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RESEARCH PAPER

Potent endogenous allelopathic compounds in *Lepidium sativum* seed exudate: effects on epidermal cell growth in *Amaranthus caudatus* seedlings

Amjad Iqbal and Stephen C. Fry*

The Edinburgh Cell Wall Group, Institute of Molecular Plant Sciences, School of Biological Sciences, The University of Edinburgh, Daniel Rutherford Building, The King's Building, Mayfield Road, Edinburgh, EH9 3JH, UK

* To whom correspondence should be addressed. E-mail: s.fry@ed.ac.uk

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Abstract

Many plants exude allelochemicals – compounds that affect the growth of neighbouring plants. This study reports further studies of the reported effect of cress (*Lepidium sativum*) seed(ling) exudates on seedling growth in *Amaranthus caudatus* and *Lactuca sativa*. In the presence of live cress seedlings, both species grew longer hypocotyls and shorter roots than cress-free controls. The effects of cress seedlings were allelopathic and not due to competition for resources. *Amaranthus* seedlings grown in the presence of cress allelochemical(s) had longer, thinner hypocotyls and shorter, thicker roots – effects previously attributed to lepidimoide. The active principle was more abundant in cress seed exudate than in seedling (root) exudates. It was present in non-imbibed seeds and releasable from heat-killed seeds. Release from live seeds was biphasic, starting rapidly but then continuing gradually for 24 h. The active principle was generated by aseptic cress tissue and was not a microbial digestion product or seed-treatment chemical. Crude seed exudate affected hypocotyl and root growth at ~ 25 and $\sim 450 \mu\text{g ml}^{-1}$ respectively. The exudate slightly (28%) increased epidermal cell number along the length of the *Amaranthus* hypocotyl but increased total hypocotyl elongation by 129%; it resulted in a 26% smaller hypocotyl circumference but a 55% greater epidermal cell number counted round the circumference. Therefore, the effect of the allelochemical(s) on organ morphology was imposed primarily by regulation of cell expansion, not cell division. It is concluded that cress seeds exude endogenous substances, probably including lepidimoide, that principally regulate cell expansion in receiver plants.

Key words: Allelopathy, *Amaranthus*, cell division, cell expansion, 4-deoxy- β -L-threo-hex-4-enopyranuronosyl-(1 \rightarrow 2)-L-rhamnose, growth regulator, lepidimoide, *Lepidium sativum*, oligosaccharins, seed mucilage.

Introduction

Plants are sources of diverse natural products, some of which have biological activity such as phytotoxins, antimicrobial agents, phytoalexins, and signalling agents (Fry *et al.*, 1993; Deng *et al.*, 2004; Muscolo *et al.*, 2005; Hegab *et al.*, 2008) and some of which provide nutrition to soil microbes (Braga and Dietrich, 1998; Braga *et al.*, 1998; Brigham *et al.*, 1999; Beninger *et al.*, 2004; Meepagala *et al.*, 2005; Isfahan and Shariati, 2007). The exudates from various plant organs contain low-molecular-weight compounds (such as sugars, inorganic ions, vitamins, nucleotides, amino acids, and phenolics), high-molecular-weight

substances (polysaccharides and enzymes, and other proteins), and root border cells (Campbell *et al.*, 1995; Dakora and Phillips, 2002; Macario *et al.*, 2003; Prakash *et al.*, 2003; Muscolo *et al.*, 2005; Bais *et al.*, 2006). For example, the polysaccharides of cress root epidermal mucilage were found to be rich in uronic acid, galactose, rhamnose, and arabinose residues (Ray *et al.*, 1988). Thus plant exudates consist of complex mixtures of large and small molecules, and cells, any of which might be of biological significance.

The secondary metabolites that are released into the rhizosphere by plant organs, such as roots, rhizomes, leaves,

stems, and seeds, and that affect neighbouring plants are known as allelochemicals. They enter the environment through various routes such as leaching, volatilization, root exudation, seed-coat exudation after imbibition, and decomposition of diverse parts of the plant (Rice, 1984; Higashinakasu *et al.*, 2004). When seeds of susceptible (receiver) plant species are exposed to allelochemicals, germination may be inhibited. If germination does occur, the seedlings may show abnormal growth, development, and metabolism. The most visible effects observed are retarded germination, short or no roots, lack of root-hairs, abnormally long or short shoots, swollen seeds, and low reproductive ability (Rice, 1979).

Hasegawa *et al.* (1992) reported that cress seedlings significantly affect the elongation of hypocotyls and roots in neighbouring *Amaranthus* seedlings. Exudates of 2-d-old cress seedlings have a similar effect on *Amaranthus* in the absence of living cress, and Hasegawa *et al.* (1992) initially suggested that the roots of cress seedlings exude a potent allelopathic substance that overstimulates shoot growth and inhibits root growth of potentially competing neighbouring plants. An active principle in cress seedling exudate was identified as lepidimoide [the sodium salt of 4-deoxy- β -L-threo-hex-4-enopyranuronosyl-(1 \rightarrow 2)-L-rhamnose], an unsaturated disaccharide (Hasegawa *et al.*, 1992) conveniently represented as Δ UA \rightarrow Rha. Based on its structure, lepidimoide was suggested to be derived from a pectic cell-wall component, rhamnogalacturonan-I (RG-I) (Fry *et al.*, 1993). Later, it was indeed successfully synthesized by digestion of RG-I-rich okra mucilage (Tanaka *et al.*, 2002). Lepidimoide has sometimes been inaccurately called 2-O-rhamnopyranosyl-4-deoxy- α -L-threo-hex-4-enopyranosiduronate, a name which wrongly implies Rha \rightarrow Δ UA rather than Δ UA \rightarrow Rha; this distinction is important because the rhamnose moiety is the reducing terminus of the disaccharide and the bio-activity of lepidimoide is lost if this position is converted to a methyl rhamnoside group (Hirose *et al.*, 2003). The 2-epimer of lepidimoide, Δ UA-(1 \rightarrow 2)-6-deoxy-L-glucose, does possess activity (Hirose *et al.*, 2004), as does Δ UA-(1 \rightarrow 2)-D-glucose (Yamada *et al.*, 1996).

Purified lepidimoide promoted hypocotyl elongation at concentrations above 3 μ M and inhibited root elongation above 100 μ M (Hasegawa *et al.*, 1992). Above about 300 μ M, lepidimoide increased the whole-seedling concentration of fructose 2,6-bisphosphate, a potent endogenous regulator of central metabolism which might possibly mediate changes in growth rate (Kato-Noguchi *et al.*, 2001); however, no significant effect on fructose 2,6-bisphosphate was detected at 3–100 μ M lepidimoide, concentrations sufficient for growth effects. Lepidimoide also enhanced the light-induced accumulation of chlorophyll and its precursor 5-aminolaevulinic acid in sunflower seedlings (Yamada *et al.*, 1998), delayed the loss of chlorophyll during senescence in oat leaf segments (Miyamoto *et al.*, 1997a), and mimicked auxin in inhibiting the abscission of bean petiole explants (Miyamoto *et al.*, 1997b).

Lepidimoide is not confined to cress seeds. It was exuded by surface-sterilized seeds of 24 species, including 17 dicots,

asparagus, leek, and five members of the Poaceae, when each was soaked in water at 25 °C for 2 d (Yamada *et al.*, 1995). Its high abundance in maize and oat is interesting because RG-I is relatively scarce in these plants. *Arabidopsis thaliana* (seeds soaked at 5 °C for 1 d) was later added to the list; its lepidimoide was isolated as the free acid rather than as the Na⁺ salt and given a different name, ‘lepidimoic acid’ (Yokotani-Tomita *et al.*, 1998). However, *in vivo* the ionic form applied is immaterial; therefore this report uses the name ‘lepidimoide’ for the free acid and any of its salts.

There are numerous reports of oligosaccharins (oligosaccharides exhibiting hormone-like biological effects) derived from plant and fungal cell-wall polysaccharides (Darvill *et al.*, 1992; Aldington and Fry, 1993). Examples include xyloglucan oligosaccharides (York *et al.*, 1984; McDougall and Fry, 1991; Kaida *et al.*, 2010), oligogalacturonides (Aziz *et al.*, 2004), galactoglucomannan oligosaccharides (Auxtová *et al.*, 1995; Benová-Kákosová *et al.*, 2006), gentiobiose (Dumville and Fry, 2003), and fungal oligo- β -glucans (Sharp *et al.*, 1984; Jamois *et al.*, 2005). Lepidimoide is of particular interest as it is one of the few postulated examples of an oligosaccharide with allelochemical activity (thus an interspecific oligosaccharin). Therefore, the physiology of the cress–*Amaranthus* interaction needed to be explored in more depth.

Several aspects of the cress–*Amaranthus* interaction remained poorly understood. For example, it was not certain whether cress tissues themselves directly manufacture lepidimoide by plant-genome-encoded activities or if lepidimoide is formed by the partial digestion of cress RG-I by microbial lyases, e.g. those from fungi (Saranpuetti *et al.*, 2006), including endophytic strains (Tanaka *et al.*, 2002), or bacteria (Ochiai *et al.*, 2007). Some previous studies (Yamada *et al.*, 1995, 1997) but not others (Yokotani-Tomita *et al.*, 1998) had used surface-sterilized seeds as a source of lepidimoide. The current study therefore directly compared aseptic and non-aseptic cress. Also, although lepidimoide was first described as originating from 2-d-old cress roots (Hasegawa *et al.*, 1992) and from 2-d-old seedlings of numerous species (Yamada *et al.*, 1995), later work on sunflower and buckwheat suggested that much of it actually exudes from the seed-coat and embryo prior to germination (Yamada *et al.*, 1997); the current study therefore compared seed exudates with seedling (root) exudates. Another question was whether a heat-stable allelochemical agent was solely responsible for the effect of cress seedlings on *Amaranthus* growth or whether interspecific competition also plays a role. An additional possibility was that the effect on hypocotyl and root elongation was due to seed-treatment chemicals present on commercially available cress seed and not due to natural cress exudates. Finally, this study investigated whether the cress allelochemicals influence *Amaranthus* seedling growth by regulating cell expansion alone or also cell division.

Materials and methods

Surface sterilization of seeds

Where specified in the Results, seeds were sterilized by shaking in sodium hypochlorite (containing 0.13%, w/v, active chlorine) at

room temperature for 10 min, then washed with 0.01 M HCl followed by sterile water. Seed sterility was verified by incubation in sterile water followed by nutrient broth (each 23 °C for 2 d). No microbial colonies were observed.

Germination of seeds in Petri dishes

In all experiments, unless otherwise stated, seeds were placed in 15 ml water or aqueous solution of exudates (with no filter-paper disc) in a 9-cm Petri dish, which was sealed with Parafilm and maintained at 23 °C for 5 d in the dark without shaking. In some experiments, 0.9 ml aqueous solution per 3-cm Petri dish was used. The seedlings were then surface-dried with paper towels, gently flattened between a sheet of glass and a piece of card, and photographed. The length of each seedling's hypocotyl and root was measured on the digital image by use of LabWorks software.

Growing cress seedlings with other species

Twenty seeds of *Amaranthus caudatus* (Love-lies-bleeding) plus 20 cress seeds were randomly placed in a Petri dish; 40 seeds of *A. caudatus* alone were used as a control. Similar experiments were repeated with lettuce (*Lactuca sativa*) in place of *Amaranthus*.

Growing *Amaranthus* seedlings in cress seedling exudates

Forty cress seeds were incubated as normal but for only 3 d; the seedlings were then removed and the water+exudate was left in the Petri dish. *Amaranthus* seeds (20) were then added and incubated for the next 5 d.

Growing *Amaranthus* seedlings in cress seed-coat exudates

Cress seeds (40) were imbibed in 15 ml water (for 24 h unless otherwise stated) at 4 °C, which allows imbibition but not germination and minimizes any contamination from micro-organisms. The seeds were then removed and *Amaranthus* seeds were placed in the same water and incubated for the next 5 d. Similar experiments were performed with so-called 'organic' cress seeds (free of pesticides) and with cress seeds collected from plants grown in a private garden in Edinburgh (receiving only rainwater). In other

experiments, the 24-h cress seed exudate was collected and centrifuged and the supernatant was freeze-dried; the dried exudate was then diluted in water at various concentration prior to a standard 5-d bioassay on *Amaranthus*. Finally, in a test of whether seeds needed to be viable in order to release a biologically active exudate, 1 g dry cress seeds was plunged into 200 ml boiling water, maintained at 100 °C for 15 min, and then incubated at 4 °C for 24 h in the same water, and then the effect of the exudate on *Amaranthus* seeds was bioassayed.

Measurements of epidermal cells

A 1-cm segment of hypocotyl from the middle of the hypocotyl was placed on a slide with a scale, and then the epidermal cells were examined under a transmitted light compound microscope ($\times 40$ objective). The photomicrographs were analysed by use of LabWorks software.

Results and discussion

Cress seedlings affect the elongation of receiver seedlings

Hasegawa *et al.* (1992) reported that cress root exudate has an allelopathic effect on *Amaranthus* seedlings. To extend this observation, the current study initially tested the effect of cress seedlings on the germination and seedling elongation of two receiver species in the same Petri dish. Twenty seeds of either *Amaranthus* or lettuce were incubated with 20 cress seeds. As a control, 40 seeds of each receiver species were incubated without cress seeds. Germination was unaffected (data now shown). However, seedlings of both receiver species that had been incubated with cress had significantly longer hypocotyls and shorter roots than the controls (Fig. 1). These results are in close agreement with the findings of Hasegawa *et al.* (1992).

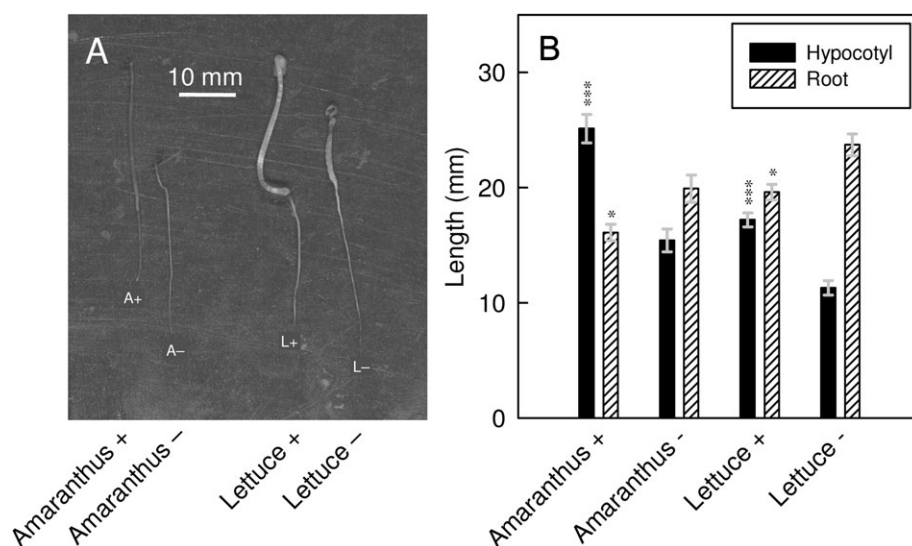


Fig. 1. Effect of cress (donor) seedlings on growth of *Amaranthus* or lettuce (receiver) seedlings. *Amaranthus* or cress seedlings were grown alone (-) or mixed with cress seedlings (+). After 5 d at 25 °C in the dark, representative receiver seedlings were photographed (A) and their hypocotyl and root lengths were measured (B). Data represent means from triplicate 9-cm Petri dishes \pm inter-dish SE. Significant differences from the relevant '-' seedlings are indicated: * $P < 0.05$; *** $P < 0.01$.

Cress seedlings also affect radial swelling of receiver seedlings and the effect persists under aseptic conditions

Live cress seedlings affected not only *Amaranthus* seedlings' growth in length (Fig. 2A) but also their growth in girth, inhibiting and promoting the swelling of hypocotyls and roots, respectively (Fig. 2B). Consequently, there was a large effect on the length:width ratio (Fig. 2C).

The effects of cress seedlings on the receiver species could in principle be due either purely to the cress seedlings themselves or to cress tissue plus associated micro-organisms, which might for example generate lepidimoide by partially digesting RG-I (Tanaka *et al.*, 2002; Saranpuetti *et al.*, 2006; Ochiai *et al.*, 2007). In a test of this, cress and *Amaranthus* seeds, or *Amaranthus* seeds alone, were incubated in both aseptic and non-aseptic conditions. After 5 d of incubation, *Amaranthus* seedlings from both aseptic and non-aseptic treatments had longer hypocotyls and shorter roots when grown with cress than when grown alone (Fig. 2A). Thus the effect of cress on *Amaranthus* seedlings was not due to surface micro-organisms.

The effect of cress seedlings on Amaranthus seedling growth is not due to competition for resources

Aseptic cress seedlings might exude allelopathic compound(s) and/or might compete with *Amaranthus* for dissolved oxygen in the medium (there were no other nutrients present in the medium, de-ionized water, for potential competition). In an experiment designed to distinguish allelopathy from competition, 20 *Amaranthus* seedlings were grown in the root exudates of 20 cress seedlings after removal of the latter. After 5 d, *Amaranthus* seedlings incubated with cress root exudates had

longer and thinner hypocotyls and shorter and thicker roots even though no living cress tissue was present (Fig. 3; root exudate data). This evidence shows that the effect of cress on a receiver species is not due to competition but must be an effect of substance(s) released from the cress seedlings.

Exudate from ungerminated cress seeds has a potent effect on Amaranthus seedling growth

Hasegawa *et al.* (1992) assumed that an allelopathic substance was exuded from the roots of cress seedlings. However, they used whole seedlings, and the same laboratory later reported that much of the allelopathic material actually originated from the seed-coat and embryo prior to germination (Yamada *et al.*, 1997). To distinguish these possibilities, the current study imbibed cress seeds for only 24 h at 4 °C, thus allowing imbibition but preventing germination, and applied the resulting seed exudate to *Amaranthus* seedlings at 25 °C. The seed exudate promoted *Amaranthus* hypocotyl elongation more strongly than the root exudate, while the effect on receiver roots was no different from that of the root exudate (Fig. 3; seed exudate data). This finding suggests that either the seed exudate contained new bioactive compound(s) different from the one (lepidimoide) found by Hasegawa *et al.* (1992) in cress seedling exudate or that the amount of lepidimoide was higher in seed exudate.

Exudate from 'organic' cress seeds also has potent allelopathic effects

Commercial seed may have come into contact with artificial plant growth regulators, fungicides, herbicides, or insecticides, which could theoretically have been responsible for

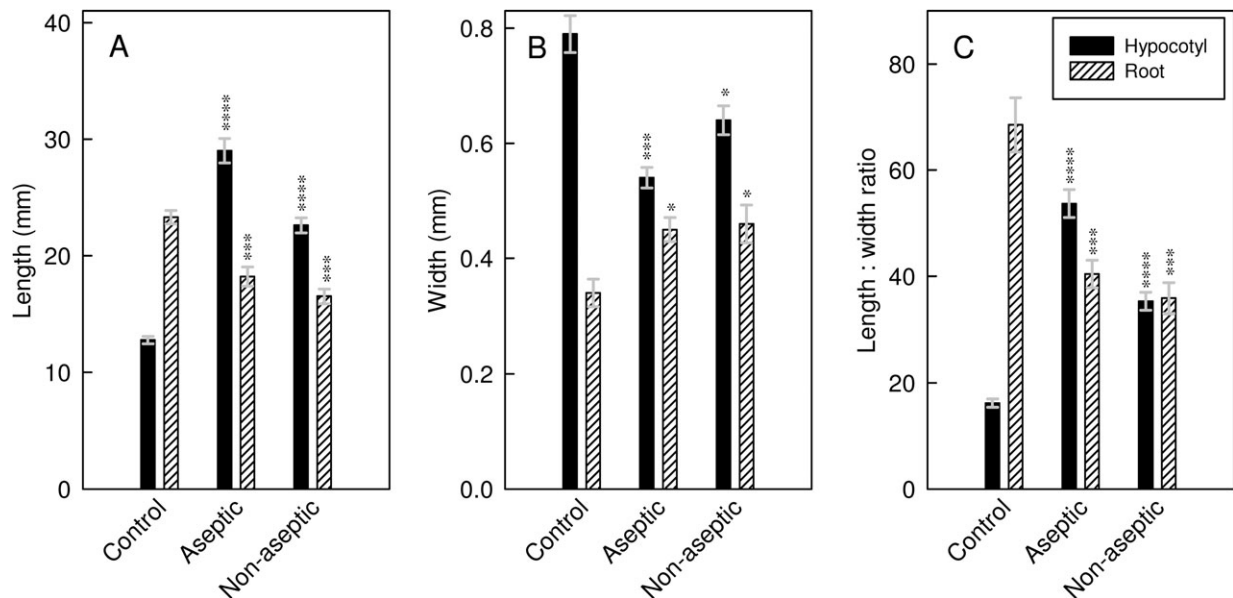


Fig. 2. Effect of cress seedlings on the growth of *Amaranthus* in aseptic and non-aseptic conditions. *Amaranthus* seedlings were grown alone (control) or mixed with cress seedlings under aseptic or non-aseptic conditions. After 5 d, the receiver (*Amaranthus*) seedlings' hypocotyls and roots were measured for length (A), width (B), and length:width ratio (C). Data represent means from triplicate 9-cm Petri dishes \pm inter-dish SE. Significant differences from the relevant control are indicated: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$.

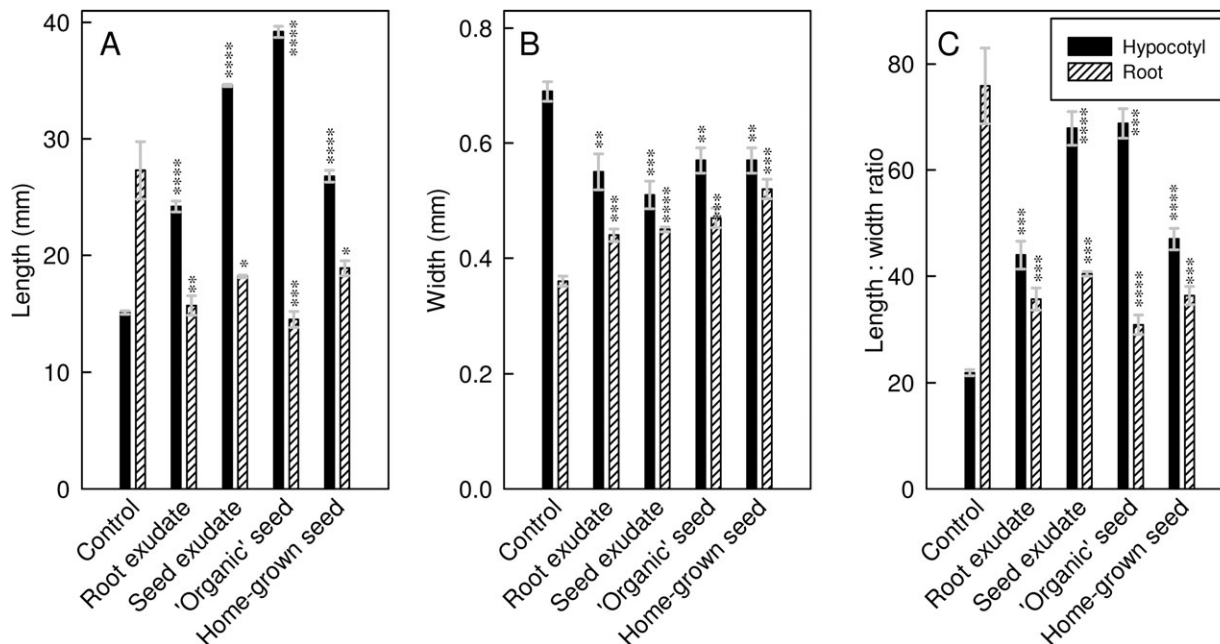


Fig. 3. Effect of root and seed exudates on *Amaranthus* seedlings in the absence of competition. *Amaranthus* seeds were incubated for 5 d in water (control), root exudate collected by the method of Hasegawa *et al.* (1992) from cress seedlings, or seed exudate from imbibed but ungerminated cress seeds. Where indicated, the exudates were from commercial 'organic' cress seed or from seed collected from 'home-grown' cress plants maintained in unfertilized garden soil with only rainwater. The 5-d-old *Amaranthus* seedlings were then measured for length (A), width (B), and length:width ratio (C). Data represent means from triplicate 9-cm Petri dishes \pm inter-dish SE. Significant differences from the relevant control are indicated: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$.

the observed effects of seed exudate on the growth of receiver seedlings. Therefore, seed exudate from commercial 'organic' cress seed (stated to be free of synthetic chemicals) was collected. *Amaranthus* seedlings grown in its presence were affected in the usual way (Fig. 3).

Although the commercial 'organic' seed was stated to be free from synthetic chemicals, there was still the possibility that it had been in contact with 'organic' preparations containing natural growth regulators (e.g. auxins and gibberellins). Cress plants were therefore grown in an unfertilized garden in Edinburgh supplied only with rainwater and the next generation of seed was collected after flowering. Exudate from this seed again affected *Amaranthus* seedling growth in the usual way (Fig. 3). It is deduced that seed-treatment chemicals are not responsible for the apparent allelopathic effect. Therefore, the effect on *Amaranthus* seedlings was due to the compound(s) which are naturally exuded from cress seeds.

Release of allelochemical(s) from cress seed is very rapid

In an experiment designed to identify when maximally bioactive material is exuded from seed, cress seeds were imbibed for various times and applied the exudates to *Amaranthus* (Fig. 4). Within the first few minutes, cress seeds released soluble bioactive compound(s) into their surroundings. Hypocotyl and root length and width were all affected by the 10-min exudate. The bioactivity towards receiver

hypocotyls reached its maximum at 24 h and thereafter started losing activity, while the activity towards *Amaranthus* roots reached its maximum at 6 h and then remained constant until at least 48 h. Thus, the bioactive compound(s) were released into aqueous solution as soon as the seeds were imbibed. This quick release of bioactivity suggests that the allelochemical(s) might be stored in or on the seed-coat and released into the environment, possibly as a defence mechanism, as soon as the seeds are imbibed; nevertheless, additional activity was gradually released given more time. Yamada *et al.* (1997) had shown that dry sunflower and buckwheat seeds contain phenol-extractable lepidimide, especially in the seed-coat. They also showed that a large amount of additional lepidimide is released by moistened seeds during 2-d imbibition, the great majority arising from the embryos rather than the seed-coats (Yamada *et al.*, 1997).

The allelopathic material is heat-stable and already present in dry cress seeds

In a test of whether the release of the bioactive principle was dependent on the cress seeds' metabolism, dry cress seeds were placed in boiling water for 15 min and then incubated in cold water for 24 h. The collected exudate from denatured seeds again resulted in *Amaranthus* hypocotyls with significantly longer hypocotyls and shorter roots than the control (Table 1). The results show that the bioactive compound(s) were heat-stable and were formed and stored by the embryo or seed-coat during seed maturation.

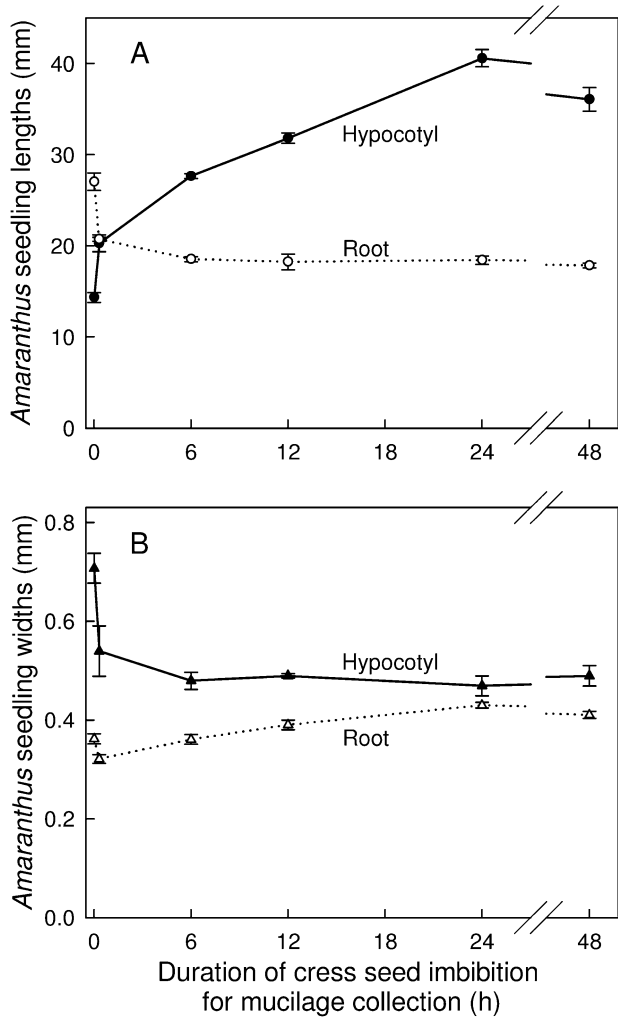


Fig. 4. Effect of cress seed exudate collected at various times of imbibition on *Amaranthus* seedling organ lengths (A) and widths (B). Cress seeds were imbibed at 4 °C for various times. *Amaranthus* seeds were then incubated in the collected exudates or in water as a control (plotted at 0 h) for 5 d. Other details are as given for Fig. 3.

Table 1. Heat-stable active principle is present in dry cress seeds. Dry cress seeds (1 g) were plunged into 200 ml boiling water and kept at 100 °C for 15 min; the seeds were removed from the hot water and incubated in 200 ml fresh water at 4 °C for 24 h. Exudate from the denatured seeds was freeze dried and redissolved in water at 0.5% (w/v). *Amaranthus* seeds were incubated in 0.9 ml of the solution or in water as a control, in 3-cm Petri dishes for 5 d, and then measured. Data are means from triplicate 9-cm Petri dishes ± inter-dish SE.

Treatment	Hypocotyl length (mm)	Root length (mm)
Control	15.8±0.6	29.7±0.5
Exudate from heat-killed cress seeds	29.0±1.0 ^a	16.5±0.9 ^a

^a Effect of treatment significant ($P < 0.001$).

Potency of cress seed exudate

In a test of the minimal effective concentration cress seed exudate, a sample was dried, redissolved in water at various concentrations, and bioassayed on *Amaranthus* as the receiver species. The effect on both hypocotyl and root elongation was concentration-dependent (Fig. 5). The minimum effective concentrations on hypocotyl and root growth were ~25 and ~450 µg ml⁻¹ respectively. This ~18-fold difference is comparable to the ~33-fold difference between hypocotyl- and root-sensitivity to lepidimoide reported by Hasegawa *et al.* (1992).

Effect of cress seed exudate on epidermal cell expansion and division

The promotion of hypocotyl elongation by seed exudate axiomatically involves a promotion of side-wall elongation.

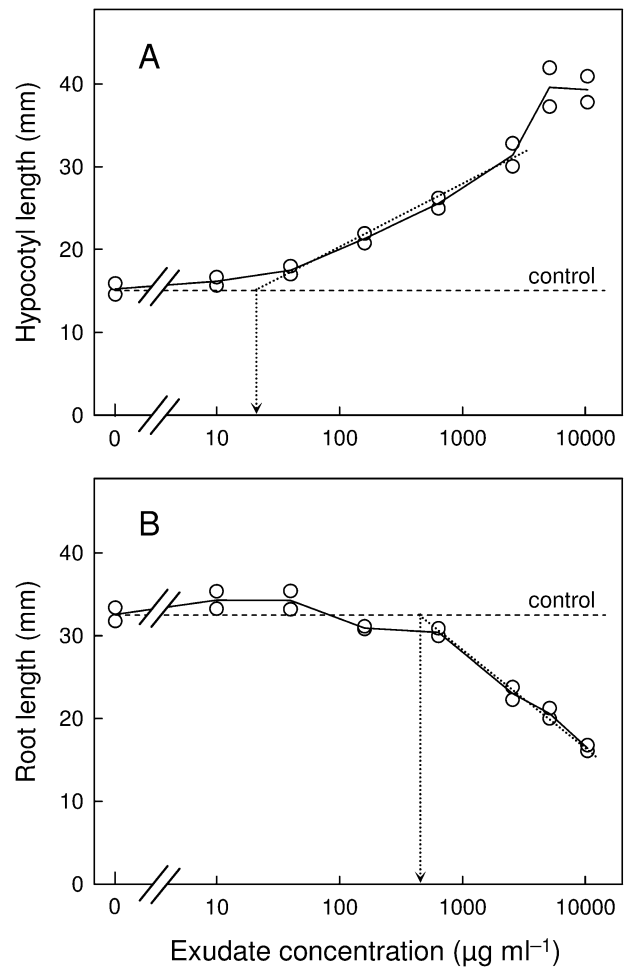


Fig. 5. Concentration-dependence of effect of cress seed exudate on *Amaranthus* seedling hypocotyl (A) and root (B) length. Dried cress seed exudate was redissolved water to give the concentrations indicated. *Amaranthus* seeds were incubated in 0.9 ml of each solution or water (control; dashed line), for 5 d in 3-cm Petri dishes and the lengths were then measured. Data are single measurements from duplicate Petri dishes. Other details are as given from in Fig. 3. Dotted arrows indicate the approximate minimum effective concentration.

In principle, this could be accompanied or unaccompanied by a matching promotion of cell division. Likewise, the inhibition of growth in hypocotyl girth by seed exudate

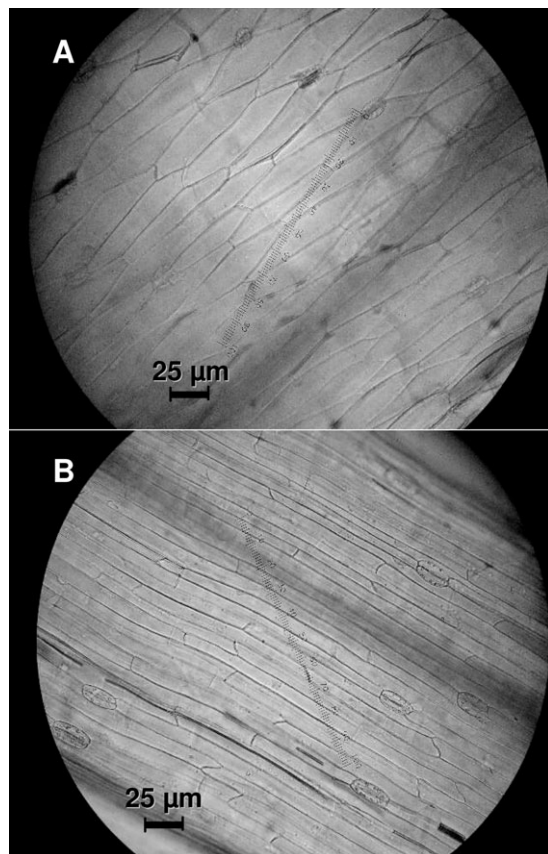


Fig. 6. Effects of cress seed exudate on epidermal cells of *Amaranthus* hypocotyls. *Amaranthus* seeds were incubated for 5 d in the dark at 25 °C in water (A) or cress seed-coat exudate (B). A 1-cm segment from the middle of each hypocotyl was placed on a slide and the epidermal cells were photographed under the $\times 40$ objective. The pictures are representative of the respective cell populations. Bars, 25 μm .

could be accompanied or unaccompanied by a matching inhibition of cell division. In an experiment designed to distinguish these possibilities in the case of the epidermis, which is usually the growth-limiting tissue in aerial plant organs (Kutschera, 2008), the final cell dimensions were measured (Fig. 6). Epidermal cells of *Amaranthus* hypocotyls that had been incubated with cress seed exudate were narrower and longer than water-treated controls (Fig. 6; Table 2). These cell patterns were consistent along the lengths of the hypocotyls with and without exudate. Epidermal cells of exudate-treated hypocotyls were 1.78 times the length and 0.49 times the width (and thus circumference) of controls (Table 2). In the same experiment, the whole hypocotyls were 2.29 times the length and 0.74 times the width of controls (Table 2). The effects on hypocotyl dimensions in this experiment were thus similar to those in all comparable experiments [mean \pm SE 2.30 ± 0.14 times the length ($n=7$) and 0.71 ± 0.01 times the width ($n=5$)].

Thus, treatment with exudate increased epidermal cell division in both planes of the epidermal sheet (Table 2). However, although causing a 129% increase in hypocotyl elongation, it caused only a 28% increase in epidermal cell number along the long axis of the organ (Table 2). Thus the principal effect of exudate on processes involved in hypocotyl elongation is to promote cell elongation and the effect on cell division in this dimension is inadequate to explain the observed growth. In the lateral dimension, the exudate resulted in a final hypocotyl circumference 26% less than in the controls, but a 55% greater cell number counted round the circumference (Table 2). This is therefore another clear case of organ growth being determined by cell expansion, not division.

Conclusion

These observations support the findings of Hasegawa's group (e.g. Hasegawa *et al.*, 1992; Yamada *et al.*, 1997). The results show that the bioactive material is released as soon as the cress seed is imbibed, without the need for metabolism or

Table 2. Effect of cress seed exudate on epidermal cell size, shape and number in *Amaranthus* hypocotyls

Amaranthus seeds were incubated in water (control) or in a solution of cress seed exudate for 5 d. From images such as those shown in Fig. 6, the length and width of the epidermal cells were estimated. In addition, the hypocotyls' lengths and widths were measured and their circumferences estimated ($\pi \times$ width). The number of epidermal cells along the long axis of the hypocotyl was approximated (hypocotyl length:cell length), as was the number of epidermal cells around a circumference of the hypocotyl (hypocotyl circumference: cell width). The total epidermal number per hypocotyl was then approximated (n along length \times n around circumference). Data are means from triplicate 9-cm Petri dishes \pm inter-dish SE. ****Effect of treatment significant ($P < 0.001$); ***Effect of treatment significant ($P < 0.01$). Ratio is calculated as treated:untreated.

Treatment	Hypocotyl length (mm)	Epidermal cell length (μm)	Hypocotyl circumference (mm)	Epidermal cell width (μm)	Estimated epidermal cell number per:		
					Hypocotyl length	Hypocotyl circumference	Whole hypocotyl (10^3)
Control	15.1 \pm 0.2	111 \pm 3	2.17 \pm 0.05	21.3 \pm 0.6	136 \pm 4	103 \pm 4	14.0 \pm 0.7
Exudate	34.6 \pm 0.1****	198 \pm 6****	1.60 \pm 0.08***	10.4 \pm 0.4****	175 \pm 5***	160 \pm 10***	28.0 \pm 1.9***
Ratio	2.29 \pm 0.02	1.78 \pm 0.07	0.74 \pm 0.04	0.49 \pm 0.02	1.28 \pm 0.05	1.55 \pm 0.11	1.99 \pm 0.17

active secretion. The biological potency of the seed exudate is very high, measurably promoting hypocotyl growth at $25 \mu\text{g ml}^{-1}$ despite the crude nature of this exudate preparation, which is likely to contain numerous inert carbohydrates, etc. besides the bioactive principle(s).

Any agent that promotes the longitudinal growth of a plant organ must axiomatically increase the total elongation of its cells' side-walls (regardless of the number of cells and thus cross-walls). *A priori*, cell division along the long axis could remain unchanged (Fig. 7A), concurrently increase (Fig. 7B), or even decrease. The scenario shown in Fig. 7C is not an option because it would not cause the effects reported in Figs. 1–5. The data indicate that cell division increases only slightly during the dramatic promotion of

Amaranthus hypocotyl elongation by cress seed exudate. Therefore, it is not a question of enhanced cell division being followed by a matching elongation. On the contrary, an enhancement of cell elongation is the prime mechanism leading to a greater hypocotyl length (Fig. 7A); the small promotion of cell division noted does not keep pace with the elongation. In the case of growth in hypocotyl width, it is even clearer that the inhibitory effect of cress seed exudate on cell-wall expansion is the primary mechanism, since cell division in the tangential plane was actually promoted (Fig. 7D, E).

The biological role of the allelopathic material in cress seed exudates is difficult to ascertain: it could possibly protect the donor plant from competitors by overstimulating the

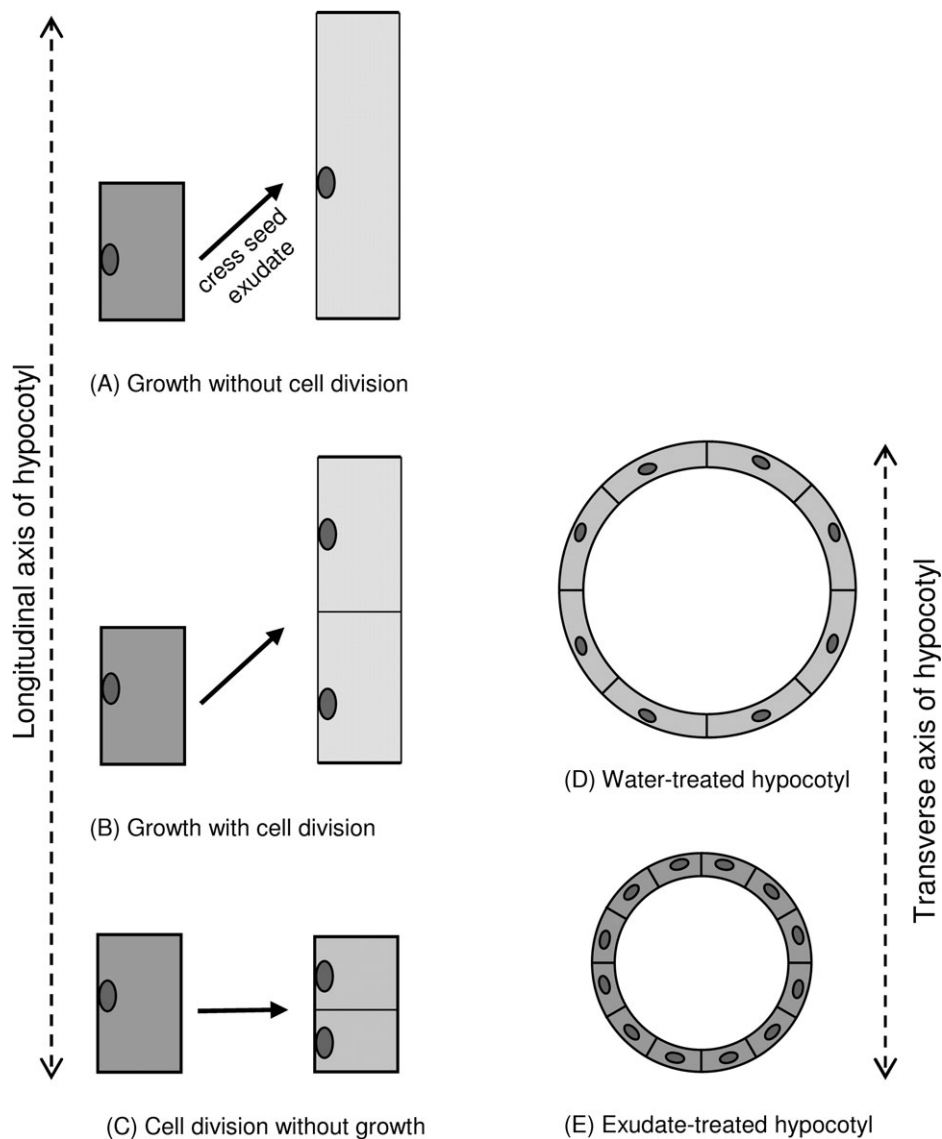


Fig. 7. Interpretative diagrams of the epidermis of the *Amaranthus* hypocotyl and its response to cress seed exudate. (A–C) Epidermal cells seen in tangential longitudinal section, to show hypocotyl elongation. Three theoretically possible stimulatory effects are shown: (A) growth without cell division, (B) growth with cell division, and (C) cell division without growth. The effect of cress seed exudate on *Amaranthus* hypocotyls is best represented by (A). (D–E) Epidermal cells seen in transverse section, to show the hypocotyl's girth. Treatment with exudate (E) results in a more slender hypocotyl despite increasing the cell count in the tangential plane, compared with water treatment (D).

hypocotyl growth of the latter to produce excessively tall, weak, 'foolish' seedlings similar to those overstimulated by gibberellin, as well as by inhibiting root growth (Rice, 1979). Alternatively, however, the presence of lepidimoides in the seed(ling)s of numerous species (Yamada *et al.*, 1995) may suggest that it is a normal component of the growth-regulator repertoire of plants in general, additional to the better-known auxins, gibberellins, cytokinins, etc. Either way, a possible application of the seed exudate phenomenon explored here could be as the basis of novel natural agrochemicals.

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References

- Aldington S, Fry SC.** 1993. Oligosaccharins. *Advance Botany Research* **19**, 1–101.
- Auxtová O, Lišková D, Kákoniová D, Kubačková M, Karácsonyi Š, Bilisics L.** 1995. Effect of galactoglucomannan-derived oligosaccharides on elongation growth of pea and spruce stem segments stimulated by auxin. *Planta* **196**, 420–424.
- Aziz A, Heyraud A, Lambert B.** 2004. Oligogalacturonide signal transduction, induction of defense-related responses and protection of grapevine against *Botrytis cinerea*. *Planta* **218**, 767–774.
- Bais HP, Weir TL, Perry GL, Gilroy S, Vivanco JM.** 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review Plant Biology* **57**, 233–266.
- Beninger CW, Abou-Zaid MM, Kistner ALE, Hallett RH, Iqbal MJ, Grodzinski B, Hall JC.** 2004. A flavanone and two phenolic acids from *Chrysanthemum morifolium* with phytotoxic and insect growth regulating activity. *Journal of Chemical Ecology* **30**, 589–606.
- Benová-Kákosová A, Digonnet C, Goubet F, et al.** 2006. Galactoglucomannans increase cell population density and alter the protoxylem/metaxylem tracheary element ratio in xylogenetic cultures of *Zinnia*. *Plant Physiology* **142**, 696–709.
- Braga MR, Dietrich SMC.** 1998. Phytoalexin-inducing oligosaccharins from the cell walls of tropical Rubiaceae. *Revista Brasileira de Fisiologia Vegetal* **10**, 71–78.
- Braga MR, Rosemeire A, Pessoni B, Dietrich SMC.** 1998. Cell wall polysaccharide composition of leaves of tropical Rubiaceae differing in phytoalexin response. *Revista Brasileira de Fisiologia Vegetal* **10**, 71–78.
- Brigham LA, Michaels JP, Flores HE.** 1999. Cell-specific production and antimicrobial activity of naphthoquinones in roots of *Lithospermum erythrorhizon*. *Plant Physiology* **119**, 417–428.
- Campbell J, Drake A, Lee V, Strother S.** 1995. A putative oligosaccharin growth promoter from *Vitis vinifera* L. primary cell walls. *Annals of Botany* **75**, 359–363.
- Dakora FD, Phillips DA.** 2002. Root exudates as mediators of mineral acquisition in low nutrient environments. *Plant and Soil* **245**, 35–47.
- Darvill A, Augur C, Bergmann C, et al.** 1992. Oligosaccharins – oligosaccharides that regulate growth, development and defense responses in plants. *Glycobiology* **2**, 181–198.
- Deng F, Aoki M, Yogo Y.** 2004. Effect of naringenin on the growth and lignin biosynthesis of gramineous plants. *Weed Biology and Management* **4**, 49–55.
- Dumville JC, Fry SC.** 2003. Gentiobiose: a novel oligosaccharin in ripening tomato fruit. *Planta* **216**, 484–495.
- Fry SC, Aldington S, Hetherington PR, Aitken J.** 1993. Oligosaccharides as signals and substrates in the plant cell wall. *Plant Physiology* **103**, 1–5.
- Hasegawa K, Mizutani J, Kosemura S, Yamamura S.** 1992. Isolation and identification of lepidimoides, a new allelopathic substance from mucilage of germinated cress seeds. *Plant Physiology* **100**, 1059–1061.
- Hegab MM, Khodary SEA, Hammouda O, Ghareib HR.** 2008. Autotoxicity of chard and its allelopathic potentiality on germination and some metabolic activities associated with growth of wheat seedlings. *African Journal of Biotechnology* **7**, 884–892.
- Higashinakasu K, Yamada K, Shigemori H, Hasegawa K.** 2004. Effects of seed exudates of several plant species during germination stage. *Weed Biology and Management* **4**, 171–175.
- Hirose K, Endo K, Hasegawa K.** 2004. A convenient synthesis of lepidimoides from okra mucilage and its growth-promoting activity in hypocotyls. *Carbohydrate Research* **339**, 9–19.
- Hirose K, Kosuge Y, Otomatsu T, Endo K, Hasegawa K.** 2003. Preparation of a growth-promoting substance, lepidimoic acid, from okra pectic polysaccharide. *Tetrahedron Letters* **44**, 2171–2173.
- Isfahan MN, Shariati M.** 2007. The effect of some allelochemicals on seed germination of *Coronilla varia* L. seeds. *American-Eurasian Journal of Agriculture and Environmental Science* **2**, 534–538.
- Jamois F, Ferrières V, Guégan J, Yvin J, Plusquellec D, Vetvicka V.** 2005. Glucan-like synthetic oligosaccharides: iterative synthesis of linear oligo- β -(1,3)-glucans and immunostimulatory effects. *Glycobiology* **15**, 393–407.
- Kaida R, Sugawara S, Negoro K, Maki H, Hayashi T, Kaneko TS.** 2010. Acceleration of cell growth by xyloglucan oligosaccharides in suspension-cultured tobacco cells. *Molecular Plant* **3**, 549–554.
- Kato-Noguchi H, Yamada K, Hasegawa K.** 2001. Lepidimoic acid increases fructose 2,6-bisphosphate in *Amaranthus* seedlings. *Phytochemistry* **56**, 499–503.
- Kutschera U.** 2008. The growing outer epidermal wall: design and physiological role of a composite structure. *Annals of Botany* **101**, 615–621.
- Macario BJ, Aguilar-Flores S, Ventura-Zapata E, Perez-Campos E, Bouquelet S, Zenteno E.** 2003. Chemical characterization of root exudates from rice (*Oryza sativa*) and their effects on the chemotactic response of endophytic bacteria. *Plant and Soil* **249**, 271–277.
- McDougall JG, Fry SC.** 1991. Xyloglucan nonasaccharide, a naturally-occurring oligosaccharin, arises *in vivo* by polysaccharide breakdown. *Journal of Plant Physiology* **137**, 332–336.

- Meepagala K, Schader K, Wedge D, Duke S.** 2005. Algicidal and antifungal compounds from the roots of *Ruta graveolens* and synthesis of their analogs. *Phytochemistry* **66**, 2689–2695.
- Miyamoto K, Ueda J, Yamada K, Kosemura S, Yamamura S, Hasegawa K.** 1997a. Inhibitory effect of lepidimoide on senescence in *Avena* leaf segments. *Journal of Plant Physiology* **150**, 133–136.
- Miyamoto K, Ueda J, Yamada K, Kosemura S, Yamamura S, Hasegawa K.** 1997b. Inhibition of abscission of bean petiole explants by lepidimoide. *Journal of Plant Growth Regulation* **16**, 7–9.
- Muscolo A, Sidari M, Rosaria PM, De Santis C, Finocchiaro A.** 2005. Early effects of phenolic compounds, extracted from two forest litters, on ammonium uptake and assimilation in *Pinus laricio* and *Pinus pinaster* seedlings. *Plant and Soil* **269**, 309–320.
- Ochiai A, Itoh T, Kawamata A, Hashimoto W, Murata K.** 2007. Plant cell wall degradation by saprophytic *Bacillus subtilis* strains: Gene clusters responsible for rhamnogalacturonan depolymerization. *Applied Environmental Microbiology* **73**, 3803–3813.
- Prakash S, Koul S, Bhalla-Sarin N.** 2003. Germinating pigeonpea (*Cajanus cajan*) seeds secrete factor(s) having antiethylene-like effects. *Physiologia Plantarum* **118**, 589–596.
- Ray TC, Callow JA, Kennedy JF.** 1988. Composition of root mucilage polysaccharides from *Lepidium sativum*. *Journal of Experimental Botany* **39**, 1249–1261.
- Rice EL.** 1979. Allelopathy. An update. *Botanical Review* **45**, 15–109.
- Rice EL.** 1984. *Allelopathy*, 2nd Edition. Orlando: Academic Press, Incharge.
- Saranpuetti C, Tanaka M, Sone T, Asano K, Tomita F.** 2006. Determination of enzymes from *Colletotrichum* sp AHU9748 essential for lepidimoide production from okra polysaccharide. *Journal of Bioscience and Bioengineering* **102**, 452–456.
- Sharp JK, Albersheim P, Ossowski P, Pilotti A, Garegg P, Lindberg B.** 1984. Host–pathogen interactions. 28. Comparison of the structures and elicitor activities of a synthetic and a mycelial-wall-derived hexa- β -D-glucopyranosyl)-D-glucitol. *Journal of Biological Chemistry* **259**, 11341–11345.
- Tanaka M, Yoshimura M, Suto M, Yokota A, Asano K, Sukara E, Tomita F.** 2002. Production of lepidimoide by an endophytic fungus from polysaccharide extracted from *Abelmoschus* sp.: identification of the product and the organism producing it. *Journal of Bioscience and Bioengineering* **93**, 531–536.
- Yamada K, Anai T, Hasegawa K.** 1995. Lepidimoide, an allelopathic substance in the exudates from germinated seeds. *Phytochemistry* **39**, 1031–1032.
- Yamada K, Anai T, Kosemura S, Yamamura S, Hasegawa K.** 1996. Structure–activity relationship of lepidimoide and its analogues. *Phytochemistry* **41**, 671–673.
- Yamada K, Kosemura S, Yamamura S, Hasegawa K.** 1997. Exudation of an allelopathic substance lepidimoide from seeds during germination. *Plant Growth Regulation* **22**, 189–192.
- Yamada K, Matsumoto H, Ishizuka K, Miyamoto K, Kosemura S, Yamamura S, Hasegawa K.** 1998. Lepidimoide promotes light-induced chlorophyll accumulation in cotyledons of sunflower seedlings. *Journal of Plant Growth Regulation* **17**, 215–219.
- Yokotani-Tomita KY, Goto N, Kosemura S, Yamamura S, Hasegawa K.** 1998. Growth-promoting allelopathic substance exuded from germinating *Arabidopsis thaliana* seeds. *Phytochemistry* **47**, 1–2.
- York WS, Darvill AG, Albersheim P.** 1984. Inhibition of 2,4-dichlorophenoxyacetic acid-stimulated elongation of pea stem segments by a xyloglucan oligosaccharide. *Plant Physiology* **75**, 295–297.