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Effect of Decapitation on Absorption, Translocation, and Phytotoxicity of Imazamethabenz in Wild Oat (*Avena fatua* L.)

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Abstract. The release of apical dominance by the physical destruction *in situ* of the apical meristem and associated leaf primordia (decapitation) promoted the growth of tillers in non-herbicide-treated wild oat plants, as indicated by increased tiller lengths and fresh weights. At 96 h after [^{14}C] herbicide treatment following decapitation, the absorption of [^{14}C]imazamethabenz and total translocation of radioactivity were respectively increased by 28% and 49%. By 96 h after [^{14}C]imazamethabenz application, the radioactivity detected in the roots of decapitated plants was 45% higher than that in the roots of nondecapitated plants while the radioactivity in tillers of decapitated plants was 2.6-fold that in tillers of intact plants. Decapitation together with foliar spraying of imazamethabenz at 200 g ha^{-1} further reduced tiller fresh weight, greatly decreased the total tiller number, and thereafter significantly increased overall phytotoxicity by 32% as measured by total shoot fresh weight. The results of this study support the hypothesis that main shoot apical dominance limits translocation of applied imazamethabenz to lateral shoots, rendering tillers less susceptible to growth inhibition by the herbicide.

Wild oat (*Avena fatua* L.) is one of the most common, persistent agricultural weeds in the prairies of Canada. Imazamethabenz, (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl)-4 (and 5)-methylbenzoic acid (3:2), is a selective postemergent herbicide for control of wild oat and several other weeds in cereal crops (Kneeshaw et al. 1983, Shaner et al. 1982). The recommended field rate of imazamethabenz for control of wild oat in cereals in Canada is 400–500 g ha^{-1}. The primary mode of action of imazamethabenz is the inhibition of biosynthesis of branched chain amino acids, valine, isoleucine and leucine through the inhibition of acetohydroxyacid synthase (Pillmoor and Caseley 1987). Translocation of imazamethabenz from the treated leaf appears to follow the mass flow of photoassimilates (Little and Shaner 1991). It has been reported that imazamethabenz stops the main shoot growth or kills the main shoot but has only a limited inhibitory effect on wild oat tillers (Little and Shaner 1991). Treatment with imazamethabenz at 100 and 200 g ha^{-1} enhanced wild oat tillering (Chao et al. 1993). We hypothesized that the limited inhibition of tillers was due to reduced translocation of imazamethabenz to lateral shoots, under the influence of apical dominance. The enhanced tillering following sublethal doses of imazamethabenz was associated with the release of apical dominance (Chao et al. 1993).

To test this hypothesis, decapitation by physical destruction *in situ* of the apical meristem and associated leaf primordia was employed to remove apical dominance. The specific objectives of this study were to (a) determine whether apical dominance inhibited the growth of wild oat tillers, (b) examine whether decapitation affected [^{14}C]imazamethabenz absorption and whether elimination of apical dominance by decapitation increased the ^{14}C translocated to tillers, and (c) determine if decapitation improved imazamethabenz phytotoxicity.

Materials and Methods

Plant Materials

CS 40, a genetically uniform line of *Avena fatua* L. was used in all cases. Seeds were germinated at room temperature on moist filter paper. For the decapitation studies that did not involve

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radioactivity, five germinated seeds were planted 2 cm deep in sandy loam soil in 12.5-cm diameter plastic pots. At the 1-leaf stage, plants were thinned to three per pot and fertilized with 50 ml 20:20:20 NPK at 3 g L^{-1}. All plants were maintained in a greenhouse and watered daily on the soil surface. Greenhouse temperatures ranged from 22 to 27 and from 19 to 22°C for day and night, respectively. Natural light was supplemented with high-pressure sodium lamps, with a 16-h photoperiod at an average photosynthetic photon flux density (PPFD) of 400–500 μE $m^{-2}s^{-1}$. The relative humidity was 30–70%.

For the radioisotope study, three wild oat seeds were planted at a depth of 2 cm in silica sand-filled styrofoam cups (6.7 cm diameter, 8.0 cm depth, fitted with drainage holes). The sand was thoroughly moistened with distilled water and then saturated with approximately 30 ml one-quarter strength standard Hoagland's solution (Hoagland and Arnon 1939). Plants were placed in the growth chamber at 20 ± 1 and 15 ± 1°C for day and night, respectively, 50% relative humidity under a 16-h photoperiod of approximately 425 μE $m^{-2}s^{-1}$ PPFD. On emergence, the seedlings were watered daily with one-quarter strength Hoagland's solution. Plants were thinned to one per pot at the 1-leaf stage.

Effect of Decapitation on the Growth of Tillers in Non-Herbicide-Treated Plants

The primary tillers were the coleoptilar tiller T0 and main stem tillers T1, T2, T3, etc., which were produced, respectively, in coleoptilar node and first, second, and third leaf nodes. The secondary tillers were designated T01, T11, and so on. T01 was the tiller from the axil of the prophyll in the coleoptilar tiller T0, and T11 was the tiller from the axil of the prophyll in the first leaf node tiller T1. The method of decapitation was adapted from Leopold (1949) and was applied to wild oat at Zadoks' growth stage 13,22 (Zadoks et al. 1974). At this stage, the plants had two visible tillers T0 and T1, four tiller buds T2, T3, T01, and T11, and the length of the main stem was 0.65–1 cm. Decapitation was carried out by removing the soil near the shoot base and using a needle to puncture and destroy *in situ* the apical portion of the main stem. The length of T0 was measured every second day following decapitation for 20 days. The lengths of the main shoot, T01, T1, and T11, and the number of tillers were determined 3 weeks after decapitation. Effects of imazamethabenz on tillering were also assessed 3 weeks after application. A completely randomized design with 15 replicates per treatment was used. The experiment was repeated twice.

Radioisotope Study of Absorption and Translocation

[^{14}C]Imazamethabenz (labelled in the fourth position carbon of the imidazolin ring, specific activity 39.55 μCi mg^{-1}) with a radiochemical purity >98% was obtained from American Cyanamid Inc. Solid [^{14}C]imazamethabenz was dissolved in 10 ml methanol as a stock solution containing 8.7 μCi ml^{-1} and 220 μg of imazamethabenz ml^{-1}. The required volume containing 5.625 μCi was taken from the stock solution, evaporated to dryness, and redissolved into 0.5 ml 3000 ppm commercial suspension concentrate formulated imazamethabenz (300 g a.i. L^{-1} or 10 mM) solution diluted with distilled water. Plants were decapitated at Zadoks' growth stage 13,22 just before [^{14}C]imazamethabenz application. Two 2-μl droplets of [^{14}C]imazamethabenz solution containing 0.01125 μCi μl^{-1} and 3.284 μg μl^{-1} were applied by micropipettes to the adaxial midsection of the first leaf lamina of the main shoot (one 2-μl droplet each side of the middle vein). After treatment of plants, the sand was kept moist but not leached. Plants were harvested 24, 48, and 96 h after treatment and divided into four parts: treated leaf (the first leaf of the main shoot), the remainder of the main shoot, tillers, and roots. The surface residues were washed off with three rinses of 10 ml of 0.1% (v/v) Tween-20 solution [polyoxyethylene (20) sorbitan monolaurate] over the treated area. The ^{14}C in each rinse was assayed by liquid scintillation. Separate analyses of standards revealed that this procedure removed all unabsorbed ^{14}C. The sum of radioactivity from these washes was considered to constitute unabsorbed herbicide. Each plant part was cut into strips and combusted in a biological sample oxidizer (Model OX 500, R. J. Harvey Instrument Corp.). The roots were washed under a stream of water to remove sand, then dried at room temperature before oxidization. The trapped $^{14}CO_2$ was quantified by liquid scintillation. The overall recovery of ^{14}C in the experiment was 90 ± 4%.

A completely randomized design with five replicates per treatment was used at each harvest time. The experiment was repeated once.

Phytotoxicity Study

The commercial suspension concentrate formulation of imazamethabenz, Assert 300 SC (Cyanamid Canada Inc., Markham, Ontario, Canada), was used in this study. It contained 300 g a.i. L^{-1}. Imazamethabenz was applied to the foliage of plants at Zadoks' growth stage 13,22 with a moving-nozzle cabinet-type sprayer calibrated to deliver 100 L ha^{-1} at 210 kpa. Half the plants were decapitated prior to spraying. Imazamethabenz was applied at the sublethal dose of 200 g ha^{-1}. Before herbicide application, the soil surface was covered with about 0.7 cm of coarse vermiculite to shield the soil from contact with the herbicide. The vermiculite was removed after spraying. At 3 weeks after imazamethabenz treatment, fresh weights of the main shoot, T0, T1, and total shoots, and tiller number were individually determined.

The experiment was a completely randomized design with five replicates per treatment. The experiment was repeated three times.

Pooled data were subjected to analysis of variance. Treatment differences were determined by Fisher's Protected LSD Test at the 5% level of significance.

Results and Discussion

Effect of Decapitation on the Growth of Tillers in Non-Herbicide-Treated Plants

Decapitation and the concomitant release of apical dominance significantly promoted the growth of T0 [Table 1; Fig. 1(A)]. At 3 weeks after removal of the main shoot apex, the lengths of T01, T1, and T11 had increased [Fig. 1(B)]. There was little growth of the main shoot in decapitated plants, and the length of the main shoot was actually the length of the leaves existing in the main shoot at the time of decapitation. Following the removal of the main shoot

Table 1. Effect of decapitation on the phytotoxicity of imazamethabenz in wild oat.[a]

Dose (g/ha)	Decapitation	Fresh weight per plant (g)				Tiller no. per plant
		Main shoot	T0	T1	Total shoots	
0	Yes	0.2	0.9	1.4	3.1	3.7
	No	3.7	0.1	0.7	4.9	3.0
200	Yes	0.2	0.1	0.1	0.9	2.7
	No	1.5	0.2	0.7	3.0	7.7
LSD (0.05)		0.3	0.3	0.3	0.9	4.5

[a] Fresh weight of each plant part and the number of total tillers determined 3 weeks after imazamethabenz application. Each value represents the mean of 13 measurements.

apex, there was a significant increase in secondary tillers but no significant increase in the number of total tillers [Fig. 1(C)]. These results indicate that inhibition of growth by apical dominance could limit imazamethabenz translocation to tillers.

Radioisotope Absorption and Translocation

Decapitation increased imazamethabenz absorption measured at 96 h following [^{14}C]imazamethabenz application [Fig. 2(A)]. This increase in absorption may be due to the greater longevity of the treated leaf (the first leaf of the main shoot) resulting from the loss of apical dominance. It is well known that shoot apex removal can rejuvenate senescing leaves, or extend leaf longevity (e.g., Krul 1974, Leopold 1962, Mothes and Baudisch 1958, Newman et al. 1973, Raafat and Herwig 1975). At 96-h after [^{14}C]imazamethabenz application, wild oat plants were at the 4- to 5-leaf stage with three visible tillers (T0, T1, and T2). During this time, the first main shoot leaves of nondecapitated wild oat plants had begun senescence as indicated by their yellow color, while the first leaves in decapitated plants remained green and vigorous much longer.

Decapitation increased translocation of radioactivity 96 h after [^{14}C]imazamethabenz application [Fig. 2(B, C)]. The amount of [^{14}C] detected within the analyzed plant parts increased significantly 96-h after application with the exception of the main shoot excluding the treated first leaf [Fig. 3(A–D)]. The radioactivity in the roots of decapitated plants was 145% of that in nondecapitated plant roots [Fig. 3(C)], while the radioactivity in tillers of decapitated plants was 2.6-fold that in tillers of intact plants [Fig. 3(D)].

The increase in total translocation of radioactivity in decapitated plants may be partially due to increased absorption. A second possibility is that the removal of the shoot apex could increase photosynthetic activity of the underlying leaves (Binnie and Clifford 1980). This would lead to the concomitant increase in the export of photoassimilate and the dissolved-imazamethabenz transport from the treated leaves, since imazamethabenz translocation out of the treated leaves moves with the carbohydrate flow (Little and Shaner 1991). However, these two effects do not fully explain the greatly increased translocation of radioactivity in decapitated plants. Since the removal of main shoot apex—the primary sink—temporarily decreases sink strength, this would result in the reduction of photosynthate translocated and thereby the decrease of dissolved-herbicide translocation. This inference is supported in this study by the trend for decreased translocation of imazamethabenz to roots and main shoot 24 and 48 h after [^{14}C]imazamethabenz treatment [Fig. 3(B, C)]. Thus, the increased translocation of both photoassimilate and herbicide would occur only when the decapitated plant re-establishes or even increases its total metabolic sink.

In the present study, the actively growing points responsible for the increased sink strengths following decapitation were probably the developing tillers, activated by the removal of apical dominance. Similar activation, as indicated by an increase in cell division was noted in *Glycine max* L. from about 25 h after shoot decapitation (Ali and Fletcher 1970). In *Pisum,* enhanced mitotic division was noted within 12 h of decapitation (Nagao and Rubinstein 1976). Increased mitotic activity probably occurs in wild oat tillers after decapitation. This increase could cause more imazamethabenz to translocate into meristematic sites of these activated tillers. The greater amount of imazamethabenz found in tillers after decapitation supports this inference. The response of tillers to decapitation is so rapid that the increased radioactivity in tillers is detected by 24 h following decapitation, although this increase is not yet statistically significant [Fig. 3(D)]. The low radioactivity in tillers of intact plants in this study confirms the results reported by Smith and Chow (1990). Apical dominance, through its inhibition of the growth of young tillers, limits imazamethabenz translocation to these lateral

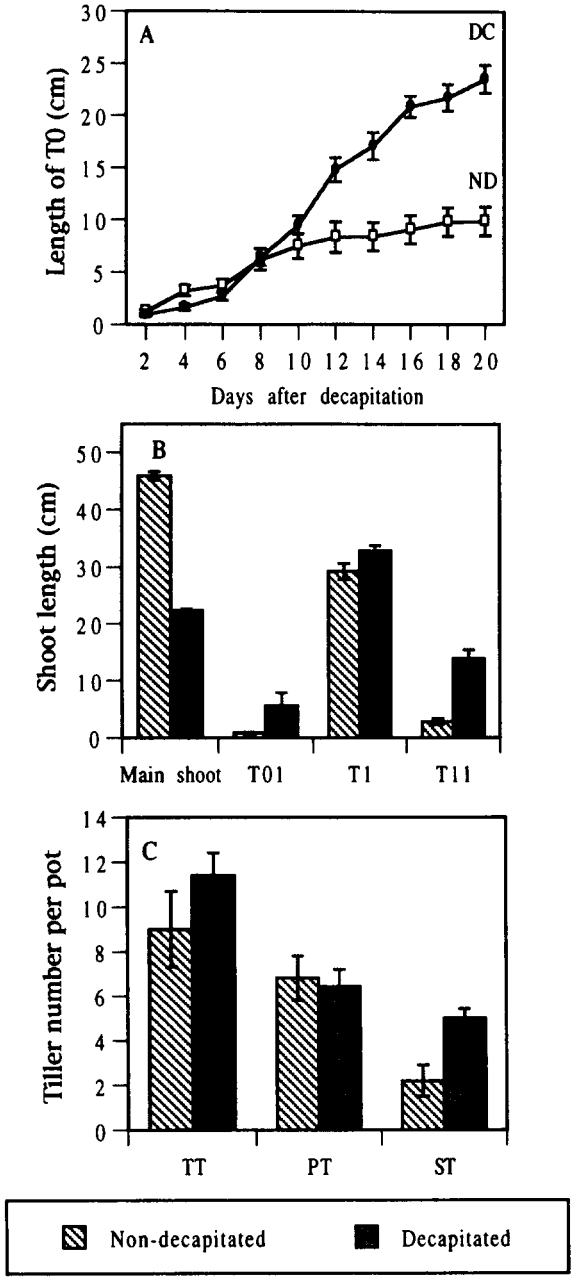

Fig. 1. Effect of decapitation on the shoot length (**A, B**) and on the number of tillers (**C**). The coleoptilar tiller, T0 (**A**) was measured every other day for 20 days, and the length of other shoots (**B**) and tiller number (**C**) were determined 3 weeks after decapitation. ND and DC represent the nondecapitated and decapitated treatments, respectively. TT, PT, and ST stand for total tillers, primary tillers, and secondary tillers, respectively. Each vertical bar represents two standard errors.

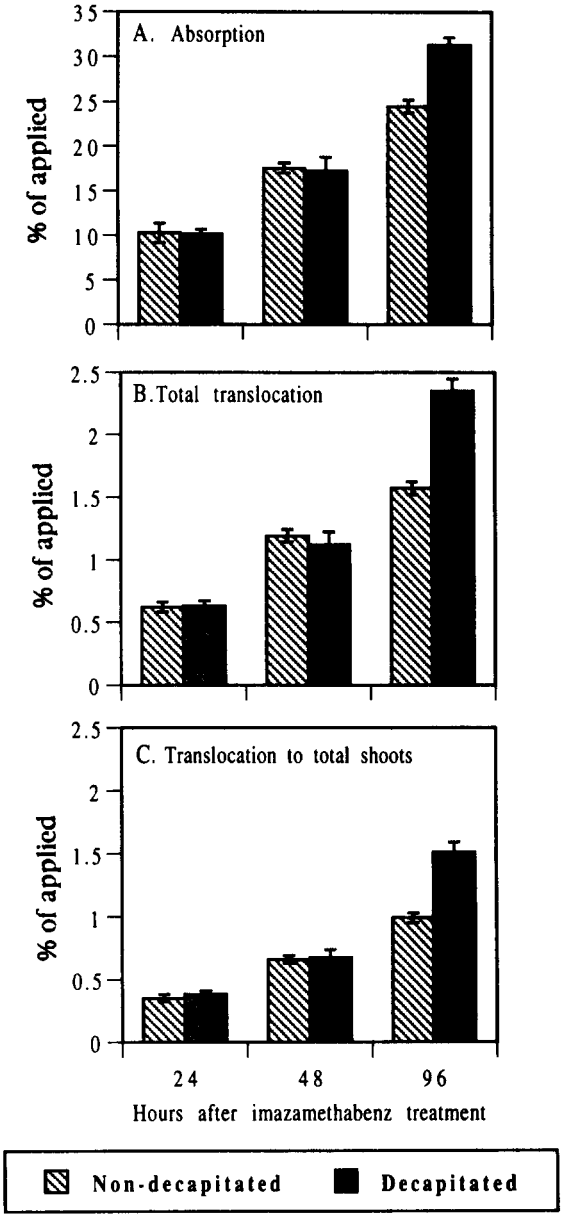

Fig. 2. Effect of decapitation on the absorption (**A**) and translocation (**B, C**) of imazamethabenz in wild oat. Each vertical bar represents two standard errors.

shoots, because the main site of action of imazamethabenz is the active growing point of the main apex. The low radioactivity in tillers of intact plants and the increase following decapitation explain why addition of a surfactant does not necessarily increase imazamethabenz translocation to tillers, although it does increase the total radioactivity absorbed and translocated out of the treated leaves (Smith and Chow 1990).

Phytotoxicity Study

Although the removal of the main shoot apex increases the fresh weights of T0 and T1 tillers in non-herbicide-treated plants, it greatly reduces

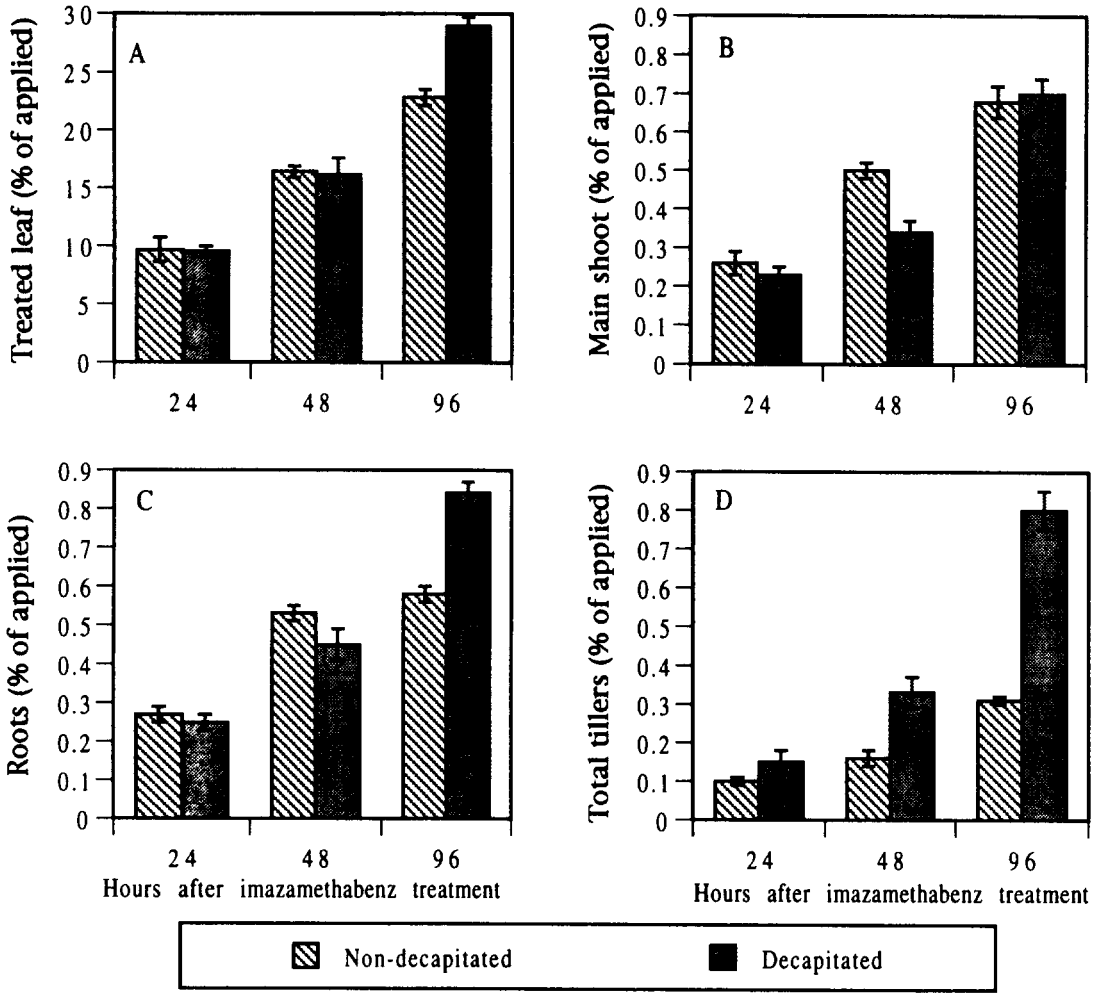

Fig. 3. Distribution of [^{14}C] in the whole plant of wild oat **(A–D)**. Each vertical bar represents two standard errors.

their fresh weight and the total number of tillers in imazamethabenz-treated wild oat plants (Table 1). Thus, decapitation increases overall phytotoxicity of imazamethabenz as indicated by greater reduction in fresh weight of total shoots in decapitated plants than that in nondecapitated plants (Table 1). This increase is most likely due to the increased herbicide accumulation in tillers as well as in roots. The results of the present study support the hypothesis that the limited ability of imazamethabenz to control wild oat tillers is due to the limited translocation of imazamethabenz to emerging tillers, which because of apical dominance posses relatively weak sink strengths.

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