0971-4693/94 US $ 5.00
Allelopathy Journal 33 (2): 163-174 (2014) *International Allelopathy Foundation* 2014
Tables: 2, Figs : 5

Effects of allelochemical stress on response of cucumber to UV-B radiation and *Botrytis cinerea* infection

A. JÓŹWIAK-ŻUREK*, M. PIETROWSKA-BOREK and M. KOZŁOWSKA

Department of Plant Physiology, Poznań University of Life Sciences,
Wołyńska 35, 60-637 Poznań, Poland
E. Mail: jozwiak.anna@yahoo.com
(Received in revised form: January 14, 2014)

ABSTRACT

Using tolerant (Genoype-1) and susceptible (Genoype-14) genotypes of cucumber, which differed in their stress tolerance, we studied the effects of allelochemical stress. The application of ferulic acid (FA) as allelochemical on cucumber enhanced its resistance to UV-B irradiation and *B. cinerea* infection. Stress was imposed either alone and in combination. FA enhanced the 4-coumarate:CoA ligase (4CL) activity and this was correlated to plant's tolerance but the activity of chalcone synthase was not enhanced. Double stress (FA + UV-B) restricted the 4CL activity but did not affect the accumulation of flavonols. The effect on lignin content was negligible. Inoculation of seedlings with *B. cinerea* subjected earlier to FA + UV-B irradiation reduced the level of 4CL and flavonols. The results did not suggest cross-tolerance, as both abiotic stresses inhibited the response to *B. cinerea*.

Key words: Allelochemical, *Botrytis cinerea,* chalcone synthase, 4-coumarate: CoA ligase, cucumber, flavonols, ferulic acid, UV-B radiation

INTRODUCTION

Under natural environmental conditions, plants face stresses simultaneously or sequentially and their effects can be temporary, concurrent or synergic (16,34). The response to combined stress factors can differ from a single factor (29). However, some responses are similar or even common, e.g. protection against dehydration connected with drought and freezing, or oxidative stress and the induction of antioxidant response in majority of events (3,22,24). Stress factors can activate various signalling pathways and lead to different responses. A pathway stimulated by one stress can positively or negatively regulate another signalling pathway (22). The combined impact of UV-B and drought reduces the water potential in wheat and causes the accumulation of flavonoids (16). An *et al.* (1) demonstrated that 0.2% quercetin reduces the SOD activity in soybean under the influence of UV radiation. Low level of UV radiation increases the freezing tolerance in winter wheat (43), while combination of heat and drought enhances the respiration, suppresses photosynthesis, influences the transcription level and sucrose accumulation (34). Besides, there are some cross-reactions between the abiotic and biotic stresses (6,36).

*Correspondence author

Allelochemicals are important inhibitory/stimulatory factors released from the donor plant into the environment and they influence the growth and development of other plants (26). The inhibitory factors are synthesized via the phenylpropanoid pathway. Ferulic acid (FA) as feruloyl CoA is an intermediate product of this pathway (2,14) and also activates this pathway (11,32,39). Besides, FA reduces the water use, inhibits foliar expansion and root elongation, reduces the rates of photosynthesis and induces lipid peroxidation (11,32).
One of the essential enzymes in phenylpropanoid pathway is 4-coumarate:CoA ligase (4CL EC 6.2.1.12), catalyzing the conversion of 4-coumaric, caffeic, ferulic, 5-hydroxyferulic and sinapic acids to their corresponding CoA esters in a two-steps, ATP and Mg^{2+}-dependent reaction, in which 4-hydroxycinnamoyladenylate is formed as an intermediate (9,45). The 4CL is site of plant growth suppression by some phenolic compounds [chalcone and naringenin (10)] and other plant growth inhibitors (44), its activity could vary under the influence of allelochemical substances.

Chalcone synthase (CHS, EC 2.3.1.74) catalyzes the condensation of 3-molecules of malonyl-CoA with 4-coumaryl-CoA, and leads to the formation of flavonoids (8,17). The activation and expression of CHS occurs at low temperature, infection by pathogens, UV radiation, etc. (27,30,41). CHS mRNA level increased in *Arabidopsis thaliana* under low temperature (4°C) and light induction (27). Furthermore, CHS gene is stimulated by pathogen infection (5,30).

Flavonoids protects the plants from excessive UV irradiation (13,21,40). While, antimicrobial chalcone, phytoalexins and lignins protects the plants from fungal attacks (25). This study aimed to determine the effects of the stress allelochemical namely ferulic acid on (i) cucumber exposed to enhanced UV-B radiation and *Botrytis cinerea* infection, (ii). activity of 4CL and CHS and (iii). accumulation of flavonols and lignin.

MATERIALS AND METHODS

I. Plant material

Two genotypes of cucumber (Genotype 1 - Tolerant, genotype 14 - Susceptible) differing in response to ferulic acid were used for examining the stress tolerance. Seeds were purchased from Nochowo "Spójnia" Polish Seeds Company. Seeds were presoaked in distilled water for 24 h and then sown in vermiculite (Not Sterilised}, in plastic pots (10-12 cm dia). Seedlings were grown in a growth chamber [day/night 25/22ºC, relative humidity: 60-65% and 12-h light period at 120 µmol m^{-2} $s^{-}1$ photosynthetic photon flux density (PPFD), using fluorescence Philips 58 W/84 sun lamps]. Plants were weekly watered and fertilized with complete Knop solution (50 cm^3/pot). Twenty days old seedlings were subjected to allelochemical stress and UV-B radiation was applied either alone or simultaneously and inoculated with *B. cinerea*.

II. Allelochemical stress and quantification of ferulic acid

Seedlings were subjected to allelochemical stress by adding 2 mM aqueous solution of ferulic acid (50 cm^3 per pot) to vermiculite. The ferulic acid was from Sigma-Aldrich Chemical Co. Total ferulic acid (free and conjugated) concentration in cucumber leaves was determined as per Lu *et al.* (28). Fresh plant material (0.5 g) was crushed in mortar and transferred to 25 cm^3 flask, to which gradually methanol with 2% sodium

bicarbonate extraction buffer (95:5 v/v) was added. Samples were placed in an ultrasonic bath for 100 min at room temperature, then supplemented with extraction buffer to volume of 25 cm^3. Extracts were filtered through a 0.2 µm mesh size filter and placed in autosampler chamber. Total ferulic acid was determined using high performance liquid chromatography (HPLC), with a 100 RP-C18 column (4.6 mm x 250 mm) (Chrompack) and a linear gradient of eluent: A [methanol: acetic acid: water (10:2:88 v/v/v)] and B [methanol: acetic acid: water (90:2:8 v/v/v)].

III. UV-B irradiation

UV radiation was supplied for 6 h daily by a TL 20W/01 RS Philips lamp with 18 kJ m^{-2} d^{-1} (750 mW m^{-2}) irradiance at the canopy level and photon flux density of 3.25 µmol m^{-2} s^{-1} at 315 nm. The irradiance level was measured with a VLX 3W radiometer (Vilber Lourmat, France).

IV. Extraction and quantification

(i). 4-coumarate:CoA ligase: Leaf samples were collected at 2, 4 and 8 days after FA and UV-B treatment and after 2 and 4 days of *B. cinerea* inoculation. Third and fourth leaves from the top were harvested during the light period. Extraction was done as per method of Knobloch and Hahlbrock (23) with some modifications, using a 100 mM Tris-HCL buffer (pH 7.8) containing 5 mM mercaptoethanol and 5% glycerol. Homogenates were mixed with Dowex AG 1-X2 (0.1 g/ml) at 4ºC for 15 min and centrifuged at 23 000 g for 30 min. Supernatants were used for the determination of the enzymes. The reaction mixture contained 0.3 mM coenzyme-A, 100 µM p-coumaric acid, 100 mM Tris-HCL buffer, 0.5 mM ATP, 5 mM $MgCl_2$ and 100 µl of the extract. The increase in p-coumarate-CoA content for 10 min was determined spectrophotometrically at 333 nm (Jasco V-530 UV-VIS Spectrophotometer) and the activity was expressed in [pkat x mg^{-1} protein. Protein content of extract was determined colorimetrically as per method of Bradford (4).

(ii). **Chalcone synthase:** Leaf samples were collected at 2 and 8 days after the FA and UV-B treatment and extracted as per Fisher *et al.* (18) using 100 mM KH_2PO_4/K_2HPO_4 pH 8.0 buffer with 18 mM L-cysteine, 20 mM ascorbic acid and Dowex-1 (0.1 g/ml). After centrifugation (30 min at 23 000xg), supernatants were collected. The reaction mixture contained 50 mM KH_2PO_4/K_2HPO_4 pH 8.0, 20 mM L-cysteine, 2% BSA, 200 µM 4-coumaroyl-CoA, 200 µM [2-^{14}C] malonyl-CoA and 10-20 mg of enzyme protein.

Samples were incubated for 1 h at 35°C. The reaction was terminated with 20% HCL and after that shaken for 40 min with 200 µL ethyl acetate. The ethyl acetate layer was dried in a SpeedVac concentrator. Samples were redissolved in 20 µl methanol and separated by thin layer chromatography on silica plates (TLC Silica gel 60, Merck Cat. No 5554), using chloroform : ethanol eluent (3:1 v/v). After drying the naringenin was visualized under a short-wave ultraviolet lamp. The spots of naringenin were cut out and radioactivity was determined by scintillation counter. Activity was expressed in pmol of naringenin x min^{-1} x mg^{-1} protein.

(iii). **Flavonols:** Flavonols in leaves were determined according to Kinnunen *et al*. (20) and Stefova *et al.* (37). Fresh leaf samples were extracted with methanol : hydrochloric acid : water solution (90:1:1 v/v/v) at a 10:1 solution/tissue fresh weight ratio. The homogenates were incubated in a water bath at 60^0C for 20 min. Samples were centrifuged (Beckman J20 centrigfuge) at 18 000 x g at ± 22^0C for 30 min. Absorbance of the supernatant was determined spectrophotometrically (Jasco V-530 UV-VIS Spectrophotometer) at 254 nm. The flavonol content was calculated using quercetin as standard (Sigma-Aldrich, cat. no Q0125) and expressed as µg x g^{-1} of F.W.

(iv). **Lignin:** Lignin was quantified using the lignin thioglycolic acid (LTGA) procedure according to Doster and Bostock (12). Powdered air dried samples (10 mg) were placed in a Teflon bottle and 5 cm^3 of 2 N HCl and 0.5 cm^3 of thioglycolic acid was added to each sample and incubated at 95°C for 4 h. The pellets were rinsed in 5 cm^3 of deionized water, incubated in 5 cm^3 of 0.5 N NaOH for 12 h at room temperature and centrifuged at 850x g for 30 min. The supernatants were acidified with 1 cm^3 of concentrated HCl, incubated at 4°C for 12 h and centrifuged at 850*g* for 30 min. The pellets were resuspended in 5 cm^3 of 0.5 N NaOH and the absorbance was measured at λ=280 nm using a Jasco UV/VIS spectrophotometer. The lignin content was expressed in mg of alkali lignin x g^{-1} of F.W. (Sigma-Aldrich, 370959)

Statistical analysis

All experiments were repeated and analyzed thrice. Results were subjected to Anova statistical analysis and Tukey's test using the MS Excel 2007 package and Sigma Plot 11.0. Correlation coefficients for the relationship between enzymes activity and flavonols content were also determined.

RESULTS AND DISCUSSION

Concentration of ferulic acid: The accumulation of ferulic acid in cucumber leaves after its application as allelochemical substance was less in genotype 1 (170 and 240 % at 2 and 8 d after treatment, respectively) than in genotype 14 (reaching 300 % over control at 8 d). The absorption was slightly stimulated by UV-B exposure (Fig. 1 and Table 1).

Table 1. The accumulation of ferulic acid (mg x g^{-1}f.w.) in cucumber genotypes

Treatment	Days after treatment			
	2	8	2	8
	Genotype 1		Genotype 14	
Control	0.29	0.57	0.70	0.96
FA	0.79 (+172.4)	1.96 (+243.9)	0.86 (+22.8)	3.80 (+295.8)
FA + UV-B	1.07 (+269.0)	2.01 (+252.6)	0.90 (+28.6)	4.31 (+349.0)

Phenylpropanoid pathway enzymes: After stress factors were applied, the activity of 4-coumarate:CoA ligase increased and the response varied with cucumber genotype (Fig. 2).

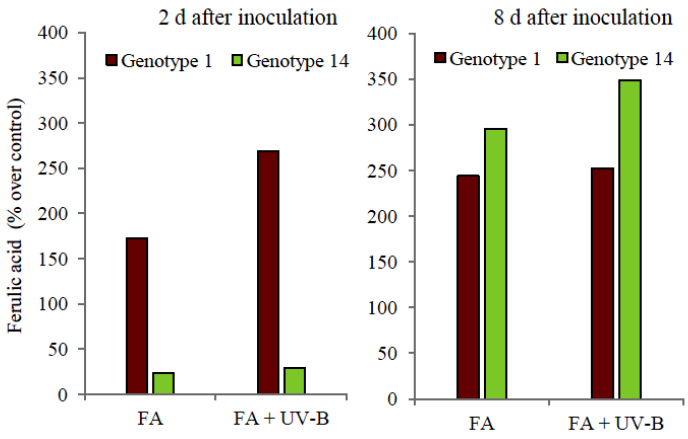

Figure 1. Effect of FA and FA + UV-B application on ferulic acid accumulation in cucumber genotypes at 2 and 8-days after treatment.

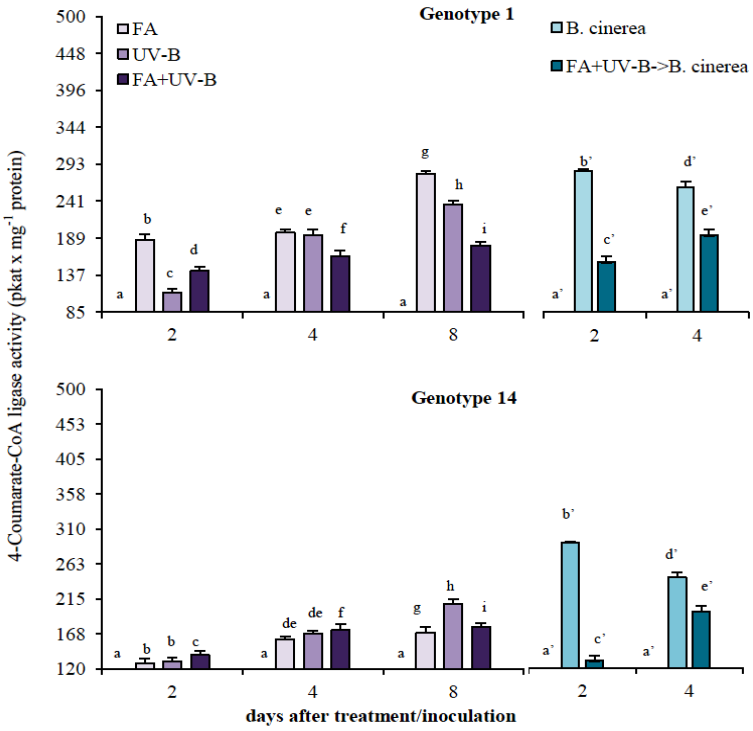

Figure 2. Effect of FA, UV-B, FA + UV-B and *B.cinerea* inoculation on the activity of 4-coumarate-CoA ligase (4CL) in cucumber seedlings (genotype 1 - FA tolerant, genotype 14 - FA sensitive); x-axis – indicate the control. Values marked with different letters are significantly at P<0.05.

The 3-folds increase in activity was recorded in genotype 1 but approximately only 50% increase in genotype 14, especially under FA and UV radiation separately. Under simultaneous double stress (FA+ UV), 4CL activity increased slightly than under single stress. *Botrytis cinerea* infection induced 4CL activity, which was stronger in genotype 1 than in 14, but the level of activity was 3-folds higher than in control. Inoculation of seedlings subjected to FA and UV radiation reduced the enzyme activities.

The chalcone synthase activity was not induced by ferulic acid but was activated by UV-B radiation: 3-folds in genotype 1 and 2-folds in genotype 14 (Fig. 3). When both stresses were applied simultaneously, the activity peaks were lower than when only UV was applied.

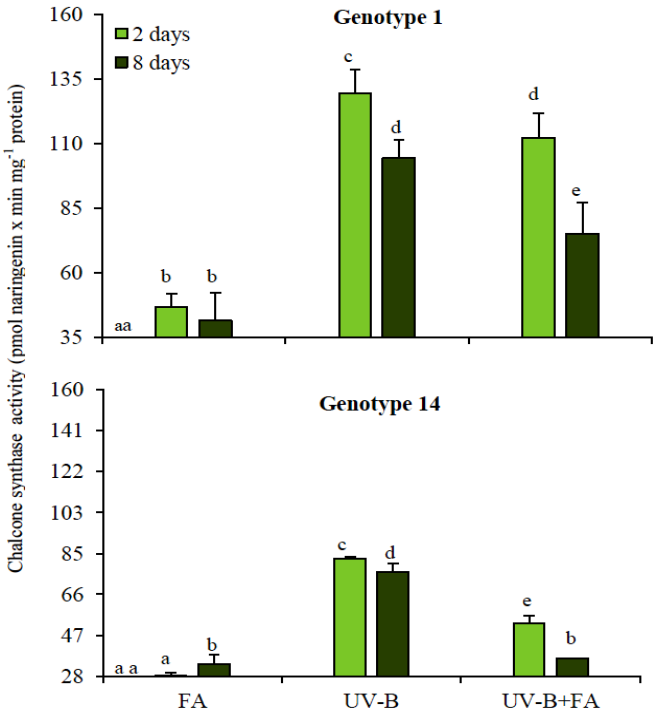

Figure 3. Effect of FA, UV-B, FA + UV-B on the activity of chalcone synthase in cucumber seedlings (genotype 1 - FA tolerant, genotype 14 - FA sensitive); x-axis – indicate the control. Values marked with different letters are significantly at P<0.05.

These results confirmed that phenylpropanoid pathway enzymes are modified by FA, enhanced by UV-B or under combined treatment (39,40,41). Moreover, a stronger response was found in genotype 1, which was more tolerant to FA and UV than genotype 14 (19). Activation of 4CL under the influence of FA and UV-B was markedly weaker than exposure to only a single stress. Similarly, both abiotic stresses decreased the 4CL activation as a defense response to *B. cinerea*. These observations suggest that one stress factor may be inhibiting the second one, or under both stresses, defence was weakened.

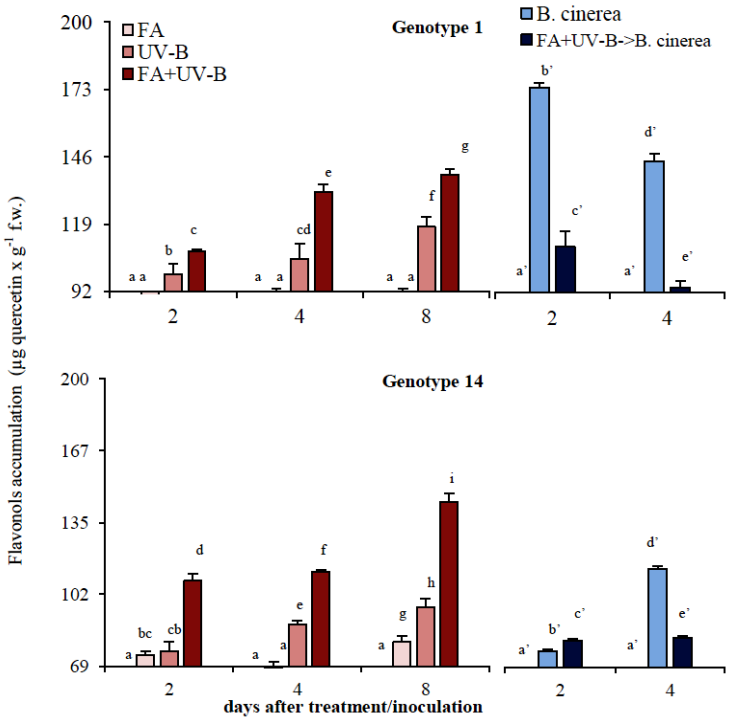

Figure 4. Effect of FA, UV-B, FA + UV-B and *B.cinerea* inoculation on flavonols accumulation in cucumber seedlings (genotype 1 - FA tolerant, genotype 14 - FA sensitive); x-axis – indicate the control. Values marked with different letters are significantly at P<0.05.

The stimulatory effects of ferulic acid on 4CL activity are more complex because many phenolic substances are potential inhibitors of this enzyme (7,10). Even 4CL was considered as a potential action site of plant growth inhibitors (44), while FA, as shown in our earlier studies (19) markedly inhibited the growth in both cucumber genotypes. In a similar experiment on *Arabidopsis thaliana* (unpublished data), UV irradiation stimulated the 4CL activity, while p-coumaric and ferulic acids at 0.1 and 1 mM concentrations did not modify the enzyme activity. Moreover, the interaction of UV radiation and p-coumaric acid was synergistic in character.

Under the influence of FA and FA+UV, the increase in 4CL activity was lower in genotype 14 than in genotype 1, while the absorption of ferulic acid was 2-folds higher in plants of genotype 14 than genotype 1. It may be concluded that FA caused inhibitory effects in genotype 14. Such dependence may also result from a feedback reaction - products inhibition. Under *in- vitro* conditions, the activity of 4CL was inhibited by chalcone and naringenin (46).

As seen here, drastic suppression of enzyme was caused by *B. cinerea* of plants previously subjected to FA+UV. If activation of 4CL could be related to the defense

responses and the synthesis of metabolites limiting the spread of pathogen, the pre-treatment with abiotic stresses would inhibit this mechanism. However, Chassot *et al.* (6) showed that although mechanical cell damage increased the resistance to *B. cinerea*, it was not necessarily through induction of phenylpropanoid pathway.

Chalcone synthase is directly related to the synthesis of flavonoids. Significant changes in activity were found under the influence of enhanced UV irradiation in a combination of FA + UV-B, especially in genotype 1. Ferulic acid did not alter the activity.

Flavonols and lignin content: Among flavonoids, only the flavonols were identified in cucumber (15). The content of flavonols increased during the experimental period in plants exposed to UV-B and under double stress, reaching the highest value on 8th day after treatment (Fig. 4). Similarly, *B. cinerea* infection induced the flavonols accumulation. However, the inoculation of cucumber plants earlier and then subjected to FA+UV treatment, had no effects on flavonols contents. This was similar to FA treatment.

Flavonols content increases under the influence of many stress factors, including sunlight and drought (38), enhanced UV radiation (35) and pathogen infection (42). In cucumber tissues, a slight accumulation of flavonols was found under UV treatment and the changes were partly correlated with 4CL activity. The highest level of these metabolites was found due to double stress. This indicates a synergistic effect, though the FA did not influence the level of flavonols. However, the accumulation of flavonols in *B. cinerea* inoculated tissues occurred only in plants not exposed to abiotic factors. This effect was even stronger than that observed in 4CL activity and confirms suppression of disease.

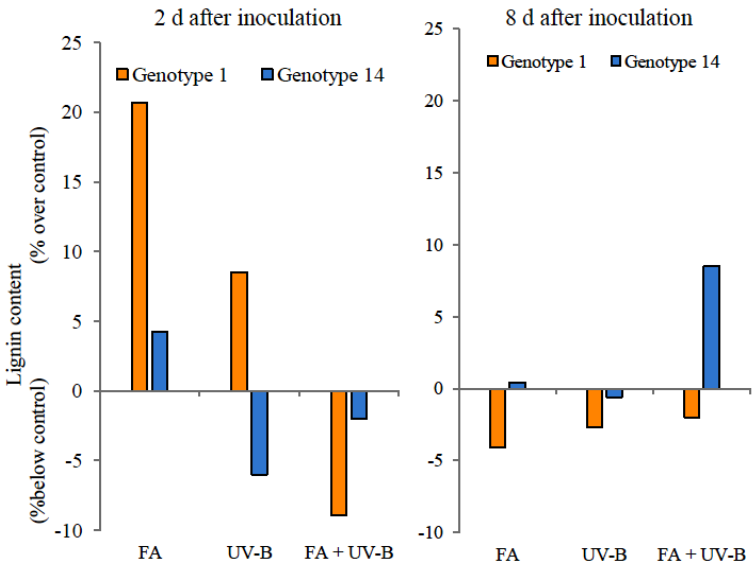

Figure 5. Effect of FA, UV-B and FA + UV-B application on lignin content in cucumber genotypes at 2 and 8-days after treatment.

Table 2. The lignin level [mg alkalilignin x g^{-1} f.w.] under FA, UV-B and FA + UV-B in cucumber genotypes . Values marked with different letters are significantly at P<0.05

Treatment	Days after treatment			
	2	8	2	8
	Genotype 1		Genotype 14	
Control	26.71 a	26.90 ab	25.24 ab	23.50 a
FA	32.24 (+20.7) c	25.81(-4.1) ab	26.29 (+4.2) a	23.60 (+0.4) a
UV-B	28.99 (+8.5) d	26.18(-2.7) ab	23.73 (-6.0) b	23.37 (-0.6) a
FA + UV-B	24.34 (-8.9) b	26.43 (-2.0)ab	24.75 (-2.0) ab	25.05 (+8.5) ab

Potentially crucial phenylpropanoid metabolites include lignin. Exposure of cucumber genotypes to FA and UV-B irradiation, did not effect their lignin content (Fig. 5 and Table 2). Changes in the lignin measured with thioglycol acid method were negligible, except 2-days after FA treatment in genotype 1 (20% stimulation). However, Yamasaki *et al.* (42) showed that UV radiation affects the lignin accumulation in cucumber leaf trichomes, while ferulic acid influenced the level of these compounds in cucumber roots (33). It is likely therefore, that lignin content probably could be increased only in specific cells or tissues.

CONCLUSIONS

The allelochemical stress caused by ferulic acid, modified the response of cucumber to UV and to *B. cinerea* inoculation. Activation of 4CL and CHS and the accumulation of flavonols were correlated to the genotype tolerance to FA and UV; a stronger response was found in genotype 1 (more tolerant to allelochemicals and UV) than genotype 14. The results do not show occurrence of cross-tolerance and even both abiotic stresses inhibited the response to *B. cinerea*.

REFERENCES

1. An, Y.Y., Qun, Z.Y. and Yuan, L. (2006). Effects of quercetin and enhanced UV-B radiation on the soy bean (*Glycine max*) leaves. *Acta Physiologiae Plantarum* **28**: 49-57.
2. Blokker, P., Boelen, P., Broekman, R. and Rozema, J. (2006). The occurrence of p-coumaric and ferulic acids in fossil plant materials and the use as UV-proxy. *Plant Ecology* **182**: 197-207.
3. Bowler, C. and Fluhr, R. (2000). The role of calcium and activated oxygens as signals for controlling cross-tolerance. *Trends in Plant Science* **5**: 241-246.
4. Bradford, M.M. (1976). A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72:** 248-254.
5. Campos, A.D., Ferreira A.G., Hampe, M.M.V., Antunes, I.F., Brancão, N., Silveira, E.P., Silva, J.B. and Osório, V.O. (2003). Induction of chalcone synthase and phenylalanine ammonia-lyase by salicylic acid and *Colletotrichum lindemuthianum* in common bean. Brazilian *Journal of Plant Physiology* **15**: 129-134.
6. Chassot, C., Buchala, A., Schoonbeek, H., Métraux, J.P. and Lamotte, O. (2008). Wounding of Arabidopsis leaves causes a powerful but transient protection against *Botrytis* infection. *Plant Journal* **55:** 555-567.
7. Chen, W.J., Yun, M.S., Deng, F. and Yogo, Y. (2004). Effects of root-applied naringenin and chalcone on the growth of annual plants. *Weed Biology and Management* **4**: 235-238.

8. Claudot, A.C., Ernst, D., Sandermann, H. and Drouet, A. (1997). Chalcone synthase activity and polyphenolic compounds of shoot tissues from adult and rejuvenated walnut trees. *Planta* **203**: 275-282.
9. Costa, M.A., Bedgar, D.L., Moinuddin, S.G.A., Kim, K.W., Cardenas, C.L., Cochrane, F.C., Shockey, J.M., Helms, G.L., Amakura, Y., Takahashi, H., Milhollan, J.K., Davin, L.B., Browse, J. and Lew, N.G. (2005). Characterization *in vitro* and *in vivo* of the putative multigene 4-coumarate:CoA ligase network in Arabidopsis: syringyl lignin and sinapate/sinapyl alcohol derivative formation. *Photochemistry* **66**: 2072-2091.
10. Deng, F., Aoki, M. and Yogo, Y. (2004). Effects of naringenin on the growth and lignin biosynthesis of gramineous plants. *Weed Biology and Management* **4**: 49-55.
11. Dos Santos, W., Ferrarese, M., Nakamura, C., Mourão, K., Mangolin, C. and Ferrarese-Filho, O. (2008). Soybean (*Glycine max*) root lignification induced by ferulic acid. The possible mode of action. *Journal of Chemical Ecology* **34:** 1230-1234.
12. Doster, M.A. and Bostock, R.M. (1988). Quantification of lignin formation in almond bark in response to wounding and infection by *Phytophthora* species. *Phytopathology* **78:** 473-477.
13. Downey, M.O., Dokoozlian, N.K. and Krstic, M.P. (2006). Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *American Journal of Enology and Viticulture* **57**: 257-268.
14. Duran-Serantes, B., Gonzalez, L. and Reigosa, M. (2002). Comparative physiological effects of three allelochemicals and two herbicides on *Dactylis glomerata*. *Acta Physiologiae Plantarum* **24**: 385-392.
15. Eldridge, A.L., Haytowitz, D.B., Bhagwat, S., Gebhardt, S.E., Holden, J.M., Beecher, G.R., Peterson, J. and Dwyer, J. (2003). *Flavonoid Content of Vegetables: The USDA's Flavonoid Database*. USDA-ARS, Beltsville Human Nutr Res Center, Food Composition Lab., Nutrient Data Laboratory, accessed: http://www.nal.usda.gov//fnic/ foodcomp.
16. Feng, H., Li, S., Xue, L. and Wang, X. (2007). The interactive effects of enhanced UV-B radiation and soil drought on spring wheat. *South African Journal of Botany* **73**: 429-434.
17. Ferrer, J.L., Austin, M.B., Stewart, C.Jr. and Noel, J.P. (2008). Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. *Plant Physiology and Biochemistry* **46:** 356-370.
18. Fischer, S., Böttcher, U., Reuber, S., Anhalt, S. and Weissenböck, G. (1995). Chalcone synthase in the liverwort *Marchantia polymorpha. Phytochemistry* **39:** 1007-1012.
19. Jóźwiak-Żurek, A., Kozłowska, M. and Nuc, K. (2011). Phenylalanine ammonia lyase under combined effects of enhanced UV-B radiation and allelopathy stress. *Acta Biologica Cracoviensia Series Botanica* **53:** 73-78.
20. Kinnunen, H., Laakso, K. and Huttunen, S. (1999). Methanol-extractable UV-B-absorbing compounds in Scots pine needles. *Chemosphere-Global Change Science* **1:** 455-460.
21. Kliebenstein, D.J. (2004). Secondary metabolites and plant/environment interactions: a view through *Arabidopsis thaliana* tinged glasses. *Plant Cell and Environment* **27**: 675-684.
22. Knight, H. and Knight, M.R. (2001). Abiotic stress signalling pathways: specificity and cross-talk. *Trends in Plant Science* **6**: 262-267.
23. Knobloh, K.H. and Hahlbrock, K. (1977). 4-Coumarate:CoA ligase from cell suspension cultures of *Petroselinum hortense* Hoffm.: partial purification, substrate specificity, and further properties. *Archives of Biochemistr*y and *Biophysics* **184**: 237-248.
24. Kozłowska, M., Jóźwiak, A., Szpakowska, B. and Golinski, P. (2009). Biological aspect of cadmium and lead uptake by *Phragmites australis* (Cav. Trin ex Steudel) in natural water ecosystems. *Journal of Elementology* **14**: 299-312.
25. Kuc, J. (1997). Molecular aspects of plant responses to pathogens. *Acta Physiologiae Plantarum* **19**: 551-559.
26. Lara-Nunez, A., Romero-Romero, T., Ventura, J.L., Blancas, V., Anaya, A.L. and Cruz-Ortega, R. (2006). Allelochemical stress caused inhibition of growth and oxidative damages in *Lycopersicon esculentum* Mill. *Plant Cell and Environment* **29**: 2009-2016.
27. Leyva, A., Jarillo, J.A., Salinas, J. and Martinez-Zapater, J.M. (1995). Low temperature induces the accumulation of phenylalanine ammonia-lyase and chalcone synthase mRNAs of *Arabidopsis thaliana* in a light-dependent manner. *Plant Physiology* **108**: 39-46.
28. Lu, G.H., Chan, K.C., Leung, K., Chan, C.L., Zhao, Z.Z. and Jaing, Z.H. (2005). Assay of free ferulic acid and total ferulic acid for quality assessment of *Angelica sinensis*. *Journal of Chromatography A* **1068**: 209-219.

29. Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in Plant Science* **11**: 15-19.
30. Nagy, N.E., Fossdal, C.G., Krokene, P., Krekling, T., Lönneborg, A. and Solheim, H. (2004). Induced responses to pathogen infection in Norway spruce phloem: changes in polyphenolic parenchyma cells, chalcone synthase transcript levels and peroxidase activity. *Tree Physiology* **24:** 505-515.
31. Poiatti, V.A.D., Dalmas, F.R. and Astarita, L.V. (2009). Defense mechanisms of *Solanum tuberosum* L. in response to attack by plant-pathogenic bacteria. *Biological Research* **42**: 205-215.
32. Politycka, B. and Mielcarz, B. (2007). Involvement of ethylene in growth inhibition of cucumber roots by ferulic and p-coumaric acids. *Allelopathy Journal* **19:** 451-460.
33. Politycka, B. (1999). Ethylene-dependent activity of phenylalanine ammonia-lyase and lignin formation in cucumber roots exposed to phenolic allelochemicals. *Acta Societatis Botanicorum Poloniae* **68**: 123-127.
34. Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davetova, S. and Mittler, R. (2004). When defense pathways collide: the response of *Arabidopsis* to a combination of drought and heat stress. *Plant Physiology* **134**: 1-14.
35. Ryan, K.G., Swinny, E.E., Markham, K.R. and Winefield, C. (2002). Flavonoid gene expression and UV photoprotection in transgenic and mutant *Petunia* leaves. *Phytochemistry* **59**: 23-32
36. Sharma, Y.K., Leon, J., Raskin, I. and Davis, K.R. (1996). Ozone-induced responses in *Arabidopsis thaliana*: the role of salicylic acid in the accumulation of defense-related transcripts and induced resistance. *Proceedings, National Academy of Sciences* **93**: 5099-5104.
37. Stefova, M., Kulevanova, S. and Stafilov, T. (2001). Assay of flavonols and quantification of quercetin in medicinal plants by HPLC with UV-diode array detection. *Journal of Liquid Chromatography and Related Technologies* **24**: 2283-2292.
38. Tattini, M., Galardi, G., Pinelli, P., Massai, R., Remorini, D. and Agati, G. (2004). Differential accumulation of flavonoids and hydroxycinnamates in leaves of *Ligustrum vulgare* under excess light and drought stress. *New Phytologist* **163**: 547-561.
39. Weir, T.L., Park, S. and Vivanco, J. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biolology* **7:** 427- 479.
40. Winkel-Shirley, B. (2002). Biosynthesis of flavonoids and effects of stress. *Current Opinion in Plant Biology* **5**: 218- 223.
41. Wolf, L., Rizzini, L., Stracke, R., Ulm, R. and Rensing, S. (2010). The molecular and physiological response of *Physcomitrella patens* to UV-B radiation. *Plant Physiology* **153:** 1123-1134.
42. Yamasaki, S., Noguchi, N. and Mimaki, K. (2007). Continuous UV-B irradiation induces morphological changes and the accumulation of polyphenolic compounds on the surface of cucumber cotyledons. *Journal of Radiation Research* **48:** 443-454.
43. Yang, S.H., Wang, L.J. and Li, S.H. (2007). Ultraviolet-B irradiation-induced freezing tolerance in relation to antioxidant system in winter wheat (*Triticum aestivum* L.) leaves. *Environmental and Experimental Botany* **60**: 300-307.
44. Yun, M.S., Chen, W., Deng, F., Kiyokawa, T., Mametsuka, K. and Yogo, Y. (2006). An *in vitro* screening assay to discover novel inhibitors of 4-coumarate: CoA ligase. *Pest Management Science* **62**:1065-1071
45. Yun, M.S., Chen, W., Deng, F. and Yogo, Y. (2007). Propanil and swep inhibit 4-coumarate: CoA ligase activity *in vitro. Pest Management Science* **63**: 815-820.
46. Yun, M.S,, Chen, W., Deng, F. and Yogo, Y. (2009). Selective growth suppression of five annual plant species by chalcone and naringenin correlates with the total amount of 4-coumarate: coenzyme A ligase. *Weed Biology and Management* **9:** 27-37.