Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 69 (2008) 9–17
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

cis-Jasmone induces accumulation of defence compounds in wheat, *Triticum aestivum*

Maria C. Blassioli Moraes [a,b], Michael A. Birkett [b,*], Ruth Gordon-Weeks [b], Lesley E. Smart [b], Janet L. Martin [b], Barry J. Pye [b], Richard Bromilow [b], John A. Pickett [b]

[a] *Embrapa Genetic Resources and Biotechnology, Cx. Postal: 02372, Cep. 70849-970 Brasília-DF, Brazil*
[b] *Biological Chemistry Department, Rothamsted Research, Harpenden, Herts. AL5 2JQ, United Kingdom*

Received 20 April 2007; received in revised form 14 June 2007
Available online 2 August 2007

Abstract

Liquid phase extraction (LPE) and vapor phase extraction (VPE) methodologies were used to evaluate the impact of the plant activator, *cis*-jasmone, on the secondary metabolism of wheat, *Triticum aestivum*, var. Solstice. LPE allowed the measurement of benzoxazinoids, i.e. 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA), 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one (HMBOA) and 6-methoxy-benzoxazolin-2-one (MBOA), and phenolic acids such as *trans*-*p*-coumaric acid, syringic acid, *p*-hydroxybenzoic acid, vanillic acid and *cis*- and *trans*-ferulic acid. Using LPE, a significantly higher level of DIMBOA was found in aerial parts and roots of *T. aestivum* following treatment with *cis*-jasmone, when compared with untreated plants. Similar results were obtained for phenolic acids, such as *trans*-ferulic acid and vanillic acid in roots. Using VPE, it was possible to measure levels of 2-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HBOA), benzoxazolin-2(3H)-one (BOA), ferulic acid, syringic acid and coumaric acid. The levels of HBOA in aerial parts and roots were significantly greater in *cis*-jasmone treated plants compared to untreated plants. *cis*-Jasmone is known to be a plant activator in terms of production of defence-related volatile semiochemicals that repel aphids and increase the foraging activity of aphid parasitoids. These results show, for the first time, that *cis*-jasmone also induces selective production of secondary metabolites that are capable of directly reducing development of pests, diseases and weeds.

Keywords: *Triticum aestivum*; Gramineae; Wheat; Liquid phase extraction; Vapor phase extraction; *cis*-Jasmone; Allelochemicals; Benzoxazinoids; Phenolic acids

1. Introduction

A substantial number of plant secondary metabolites are involved in direct and indirect plant defence against insect herbivores and pathogens. Wheat, *Triticum aestivum* L. (Gramineae) has been found to possess allelopathic potential, and this allelopathy has been shown to be associated with the presence of benzoxazinoids, such as 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA), and phenolic acids (Perez and Ormenonunez, 1991; Understrup et al., 2005).

It has been well documented that jasmonic acid (JA) and methyl jasmonate are activators of plant defence (Koch et al., 1999; Kessler and Baldwin, 2002). However, by comparison, few studies have been conducted to evaluate the impact of *cis*-jasmone, which is considered to be the final product in the jasmonic acid biosynthetic pathway from α-linolenic acid (Koch et al., 1997). *cis*-Jasmone is highly volatile, when compared with the other compounds of the JA pathway, and is an activator of chemical defence

Abbreviations: *BOA, benzoxazolin-2(3H)-one; HBOA, 2-hydroxy-7-methoxy-(2H)*-1,4-benzoxazin-3(4*H*)-one; DIBOA, 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one; DIMBOA, 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one; MBOA, 6-methoxy-benzoxazolin-2(3H)-one; HMBOA, 2-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one; PHB, *p*-hydroxybenzoic acid; VAN, vanillic acid; SYR, syringic acid; *cis*-FER, *cis*-ferulic acid; *trans*-*p*-COU, *trans*-*p*-coumaric acid; *trans*-FER, *trans*-ferulic acid.

* Corresponding author. Tel.: +44 1582 763133; fax: +44 1582 762595.
E-mail address: mike.birkett@bbsrc.ac.uk (M.A. Birkett).

0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.06.020

in plants, causing the release of volatile semiochemicals (Birkett et al., 2000). Bean plants, *Vicia faba*, treated with *cis*-jasmone showed a significant increase in the production of (*E*)-ocimene over a period of at least eight days, whereas when plants were exposed in the same way to methyl jasmonate, although there was an increase in the level of (*E*)-ocimene, the effect was short-lived (Birkett et al., 2000). In addition, changes in gene expression levels from *cis*-jasmone treated *V. faba* were recorded (Birkett et al., 2000). Bruce et al. (2003a, 2003b) showed in field studies that when *T. aestivum* is treated with *cis*-jasmone, there is a significant reduction in the development of grain aphid, *Sitobion avenae*, populations. This negative impact on aphids could be related to the induction of benzoxazinoid and phenolic acid production as a direct defence mechanism, as these compounds are known to confer plant resistance to insects and allelopathic effects (Guenzi and McCalla, 1966; Niemeyer, 1988).

The impact of plant activators upon induced plant secondary metabolism has been studied up to now using a variety of separate and complex analytical techniques on different parts of the plant. Volatile organic compounds (VOCs) emitted by plants are typically studied by the collection of the headspace of parts of, or the whole plant, and are analysed by high-resolution gas chromatography (GC) (e.g. Agelopoulos et al., 1999). However, the impact of external signals on internal plant physiology typically is studied by extraction of plant tissue (roots and/or leaves), with different solvents sometimes followed by chemical derivatization and analysis by GC or by high-pressure liquid chromatography (HPLC). This has been applied to hydroxamic acid analysis in cereals (Wu et al., 1999). Qualitative and quantitative changes in plant metabolism are assessed by mass spectrometry, coupled to either GC or LC, using conventional electron impact mass spectrometry (EI-MS), or by more sophisticated techniques such as electrospray-ionization-mass spectrometry (ESI-MS). In either case, identification can be enhanced by the use of tandem mass spectrometry techniques (MS–MS). However, few attempts have been made to assess the impact of external signals on the secondary metabolites of the whole plant. Schmelz et al. (2003, 2004) proposed the simultaneous quantification of phytohormone, phytotoxin and VOC production in plants using vapor phase extraction (VPE) in various model systems: thale cress, *Arabidopsis thaliana*, following infection with the pathogen, *Pseudomonas syringe*; maize, *Zea mays*, during herbivory by the corn earworm, *Helicoverpa zea*; tobacco, *Nicotiana tabacum*, after mechanical damage, and tomato, *Lycopersicon esculentum*, during drought stress. VPE offers advantages and potential applicability in the preparation and simultaneous GC–MS analysis of phytotoxins (coronatine), phytohormones (salicylic acid, jasmonic acid, indole-3-acetic acid and abcisic acid) and VOCs from low levels (milligrams) of plant tissue. This approach enabled the exploration of interactions between physiologically and ecologically relevant chemical signals at the level of production, and to rationalise previous findings on induced plant defence.

The aim of this study was to investigate the impact of *cis*-jasmone upon secondary metabolism in wheat, *T. aestivum* (var. Solstice). For comparison, liquid phase extraction (LPE), which uses different solvents (polar and non-polar) to obtain the partition of the compounds with different polarities from biological matrices, was used alongside VPE. For both techniques, derivatization was carried out prior to analysis, with the reagents *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) and trim-

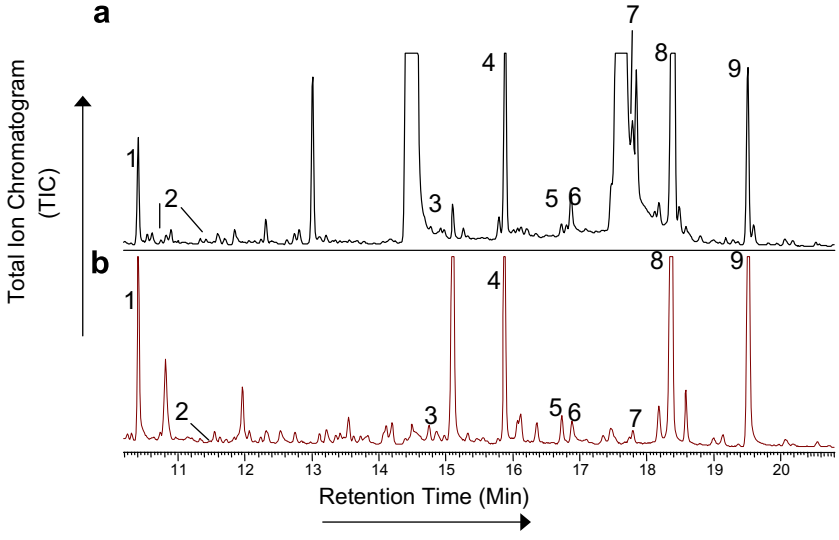

Fig. 1. Typical GC–MS profiles of extracts of aerial parts (a) and roots (b) obtained from wheat, *T. aestivum,* obtained by liquid-phase extraction (LPE). The compounds were identified as TMSi derivatives. MBOA (1), *p*-hydroxybenzoic acid (2), vanillic acid (3), HMBOA (4), syringic acid, (5), *cis*-ferulic acid, (6), *trans*-*p*-coumaric acid (7), DIMBOA (8), *trans*-ferulic acid (9).

ethylsilyldiazomethane ($TMSCHN_2$) being used for LPE and VPE, respectively.

2. Results

2.1. Liquid phase extraction (LPE)

Coupled GC–mass spectrometry (GC–MS) analysis of TMSi-derivatized aerial and root extracts of untreated wheat, *T. aestivum*, prepared using liquid phase extraction (LPE), confirmed the presence of several benzoxazinoids and phenolic acids. These included DIMBOA, HMBOA, MBOA, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, *cis*-ferulic acid, *trans*-*p*-coumaric acid and *trans*-ferulic acid (Fig. 1).

Quantitative analysis of *T. aestivum* treated with *cis*-jasmone showed that for aerial parts and roots, there were higher levels of some benzoxazinoids compared to phenolic acids (Figs. 2 and 3). Furthermore, extracts obtained from *cis*-jasmone treated plants showed significantly higher levels of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) when compared with untreated plants ($F_{2,76} = 7.31$, $p < 0.0010$) (Fig. 2) (pairwise Student–Newman–Keuls test $P > 0.05$). The level of DIMBOA in aerial parts treated with *cis*-jasmone was 108.1 ± 15.2 mg/kg of fresh weight, and in untreated leaves was 42.9 ± 12.2 mg/kg. In roots, the levels of DIMBOA were 729.2 ± 156.1 mg/kg for treated plants and 162.3 ± 37.3 mg/kg for untreated plants. MBOA was observed in aerial parts and roots, but the levels found in aerial parts were lower than the GC detection limit, thus precluding quantification. The levels in roots were very low but quantifiable. Statistical analysis did not show any difference between the treatments (Fig. 2). The levels found in roots were 4.6 ± 1.34 mg/kg for *cis*-jasmone treated plants and 3.6 ± 1.2 mg/kg for untreated plants. Similar results were obtained for HMBOA, with a level of 9.9 ± 4.2 mg/kg for *cis*-jasmone treated plants and 2.7 ± 1.5 mg/kg for untreated plants. The levels acquired from aerial parts for HMBOA were 6.2 ± 1.4 mg/kg for *cis*-jasmone treated plants, and 5.6 ± 1.2 mg/kg for untreated plants, respectively.

Root extracts from *cis*-jasmone treated plants showed significantly higher levels of vanillic acid ($F_{2,15} = 4.08$, $p = 0.04$) and *trans*-ferulic acid ($F_{2,19} = 6.56$, $p = 0.009$) (Fig. 3). Levels of syringic acid and *trans*-*p*-coumaric acid appeared to be higher in treated plants, but this difference was not statistically significant, which could be explained by the high variability of the levels and low quantities measured. For aerial parts, *cis*-jasmone treated plants contained significantly higher levels of *trans*-*p*-coumaric acid ($F_{2,20} = 4.33$, $p = 0.04$) and syringic acid ($F_{2,20} = 4.43$, $p = 0.04$).

2.2. Vapor phase extraction (VPE)

Coupled GC–MS analysis of *T. aestivum* extracts, prepared using vapor phase extraction (VPE) of aerial and root parts of untreated plants, confirmed the presence of benzoxazinoids and phenolic acids (Fig. 4).

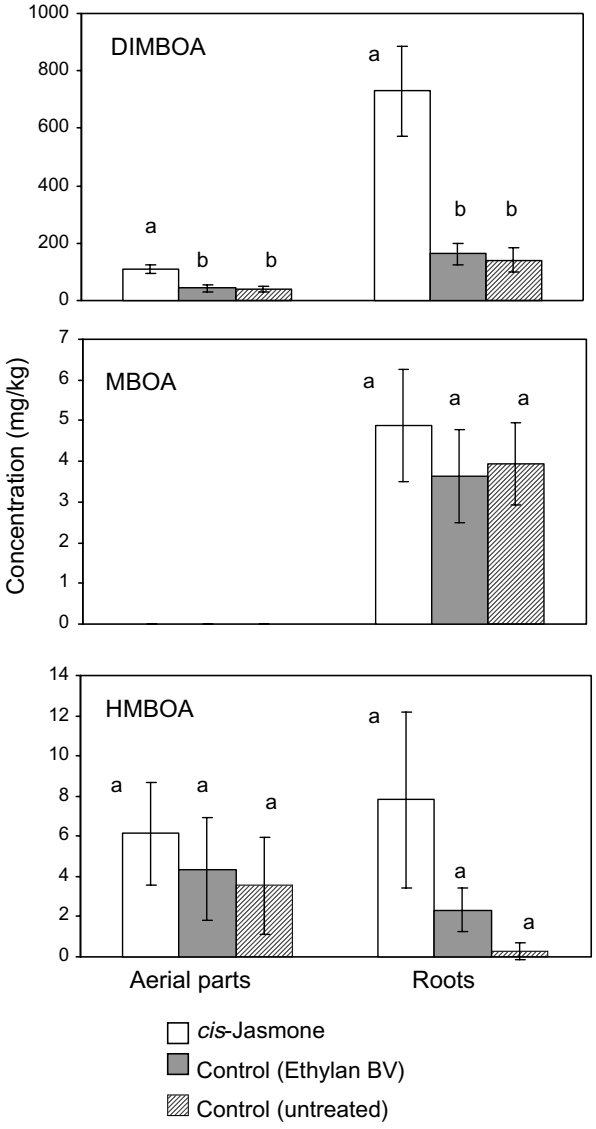

Fig. 2. Mean amount (±SE) of DIMBOA, MBOA and HMBOA extracted from root and aerial parts of wheat, *T. aestivum*, using LPE. All compounds were identified as TMSi derivatives and reported in mg/kg fresh weight. Means followed by the same letter are not significantly different (95% level).

Quantitative analyses showed that for root extracts, plants treated with *cis*-jasmone contained higher levels of HBOA ($F_{2,16} = 13.98$, $p = 0.006$), *trans*-ferulic acid, methyl ester ($F_{2,16} = 22.04$, $p = 0.002$) and syringic acid, methyl ester ($F_{2,16} = 7.99$, $p = 0.02$). For aerial parts, *cis*-jasmone treated plants had higher levels of HBOA ($F_{2,16} = 30.4$, $p < 0.01$), *trans*-ferulic acid, methyl ester ($F_{2,16} = 14.2$, $p = 0.005$), syringic acid, methyl ester ($F_{2,16} = 5.06$, $p = 0.05$) and *trans*-coumaric acid, methyl ester ($F_{2,16} = 5.76$, $p = 0.04$) (Fig. 5). For BOA, which was identified in roots and aerial parts, there was no significant difference in levels between treatments.

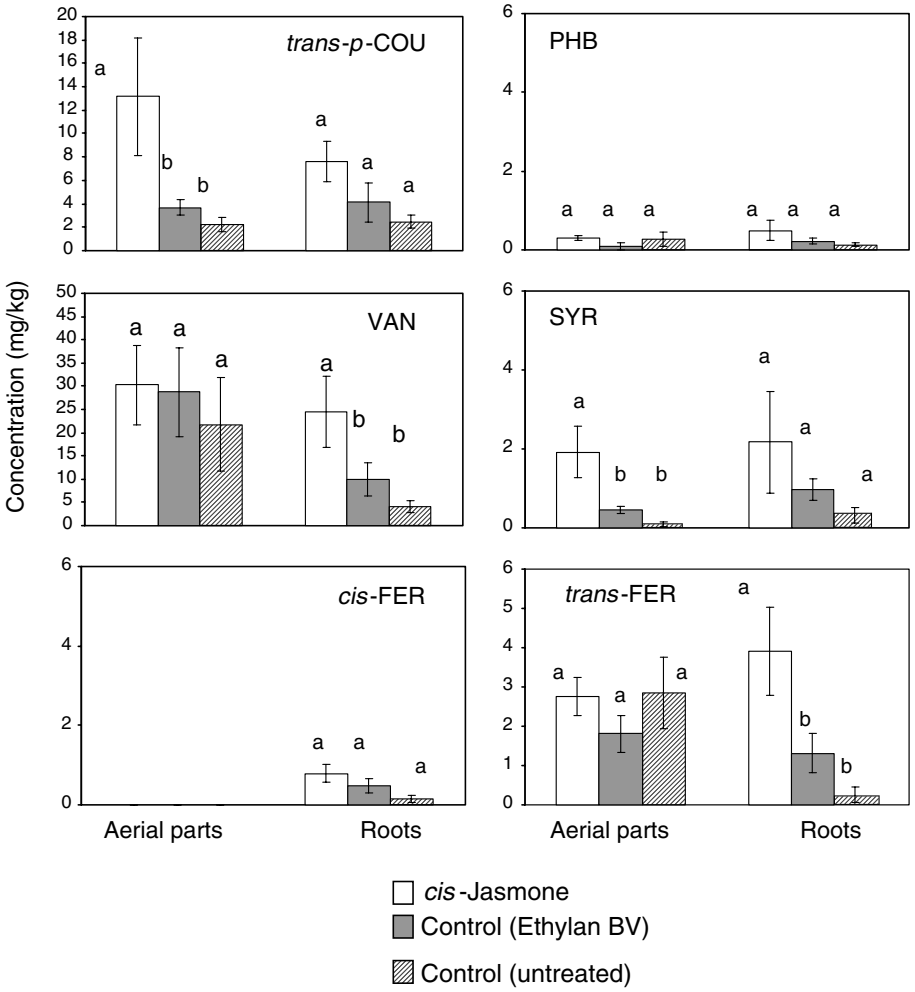

Fig. 3. Mean amount (±SE) of phenolic acids extracted from root and aerial parts of wheat, *T. aestivum*, using LPE. All compounds were identified as TMSi derivatives and amounts reported are in mg/kg fresh weight. Means not followed by the same letter are not significantly different (95% level).

3. Discussion

A large number of secondary metabolites in plants have a role in direct plant defence. For wheat, *T. aestivum*, these include benzoxazinoids and phenolic acids. Levels of benzoxazinoids have been shown to vary between cultivars. Wu et al. (1999, 2000, 2001) and Ni and Quisenberry (2000) studied different varieties and isolines of roots and aerial parts of wheat, and showed that for the latter, DIMBOA was found in concentrations as low as 39.3 ± 7.6 mg/kg, and as high as 730.4 ± 1.4 mg/kg of dry mass. In the roots, levels of DIMBOA as low as 48.3 ± 3.1 mg/kg and as high as 733.9 ± 1.4 mg/kg of dry mass for wild types and PI lines were recorded. In our work, the concentration of DIMBOA did not vary between untreated plants. However, when treated with *cis*-jasmone, levels of DIMBOA in roots and aerial parts of wheat were significantly increased ($N = 38$, $F_{2,36} = 21.43$, $p = 0.001$).

Phenolic acids such as *p*-coumaric acid, syringic acid and ferulic acid have been regarded as some of the major phenolic acids present in different cultivars. Wu et al. (1999, 2000, 2001) showed that there is variability in levels of phenolic acids between varieties of wheat. For example, vanillic acid was found in aerial parts in concentrations as low as 0.6 ± 1.2 mg/kg, and as high 17.5 mg/kg (dry weight) in cultivar AUS 12627. The variety of wheat (Solstice) used in our study contained lower levels of phenolic acids, when compared with varieties studied by Wu et al. (1999, 2000, 2001). This could be due to differences in the age of the plants used. In our study, six day old plants were used, compared to the study of Wu et al. where 17 day old plants were used.

The variability in the amount of secondary metabolites in plants is clearly linked with genetic characteristics, although biotic and abiotic factors can provoke changes in this variability (Villagrasa et al., 2006; Oikawa et al., 2001, 2004). Villagrasa et al. (2006) reported that for different varieties of wheat in conventional and organic cultivation, different levels of benzoxazinoids were observed. The effect of different types of biotic factors has been shown to be very similar, provoking changes in secondary metabolite production in wheat, maize and rye. For example, when

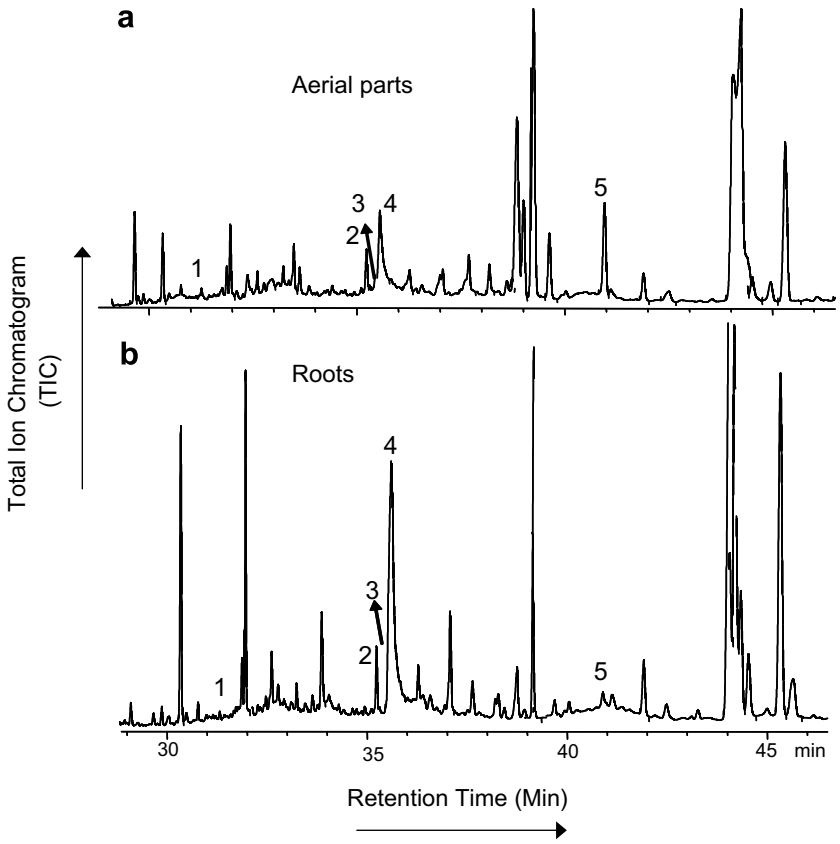

Fig. 4. Typical GC–MS profiles of extracts of aerial parts (a) and roots (b) of wheat, *T. aestivum*, prepared using vapour-phase extraction (LPE). The acidic compounds were identified as methyl ester (ME) derivatives. BOA (1), syringic acid, ME (2), *trans*-*p*-coumaric acid, ME (3), HBOA (4), *trans*-ferulic acid and ME (5).

plants were treated with either jasmonic acid or a pathogen, there was a similar significant change in the levels of benzoxazinoids (Oikawa et al., 2001, 2004).

Wu et al. (2001) showed that of 58 accessions, 50 contained higher levels of DIMBOA in roots than in aerial parts, and only two accessions had less than 260 mg/kg of DIMBOA in their roots. Our results suggest that DIMBOA is stored mainly in roots, or that the production of DIMBOA occurs in the roots, and that *cis*-jasmone is either eliciting higher production of DIMBOA, or provoking the degradation of DIMBOA – glucoside. Further work is underway to establish which of these processes is taking place following *cis*-jasmone treatment.

In this work, it was possible to identify and quantify three main compounds in the benzoxazinoid biosynthetic pathway, MBOA, HMBOA and DIMBOA, in extracts of aerial parts and roots from wheat, *T. aestivum* using LPE methodology. These data are in agreement with previous results (Villagrasa et al., 2006). These compounds are related to DIMBOA by biosynthesis, i.e. HBOA or degradation, i.e. HMBOA. In the first case, then the pathway to its biosynthesis could be different from that one proposed in maize by Frey et al. (2003), but this needs to be studied further.

The extracts obtained from roots of plants treated with *cis*-jasmone using LPE methodology contained a higher level of DIMBOA, when compared with untreated plants. HBOA was tentatively identified using VPE methodology in aerial parts and roots, and in both parts, a higher level of HBOA was observed when the plant was treated with *cis*-jasmone, compared with untreated plants. In the LPE methodology, HBOA was not identified. However, it is possible that under the GC–MS conditions applied, which involved magnet scanning across a wide mass range, HBOA may have been present below the limit of detection. Studies are underway to repeat the GC–MS analysis using single ion monitoring (SIM), which can considerably lower the limit of detection.

cis-Jasmone is known to be a plant activator in terms of production of defence-related volatile semiochemicals that repel aphids and increase the foraging activity of aphid parasitoids (Birkett et al., 2000). These include the isoprenoid (*E*)-ocimene, and also the isoprenoid oxidation product 6-methyl-5-hepten-2-one (Pickett et al., 2005). The results in this study show, for the first time, that *cis*-jasmone also induces selective production of secondary metabolites that are capable of directly reducing development of pests, diseases and weeds. Thus, it appears that the different biosynthetic pathways associated with isoprenoid, benzoxazinoid and phenolic acid biosynthesis are all affected when plants are treated with this compound. However, for the case of DIMBOA, which is also stored as the glycoside in vacuoles, it cannot be ruled out that *cis*-jasmone affects not the biosynthetic pathway, but may instead provoke the

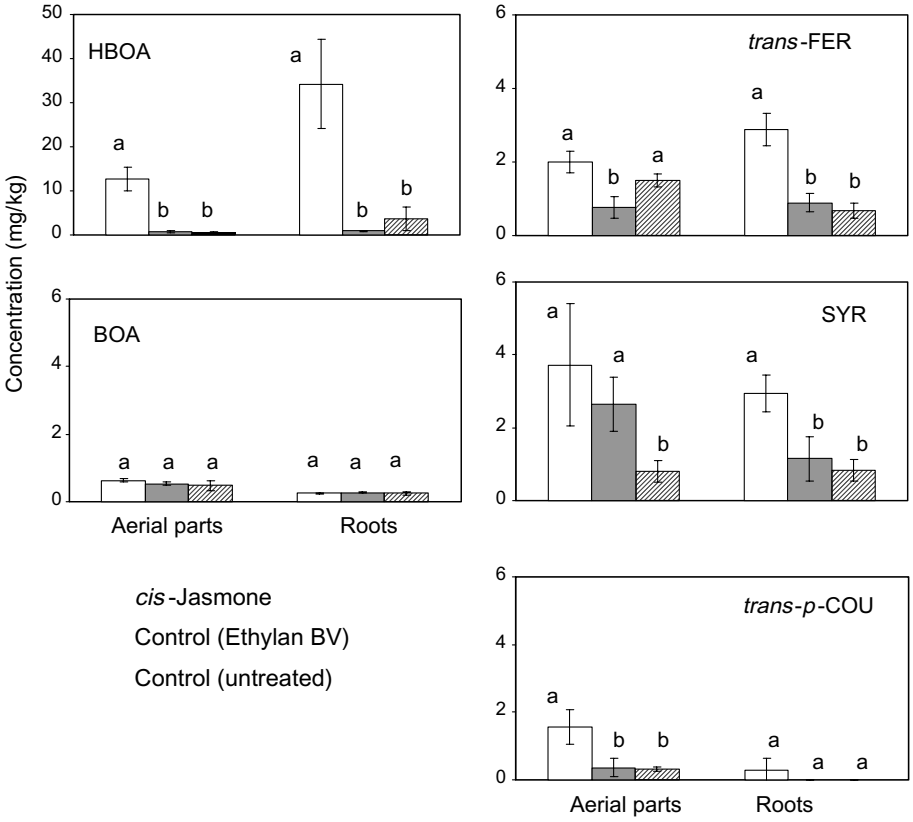

Fig. 5. Mean amount (±SE) of HBOA, BOA and phenolic acids extracted from root and aerial parts of wheat, *T. aestivum*, using VPE. The phenolic acids were identified as methyl ester (ME) derivatives and amounts reported are in mg/kg fresh weight. Means not followed by the same letter are not significantly different (95% level).

release of the aglycone *via* a hydrolytic process. However, an increase of HBOA observed in the aerial part and root extracts prepared *via* VPE extraction suggests that *cis*-jasmone could be inducing the benzoxazinoid pathway, because there is no evidence that HBOA is stored in vacuoles. Similarly, Wang et al. (2006) recorded that methyl jasmonate induced maize into higher production of DIMBOA and phenolic acids, whereas Oikawa et al. (2002) showed that wheat leaves treated with jasmonic acid induced the accumulation of HDMBOA-glucoside. Studies with maize showed that HBOA is known to be a precursor of DIBOA (Tanabe et al., 1999; Frey et al., 1997, 2000), and there is a cluster of four closely related genes (*Bx*2-5), which are involved with the biosynthesis of the benzoxazinoids in maize. Studies are underway to verify if the same genes and pathways are implicated in wheat following *cis*-jasmone treatment.

The LPE and VPE methodologies provide different and important information about the profile of secondary metabolites produced by wheat when exposed to *cis*-jasmone. In this work, LPE was crucial for the identification of DIMBOA, MBOA and HMBOA, whereas VPE was crucial for the identification of HBOA. Further analytical studies are required to make accurate comparative measurements of levels of these compounds in different wheat varieties following *cis*-jasmone treatment. Furthermore, molecular genetic studies associated with treatment need to be carried out, in order to detect changes in gene expression. These studies will enable investigations into generating inducible varieties by breeding, and will allow testing of the hypothesis that genes underlying variation in benzoxazinoid levels are correlated with the desirable phenotype of reduced aphid colonisation, in a uniform genetic background.

4. Experimental

4.1. Plant material

Wheat seedlings, *T. aestivum* (var. Solstice), were grown in a glasshouse with supplementary lighting to give a 16 h day at a temperature of 20 °C, until they reached 10 cm height. At this stage, the plants were sprayed with one of three different treatments as described in Section 4.2.

4.2. cis-Jasmone treatment

T. aestivum seedlings, prepared as described in Section 4.1, were subjected to spray treatments using a hydraulic system at an application rate of 200 l/h during 30 s. Three days after spraying, the plants were harvested, frozen under liquid nitrogen, and kept at −80 °C until required for extraction and analysis. The following spray treatments

were used: (1) distilled water as a control treatment; (2) ethylan BV (EBV), a non-ionic surfactant, in distilled water (0.1% v:v); 3. *cis*-jasmone in distilled water (0.025% v:v), solubilised by EBV (0.1% v:v). Preparation of the *cis*-jasmone formulation was carried out by pre-mixing (25 μl) with EBV (100 μl), then addition of the concentrate to distilled water (100 ml), accompanied with vigorous shaking.

4.3. Liquid phase extraction (LPE)

T. aestivum tissue was frozen and ground in liquid nitrogen using a pestle and mortar directly after harvesting. Aerial parts and roots were sampled separately. A sample of each material (100 mg) was transferred to a 10 ml screw cap vial, HCl (3 ml, 0.001 M) was added, and the sample was homogenised for 10 min in an ultrasonic bath. The samples were extracted with diethyl ether (3 × 10 ml), and the upper layers were transferred to a 30 ml screw cap vial. The solvent was evaporated to dryness under a gentle flow of filtered nitrogen. An internal standard (100 μl, 50 ng/μl of *n*-tetracosane) was added to each sample. The solution was evaporated again, and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), (100 μl) added to each sample. The samples were heated in a water bath at 60 °C for 1 h, then analysed directly by gas chromatography and coupled gas chromatography–mass spectrometry.

4.4. Vapor phase extraction (VPE)

T. aestivum tissue was frozen and ground in liquid nitrogen using a pestle and mortar directly after harvesting. Samples (100 mg) were weighed and transferred to 1.5 ml Eppendorf tubes. Aerial parts and roots were sampled separately. The samples were extracted with *n*-PrOH:H_2O:HCl (300 μl, 2:1:0.005). An internal standard (100 μl, 50 ng/μl of *n*-tetracosane) was then added to each sample, which was then shaken for 30 s in a vortex. Methylene chloride ($MeCl_2$), (600 μl) was added to each sample, re-shaken for 5 s in the vortex, and centrifuged at 11,300*g* for 30 s. The bottom $MeCl_2$:*n*-PrOH layers were pipetted, without mixing the layers, transferred to 4 ml glass vials and sealed with Teflon septum screw caps (Chromacol, UK). The samples were derivatized by the addition of trimethylsilyldiazomethane in hexane (2 μl, 2 M). The vials were then capped and allowed to sit at room temperature for 30 min. Excess trimethylsilyldiazomethane was destroyed by adding acetic acid in hexane (2 μl, 2 M) to each sample, and allowing them to sit at room temperature for 30 min. To collect the volatiles, PTFE tubing carrying a low pressure, charcoal filtered N_2 stream (0.2 ml/min) was used. Volatile analytes were trapped on Super Q (~50 mg, 80/100 mesh) adsorbent. Super Q tubes were constructed from the following: glass tubing (3.2 mm ID × 4.5 mm OD, and 7.0 cm of length) with supporting silanized glass wool. To aid volatile collection, the vials were placed into the heating block at 70 °C until all the solvent evaporated. When the vial was dried, it was moved to another heating plate at 200 °C for 2 min. The trapped volatiles were eluted from the Super Q tube using $MeCl_2$ (500 μl), and the eluates stored in tightly capped microvials at −20 °C until required for analysis.

4.5. Gas chromatography (GC)

The *T. aestivum* extracts obtained from LPE and VPE were analysed by gas chromatography using a HP-6890 GC, equipped with a cool on-column injector and a flame ionization detector (FID) at 270 °C. For LPE extracts, an HP-5 capillary column (30 m × 0.32 mm ID, 0.25 μm film, J&W Scientific, Folsom, CA, USA) was used, with a temperature programme of 80 °C/1 min, 10 °C/min to 160 °C/0.1min, 5 °C/min to 235 °C/0.1 min, 50 °C/min to 280 °C/30 min. For VPE extracts, an HP-1 capillary column (50 m × 0.32 mm ID, 0.52 μm film, J&W Scientific, Folsom, CA, USA) was used, with a temperature programme of 30 °C/1 min, 5 °C/min to 150 °C/0.1 min, 10 °C/min to 250 °C/20 min. The carrier gas was hydrogen (flow-rate 1 ml/min). Data were collected with HPChem software, and were handled using Origin 5.0 (Microcal Software). The peaks were quantified on the basis of individual peak areas and response factors for the individual compounds. Response factors were calculated from injections of known amounts of each compound (50 ng) containing an internal standard (*n*-tetracosane, 50 ng), apart from HBOA and HMBOA, which were calculated by comparison of peak areas with other benzoxazinoids.

4.6. Coupled GC–mass spectrometry (GC–MS)

All extracts were analysed using a Thermo-Finnigan MAT95XP magnetic sector mass spectrometer that was directly coupled to a TRACE 2000 GC. For LPE extracts, the GC was equipped with a DB-5 capillary column (30 m × 0.32 mm ID, 0.25 μm film, J&W Scientific, Folsom, CA, USA), and a split–splitless injector. The oven temperature programme was set at 80 °C for 1 min, then 10 °C/min to 160 °C/0.1min, 5 °C/min to 235 °C/0.1 min, 50 °C/min to 280 °C/30 min. For the VPE extracts, the GC was equipped with a DB-1 capillary column (50 m × 0.25 mm ID, 0.52 μm film, J&W Scientific, Folsom, CA, USA), and a cool on-column injector (Gerstel, CIS 4). Helium was used as the carrier gas (flow-rate 1 ml/min). Ionization was by electron impact (70 eV, source temperature 200 °C). Data were collected and analysed with Xcalibur software. Identifications were made by comparison of spectra with library databases (NIST) or with published spectra in the literature. The identification of $TMSi_2$–DIMBOA was made by comparing the mass spectra and GC retention time with an authentic standard of DIMBOA, derivatized with the same reagent and the identification of TMSi–MBOA and $TMSi_2$–HMBOA was made by comparing the spectra with published data (Woodward et al., 1978).

4.7. Statistical analysis

In the experiments with *T. aestivum* using three treatments, the concentrations of each compound identified were compared using ANOVA and pairwise comparisons Student–Newman–Keuls test $p > 0.05$ (Genstat).

4.8. Chemicals

Super Q (80/100 mesh) was purchased from Alltech (PA, USA). Tenax TA (60/80 mesh) was purchased from Supelco (Bellefonte, PA, USA). *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was purchased from Fluka (Germany). Hexane (95% pesticide residue) was purchased from Fisher Scientific (Loughborough, Leicestershire, UK). The following chemicals were purchased from Sigma–Aldrich (Gillingham, Dorset, UK); ferulic acid, vanillic acid, *trans*-*p*-coumaric acid, *p*-hydroxybenzoic acid, benzoxazolin-2-one (BOA), syringic acid, *n*-tetracosane (99%), trimethylsilyldiazomethane (2 M in hexane), acetic acid (99%). 1-propanol (99.5% HPLC grade), diethyl ether (purris.), methylene chloride (99%). Ethylan BV was purchased from Acros Chemicals (Manchester, UK). *cis*-Jasmone was purchased from Avocado, Morecombe, UK. DIMBOA was isolated from wheat seedlings using a previously reported protocol (Woodward et al., 1978).

Acknowledgements

This work was funded by a Rothamsted International Fellowship to MCBM. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the United Kingdom.

References

Agelopoulos, N.G., Hooper, A.M., Maniar, S.P., Pickett, J.A., Wadhams, L.J., 1999. A novel approach for isolation of volatile chemicals released by individual leaves of a plant in situ. Journal of Chemical Ecology 25, 1411–1425.

Birkett, M.A., Campbell, C.A.M., Chamberlain, K., Guerreri, E., Hick, A.J., Martin, J.L., Matthes, M., Napier, J.A., Pettersson, J., Pickett, J.A., Poppy, G.M., Pow, E.M., Pye, B.J., Smart, L.E., Wadhams, G.H., Wadhams, L.J., Woodcock, C.M., 2000. New roles for *cis*-jasmone as an insect semiochemical and in plant defense. Proceedings of the National Academy of Sciences USA 97, 9329–9334.

Bruce, T.J.A., Martin, L.J., Pickett, J.A., Pye, B.J., Smart, L.E., Wadhams, L.J., 2003a. *cis*-Jasmone treatment induces resistance in wheat plants against the grain aphid, *Sitobion avenae* (Fabricius) (Homoptera:Aphididae). Pest Management Science 59, 1031–1036.

Bruce, T.J., Pickett, J.A., Smart, L.E., 2003b. *cis*-Jasmone switches on plant defence against insects. Pesticide Outlook 14, 96–98.

Frey, M., Chomet, P., Glawischning, E., Stettner, C., Grun, S., Winklmair, A., Eisenreich, W., Bacher, A., Meeley, R.B., Briggs, S.P., Gierl, K., Simcox, A., 1997. Analysis of a chemical plant defense mechanism in grasses. Science 277, 696–699.

Frey, M., Stettner, C., Pare, P.W., Schmelz, E.A., Tumlinson, J.H., Gierl, A., 2000. An herbivore elicitor activates the gene for indole emission in maize. Proceedings of the National Academy of Sciences 97, 14801–14806.

Frey, M., Huber, K., Sicker, D., Lindberg, P., Meeley, R.B., Simmons, C.R., Yalpini, N., Gierl, A., 2003. A 2-oxoglutarate-dependent dioxygenase is integrated in DIMBOA-biosynthesis. Phytochemistry 62, 371–376.

Guenzi, W.D., McCalla, T.M., 1966. Phenolic acids in oats, wheat, sorghum, and corn residues and their phytotoxicity. Agronomy Journal 58, 303–304.

Kessler, A., Baldwin, I.T., 2002. Plant responses to insect herbivory: the emerging molecular analysis. Annual Review of Plant Biology 53, 299–328.

Koch, T., Bandemer, K., Boland, W., 1997. Biosynthesis of *cis*-jasmone: a pathway for the inactivation and the disposal of the plant stress hormone jasmonic acid to the gas phase? Helvetica Chimica Acta 80, 838–850.

Koch, T., Krumm, T., Jung, V., Engelberth, J., Boland, W., 1999. Differential induction of plant volatile biosynthesis in the lima bean by early and late intermediates of the octadecanoid-signaling pathway. Plant Physiology 121, 153–162.

Ni, X., Quisenberry, S.S., 2000. Comparison of DIMBOA concentrations among wheat isolines and corresponding plant introduction lines. Entomologia Experimentalis et Applicata 96, 275–279.

Niemeyer, H.M., 1988. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-e-ones), defence chemicals in the Gramineae. Phytochemistry 27, 3349–3358.

Oikawa, A., Ishihara, A., Tanaka, C., Mori, N., Tsuda, M., Iwamura, H., 2004. Accumulation of HDMBOA-Glc is induced by biotic stresses prior to the release of MBOA in maize leaves. Phytochemistry 65, 2995–3001.

Oikawa, A., Ishihara, A., Hasegawa, M., Kodama, O., Iwamura, H., 2001. Induced accumulation of 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one glucoside (HDMBOA-Glc) in maize leaves. Phytochemistry 56, 669–675.

Oikawa, A., Ishihara, A., Iwamura, H., 2002. Induction of HDMBOA-Glc accumulation and DIMBOA-Glc 4-*O*-methyltrasnferase by jasmonica acid in poaceous plants. Phytochemistry 61, 331–337.

Perez, F.J., Ormenonunez, J., 1991. Difference in hydroxamic acid content in roots root exudates of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.): possible role in allelopathy. Journal of Chemical Ecology 17, 1037–1043.

Pickett, J.A., Birkett, M.A., Bruce, T.J.A., Chemaberlain, K.C., Gordon-Weeks, R., Matthes, M., Moraes, C.B., Napier, J.A., Smart, L.E., Wadhams, L.J., Woodcock, C.M., 2005. *cis*-Jasmone as an allelopathic agent through plant defence induction. In: Proceedings of the Fourth World Congress on Allelopathy, Wagga Wagga, Australia, 21–26 August 2005, pp. 122–127.

Schmelz, E.A., Engelberth, J., Alborn, H.T., O'Donnell, P., Sammons, M., Toshima, H., Tumlinson, J.H., 2003. Simultaneous analysis of phytohormones, phytotoxins, and volatile organic compounds in plants. Proceedings of the National Academy of Sciences USA 100, 10552–10557.

Schmelz, E.A., Engelberth, J., Tumlinson, J.H., Block, A., Alborn, T., 2004. The use of vapour phase extraction in metabolic profiling of phytohormones and other metabolites. Plant Journal 39, 790–808.

Tanabe, J., Sue, M., Isihara, A., Iwamura, H., 1999. Occurrence characterization of 2-hydroxy-1,4-benzoxazin-3-one and indole hydroxylases in juvenile wheat. Bioscience and Biochemistry 63, 1614–1617.

Understrup, A.G., Ravnskov, S., Hansen, H.C.B., Fomsgaard, I.S., 2005. Biotransformation of 2-benzoxazolinone to 2-amino-(3*H*)-phenoxazin-3-one and 2-acetylamino-(3*H*)-phenoxazin-3-one in soil. Journal of Chemical Ecology 31, 1205–1222.

Villagrasa, M., Guillamon, M., Labandeira, A., Taberner, A., Elijarrat, E., Barcelo, D., 2006. Benzoxazinoid allelochemicals in wheat: distribution among foliage, roots, and seeds. Journal of Agricultural and Food Chemistry 54, 1009–1015.

Wang, J., Xu, T., Zhang, L., Zhong, Z., Luo, S., 2006. Effect of methyl jasmonate on hydroxamic acid and phenolic acid content in maize and its allelopathic activity to *Echinochloa crusgalli* (L.). In: Proceedings of the Fourth World Congress on Allelopathy, 21–26 August 2005, Charles Sturt University, Wagga Wagga, NSW, Australia. <http://www.regional.org.au/au/allelopathy/2005/2/3/2735_jianwuw.htm>.

Woodward, M.D., Corcuere, L.J., Helgeson, J.P., Upper, C.D., 1978. Decomposition of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one in aqueous solutions. Plant Physiology 61, 796–802.

Wu, H., Haig, T., Pratley, J., Lemerle, D., An, M., 1999. Distribution and exudation of allelochemicals in wheat, *Triticum aestivum*. Journal of Chemical Ecology 26, 2141–2154.

Wu, H., Haig, T., Pratley, J., Lemerle, D., An, M., 2000. Allelochemicals in wheat (*Triticum aestivum* L.): variation of phenolic acids in root tissues. Journal of Agricultural and Food Chemistry 48, 5321–5325.

Wu, H., Haig, T., Pratley, J., Lemerle, D., An, M., 2001. Allelochemicals in wheat (*Triticum aestivum* L.): variation of phenolic acids in shoot tissues. Journal of Chemical Ecology 27, 125–135.