Secondary metabolite profiling of wheat, *Triticum aestivum* , following treatment with *cis*-jasmone

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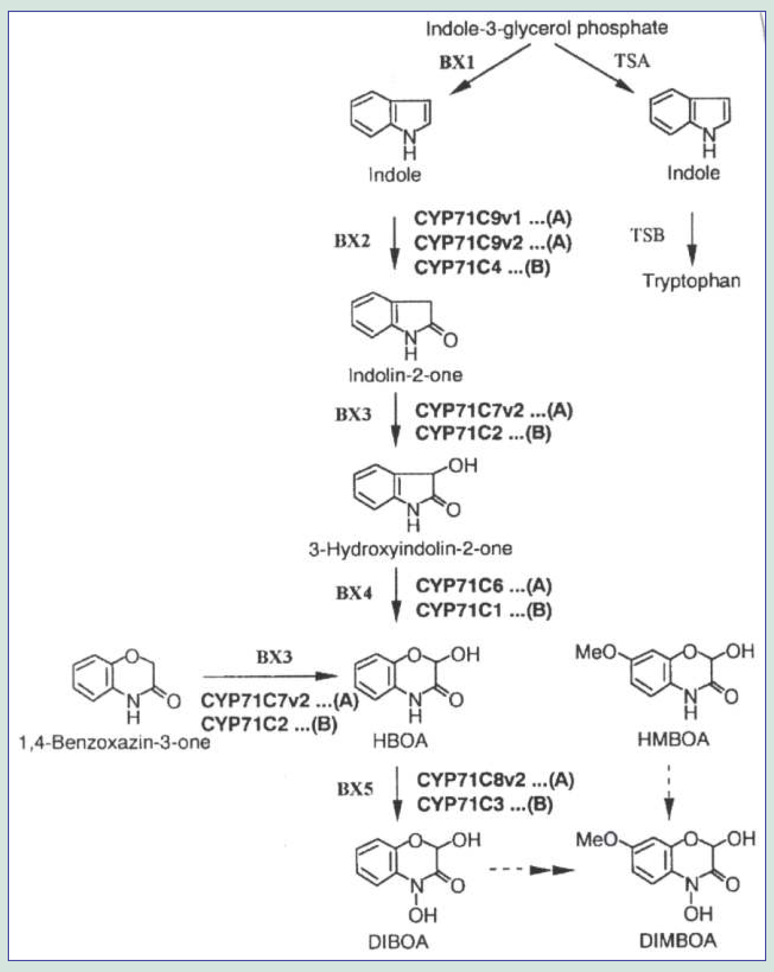
INTRODUCTION

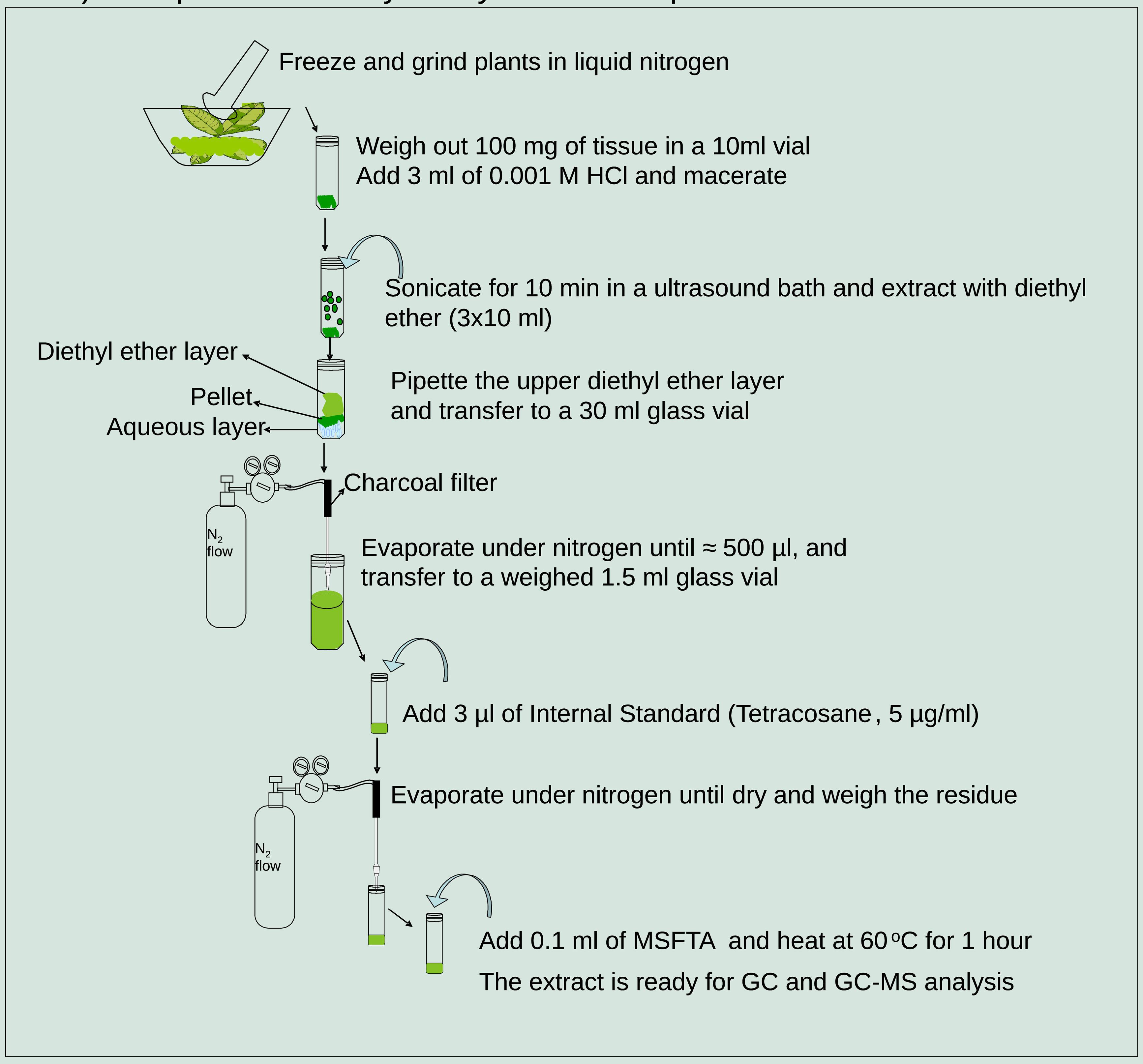
Induced plant defence responses to pest attack are seen as offering opportunities for novel approaches to the protection of crop plants. This has been demonstrated in the field, where the spraying of winter wheat crops with *cis*-jasmone formulations led to statistically significant reductions in cereal aphid, *Sitobion avenae*, populations over a number of field seasons (Bruce et al., 2003a). In addition, statistically significant reductions in the mean intrinsic rate of population increase and in nymph production by *S. avenae* on certain wheat varieties previously treated with *cis*-jasmone were observed (Bruce et al., 2003b). This appears to relate to induction of antibiotic secondary metabolites such as from the hydroxamic acid pathway, including 2,4-dihydroxy-7-methoxy-1,4-benzooxazin-3-one (DIMBOA). To provide evidence for this hypothesis, levels of allelopathic compounds in wheat were investigated following *cis*-jasmone treatment, using a liquid-phase extraction approach.



Biosynthesis of hydroxamic acids in wheat and maize

MATERIALS AND METHODS

Wheat seedlings, *Triticum aestivum* (Solstice variety) were subjected to the following treatment sprays:- i) *cis*-Jasmone (25 ?l) solubilized in deionized water (100 ml) by means of a non-ionic surfactant (ethylan BV, 0.1%); ii) water with 0.1 % ethylan EBV; iii) water. Treated plants were extracted using the approach detailed below, and derivatized using *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSFTA). Samples were analysed by GC and coupled GC-MS.



GC-MS conditions: Thermo-Finnigan MAT95XP magnetic sector mass spectrometer, equipped with an ion trap, directly coupled to a Finnigan Trace 2000 GC. The GC was equipped with a DB-5 column (30 m X 0.25 mm ID, 0.25 µm film, J&W Scientific) and a split-splitless injector. The GC oven was programmed to start at 80°C for 1 min, then 5°C/min to 180°C, and 50°C/min to 280°C, with a final hold time of 30 min. The carrier gas was helium. Ionization was by electron impact (70eV, source temperature 200°C). Data were collected and analysed with Xcalibur software. Identifications were made by comparison of spectra with library databases (NIST).

RESULTS

GC and GC-MS analysis of wheat extracts confirmed the presence of trimethylsilyl (TMS)-derivatised DIMBOA in leaf and root samples (Fig. 1). Comparison of treatments showed that there was a statistically significant higher levels of DIMBOA in the *cis*-jasmone/EBV treatments, compared to the EBV and control treatments (Fig. 2).

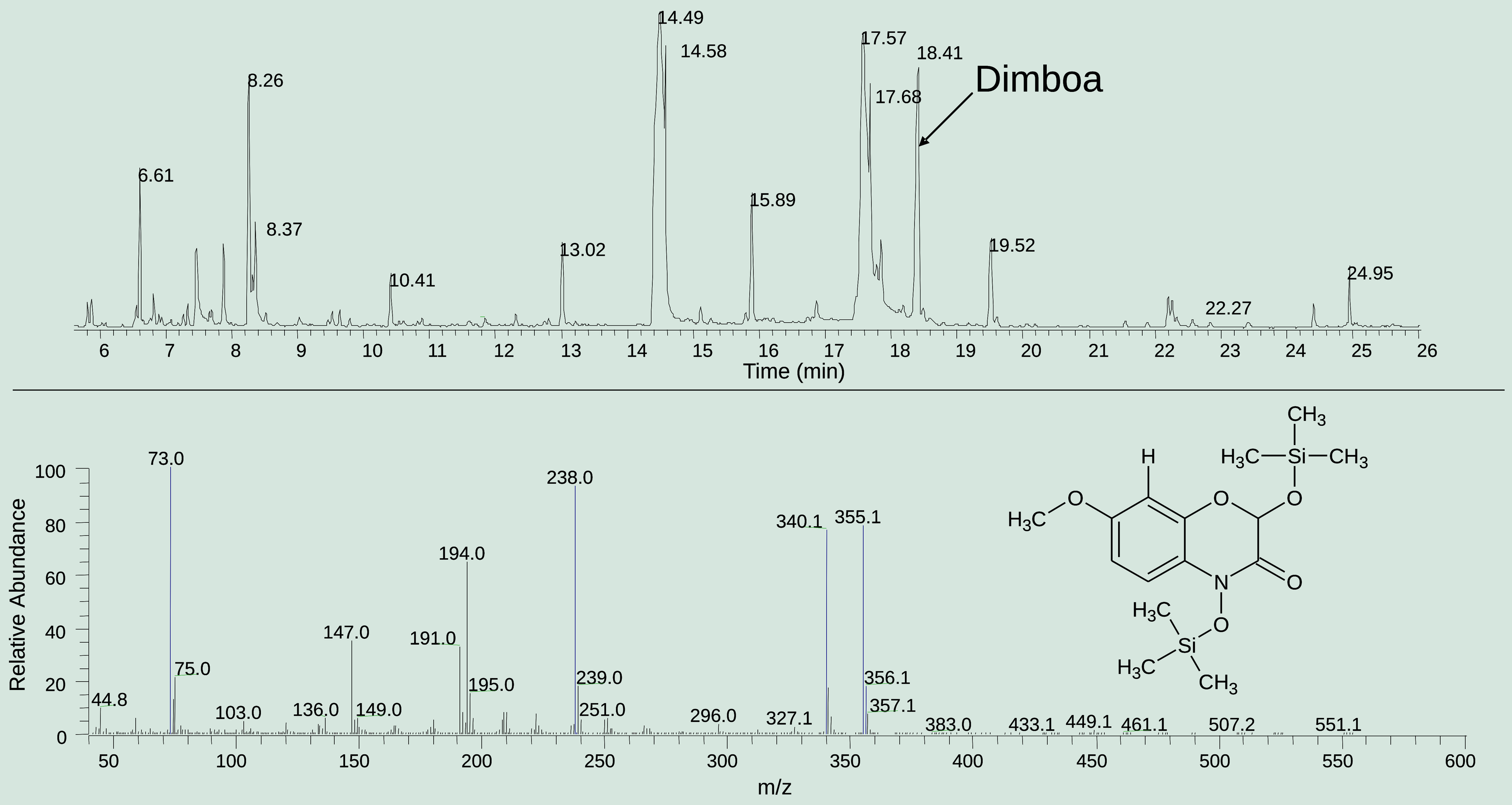


Figure 1. A. GC-MS analysis of extract obtained from leaves of wheat treated with *cis*-jasmone and B. Mass spectrum of peak at 18.41 min, confirming the presence of trimethylsilyl (TMS)-derivatized DIMBOA.

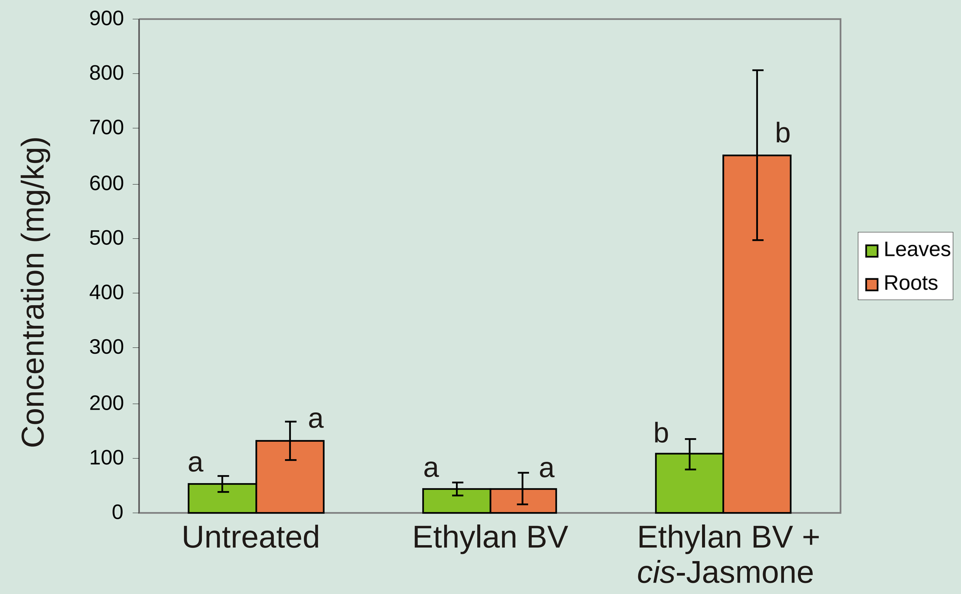


Figure 2. Concentration of DIMBOA released by wheat *T. aestivum* when treated with *cis*-jasmone + EBV, EBV alone and untreated. Bars in a graph followed by the same small letter indicate no significant differences (pairwise Newman-Keuls test P>0.05;. ■ *df* =78, F=7.31, p<0.001;. ■ *df*=4922, F=3.34, *p*<0.05).

Further evaluation of wheat leaf extracts showed that production of the phenolic acids syringic acid and 4-hydroxycinnamic acid was also increased following *cis*-jasmone treatment (Fig. 3). These results indicate that *cis*-jasmone has a role as an allelopathic agent, inducing production of hydroxamic and phenolic acids in wheat seedlings.

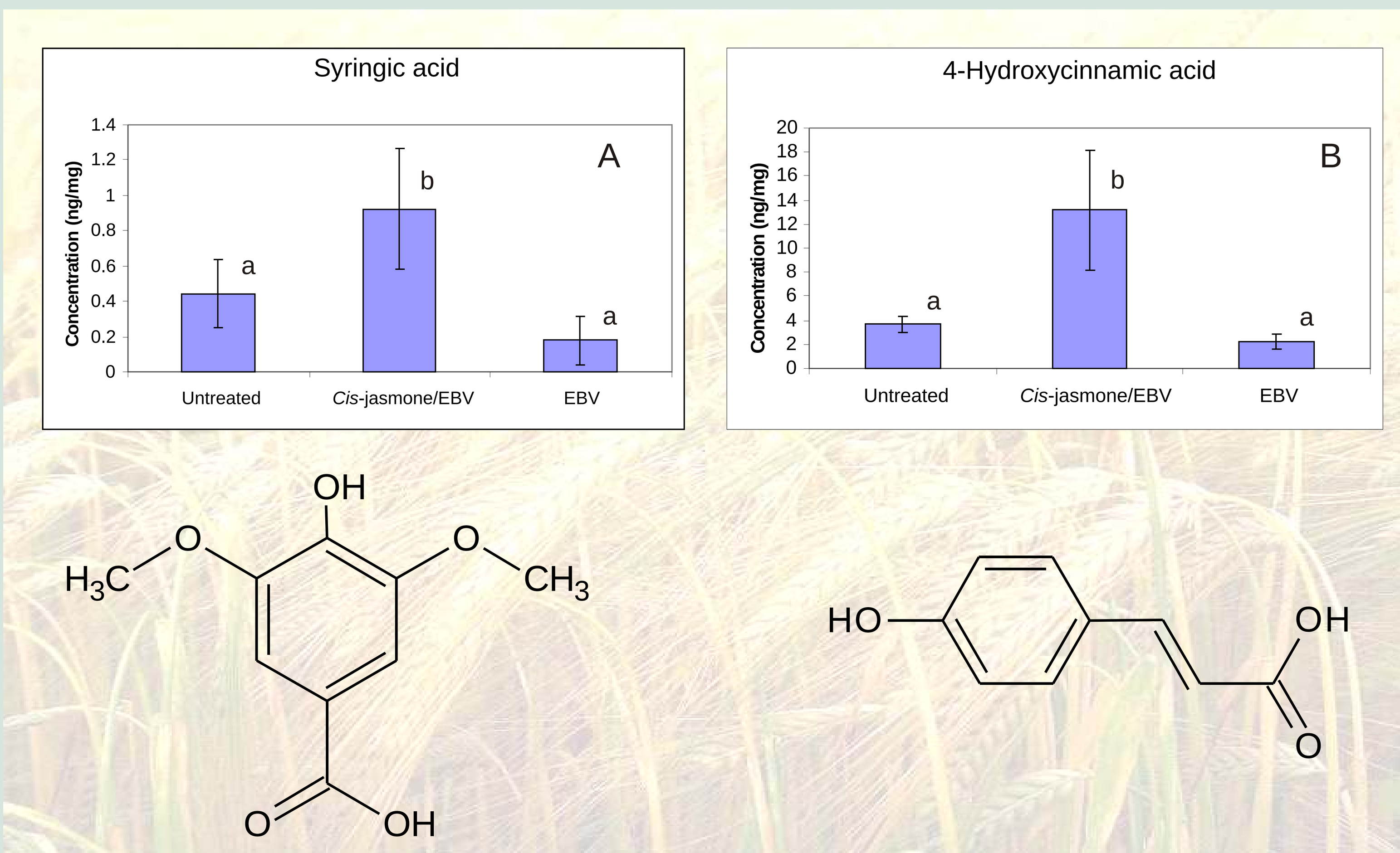


Figure 3. Concentration of phenolic acids in wheat, *T. aestivum,* when treated with *cis*-jasmone + EBV, EBV alone and untreated. Bars in a graph followed by the same small letter indicate no significant differences (pairwise Student-Newman-Keuls test P>0.05; **A.** *df*=22, F=4.33, p<0.05; **B.** *df*=22, F=4.43, p<0.05.

References
Bruce, T.J., Martin, J.L., Pickett, J.A., Pye, B.J., Smart, L.E. and 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., Pickett, J. and Smart, L. (2003b) *Cis*-jasmone switches on plant defence against insects. *Pesticide Outlook* June 2003 96-98.

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