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PLANTS RESPONSE TO ISOXAFLUTOLE RESIDUES IN SOIL

Maria K. Swarcewicz,[1] Prasanta C. Bhowmik,[2] Sowmya Mitra[3]

[1]*Department of General Chemistry, Agricultural University of Szczecin, Poland*
[2]*Department of Plant and Soil Sciences, University of Massachusetts, USA*
[3]*Department of Horticulture/Plant and Soil Science, California State Polytechnic University, USA*

ABSTRACT

A plant bioassay technique was developed for detecting residues of isoxaflutole in the soil. Radish (*Raphanus sativus* L.), oilseed rape (*Brassica napus* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) were grown in treated soil with various concentrations of isoxaflutole. A Hadley fine sandy loam (Typic Udifluvents) soil (South Deerfield, MA) was used for bioassay study. Bioassay was conducted in a controlled growth chamber. Isoxaflutole inhibited the growth of radish and oilseed rape. Wheat was more tolerant to isoxaflutole even at concentrations several orders of magnitude higher than the concentrations used for radish and oilseed rape. Maize was tolerant to isoxaflutole in a fine sandy loam soil. The amount of the herbicide required to reduce the above-ground growth of test species by 50%, GR_{50} was estimated from dose-response data, using logistic regression curves.

Key words: isoxaflutole, plant bioassay, residues in soil, herbicides

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INTRODUCTION

Isoxaflutole [5-cyclopropyl-4-(2-methylsulphonyl-4-trifluoromethylbenzoyl isoxazole] is a member of isoxazole herbicides. Isoxaflutole disrupts pigment biosynthesis and its inhibition in meristematic tissues gives rise to bleaching symptoms in both grass and broadleaf weeds [1,2,3]. Isoxaflutole residues in soil often can be accurately analysed by instrumental methods [4,5,6]. Bioassay method is relatively simple and straightforward and may detect total residual phytotoxicity of herbicides [7]. Plants response to sulfonylurea and imazethapyr residues in soils has been studied [8,9]. The plants used were turnip (*Brassica rapa* L.) and sunflower (*Helianthus annus* L.) which were the most sensitive species to rimsulfuron residues, whereas sugar beet (*Beta vulgaris* L.), turnip and oilseed rape proved to be most sensitive to imazethapyr. Hence different species responded differently to a particular herbicide applied at the same rate. The analysis of residue of some of the highly active sulfonylurea herbicides was more satisfactory using a root growth bioassay of maize and a shoot fresh weight for several gramineous species [9,10,11,12]. Our previous studies showed that oilseed rape was the most sensitive species to isoxaflutole followed by radish, wheat, and maize treated seeds [13]. The growth response of tested species depends on the concentration isoxaflutole in the soil. Isoxaflutole is absorbed by root and foliage. The level of herbicide residue in soil can influence the sensitivity and tolerance of plant species.

The objective of this research was to develop a plant bioassay to detect isoxaflutole residue in the soil. Various plant parameters for plant bioassay were compared. Regression curves of the shoot dry weights of the different plants as affected by isoxaflutole in the soil were determined. After isoxaflutole bioassay by soaked seeds of tested plants [13], a similar reaction to plant species was searched for various concentrations of herbicide in soil.

MATERIAL AND METHODS

The experiment was conducted in the growth chamber in the Department of Plant and Soil Science, University of Massachusetts in 1997, Amherst MA, USA. Isoxaflutole was obtained from Rhône-Poulenc Agrochimie Company (at present Aventis Crop Sciences). It is formulated as a 75% water dispersible granule and marked under the trade name of Balance ™, USA (Merlin 750 WG – Aventis Crop Sciences in Poland). 'Canola NS 45A71' oilseed rape, 'Cherry Belle' radish, 'Geneva' wheat and 'Max 747' maize were used for plant bioassay. Seeds of wheat, radish, maize and oilseed rape were grown in isoxaflutole treated soil. The treated soil was placed in 250-ml styrofoam cups containing 270 g of Hadley fine sandy loam (Typic Udifluvents) containing sand, silt and clay in 50.2%, 44.6% and 5.1%, respectively. The soil had a pH of 6.3 with an organic matter content of 1.5% and a cation exchange capacity of 68 $mmol \cdot kg^{-1}$. The soil was air-dried, sieved at 2.0 mm, and the moisture level was raised to 40% of maximum water holding capacity and the soil was equilibrated at this moisture level for 48 h. The previous work showed that the addition of herbicide to air-dried soil reduced the availability of the chemical to plants. For each plant bioassay seven concentrations of isoxaflutole in soil were prepared. The seeds were placed from 1.0 cm (oilseed rape, radish) to 3.0 cm (wheat, maize) below the soil surface. The pots were lined with aluminium foil to prevent soil and moisture loss through the air. The initial weight of each pot was recorded and the soil was watered to 70% field capacity. On a daily basis, the pots were weighed, watered to 70% field capacity for 2-4 weeks. Following watering each treatment of herbicide concentration was applied to the soil with a 5-ml disposable syringe. The pots were placed in a growth chamber under constant light and temperature regimes. The average temperature was 25 ± 5°C (day/night) and the day length was a 12-h photoperiod at an intensity of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The treatments were in a randomised block design with four replications and the experiments were repeated twice. Radish and wheat were harvested 14 and 21 days after treatment, while oilseed rape and maize - 25 days after treatment. Height, fresh weight and dry weight of shoots were determined. The roots were carefully washed with water and the length, fresh weight and dry weight were recorded.

The results were expressed as the growth reduction (GR_{50}) obtained from concentration of herbicide in soil required to reduce the growth of plant dry weight shoots by 50% and compared with the value GR_{80}.

General linear model (GLM) program of SAS [14] was used to analyse the data. All the data were subjected to analysis of variance (ANOVA) and appropriate mean separation techniques were used. Standard error of mean for each concentration was chosen for determining mean separation for each parameter. The SAS software linear regression analyses ($Y = bo + b_1 x$) or a quadratic polynomial regression analysis were used where Y was predicted shoot/root growth expressed as weight of shoot/root (mg), bo, b_1, were partial regression coefficients, and x was herbicide concentration expressed as µg a.i. per kg of dry soil. All the data was verified at the $\alpha = 0.05$ level.

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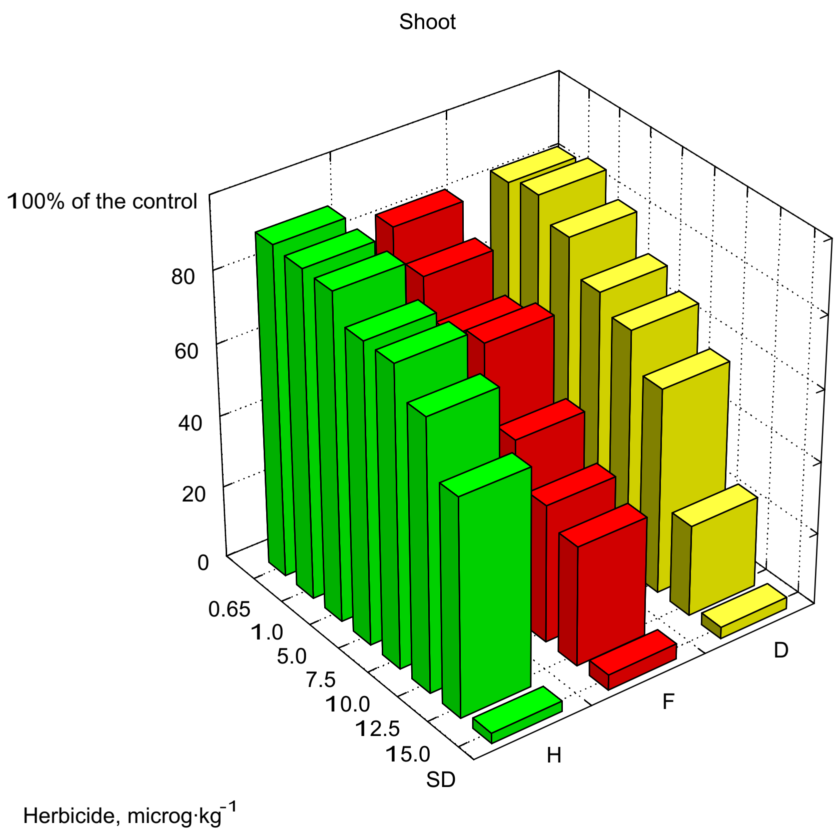
RESULTS AND DISCUSSION

Radish

Isoxaflutole from 0.65 to 15.0 µg a.i. kg^{-1} inhibited both shoot and root growth of radish (Fig. 1). Radish root growth was more sensitive to soil-applied isoxaflutole than radish shoot growth (fresh or dry weight). Herbicidal activity of isoxaflutole at 15.0 µg a.i. kg^{-1} was more pronounced than the other concentrations. The percentage reduction of height, fresh weight and dry weights of radish were 39.4, 66.6, and 74.7%, respectively, 14 days after treatment (DAT) as compared to the untreated check when herbicide concentration in soil was at 15.0 µg a.i. kg^{-1}. The growth reductions of the root length, fresh weight and dry weight of radish were from 71 to 78%, respectively, at 15.0 µg a.i. kg^{-1}. With the increase in concentration of isoxaflutole the dry weights of both shoot and root decreased, which could be explained with linear or quadratic regressions. The reduction in dry weight of the shoots followed the equation: ShtWt. = 15.84 – 1.52 (concn) + 0.34 $(concn)^2$ with an R^2 = 0.980 (Fig. 2). While the reduction in dry weight of roots could be explained with equation: RtWt. = 3.28 + 4.97 (concn), where R^2 = 0.975. Radish was sensitive to isoxaflutole and the GR_{50} value was determined as 12.6 µg a.i. kg^{-1} for shoots and 9.4 µg a.i. kg^{-1} for roots (Table 1). The GR_{80} values were obtained from extrapolation of experimental data.

Fig. 1. Growth response of radish to soil-applied isoxaflutole at various concentrations
H - height of shoot as % of the control, F - fresh weight as % of the control,
D - dry weight as % of the control, L - length of root as % of the control,
SD - standard error of the experiment



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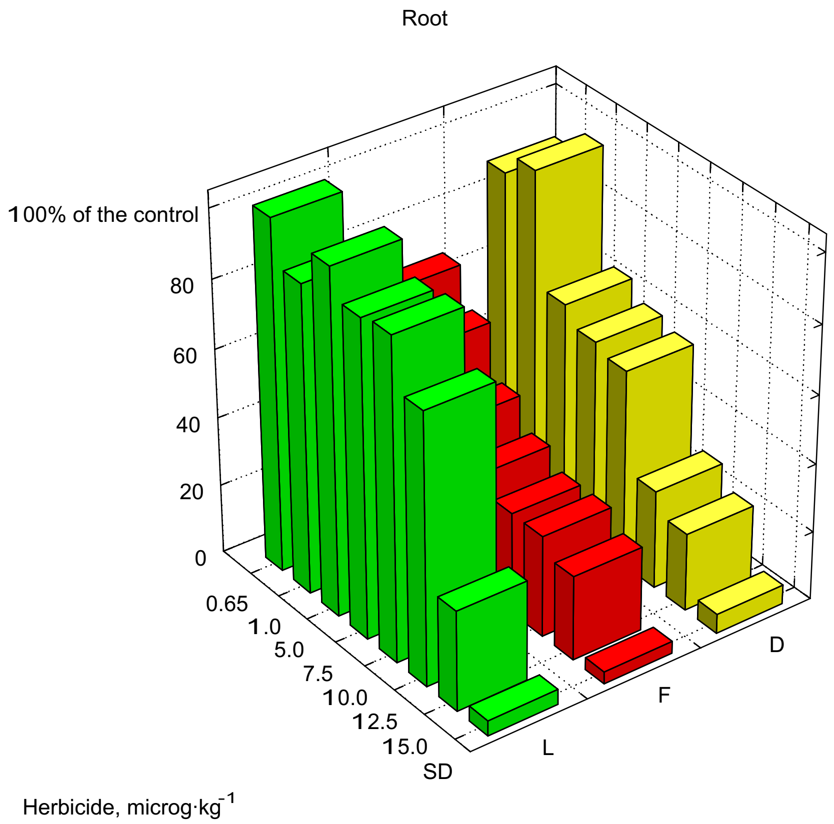
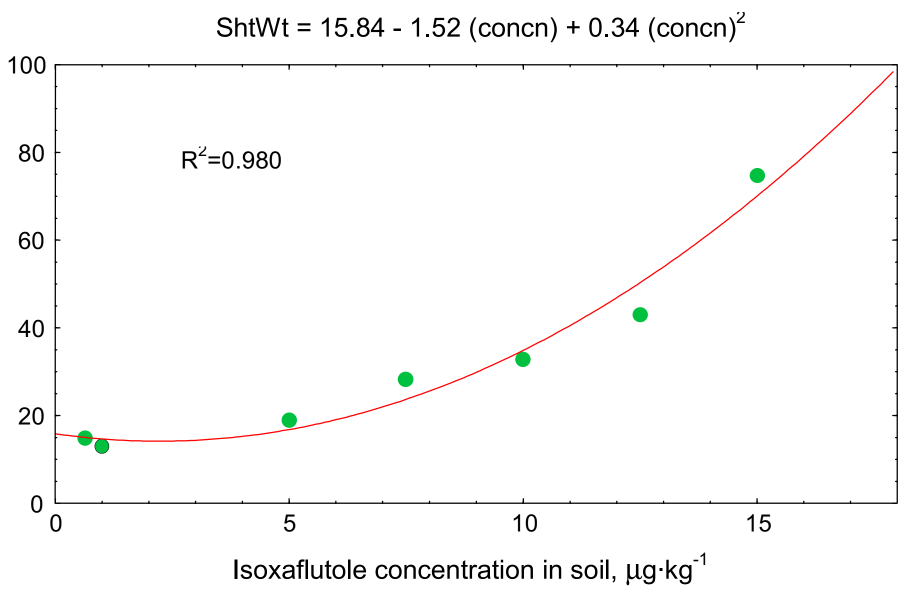


Fig. 2. Percent reduction in radish shoot and root dry weights 14 days after treatment as affected by various concentrations of isoxaflutole
Each point represents mean values observed, while each line is fitted to the regression model for shoot or root



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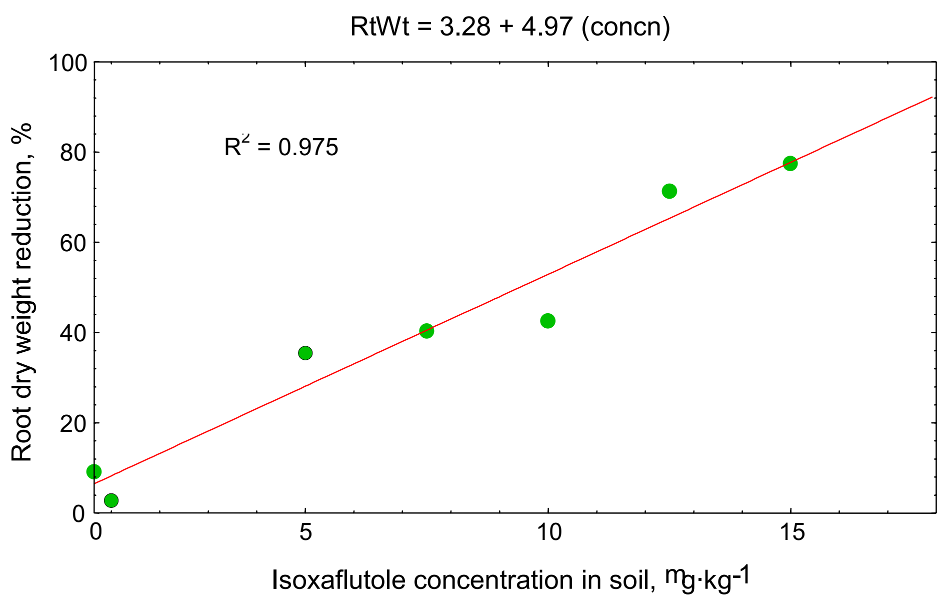


Table 1. Isoxaflutole concentration required to reduce 50% and 80% (GR_{50}, GR_{80}) of the dry weight of shoot and root of three crops

Crop	Estimates of GR_{50} and GR_{80} value of isoxaflutole, µg a.i.·kg^{-1} of soil			
	Shoot		Root	
	GR_{50}	GR_{80}	GR_{50}	GR_{80}
Radish	12.6	16.2*	9.4	15.7*
Oilseed rape	5.0	10.9	4.8	10.5
Wheat	414.3*	682.1*	266.9	417.6*

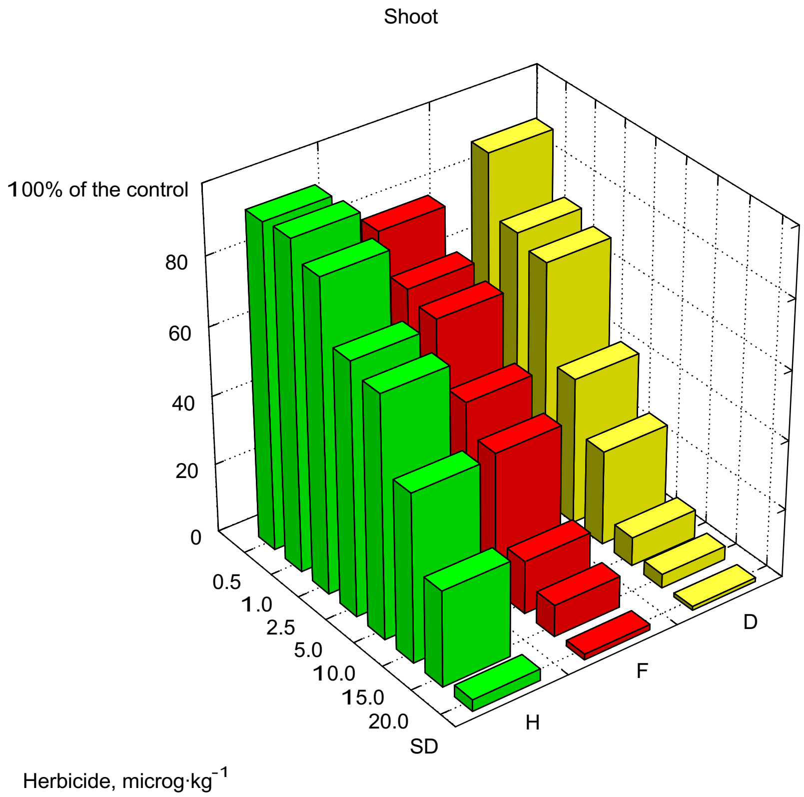
*** values obtained by data extrapolation**

Plants susceptible to isoxaflutole displayed bleached symptoms. As a result of the herbicide action the chlorophyll is destroyed and consequently the chloroplast. Finally the plants appear totally bleached and die to the lack of photosynthetic nutrients [2]. Radish showed a general reduction of true leaf size, proportional to the concentration of the herbicide in the soil but overall radish shoots were less sensitive to isoxaflutole compared to the roots. Radish treated with high doses of isoxaflutole showed bleached symptoms.

Oilseed rape

Isoxaflutole from 0.5 to 20.0 µg a.i. kg^{-1} inhibited both shoot and root growth of oilseed rape (Fig. 3). Isoxaflutole at 15.0 µg a.i. kg^{-1} applied to the soil reduced height, fresh weight and dry weight of oilseed rape by 51.0, 84.6, and 91.7%, respectively, as compared with the untreated soil check. The growth reduction in the root length was 63.6%, fresh weight 90.8%, and dry weight 93.6%, at the same concentration of isoxaflutole in soil. The dry weights of both shoot and root decreased as a result of plant injury (Fig. 3). This relationship was highly correlated as demonstrated by the quadratic regression equation: ShtWt. = 12.01 + 8.85 (concn) – 0.23 (concn)^2 with an R^2 = 0.988 at 25 DAT (Fig. 4). The reduction in dry weight of roots could be explained with equation: RtWt. = 12.26 + 9.23 (concn) – 0.26 (concn)^2 with an R^2 = 0.990 (Fig. 4). The GR_{50} values were determined as 5.0 µg a.i. kg^{-1} for shoots dry weight and 4.8 µg a.i.·kg^{-1} for dry weight roots, while the GR_{80} values were determined as 10.9 for shoot and 10.5 µg a.i. kg^{-1} for root (Table 1). Concerning the trend of phytotoxicity of isoxaflutole was similary for radish and oilseed rape.

Fig. 3. Growth response of oilseed rape to soil-applied isoxaflutole at various concentrations
H - height of shoot as % of the control, F - fresh weight as % of the control,
D - dry weight as % of the control, L - length of root as % of the control,
SD - standard error of the experiment



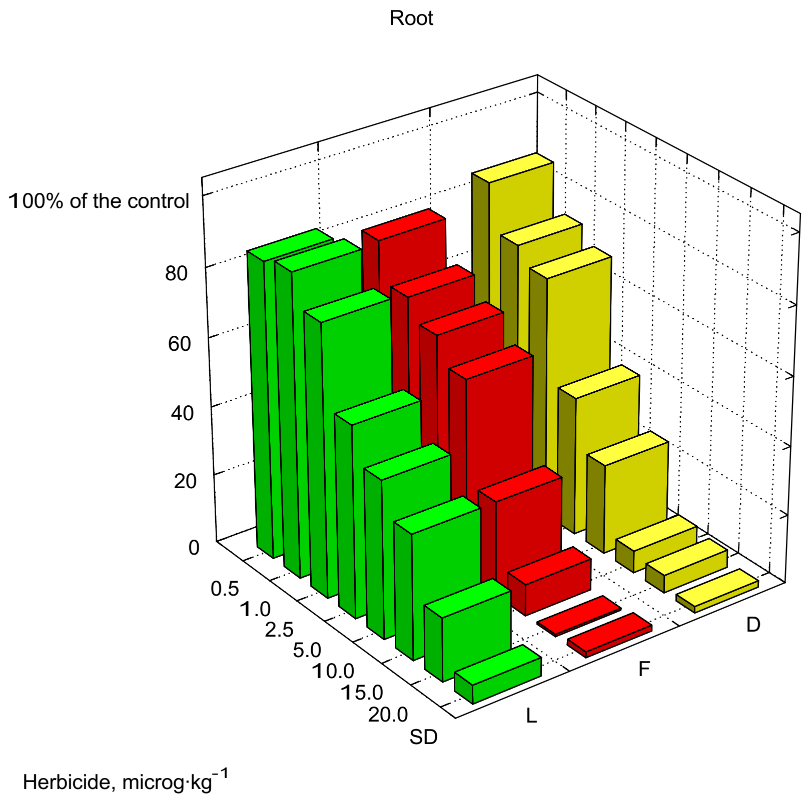


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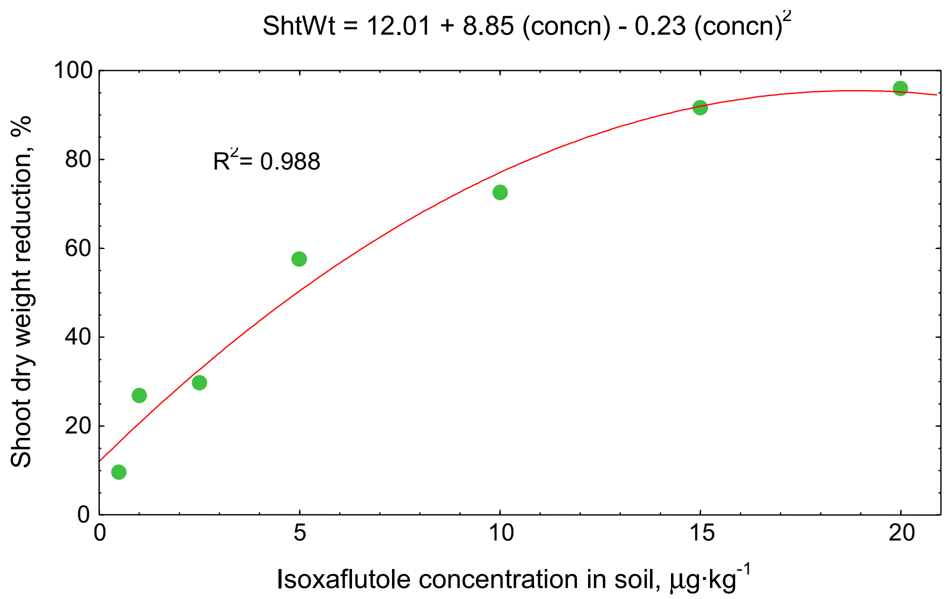
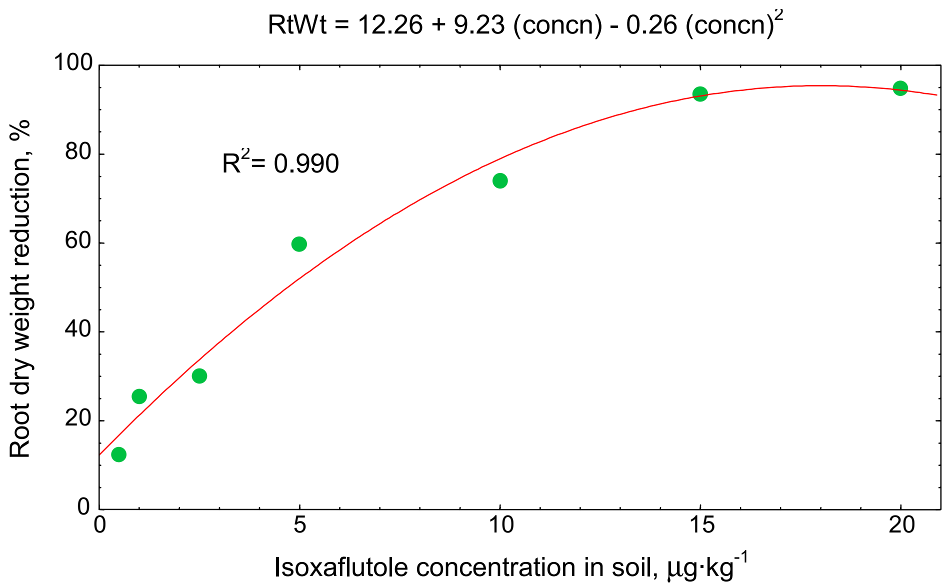
Fig. 4. Percent reduction of oilseed rape shoot and root dry weight 25 days after treatment as affected by various concentration of isoxaflutole
Each point represents mean values observed, while each line is fitted to the regression model for shoot or root





Wheat

Wheat was more tolerant to isoxaflutole than radish or oilseed rape. Herbicidal activity of isoxaflutole from 100 to 400 µg a.i. kg^{-1} in a fine sandy loam reduced the wheat growth (Fig. 5). Isoxaflutole at 400 µg a.i. kg^{-1} resulted in a growth reduction in shoot height by 19%, while fresh and dry weights of shoot were reduced by 45 - 47%, as compared with the untreated soil check at 21 DAT. Wheat roots were more sensitive to isoxaflutole than shoots (Fig. 5). Growth reduction in root fresh and dry weight was similar in value (71%). The linear regression line depicted shoot dry weight reduction: ShtWt.= 3.579 + 0.112 (concn), R^2 = 0.984 at 25 DAT (Fig. 6). Growth reduction in root weight was represented by the linear regression line: RtWt. = – 3.071 – 0.199 (concn), R^2 = 0.974. The GR_{50} value was determined as 414 µg a.i. kg^{-1} for wheat shoot and 267 µg a.i. kg^{-1} for wheat root (Table 1). A slight growth of bleaching symptoms due to high isoxaflutole doses was observed. Overall wheat roots were more sensitive to the herbicide compared to the shoots as noticed with other plants (i.e. oilseed rape and radish). Maize showed no visual effects of isoxaflutole in this soil type. The maize was tolerant to the herbicide, and maize growth reduction at 500 µg a.i. kg^{-1} was only 10% in a fine sandy loam (data not presented).

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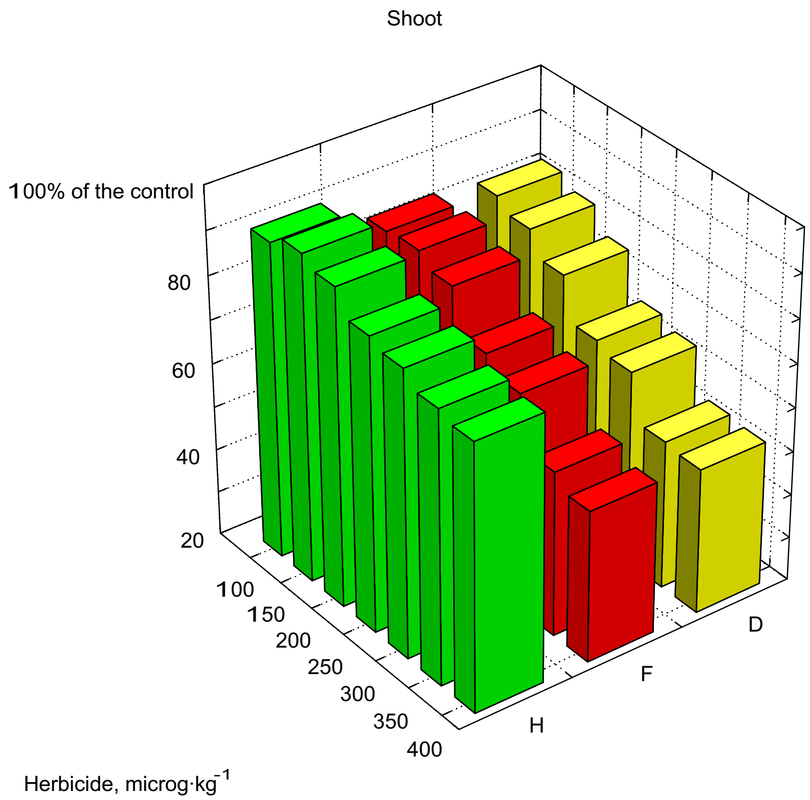
Fig. 5. Growth response of wheat to soil-applied isoxaflutole at various concentrations
H - height of shoot as % of the control, F - fresh weight as % of the control, D - dry weight as % of the control, L - length of root as % of the control, SD - standard error of the experiment



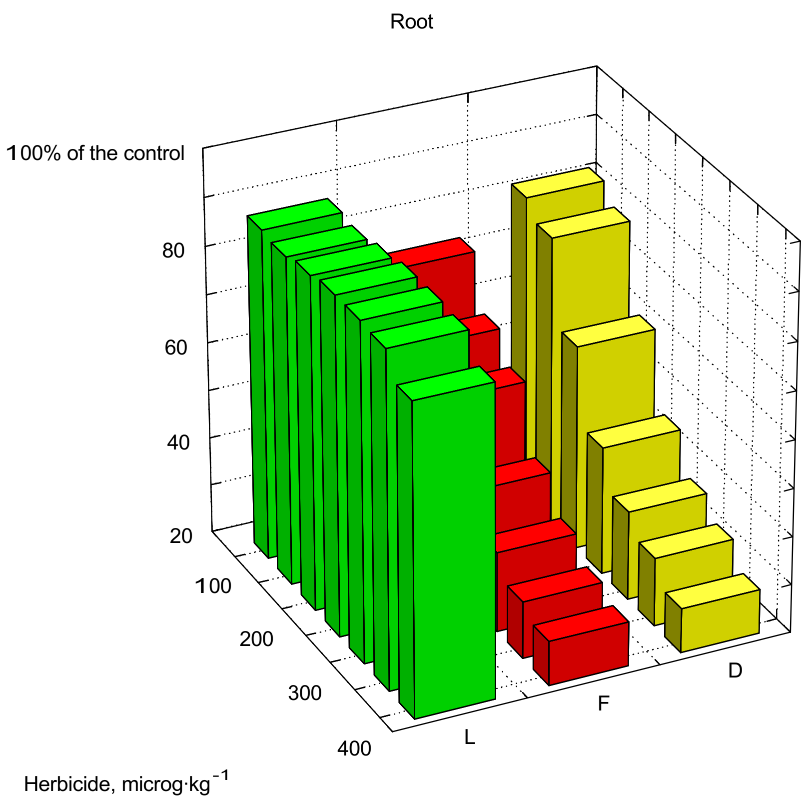


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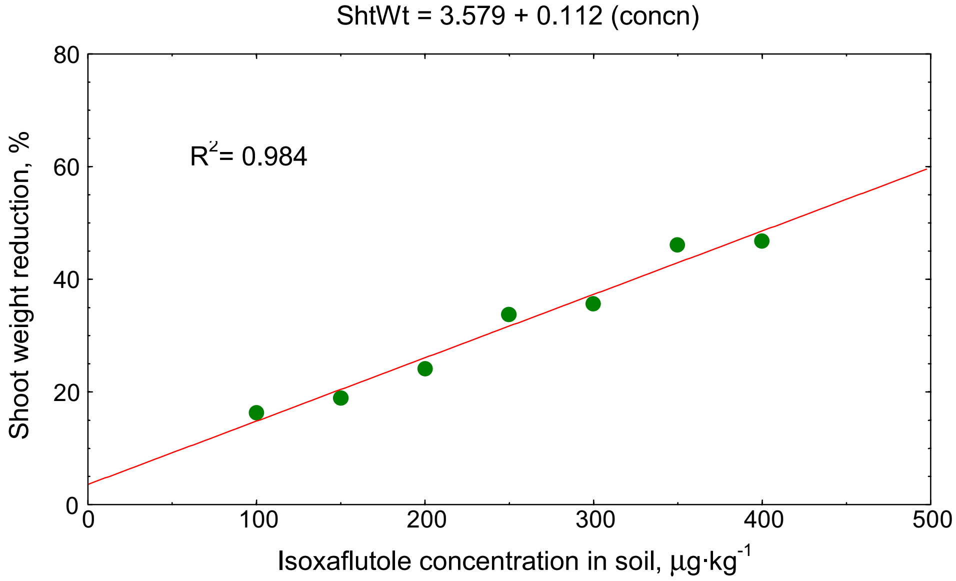
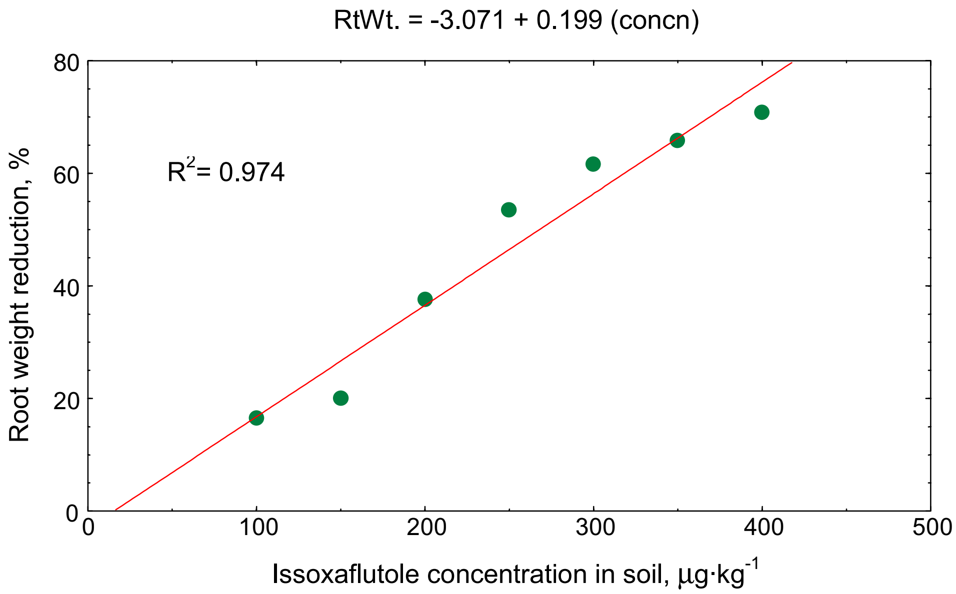
Fig. 6. Percent reduction in wheat shoot and root dry weights 21 days after treatment as affected by various concentrations of isoxaflutole
Each point represents mean values observed, while each line is fitted to the regression model for shoot or root





Short-term laboratory bioassays are used for detecting herbicide residues in soil. Applying isoxaflutole to soil before the seedling emerges is suitable for detecting herbicide that can be taken up by the root, and interfere with physiological and biochemical processes in the plant. In general, radish shoot and root were similarly sensitive to isoxaflutole, in contrary to bioassay of soaked seeds, where the GR_{80} value for radish root was twice the value for radish shoot [13]. Oilseed rape, like radish, was also sensitive to isoxaflutole. These results showed similar trends for soaked seeds as published earlier [13]. The response of root of oilseed rape to isoxaflutole was almost the same. Our data indicate that oilseed rape was a better plant species for bioassay than radish as the standard error of the experiment was smaller than that of radish. Wheat was much more tolerant to isoxaflutole. In this case, a differential response between shoot and root was observed.

The regression model could explain decreased dry weights of root and shoot. The coefficient value of multiple determinations (R^2) was high for all the shoot and root dry weights. *R*-squared for the regression increased from 97.4% to 99.0% depending on the test plant and part of plant. The linear regression analysis described growth response of radish root; wheat shoot/root indicates significance at 0.01 level. The quadratic polynomial regression had a good fit for data from radish shoot and oilseed rape shoot/root indicating significance at 0.01 level.

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These research results should help to determine the sensitivity and tolerance of plant species to isoxaflutole. The amount of isoxaflutole required to reduce the above–ground of oilseed rape as GR_{50} was 5.0 µg a.i. kg^{-1}, radish - 12.6 µg a.i. kg^{-1}, and wheat - 414.3 µg a.i. kg^{-1}. Similarly, Clay (1993) suggested using a wide range rates in order to obtain the best curve fit to the rate–response data. The variation of the shape of the dose-response curves with bioassay might be explained by differences in the growth rates of plants treated at different herbicide concentrations [12]. The response of a plant–species as oilseed rape and wheat to isoxaflutole residues in soil is a result of several complex dynamic processes, such as plant growth [2], herbicide metabolism [5] and degradation in soil [4,6], and, finally, degradation in plant.

Future research should examine quantitative understanding of dose-time-response relationships between isoxaflutole and soil.

CONCLUSIONS

1. Isoxaflutole inhibited the growth of radish and oilseed rape, wheat was more tolerant to herbicide of higher concentrations, while maize was tolerant to herbicide in a fine sandy loam soil.
2. The quadratic or linear regression model could explain decreased dry weights of root and shoot of radish, oilseed rape and wheat.
3. The ranking plants species based on sensitivity to isoxaflutole residues in soil included oilseed rape >radish>wheat, but differences between oilseed rape and radish were very small.
4. Oilseed rape showed the best plant parameter for bioassay method applied to detect residues of isoxaflutole in soil.

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Maria K. Swarcewicz
Department of General Chemistry, Agricultural University
Słowackiego 17, 71-434 Szczecin, Poland,
e-mail: mswar@go2.pl
Prasanta C. Bhowmik
Department of Plant and Soil Sciences
University of Massachusetts, Amherst MA 01003, USA
e-mail: pbhowmik@pssci.umass.edu
Sowmya Mitra
Department of Horticulture/Plant and Soil Science
California State Polytechnic University
3801 West Temple Avenue, Pomona, CA, USA