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# Effects of Imazamethabenz on the Main Shoot Growth and Tillering of Wild Oat (*Avena fatua* L.)

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**Abstract.** Foliar application of imazamethabenz at sublethal doses of 100 and 200 g a.i./ha to wild oat plants at the two-leaf stage without tillers greatly inhibited the growth of the main shoot but increased tillering. The near cessation of sheath and the main stem elongation indicated that the major effect of imazamethabenz on the main shoot was inhibition of intercalary growth. Low doses of imazamethabenz treatment resulted in more leaves (including leaf primordia) in the main stem but did not affect mature first and second leaves. Sublethal doses of imazamethabenz only briefly inhibited tiller growth. A later increase in tillering in treated plants resulted from the stimulated resumed growth of tillers and the increased initiation of tiller buds. Such enhanced tillering mainly resulted from the release of apical dominance due to the inhibition or cessation of the main stem growth with imazamethabenz treatment. Both doses of imazamethabenz (100 and 200 g a.i./ha) significantly reduced the biomass of shoots and roots, but increased the ratio of roots/shoots dry weight.

Many herbicides cause growth abnormalities in both crops and weeds. 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 an imidazolinone herbicide selectively used for the control of wild oats (*Avena fatua* L.) and several other weeds in wheat and barley (Kneeshaw et al. 1983, Pillmoor and Caseley 1984, Shaner et al. 1982). The primary mode of action of imazamethabenz is the inhibition of acetohydroxyacid synthase (AHAS), an enzyme common to the biosynthesis of branched chain amino acids, valine, leucine, and isoleucine (Pillmoor and Caseley 1987). Application of imazamethabenz to wild oat plants inhibited and stunted the main shoot growth (Pillmoor and Caseley 1984). However, following application at sublethal doses, the growth inhibition was transient with extensive subsequent regrowth (Pillmoor and Caseley 1984). In our preliminary studies, prolific tillering was caused when such sublethal applications (below recommended field doses of 400–500 g a.i./ha) were applied to *A. fatua* foliage under optimum growing conditions. Similar results have been reported for other imidazolinone compounds (Bhalla and Shehata 1991, Risley 1986). Such increased tillering affects the initial reduction in wild oat biomass. It is not clear whether and how such tillering effect is related to the main shoot inhibition and whether increase in tillering following imazamethabenz application results from the stimulation of existing tillers, or tiller bud initiation, or both.

To our knowledge, there are no published reports on the effects of imazamethabenz on wild oat tillering. The objective of this study was to determine effects of sublethal doses of imazamethabenz on the main shoot growth and the tillering of wild oat.

## Materials and Methods

### *Growth of the Main Shoot and Tillering*

A genetically uniform line of *Avena fatua* L., CS 40, was used in this study. Wild oat seeds were germinated on moist filter paper in Petri dishes at room temperature. After 4 days, five germinated seeds with radicals just emerging were planted to a depth of 2 cm in 12.5 cm pots containing sandy loam soil. At the one-leaf stage, the plants were thinned to three per pot and each pot was given 50 ml of a water soluble N:P:K (20:20:20) at a rate of 3 g/L. All plants were maintained in the greenhouse and watered daily

to field capacity with tap water. Temperatures in the greenhouse were 22–27/19–22°C day/night. Natural light was supplemented with high pressure sodium lamps, with a 16 h photoperiod at an average photosynthetic photon flux density of 400–500 μE $m^{-2}s^{-1}$. The relative humidity was 30–70%.

A commercial suspension concentrate formulation of imazamethabenz, containing 300 g active ingredient (a.i.) per liter (American Cyanamid Company) was used in this study. Foliar application of imazamethabenz at doses of 0, 100, and 200 g a.i./ha was made with a moving-nozzle cabinet-type sprayer to wild oat plants at Zadoks' growth stage 12,20 (Zadoks et al. 1974). The flat fan-type sprayer nozzle (730039 # 11) was calibrated to deliver 100 L/ha at 210 kPa. Prior to spraying, the soil surface was covered with a layer of coarse vermiculite to prevent root absorption of the herbicide. The vermiculite was discarded 3 h after spraying.

Effect of imazamethabenz on the main shoot growth was evaluated on the following parameters: lengths of sheath, lamina, and the main stem; total leaf (including leaf primordia) number of the main shoot; and fresh weights of each lamina and total main shoot. Effect of imazamethabenz on tillering was determined on the length and fresh weight of each tiller, and on the numbers of tillers and tiller buds. In this study, tillers were defined as those branches which had already grown out of leaf sheaths and had become visible, while tiller buds were defined as all buds produced in the plants including both concealed buds and visible tillers. After herbicide application, the lengths of each lamina and tiller, and the number of visible tillers on the same 15 plants of five pots in each dose treatment were recorded on every other day for 24 days. On the 24th day after imazamethabenz application, the same 15 plants from five pots in each treatment were harvested and the length of each sheath, and the fresh weights of each lamina, each tiller, and the total main shoot were determined.

After imazamethabenz application, the length of the main stem, and the total number of tiller buds were recorded on alternate days over a 20-day period. During the recording, the leaf sheaths were removed first and then the main stem length and the number of tiller buds were measured and counted under a 12 × dissection microscope. At each time of measuring, four plants from four different pots in each treatment were examined. On the 20th day of recording after imazamethabenz treatment, when younger flag leaves and inflorescence had formed, the final leaf (and leaf primordia) number was counted. During each counting of tiller buds, the primary, secondary, and tertiary tiller buds were identified. Tiller designation followed the system proposed by Mitchell (1953). The primary tillers were the coleoptilar tiller T0, the main stem tillers T1, T2, T3, etc. which were produced, respectively, in the coleoptilar node and in the first, second, and third leaf nodes. The secondary tillers were designated T01, T11, etc. T01 was the tiller from the axil of the prophyll in coleoptilar tiller T0, and T11 was the tiller from the axil of the prophyll in the first leaf node tiller T1. The tertiary tillers were those produced from secondary tillers.

### *Overall Phytotoxicity on the Whole Plant*

Wild oat CS 40 seeds were first germinated and grown in vermiculite saturated with ¼ strength standard Hoagland's solution (Hoagland and Arnon 1939). At the one-leaf stage, three seedlings were transferred to 1-liter foil-covered bottles filled with ¼ strength standard Hoagland's solution, and placed in the growth chamber at 20 ± 1/15 ± 1°C day/night, 50–80% relative humidity under a 16-h photoperiod of approximately 425 μE $m^{-2}s^{-1}$. The culture solution was changed weekly, with nutrient level in the bottles adjusted daily with distilled water.

Imazamethabenz at doses of 0, 100, and 200 g a.i./ha was sprayed on the foliage of wild oat plants at Zadoks' growth stage 12,20 with the same application method as that used in the growth of the main shoot and tillering. Four weeks after imazamethabenz application, plants were harvested. The fresh weights of the main shoot and total tillers, dry weights of total shoots and roots, and the ratio of roots/shoots dry weight were determined.

### *Experiment Design and Statistical Analysis*

A completely randomized design was employed in all studies. Assessments on the growth of the main shoot and tillering in the same experiment were made with a total of 25 pots (three plants per pot) in each dose treatment. For the experiment on overall phytotoxicity in whole plants, there were five replicates in each dose treatment. Each experiment was repeated once. All data were subjected to analysis of variance (ANOVA) using General Linear Model procedure (GLM) of Statistical Analysis System (SAS Institute Inc., Cary, North Carolina, USA). Means were compared at the 5% level of significance using Fisher's least significant difference (lsd) test.

## Results

### *Effect on the Main Shoot Growth*

Imazamethabenz treatment inhibited the sheath elongation of all main shoot leaves except the first leaf sheath which had already elongated at time of spraying (Table 1). The growth of all laminae (from third to sixth) developed after herbicide application was significantly reduced (Fig. 1A–C, Table 1). However, visible injury to lamina was not observed until 1 week after imazamethabenz application, when distortion and marginal serration appeared in developing leaves. The most obvious trauma was the interveinal transparent striping present in younger lamina. Imazamethabenz at doses of 100 and 200 g a.i./ha had little inhibition on mature first and second leaves but delayed their senescence, as evidenced by the greener coloration and greater fresh weight of these two leaves in all herbicide-treated plants at time of harvest as compared to the first and second leaves of untreated plants (Table 1). Plants treated with imazamethabenz produced more leaves (including leaf primordia) (Table 2), although the growth of the additional leaves was very

**Table 1.** Effect of imazamethabenz on the sheath length and the lamina fresh weight of the wild oat main shoot 24 days after imazamethabenz application.

| Dose (g/ha) | Leaf[a] 1st | 2nd | 3rd | 4th | 5th | 6th |
|---|---|---|---|---|---|---|
| | | | Sheath length (cm) | | | |
| 0 | 3.8 a | 7.4 a | 9.3 a | 9.4 a | 9.3 a | 12.1 a |
| 100 | 3.6 a | 2.7 b | 4.4 b | 6.4 b | 8.0 a | 8.6 b |
| 200 | 4.2 a | 0.9 c | 0.9 c | 2.3 c | 4.4 b | 5.1 c |
| | | | Lamina fresh weight (mg/plant) | | | |
| 0 | 16 b | 89 a | 380 a | 374 a | 307 a | 249 a |
| 100 | 39 a | 119 a | 96 b | 120 b | 196 b | 270 a |
| 200 | 45 a | 105 a | 29 c | 11 c | 54 c | 129 b |

[a] Within columns, means followed by the same letter do not differ at the 0.05 level based on Fisher's protected LSD test.

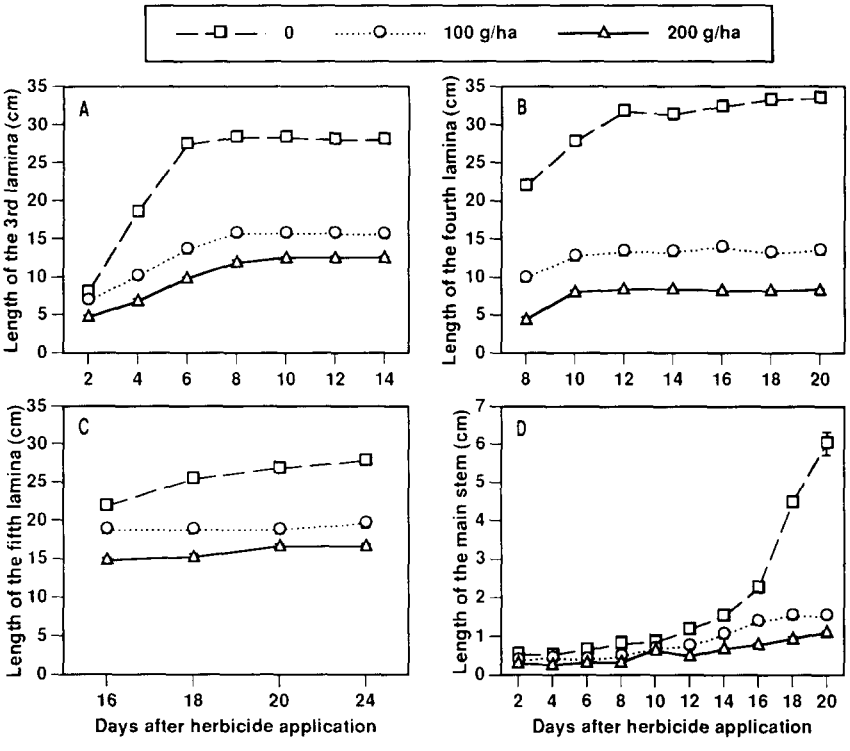


**Fig. 1.** Effect of imazamethabenz on the growth of main shoot lamina (A–C) and on the length of the main stem (D). Vertical bars represent the standard error (SE) of the means. Most SE bars are within the symbols.

limited. The growth inhibition by imazamethabenz on the main stem was highly significant. During the 3-week observation period, no apparent stem elongation occurred (Fig. 1D). The fresh weight of the main shoot was greatly reduced with imazamethabenz treatment (Table 2).

### *Effect on Tillering*

Imazamethabenz inhibited the growth of the earliest tillers, T0 and T1, but such inhibition was very limited and only temporary (Fig. 2A and B). Around 10 days after imazamethabenz application, the growth of T0 resumed (Fig. 2A). Twenty-four days after imazamethabenz application, the length (Fig. 2B) and fresh weight (Table 3) of the inhibited T1 tiller in the herbicide-treated plant attained the development of T1 in the untreated plant. In herbicide-treated plants, the growth of all later-developed tillers, T2, T3, T01, and T11 was stimulated (Fig. 2C–F). Apart from the stimulation in the growth of existing tillers, imazamethabenz treatment also resulted in an increase in the initiation of tiller buds, especially of secondary tiller buds (Fig. 2H, Table

**Table 2.** Effect of imazamethabenz on total leaf number and fresh weight of wild oat main shoot.[a]

| Dose (g/ha) | Main shoot[b] Leaf number per plant | Fresh weight g per plant |
|---|---|---|
| 0 | 7.2 b | 2.4 a |
| 100 | 9.3 a | 1.9 b |
| 200 | 9.5 a | 1.3 c |

[a] Total leaf number includes leaf primordia. Total leaf number and total main shoot fresh weight were determined, respectively, 20 and 24 days after imazamethabenz application.
[b] Within columns, means followed by the same letter do not differ at the 0.05 level based on Fisher's protected LSD test.

3). In some herbicide-treated plants, two or three collateral or overlapped buds appeared on the same nodes of main or tiller stems. This phenomenon did not occur in control plants. Thus, the treated plants had more tillers than untreated plants (Fig. 2G).

### *Overall Phytotoxicity on the Whole Plant*

Imazamethabenz at low dose (100 g a.i./ha) greatly reduced the fresh weight of the main shoot but increased the total tiller fresh weight (Table 4). The dry weights of both roots and total shoots were decreased with imazamethabenz treatment. However, the dry weight root/shoot ratio increased after imazamethabenz treatment at a dose of 200 g/ha (Table 4).

## Discussion

Imazamethabenz at the sublethal doses used in this study was active against the main shoot. All developing tissues of the main shoot were significantly inhibited. The lesser inhibition of the later-formed fifth and sixth leaves was due to the recovery of the main shoot from the herbicidal effect, confirming the results by Pillmoor and Caseley (1984). The lack of elongation in leaf sheath and the main stem in herbicide-treated plants implied that intercalary growth was almost completely stopped and that this was the major inhibitory site of imazamethabenz. This growth inhibition on intercalary tissue may be due in part to an inhibition of cell division in intercalary meristems, since imazamethabenz and other AHAS inhibitors have no direct effect on auxin, or gibberellin-induced cell elongation (Pillmoor and Caseley 1987, Ray 1982). Imazamethabenz at low doses had little effect on matured first and second leaves where no visible symptoms were observed. The greater longevity and increased biomass of these mature leaves in treated plants were probably the result of the inhibition or cessation of the main apical growing point by imazamethabenz. This effect was much like the rejuvenation of senescing leaves produced by shoot apex removal, or the regreening of yellowing leaves produced by decapitation (Leopold 1961, Mothes and Baudish 1958, Newman et al. 1973, Raafat and Herwig 1975). However, this interpretation did not fully explain why matured leaves were unaffected by imazamethabenz, because AHAS inhibition was almost certainly complete in mature leaves.

Sublethal doses of imazamethabenz treatment resulted in prolific tillering due to both the stimulated growth of existing tillers and the enhanced initiation of tiller buds. It is known that imazamethabenz translocated out of the treated leaves concentrates mostly in the main stem apex (Pillmoor and Caseley 1984, Smith and Chow 1990), because the pattern of herbicide distribution is determined by the relative sink strength. The main apical meristem is the primary metabolic sink and the apices of the tillers are weak ones. As a result, the main culm is killed or its growth is completely stopped by imazamethabenz. Translocation of applied imazamethabenz to the tillers is limited (Smith and Chow 1990) because the growth of tillers or tiller buds is subjected to and suppressed by the main stem apex. We demonstrated that loss of apical control through destroying the main stem apex resulted in a threefold increase in the amount of $^{14}$C-imazamethabenz translocated to the tillers (unpublished data). Thus, the low amount of imazamethabenz translocated to tillers due to the apical dominance of the main stem limited the herbicidal effect on these lateral shoots. Inhibition of T0 growth in treated plants was maintained for only about 8–10 days (Fig. 2A). The results of cessation of the main stem growth were releasing of apical dominance and diverting of assimilate partition. Because imazamethabenz and other AHAS inhibitors do not primarily affect photosynthesis, the plant is not short of photosynthates. It could be expected that more photosynthates are partitioned to tillers and tiller buds. Consequently, the initially inhibited growth of tillers or tiller buds was transient and subsequently the resumed growth was accelerated.

The increased leaf (including leaf primordia) number in the main shoot, and the growth inhibition or cessation of the main stem elongation may increase the initiation of primary tiller buds, because normally tiller production stops when plants enter

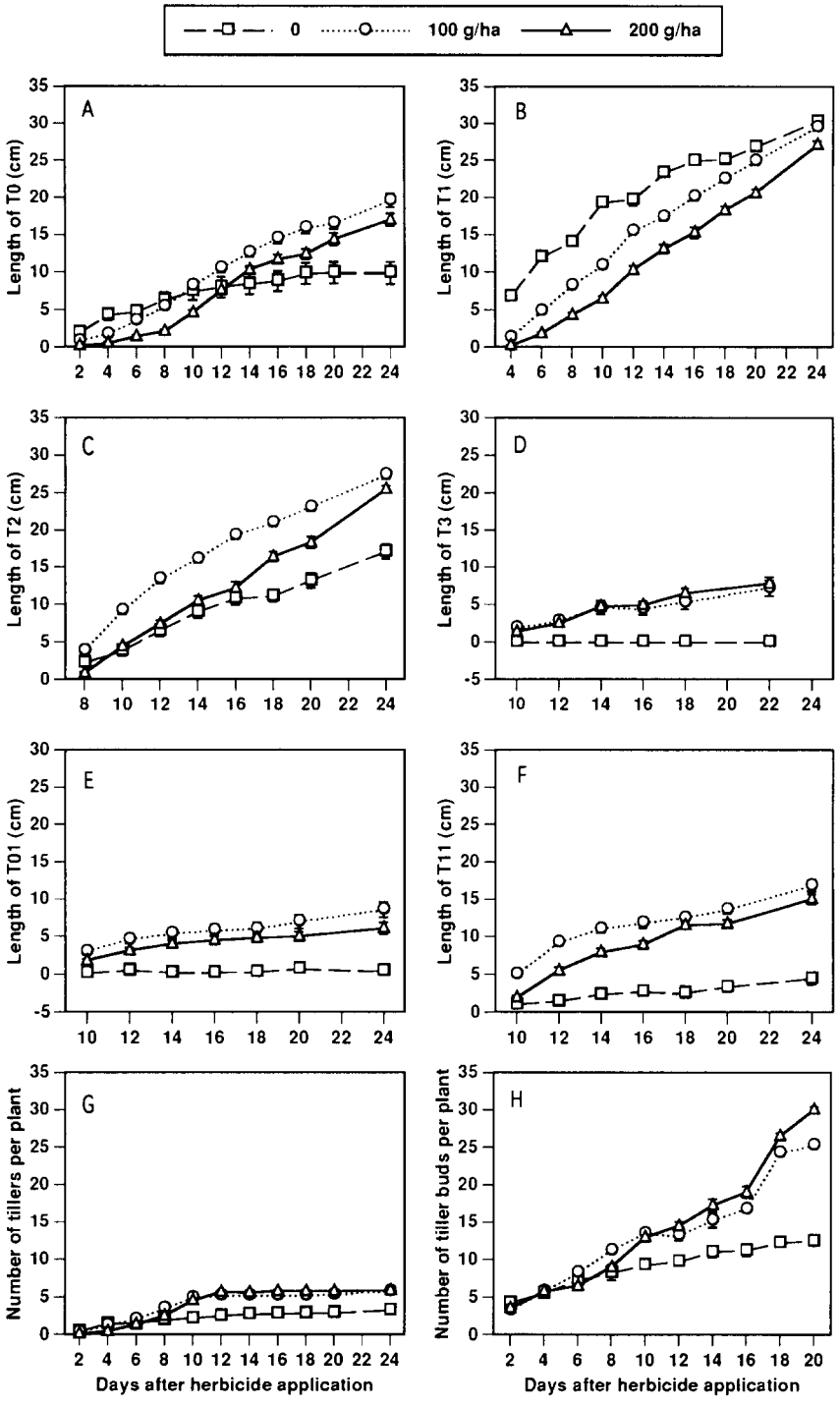


**Fig. 2.** Effect of imazamethabenz on the length of primary (A–D) and secondary (E,F) tillers, and on the number of tillers (G) and tiller buds (H) in wild oat. Vertical bars represent the standard error (SE) of the means. Most SE bars are within the symbols.

the stem elongation stage. The increased growth of primary and secondary tillers after imazamethabenz treatment may be partially responsible for the increase in number of secondary and tertiary tiller buds initiated on the parent tillers.

Inhibition of the growth and development of the main shoot growing point, and reduction in root biomass will influence the endogenous hormone balance of the plant, because these growing points are the source and site of action of many hormones [e.g., auxin is produced in apices and plays a role in apical dominance (Goodwin et al. 1978, Hillman 1984); and cytokinins are produced in root tips and stimulate meristematic activity (Wareing 1977)].

**Table 3.** Effect of imazamethabenz on the fresh weight of each tiller and on the number of tiller buds in each order.[a]

| Dose (g/ha) | Growth of tillers[b] | | | | | |
|---|---|---|---|---|---|---|
| | Fresh weight of each tiller (mg) | | | | | |
| | T0 | T1 | T2 | T3 | T01 | T11 |
| 0 | 139 b | 694 a | 279 c | 0 b | 1 b | 34 b |
| 100 | 318 a | 750 a | 653 a | 80 a | 79 a | 185 a |
| 200 | 208 ab | 545 a | 478 b | 55 a | 39 ab | 131 a |
| | Number of tiller buds in each order | | | | | |
| | Primary | | Secondary | Tertiary | | Total |
| 0 | 3.7 b | | 6.7 b | 1.7 b | | 12.2 b |
| 100 | 5.7 a | | 12.3 a | 5.0 ab | | 23.0 a |
| 200 | 6.5 a | | 16.0 a | 6.8 a | | 29.3 a |

[a] Fresh weight of each tiller and the number of tiller buds in each order were determined, respectively, 24 and 20 days after imazamethabenz.
[b] Within columns, means followed by the same letter do not differ at the 0.05 level based on Fisher's protected LSD test.

**Table 4.** Phytotoxicity of imazamethabenz on whole plants grown in hydroponic culture.[a,b]

| Dose (g/ha) | Main shoot | Total tillers | Roots | Total shoots | Root/shoot ratio |
|---|---|---|---|---|---|
| | –(g fresh wt per plant)– | | –(g dry wt per plant)– | | (g/g) |
| 0 | 9.87 a | 9.27 b | 0.88 a | 4.13 a | 0.21 b |
| 100 | 5.99 b | 13.02 a | 0.78 a | 3.42 b | 0.25 b |
| 200 | 3.41 c | 7.64 c | 0.49 b | 1.80 c | 0.40 a |

[a] Fresh weights of the main shoot and total tillers, dry weights of total shoots and roots, and ratio of roots/shoots dry weight were determined 4 weeks after imazamethabenz application.
[b] Within columns, means followed by the same letter do not differ at the 0.05 level based on Fisher's protected LSD test.

Plants treated with imazaquin, another imidazolinone herbicide, showed leaf epinasty, proliferation of axillary buds, and shortening of internodes (Risley 1986). Risley (1986) found that imazaquin inhibited the release of auxin-induced ethylene from leaf discs in *Xanthium strumarium* and soybeans. The occurrence of unusual collateral and overlapped tiller buds in imazamethabenz-treated wild oat in this study may imply changes in endogenous hormonal balance. However, our present research did not involve this investigation. Studies on this aspect are required to fully elucidate the effect of imazamethabenz on tillering.

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