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Short communication

Isothermal calorimetry as a tool for estimating resistance of wild oat (*Avena fatua* L.) to aryloxyphenoxypropionate herbicides

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Abstract

The application of isothermal calorimetry for the early detection of the resistance of wild oat to fenoxaprop[1] and diclofop[2] was investigated. In the first test, three leaf tillers were sprayed with field doses of fenoxaprop or diclofop. For resistant biotypes, the rate of heat flow after 48 h was similar to that in control plants. In susceptible biotypes, fenoxaprop significantly reduced and diclofop significantly increased the rate of heat flow. In the second test, 3-day-old seedlings were put into calorimetric ampoules on filter paper moistured with herbicide solution (152% and 40% of the field dose for fenoxaprop and diclofop, respectively). Rate of heat flow was measured for 72 h, however, differences were already visible in the first hours of germination on each herbicide. Rate of heat flow for seedlings resistant to both herbicides was higher than for susceptible ones. The most evident differences between susceptible and resistant biotypes were noticed after 10–20 h and 25–40 h (of the seedlings' growth) on fenoxaprop and diclofop, respectively, when a sharp increase of rate of heat flow was observed. In conclusion, calorimetry may be used as a rapid test for the detection of the resistance of wild oat biotypes to fenoxaprop and diclofop.

Keywords: *Avena fatua*; Herbicides; Isothermal calorimetry; Resistance

1. Introduction

Wild oat (*Avena fatua* L.) is one of the most troublesome grass weeds in temperate climates. It mostly infests spring cereals, like wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). The use of mechanical cultivation to reduce wild oat infestation in fields is ineffective, because of the unequal germination of seeds [1]. It is the reason for the prevailing usage of postemergence herbicides. Some of the most common active compounds of herbicides used in the field to fight with wild oat are aryloxyphenoxypropionates—fenoxaprop and diclofop. The limited number of herbicides able to prevent wild oat in cereals is a serious difficulty. Continuous employment of herbicides with the same mode of action may lead to the resistance of wild oat. Herbicide resistance denotes the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to a wild type [2]. Worldwide *A. fatua* is highly resistant to a number of herbicides, mostly to ACC-ase inhibitors [2,3] from A group [4]—inhibitors of lipid biosyntheis [5]. The most popular methods for detecting herbicide resistance are glasshouse pot experiments and laboratory tests [6,7]. But these methods are time and space consuming. Nowadays, according to Ridley et al. [8], when resistance to herbicides in many countries has become a serious problem, rapid tests for herbicide resistance detection are needed. Compared to laboratory tests and field experiments, calorimetric methods appear to be faster for detecting many different stressors [9]. Measurements of rate of heat flow from various kinds of biological material enables rapid studies of pathogenesis [10], salinity [11,12] and drought [13] stresses, and may be useful for determination of temperatures for the optimal growth of plants [14–17]. Calorimetry may be used in the studies of herbicide resistance (Suwanagul 1995, cited in [18]). The purpose of this study was

Abbreviations: fenoxaprop, (±)-2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoic acid; diclofop, (±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid

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[1] Trade formulation: Puma Uniwersal 69 g a.i. L^{-1}; Aventis CropScience.
[2] Trade formulation: Illoxan 360 g a.i. L^{-1}; Aventis CropScience.

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to investigate the usefulness of calorimetry in the early detection of the resistance of wild oat to fenoxaprop and diclofop.

2. Materials and methods

2.1. Plant material

Biotypes: MIECH resistant to fenoxaprop and SPYT resistant to diclofop, and a susceptible biotype: PRAND, were chosen for the experiment [19]. Seeds were collected from fields in south-eastern Poland, from 1 m^2 of wild oat patch on each field. Resistant seeds were taken from fields where, according to farmers, these herbicides were ineffective.

2.2. Laboratory test of resistance

Seeds of resistant and susceptible wild oat biotypes were dehulled by hand and germinated in Petri dishes for 48 h at room temperature, in darkness. Susceptibility to herbicides was tested by a modified Letouze et al. method [20]. Germinated seeds of each population were put on thick filter paper in 500 cm^3 transparent, plastic boxes with three layers of glass granules on the bottom. The experiment was a totally randomized design with three replications (10 seeds in each box). Water solutions (25 cm^3) of 10.5 mg of fenoxaprop or 36 mg of diclofop was put into each box. Concentrations of both herbicides were established as lethal doses in earlier experiments. The boxes were placed in a growth room with a 16 h photoperiod, at 21/15 °C (day/night) temperatures. After 6 days the percentage of seedlings alive in each biotype and their coleoptile length were estimated.

2.3. Calorimetric measurements

Analyses of rate of heat flow from the leaves and germinated seeds of wild oat were conducted in an isothermal calorimeter (BioActivity Monitor 2277) at 20 °C.

2.3.1. Leaves

A pot experiment in a totally randomized design was established. Five germinated seeds were planted in five 10 cm diameter plastic pots filled with a 2:1 (v/v) peat/sand mixture. The pots were placed in a growth room with a 10 h photoperiod and at 15/10 °C (day/night). Plants in the three to four leaf stage were sprayed with herbicide, at the recommended field rate. Control plants were sprayed with water. Measurements on the second fully developed leaf were conducted at 24 and 48 h after spraying. A 3 cm long leaf was put into an ampoule, equilibrated for 25 min and then the rate of heat flow was recorded for 20 min. Comparisons were made between the same biotype treated with herbicide or with water (control).

2.3.2. Seedlings

The following combinations were studied: seeds of MIECH + fenoxaprop, seeds of SPYT + diclofop, seeds of PRAND + fenoxaprop, seeds of PRAND + diclofop, seeds of these biotypes + water (control).

Twenty cubic centimeter ampoules equipped with lids which enable natural air exchange were used. Three seeds (germinated as described in Section 2.2) were put into ampoule on filter paper (Ø 18 mm) moistured with 300 μl of solution containing 126 μg fenoxaprop or 432 μg of diclofop (doses similar to those in the laboratory test). The reference ampoule contained only the same solution. Seeds germinating on water moistened paper were taken as a control. Heat rate data were collected for 72 h. Rate of heat flow was recalculated per 1 g of seedlings dry weight, thus converted into specific rate of heat flow.

2.4. Statistical analyses

Statistical analyses of the laboratory test was done by a non-parametric Kolmogorov–Smirnov test [21], for the comparison of two independent samples. The percentages were compared with χ^2 Pearson's test. A comparison was made between the susceptible biotype PRAND and resistant biotypes—MIECH and SPYT. Data from the calorimetric measurements on leaves ($n = 5$) were subjected to analysis of variance (ANOVA), and means were compared by Tukey's test [STATISTICA 6.0 for Windows, StatSoft Inc.]. For calorimetric measurements on seeds, the data presented as graphs are mean values of five repetitions for each treatment.

3. Results

3.1. Resistance to herbicides

A significantly higher percentage of seedlings resistant to herbicides survived, Table 1. Germination of susceptible seedlings was 60% lower than resistant ones. Resistant seedlings also developed significantly longer coleoptiles than susceptible ones.

3.2. Leaf heat production

Twenty-four hours after treating wild oat plants with fenoxaprop, a significant decline of rate of heat flow in both resistant and susceptible biotypes, in comparison to control, was observed, Table 2. Treating with diclofop increased heat rate of leaves in resistant biotypes by about 39%. In the case of susceptible biotypes, the heat produced was the same as the control. Less ambiguous results were observed after 48 h. Both resistant biotypes had heat rates similar to their controls, but susceptible

Table 1
The influence of fenoxaprop and diclofop on survivability and growth of wild oat seedlings

Herbicide	Biotype	Population	Seedlings alive (%)	Coleoptile length (mm)
fenoxaprop	Resistant	MIECH	83*	10.0**
	Susceptible	PRAND	27	2.1
diclofop	Resistant	SPYT	83*	6.5*
	Susceptible	PRAND	23	2.8

* Differences significant at $p \leq 0.05$.
** Differences significant at $p \leq 0.01$.

Table 2
The influence of diclofop and fenoxaprop on heat production (mW g^{-1}_{DW}) from leaves of resistant and susceptible wild oat (*Avena fatua* L.) biotypes

Herbicide	Time after treatment (h)	Heat production (mW g^{-1}_{DW})			
		Resistant biotypes MIECH		Susceptible biotypes PRAND	
		Control	Treated	Control	Treated
fenoxaprop	24	7.35	5.56* (75.6)	9.44	6.11* (64.7)
	48	8.15	7.42 (91.0)	9.20	6.52* (70.9)
Herbicide	Time after treatment (h)	Heat production (mW g^{-1}_{DW})			
		Resistant biotypes SPYT		Susceptible biotypes PRAND	
		Control	Treated	Control	Treated
diclofop	24	7.53	10.43* (138.5)	10.34	10.54 (101.9)
	48	8.43	7.61 (90.3)	8.73	10.34* (118.4)

The figures in parentheses are changes in heat production rate as a percentage of control.
* Differences significant at $p \leq 0.05$.

biotypes were significantly different from controls. Biotype susceptible to fenoxaprop showed heat flow lower than control by about 29%. On the contrary, biotype susceptible to diclofop had values of heat higher by about 18% in comparison to its control.

3.3. Seedling heat emission

The rate of heat flow from control seedlings continuously increased during 72 h of growth, Fig. 1. The rate of heat flow from herbicide treated seedlings went through a maximum between 10 and 20 h for seedlings treated with fenoxaprop (Fig. 1A) and for plants growing on diclofop between 25 and 40 h (Fig. 1B). Differences in the rate of heat flow between resistant and susceptible biotypes were already apparent in the first hours of germination on each herbicide. Resistant plants had higher values of metabolic activity than susceptible ones. In general, the peak of metabolic heat of susceptible biotypes was lower by about 50% than the peak for herbicide resistant plants (Fig. 1A and B) The highest value for resistant biotype growing with fenoxaprop was 8.9 mW g^{-1}_{DW}. In comparison, the highest value for susceptible biotype was 4.4 mW g^{-1}_{DW}. As for diclofop, the maximum value for resistant plants was 8.4 mW g^{-1}_{DW} and for susceptible ones 5.7 mW g^{-1}_{DW}. Moreover, the peak of heat emitted by susceptible biotypes occurred later than in resistant ones. Qualitatively identical results were obtained in all replications.

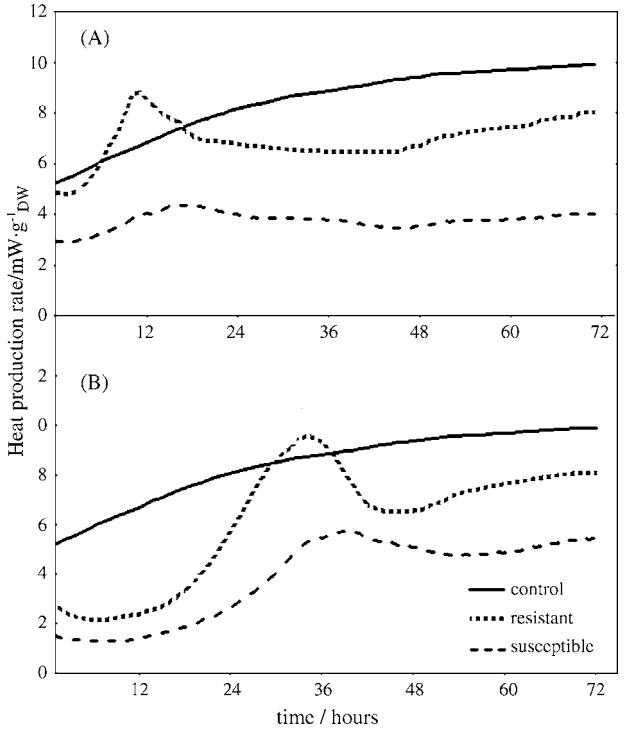

Fig. 1. Heat production rates of wild oat resistant and susceptible seedlings in the presence of fenoxaprop (A) and diclofop (B). The control curve applies for both resistant and susceptible biotypes, as the differences in heat flow between them were less than 4%.

4. Discussion

In the case of measurements conducted on leaves, results indicating differences between wild oat biotypes resistant or susceptible to diclofop or fenoxaprop, are found 48 h after leaf spraying. According to Maneechote et al. [22] 48 h after herbicide treatment is the first optimal time of herbicide action within the plant tissues. Calorimetric monitoring of seedlings germinating on herbicide solutions seems to be a much more promising test. The time to obtain information about a biotype's resistance could be considerably shortened. Differences in heat rates are visible in the first few hours of germination. Use of a multi-position calorimeter would provide results at once in a few replications. In these tests, both herbicides were applied in controlled laboratory conditions, which allows them to effectively enter and translocate within plant tissues [7]. According to Heap et al. [23] and Murray et al. [24] ACC-ase inhibitors used in such conditions are much more active, in comparison with field experiments.

Another problem is with the biochemical processes running in leaves and seedlings treated with the examined herbicides. The explanation of the nature of differences and time-dependent changes of heat flow from wild oat tissue, as a reaction to herbicides is an open question. Heat energy is largely evolved in oxidative processes of respiration, as well as (in much smaller extend) in biosynthesis and membrane transport, etc. [25]. Calorimetric measurements describe only the general metabolic activity of tissue. Probably, changes in heat emission are not the cause of respiration perturbations, as this process was not disturbed in herbicide treated leaves (data not shown). Increases in heat rates in resistant biotypes may result from the activation of metabolic pathways required for defense against stress caused by herbicides.

4.1. Conclusion

Calorimetric methods are useful tests for the detection of resistance to fenoxaprop and diclofop in wild oat biotypes. Calorimetric monitoring of seedling germination is proposed as a rapid test, with a large practical application in agriculture. The differences between resistant and susceptible biotypes are visible after the first few hours, and this effect deepens. Calorimetric measurement conducted on leaves, can be used to complement dose–response tests. Examined biotypes may continue their growth and reach the generative stage of development, seed collections and molecular studies. It is possible that both tests could be useful for estimation of the resistance of other Gramineae weeds against a herbicide's active ingredients.

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