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Strong endothermic effects caused by allelopathic interactions during growth of mustard, rape, wheat and clover seedlings

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Abstract In order to better understand the processes of allelopathic interactions and to elucidate the impact of various herbal extracts on seedling growth, investigations were initiated using isothermal calorimetry as a monitor. Seeds of wheat, mustard, rape and clover were germinated on aqueous herbal extracts from arnica, hypericum, milfoil, ribwort, sage and sunflower for 24 h (until the root was visible). Then, five seedlings were put into a calorimeter ampoule with herbal extracts. The specific thermal power (=heat production rate) of the seedlings during their growth was measured by isothermal calorimetry at 20 °C. Heat rate data were collected for 48 h. As a control seedlings were grown on water. The patterns of the thermal power–time curve during seedling growth on the herbal extracts and on water were completely different. In comparison with the water control, seedling growth on the herbal extracts was accompanied by a strong exothermic peak (first phase), whereas in the second phase distinct endothermic peaks were observed. The time after which the maxima of exo- and endothermic peaks occurred strongly depended on the seedling species and the origin of herbal extract. Similarly, the total thermal effect connected with seedling growth was correlated with the seedling species and herbal extract type.

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Introduction

Allelopathy is defined as the effect(s) of one plant on other plants through the release of chemical compounds (allelochemicals) into the environment [1]. This definition is largely accepted and includes both positive (growth promoting) and negative (growth inhibiting) effects. However, many ecologists favour definitions including only the negative effects in interactions between plants. For example, allelopathy is defined as growth suppression of one plant species by another due to the release of toxic compounds [2]. Although allelopathy has been known for many years, it has only recently been accepted as a legitimate area of research. One of the reasons for questioning allelopathy has been the lack of clearly identified allelochemicals and the use of inappropriate bioassays to identify the phenomenon of allelopathy [3]. Over the years, the procedures of chemical identification have become much more advanced and biologically active substances have been found [4]. Many of these chemicals are toxic to higher plants and for this reason they can be used as natural herbicides [5]. Products of the secondary metabolism of plants can be a source of natural phytotoxins. These may be compounds which have an allelopathic potential [6] and which are secreted as substances to aid competition between species [7]. These can also be compounds stored in plant glands [8] which serve to defend them against herbivores. Recently, compounds which release from roots to soil are commonly regarded as substances, which have a great importance in allelopathic interactions [9, 10].

Therefore, the impact of various water herbal extracts on changes of specific thermal power, connected with the early stages of seedling growth, were examined in this article. Investigations were performed on plants (seedlings) with different storage materials in their seeds, i.e. monocotyledonous (*Triticum aestivum* L.), dicotyledonous crucifers (*Sinapis alba* L. and *Brassica napus* var. *oleifera* L.) and dicotyledonous legume (*Trifolium repens* L.).

Thermal effects (heat flow) during seedling growth were continuously monitored by isothermal calorimetry.

Experimental

Plant material

Mustard seeds cultivars 'Nakielska' were received from the Plant Breeding and Acclimatisation Institute—Oil Seed Department in Poznań (Poland), seeds of rape cultivars 'Huzar' from the Plant Breeding Institute in Strzelce—Małyszyn Branch (Poland), wheat seeds cultivars 'Trend' from the Plant Breeding Institute in Poznań—Tulce Branch (Poland) and seeds of clover cultivars 'Huia' from "Plantator-agro" in Tarnów (Poland).

A total of 50 seeds from each species were germinated for 24 h (until the root was visible) in a plastic Petri dish on filter paper moistened with aqueous herbal extracts or distilled water (as a control) in darkness at 20 °C. Next, the obtained seedlings were used for the future experiments (as described below).

Preparation of extracts

The flowers of arnica, leaves of hypericum, milfoil, ribwort, sage and sunflower were received from "Herbapol" in Kraków. Then, the tissues were ground to powder. The extracts were prepared by shaking 5 g of powdered tissue with 95-mL distilled water in darkness at room temperature for 24 h.

Calorimetric measurements

The specific thermal power (STP) was measured (in mW) in an isothermal calorimeter (TAM III, TA Instruments). Ampoules (volume 20 mL) with lids were used in the experiment, which enabled air exchange. Five seedlings of mustard, rape and wheat and twenty seedlings of clover were put into an ampoule on filter paper with aqueous herbal extract. The volumes of extracts were 300 µL for mustard and rape, 500 µL for wheat and 250 µL for clover seedlings. In the reference ampoule there was only filter paper moistened with the same volume of extract. Seedlings on the filter paper with water were taken as an absolute control. Measurements of thermal power emitted during seedling growth were recorded continuously for 48 h at 20 °C. The heat production rate was expressed per gram of dry mass. The presented data are representative for ten independent biological replicates.

Results and discussion

Seedling heat production

Calorimetric measurements give information about plant tissue response to stress conditions, in this case these being allelopathic interactions. The value of STP shows the viability of seedlings during their growth. The STP of the control seedlings (growing on water) of each investigated

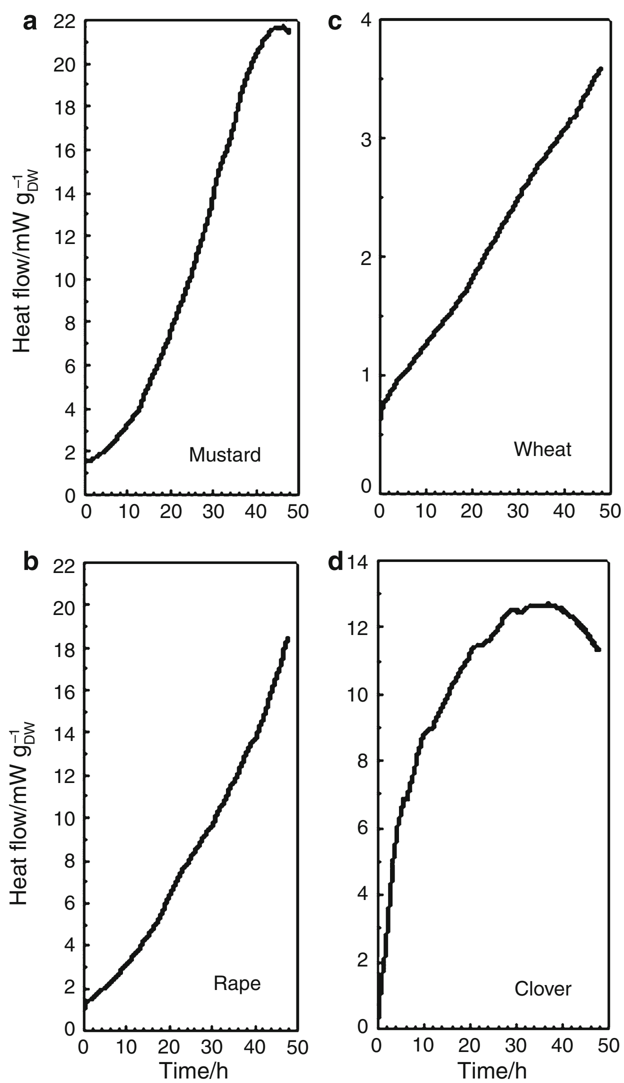

Fig. 1 Heat flow of mustard, rape, wheat and clover seedlings during growth on water (control)

species increased continuously during the 48 h of growth (Fig. 1a–d).

The growth of mustard and rape seedlings on water is characterised by a very similar pattern of thermal power–time curves (Fig. 1a, b). This could probably be connected with the same type of storage materials in the seeds and similar kinds of biochemical reactions taking place during seedling growth. On the other hand, the growth of wheat

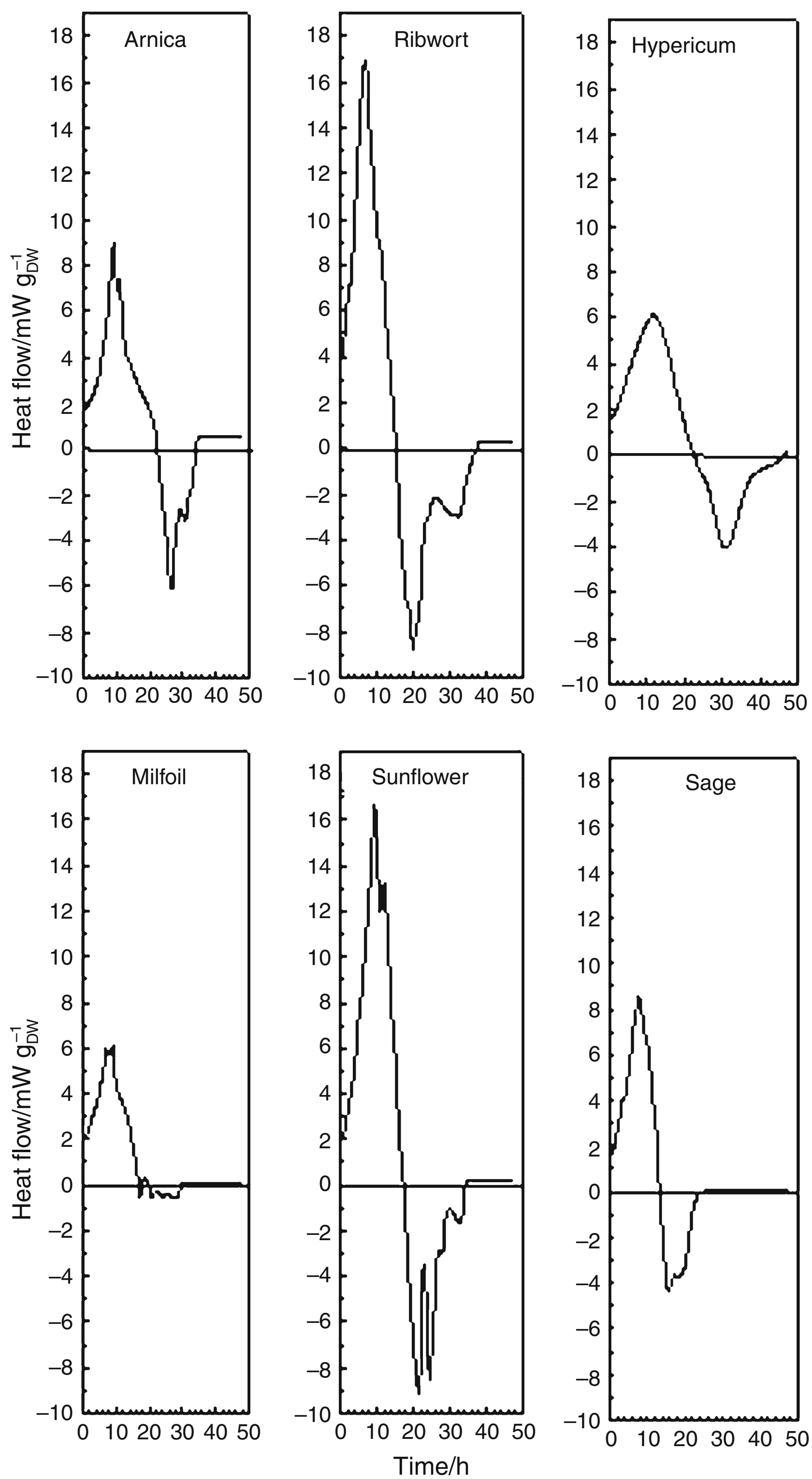

Fig. 2 Heat flow power of mustard seedlings during growth on herbal extracts

and clover seedlings is described by a dissimilar pattern of thermal power–time curves (Fig. 1c, d), which showed differences in biochemical processes connected with the breakdown of storage materials.

The differences in patterns of the thermal power–time curves for mustard seedlings (Fig. 2), which were grown on herbal extracts in comparison with water (Fig. 1a), are very impressive. Two distinct peaks are visible—the first one is exothermic and the second is endothermic.

This provides that chemical compounds which are present in the herbal extracts strongly influenced the metabolism of the seedlings. The shape of curve obtained for mustard seedlings growing on extract from arnica is very similar to the pattern of the curve for seedlings which grew on sage. The similarity between the thermal power–time curves is observed for seedlings growing on ribwort and sunflower extracts (Fig. 2). The values of thermal power in exothermic and endothermic peaks are the highest for the ribwort and sunflower extracts, whereas the lowest endothermic effect was observed during growth on the milfoil extract (Fig. 2).

Maxima of exothermic peaks appeared almost at the same time for most of all the extracts (around 8 h of measurements), with the exception of hypericum (ca. 12 h of measurements). Maximal values of endothermic peaks appeared after 30 h for hypericum and 16 h for sage extracts, whereas after ca. 23.5 h for the others (Table 1).

Differences in the STP between rape seedlings growing on herbal extracts were already evident in the first hour of measurement (Fig. 3). Also, in this case exo- and endothermic peaks were visible. The highest values (in the exothermic area) and the lowest values (in the endothermic area) of thermal power were observed for rape seedlings growing on arnica and sunflower extracts. On the other hand, values of thermal power for seedlings on hypericum, milfoil and sage extracts were similar (Fig. 3).

Maxima of exo- and endothermic peaks appeared on each extract at a similar time (ca. 8 or 17 h of measurements, respectively) with the exception of the hypericum extract, on which these maxima were observed after 13 and 24 h (Table 1).

The patterns of thermal power–time curves for wheat (Fig. 1c) and clover seedlings (Fig. 1d) which were grown on water were quite different in comparison to the pattern on herbal extracts (Figs. 4, 5, respectively).

In the case of wheat seedlings, endothermic reactions were not observed on the ribwort and milfoil extracts or they were very weak (Fig. 4). The strongest endothermic reaction was on the hypericum extract with the maximum at 47 h after seed germination (23 h of measurements). For wheat seedlings growing on the other extracts, the endothermic reaction occurred at a similar time (ca. 20 h). It is interesting to note that in the case of growth on the ribwort extract the exothermic peak reached its maximum just after 30 h of measurements (54 h after seed germination) (Table 1).

Table 1 Time (in hours) from the beginning of measurements to the appearance of maxima's exo- or endothermic peaks during growth of seedlings on herbal extracts

Species	Extract origin	Appearance time of maximum of exothermic peak/hours ± SD	Appearance time of maximum of endothermic peak/hours ± SD
Mustard	Arnica	9.0 ± 0.8^{c}	25.5 ± 1.5^{ac}
	Ribwort	7.8 ± 0.5^{bc}	26.7 ± 3.6^{ac}
	Hypericum	11.6 ± 1.0^{a}	29.6 ± 2.3^{a}
	Milfoil	6.1 ± 0.4^{b}	20.7 ± 1.6^{bc}
	Sunflower	8.5 ± 0.8^{c}	21.3 ± 2.9^{bc}
	Sage	7.1 ± 0.3^{bc}	16.0 ± 0.8^{b}
Rape	Arnica	8.9 ± 1.1^{b}	19.6 ± 1.8^{c}
	Ribwort	9.8 ± 1.0^{b}	19.3 ± 1.8^{c}
	Hypericum	13.0 ± 1.3^{a}	24.3 ± 0.9^{a}
	Milfoil	6.9 ± 1.0^{b}	16.4 ± 0.5^{bc}
	Sunflower	7.4 ± 1.0^{b}	16.0 ± 0.7^{bc}
	Sage	7.8 ± 1.0^{b}	15.3 ± 0.6^{b}
Wheat	Arnica	8.2 ± 1.0^{c}	21.6 ± 1.9^{ab}
	Ribwort	30.1 ± 1.3^{a}	–
	Hypericum	11.4 ± 1.0^{b}	23.1 ± 1.6^{a}
	Milfoil	4.5 ± 0.3^{d}	–
	Sunflower	5.1 ± 0.2^{d}	22.1 ± 1.5^{a}
	Sage	6.7 ± 0.8^{cd}	17.0 ± 1.5^{b}
Clover	Arnica	6.2 ± 0.9^{b}	15.8 ± 1.0^{b}
	Ribwort	12.9 ± 1.4^{a}	–
	Hypericum	10.9 ± 1.4^{a}	20.9 ± 1.9^{a}
	Milfoil	7.0 ± 0.2^{b}	16.0 ± 0.6^{b}
	Sunflower	6.5 ± 0.7^{b}	16.7 ± 1.3^{b}
	Sage	7.4 ± 0.7^{b}	15.4 ± 1.5^{b}

Mean values from 10 replicates ± SD. Values (for particular species) marked by the same letters do not significantly differ according to Duncan's test ($p < 0.05$)

In the case of clover seedlings (Fig. 5), strong endothermic reactions were visible on all of the herbal extracts with the exception of ribwort. Maxima of exothermic peaks appeared at the same time for arnica, milfoil, sunflower and for the sage extracts (ca. 7 h), whereas for ribwort and hypericum this was much later (after ca. 12 h). Maxima of endothermic peaks occurred two times longer than the maxima of the exothermic peak. Nevertheless, the time in which the maximum of the endothermic peak for the hypericum extract appeared was significantly different as compared to the others (Table 1).

Differences in the patterns of the thermal power–time curves for the investigated species proved that allelochemicals which are present in herbal extracts have a

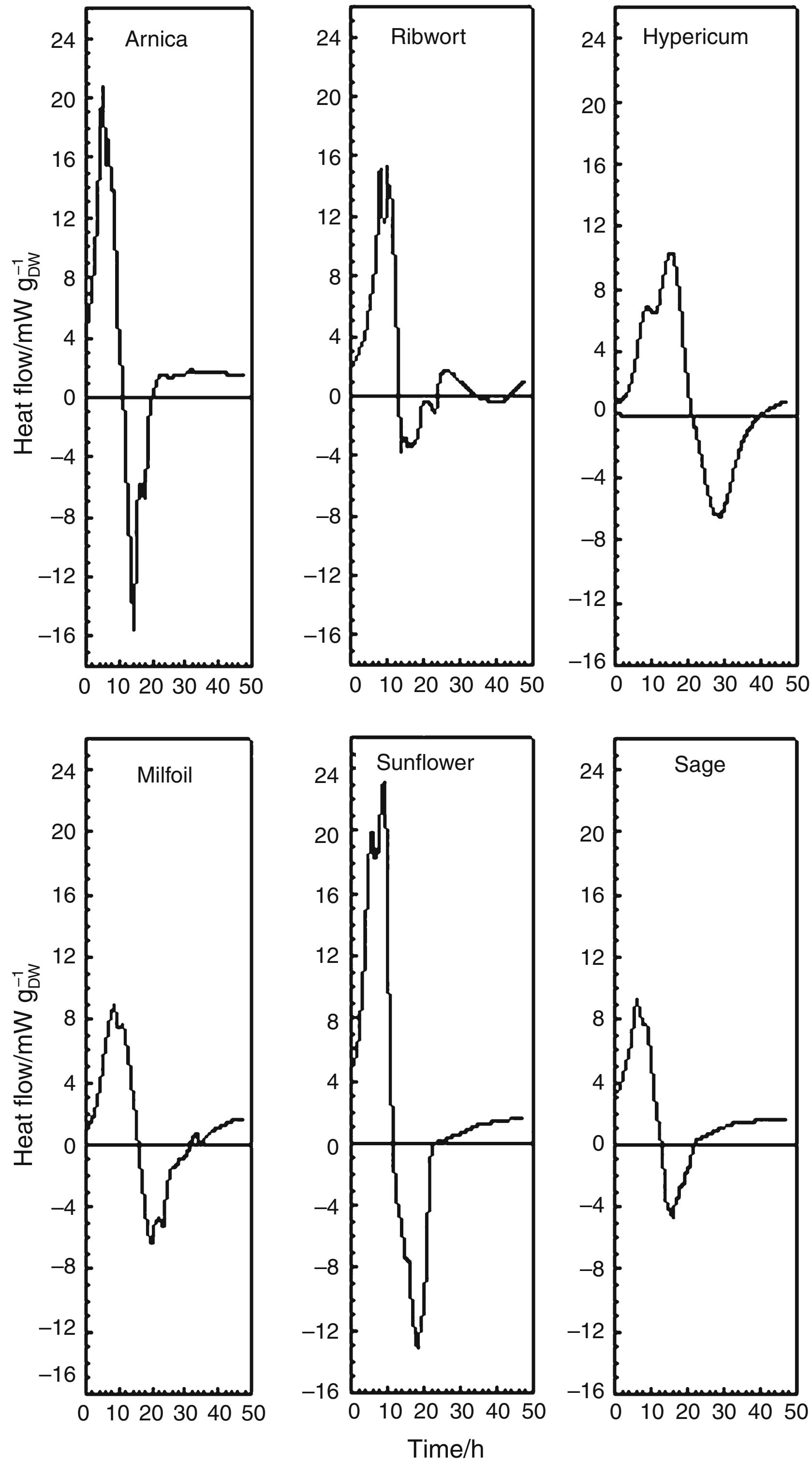

Fig. 3 Heat flow of rape seedlings during growth on herbal extracts

strong influence on the physiological processes and metabolism of seedlings.

The appearance of endothermic reactions during seedling growth on herbal extracts was quite surprising. Admittedly, similar endothermic reactions were observed earlier during the growth of mustard seedlings on selected herbal extracts (Skoczowski and Troć, unpublished data). Moreover, Schabes and Sigstad [11] described endothermic reactions which occurred during the germination of soybean seeds. Mass specific thermal power values for this reaction were incomparably lower than shown in this article. Sigstad and Prado [12] also indicated small

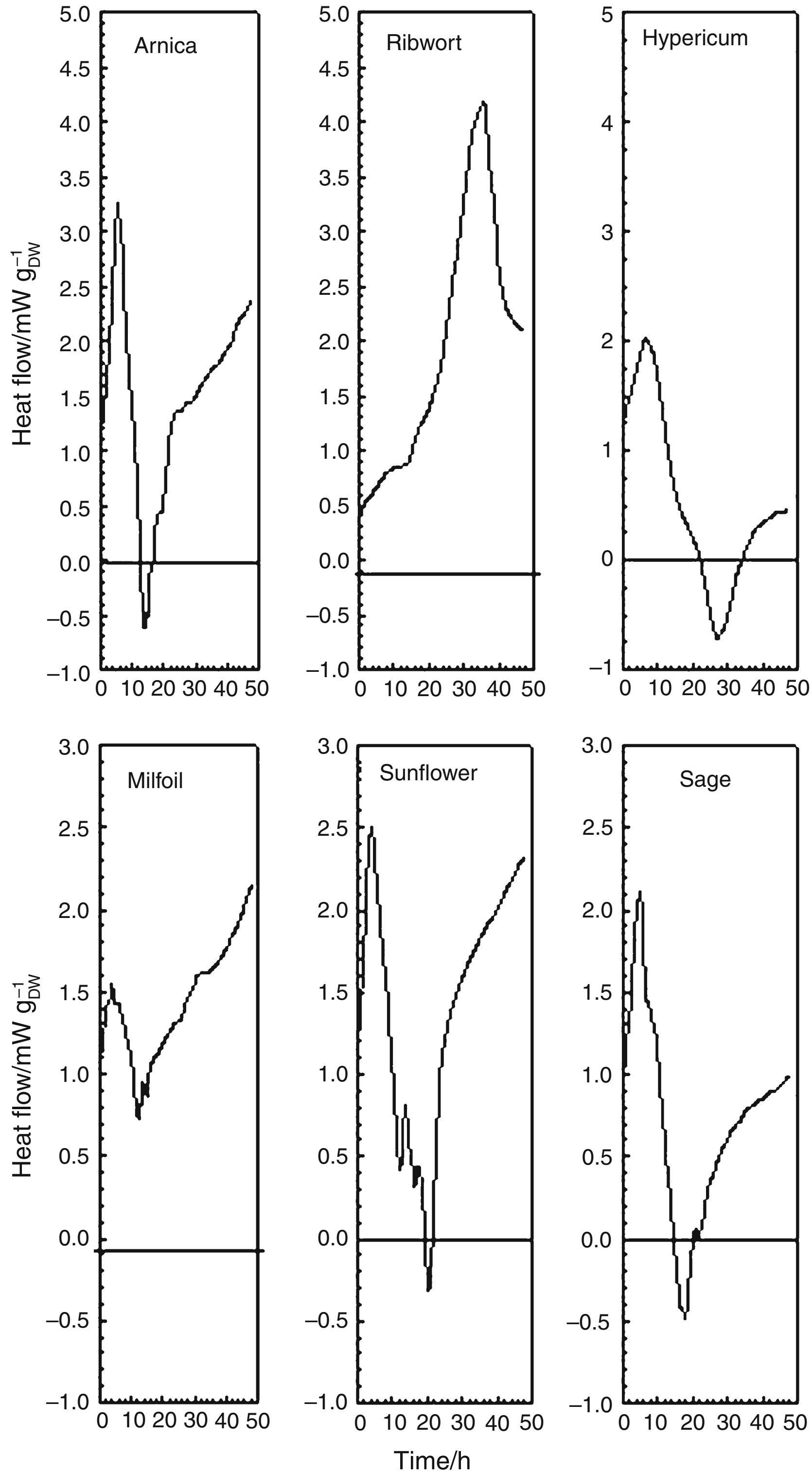

Fig. 4 Heat flow of wheat seedlings during growth on herbal extracts

endothermic peaks observed during the imbibition of *Chenopodium quinoa* seeds. Yet, according to the cited authors, these endothermic peaks were connected with the moment at which the root protrudes [12].

At this stage of experiments providing a physiological explanation of the phenomenon described in this article is very difficult. The investigated seedlings are autotrophic, characterised by high dark respiration intensity, connected

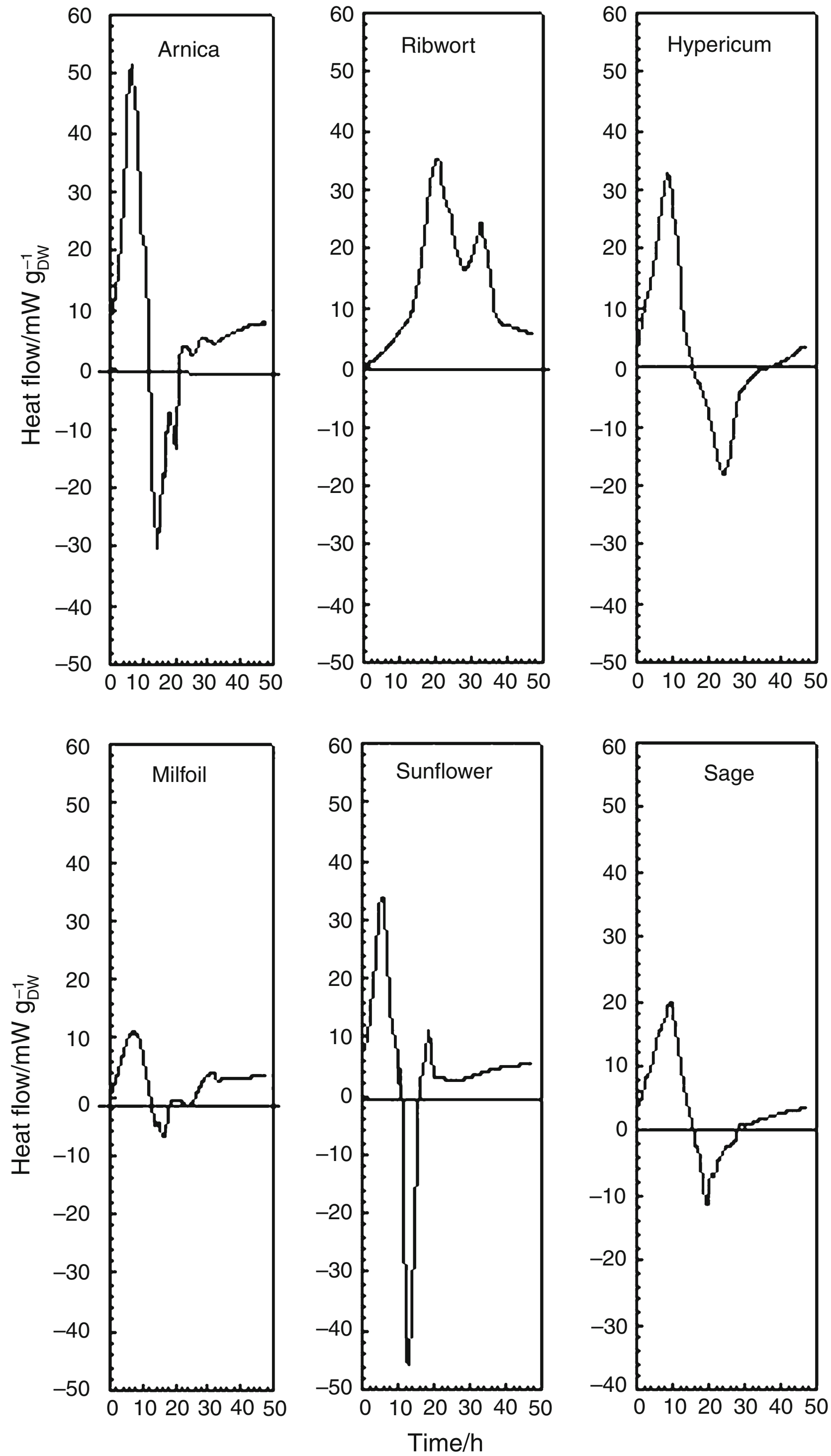

Fig. 5 Heat flow of clover seedlings during growth on herbal extracts

with a breakdown of storage materials and anabolic processes. Thus, high thermal power emission is expected. Therefore, perhaps the endothermic reactions take place out of plant tissue and are the results of interactions between exudates from the root system and chemical compounds which are present in the herbal extracts. An increase in permeability of the plasma membranes in seedlings growing on the herbal extracts could point to this supposition (data not shown). Moreover, when the mustard seedlings were transferred onto the water (immediately after appearance of the exothermic peak), endothermic reactions were not observed. Simultaneously, measurements of residues in the calorimetric ampoules (exudates + herbal extract) gave endothermic reactions (data not shown).

Conclusions

The thermal power–time curves obtained for mustard, rape, wheat and clover seedlings which were grown on herbal extracts indicated to which extent allelopathic compounds present in herbal extracts influence the metabolism of the growing seedlings. The results demonstrate the power of isothermal calorimetry as a tool to investigating the phenomena of allelopathy.

The other methods used with this purpose mainly show inhibition of growth (length, weight) but they do not show the changes of metabolic activity. Only with the use of calorimetry we can see the endothermic reaction during seedling growth. It is not possible to use any other method. Moreover, this method precisely showed the moment when additional detailed analytical methods should be used. In other words, it might be desirable to combine isothermal calorimetry with specific analytical techniques [12]. This trend was proposed earlier by Wadsö [13] and is also recommended by Skoczowski et al. [14]. The similarity of patterns of thermal power–time curves for mustard and rape seedlings which grew on the same herbal extract suggests that the kind of storage material in seeds plays an important role in the metabolic response of seedlings to stress factors such as allelochemical compounds.

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