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PINTO BEAN TOLERANCE TO AND WEED CONTROL WITH POSTEMERGENCE IMAZETHAPYR AND BENTAZON

By

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ABSTRACT

PINTO BEAN TOLERANCE TO AND WEED CONTROL WITH POSTEMERGENCE IMAZETHAPYR AND BENTAZON

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Dry bean producers have limited postemergence broadleaf weed control options. Research determined (1) if bentazon improved dry edible bean tolerance to postemergence imazethapyr, (2) if weed control was antagonized when imazethapyr and bentazon were tank-mixed, and (3) the basis and inheritance of varietal pinto bean tolerance to postemergence imazethapyr. Imazethapyr and bentazon were applied with various adjuvants to 'Olathe' pinto bean. Imazethapyr visually injured pinto beans 7 DAT in both field and greenhouse research. When 840 g ha^{-1} of bentazon was tank-mixed with 53 g ha^{-1} of imazethapyr, visual injury decreased as compared to imazethapyr alone in both studies. Fifty three g ha^{-1} of imazethapyr delayed physiological maturity by 8 and 15 days compared to the untreated control in 1991 and 1992, respectively. Pinto bean seed yields, however were not reduced compared to the untreated control. When 840 g ha^{-1} of bentazon was tankmixed with 53 g ha^{-1} of imazethapyr, maturity was not delayed. ^{14}C-Imazethapyr absorption decreased by more than 40% and translocation from the treated pinto bean leaflet by more than 50% when tank-mixed with bentazon compared to ^{14}C-imazethapyr alone. The

addition of 20 mM Na-acetate inhibited absorption of ^{14}C-imazethapyr similar to tank-mixing with bentazon, but did not inhibit translocation.

Imazethapyr and bentazon were applied with POA in a factorial arrangement to weed species. Tank-mixing 840 g ha^{-1} of bentazon with 13 or 27 g ha^{-1} of imazethapyr increased redroot pigweed and eastern black nightshade dry weight as compared to Colby's expected values in the greenhouse. However, weed control was not antagonized in field studies. Subsequent greenhouse studies indicated that soil interception of imazethapyr increased redroot pigweed control. Bentazon decreased redroot pigweed leaf absorption of ^{14}C-imazethapyr by 15% and translocation from the treated leaf by greater than 50% as compared to ^{14}C-imazethapyr alone.

All rates of postemergence imazethapyr injured Olathe, Sierra, UI114, P89405, Aztec, and P90570 pinto bean varieties 7 DAT in 1991 and 1992 except 53 g ha^{-1} of imazethapyr applied to Sierra pinto bean in 1991. Olathe was injured more than other varieties in 1991, and physiological maturity was delayed more than the maturity of Sierra in both years. However, seed yields of any variety were not reduced in 1991, and only P90570 had reduced seed yields from 53 g ha^{-1} of imazethapyr in 1992. Olathe pinto bean absorbed and translocated more than 1.4 and 1.3 times, respectively, ^{14}C-imazethapyr as Sierra pinto bean 24 h after application.

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CHAPTER 1

Introduction

Dry edible beans (*Phaseolus vulgaris* L.) are a major specialty crop in the state of Michigan with 350,000 acres of production in 1990. Michigan dry bean producers led the nation in total acreage and ranked 12th in the production of pinto beans (33). With the loss of chloramben[1] (3-amino-2,5-dichlorobenzoic acid) and dinoseb (2-1(1-methylpropyl)-4,6-dinitrophenol), Michigan dry bean producers have been searching for broadleaf weed control options. Standard weed control programs have included metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide) plus chloramben or dinoseb applied preemergence or trifluralin (2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl) benzenamine) plus EPTC (*S*-ethyl dipropylcarbamothioate) preplant incorporated followed by chloramben preemergence (usually banded). The loss of chloramben severely reduced broadleaf weed control options.

Bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-(4(3*H*)-one 2,2 dioxide] is the only postemergence broadleaf herbicide available and dry beans are very tolerant of bentazon (28, 72). Bentazon controls common cocklebur (*Xanthium strumarium* L.), jimsonweed (*Datura strumarium* L.), velvetleaf (*Abutilon theophrasti* Medicus), wild mustard (*Brassica kaber* (DC.)

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[1]Prairie Farmer. 1990. Amiben's loss limits dry bean herbicide options. Jan. 2, pp 8-9.

L.C.Wheeler), and common ragweed (*Ambrosia artemisiifolia* L.). However, bentazon does not control redroot pigweed (*Amaranthus retroflexus* L.) or eastern black nightshade (*Solanum ptycanthum* Dun.) (72), two problem weeds in dry edible beans.

Imazethapyr (2-[4,5-dihydro-4-methyl-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid) effectively controls redroot pigweed, eastern black nightshade, wild mustard, and common cocklebur. The activity on redroot pigweed and eastern black nightshade would complement bentazon's weed spectrum. Soybeans (*Glycine max* L.) and other legumes are tolerant of postemergence imazethapyr (13, 65, 71). However, dry edible beans have shown susceptibility to imazethapyr applications (16, 70), and the pinto bean class of dry beans appears to be particularly sensitive to postemergence imazethapyr (18). Early season injury symptoms include stunting, leaf crinkling, and interveinal chlorosis, while late season injury is characterized by reduced plant height and delayed maturity. Wilson and Miller (70) observed seed yield reductions one year following the application of 100 g ha^{-1} (a 2X standard application) of imazethapyr.

While screening for postemergence herbicide programs for dry bean producers, researchers (48) noted that bentazon safened dry edible bean response to imazethapyr. However, reductions in weed control have been reported (11) when bentazon is tank-mixed with imazethapyr.

Two or more herbicides are tank-mixed to increase the weed control spectrum over that achieved from either herbicide applied alone. When herbicides applied in a tank-mix act independently and the weeds controlled predicted by the performance of each herbicide applied alone, the effect is considered additive (21). However, the weed control spectrum may not follow the predicted performance. Hatzios and Penner (1985) described an interaction as antagonistic when biological activity decreased compared to that of each herbicide applied alone. Conversely, an interaction that resulted in enhanced of biological activity was termed synergistic.

Numerous studies have determined herbicide interactions on various species. Many have reported antagonized grass control when bentazon was tank-mixed with sethoxydim (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) (9, 20, 51). Wanamarta and Penner (1989) concluded that reduced quackgrass (*Elytrigia repens* (L.) Nevski) control was due to inhibited absorption of ^{14}C-sethoxydim in the presence of the sodium salt of bentazon. Conversely, pitted morningglory (*Ipomoea lacunosa* L.) control synergistically increased from combinations of imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolincarboxylic acid) and imazethapyr (54). An increase in morningglory control was also observed when imazapyr ((±)-2-[4,5-dihydro-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid) was combined with either imazaquin or imazethapyr (53,

69).

Formulated bentazon has been implicated as an antagonist when tank-mixed with a number of other herbicides (9, 16, 20, 23, 51, 58). Bentazon is formulated as a sodium salt (Na^+) (72). Sodium ions in the bentazon formulation appear responsible for many instances of antagonism by contributing alkaline cations that can form salts of acidic herbicides such as imazethapyr and sethoxydim which are not readily absorbed by plants (66). The Na^+ ion added as Na-acetate has been shown to inhibit uptake of sethoxydim and imazethapyr on quackgrass and pinto bean, respectively (5, 66). However, in both cases and others the absorption can be restored to levels previously observed by adding NH_4 in the form of either UAN or $(NH_4)_2SO_4$ (16, 23). This addition of an organic acid to the spray solution can overcome the antagonism by preventing the formation of the sodium salt. Adding abundant ammonium may also prevent or overcome this antagonism (44).

Imazethapyr. Imazethapyr is a member of the imidazolinone herbicide family of which imazapyr, imazamethabenz ((±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-4(and 5)-methylbenzoic acid (3:2)), and imazaquin also belong. Imazethapyr can be applied pre-plant incorporated, preemergence, or postemergence for selective control of various weed species in soybeans and other legumes. Imazethapyr is absorbed by both roots and shoots, and is translocated

to the meristematic tissues, characteristic of a phloem mobile herbicide (13, 56). The primary site of imazethapyr and other imidazolinone herbicide action has been reported to be the inhibition of the acetolactate synthase (ALS, also called acetohydroxyacid synthase) (2, 38). ALS is the first common enzyme in the synthesis of the branched chain amino acids valine, leucine, and isoleucine. The ALS enzyme is believed to reside in the plastids of plant cells (35). The plant genes coding for ALS have been isolated and characterized (31). The regulation of ALS is believed to be feedback inhibition by its products (32, 34). Both valine and leucine are inhibited by their own synthesis but are more inhibitive when supplied together (36). The sulfonylurea and the triazolopyrimidine sulfonanilide herbicides have also been reported as potent inhibitors of ALS (24, 47, 62)

It has been proposed that plants treated with the imidazolinone herbicides are slowly starved of valine, leucine, and isoleucine (57). However, subsequent research has been proposed suggesting that the death of the plant may be due to the toxic buildup of intermediates. The buildup of α-ketobutyrate has been triggered in plants treated with chlorosulfuron and sulfometuron methyl (50). Subsequent research has shown that α-ketobutyrate accumulation can be toxic (25).

Selectivity of imazethapyr is based primarily on metabolism (13). The half-life of imazethapyr in soybeans, a tolerant species, is 1.6 days while in redroot

pigweed, a susceptible species, it is 32.1 days[2]. Wilcut et al. (1988) determined the half-life of imazaquin, another imidazolinone, in tolerant soybean, to be 4.4 days while in susceptible cocklebur the half life was 39.8 days (67). Selectivity of the sulfonylurea herbicides has also been shown to be primarily based on metabolism (68).

Soybean, corn (*Zea mays* L.), and dry edible bean convert imazethapyr to 5-hydroxyethyl-imazethapyr followed by glucose conjugation (Figure 1). The rate of conversion to the glucose conjugate follows the order soybean > dry edible bean > corn, which results in reduced dry bean and corn tolerance[2,3](6). Five-hydroxyethyl-imazethapyr is herbicidally active, but five times less active than imazethapyr[2].

Some plants possess ALS isozymes that are less sensitive to imidazolinone and sulfonylurea inhibition, thereby increasing their tolerance (3, 19). Genetic studies of sulfonylurea resistance in soybean (55), *Lactuca* spp. (41), and imidazolinone resistant corn (30) has indicated that the resistance trait is inherited as a single nuclear gene in a semidominant fashion.

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[2]AC-263-499 Technical Information Report. 1985. American Cyanamid Co., Princeton, NJ 08540.

[3]Personal communication. Dale Shaner, American Cyanamid Co., Princeton, NJ 08540.

Bentazon. Bentazon is a selective postemergence herbicide commonly used in soybean, dry bean, and corn for control of several weed species (28, 72). The primary site of bentazon action is the inhibition of non-cyclic electron transport while photosystem I is unaffected (7). Bentazon was also shown to inhibit the Hill reaction activity of isolated chloroplast from both hot pepper (*Capsicum chinense* L.) and sweet pepper (*Capsicum annuum* L.) (4). More specifically, the site of bentazon inhibition of the photosynthetic electron transport is at the reducing side of photosystem II, between the primary electron acceptor Q and plastoquinone (63). Others (46) have shown that light was required for necrosis to develop in bentazon treated leaves.

The mechanism of bentazon selectivity is based primarily on metabolism (37). Large amounts of metabolites were detected in bentazon tolerant plants, but only small amounts were present in plants susceptible to bentazon (37). Differences in spray retention and the rate of bentazon metabolism contributed to the selective of bentazon between soybean and Canada thistle (*Cirsium arvense* (L.) Scop.) (43). The rapid metabolism of bentazon in navy bean accounted for its tolerance as compared to more sensitive cocklebur and black nightshade (29). The unifoliate navy bean leaf metabolized bentazon more slowly than the trifoliolate leaf (28).

Differential susceptibility between corn inbreds and soybean genotypes is the result of differential metabolism (8, 10). At 72 h after treatment, 63% of the absorbed ^{14}C-bentazon remained as parent in the susceptible corn inbred as

compared to 25% in the tolerant (8). Neither absorption nor translocation could account for tolerance differences between soybean genotypes. However, tolerant genotypes metabolized 80 to 90% of the absorbed bentazon within 24 h as compared to only 10 to 15% metabolism in susceptible genotypes (10). Analysis of bentazon sensitivity among various corn inbreds and single crosses suggested this trait is recessive and controlled by a single gene designated *ben* (15).

Bentazon tolerant crops first hydroxylate and then conjugate bentazon to glucose (Figure 2). In bentazon tolerant crops such as rice (*Oryza sativa* L.), rye (*Hordeum vulgare* L.), barley (*Secale cereale* L.), corn, wheat (*Triticum aestivum* L.), and peas (*Pisum sativa* L.), the glucose conjugate is formed primarily from 6-hydroxy-bentazon (37, 42, 49). Small amounts of 8-hydroxy-bentazon are also formed but in much smaller concentrations. Soybean is unique because it forms both glucose conjugates in a 1:1 ratio (42).

Interactions. In recent years, crop producers often combine two or more herbicides in the same spray tank to reduce the number of 'passes' across the field and to increase the number of weed species controlled. Reducing the number of passes reduces application costs and soil compaction. However, in some instances, a herbicide may control a weed when applied alone, but not when tank-mixed with a second herbicide. In a different scenario, two herbicides could be tank-mixed, and control a weed better than when either herbicide is applied alone. These

phenomena are referred to as interactions. Hatzios and Penner (1985) described an interaction as antagonistic when biological activity decreased compared to the performance of each herbicide alone. Conversely, an interaction that enhanced biological activity was synergistic.

An interaction has been defined and redefined for several different research areas. The strict statistical definition is when the effect of a factor deviates more than can be attributed to chance, then the differential response is called the interaction of two factors (59). Drury (14) stated that an interaction was a calculus phenomena and the statistical concept of an interaction was over-simplistic. Drury's calculus method for the study of an interaction was based on the use of a multiple regression polynomial model for fitting data and determining the partial derivative of each equation with respect to each factor and the second partial derivative with respect to both factors (as their interaction). The numerical factors were then calculated and plotted and finally comparisons of the sign of the interaction used to determine the synergism or antagonism of each factor at a specific point. To analyze an interaction by this method, one must construct an isobole. A range of data from around each herbicide's I_{50} value must be collected and graphed, and the I_{50} value interpolated.

Several others (1, 64) have also proposed the use of isoboles to present interaction data. Others (26, 39, 40) have utilized regression estimate analysis to evaluate interaction studies. Clearly, most of these methods for the presentation

of interaction data require computer analysis, since the calculations and plotting are very complex. These methods have failed to gain widespread acceptance because of the mathematical intricacies involved. Another reason these methods have not gained widespread acceptance is that many studies are conducted with herbicide rates approximating field rates, which generally exceed greater than 50% control.

Most methods of describing and quantifying interactions have failed because of their complexities. However, Colby (1967) proposed a formula used frequently by weed scientists in describing the effect of a herbicide mixture on plants. The formula is:

$$E = \frac{X * Y}{100}$$

where E, X, and Y are the expected growth as a percent of control with herbicides, growth as a percent of control with herbicide A at p lb A^{-1}, and growth as a percent of control with herbicide B at q lb A^{-1}, respectively. When the observed response is greater than expected, the combination is synergistic and when less than expected, it is antagonistic. If the observed responses are equal, the combination is additive. Statistical significance between the actual value and the expected value can be determined by using the modified Least Significance Difference equation developed by Hamill and Penner (17). However, when values

approach extremes (either 0 or 100 %), this method is too discretionary (personal observation). More recently, significance has been determined by using Fisher's protected LSD to compare Colby's expected with the observed (53, 54, 69). This method appears to provide the optimum amount of discretion and separation.

Analyzing and quantifying herbicide interactions remains a confusing and complex research. More statistical research is needed to help identify methods to analyze herbicide interactions. With increased use of postemergence herbicides for broad spectrum weed control, there will continue to be a need to understand herbicide interactions both statistically and biologically.

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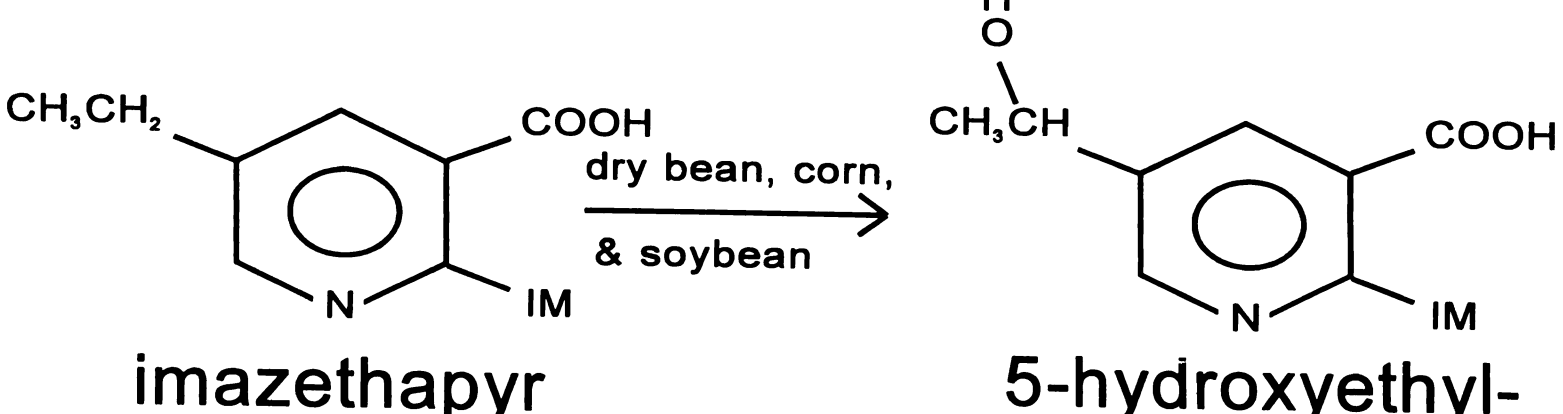
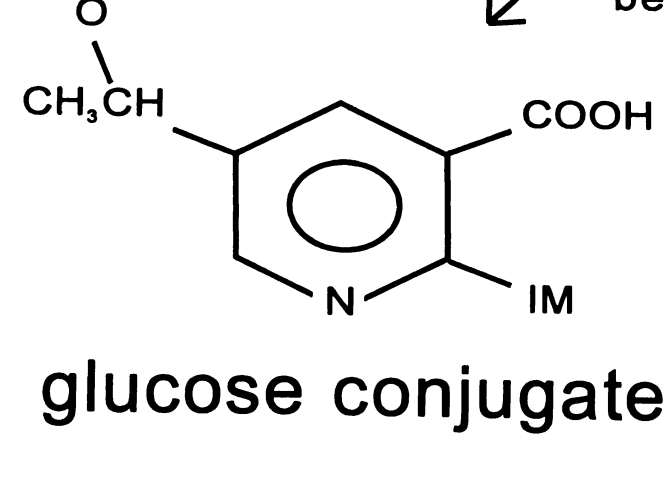
Figure 1. Schematic diagram of initial imazethapyr metabolism in corn, soybean, and dry bean. IM represents the imidazolinone chemical structure.



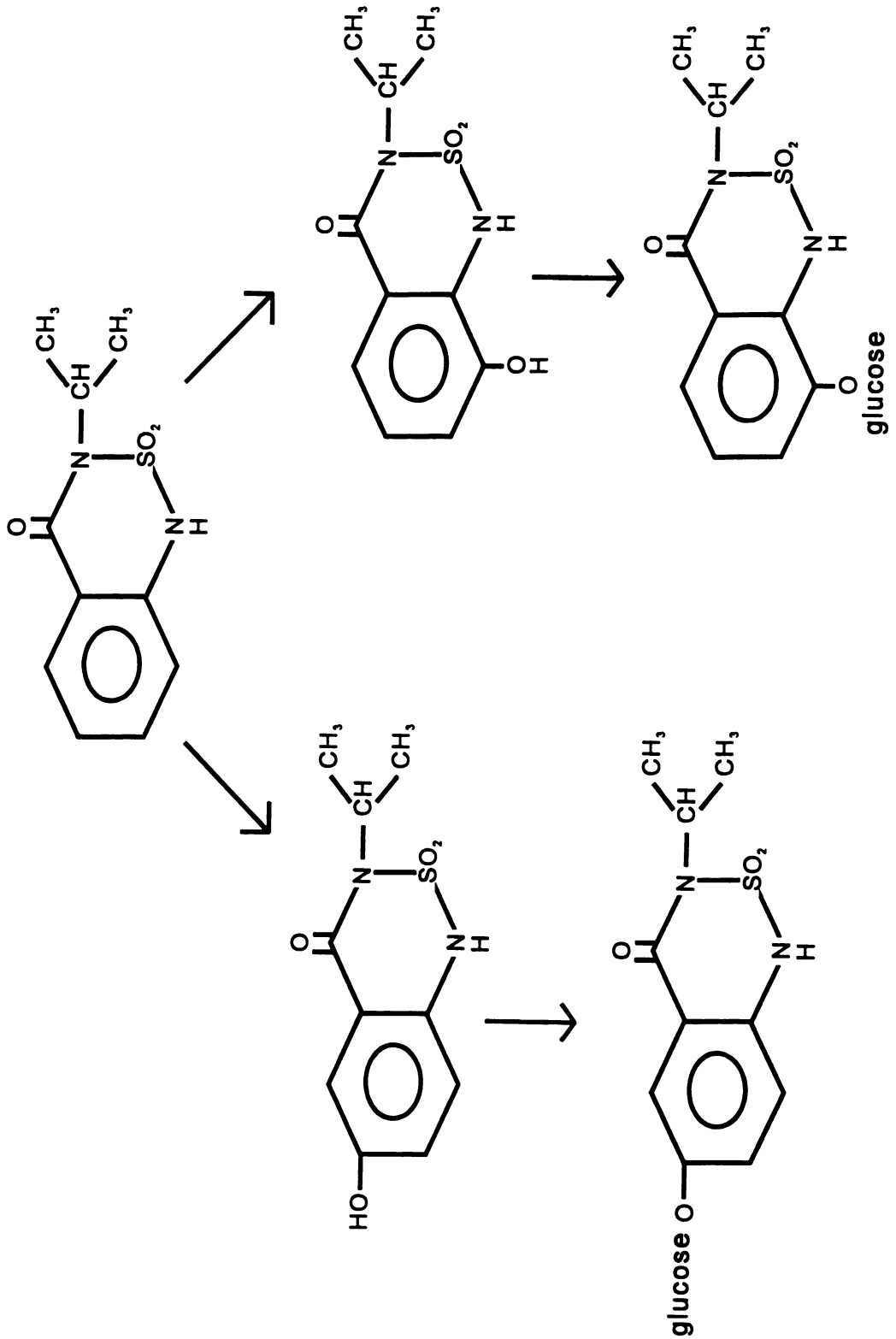
imazethapyr

5-hydroxyethyl-imazethapyr



glucose conjugate

Figure 2. Schematic diagram of bentazon metabolism in tolerant higher plants. Structures of bentazon and its metabolites are (top) bentazon, (upper left) 6-OH-bentazon, (lower left) 6-O-ß-glucose-bentazon, (upper right) 8-OH-bentazon, and (lower right) 8-O-ß-glucose-bentazon (Modified from Sterling and Balke (61)).


CH3
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glucose
HO
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'Olathe' Pinto Bean (*Phaseolus vulgaris*) Response to Postemergence Imazethapyr and Bentazon[1]

T. A. BAUER, K. A. RENNER, and D. PENNER[2]

Abstract. Dry bean producers have limited postemergence broadleaf weed control options. Research determined whether bentazon increased dry edible bean tolerance to postemergence imazethapyr applications. Imazethapyr and bentazon were applied with POA in a factorial arrangement to 'Olathe' pinto bean in field and greenhouse research. In a separate study, imazethapyr with various adjuvants was applied alone and in combination with bentazon and pinto bean response observed. Imazethapyr visually injured pinto bean 7 DAT in both field and greenhouse research. Chlorophyll *a* content, a quantitative measure of bean chlorosis, decreased compared to the untreated control following imazethapyr application. Chlorophyll *a* content decreased with imazethapyr plus POA or Dash, but not when imazethapyr was applied with Sunit II or Sylgard 309. When 840

[1]Received for publication _____ and in revised form _____.

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g ha^{-1} of bentazon was tank-mixed with 53 g ha^{-1} of imazethapyr, visual bean injury decreased and chlorophyll *a* increased as compared to imazethapyr alone in both studies. Fifty three g ha^{-1} of imazethapyr delayed physiological maturity by 8 and 15 days compared to the untreated control in 1991 and 1992, respectively. Pinto bean seed yields, however were not reduced compared to the untreated control. When 840 g ha^{-1} of bentazon was tankmixed with 53 g ha^{-1} of imazethapyr, maturity was not delayed. ^{14}C-Imazethapyr absorption decreased by more than 40% and translocation from the treated leaf by more than 50% when tank-mixed with bentazon compared to ^{14}C-imazethapyr alone. The addition of 20 mM Na-acetate inhibited absorption of ^{14}C-imazethapyr, but did not inhibit translocation. The decreased absorption and translocation of imazethapyr when tank-mixed with bentazon likely accounts for the safening effect observed in greenhouse and field studies.

Nomenclature: Dry bean (*Phaseolus vulgaris* L.) #[3] PHAVU; bentazon, [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-(4(3*H*)-one 2,2 dioxide]; imazethapyr, 2-[4,5-dihydro-4-methyl-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid.

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[3]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

Additional index words. Interaction, antagonism, PHAVU, AMARE, ABUTH.

Introduction

With the recent loss of chloramben (3-amino-2,5-dichlorobenzoic acid), dry edible bean producers lack herbicide options for broadleaf weed control[4]. Bentazon is the only postemergence broadleaf weed control option available. Dry edible beans are tolerant of bentazon (11, 22), which controls cocklebur (*Xanthium strumarium* L.), jimsonweed (*Datura strumarium* L.), velvetleaf (*Abutilon theophrasti* Medicus), and common ragweed (*Ambrosia artemisiifolia* L.). However, bentazon does not control redroot pigweed (*Amaranthus retroflexus* L.) or eastern black nightshade (*Solanum ptycanthum* Dun.) (22), two common weed species in dry edible beans.

Imazethapyr effectively controls redroot pigweed, eastern black nightshade, and common cocklebur. The activity on these weed species could complement bentazon's spectrum of control when tank-mixed. Soybeans and other legumes are tolerant of postemergence applications of imazethapyr (5, 18, 21). However, dry edible beans have shown susceptibility (20). Early season injury symptoms include stunting, leaf crinkling, and interveinal chlorosis. Soybeans and dry beans first convert imazethapyr to 5-hydroxyethyl-imazethapyr and then to the glucose

[4]Amiben's loss limits dry bean herbicide options. Prairie Farmer. Jan. 2, 1990. pp 8-9.

conjugate (1). The conversion to the glucose conjugate is faster in soybean than in dry bean, which may result in the lower dry bean tolerance observed since 5-hydroxyethyl-imazethapyr is herbicidally active[5]. However, 5-hydroxyethyl-imazethapyr is five times less herbicidally active than imazethapyr[6].

Reductions in weed control from the addition of bentazon to imazethapyr applications have been reported (3). Numerous papers have been published on the antagonized grass control resulting when bentazon is applied jointly with sethoxydim (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) (2, 8, 14). Wanamarta and Penner (1989) concluded that reduced quackgrass control was due to inhibited absorption of ^{14}C-sethoxydim in the presence of the sodium salt of bentazon. The absorption reduction caused by Na-bentazon was mimicked by mixing Na-acetate with ^{14}C-sethoxydim. Conversely, UAN increased absorption of ^{14}C-sethoxydim in the presence of both the sodium salt of bentazon and Na-acetate (12, 19).

In preliminary research (13), bentazon reduced injury to dry edible beans when tank-mixed with imazethapyr compared to imazethapyr alone. Twenty eight percent UAN increased dry bean response from 12 to 24% as compared to POA (13). The objectives of this research were to (1) determine if bentazon improved

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[5]Personal communication. Dale Shaner, American Cyanamid Co., Princeton, NJ 08540.

[6]AC-263-499 Technical Information Report. 1985. American Cyanamid Co., Princeton, NJ 08540.

dry edible bean tolerance to imazethapyr in greenhouse and field studies, (2) determine if adjuvant selection influenced dry edible bean response, (3) determine if bentazon decreased absorption and/or translocation of ^{14}C-imazethapyr when the two herbicides were tank-mixed, and (4) assess if Na-acetate influenced the absorption and/or translocation of ^{14}C-imazethapyr similar to bentazon, since it is postulated than the Na-ion in the bentazon formulation inhibits absorption of some herbicides (14, 19).

Materials & Methods

Greenhouse Study. Olathe pinto bean seed were planted in BACCTO[7] greenhouse potting soil in 946 ml plastic pots. Environmental conditions were maintained at 25 C ± 4 C, and plants were grown in a 16 h photoperiod of natural and supplemental metal halide lighting with a midday photosynthetic photon flux density of 1000 μE m^{-2} s^{-1}. After emergence, plants were thinned to one per pot. Plants were surface watered as needed and fertilized weekly with 0.1 g of water soluble fertilizer solution (20% N, 20% P_2O_5, 20% K_2O). All herbicide treatments were applied postemergence with a continuous belt-link sprayer equipped with a

[7]Baccto is a product of Michigan Peat Co. Houston, TX 77098.

single 8001 even flat fan nozzle[8] calibrated to deliver 205 L ha^{-1} at a spray pressure of 210 kPa. Belt speed was set at 1.5 km h^{-1}. Olathe pinto bean was treated at the 1st trifoliolate leaf stage (9 cm).

Imazethapyr at 0, 53, 106, and 212 g ha^{-1}, bentazon at 0, 420, 840, and 1680 g ha^{-1}, and their combinations were applied to Olathe pinto bean to observe sensitivity. Treatments were applied in a factorial arrangement with 1.2 L ha^{-1} POA[9]. Olathe was selected as the dry bean variety since preliminary studies (data not reported) demonstrated that this variety was particularly sensitive to imazethapyr. All pots were arranged in a completely randomized design and the experiments were repeated twice in time with four replications. Data were subjected to ANOVA. Interactions were not present between experiments and treatments, and therefore data were combined over time. For the herbicide combinations, the expected herbicide injury value was calculated following Colby's method (4). This calculation was determined for greenhouse data only since the expected value is most useful when approximating the IG_{50} value (4). Mean comparisons were made using Fisher's Protected $LSD_{\alpha=0.05}$.

Injury to Olathe pinto bean was visually evaluated 7 DAT on a scale ranging from 0 (no visible injury) to 10 (total plant necrosis). To quantitatively measure

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[8]Teejet flat fan tips. Spraying Systems Co., North Ave. and Schmale Road, Wheaton, IL 60188.

[9]Herbimax, 83% petroleum oil, 17% adjuvant, Loveland Industries, Inc. Greeley, CO 80632.

pinto bean chlorosis, three leaf discs, 6.5 mm in diameter, were harvested from the middle leaflet of the third trifoliolate from each bean plant. Chlorophyll was extracted with 3 ml of *N*,*N*-dimethylformamide (10). Total chlorophyll, chlorophyll *a*, and chlorophyll *b* levels were determined using UV-VIS spectrophotometry as described by Inskeep and Bloom (1985). Through statistical analysis, chlorophyll *a* levels were determined to be the most sensitive indicator of herbicide injury, and only chlorophyll *a* levels will be reported. Trifoliolate dry weights were measured as an additional indication of herbicide injury. The trifoliolates were cut next to the stem and dried. The first trifoliolate was not included.

Field Study. Field experiments were conducted in 1991 and 1992. Plots were maintained weed-free the entire season to eliminate the confounding factor of weed interference on pinto bean maturity and yield. Soil characteristics and locations for each experiment are summarized in Table 1.

Seedbed preparation consisted of fall moldboard plowing followed by two passes with a Danish S-tine field cultivator[10] in the spring, the second pass perpendicular to the first pass in both years. Pinto beans were planted June 13, 1991 and June 9, 1992 in plots 2.8 m by 6.1 m with crop row spacings of 71 cm at a seeding rate of 172,000 seeds ha^{-1}. Herbicide treatments were applied on July

[10]Kongskilde. Kongskilde Corp. Bowling Green, OH 43402.

3, 1991 and July 6, 1992 when beans were at the second trifoliolate growth stage.

Pinto bean injury was measured 7 and 14 DAT on a scale ranging from 0 (no visible injury) to 100 (total plant necrosis). To quantitatively measure pinto bean chlorosis 7 DAT, a single leaf disc 6.5 mm in diameter was harvested from the middle leaflet of the third trifoliolate from three randomly selected bean plants in the middle two rows of each plot. Chlorophyll was extracted as previously described for the greenhouse studies. The number of growing days required to reach physiological maturity was recorded. Plots were considered physiologically mature when 90% of the pods had turned from green to a golden-bronze color. Yields were measured by hand harvesting two, 4.6 m lengths from the middle two rows of each plot. Yields were adjusted to 18% moisture.

Imazethapyr was applied at 0, 53, 106, and 212 g ha^{-1}, while bentazon was applied at 0, 420, 840, and 1680 g ha^{-1}. All postemergence herbicides were applied with a tractor-mounted compressed air sprayer in a total volume of 205 L ha^{-1} at a spray pressure of 210 kPa. The boom was adjusted to 61 cm above the soil surface and equipped with 8002 flat fan nozzles spaced 51 cm apart. Herbicide treatments were applied in a factorial arrangement with 1.2 L ha^{-1} POA. Treatments were arranged in a randomized complete block design with four replications. All data were subjected to ANOVA. Year by treatment interactions were present so 1991 and 1992 data are presented separately. Means comparisons were made by Fisher's Protected $LSD_{\alpha=0.05}$.

Adjuvant study. Soil characteristics for 1991 and 1992 field experiments are summarized in Table 1. Prior to planting, the soil was moldboard plowed in the spring followed by a single pass with a Danish S-tine field cultivator. Olathe pinto beans were planted on June 4, 1991 and June 12, 1992 in 3 m by 7.6 m plots with crop row spacings of 76 cm at a seeding rate of 203,000 seeds ha^{-1} and 172,000 seeds ha^{-1} in 1991 and 1992, respectively. Herbicide treatments were applied on June 24, 1991 and July 6, 1992 when beans were at the second trifoliolate growth stage.

Visual injury to Olathe pinto bean was measured 7 DAT on a scale ranging from 0 (no visible injury) to 100 (total plant necrosis). To quantitatively measure pinto bean chlorosis 7 DAT, a single leaf disc 6.5 mm in diameter was harvested from the middle leaflet of the third trifoliolate from three randomly selected bean plants in the middle two rows of each plot. Chlorophyll was extracted as previously described.

Imazethapyr at 0 and 53 g ha^{-1} and bentazon at 0 and 840 g ha^{-1}, and various combinations thereof, were applied with POA, NIS[11], DASH[12], MSO[13], and

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[11]X-77 Non-Ionic Surfactant. A mixture of alkylarylpolyoxyethleneglycols, free fatty acids and isopropanol. Valent U.S.A. Corp., Walnut Creek, CA 94956.

[12]Dash is a commercial adjuvant product marketed by BASF, 100 Cherry Hill Rd., Parsippany, NJ 07054.

[13]Sunit II methylated seed oil marketed by American Cyanamid Co., Princeton, NJ 08540.

Sylgard 309[14]. All postemergence herbicides were applied with a tractor-mounted compressed air sprayer in a total volume of 205 L ha^{-1} at a spray pressure of 210 kPa. The boom was adjusted to 61 cm above the soil surface and equipped with 8002 flat fan nozzles spaced 51 cm apart. Herbicide treatments were applied in a factorial arrangement with the various adjuvants. Treatments were arranged in a randomized complete block design with four replications. All data were subjected to ANOVA. Year by treatment interactions were present so 1991 and 1992 data are presented separately. Mean comparisons were made by Fisher's Protected $LSD_{\alpha=0.05}$.

^{14}C-Imazethapyr absorption and translocation studies. ^{14}C-Imazethapyr (pyridine ring labelled, 6th position with specific activity = 784.4 MBq/g) absorption alone and tank-mixed with bentazon was evaluated. Olathe pinto bean was planted in BACCTO greenhouse soil in 946 ml plastic pots. All experiments were conducted in growth chambers with day/night temperatures of 26/22 C. Chambers were maintained at 68% relative humidity with a 16 h photoperiod from fluorescent and incandescent lighting with a photosynthetic photon flux density of 750 μE m^{-2} s^{-1}. Plants were top-watered as needed. A 2 μL drop containing 370

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[14]Sylgard 309 is a silicon adjuvant mixture of 2-(3-hydroxypropyl)-heptamethyltrisiloxane, ethyloxylated, acetate EO glycol, -allyl, -acetate marketed by Dow Corning Corp., Midland, MI 48686.

Bq of ^{14}C-imazethapyr was applied with a micro-syringe[15] between the leaflet mid-vein and the edge of the leaflet approximately ⅓ of the distance from the leaflet tip to the base of the leaflet. Special care was taken to not place the spot on a leaf vein. The spotting solution contained ^{14}C-imazethapyr with the appropriate amounts of formulation blank, unlabelled commercial imazethapyr[16], commercial bentazon[17] (when appropriate) POA, and water to simulate a spray solution containing imazethapyr at 53 g ha^{-1}, bentazon at 840 g ha^{-1}, and POA at 1.2 L ha^{-1} in a total volume of 205 L ha^{-1}.

The treated leaflet for Olathe pinto bean was the middle leaflet of the first trifoliolate. At 0, 1, 2, 4, 8, 24, and 48 h after treatment (spotting), the leaflet was excised and rinsed in a 20 ml glass scintillation vial containing 3 ml of methanol : H_2O (2:1 v/v), and gently swirled for 30 s. The leaflet was then washed with a minimal amount of rinsing solution and then 15 ml of scintillator[18] was added. The unabsorbed ^{14}C-imazethapyr was quantified by liquid scintillation spectroscopy (LSS)[19] and absorption determined by subtracting the amount of ^{14}C-

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[15]Hamilton microsyringe. Hamilton Co. Reno, NV 89520-0012.

[16]Pursuit 2AS herbicide. American Cyanamid Co. Princeton, NJ 08540.

[17]Basagran 4L, BASF Corp. Parsippany, NJ 07054.

[18]Safety-Solve. Research Products International Corp. Mount Prospect, IL 60056.

[19]Model 1500. Packard Instrument Corp. Downers Grove, IL 60515.

imazethapyr recovered in the washoff solution from the amount of ^{14}C-imazethapyr applied.

The treated leaflet was saved and combusted in a biological oxidizer[20]. The CO_2 was trapped in a solution of scintillator : CO_2 absorber[21] (2:1 v/v). Total radioactivity in the sample was then determined by LSS. The amount of ^{14}C-imazethapyr in the treated leaflet was calculated by subtracting the amount of ^{14}C recovered in the treated leaf from what was absorbed. This amount was then subtracted from 100% to calculate ^{14}C-imazethapyr translocated from the treated leaflet.

Effect of Na-acetate and UAN on ^{14}C-imazethapyr absorption and translocation. ^{14}C-Imazethapyr was applied alone and with various combinations of commercial formulated bentazon, 20 mM Na-acetate, and 4% (v/v) UAN. All treatments were applied with the appropriate amount of formulation blank, unlabelled commercial imazethapyr, commercial bentazon, POA, and water to simulate a spray solution containing imazethapyr at 53 g ha^{-1}, bentazon at 840 g ha^{-1}, and POA at 1.2 L ha^{-1} in a total volume of 205 L ha^{-1}. All absorption and translocation measurements were carried out as outlined above except only 24 h measurements were recorded.

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[20]OX-300. R. J. Harvey Instrument Corp. Patterson, NJ 07642.

[21]Carbo-Sorb II. Packard Instrument Co. Meriden, CT 06450.

All pots for the absorption and translocation studies were arranged in a completely randomized design with five replications. Absorption and translocation experiments were conducted at least twice. All data were subjected to ANOVA and data were combined over time. Mean comparisons were made by Fisher's Protected $LSD_{\alpha=0.05}$.

^{14}C-Imazethapyr absorption and translocation from the treated leaf studies were subjected to curvilinear regression analysis and coefficient values determined (15). The absorption and translocation data were fit to the curvilinear equation Y = (I*X)/(1+(I/A)*X), where Y and X are the Y - axis and X - axis coordinates, I is the percentage absorption as time approaches 0, and A is the percentage absorption as time approaches infinity. This type of regression equation is useful to explain absorption trends.

Results and Discussion

Greenhouse studies. Bentazon did not cause any visual injury to pinto bean 7 DAT (Table 2). When imazethapyr was applied at 53 g ha^{-1}, injury increased to 2 (scale of 0 to 10). When bentazon was tank-mixed with 53 g ha^{-1} of imazethapyr (1X application rate), injury was not greater than that of the untreated control.

Injury from imazethapyr at 106 and 212 g ha^{-1} (2X and 4X application rates)

increased to 2.5 and 4.5, respectively. Again, pinto bean injury decreased when bentazon was tank-mixed as compared to each respective rate of imazethapyr alone (Table 2). Colby's expected values indicated strong antagonism (safening) with all tank-mixes.

Trifoliolate dry weights followed the same pattern. Bentazon did not decrease dry trifoliolate weight, while imazethapyr at 1X, 2X, and 4X application rates decreased trifoliolate dry weights to 27, 45, and 45 %, respectively, compared to the untreated control. Trifoliolate dry weights were greater than Colby's expected values with each tank-mix combination, demonstrating the safening effect of bentazon when applied with imazethapyr on pinto bean.

Chlorophyll *a* measurements quantitatively determined pinto bean chlorosis. Chlorophyll *a* was reduced from bentazon alone at 840 and 1680 g ha^{-1} and all imazethapyr applications. The observed chlorophyll *a* levels were greater than Colby's expected values except when 420 g ha^{-1} of bentazon was tank-mixed with either 53 or 212 g ha^{-1} imazethapyr, which demonstrated less pinto bean injury when bentazon was tank-mixed.

Imazethapyr caused significant visual injury, trifoliolate dry weight reduction, and loss of chlorophyll *a* compared to the untreated control or bentazon. When bentazon was tank-mixed with imazethapyr, visual injury decreased, trifoliolate weight increased, and chlorophyll *a* levels increased over the expected values compared to the untreated control or bentazon alone, demonstrating that bentazon

safened pinto bean response to imazethapyr.

Field Studies. In 1991, postemergence bentazon did not visually injure Olathe pinto bean 7 DAT, decrease chlorophyll *a* levels, delay maturity, or influence seed yields (Table 3). Imazethapyr at 53 g ha^{-1} (1X use rate) caused 8% injury 7 DAT. These injury symptoms included leaf chlorosis, plant stunting, and proliferation of secondary shoots from plant nodes. The 7 DAT injury for the tank-mix application of imazethapyr at the 1X rate plus bentazon at the 1X rate was not different than that of the untreated control. Chlorophyll *a* levels decreased from 53 g ha^{-1} of imazethapyr compared to the untreated control. However, when 840 g ha^{-1} of bentazon was tank-mixed with 53 g ha^{-1} of imazethapyr, chlorophyll *a* levels did not differ significantly from the untreated control. Olathe pinto bean required 78 days to reach physiological maturity, and 86 days following the 1X rate of imazethapyr. Bentazon tank-mixed with imazethapyr decreased the days to maturity to that of the untreated control. No herbicide combination decreased dry bean seed yields in 1991.

In 1992, bentazon did not cause significant dry bean injury, decrease chlorophyll *a* levels, delay maturity, or reduce seed yield, indicating Olathe pinto bean tolerance to bentazon. At 7 DAT, 53 g ha^{-1} imazethapyr caused 13% visual injury (Table 4). When any rate of bentazon was tank-mixed with 53 g ha^{-1} of imazethapyr, pinto bean injury decreased to that of the untreated control. By 14

DAT, pinto bean response to the 1X rate of imazethapyr was not evident. However, injury at the 2X and 4X rates remained at 15 and 33%, respectively. Tank-mixing any rate of bentazon with either the 2X or 4X rate of imazethapyr decreased pinto bean injury.

Imazethapyr at all rates decreased chlorophyll *a* levels compared to the untreated control. Olathe pinto bean required 86 days to reach physiological maturity in 1992, 8 days longer than the previous year. This was primarily due to the less favorable growing season (cooler temperatures) in 1992. Imazethapyr applied at the 1X rate increased the days to reach physiological maturity to 101 days. When any rate of bentazon was tank-mixed with the 1X rate of imazethapyr the days required to reach physiological maturity decreased to that of the untreated control. Imazethapyr alone at 106 and 212 g ha^{-1} decreased seed yields as compared to the untreated control. When bentazon at 840 or 1680 g ha^{-1} was tank-mixed with 106 g ha^{-1} of imazethapyr, seed yields were not reduced.

In both growing seasons, imazethapyr caused significant early season injury and delayed maturity of pinto beans. The observed injury was more prominent in 1992 than 1991. Only in 1992 were the dry bean seed yields reduced from 106 or 212 g ha^{-1} of imazethapyr compared to the untreated control. However, in both years when 840 g ha^{-1} of bentazon was tank-mixed with 53 g ha^{-1} of imazethapyr, early season injury was reduced and late season injury (maturity delay and reduced seed yields) was not observed.

Adjuvant study. In 1991, POA, Dash, and Sunit II applied with imazethapyr caused more pinto bean injury 7 DAT than NIS (Table 5). All adjuvants applied with imazethapyr in 1992 caused similar injury. Less pinto bean injury was observed 7 DAT in both years when bentazon was tank-mixed with imazethapyr. In 1991, chlorophyll *a* levels were reduced by imazethapyr plus POA, Dash, or NIS. In 1992, chlorophyll *a* levels were reduced by imazethapyr plus Dash or NIS. Regardless of adjuvant, bentazon safened pinto bean to imazethapyr applications.

^{14}C-Imazethapyr absorption and translocation in Olathe pinto bean. At 1, 2, 4, 8, and 24 h after treatment, the presence of bentazon in the spray solution decreased absorption of ^{14}C-imazethapyr in dry edible bean as compared to ^{14}C-imazethapyr alone (Figure 1). By 8 h after treatment, ^{14}C-imazethapyr absorption when applied alone was greater than 75% of applied. The asymptotic value for ^{14}C-imazethapyr absorption was 82% of applied. By contrast, when ^{14}C-imazethapyr was tank-mixed with bentazon, absorption at 8 h after treatment was less than 45% of applied and the asymptotic value was 48%. At 8 h or greater, ^{14}C-imazethapyr absorption decreased by $\approx$ 40% when tank-mixed with bentazon compared to ^{14}C-imazethapyr applied alone.

More ^{14}C-imazethapyr was translocated from the treated leaf at 4, 8, 24, and 48 h after treatment when applied alone versus tank-mixed with bentazon (Figure

2). At 24 and 48 h after treatment, 28% and 38% of absorbed ^{14}C-imazethapyr, respectively, translocated from the treated leaf. Translocation from the treated leaf decreased to 12% and 17% of absorbed at 24 and 48 h after treatment, respectively, when bentazon was tank-mixed with ^{14}C-imazethapyr. This is greater than a 50% reduction in ^{14}C translocation from the treated leaf compared to that of ^{14}C-imazethapyr applied alone.

The reduction in translocation of ^{14}C-imazethapyr from the treated leaf by tank-mixing with bentazon may also contribute to the safening in Olathe pinto bean. The translocation of a phloem mobile herbicide, such as imazethapyr, may be inhibited when the production and translocation of photoassimilate is also inhibited (6). Bentazon is known to reduce photoassimilate production and translocation by inhibition of electron transport in photosystem II (7, 17). It is therefore reasonable to assume that bentazon will reduce the phloem transport of imazethapyr.

Bentazon decreased both absorption and translocation of imazethapyr in Olathe pinto bean. Bentazon has been noted to decrease absorption of both sethoxydim and acifluorfen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid) (14, 16, 19). The decrease in absorption and translocation of imazethapyr apparently accounts for the safening effect that bentazon exerts on imazethapyr when applied postemergence to Olathe pinto bean in greenhouse and field studies.

Effect of Na-acetate and UAN on ^{14}C-imazethapyr absorption and translocation. When ^{14}C-imazethapyr was applied alone, 82% of the herbicide was absorbed by 24 h (Table 6). When bentazon or Na-acetate was tank-mixed with ^{14}C-imazethapyr, absorption decreased to 61% and 58%, respectively. UAN did not increase absorption over that of ^{14}C-imazethapyr applied alone, but overcame the decreased ^{14}C-imazethapyr absorption when bentazon or Na-acetate were in the solution.

When ^{14}C-imazethapyr was applied alone, 29% of the absorbed herbicide was translocated from the treated leaf. Bentazon tank-mixed with ^{14}C-imazethapyr decreased translocation to 18%. Neither UAN or Na-acetate altered translocation when applied with bentazon and ^{14}C-imazethapyr or with ^{14}C-imazethapyr alone.

Previous research (19) has shown that Na-acetate can inhibit the absorption of sethoxydim similar to the inhibited absorption caused by bentazon. Similarly, ^{14}C-imazethapyr absorption decreased with the addition of 20 mM Na-acetate similar to the addition of bentazon. The addition of UAN to each tank-mix previously mentioned reversed the decreased absorption observed (19).

Imazethapyr can cause early season visual injury as well as delay maturity of Olathe pinto beans. However, when bentazon is tank-mixed with imazethapyr, early and late season injury (maturity delay and decreased seed yields) was not observed. Studies utilizing ^{14}C-imazethapyr indicate that formulated bentazon decreases both absorption and translocation of imazethapyr, resulting in increased

tolerance of Olathe pinto bean to imazethapyr. However, Na-acetate did not alter translocation as did bentazon. We concur with Wanamarta and Penner who hypothesized that the Na-ion, with which bentazon is formulated, disassociates from bentazon and associates with imazethapyr, altering the polarity of this molecule and decreasing its absorption (12, 19). This decreased absorption can be overcome by the use of UAN which supports this hypothesis.

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Table 1. Soil characteristics of field studies conducted in 1991 and 1992.

	Year	
Study	1991	1992
Pinto Bean Herbicide Tolerance	sandy clay loam 2.9% OM pH 7.1 51% sand 19% silt 30% clay Parkhill sandy clay loam (Mollic Haplaquept, fine-loamy, mixed, mesic)	clay 2.4% OM pH 6.5 23% sand 16% silt 61% clay Misteguay clay (Aeric Haplequept, fine-loamy, mixed, mesic)
Adjuvant Study	loamy sand 1.7% OM pH 5.5 82% sand 10% silt 7% clay Capac loamy sand (Aeric Ochraqualfs, fine-loamy, mixed, mesic)	sandy clay loam 3.4% OM pH 7.0 55% sand 24% silt 21% clay Capac sandy clay loam (Aeric Ochraqualfs, fine-loamy, mixed, mesic)

Table 2. Olathe pinto bean response to bentazon and imazethapyr applied alone and in combination in the greenhouse. A * indicates significant antagonism when comparing the observed data to the predicted value[a].

Treatment	Rate	Visual injury 7 DAT[b]		Dry trifoliolate wt		Chlorophyll *a*	
	--g ha^{-1}--			----g----		-----mg L^{-1}-----	
Untreated	- -	0		1.1		11.4	
Bentazon	420	0		1.0		10.9	
Bentazon	840	0		1.1		9.6	
Bentazon	1680	0		1.1		8.6	
Imazethapyr	53	2		0.8		8.1	
Imep+bent[c]	420	0	(2)*	0.9	(0.7)*	8.3	(7.2)
Imep+bent	53+840	0	(2)*	1.0	(0.8)*	7.9	(6.1)*
Imep+bent	53+1680	0	(2)*	1.0	(0.8)*	8.3	(5.3)*
Imazethapyr	106	2.5		0.6		6.3	
Imep+bent	106+420	1	(2.5)*	0.9	(0.6)*	7.6	(5.9)*
Imep+bent	106+840	1	(2.5)*	0.9	(0.6)*	8.0	(5.2)*
Imep+bent	106+1680	0	(2.5)*	1.0	(0.7)*	8.0	(4.6)*
Imazethapyr	212	4.5		0.6		5.8	
Imep+bent	212+420	3	(4.5)*	0.7	(0.5)*	6.4	(5.5)
Imep+bent	212+840	2	(4.5)*	0.8	(0.6)*	6.9	(4.8)*
Imep+bent	212+1680	1.5	(4.5)*	0.7	(0.6)*	8.7	(4.3)*
$LSD_{\alpha=0.05}$		0.5		0.1		1.7	

[a]The predicted values, enclosed in parenthesis, are calculated using Colby's method.

[b]Visual injury was rated on a scale of 0 (no visible injury) to 10 (total plant necrosis).

[c]Imep and bent are the approved codes for imazethapyr and bentazon, respectively. 1991. NCWSS Proc. 46:164.

Table 3. Response of Olathe pinto bean to bentazon and imazethapyr applied alone and in combination in 1991 in the field.

Treatment	Rate	7 DAT	14 DAT	Chlorophyll *a*	Physiological maturity	Seed yields
	--g ha^{-1}--	--% visual injury--		mg L^{-1}	days	kg ha^{-1}
Untreated	- -	0	0	5.1	78	2690
Bentazon	420	0	0	5	79	2810
Bentazon	840	0	0	4.9	79	2190
Bentazon	1680	3	3	5.4	79	2440
Imazethapyr	53	8	5	4.3	86	2270
Imep+bent	53+420	3	3	4.7	78	1990
Imep+bent	53+840	0	0	4.7	81	2620
Imep+bent	53+1680	5	3	4.8	81	2340
Imazethapyr	106	8	5	3.5	90	2190
Imep+bent	106+420	3	3	4.2	82	2310
Imep+bent	106+840	5	3	4.2	80	1984
Imep+bent	106+1680	5	3	4.1	80	2174
Imazethapyr	212	15	13	3.4	91	1900
Imep+bent	212+420	10	5	3.6	87	1990
Imep+bent	212+840	5	5	4.1	86	2210
Imep+bent	212+1680	5	3	4.1	85	2020
$LSD_{\alpha=0.05}$		3	3	0.9	4	NS

Table 4. Response of Olathe pinto bean to bentazon and imazethapyr applied alone and in combination in 1992 in the field.

Treatment	Rate	7 DAT	14 DAT	Chlorophyll *a*	Physiological maturity	Seed yield
	--g ha^{-1}--	--% visual injury--		mg L^{-1}	days	kg ha^{-1}
Untreated	- -	0	0	3.9	86	2520
Bentazon	420	0	0	4.3	86	2180
Bentazon	840	0	0	3.9	87	2190
Bentazon	1680	0	0	4.3	88	2120
Imazethapyr	53	13	5	3.3	101	2280
Imep+bent	53+420	3	3	3.8	87	1750
Imep+bent	53+840	3	0	4.2	87	2190
Imep+bent	53+1680	0	0	4.2	87	1940
Imazethapyr	106	18	15	2.8	103	1880
Imep+bent	106+420	10	5	3.3	93	1900
Imep+bent	106+840	10	5	3.3	91	2060
Imep+bent	106+1680	10	5	3.3	88	2060
Imazethapyr	212	28	33	2.5	110	1380
Imep+bent	212+420	20	18	2.4	103	1400
Imep+bent	212+840	18	18	2.8	103	1610
Imep+bent	212+1680	18	13	2.7	98	1550
$LSD_{\alpha=0.05}$		3	3	0.6	3	660

Table 5. Response of Olathe pinto bean to bentazon and imazethapyr applied alone and in combination in the field as influenced by various adjuvants.

Treatment	1991			1992		
	7 DAT	14 DAT	chl *a*	7 DAT	14 DAT	chl *a*
	-------% injury-------		mg L^{-1}	-------% injury-------		mg L^{-1}
Imazethapyr + POA (1.2 L)	23	5	2.9	15	8	2.6
Imazethapyr + POA (2.4 L)	20	5	3.0	15	8	2.5
Imazethapyr + NIS	15	5	2.9	15	8	2.0
Imazethapyr + Dash	20	8	2.9	15	8	2.0
Imazethapyr + Sunit II	23	8	3.4	15	10	2.4
Imazethapyr + Sylgard 309	18	5	3.5	18	8	2.8
Bentazon + POA (1.2 L)	0	0	4.6	0	0	3.6
Bentazon + POA (2.4 L)	0	0	4.5	0	3	3.2
Bentazon + NIS	0	0	4.0	0	0	3.6
Bentazon + Dash	0	0	4.2	0	0	2.9
Bentazon + Sunit II	0	0	3.9	0	0	3.9
Bentazon + Sylgard 309	0	0	4.4	0	0	3.9
Imazethapyr + bentazon + POA (1.2 L)	5	3	4.8	3	0	3.1
Imazethapyr + bentazon + POA (2.4 L)	5	0	3.4	3	0	2.9
Imazethapyr + bentazon + NIS	8	3	4.0	3	0	3.3
Imazethapyr + bentazon + Dash	8	3	3.8	3	0	2.8
Imazethapyr + bentazon + Sunit II	5	3	3.3	5	0	2.7
Imazethapyr + bentazon + Sylgard 309	8	3	4.1	8	5	2.9
Untreated control	0	0	4.1	0	0	3.3
$LSD_{\alpha=0.05}$	5	3	0.9	5	3	0.9

Table 6. The effect of Na-acetate and UAN on ^{14}C-imazethapyr absorption and translocation 24 h after treatment when applied alone and tank-mixed with bentazon. All treatments were applied with the equivalent of 1.2 L ha^{-1} POA.

Treatment	Absorption	Translocation
	--% of applied--	--% of absorbed--
^{14}C-imazethapyr	82	29
^{14}C-imazethapyr + bentazon	61	18
^{14}C-imazethapyr + Na-acetate	58	26
^{14}C-imazethapyr + UAN	92	24
^{14}C-imep + bentazon + UAN	86	19
^{14}C-imep + Na-acetate + UAN	92	22
$LSD_{\alpha=0.05}$	11	5

Figure 1. ^{14}C-Imazethapyr absorption when applied alone and tank-mixed with bentazon in Olathe pinto bean. Regression equation for ^{14}C-imazethapyr when applied alone is $Y = (113*X)/(1+(113/82)*X)$ ($R^2=0.71$) and when tank-mixed with bentazon is $Y = (73*X)/(1+(73/48)*X)$ ($R^2=0.84$). $LSD_{\alpha=0.05}$ bars are centered over data points.

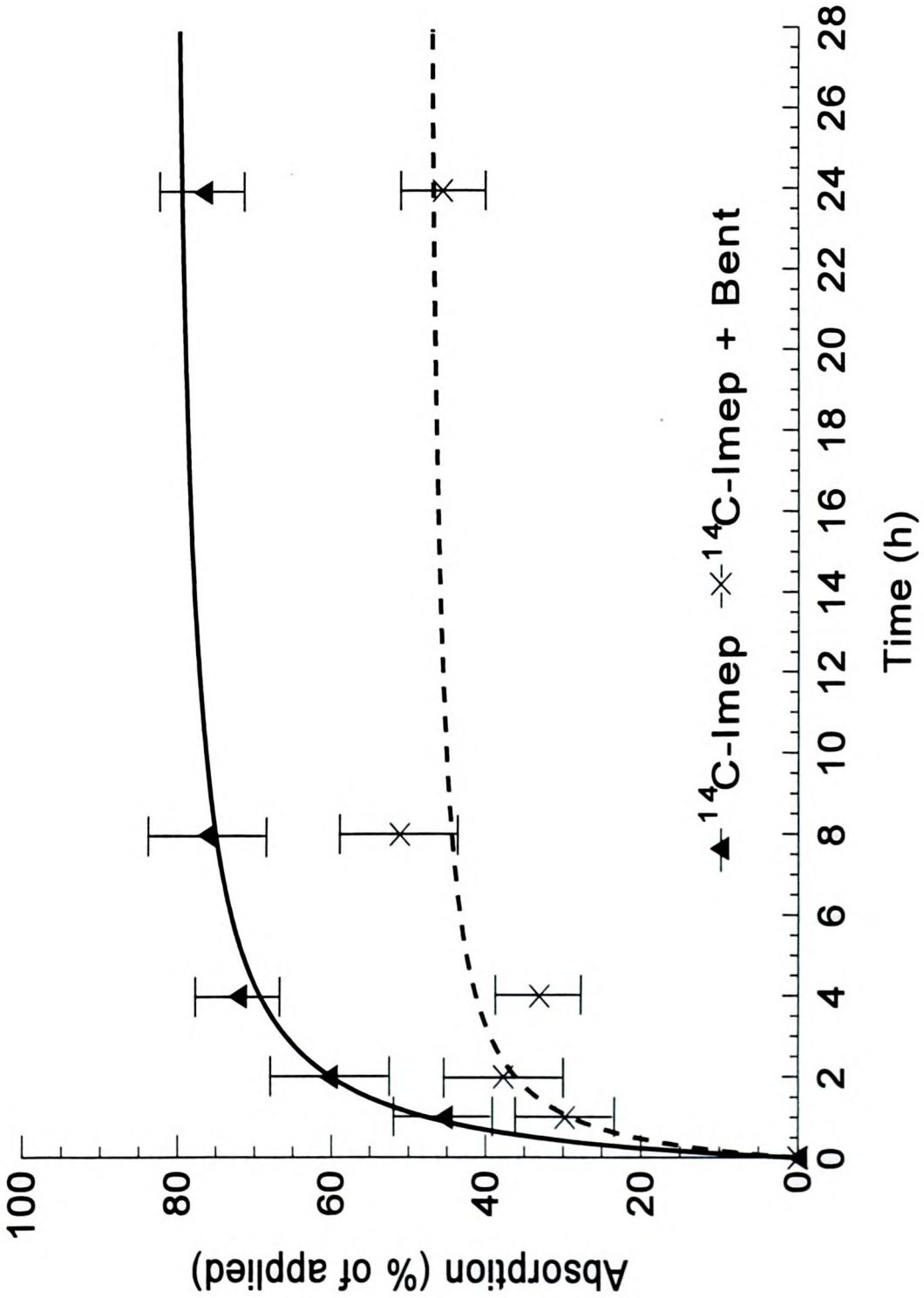
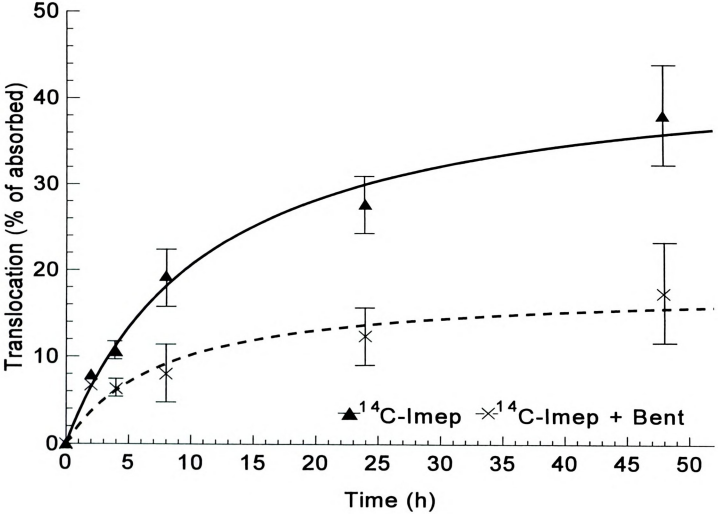

Absorption (% of applied)
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
Time (h)
^{14}C-Imep
^{14}C-Imep + Bent

Figure 2. ^{14}C-Imazethapyr translocation from the treated leaf when applied alone and tank-mixed with bentazon in Olathe pinto bean. Regression equation for ^{14}C-imazethapyr when applied alone is $Y = (3.7*X)/(1+(3.7/45)*X)$ ($R^2=0.82$) and when tank-mixed with bentazon is $Y = (2.3*X/(1+(2.3/18)*X)$ ($R^2=0.74$). $LSD_{\alpha=0.05}$ bars are centered over data points.


Translocation (% of absorbed)
Time (h)
0
10
20
30
40
50
0
5
10
15
20
25
30
35
40
45
50
14C-Imep
14C-Imep + Bent

Response of Selected Weed Species to Postemergence Imazethapyr and Bentazon[1]

T. A. BAUER, K. A. RENNER, and D. PENNER[2]

Abstract. Imazethapyr and bentazon were applied with POA in a factorial arrangement to weed species in greenhouse and field research to determine if postemergence weed control by imazethapyr was antagonized when bentazon was tank-mixed. Tank-mixing 840 g ha^{-1} of bentazon with 13 or 27 g ha^{-1} of imazethapyr increased redroot pigweed and eastern black nightshade dry weight as compared to Colby's expected values in the greenhouse. However, weed control was not antagonized in field studies. Subsequent greenhouse studies indicated that soil interception and resulting root uptake of imazethapyr increased redroot pigweed control. Bentazon decreased absorption of ^{14}C-imazethapyr by 15% and translocation from the treated leaf by greater than 50% as compared to ^{14}C-imazethapyr alone.

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[1]Received for publication _____ and in revised form _____.

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Nomenclature: Bentazon, [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-(4(3*H*)-one 2,2 dioxide]; imazethapyr, 2-[4,5-dihydro-4-methyl-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid; dry bean (*Phaseolus vulgaris* L.) #[3] PHAVU; redroot pigweed (*Amaranthus retroflexus* L.) # AMARE; eastern black nightshade (*Solanum ptycanthum* Dun.) # SOLPT; common ragweed (*Ambrosia artemisiifolia* L.) # AMBEL; common lambsquarters (*Chenopodium album* L.) # CHEAL; velvetleaf (*Abutilon theophrasti* Medicus) # ABUTH.

Additional index words. Interaction, antagonism, AMARE, ABUTH.

[3]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

Introduction

Two or more herbicides are often tank-mixed to increase the weed control spectrum over that achieved when each herbicide is applied alone. When herbicides applied in a tank-mix combination act independently and the weed spectrum controlled can be predicted by the performance of each herbicide applied alone, the effect is termed additive (8). However, weed control may not follow the predicted performance. Hatzios and Penner (1985) described an interaction as antagonistic when the observed activity of the combination decreased compared to the performance of each herbicide applied alone. Conversely, an interaction that enhanced biological activity was referred to as synergistic.

Numerous studies have determined herbicide interactions on various weed species. Bentazon applied jointly with sethoxydim (2-[1-(ethoxyimino)butyl]-5-[2-ethylthio)propyl-3-hydroxy-2-cyclohexen-1-one) resulted in antagonized grass control (1, 7, 11). Wanamarta and Penner (1989) concluded that reduced quackgrass (*Elytrigia repens* (L.) Nevski) control was due to inhibited diffusion of ^{14}C-sethoxydim into quackgrass in the presence of the sodium salt of bentazon. Conversely, pitted morningglory (*Ipomoea lacunosa* L.) control increased synergistically from combinations of imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl-3-quinolincarboxylic acid) and imazethapyr

(14). An increase in morningglory control was also observed when imazapyr ((±)-2-[4,5-dihydro-4-methyl-4-(1-methyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid) was applied in combination with either imazaquin or imazethapyr (13, 19).

With the recent loss of chloramben (3-amino-2,5-dichlorobenzoic acid), dry edible bean producers have limited herbicide options for broadleaf weed control[4]. Bentazon is the only postemergence broadleaf weed control option, and dry edible beans are very tolerant of bentazon (9, 22), which controls cocklebur (*Xanthium strumarium* L.), jimsonweed (*Datura strumarium* L.), velvetleaf, and common ragweed. However, bentazon does not control redroot pigweed or eastern black nightshade.

Imazethapyr effectively controls redroot pigweed, eastern black nightshade, and common cocklebur. Activity on these weed species complements the weed control spectrum of bentazon. Soybeans (*Glycine max* L.) and other legumes are tolerant of postemergence applications of imazethapyr (4, 17, 21). However, dry edible beans have shown susceptibility to imazethapyr applications (20). Early season injury symptoms include stunting, leaf crinkling, and interveinal chlorosis.

In earlier research (10), bentazon improved dry edible bean tolerance to postemergence imazethapyr. However, decreased weed control from addition of

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[4]Amiben's loss limits dry bean herbicide options. Prairie Farmer. Jan. 2., 1990. pp 8-9.

bentazon to imazethapyr has been reported (2). The objectives of this research were to (1) investigate whether weed control in greenhouse studies and field studies was enhanced or reduced when imazethapyr and bentazon were tank-mixed, (2) assess if soil interception of imazethapyr increased redroot pigweed control, and (3) determine if tank-mixing bentazon altered absorption and/or translocation of ^{14}C-imazethapyr in redroot pigweed.

Materials and Methods

Greenhouse Studies. Locally collected seed of redroot pigweed, eastern black nightshade, velvetleaf, and common ragweed were planted in BACCTO[5] greenhouse potting soil in 946 ml plastic pots. Environmental conditions were maintained at 25 C $\pm$ 4 C, and plants were grown in a 16 h photoperiod of natural and supplemental metal halide lighting with a midday photosynthetic photon flux density of 1000 μE m^{-2} s^{-1}. After emergence, plants were thinned to one per pot. Plants were surface watered as needed and fertilized weekly with 0.1 g of water soluble fertilizer solution (20% N, 20% P_2O_5, 20% K_2O). All herbicide treatments were applied postemergence with a continuous belt-link sprayer equipped with a

[5]Baccto is a product of Michigan Peat Co. Houston, TX 77098.

single 8001 even flat fan nozzle[6] calibrated to deliver 205 L ha^{-1} at a spray pressure of 210 kPa. Belt speed was set at 1.5 km h^{-1}. Redroot pigweed, eastern black nightshade, velvetleaf, and common ragweed were treated at the fifth leaf (6 cm), fourth to fifth leaf (2.5 to 4 cm), third to fourth leaf (6 to 7.5 cm), and second to fourth leaf (5 cm) stage, respectively.

Imazethapyr was applied at 0, 13, 27, and 53 g ha^{-1}, while bentazon was applied at 0, 210, 420, and 840 g ha^{-1}. The treatments were applied in a factorial arrangement with 1.2 L ha^{-1} POA[7]. To measure weed control, the plants were excised at the soil surface 14 DAT, dried, and weights recorded. All pots were arranged in a completely randomized design and the experiments were repeated in time with four replications in each experiment. All data were subjected to ANOVA. Interactions were not present between experiments and treatments so the experimental data were combined over time. For the herbicide combinations, the expected weed control value was calculated following Colby's method (3). This calculation was carried out only on the greenhouse data since the expected value is most useful at the GR_{50} values (3), which the greenhouse data approached. Mean comparisons were made using Fisher's Protected $LSD_{\alpha=0.05}$.

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[6]Teejet flat fan tips. Spraying Systems Co., North Ave. and Schmale Road, Wheaton, IL 60188.

[7]Herbimax. 83% petroleum oil, 17% adjuvant. Loveland Industries, Inc. Greeley, CO 80632.

Field Studies. Field experiments were conducted in 1991 and 1992 in a conventional row dry edible bean crop. Soil characteristics for each experiment are summarized in Table 1. Locally collected velvetleaf and redroot pigweed seeds were spread uniformly across the field site both years to ensure adequate weed populations. Weed seeds were shallowly incorporated 2 to 3 cm using a Danish S-tine field cultivator[8]. Immediately following weed seed incorporation, Olathe pinto beans were planted in 3 m by 7.6 m plots with crop row spacings of 76 cm at a population of 203,000 seeds ha^{-1} and 172,000 seeds ha^{-1} in 1991 and 1992, respectively. Planting dates were June 4, 1991 and June 12, 1992. Herbicides were applied on June 24, 1991 and July 6, 1992 when weeds were at the desired growth state.

Imazethapyr was applied at 0, 53, and 106 g ha^{-1}, while bentazon was applied at 0, 420, and 840 g ha^{-1}. In 1992, 27 g ha^{-1} of imazethapyr was also applied. Herbicides were applied with a tractor-mounted compressed air sprayer in a total volume of 205 L ha^{-1} at a spray pressure of 210 kPa. The boom was adjusted to 61 cm above the soil surface and equipped with 8002 flat fan nozzles spaced 51 cm apart. Treatments were applied in a factorial arrangement with 1.2 L ha^{-1} POA. Treatments were arranged in a randomized complete block design with four replications. All data were subjected to ANOVA. Year by treatment interactions

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[8]Kongskilde. Kongskilde Corp. Bowling Green, OH 43402.

were present so 1991 and 1992 data are presented separately. Mean comparisons were made by Fisher's Protected $LSD_{\alpha=0.05}$.

Plots were visually evaluated 14 DAT to measure weed control. Control was measured on a scale of 0 (no control) to 100 (total plant necrosis). To quantitatively measure weed control, four uniformly sized redroot pigweed and velvetleaf plants between the center two rows were marked using a plastic garden stake 3 to 5 days prior to herbicide application. At 21 DAT, the plants were harvested at ground level and the dry weight of each species was averaged for each herbicide treatment.

Weed seedling densities in 1991 for redroot pigweed, velvetleaf, and common lambsquarters were established at 60 to 100, 40 to 60, and 10 to 50 plants m^{-2}, respectively, and in 1992, 40 to 110, 50 to 90, and 10 to 90, respectively. The redroot pigweed, velvetleaf, and common lambsquarters were 4 to 6 leaves (6 to 8 cm), 3 to 5 leaf (7 to 9 cm), and 4 to 6 leaf (5 to 7 cm), respectively.

Herbicide soil activity studies. Redroot pigweed seeds were planted in a Capac loamy sand (Aeric Ochraqualfs, fine-loam, mixed, mesic) soil as previously described in the greenhouse studies section.

Imazethapyr was applied at 0 and 53 g ha^{-1} while bentazon was applied at 0 and 840 g ha^{-1}. Herbicides were applied with 1.2 L ha^{-1} POA with a continuous belt-link sprayer equipped with an 8001 even flat fan nozzle calibrated to deliver

205 L ha^{-1} at 210 kPa. Belt speed was set at 1.5 km h^{-1}. Treatments were applied to redroot pigweed at the fifth leaf stage (6 cm) in greenhouse pots both with and without 1.5 cm of vermiculite on the soil surface. By placing vermiculite on the soil surface, soil interception of the spray solution was limited, thus restricting the soil activity of the herbicides in the spray solution. To measure weed control, the plants were excised at the soil surface 14 DAT, dried, and weights recorded. The experimental design was a split-plot with the vermiculite and no vermiculite treatments as the main plots and the herbicide treatments as the sub-plots. The pots were completely randomized and the experiment repeated in time with four replications each time. Interactions were not present between experiments and treatments, and the data were combined for analysis.

^{14}C-imazethapyr absorption and translocation studies. Absorption of ^{14}C-imazethapyr (pyridine ring labelled, 6th position with specific activity = 784.4 MBq/g) applied alone and tank-mixed with bentazon was determined. Redroot pigweed seed were planted in BACCTO greenhouse soil in 946 ml plastic pots. All experiments were conducted in growth chambers with day/night temperatures of 26/22 C. Chambers were maintained at 68% relative humidity with a 16 h photoperiod from fluorescent and incandescent lighting with a photosynthetic photon flux density of 750 μE m^{-2} s^{-1}. Plants were surface-watered as needed. A

2 μL drop containing 370 Bq of ^{14}C-imazethapyr was applied with a micro-syringe[9] between the fifth leaf mid-vein and the edge of the leaf approximately ⅓ of the distance from the leaf tip to the base of the leaf. Special care was taken to not place the spot on a leaf vein. The spotting solution contained ^{14}C-imazethapyr with the appropriate amounts of formulation blank, unlabelled commercial imazethapyr[10], commercial bentazon[11] (when appropriate), POA, and water to simulate a spray solution containing imazethapyr at 53 g ha^{-1}, bentazon at 840 g ha^{-1}, and POA at 1.2 L ha^{-1} in a total volume of 205 L ha^{-1}.

At 0, 1, 2, 4, 8, 24, and 48 h after treatment (spotting), the leaf was rinsed in a 20 ml glass scintillation vial containing 3 ml of methanol : H_2O (2:1 v/v), and gently swirled for 30 s. The leaf was then washed with a minimal amount of rinsing solution and 15 ml of scintillator[12] was added. The unabsorbed ^{14}C-imazethapyr was quantified by liquid scintillation spectrometry (LSS)[13] and absorption calculated by subtracting the amount of ^{14}C-imazethapyr recovered in the washoff solution from the amount of ^{14}C-imazethapyr applied.

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[9]Hamilton microsyringe, Hamilton Co. Reno, NV 89520-0012.

[10]Pursuit 2AS herbicide. American Cyanamid Co. Princeton, NJ 08540.

[11]Basagran 4L, BASF Corp. Parsippany, NJ 07054.

[12]Safety-Solve. Research Products International Corp. Mount Prospect, IL 60056.

[13]Model 1500. Packard Instrument Corp. Downers Grove, IL 60515.

The treated leaf was saved and combusted in a biological oxidizer[14]. The CO_2 was trapped in a solution of scintillator : CO_2 absorber[15]. Total radioactivity in the sample was then determined by LSS. The amount of ^{14}C-imazethapyr in the treated leaf was calculated by subtracting the amount of ^{14}C recovered in the treated leaf from what was absorbed. This amount was then subtracted from 100% to calculate ^{14}C-imazethapyr translocated from the treated leaf.

Results and Discussion

Greenhouse studies. Imazethapyr at 13, 27, and 53 g ha^{-1} reduced dry weight of redroot pigweed by 82, 87, and 85%, respectively, as compared to the untreated control (Table 2). Bentazon at 210, 420, and 840 g ha^{-1} decreased redroot pigweed dry weight by 36, 25, and 42%, respectively. When 210 or 420 g ha^{-1} of bentazon was tank-mixed with 13 g ha^{-1} of imazethapyr or when 420 g ha^{-1} of bentazon was tank-mixed with 27 g ha^{-1} of imazethapyr, redroot pigweed dry weights increased as compared to each respective rate of imazethapyr. When dry weight data were compared to Colby's expected values, antagonized weed control

[14]OX-300. R. J. Harvey Instrument Corp. Patterson, NJ 07642.

[15]Carbo-Sorb II. Packard Instrument Co. Meriden, CT 06450.

was noted when any bentazon was tank-mixed with 13 or 27 g ha^{-1} of imazethapyr (Table 2). When imazethapyr was applied at 53 g ha^{-1}, antagonism from bentazon was not observed.

Bentazon or imazethapyr applied alone at the highest rates decreased eastern black nightshade dry weight by 68 and 62%, respectively (Table 2). A significant increase in plant dry weight occurred only when 53 g ha^{-1} of imazethapyr was tank-mixed with 210 g ha^{-1} of bentazon as compared to 53 g ha^{-1} of imazethapyr alone. Six of the nine tank-mix combinations resulted in less plant dry weight reduction than expected as calculated by Colby's method. However, a loss in eastern black nightshade control by imazethapyr when tank-mixed with bentazon was not evident, indicating the increased sensitivity of this species to bentazon.

Similar trends were noted with common ragweed. Bentazon and imazethapyr applied alone at the highest rates decreased dry weight by 74 and 50%, respectively. A loss of common ragweed control was not observed when imazethapyr and bentazon were tank-mixed, again indicating the sensitivity of this weed species to bentazon.

Bentazon and imazethapyr applied alone at the highest rates decreased velvetleaf dry weight by 30 and 38%, respectively. Velvetleaf control was less than expected in the greenhouse possibly due to vigorous growth (personal observation) under greenhouse conditions. No reduction or enhancement of weed control occurred with any tank-mix combination.

Redroot pigweed and eastern black nightshade displayed antagonistic responses as indicated by Colby's expected values when bentazon was tank-mixed with imazethapyr. No antagonism was observed in common ragweed control and velvetleaf control was poor with all herbicide treatments. Redroot pigweed and eastern black nightshade are both controlled by imazethapyr applied at field rates but are not controlled by bentazon. Control of common ragweed and velvetleaf is better with bentazon than with imazethapyr (2). Thus, weed control may be antagonized when imazethapyr and bentazon are tank-mixed if the weed species are more susceptible to imazethapyr than bentazon.

Field Studies. Redroot pigweed control in the field was not reduced either year when bentazon was tank-mixed with imazethapyr. Imazethapyr at 53 g ha^{-1} or 106 g ha^{-1} provided 98 to 99% control of redroot pigweed 14 DAT both years (Tables 3 and 4). Tank-mixing 420 or 840 g ha^{-1} of bentazon with any rate of imazethapyr did not reduce redroot pigweed control. Redroot pigweed dry weight responded similarly. Imazethapyr at 53 or 106 g ha^{-1} reduced redroot pigweed dry weight 87 and 93%, respectively, in 1991 and 84 and 93%, respectively, in 1992. Tank-mixing bentazon at 420 or 840 g ha^{-1} with either rate of imazethapyr did not effect redroot pigweed dry weight as compared to each respective rate of imazethapyr applied alone in 1991. However in 1992, adding 840 g ha^{-1} of bentazon improved redroot pigweed control with each respective rate of imazethapyr, compared to

imazethapyr alone (Table 4).

Velvetleaf control increased both years when bentazon was tank-mixed with imazethapyr as compared to imazethapyr or bentazon alone. Bentazon at 420 and 840 g ha^{-1} provided 18 and 78% velvetleaf control, respectively, 14 DAT in 1991 and 78 and 92% velvetleaf control, respectively, in 1992. Imazethapyr provided 75 to 80% velvetleaf control in 1991, and 60 to 68% control in 1992. Velvetleaf control from 420 g ha^{-1} of bentazon increased when 53 or 106 g ha^{-1} of imazethapyr was tank-mixed in 1991 and 1992.

In 1991, velvetleaf dry weight reduction was improved 60 and 51% when 420 g ha^{-1} of bentazon was tank-mixed 53 or 106 g ha^{-1} of imazethapyr, respectively. In 1992, bentazon at 420 and 840 g ha^{-1} decreased velvetleaf dry weight by 92 and 100%, respectively. When bentazon at 840 g ha^{-1} was tank-mixed with either rate of imazethapyr, a reduction in plant dry weight was observed as compared to each respective rate of imazethapyr applied alone.

Bentazon applied alone at 840 g ha^{-1} provided 90% control of common lambsquarters in 1991 and 98% control in 1992. Imazethapyr alone gave poor control of common lambsquarters. Adding imazethapyr to the bentazon treatment did not improve or reduce common lambsquarters control in 1991 and 1992.

In greenhouse studies, redroot pigweed and eastern black nightshade control was reduced when bentazon was tank-mixed with 13 or 27 g ha^{-1} of imazethapyr. However, this antagonized redroot pigweed control was not observed in the field.

Control of weed species that are controlled by bentazon as well as by imazethapyr, such as velvetleaf, common ragweed, and common lambsquarters, was not antagonized in field or greenhouse studies. Further greenhouse research determined the possible mechanism for the reduction in redroot pigweed control.

Herbicide soil activity studies. Greenhouse studies were designed to determine if the soil interception of the spray solution played a role in redroot pigweed control by imazethapyr. When vermiculite was not spread on the soil surface, bentazon at 840 g ha^{-1} reduced redroot pigweed dry weight 21% as compared to the untreated control (Table 5). Imazethapyr alone or tank-mixed with bentazon reduced redroot pigweed dry weight by 86 and 79%, respectively, as compared to the untreated control plant.

When comparing across soil treatments, dry weight of the untreated redroot pigweed in the presence of vermiculite was lower than where vermiculite was not placed on the soil surface (Table 5). This was unexpected but may be due to the increased handling of the greenhouse pots with vermiculite on the soil surface and subsequent removal of the vermiculite. When imazethapyr was applied alone or tank-mixed with bentazon, redroot pigweed dry weight was greater when vermiculite was placed on the soil surface, demonstrating that soil activity from imazethapyr increased redroot pigweed control.

^{14}C-Imazethapyr absorption and translocation in redroot pigweed. At 4, 8, and 24 h after treatment, the presence of bentazon in the spray solution decreased absorption of ^{14}C-imazethapyr by redroot pigweed as compared to ^{14}C-imazethapyr applied alone (Figure 1). By 8 h after treatment, ^{14}C-imazethapyr absorption when applied alone was greater than 95% of applied. When bentazon was applied with ^{14}C-imazethapyr at 8 h, absorption was $\leq$ 80% of applied. Redroot pigweed are extremely sensitive to imazethapyr. A 15% decrease in absorption may not totally account for the reduced weed control observed in earlier greenhouse studies.

More ^{14}C-imazethapyr was translocated from the treated leaf 24 and 48 h after treatment when ^{14}C-imazethapyr was applied alone than when bentazon was present (Figure 2). At 24 and 48 h after treatment, 27% and 30% of absorbed ^{14}C was translocated from the treated leaf. However, when bentazon was tank-mixed with ^{14}C-imazethapyr, translocation was only 11% and 19% of absorbed ^{14}C at 24 and 48 h after treatment, respectively. This is greater than a 50% reduction in ^{14}C translocated from the treated leaf compared to ^{14}C-imazethapyr alone.

The reduction in translocation of ^{14}C-imazethapyr from the treated leaf by tank-mixing with bentazon may contribute to the antagonized redroot pigweed control in initial greenhouse studies. The translocation of a phloem mobile herbicide, such as imazethapyr, may be inhibited when the production and translocation of photoassimilate is also inhibited (5). Bentazon is known to reduce photoassimilate production and translocation by inhibition of electron transport in

photosystem II (6, 16). It is therefore reasonable to assume that bentazon will reduce the phloem transport of imazethapyr.

Bentazon decreased both absorption and translocation of ^{14}C-imazethapyr in redroot pigweed. Bentazon has also been observed to decrease absorption of sethoxydim and acifluorfen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid) in selected plant species (12, 15, 18). The decrease in absorption and translocation of ^{14}C-imazethapyr when tank-mixed with bentazon probably contributed to decreased redroot pigweed control in greenhouse studies. Also, soil interception of the spray solution may have played an important role in controlling redroot pigweed in field studies.

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Table 1.. Soil characteristics of field studies in 1991 and 1992.	
----------Year----------	
1991	1992
loamy sand 1.7% OM pH 5.5 82% sand 10% silt 8% clay Capac loamy sand (Aeric Ochraqualfs, fine-loamy, mixed, mesic)	sandy clay loam 3.4% OM pH 7 55% sand 25% silt 20% clay Capac sandy clay loam (Aeric Ochraqualfs, fine-loamy, mixed, mesic)

Table 2. Weed dry weight 14 DAT from bentazon and imazethapyr applied alone and in combination in the greenhouse. A * indicates significant antagonism when comparing the observed data to the predicted value[a].

Treatment	Rate	AMARE		SOLPT		AMBEL		ABUTH	
	--g ha^{-1}-	----------g plant^{-1}----------							
Untreated	- -	5.5		3.4		3.4		2.6	
Bentazon	210	3.5		3.0		2.5		2.4	
Bentazon	420	4.1		2.4		1.3		2.6	
Bentazon	840	3.2		1.1		0.9		1.9	
Imazethapyr	13	1.0		2.3		2.8		2.4	
Imep+bent[b]	13+210	2.5	(0.7)*	2.1	(2.0)	2.2	(2.1)	2.2	(2.2)
Imep+bent	13+420	2.3	(0.8)*	2.4	(1.7)*	1.8	(1.1)*	2.4	(2.3)
Imep+bent	13+840	1.8	(0.6)*	2.0	(0.8)*	0.5	(0.7)	1.7	(1.7)
Imazethapyr	27	0.7		1.7		2.1		1.9	
Imep+bent	27+210	1.6	(0.5)*	2.3	(1.4)*	2.2	(1.6)	2.1	(1.7)
Imep+bent	27+420	2.1	(0.6)*	1.7	(1.2)	1.3	(0.8)	2.1	(1.8)
Imep+bent	27+840	1.6	(0.4)*	1.5	(0.5)*	0.9	(0.6)	1.7	(1.3)
Imazethapyr	53	0.8		1.3		1.7		1.6	
Imep+bent	53+210	0.8	(0.6)	2.2	(1.1)*	1.4	(1.3)	1.7	(1.4)
Imep+bent	53+420	0.6	(0.6)	1.0	(0.9)	0.6	(0.7)	1.6	(1.6)
Imep+bent	53+840	1.0	(0.6)	1.7	(0.4)*	0.3	(0.5)	1.5	(1.1)
$LSD_{\alpha=0.05}$		0.9		0.7		0.6			

[a]The predicted values, enclosed in parenthesis, are calculated using Colby's method.

[b]Imep and bent are the approved codes for imazethapyr and bentazon, respectively. 1991. NCWCC Proc. 46:164.

Table 3. Response of redroot pigweed, velvetleaf, and common lambsquarters to bentazon and imazethapyr applied alone and in combination in 1991 in the field.

Treatment	Rate	Visual control 14 DAT			Dry weight 21 DAT	
		AMARE	ABUTH	CHEAL	AMARE	ABUTH
	--g ha^{-1}--	----------%			---------g plant^{-1}--------	
Untreated	- -	0	0	0	12.1	5.6
Bentazon	420	8	18	73	16.5	5.3
Bentazon	840	38	78	90	6.7	1
Imazethapyr	53	99	75	60	1.6	2.7
Imep+bent	53+420	93	78	80	1.9	2.1
Imep+bent	53+840	95	90	90	2.2	0.6
Imazethapyr	106	99	80	70	0.9	3.0
Imep+bent	106+420	98	80	80	0.7	2.6
Imep+bent	106+840	95	90	88	0.5	0.4
$LSD_{\alpha=0.05}$		13	20	13	2.1	1.7

Table 4. Response of redroot pigweed, velvetleaf, and common lambsquarters to bentazon and imazethapyr applied alone and in combination in 1992 in the field.

Treatment	Rate	Visual control 14 DAT			Dry weight 21 DAT	
		AMARE	ABUTH	CHEAL	AMARE	ABUTH
	--g ha^{-1}--	----------%----------			--------g $plant^{-1}$--------	
Untreated	- -	0	0	0	32.2	22.4
Bentazon	420	30	78	97	15.6	1.7
Bentazon	840	33	92	98	10.4	0
Imazethapyr	27	88	60	45	6.3	8.3
Imep+bent	27+420	83	83	93	3.5	0.8
Imep+bent	27+840	83	93	93	2.3	0
Imazethapyr	53	98	63	65	5.3	6.5
Imep+bent	53+420	93	90	83	2.6	0.5
Imep+bent	53+840	95	93	90	1.4	0.1
Imazethapyr	106	98	68	65	3.5	4.9
Imep+bent	106+420	99	88	83	1.4	1.9
Imep+bent	106+840	98	93	88	0.6	0.1
$LSD_{\alpha=0.05}$		8	10	15	2.9	1.5

Table 5. Redroot pigweed dry weight 21 DAT as influenced by imazethapyr and bentazon with and without soil interception of the spray solution.

Imazethapyr	Bentazon	No vermiculite	Vermiculite	$LSD_{\alpha=0.05}$[c]
--g ha^{-1}--	--g ha^{-1}--	----------g $plant^{-1}$----------		
0	0	1.4	1.2	0.2
0	840	1.1	1.1	
53	0	0.2	0.5	
53	840	0.3	0.5	
	$LSD_{\alpha=0.05}$	0.2		

[c]A split plot $LSD_{\alpha=0.05}$ was calculated for comparing values across soil treatment (value at right) or comparing values across herbicide treatment (value at bottom).

Figure 1. ^{14}C-Imazethapyr absorption when applied alone and tank-mixed with bentazon in redroot pigweed. Regression equation for ^{14}C-imazethapyr when applied alone is $Y = (784*X)/(1+(784/96)*X)$ ($R^2=0.82$) and when tank-mixed with bentazon is $Y = (738*X)/(1+(738/79)*X)$ ($R^2=0.75$). $LSD_{\alpha=0.05}$ bars are centered over data points.

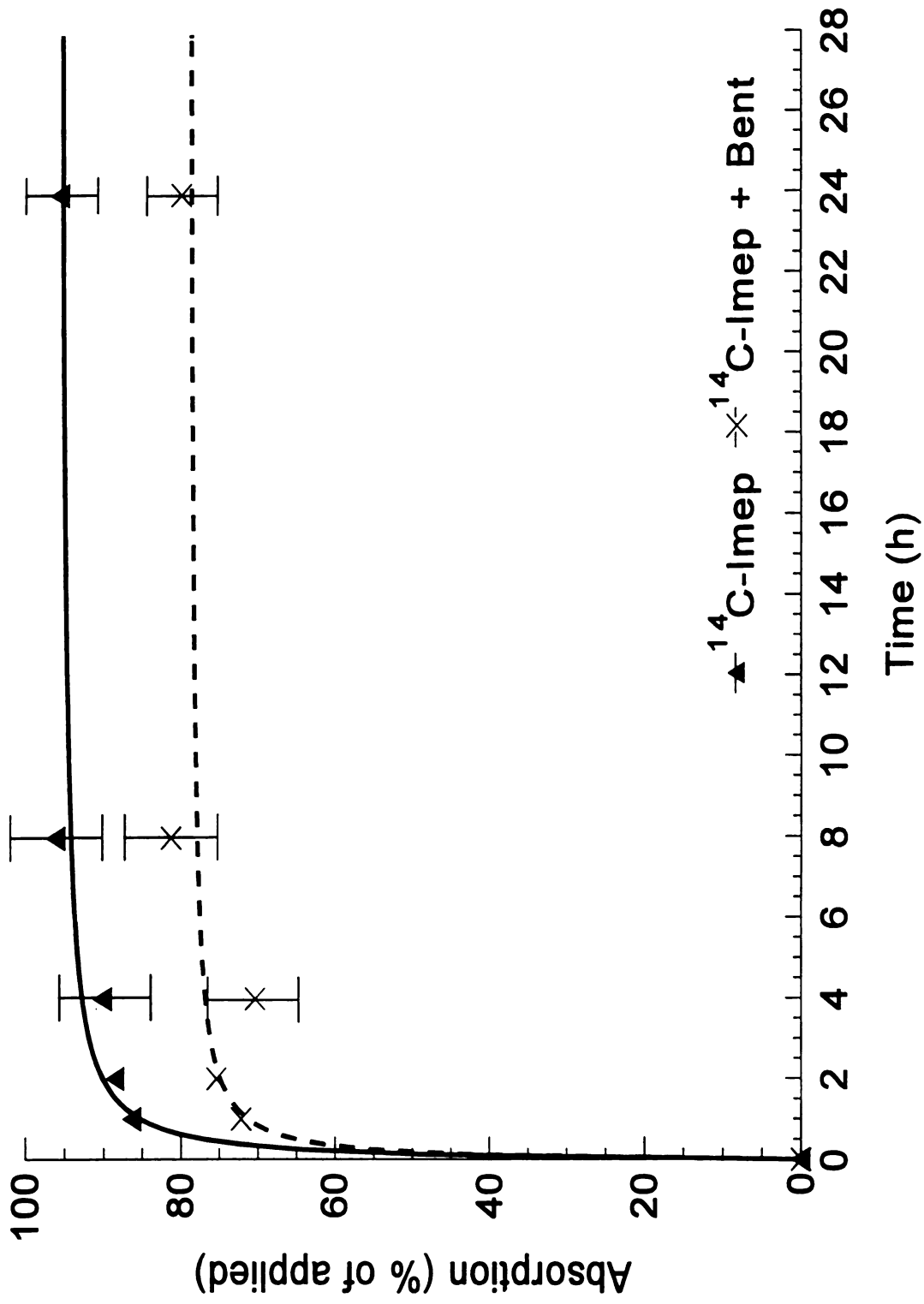
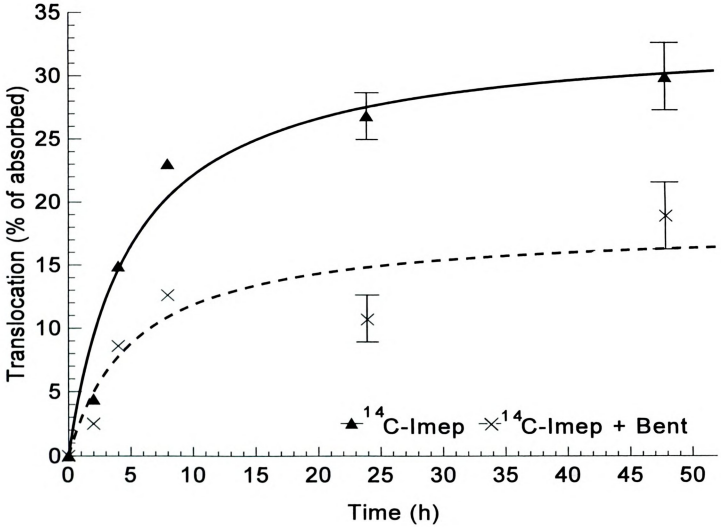

Absorption (% of applied)
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
Time (h)
14C-Imep
14C-Imep + Bent

Figure 2. ^{14}C-Imazethapyr translocation from the treated leaf when applied alone and tank-mixed with bentazon in redroot pigweed. Regression equation for ^{14}C-imazethapyr when applied alone is $Y = (6.5*X)/(1+(6.5/33)*X)$ ($R^2=0.86$) and when tank-mixed with bentazon is $Y = (3.4*X/(1+(3.4/18)*X)$ ($R^2=0.85$). $LSD_{\alpha=0.05}$ bars are centered over data points.


Translocation (% of absorbed)
Time (h)
0
5
10
15
20
25
30
35
40
45
50
^{14}C-Imep
^{14}C-Imep + Bent

Pinto Bean (*Phaseolus vulgaris*) Varietal Tolerance to Postemergence Imazethapyr[1]

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Abstract. Preliminary greenhouse studies indicated pinto bean varietal tolerance differences to postemergence imazethapyr. The objectives of this research were to determine (1) if differences existed in pinto bean varietal tolerance to postemergence imazethapyr under field conditions, (2) if tolerance differences were due to differential acetolactate synthase enzyme sensitivity or differences in ^{14}C-imazethapyr absorption, translocation, and/or metabolism, and (3) the number of genes involved and the heritability of imazethapyr tolerance in pinto bean. All rates of postemergence imazethapyr injured Olathe, Sierra, UI114, P89405, Aztec, and P90570 pinto bean varieties 7 DAT in 1991 and 1992 except 53 g ha^{-1} of imazethapyr applied to Sierra pinto bean in 1991. Olathe was injured more than other varieties in 1991, and physiological maturity was delayed more than the maturity of Sierra in both years. However, seed yields of all varieties were not

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reduced in 1991, and only P90570 had reduced seed yields from 53 g ha^{-1} of imazethapyr in 1992. Differential sensitivity of the ALS enzyme to imazethapyr was not the mechanism of differential varietal response. Olathe pinto bean absorbed and translocated more than 1.4 and 1.3 times, respectively, ^{14}C-imazethapyr as Sierra pinto bean 24 h after application. No differences in ^{14}C-imazethapyr metabolism were detected between Olathe and Sierra pinto beans. Broad sense heritability of imazethapyr tolerance in pinto bean was calculated to be 0.85. The number of genes controlling the inheritance of imazethapyr tolerance in pinto beans was greater than one.

Nomenclature: Dry bean (*Phaseolus vulgaris* L.) #[3] PHAVU, imazethapyr, 2-[4,5-dihydro-4-methyl-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid.

Additional index words. Imidazolinone, acetolactate synthase, differential tolerance, foliar absorption, translocation, metabolism, inheritance.

[3]Letters following this symbol are a WSSA-approved computer code from the Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

Introduction

The only postemergence broadleaf weed control option for Michigan dry edible bean producers is bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-(4(3*H*)-one 2,2 dioxide] which does not control redroot pigweed or eastern black nightshade (26). Imazethapyr controls these two weed species (3, 26) and many legumes are tolerant of postemergence imazethapyr applications (3, 21, 24). However, dry edible beans were susceptible to imazethapyr in previous research (16, 23). Early season injury symptoms included bean stunting, leaf crinkling, and interveinal chlorosis while late season injury was characterized by reduced plant height and delayed maturity. Wilson and Miller (23) observed seed yield reductions one year following the application of 100 g ha^{-1} of imazethapyr (a 2X application rate). The authors noted bean cultivar by herbicide interactions, but the interactions varied with year and location.

Imazethapyr is absorbed by both roots and shoots, and is translocated to the meristematic tissues, characteristic of a phloem mobile herbicide (3, 18). The primary site of imazethapyr action has been reported to be the inhibition of the acetolactate synthase (ALS, also referred to as acetohydroxyacid synthase), the first common enzyme in the synthesis of the branched chain amino acids valine, leucine, and isoleucine (2, 12). The sulfonylurea herbicides have also been

reported to inhibit the ALS enzyme (8, 15).

Differential susceptibility of soybean (*Glycine max* L.), peanut (*Arachis hypogaea* L.), and several weed species was attributed primarily to differential metabolism (3). Soybean and corn (*Zea mays* L.) convert imazethapyr to 5-hydroxyethyl-imazethapyr followed by glucose conjugation. The conversion to the glucose conjugate is more rapid in soybean than in corn, which may result in the reduced corn tolerance observed[4,5]. Some plants possess ALS isozymes which are less sensitive to imidazolinone and sulfonylurea inhibition which increase their tolerance (1, 6). Genetic studies of sulfonylurea herbicide resistance in soybean (17) and *Lactuca* spp. (11), and imidazolinone resistance in corn (13) indicated that resistance was by a single nuclear gene inherited in a semidominant fashion.

The pinto bean class of dry beans appears to be particularly sensitive to postemergence imazethapyr (5). Preliminary greenhouse studies indicated pinto bean varietal tolerance differences following postemergence imazethapyr. The objectives of this research were to (1) determine if differences in pinto bean varietal tolerance to postemergence imazethapyr occurred under field conditions, (2) determine if tolerance differences were due to differential ALS enzyme

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[4]Personal communication. Dale Shaner, American Cyanamid Co., Princeton, NJ 08540.

[5]AC-263-499 Technical Information Report. 1985. American Cyanamid Co., Princeton, NJ 08540.

sensitivity or differences in ^{14}C-imazethapyr absorption, translocation, and/or metabolism, and (3) assess the number of genes involved and the heritability of imazethapyr tolerance in pinto bean.

Materials and Methods

Field Research. Soil characteristics for 1991 and 1992 field experiments are summarized in Table 1. Plots were maintained weed-free the entire season by hand hoeing to eliminate the confounding factor of weed interference on pinto bean maturity and yield.

Seedbed preparation consisted of fall moldboard plowing followed by two passes with a Danish S-tine field cultivator[6] in the spring, the second pass perpendicular to the first. Pinto beans were planted on June 13, 1991 and June 9, 1992 at a seeding rate of 172,000 seeds ha^{-1} into plots 2.03 m by 4.57 m with crop row spacings of 51 cm. Herbicide treatments were applied on July 3, 1991 and July 6, 1992 when dry beans had reached the second trifoliolate growth stage.

Plots were visually evaluated 7 and 14 DAT to assess pinto bean injury. Injury was measured on a scale ranging from 0 (no visible injury) to 100 (total

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[6]Kongskilde. Kongskilde Corp. Bowling Green, OH 43402.

plant necrosis). To quantitatively measure pinto bean chlorosis, three leaf discs, 6.5 mm in diameter, were harvested from the middle leaflet of the third trifoliolate from three randomly selected bean plants in the middle two rows of each plot 7 DAT. Chlorophyll was extracted with 3 ml of *N,N*-dimethylformamide (7). Total chlorophyll, chlorophyll *a*, and chlorophyll *b* levels were determined using UV-VIS spectrophotometry as described by Inskeep and Bloom (1985). Through statistical analysis, chlorophyll *a* levels were determined to be the most sensitive indication of herbicide injury, so only chlorophyll *a* levels will be reported. The number of growing days required to reach physiological maturity was recorded. Plots were considered physiological mature when 90% of the pods had turned from green to a golden-bronze (buck-skin) color. Yields were measured by hand harvesting and threshing two, 3.05 m sections from the middle two rows of each plot. Yields were adjusted to 18% moisture.

Imazethapyr was applied at 0, 53, and 106 g ha^{-1} to Olathe, Sierra, UI114, Aztec, P89405, P90570 pinto beans. All postemergence herbicides were applied with a tractor-mounted compressed air sprayer in a total volume of 205 L ha^{-1} at a spray pressure of 210 kPa. The boom was equipped with 8002[7] flat fan nozzles spaced 51 cm apart adjusted to 61 cm above the soil surface. Herbicide treatments

[7]Teejet flat fan tips. Spraying Systems Co., North Ave. and Schmale Road, Wheaton, IL 60188.

were applied in a factorial arrangement with ¼ % (v/v) NIS[8]. Treatments were arranged in a randomized complete block design with four replications. All data were subjected to ANOVA. Year by treatment interactions were present so 1991 and 1992 data are presented separately. Mean comparisons were made using Fisher's Protected $LSD_{\alpha=0.05}$. Pinto bean seed yields were converted to a percent of the untreated control for mean separations. Values presented are actual seed yields.

ALS enzyme assay. ALS enzyme activity was determined from the leaves of 21 to 28 day old Olathe and Sierra pinto beans. Pinto bean seed were planted in BACCTO[9] greenhouse potting soil in 946 ml plastic pots. Environmental conditions were maintained at 25 C ± 4 C, and plants were grown in a 16 h photoperiod of natural and supplemental metal halide lighting with a midday photosynthetic photon flux density of 1000 μE m^{-2} s^{-1}. After emergence, plants were thinned to one per pot. Plants were surface watered as needed and fertilized weekly with 0.1 g of water soluble fertilizer solution (20% N, 20% P_2O_5, 20% K_2O).

ALS enzyme was extracted and activity levels measured in the presence of

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[8]X-77 Non-Ionic Surfactant. A mixture of alkylarylpolyoxyethleneglycols, free fatty acids and isopropanol. Valent U.S.A. Corp., Walnut Creek, CA 94956.

[9]Baccto is a product of Michigan Peat Co. Houston, TX 77098.

imazethapyr as outlined by Ray (15) and Shaner (18) with the following modifications. All extraction, centrifugation, and column procedures were conducted at 4 C. Forty to 50 g of plant leaves were homogenized in a volume of cold buffer (0.1 M K_2HPO_4, pH 7.5, 1 mM sodium pyruvate, 0.5 mM MgCl, 0.5 mM thiamine pyrophosphate, 10 μM flavin adenine dinucleotide (FAD), 10% v/v glycerol) equivalent to twice the weight of the tissue. Polyvinylpolypyrrolidone (2.5 g) was added for every 10 g of plant material homogenized. The homogenate was filtered through eight layers of cheesecloth and then centrifuged at 27,000 g for 20 min. Saturated cold $(NH_4)_2SO_4$ solution was added to the supernatant to bring the final $(NH_4)_2SO_4$ concentration to 50% saturated. The solution was centrifuged at 15,000 g for 15 min and the pellet redissolved in resuspension buffer (0.1 M K_2HPO_4, pH 7.5, 20 mM sodium pyruvate, 0.5 mM MgCl) and placed on a Sephadex G-25 PD-10[10] column. The desalted protein was immediately used for enzyme assays.

ALS enzyme activity was assayed by combining 0.5 ml of enzyme preparation with 1 ml of reaction buffer (25 mM K_2HPO_4, pH 7.0, 0.625 mM MgCl, 25 mM sodium pyruvate, 0.625 mM thiamine pyrophosphate, 1.25 μM FAD) and the mixture incubated for 1 h at 35 C. Reaction tubes contained either 0, 1, 10, 100, or 1000 μM of imazethapyr. The reaction was stopped by the addition of 50 μL

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[10]PD-10 column. Pharmacia, Inc., Piscataway, NJ 08855-1327.

of 6 N H_2SO_4 and the solutions were then heated at 60 C for 15 min. Following termination of the reaction, 0.5 ml of 0.5% (w/v) creatine and 0.5 ml of 5% (w/v) α-naphthol (freshly prepared in 2.5 N NaOH) were added. The solutions were heated for an additional 15 min at 60 C and the acetoin content measured by the Westerfield method (22). Protein concentration was determined by the Lowry method (9).

The ALS enzyme assay was conducted twice with five replications of each herbicide concentration per experiment. Enzyme activity is presented as a percent of untreated control assays with the data subjected to ANOVA at each herbicide concentration. Data were subjected to linear regression analysis to determine I_{50} values (herbicide concentration required to inhibit enzyme activity by 50%).

<u>^{14}C-Imazethapyr absorption, translocation, and metabolism</u>. ^{14}C-Imazethapyr (pyridine ring labelled, 6th position with specific activity = 784.4 MBq/g) absorption, translocation, and metabolism was evaluated in Olathe and Sierra pinto beans. Pinto beans were planted in BACCTO greenhouse soil in 946 ml plastic pots. All experiments were conducted in growth chambers with day/night temperatures of 26/22 C. Chambers were maintained at 68% relative humidity with a 16 h photoperiod from fluorescent and incandescent lighting with a photosynthetic photon flux density of 750 μE m^{-2} s^{-1}. Plants were surfaced-watered

as needed.

The middle leaflet of the first trifoliolate was treated with ^{14}C-imazethapyr. Prior to spotting ^{14}C-imazethapyr on the treated leaflet, plants were sprayed with unlabelled herbicide to simulate a field application. The herbicide treatment was applied with a continuous belt-link sprayer equipped with a single 8001 even flat fan nozzle calibrated to deliver 205 L ha^{-1} at a spray pressure of 210 kPa. Preliminary spray retention studies (data not presented) indicated the treated leaflet intercepted approximately 24 μL of spray solution. The treated leaflet was covered with aluminum-foil prior to treatment with unlabelled herbicide so as to avoid any spray interception. The covered leaflet was then spotted with 12, 2 μL drops using a microsyringe[11] containing a total of 5.83 kBq of ^{14}C-imazethapyr. Drops were not spotted on a leaf vein. The spotting solution contained ^{14}C-imazethapyr with the appropriate amounts of formulation blank, unlabelled commercial imazethapyr[12], NIS, and water to simulate a spray solution containing 53 g ha^{-1} of imazethapyr, and NIS at ¼ % (v/v) in a total volume of 205 L ha^{-1}.

At 6, 24, 48, and 72 h after treatment (spotting), the treated leaflet was excised and rinsed in a 20 ml glass scintillation vial containing 15 ml of methanol : dd H_2O (2:1 v/v) and swirled for 30 s. The leaflet was then rinsed with a minimal amount of the rinsing solution. The total volume was divided into five,

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[11]Hamilton microsyringe, Hamilton Co. Reno, NV 89520-0012.

[12]Pursuit 2AS herbicide. American Cyanamide Co. Princeton, NJ 08540.

20 ml glass scintillation vials and 15 ml of scintillator[13] added to each vial. The unabsorbed ^{14}C-imazethapyr was quantified by liquid scintillation spectrometry (LSS)[14] and absorption determined by subtracting the amount of ^{14}C-imazethapyr recovered in the washoff solution from the total amount of ^{14}C-imazethapyr applied.

The treated leaflet was frozen with dry ice and stored at -30 C for metabolism analysis. The potting media was rinsed from the roots and the remainder of the plant was then sectioned into four additional parts: above the treated leaflet, lateral leaflet, below the treated leaflet, and roots. Fresh weight of each plant part was recorded and the plant samples were combusted in a biological oxidizer[15]. Radioactivity in each plant part was quantified by LSS.

Absorbed ^{14}C was extracted from the treated leaflet. The treated leaflet was homogenated in 25 ml of methanol for 2 min in a stainless steel Sorvall Omnimixer[16]. The inside of the mixer was washed with 15 ml of methanol and the residue blended for an additional 2 min. The homogenate was filtered under

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[13]Safety-Solve. Research Products International Corp. Mount Prospect, IL 60056.

[14]Model 1500. Packard Instrument Corp. Downers Grove, IL 60515.

[15]OX-300. R. J. Harvey Instrument Corp. Patterson, NJ 07642.

[16]Sorvall Omni-Mixer. Omni International, Inc. Waterbury, CT 06704.

vacuum through filter paper[17], and the homogenizer vessel thoroughly rinsed with methanol and filtered. The entire filtrate volume was recorded. Two, 0.5 ml aliquots were sampled from the filtrate, and radioactivity was quantified with LSS. The filter paper and extracted tissue were allowed to air dry and then oxidized to determine unextracted radioactivity. The filtrate was concentrated to 1 to 5 ml under a vacuum at 39 C using a rotary evaporator[18]. The flask was rinsed twice with methanol at a total volume of 3 ml and combined with the concentrated plant extract. The remaining extract was filtered through a polysulfone membrane filter[19] with a mean pore size of 0.45 μm following evaporation under a stream of nitrogen in a water bath at 35 C. The filter housing was then rinsed with 1 ml methanol, and added to the filtrate. The total volume was then reduced to 1 ml and 50 μL of concentrated extract was chromatographed using thin-layer chromatography (TLC) silica gel plates[20]. As a standard, 370 Bq of ^{14}C-imazethapyr was also spotted on each plate. TLC plates were developed in a system of n-propanol : methylene chloride : formic acid (4:5:1, v/v/v) which provided a clear separation of metabolites. Radioactivity was located and

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[17]Whatman #1. Whatman International Ltd. Maidstone, England.

[18]Büchi R110. Brinkmann Instruments, Inc. Westbury, NY 11590.

[19]Supor 200 Membrane Filter. Gelman Sciences Inc., Ann Arbor, MI 48106.

[20]Whatman Silica Gel 150 Å LK5DF, Whatman International, Ltd, Maidstone, England.

quantified by a plate scanner[21]. The amount of ^{14}C in the treated leaflet was calculated by adding the extractable and unextractable ^{14}C. To calculate the amount of ^{14}C translocated from the treated leaflet, the amount of ^{14}C recovered in the treated leaflet was subtracted from the amount absorbed. This value was then converted to a percentage and subtracted from 100%.

All pots for the ALS enzyme assay, absorption, translocation, and metabolism studies were arranged in a completely randomized design with four replications. Absorption, translocation, and metabolism experiments were repeated. All data were subjected to ANOVA and data were combined over time. Mean comparisons were made by $LSD_{\alpha=0.05}$ within each time period.

^{14}C-Imazethapyr absorption and translocation from the treated leaf studies were subjected to curvilinear regression analysis and coefficient values determined (19). The absorption and translocation data were fit to the curvilinear equation Y = (I*X)/(1+(I/A)*X), where Y and X are the Y - axis and X - axis coordinates, I is the percentage absorption as time approaches 0, and A, the asymptotic value, is the percentage absorption as time approaches infinity.

Inheritance of imazethapyr tolerance in pinto bean. F_2 crosses of Olathe (least tolerant) x Sierra (most tolerant) pinto beans were evaluated for inheritance of

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[21]Radioactivity Detecting Plate Scanner. AMBIS Inc., San Diego, CA 92123.

imazethapyr tolerance. The F_1 generation was assayed with a diaphorase isozyme to ensure they were true crosses (data not presented) using a method modified by Malburg (10). Approximately 440 F_2 seeds and 20 F_1 seeds were planted on June 22, 1992 at a 20 cm spacing within rows and 51 cm spacing between rows. Where emergence was poor and to fill the ends of rows, the parental line Sierra was hand planted 1 week later to ensure uniform interplant competition. Parental lines were included as controls on each side of the F_2 population. Imazethapyr was applied at 106 g ha^{-1} with ¼ % (v/v) NIS as previously described when beans were at the second trifoliolate stage. Visual injury to each pinto bean was assessed 14 DAT. Injury was measured on a scale ranging from 0 (no visible injury) to 100 (total plant necrosis). Broad sense heritability of imazethapyr tolerance was calculated using the following formula:

$$H = \frac{\sigma^2_{F2} - \sqrt[3]{(\sigma^2_{F1})\,(\sigma^2_{P1})\,(\sigma^2_{P2})}}{\sigma^2_{F2}}$$

where σ^2_{P1}, σ^2_{P2}, σ^2_{F1}, and σ^2_{F2} are variances of parent 1, parent 2, F_1, and F_2 generations, respectively (4). Additionally, the number of factors (genes) controlling the inheritance of imazethapyr tolerance was calculated using the equation:

$$N = \frac{\Delta^2}{8\,(\sigma^2_{F2} - \sigma^2_{F1})}$$

where N and Δ are the number of factors and the difference between the parental strains, respectively (14, 25). The symbols σ^2_{F1} and σ^2_{F2} are as described above.

Results and Discussion

Field Research. All pinto bean varieties except Sierra exhibited injury from 53 g ha^{-1} of imazethapyr 7 DAT in 1991 (Table 2). All varieties were injured from 106 g ha^{-1} of imazethapyr, and Olathe was injured more than other varieties. By 14 DAT, injury to any variety was no longer evident from 53 g ha^{-1} of imazethapyr. Injury persisted at 106 g ha^{-1} of imazethapyr on Olathe, UI114, P89405, and P90570. Chlorophyll *a* levels decreased in UI114 and Aztec from 106 g ha^{-1} of imazethapyr. Maturity of all varieties was delayed except for Sierra and UI114 treated with 53 g ha^{-1} of imazethapyr. Olathe maturity was delayed more than all varieties except Aztec from 106 g ha^{-1} of imazethapyr. However, pinto bean seed yields were not reduced by any imazethapyr application.

In 1992, imazethapyr injured all pinto bean varieties at 7 and 14 DAT (Table 3). Olathe displayed more injury than either Sierra or Aztec 7 DAT. By 14 DAT, Olathe stilled showed more injury from 53 g ha^{-1} of imazethapyr, and more injury than all varieties except Aztec from 106 g ha^{-1} of imazethapyr. Chlorophyll *a* levels were reduced in Olathe and Aztec from 106 g ha^{-1} of imazethapyr.

Physiological maturity of all varieties was delayed from both rates of imazethapyr. Olathe and Aztec were delayed more than P89405 and Sierra. P90570 seed yields were reduced by 53 and 106 g ha^{-1} of imazethapyr while UI114 and Aztec seed yields were reduced from 106 g ha^{-1} of imazethapyr.

Imazethapyr injured all pinto beans 7 DAT in 1991 and 1992 except in 1991 when applied to Sierra at 53 g ha^{-1}. However, Olathe was injured more than other varieties in 1991 and 1992, and chlorophyll *a* levels were reduced in 1991. Olathe maturity was delayed more than the maturity of Sierra in both years, although seed yields of Olathe and Sierra were not reduced. Sierra appears to be more tolerant than Olathe to postemergence imazethapyr and these two varieties were therefore used in subsequent research determining the basis of differential response to imazethapyr.

ALS enzyme assay. ALS enzyme was assayed from leaves of Olathe and Sierra pinto bean (Figure 1). Activity was measured in the presence of imazethapyr at concentrations ranging from 1 to 1000 μM. ALS activity for the untreated controls averaged 414 and 339 nM acetoin h^{-1} mg^{-1} for Sierra and Olathe, respectively. $ANOVA_{\alpha=0.05}$ revealed no differences in ALS activity between the two varieties for any imazethapyr concentration. Therefore, differential sensitivity of the ALS enzyme to imazethapyr is not the mechanism of differential varietal response.

To determine the I_{50} values, the data were combined over varieties and subjected to linear regression ($Y = 84.1 - 13*\ln(X)$ $R^2 = 0.98$). The herbicide concentration required to inhibit enzyme activity by 50% for pinto bean was 16 μM. This I_{50} value correspond closely with the I_{50} values reported for lima bean, 18.4, and other plant species (20).

^{14}C-Imazethapyr absorption, translocation, and metabolism. At 6, 24, 48, and 72 h after treatment, Olathe absorbed more ^{14}C-imazethapyr than Sierra (Figure 2). By 24 h after treatment, Olathe absorbed 74% of the applied ^{14}C-imazethapyr while Sierra absorbed only 48%. The asymptotic value for ^{14}C-imazethapyr absorption on Olathe and Sierra was 76% and 53% of the applied, respectively. At 24 h or greater, Olathe absorbed 1.4 times more ^{14}C-imazethapyr than Sierra.

At 24, 48, and 72 h after treatment, Olathe translocated more ^{14}C from the treated leaflet than Sierra (Figure 3). The asymptotic value for ^{14}C translocation from the treated leaflet of Olathe and Sierra was 41% and 30%, respectively. This indicates 1.3 times more ^{14}C translocation by Olathe than by Sierra.

At 24, 48, and 72 h after treatment, Sierra retained more ^{14}C in the treated leaflet than Olathe (Table 4), indicating Olathe translocated more ^{14}C from the treated leaflet than Sierra. At all times measured, Olathe had accumulated more ^{14}C above the treated leaflet than Sierra. By 72 h, Olathe had accumulated more than twice as much ^{14}C above the treated leaflet as Sierra. Recovery of applied

^{14}C averaged greater than 95%.

No differences in ^{14}C-imazethapyr metabolism were detected (Table 5). The ^{14}C which migrated to an R_f value of 0.75 co-chromatographed with the ^{14}C-imazethapyr standards, and was identified as parent imazethapyr. Technical grade 5-hydroxyethyl-imazethapyr was obtained[22] and was chromatographed using the same solvent system as described above and had an R_f value of 0.57. Since the least polar metabolite also chromatographed at 0.57, this metabolite was identified as 5-hydroxyethyl-imazethapyr. TLC plates with metabolite migrating to an R_f value of 0.22 were scraped and incubated with ß-glucosidase[23] at pH 5. The sample was then spotted on a TLC plate and developed as described above. The resulting R_f value was 0.57, indicating that the metabolite at an R_f value of 0.22 is the glucose conjugate of 5-hydroxyethyl-imazethapyr.

To determine the half-life of ^{14}C-imazethapyr within the plant, the data were combined over varieties and subjected to curvilinear regression ($Y = 100 - (13.3*X)/(1 + 0.133*X)$ $R^2 = 0.85$). The calculated half-life for ^{14}C-imazethapyr in pinto bean was determined to be 7.5 h.

Soybean and dry edible bean first convert imazethapyr to 5-hydroxyethyl-

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[22]American Cyanamid Co., Princeton, NJ 08543-0400.

[23]Sigma ß-glucosidase from almonds. Sigma Chemical Co., St. Louis, MO 63178.

imazethapyr followed by glucose conjugation[24]. The conversion to the glucose conjugate is faster in soybean[25] than in pinto bean as shown in the previously described studies. The 5-hydroxyethyl-imazethapyr molecule maintains herbicidal activity, however, it is five times less active than imazethapyr[24]. Because 5-hydroxyethyl-imazethapyr persists longer in dry bean than in soybean, its herbicidal activity may render dry bean less tolerant to imazethapyr than soybean.

Inheritance of imazethapyr tolerance in pinto bean. The percent injury, sample size, and predicted variances of the parent, F_1, and F_2 populations are listed in Table 6. Broad sense heritability was calculated to be 0.85, indicating that imazethapyr tolerance in pinto bean is a highly heritable trait, and selection for tolerance is possible. F_2 data indicates that dominance effects may be skewing distribution towards the susceptible parent. Dominance effects are genetic but not necessarily fixable in the pure breeding variety whereas additive effects are fixable. It is often useful to estimate the number of factors (genes) controlling the inheritance of a factor. Using an equation proposed by Wright (1934) and modified by Poehlman (1987), the minimum number of factors controlling the

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[24]AC-263-499 Technical Information Report. 1985. American Cyanamid Co., Princeton, NJ 08540.

[25]Personal communication. Dale Shaner, American Cyanamid Co., Princeton, NJ 08540.

inheritance of imazethapyr tolerance in pinto bean is greater than 1. However, this method of estimating the number of genes is based on the assumptions that the genes have equal effect, without dominance or epistasis, and that no linkage exists. If these assumptions do not hold true, the number of factors would be higher than estimated.

The inheritance of imazethapyr tolerance in pinto beans appears to be a highly heritable trait controlled by more than a single gene. However, ratings are somewhat arbitrary making individual plant rating difficult and imprecise. Since this trait is not inherited qualitatively (controlled by one major gene), it would be impractible for dry bean breeders to select for this trait in early segregating generations. Additionally, imazethapyr tolerance was difficult to evaluate on a single plant basis (personal observation) making this trait even more difficult to select in an F_2 generation. Data would suggest that selection for tolerance be delayed to a later generation when tolerance can be better accessed using replicated plots. The intermediate reaction of F_1 generation to imazethapyr would suggest the additive nature of the inheritance and support selection for tolerance in later generations when such traits are fixed.

Imazethapyr can cause early season visual injury as well as a delay in pinto bean maturity. Pinto bean varieties vary in response to postemergence imazethapyr, with Sierra being more tolerant than Olathe. Differential ALS enzyme sensitivity did not account for this tolerance difference nor did

imazethapyr metabolism differ between varieties. However, Olathe absorbed and translocated more ^{14}C-imazethapyr than Sierra, possibly accounting for the reduced tolerance exhibited by Olathe pinto bean. Imazethapyr tolerance in pinto bean appears to be a relatively highly heritable trait but under quantitative control which would suggest that individual plant selection would not be feasible.

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Table 1. Soil characteristics of field studies conducted in 1991 and 1992.

	Year	
Study	1991	1992
Pinto Bean Herbicide Tolerance	sandy clay loam 2.9% OM pH 7.1 51% sand 19% silt 30% clay Parkhill sandy clay loam (Mollic Haplaquept, fine-loamy, mixed, mesic)	clay 2.4% OM pH 6.5 23% sand 16% silt 60% clay Misteguay clay (Aeric Haplequept, fine-loamy, mixed, mesic)

Table 2. Pinto bean varietal tolerance differences to postemergence imazethapyr applications in 1991.

Variety	Imazethapyr rate	Injury 7 DAT	Injury 14 DAT	Chlorophyll *a*	Maturity delay	Yield
	--g ha^{-1}--	---------%---------		-% of control-	-days-	kg ha^{-1}
Olathe	0	0	0	100	0	3680
Sierra	0	0	0	100	0	3830
UI114	0	0	0	100	0	3200
P89405	0	0	0	100	0	3150
Aztec	0	0	0	100	0	3170
P90570	0	0	0	100	0	2600
Olathe	53	13	3	90	4	3300
Sierra	53	3	0	102	0	3450
UI114	53	5	3	88	2	3220
P89405	53	8	3	102	4	2970
Aztec	53	5	3	88	3	3260
P90570	53	5	3	103	3	2390
Olathe	106	23	5	91	7	3550
Sierra	106	10	3	103	4	3420
UI114	106	8	5	83	4	3040
P89405	106	10	5	93	4	3280
Aztec	106	10	3	80	6	3170
P90570	106	8	5	95	3	3060
$LSD_{\alpha=0.05}$		3	3	17	2	NS

Table 3. Pinto bean varietal tolerance differences to postemergence imazethapyr applications in 1992.

Variety	Imazethapyr rate	Injury 7 DAT	Injury 14 DAT	Chlorophyll *a*	Maturity delay	Yield[a]
	--g ha^{-1}--	---------%---------		-% of control-	-days-	----kg ha^{-1}----
Olathe	0	0	0	100	0	2570 a
Sierra	0	0	0	100	0	3120 a
UI114	0	0	0	100	0	2420 a
P89405	0	0	0	100	0	2770 a
Aztec	0	0	0	100	0	2640 a
P90570	0	0	0	100	0	2180 a
Olathe	53	18	18	85	14	2330 a
Sierra	53	10	10	88	8	2900 a
UI114	53	13	13	101	12	1880 abc
P89405	53	13	13	90	7	2280 ab
Aztec	53	10	13	96	15	2110 abc
P90570	53	13	13	107	9	1480 bcd
Olathe	106	28	33	77	18	2090 abc
Sierra	106	20	23	79	14	2940 a
UI114	106	23	25	90	15	1450 cd
P89405	106	25	25	97	11	2300 ab
Aztec	106	20	28	67	19	1690 bcd
P90570	106	23	25	97	15	1200 d
$LSD_{\alpha=0.05}$		5	5	22	3	

[a]Means followed by the same letter are not significantly different when compared using Fisher's protected $LSD_{\alpha=0.05}$.

Table 4. Partitioning of ^{14}C applied as ^{14}C-imazethapyr in Olathe and Sierra pinto bean.

Time after trt	Variety	Treated leaflet	Above the treated leaflet	Lateral leaflets	Below the treated leaflet	Roots	Recovery
		---------- % of recovered ----------					% of applied
6 h	Olathe	92	2	2	2	2	89
	Sierra	95	1	1	1	2	94
24 h	Olathe	83	5	3	5	4	95
	Sierra	89	3	2	3	4	94
48 h	Olathe	80	7	4	6	4	100
	Sierra	86	5	3	3	4	98
72 h	Olathe	80	7	3	6	5	98
	Sierra	86	4	3	3	3	95
$LSD_{\alpha=0.05}$ within time across variety		5	1	NS	NS	NS	
$LSD_{\alpha=0.05}$ within variety across time		7	3	3	3	3	

Table 5. Metabolism of ^{14}C-imazethapyr in Olathe and Sierra pinto bean.

Time after treatment	Variety	R_f values 0.75	R_f values 0.57	R_f values 0.22	Total accounted
		% of extractable ^{14}C			%
6 h	Olathe	69	28	1	98
	Sierra	68	30	1	99
24 h	Olathe	15	72	12	99
	Sierra	14	75	11	100
48 h	Olathe	6	68	23	97
	Sierra	6	71	22	99
72 h	Olathe	5	62	31	98
	Sierra	3	65	31	99
$LSD_{\alpha=0.05}$ within time across variety		NS	NS	NS	
$LSD_{\alpha=0.05}$ within variety across time		8	6	6	

Table 6. The injury, sample size, and variance of parent, F_1, and F_2 populations.

Population	Injury 14 DAT	n	σ^2
	-----%-----		
Olathe	15	20	0.34
Sierra	10	20	0.21
F_1	13	18	0.42
F_2	14	422	2.05

Figure 1. Olathe and Sierra pinto bean ALS enzyme activity in the presence of imazethapyr.

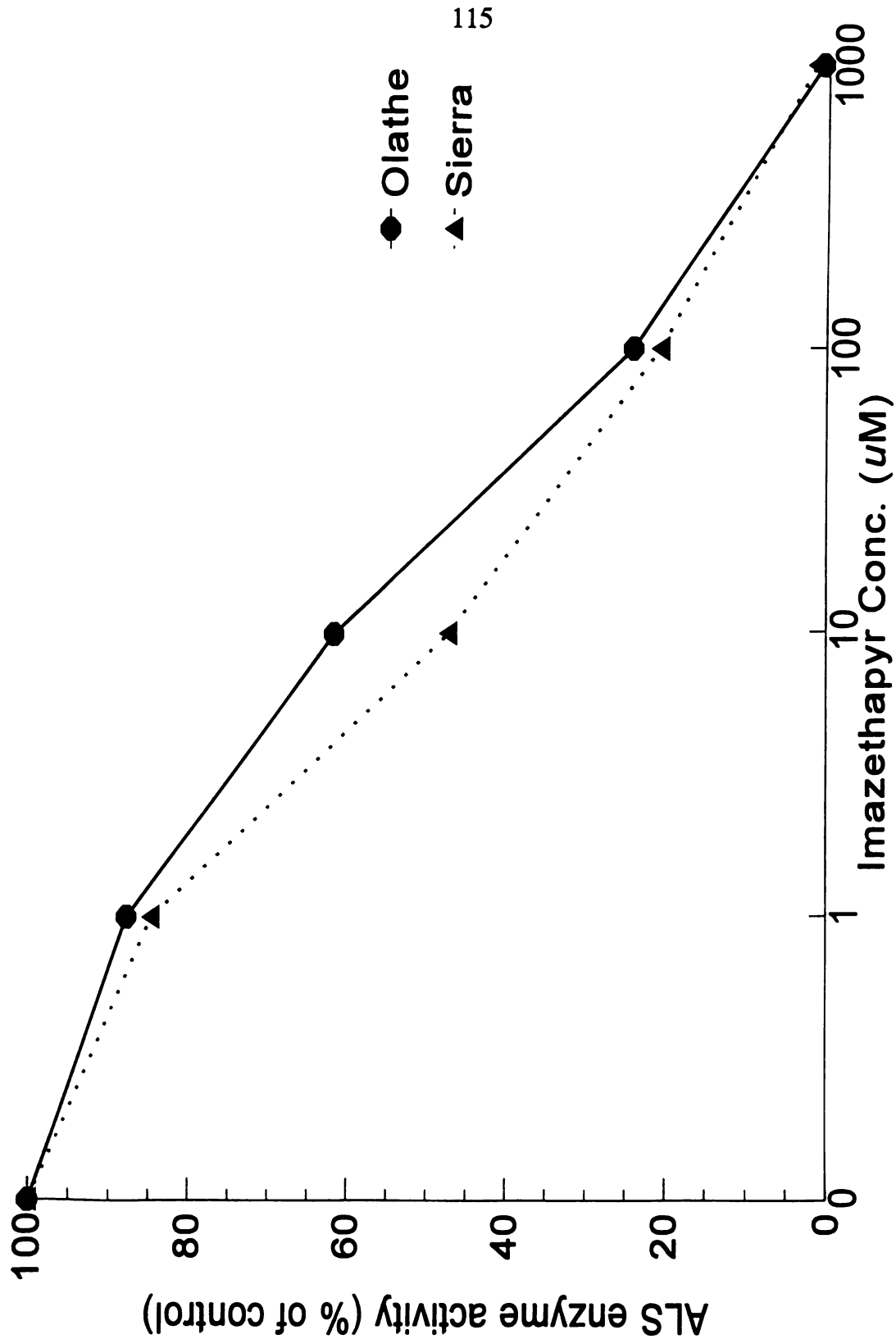

Olathe
Sierra
ALS enzyme activity (% of control)
100
80
60
40
20
0
0
1
10
100
1000
Imazethapyr Conc. (uM)

Figure 2. ^{14}C-Imazethapyr absorption in Olathe and Sierra pinto bean varieties. Regression equation for ^{14}C-imazethapyr when applied to Olathe was $Y = (51*X)/(1+(51/76)*X)$ ($R^2=0.91$) and when tank-mixed with bentazon is $Y = (36*X)/(1+(36/53)*X)$ ($R^2=0.91$). $LSD_{\alpha=0.05}$ bars are centered over data points.

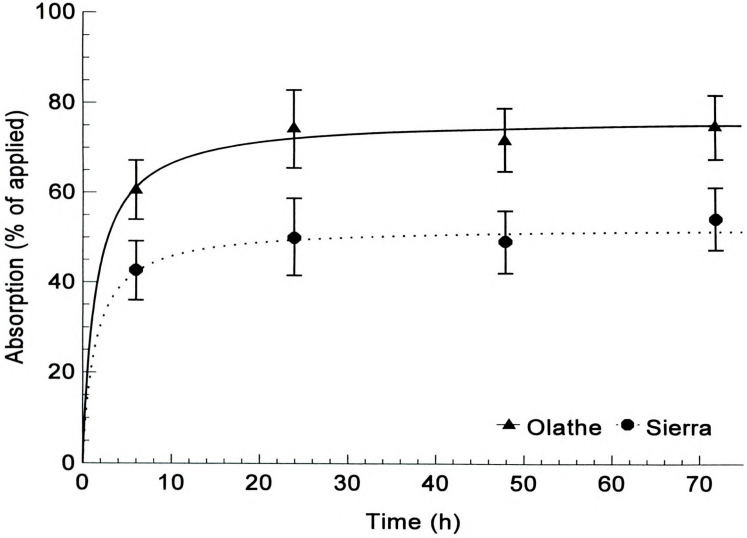
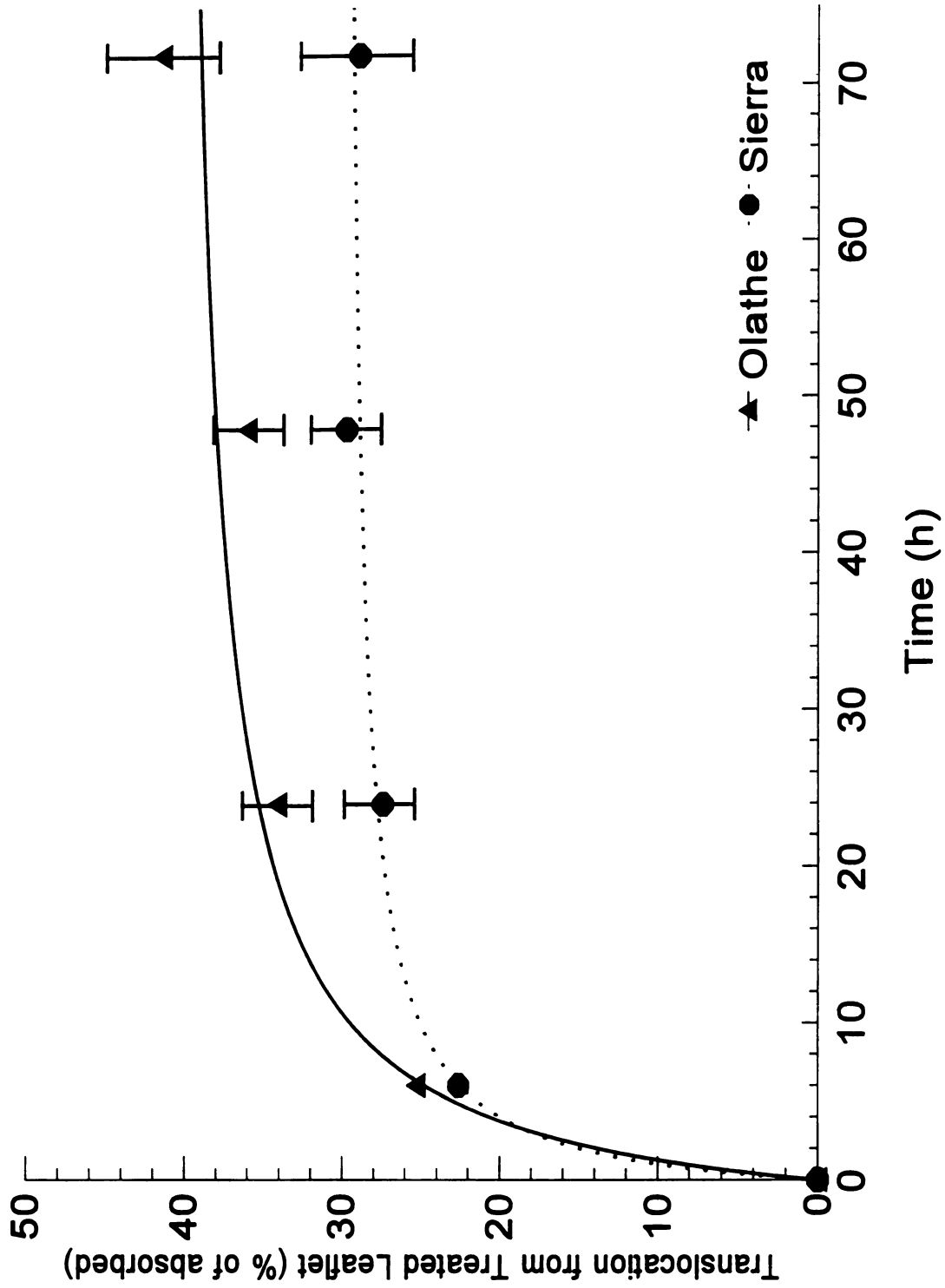

100
80
60
40
20
0
Absorption (% of applied)
0
10
20
30
40
50
60
70
Time (h)
Olathe
Sierra

Figure 3. ^{14}C-Imazethapyr translocation from the treated leaflet in Olathe and Sierra pinto bean varieties. Regression equation for ^{14}C-imazethapyr when applied alone is $Y = (10*X)/(1+(10/41)*X)$ ($R^2=0.93$) and when tank-mixed with bentazon is $Y = (15*X/(1+(15/30)*X)$ ($R^2=0.96$). $LSD_{\alpha=0.05}$ bars are centered over data points.


Translocation from Treated Leaflet (% of absorbed)
0
10
20
30
40
50
0
10
20
30
40
50
60
70
Time (h)
Olathe
Sierra