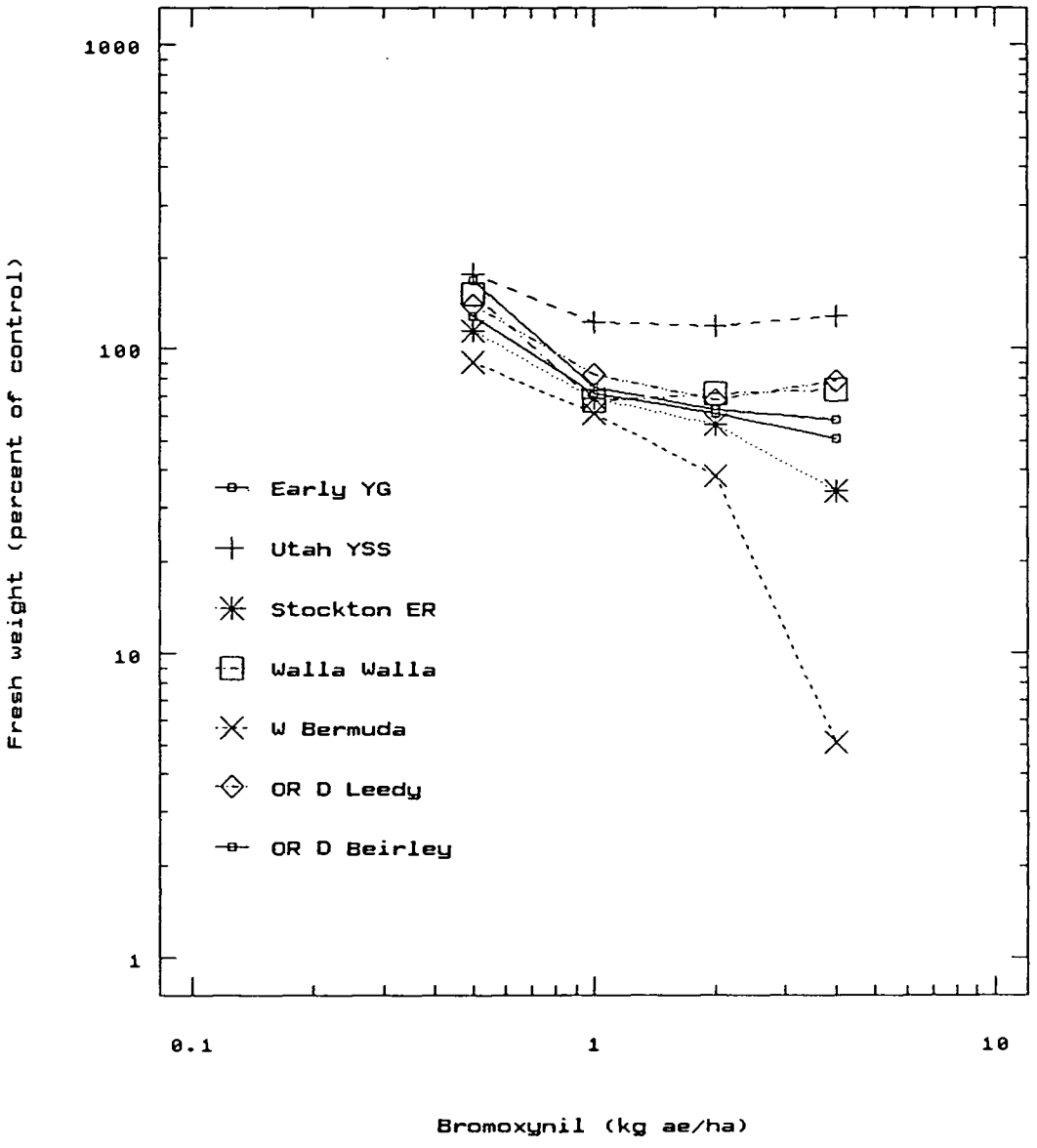

Figure 3.6 Fresh weight (percent of control) of seven cultivars.

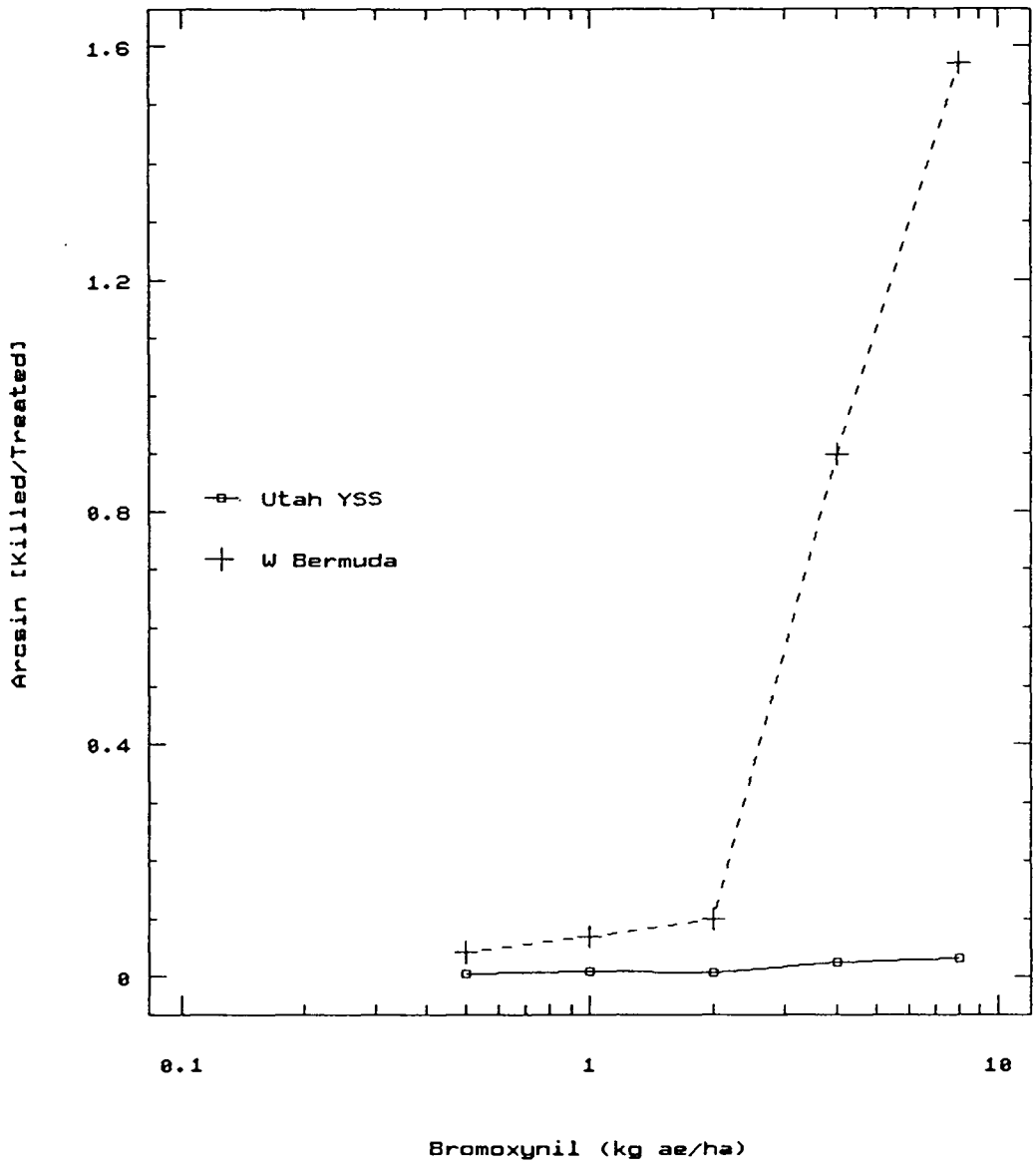

Figure 3.7 Arcsin transformed kill ratio up to 8 kg ae/ha.

Fresh weight of the surviving plants (Figure 3.8) gave similar results. Fresh weight of Utah YSS fluctuated around 80% and remained at a high level while a drastic decline with increasing bromoxynil levels was observed in White Bermuda.

Increasing bromoxynil rates up to 32 kg/ha (Figure 3.9) resulted in bromoxynil accounting for 55% of the variability in percent kill. The difference between the cultivars was statistically significant. At the highest rate, percent kill in Utah YSS was below 40% while in White Bermuda, near complete kill was reached around 16 kg/ha.

Fresh weight of the 2 cultivars with applications of bromoxynil of up to 32 kg/ha are shown in Figure 3.10. Bromoxynil explained up to 59% of the variability in fresh weight. Decline in fresh weight was gradual in Utah YSS with about 10% at the highest rate compared to a drastic decline in White Bermuda.

The use of parameters like the median lethal dose (LD_{50}) and the median effective dose (ED_{50}) for the detection of differences in tolerance is a useful quick procedure. Typically the values are derived from dose-response experiments similar to the ones reported here. The two cultivars showed differences in the maximum and to a certain degree in the minimum effects that can be detected by the method of assessment employed. The median lethal dose for White Bermuda varied from 3 kg ae/ha in Figure 3.7 to about 8 kg ae/ha in Figure 3.9. Over the

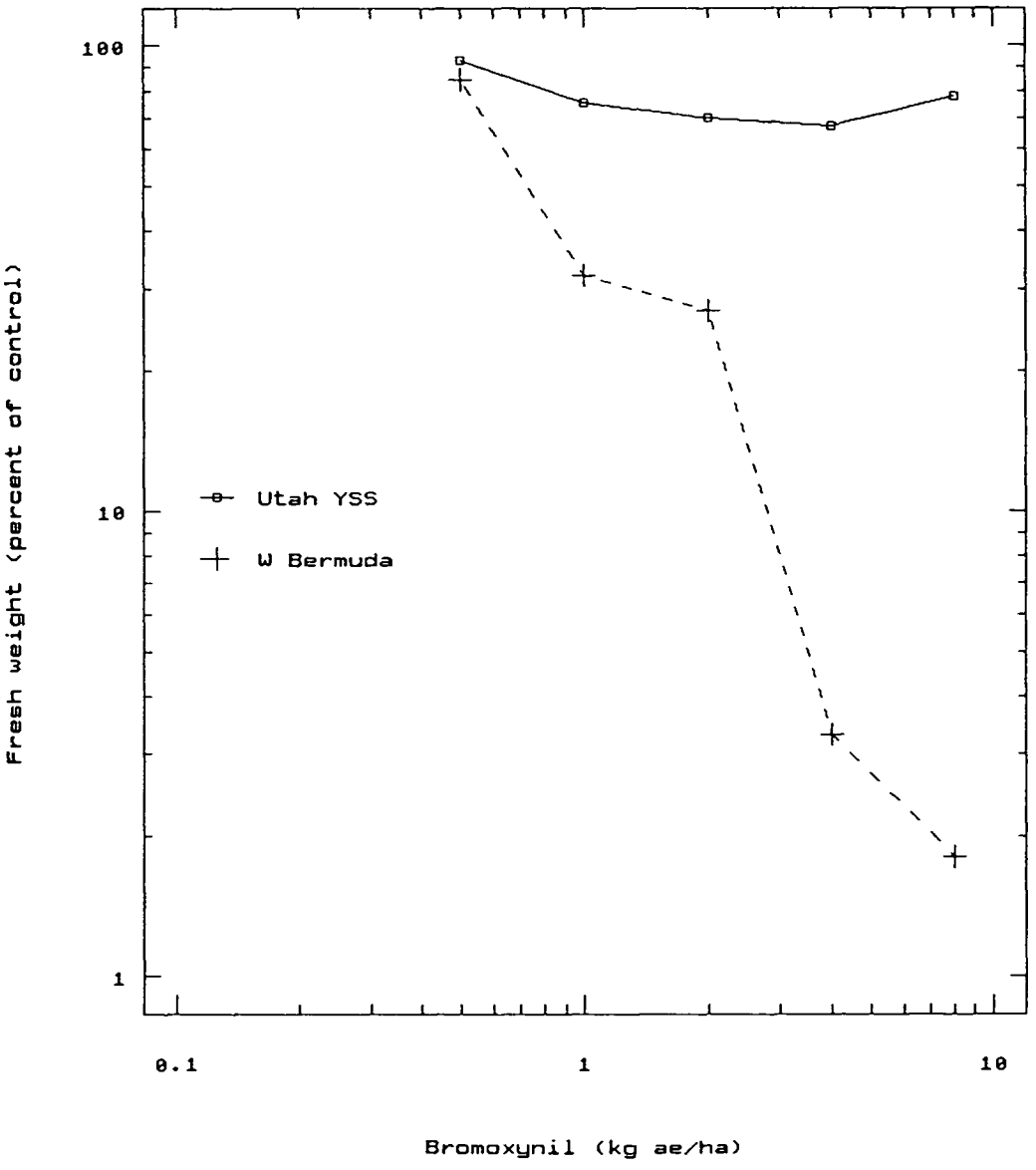

Figure 3.8 Fresh weight (percent of control) up to 8 kg ae/ha.

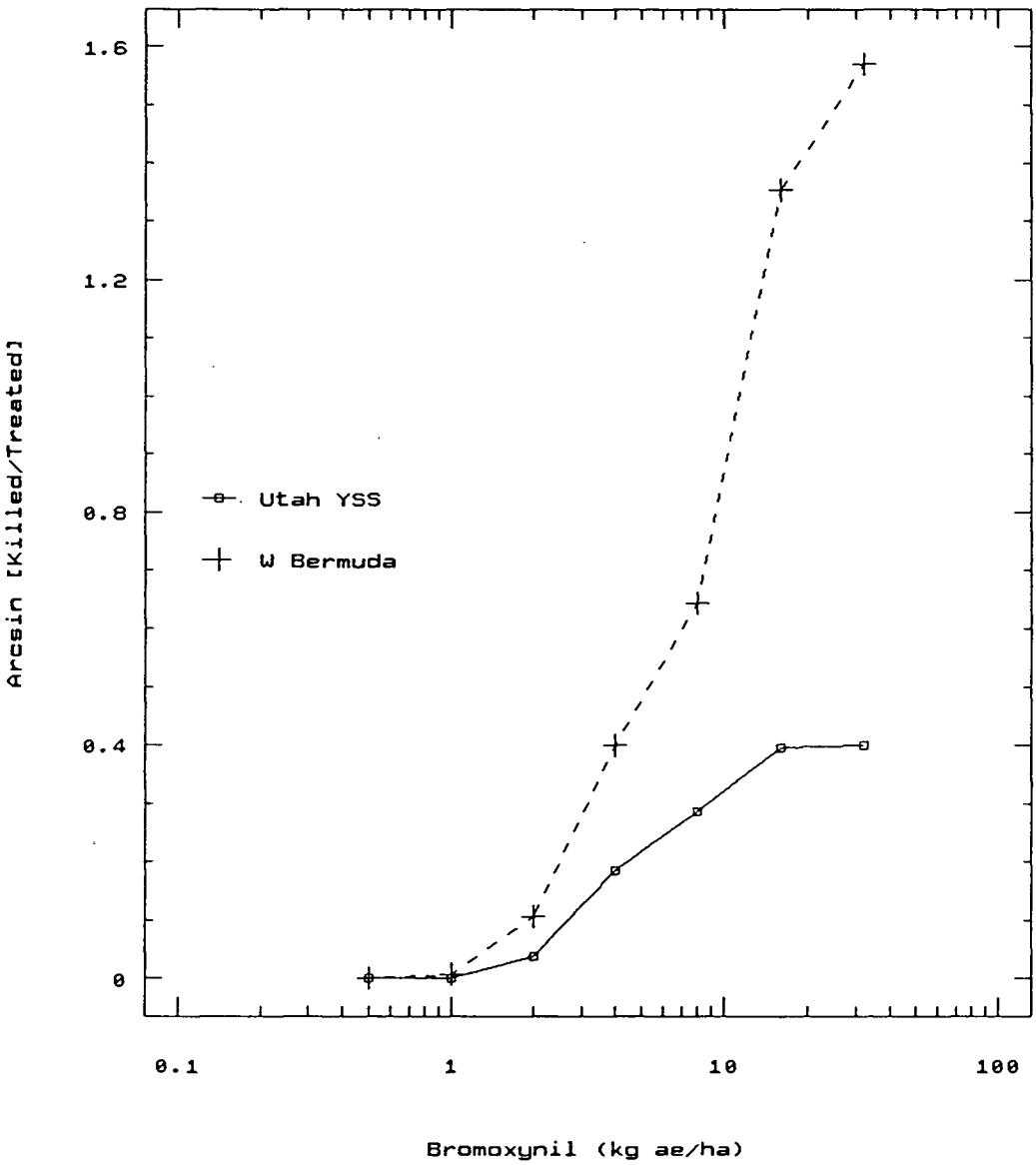

Figure 3.9 Arcsin transformed kill ratio up to 32 kg ae/ha.

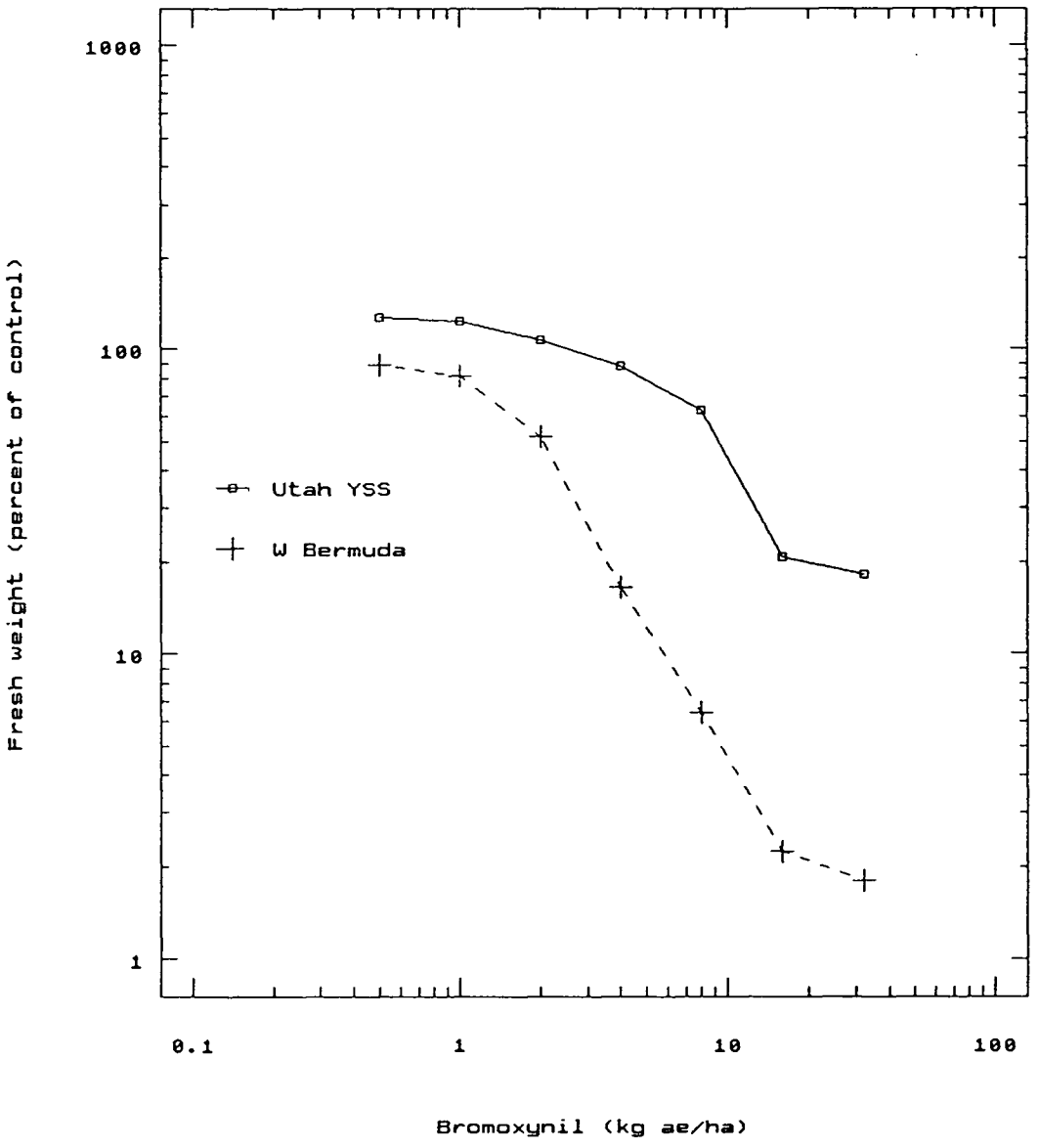

Figure 3.10 Fresh weight (percent of control) up to 32 kg ae/ha.

same range of rates, percent kill in Utah YSS ranged from 8 to 38%. This indicates that the maximum detectable effect can be used to differentiate the two cultivars is at bromoxynil application rates between 4 and 5 kg ae/ha.

Data on fresh weight of surviving plants as influenced by increasing rates of bromoxynil showed a quasi-log linear response. The decline in fresh weight of the surviving plants as a function of increasing rates was consistently shown by the sensitive cultivar White Bermuda. In Utah YSS, fresh weight of the surviving plants at sub-lethal rates was substantially greater than the untreated controls or fluctuated to about 70%.

Screening of onion cultivars for tolerance to bromoxynil

Consistency of the check cultivars, both in terms of percent survival and fresh weight of the surviving plants confirmed earlier results (Table 3.1). White Bermuda had 20% survival and the surviving plants had 7% of the fresh weight of the untreated control. Utah YSS had 90% survival and the surviving plants had up to 79% of the fresh weight of the untreated control. Survival of three other Sweet Spanish type cultivars, namely, White Sweet Spanish, Valencia YSS and Sweet Spanish were similar to Utah YSS but only White Sweet Spanish and Sweet Spanish had fresh weights comparable to Utah YSS. Oregon Danvers Beirley and Leedy, Early Yellow Globe, Currier YSS, Golden Globe and Downing Yellow Globe were intermediate in their responses. These cultivars had low survival rates and low fresh weights because of bromoxynil injury.

Table 3.1. Angular transformed percent survival and fresh weight (percent of control) of thirteen cultivars to 5.0 kg/ha bromoxynil.

Entry no.	Cultivar	Survival (degrees)	Fresh Weight (percent)
3	Yellow Bermuda	6.49 a	5.99 a
6	Crystal White Wax	17.39 ab	8.75 a
15	Crystal White Wax	19.21 ab	11.98 a
16	White Bermuda	19.93 ac	5.57 a
4	White Bermuda	20.33 ac	6.72 a
2	OR Danvers Beirley	42.67 bd	25.67 ab
7	OR Danvers Leedy	44.55 bd	23.26 ab
13	Early Yellow Globe	53.80 cd	27.56 ab
9	Currier YSS	55.89 de	22.90 ab
14	Golden Globe	59.33 de	35.53 ac
12	Downing Yellow Globe	62.85 de	32.61 ab
1	White Sweet Spanish	68.68 de	44.02 bc
11	Valencia YSS	74.47 de	35.17 ac
8	Sweet Spanish	75.68 de	46.71 bc
5	Utah YSS	90.00 e	65.18 cd
10	Utah YSS	90.00 e	79.00 d

[1]Means followed by the same letter are not significantly different at P=0.05, using Tukey's test.

The tolerant and sensitive check cultivars (Table 3.2) confirmed much of what had been found in earlier experiments. White Bermuda and Utah YSS are different and the evaluation criteria reflect this difference. White Bermuda had up to 41% survival and 38% of the fresh weight of the untreated controls while Utah YSS had 74% survival and 98% of the fresh weight of the controls. 'Winner' compared favorably with Utah YSS, both in terms of survival and fresh weight of the surviving plants.

The usefulness of visual ratings in evaluating plant response to bromoxynil is well documented (Wax, et al, 1974; Pantone, et al, 1988). Visual ratings are capable of detecting differences in rates as low as 0.12 kg ae/ha and highly reliable when response is a linear function of the logarithm of the stimulus, that is, closely following the Weber-Fechner law. The absence of an objective reference point, however, limits the value of the ratings when comparing ratings obtained from similar experiments with the same rates of application.

Data on quantal responses based on a more objective reference point, in this case, having >1 cm green tissue above the ground surface two weeks after treatment can be used to make valid comparisons of results from similar experiments. In addition to reflecting response as a linear function of the logarithm of the dose, other relationships that may be present can be detected as shown by the data presented in these studies. The data represents ability to withstand herbicide phytotoxicity expressed as survival or kill ratios and

Table 3.2. Angular transformed percent survival and fresh weight (percent of control) of eleven onion cultivars to 5.0 kg/ha bromoxynil.

Entry no.	Cultivar	Survival (degrees)	Fresh Weight (percent)
6	White Bermuda	36.97 a	37.94 a
13	White Bermuda	41.53 ab	37.71 a
11	Saturn	48.90 ac	58.38 ab
8	Sleeper	50.56 ac	66.92 ab
12	White Lisbon	53.35 ad	57.74 ab
4	Progress	54.06 ad	67.75 ab
9	Sierra	58.97 ae	73.39 ab
5	Chieftain	61.80 ce	91.99 bc
3	Walla Walla	64.17 ce	87.45 bc
1	Magnum Yellow	67.66 ce	93.91 bc
10	Yula	67.75 ce	76.58 ac
7	Utah YSS	72.46 de	96.58 bc
14	Utah YSS	73.80 e	98.49 bc
2	Winner	77.38 e	117.13 c

[1]Means followed by the same letter are not significantly different at P=0.05, using Tukey's test.

percentages. Congruence in the results from quantal and quantitative assessments highlight the achievement of the methodology developed in this study. Plotting fresh weight of surviving plants (% of control) in log scale against bromoxynil rate also in log scale results in a negative (declining) curvilinear response. Plotting arcsin transformed kill ratio in a linear scale against bromoxynil rate in log scale results in a positive (increasing) curvilinear response. At a carefully selected rate of application, however, one can decide to enhance tolerance by selecting for higher survival rate, faster recovery from herbicide injury or both.

Information obtained on the cultivars Utah YSS and Winner, identified in these experiments answers the question, Do we have onion cultivars tolerant to bromoxynil? Capable of surviving up to 10 times the field rate of application under greenhouse conditions, they can recover from herbicide injury within 2 weeks and produce fresh weights comparable to the untreated controls. Using these cultivars, one can undertake screening of the onion germplasm to identify other tolerant cultivars and possibly trace the inheritance of tolerance to bromoxynil. 'Winner' is known to have been derived from the same gene pool as Utah YSS. The cultivars Utah YSS and White Bermuda are excellent materials for studies to elucidate the mechanism of cultivar tolerance to bromoxynil in onions.

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STUDIES ON THE MECHANISMS OF CULTIVAR TOLERANCE

ABSTRACT

Effects of ethofumesate, piperonyl butoxide and the hydrolysis of cyano ^{14}C bromoxynil in tolerant and sensitive onion cultivars were studied to identify the mechanisms that confer differential tolerance to bromoxynil.

Ethofumesate did not enhance bromoxynil phytotoxicity to the tolerant cultivar Utah Yellow Sweet Spanish (YSS). Piperonyl butoxide did not modify bromoxynil phytotoxicity to either Utah YSS or White Bermuda, a sensitive cultivar. Metabolism of cyano ^{14}C bromoxynil yielded no significant differences in the amounts of unextractable radioactive residues and 3,5-dibromo-4-hydroxybenzoic acid. Hydrolysis of the cyano moiety of bromoxynil occurred in both the tolerant and sensitive cultivar. Tolerance to bromoxynil in the onion cultivar Utah YSS is not due to any quantitative difference in the formation of 3,5-dibromo-4-hydroxybenzoic acid from 3,5-dibromo-4-hydroxybenzonitrile when compared with bromoxynil transformation in White Bermuda.

INTRODUCTION

The identification of onion cultivars with markedly different responses to bromoxynil stimulated interest in the mechanisms that confer cultivar tolerance in onions. A number of factors are known to contribute to tolerance, including herbicide rate, stage of growth and environmental conditions. While tolerance often results from a complex interaction of many factors, the significance of an underlying mechanism that conditions tolerance important. The wide range of conditions in the onion growing areas of Oregon coupled with the different cultivars grown under these conditions suggests studying the effects of a wax formation inhibitor, ethofumesate; using piperonyl butoxide, which acts by inhibiting cytochrome P450 monooxygenases; and examining the hydrolysis of cyano ^{14}C bromoxynil in both the tolerant and sensitive cultivar.

REVIEW OF LITERATURE

The biotransformation of bromoxynil in a tolerant wheat plant (Buckland, et al, 1973) results in a large number of products including naturally-occurring substances. The transformations (Figure 4.1) initially involve the deesterification of the octanyl group followed by concurrent and consecutive reactions including hydrolysis of the cyano group, dehalogenation, hydrogenation and hydroxylation of the aromatic ring. Isolation of phenolic products indicate that conjugation occurs which may prevent the formation of phytotoxic quinones.

A study by Schafer and Chilcote (1970) showed differences in the amount of $^{14}CO_2$ evolved from cyano-labelled bromoxynil from wheat and coast fiddleneck. The work of Wain (1963) with the iodine analogue ioxynil, suggests that the phytotoxic action of bromoxynil may involve the liberation of Br ions which are oxidized by peroxidases and form phytotoxic bromines. These reactions could cause peroxidation of membrane lipids and possibly explain the rapid development of phytotoxicity symptoms in sensitive plants. The dehalogenated moiety has been shown to polymerize under ultra-violet light though the relevance of such reactions are still unknown. The transformations are summarized in Figure 4.2.

Hydrolysis of the nitrile group of bromoxynil in higher plants was studied by Schafer and Chilcote (1970) and Buckland, et al, (1973). Pioneering studies

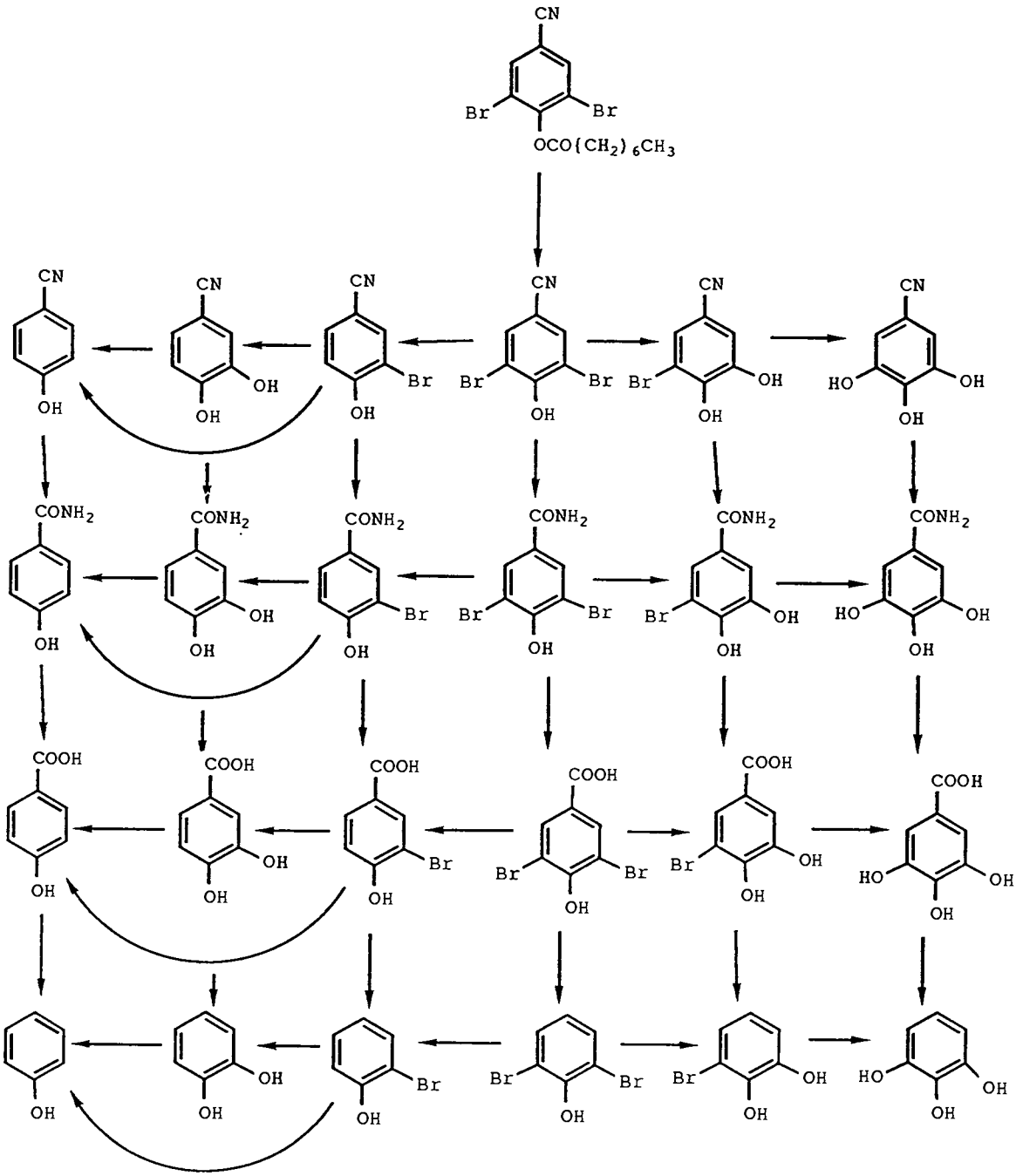

Figure 4.1 Biotransformation of bromoxynil in wheat.

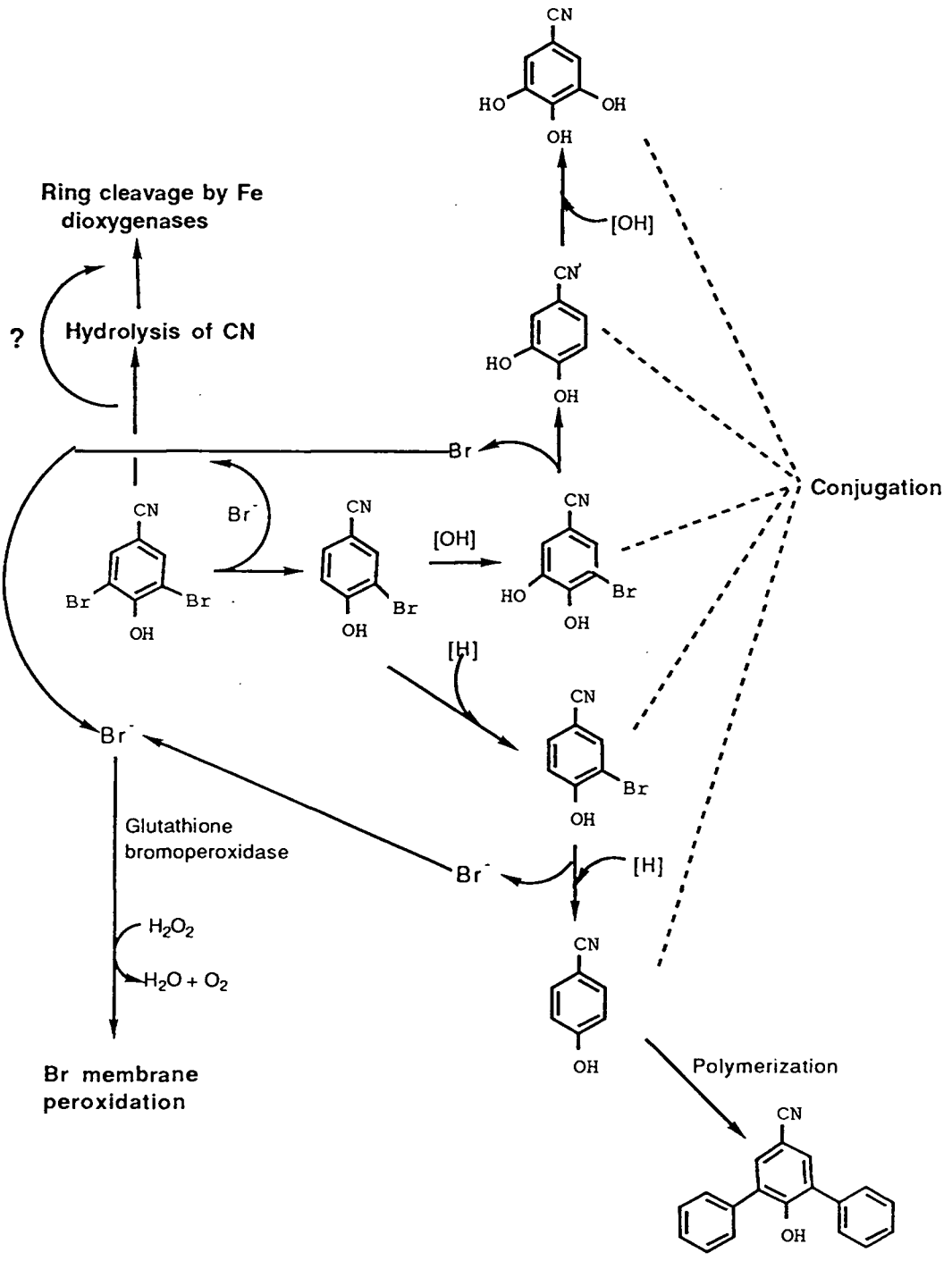

Figure 4.2 Biotransformation of bromoxynil in tolerant and sensitive organisms.

using bacterial enzymes (Harper (1977, 1985) and the later achievements of Stalker and McBride (1987) are examples of the sustained interest in this important reaction (Figure 4.3). The herbicidal action of bromoxynil may be mediated by the activity of a number of enzymes including nitrilase, peroxidase, hydrogenase, hydroxylase, amidase and those that catalyze conjugation reactions. Success with nitrilase has been demonstrated by the expression of the bromoxynil detoxifying bxn gene in transgenic tobacco and tomato (Stalker, et al, 1988). Transgenic plants tolerated up to 4 times the field rate of application.

The questions we sought to resolve were: (1) Is the bromoxynil tolerant Utah YSS adversely affected by inhibitors known to alter herbicide tolerance? and (2) Can cultivar tolerance to bromoxynil in onions be explained by differential hydrolysis of the cyano moiety? To answer these questions, experiments were undertaken to examine (1) the effects of ethofumesate, a known inhibitor of epicuticular wax formation (Verity, et al, 1981; Rubin, et al, 1986) on tolerance, (2) the effect of piperonyl butoxide, a known cytochrome P450 monooxygenase inhibitor (Gaillardon, et al, 1985) on bromoxynil tolerance, and (3) the hydrolysis of cyano ^{14}C bromoxynil in the tolerant and sensitive onion cultivars.

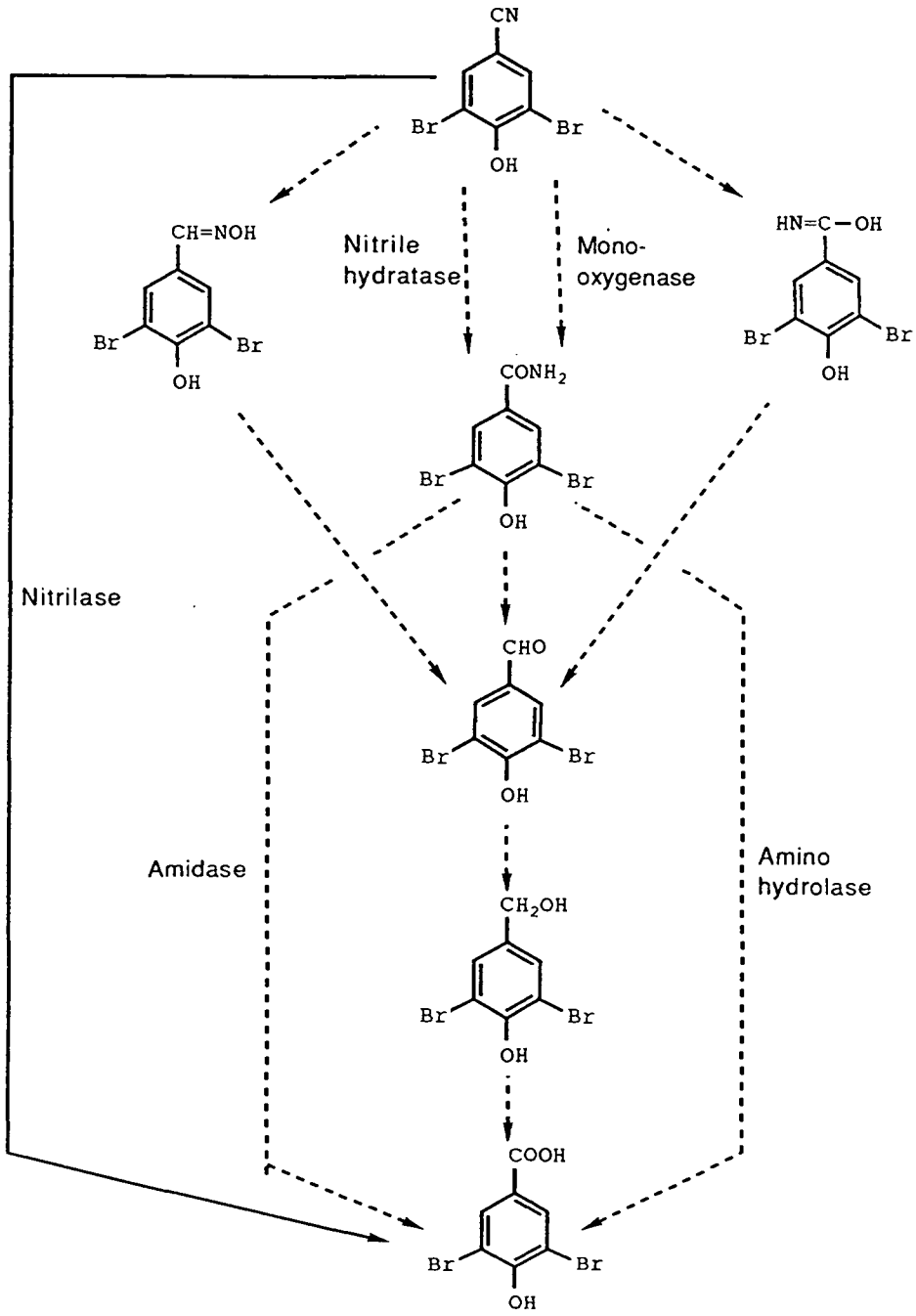

Figure 4.3 Nitrile hydrolysis - postulated and verified reactions.

MATERIALS AND METHODS

Effect of ethofumesate on cultivar tolerance to bromoxynil

Bromoxynil tolerant Utah YSS and sensitive White Bermuda onion cultivars were seeded separately in 10 cm square plastic pots filled with greenhouse mix. Ethofumesate was sprayed at rates of up to 1 kg ai/ha immediately after seeding. The pots were sub-irrigated overnight and placed on a greenhouse bench using a randomized complete block design. When the seedlings were at the 2 leaf stage, bromoxynil was sprayed at 5.0, 2.5 and 0 kg ae/ha. A factorial experiment with bromoxynil X ethofumesate X cultivar format was designed. Experiment 1 was a 2 X 2 X 2 factorial replicated five times while experiment 2 was a 3 X 3 X 3 factorial replicated four times. Data were collected on percent survival and fresh weight of the surviving plants 14 days after spraying. Angular transformed percent survival and the untransformed fresh weight of the surviving plants expressed as percent of the untreated control were subjected to analysis of variance. Mean separation was by Tukey's test.

Effect of piperonyl butoxide on tolerance to bromoxynil

Onion cultivars Utah YSS and White Bermuda at the 3, 2, 1 full leaf and the cotyledonary leaf stage were sprayed with 2.5 kg ae/ha bromoxynil with and without piperonyl butoxide.

Two factorial experiments with leaf stage, bromoxynil-piperonyl butoxide treatments and cultivars were undertaken. Experiment 1 was a 4 X 4 X 2 factorial replicated twice and experiment 2 was a 3 X 3 X 2 factorial replicated 4 times. The carrier used for applying the treatments and spraying the Untreated Controls was 16% acetone with 0.1% Oxysorbic (20 POE) (polyoxyethylene sorbitan monolaurate) at a volume equivalent to 365 l/ha. Piperonyl butoxide was used at the rate of 10 ug/ml.

Hydrolysis of cyano ^{14}C bromoxynil in tolerant and sensitive onion cultivars

Leaves of Utah YSS (tolerant) and White Bermuda (sensitive) onions were excised from seedlings at the 1 and 2 leaf stage. Using only the second fully grown leaf, leaves were infiltrated with radioactively labelled cyano ^{14}C bromoxynil under reduced pressure for 40 minutes and allowed to stand for another 20 minutes at atmospheric pressure. Leaves were transferred into petri dishes lined with moist filter paper and allowed to metabolize for 48 and 72 hours under continuous light at room temperature.

After the designated times for metabolism, leaves were cut into 1 cm lengths and shaken in 20 ml methanol for 2 minutes to remove radiolabeled bromoxynil. The leaf segments were homogenized for 3 minutes, filtered and extracted 3 times with 25 ml methanol. The residue was solubilized with NCS[1]

[1]Amersham/Searle Corp., Arlington Heights, IL

solubilizer and decolorized with 0.4 ml hydrogen peroxide. One ml aliquot was placed in a scintillation vial containing Cytoscint[1] and counted in a scintillation counter.

Extracts were evaporated first in a vacuum evaporator and later by passing nitrogen gas over the samples until they were reduced to a volume of 2 ml. Crude extracts were spotted on a TLC plate and developed in chloroform:acetic acid 19:1 v/v. Rf value of 3,5-dibromo-4-hydroxybenzoic acid was compared with an authentic standard which was developed with the extracts and detected with uv light as a spot with an Rf value of 0.62. The spots were scraped and placed in scintillation vials containing Cytoscint and counted. The experiment was a 3 factor factorial (leaf stage, metabolism time and cultivars) with 2 levels of each factor and replicated 4 times.

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[1]ICN Biochemicals, 3300 Highland Ave. Costa Mesa, CA

RESULTS AND DISCUSSION

Effect of ethofumesate on cultivar tolerance to bromoxynil

The interaction between bromoxynil and ethofumesate is of considerable theoretical and practical importance. By inhibiting epicuticular wax formation, ethofumesate should precondition onions to bromoxynil injury. If tolerance is mediated primarily by epicuticular waxes, ethofumesate may make a tolerant cultivar sensitive to bromoxynil. If ethofumesate does not modify bromoxynil activity, other mechanisms of tolerance may be operating.
Significance of the bromoxynil x ethofumesate interaction can be determined in factorial experiments by examining the probability values (P values) of the interactions and by the fact that the interaction results in the tolerant cultivar becoming sensitive to bromoxynil.

Effects of ethofumesate on the survival of Utah YSS and White Bermuda are shown in Table 4.1. At the 2 leaf stage, survival of the sensitive cv. White Bermuda were 58 and 18% at 2.5 and 5.0 kg ae/ha bromoxynil, respectively. Survival dropped to 26 and 9% with the addition of 1.0 kg/ha ethofumesate. The decline of roughly 50% in survival at both rates of bromoxynil is considerable but not statistically significant. Percent survival of Utah YSS was essentially unaffected by either bromoxynil or ethofumesate. Fresh weight of the surviving plants as percent of control (Table 4.2) showed a response similar to percent survival. Only the response of the cultivars to bromoxynil was significantly different.

Table 4.1. Angular transformed percent survival of Utah YSS and White Bermuda as influenced by ethofumesate and bromoxynil.

Bromoxynil (kg/ha)	Cultivar	Ethofumesate (kg/ha) 0	1.0
2.5	Utah YSS	90.00	87.26
	White Bermuda	57.88	25.94
5.0	Utah YSS	90.00	90.00
	White Bermuda	17.73	9.11

Main effects:[1]

Bromoxynil (kg/ha)

2.5	65.27 a
5.0	55.53 a

Ethofumesate (kg/ha)

0.0	63.91 a
1.0	56.90 a

Cultivar

Utah YSS	89.32 a
White Bermuda	31.49 b

Interactions:	**P value**
Bromoxynil x ethofumesate	0.06
Bromoxynil x cultivar	0.05
Ethofumesate x cultivar	0.30

[1]Means followed by the same letter are not significantly different at P=0.05, using Tukey's test.

Table 4.2. Fresh weight (percent of control) of Utah YSS and White Bermuda as influenced by ethofumesate and bromoxynil.

Bromoxynil (kg/ha)	Cultivar	Ethofumesate (kg/ha) 0	1.0
2.5	Utah YSS	86.35	98.05
	White Bermuda	37.50	45.00
5.0	Utah YSS	93.58	80.90
	White Bermuda	33.75	23.75

Main effects:[1]

Bromoxynil (kg/ha)

2.5	66.82 a
5.0	56.54 a

Ethofumesate (kg/ha)

0.0	62.96 a
1.0	60.40 a

Cultivar

Utah YSS	87.90 a
White Bermuda	35.45 b

Interactions:	**P value**
Bromoxynil x ethofumesate	0.22
Bromoxynil x cultivar	0.87
Ethofumesate x cultivar	0.88

[1]Means followed by the same letter are not significantly different at P=0.05, using Tukey's test.

Experiment 2 included 0.5 kg/ha ethofumesate to determine intermediate dose effects. In addition to the differences between the cultivars, effects of ethofumesate were significant. Interactions between ethofumesate and the cultivars were detected in survival (Table 4.3) and fresh weight (Table 4.4). When comparisons between the 2 cultivars are made across the rates of bromoxynil and ethofumesate, the following differences become apparent. Little or no effect on Utah YSS can be detected, while in the sensitive White Bermuda, drastic reductions in survival and fresh weights of the surviving plants occur with increasing rates of both bromoxynil and ethofumesate. Interaction between bromoxynil and ethofumesate, however is not statistically significant.

Ethofumesate affects the sensitive cultivar as shown by a reduction in percent survival of around 54% and up to 65% in fresh weight without bromoxynil. With a follow up treatment of bromoxynil, enhancement of phytotoxicity can be observed. Similar results were obtained by Rubin, et al, (1986) in Israel with the cultivar Ben Shemen.

Utah YSS did not respond to ethofumesate in the same manner as White Bermuda. Percent survival and fresh weight of Utah YSS was unaffected across all rates of ethofumesate. The conclusion that can be drawn is that ethofumesate does not alter tolerance to bromoxynil in Utah YSS. It could be that epicuticular

Table 4.3. Angular transformed percent survival of Utah YSS and White Bermuda as influenced by ethofumesate and bromoxynil.

Bromoxynil (kg/ha)	Cultivar	Ethofumesate (kg/ha) 0	0.5	1.0
0.0	Utah YSS	90.00	90.00	83.36
	White Bermuda	90.00	61.44	45.80
2.5	Utah YSS	90.00	90.00	90.00
	White Bermuda	63.34	40.47	19.01
5.0	Utah YSS	90.00	90.00	72.47
	White Bermuda	90.00	39.10	18.79

Main effects:[1]

Bromoxynil (kg/ha)

0.0	76.76 a
2.5	65.47 a
5.0	66.72 a

Ethofumesate (kg/ha)

0.0	85.56 a
0.5	68.50 b
1.0	54.91 c

Cultivar

Utah YSS	87.32 a
White Bermuda	51.99 b

Interactions:	P value
Bromoxynil x ethofumesate	0.33
Bromoxynil x cultivar	0.02
Ethofumesate x cultivar	<0.01

[1]Means followed by the same letter are not significantly different at P=0.05, using Tukey's test.

Table 4.4. Fresh weight (percent of control) of Utah YSS and White Bermuda as influenced by ethofumesate and bromoxynil.

Bromoxynil (kg/ha)	Cultivar	Ethofumesate (kg/ha) 0	0.5	1.0
0.0	Utah YSS	100	95.60	82.37
	White Bermuda	100	70.34	34.52
2.5	Utah YSS	101.97	95.50	71.05
	White Bermuda	82.05	48.18	9.54
5.0	Utah YSS	103.66	93.17	92.63
	White Bermuda	100.54	46.49	22.90

Main effects:[1]

Bromoxynil (kg/ha)

0.0	80.47 a
2.5	68.35 a
5.0	76.71 a

Ethofumesate (kg/ha)

0.0	98.11 a
0.5	74.95 b
1.0	52.47 c

Cultivars

Utah YSS	92.89 a
White Bermuda	57.48 b

Interactions:	**P value**
Bromoxynil x ethofumesate	0.80
Bromoxynil x cultivar	0.41
Ethofumesate x cultivar	<0.01

[1]Means followed by the same letter are not significantly different at P=0.05, using Tukey's test.

waxes play no significant role in the tolerance of Utah YSS to bromoxynil. Akey and Souza-Machado (1985) found increasing tolerance to oxyfluorfen inspite of decreasing epicuticular wax content in the onion cultivar Taurus.

Effect of piperonyl butoxide on tolerance to bromoxynil

Effects of piperonyl butoxide on cultivar tolerance to bromoxynil was examined using tolerant and sensitive cultivars at different growth stages. Differences in percent survival between the 2 cultivars varied with plant development. At the cotyledonary leaf stage, complete kill of White Bermuda resulted (Table 4.5). At the 1-leaf stage, 20 and 27% survived and survival was up to 72 and 82% at the 2-leaf stage. Percent survival in Utah YSS was largely unaffected by bromoxynil regardless of the stage of development. The main effects of leaf stage, treatment, cultivar and the interaction between treatment and cultivar were statistically significant.

Bromoxynil at 2.5 kg ae/ha with 10 ug/ml piperonyl butoxide tended to cause greater reduction in the fresh weight of White Bermuda compared with bromoxynil alone (Table 4.6) in experiment 1 but this tendency was not observed in experiment 2. The differences were not statistically significant and did not appear to be biologically meaningful.

Table 4.5. Angular transformed percent survival of Utah YSS and White Bermuda at different stages as influenced by piperonyl butoxide (PBO) and bromoxynil (BR).

Leaf Stage		UNTREATED	PBO	BR+PBO	BR
3	Utah YSS	90.00	90.00	82.79	82.97
	White Bermuda	90.00	90.00	81.22	90.00
2	Utah YSS	90.00	90.00	90.00	90.00
	White Bermuda	90.00	90.00	81.94	72.23
1	Utah YSS	90.00	90.00	90.00	81.63
	White Bermuda	90.00	90.00	26.60	19.80
0[1]	Utah YSS	90.00	90.00	83.15	90.00
	White Bermuda	90.00	90.00	1.81	1.81

Main effects:[2]

Leaf stage	
3	87.13 a
2	86.77 a
1	72.26 b
0	67.10 b

Treatments	
Untreated	90.00 a
Piperonyl butoxide	90.00 a
Bromoxynil + PBO	67.19 b
Bromoxynil alone	66.06 b

Cultivars	
Utah YSS	88.16 a
White Bermuda	68.46 b

Interactions:	**P value**
Leaf stage x treatment	<0.01
Leaf stage x cultivar	<0.01
Treatment x cultivar	<0.01

[1]Cotyledonary leaf stage.
[2]Means followed by the same letter are not significantly different at P=0.05, using Tukey's test.

Table 4.6. Fresh weight (percent of control) of Utah YSS and White Bermuda at different stages as influenced by piperonyl butoxide (PBO) and bromoxynil (B).

Leaf Stage		UNTREATED	PBO	BR+PBO	BR
3	Utah YSS	100	119.21	99.06	93.38
	White Bermuda	100	102.52	52.63	64.59
2	Utah YSS	100	78.77	85.09	90.45
	White Bermuda	100	100.52	31.34	38.98
1	Utah YSS	100	86.55	83.93	82.40
	White Bermuda	100	110.26	32.08	53.99
0[1]	Utah YSS	100	84.08	94.65	72.98
	White Bermuda	100	101.58	0	0

Main effects:[2]

Leaf stage

3	91.42 a
2	78.14 b
1	81.15 ab
0	69.58 b

Treatments

Untreated	100 a
Piperonyl butoxide	97.93 a
Bromoxynil + PBO	60.08 b
Bromoxynil alone	62.29 b

Cultivars

Utah YSS	91.91 a
White Bermuda	68.23 b

Interactions:	**P value**
Leaf stage x treatment	0.14
Leaf stage x cultivar	0.12
Treatment x cultivar	<0.01

[1]Cotyledonary leaf stage.
[2]Means followed by the same letter are not significantly different at P=0.05, using Tukey's test.

Experiment 2 was scaled down to a 3 X 3 X 2 factorial to focus on the differences between the 1 and 2 leaf stage with and without piperonyl butoxide. All the main effects as well as the leaf stage X treatment interaction were significant (Table 4.7). It was interesting to note that percent survival and fresh weight in Utah YSS was drastically reduced at the cotyledonary and 1 leaf stage (Table 4.8).

The effect of piperonyl butoxide on bromoxynil phytotoxicity can vary from being undetectable to causing some fluctuation in the fresh weight over bromoxynil alone in both cultivars. The more significant finding from the 2 experiments is the influence of stage of growth on the tolerance of Utah YSS and White Bermuda. Consistency in tolerance to 2.5 kg ae/ha bromoxynil can be obtained when bromoxynil is sprayed at the 2 leaf stage. At the cotyledonary and 1-leaf stage, Utah YSS may be sensitive although not to the same degree as White Bermuda.

The known effects of piperonyl butoxide on cytochrome P450 monooxygenases which confers wheat cultivar sensitivity to chlortoluron (Gaillardon, et al, 1985; Cabanne, et al, 1985) was not apparent from the effects of piperonyl butoxide on onion tolerance to bromoxynil. Possibly, cytochrome P450 monooxygenases in onions are not sensitive to piperonyl butoxide, or that in onions, they do not confer differential tolerance or sensitivity to bromoxynil.

Table 4.7. Angular transformed percent survival of Utah YSS and White Bermuda at different stages as influenced by piperonyl butoxide (PBO) and bromoxynil (BR).

Leaf Stage		UNTREATED	BR+PBO	BR
2	Utah YSS	90.00	90.00	90.00
	White Bermuda	85.13	79.65	72.46
1	Utah YSS	90.00	35.69	26.18
	White Bermuda	90.00	1.81	8.85
0[1]	Utah YSS	90.00	14.58	31.63
	White Bermuda	90.00	1.81	1.81

Main effects:[2]

Leaf Stage		
2	84.54	a
1	42.09	b
0	45.65	b
Treatment		
Untreated	89.19	a
Bromoxynil + PBO	38.49	b
Bromoxynil alone	44.61	b
Cultivar		
Utah YSS	64.46	a
White Bermuda	50.40	b

Interaction:	**P value**
Leaf Stage x treatment	<0.01
Leaf Stage x cultivar	0.79
Treatment x cultivar	0.06

[1]Cotyledonary leaf stage.
[2]Means followed by the same letter are not significantly different at P=0.05, using Tukey's test.

Table 4.8. Fresh weight (percent of control) of Utah YSS and White Bermuda at different stages as influenced by piperonyl butoxide (PBO) and bromoxynil (BR).

Leaf Stage		UNTREATED	BR+PBO	BR
2	Utah YSS	100	105.34	98.43
	White Bermuda	100	52.72	29.69
1	Utah YSS	100	37.89	18.47
	White Bermuda	100	0	3.31
0[1]	Utah YSS	100	34.50	36.45
	White Bermuda	100	0	0

Main effects:[2]

Leaf Stage	
2	78.95 a
1	43.88 b
0	43.04 b
Treatment	
Untreated	100 a
Bromoxynil + PBO	31.66 b
Bromoxynil	34.21 b
Cultivar	
Utah YSS	68.35 a
White Bermuda	42.22 b

Interactions:	**P value**
Leaf Stage x treatment	<0.01
Leaf Stage x cultivar	<0.01
Treatment x cultivar	<0.01

[1]Cotyledonary leaf stage.
[2]Means followed by the same letter are not significantly different at P=0.05, using Tukey's test.

Hydrolysis of cyano ^{14}C bromoxynil in tolerant and sensitive onion cultivars

Generally, unextractable radioactivity (Table 4.9) in the residues of treated onion leaves tended to increase with longer times allowed for metabolism. Analysis of variance indicate that the differences from leaf stage, metabolism time and cultivars were not significant. Further studies are needed to determine the nature and biological activity of the unextractable radioactive residues pooled together in these results.

Data on the extractable 3,5-dibromo-4-hydroxybenzoic acid (Table 4.10) confirm the formation of the metabolite in both the tolerant and sensitive cultivar. The relative amounts formed by the two cultivars at the different leaf stages and times allowed for metabolism failed to indicate any significant difference among the 3 factors and their interactions.

Our results strongly indicate that tolerance to bromoxynil in Utah YSS is not mediated by the hydrolytic reaction that leads to the formation of 3,5-dibromo-4-hydroxybenzoic acid. We conclude that the mechanism that confers tolerance to bromoxynil in Utah YSS may be different from that which confers tolerance in transgenic tobacco expressing the bromoxynil degrading bxn gene.

Table 4.9. Unextractable radioactivity (cpm/mg fresh weight) from residues of Utah YSS and White Bermuda as influenced by leaf stage and incubation time.

Leaf Stage		Incubation Time (hours)	
		48	72
2	Utah YSS	97.38	69.84
	White Bermuda	197.60	455.26
1	Utah YSS	471.23	716.48
	White Bermuda	92.93	228.14

Main effects:[1]

Leaf Stage		
1	377.17	a
2	205.02	a
Time (hours)		
48	214.76	a
72	367.43	a
Cultivar		
Utah YSS	338.74	a
White Bermuda	243.46	a

Interactions	**P value**
Leaf Stage x time	0.60
Leaf Stage x cultivar	<0.01
Time x cultivar	0.54

[1]Means followed by the same letter are not significantly different at P=0.05, using Tukey's test.

Table 4.10. Extractable C 3,5-dibromo-4-hydroxybenzoic acid (cpm/mg fresh weight) from Utah YSS and White Bermuda as influenced by leaf stage and incubation time.

Leaf Stage		Incubation Time (hours)	
		48	72
2	Utah YSS	150.53	94.38
	White Bermuda	92.92	60.68
1	Utah YSS	56.38	92.45
	White Bermuda	110.56	148.40

Main effects:[1]

Leaf Stage		
1	99.33	a
2	102.78	a
Time (hours)		
48	98.98	a
72	102.62	a
Cultivar		
Utah YSS	102.97	a
White Bermuda	99.15	a

Interactions	**P value**
Leaf Stage x time	0.08
Leaf Stage x cultivar	0.03
Time x cultivar	0.78

[1]Means followed by the same letter are not significantly different at P=0.05, using Tukey's test.

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GENERAL DISCUSSION

The development of bromoxynil tolerant onion cultivars is not expected to be actively pursued by either the herbicide or the seed industry because the economic rewards that could accrue are marginal compared with widely used herbicides and staple crops. Oregon onion growers, on the other hand, are faced with a lack of weed control options and sensitive onion cultivars.

Observations on the variation in response to bromoxynil indicate that there are sensitive and tolerant cultivars. When there is an adequate margin of safety over the normal range of field application rates, tolerant cultivars can be useful in a production system using bromoxynil to control weeds.

In an effort to overcome the difficulties inherent in visual rating scales, the method that was developed for assessing tolerance was a departure from the assessment techniques used by other workers. Quantal response (dead or alive) based on having >1 cm green tissue above the ground level 2 weeks after treatment was a fairly rigid criteria of survival. Quantitative response was obtained by harvesting and weighing the surviving plants and expressing fresh weight as percent of the untreated control. Quantal and quantitative responses were complementary variables that reinforce the overall assessment of tolerance, the rationale being that tolerant cultivars must not only survive but also be able to recover fairly rapidly from herbicide injury.

Utah YSS and White Bermuda were found to be tolerant and sensitive, respectively. To characterize the differences between the 2 cultivars, dose-response experiments were conducted to determine if phytotoxicological points of reference like the rates that would kill 50% of the cultivars (LD_{50}) and the rates that reduce growth by 50% (GR_{50}) would be appropriate for tolerance assessment and detection. Results indicate that the application rates that elicit the maximum and minimum responses both in terms of percent kill and fresh weight were different for the two cultivars. Differences between the cultivars were not clear because the responses were not symmetrical and predictable by a mathematical equation, indicating that there may be more than one site of action influencing tolerance. As a consequence, comparisons using LD_{50} and GR_{50} were not made as the slopes of the response variables were not parallel, a necessary condition for valid comparisons.

A discriminating rate to identify tolerant cultivars was derived from the maximum response of Utah YSS which was 38% kill at 16 kg ae/ha. This level of response in White Bermuda occurs around 5 kg ae/ha. At this rate, a number of cultivars were screened for tolerance with Utah YSS and White Bermuda as checks. The screening identified cv. Winner as comparing favorably with Utah YSS for tolerance to bromoxynil.

To elucidate the mechanism of cultivar tolerance, effects of chemicals known to alter herbicide phytotoxicity were examined. The rationale was, if tolerance can be altered, the action of the herbicide, reaction of the cultivar and the known mechanism of inhibitor action can be reconciled. Results indicate that in Utah YSS, such interactions, if they occurred, did not alter tolerance. The effects of ethofumesate and piperonyl butoxide were not detectable in the tolerant cultivar.

Hydrolysis of the cyano moiety, the mechanism that confers tolerance by detoxifying the herbicide was examined using cyano ^{14}C bromoxynil. Results indicate that hydrolysis occurs in both the tolerant and sensitive cultivars. Quantitative hydrolysis therefore, is unlikely the basis for the tolerance of Utah YSS.

The tolerant cultivars identified by the screening procedure are heterogeneous for tolerance and should be purified before they are used commercially. There is also a need to understand the genetics of tolerance to fully utilize its potential.

The finding that tolerance in Utah YSS is not due to hydrolysis of the cyano moiety in bromoxynil is significant in terms of the implications for the future development of tolerant cultivars. It implies that tolerance to bromoxynil

need not be the exclusive domain of the patent holders of the bromoxynil degrading bxn gene and that cultivars can be obtained from screening under greenhouse conditions. This can be done without huge outlays of resources which translates into higher seed costs to growers. Growers, on the other hand, can identify tolerant cultivars, purify their stock and plant bromoxynil tolerant cultivars.

These findings also point out the need to reexamine the reported sensitivity of Sweet Spanish cultivars to the fungicide chlorothalonil. This fungicide, like bromoxynil contains a nitrile moiety. Undoubtedly, it would be exciting to find out if bromoxynil tolerant cultivars are also tolerant of chlorothalonil.

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