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EFFECTS OF METHYL JASMONATE AND GUMS FORMED IN STONE FRUIT TREES ON *IN VITRO* ETHYLENE PRODUCTION BY MYCELIUM OF *VERTICILLIUM DAHLIAE* AND *ALTERNARIA ALTERNATA*

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ABSTRACT

The knowledge about the role of jasmonates in ethylene production by pathogenic fungi is ambiguous. In this study, we describe the effect of methyl jasmonate (JA-Me) and gums formed in stone fruit trees on the growth and *in vitro* ethylene production by mycelium of *Verticillium dahliae* and *Alternaria alternata*. Methyl jasmonate at concentrations of 100, 250 and 500 $\mu g \cdot cm^{-3}$ inhibited the mycelium growth of *V. dahliae* and *A. alternata*, proportionally to the concentrations used. After 8 days of incubation, JA-Me at concentration of 500 $\mu g \cdot cm^{-3}$ limited the area of mycelium of these pathogens by 7-8 times but did not entirely inhibited the pathogen growth. Addition of gums produced by trees of cherry and peach to a medium containing 40 $\mu g \cdot cm^{-3}$ JA-Me did not influence the mycelium growth of *V. dahliae*, but gums of plum and apricot trees stimulated mycelium growth, in comparison to JA-Me only. Methyl jasmonate at concentrations of 2 and 40 $\mu g \cdot cm^{-3}$ stimulated the ethylene production by mycelium of *V. dahliae* and *A. alternata*. It is possible that methyl jasmonate stimulated ethylene production in mycelium of these pathogens through interaction with some fractions of galactans formed during hydrolysis of agar. The lack of interaction of JA-Me with polysaccharides of stone fruit trees gums concerning ethylene production was documented and it needs further explanation.

Key words: *Verticillium dahliae*, *Alternaria alternata*, ethylene production, gums

INTRODUCTION

Verticillium wilt diseases are mainly caused by two species, *Verticillium albo-atrum* and *V. dahliae*, which differ in morphology, physiology and host range preference (Fradin & Thomma 2006; Robb 2007). Ethylene production is known to increase rapidly upon *Verticillium* infection, but *in vitro* the pathogens produce low level of ethylene (Tzeng & DeVay 1984). The *in vitro* rate of ethylene production is not connected with the relative virulence of *V. dahliae* in cotton (Tzeng & DeVay 1984). It has been reported that *V. dahliae* secretes glycoproteins and induces phytoalexin formation in plants (Davis et al. 1998).

Among *Alternaria* fungi, *Alternaria alternata* is the most dangerous pathogen of many host plants. *A. alternata* mycelium also produces small amounts of ethylene *in vitro* on PDA medium (Kępczyńska & Kępczyński 2005). Bashan (1994) showed that *A. alternata* and *A. macrospora* did not produce ethylene in culture, but they induced ethylene by wounding diseased tissues.

Jasmonic acid (JA), methyl jasmonate (JA-Me) and their related compounds, which are designated as jasmonates, show various important activities in the regulation of plant growth and development (Ueda & Kato 1980; Koda 1992; Sembdner & Parthier 1993). Jasmonates have been reported to control ethylene biosynthesis in intact plants and their excised organs by stimulating activities of

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ACC synthase and ACC oxidase (Saniewski 1997; Saniewski et al. 1999). It is also well known that JA and connected compounds occur in fungi, including plant pathogens, for example in *Fusarium oxysporum* f. sp. *mathiolae* (Miersch et al. 1999). The knowledge about the role of jasmonates in ethylene production in pathogenic fungi is still incomplete and ambiguous. Kępczyńska & Kępczyński (2005) showed that methyl jasmonate (JA-Me) at a concentration of 1 mM had no influence on ethylene production by *Alternaria alternata* mycelium. It is generally accepted that jasmonic acid (JA) and ethylene (ET) mediate the induced systemic resistance, which is usually associated with defense against pathogens. Several JA/ET-dependent genes that encode pathogen-related proteins, including plant defensing1.2 (*PDF1.2*) thionin2.1 (*THI2.1*), hevein-like protein (*HEL*) and chitinase B (*CHIB*) are expressed in plants (Van Loon et al. 2006).

In this study, we report the effect of methyl jasmonate and gums produced by stone fruit trees on the growth and *in vitro* ethylene production by mycelium of *V. dahliae* and *A. alternata*.

MATERIALS AND METHODS

The two pathogens: *V. dahliae* and *A. alternata* were studied in *in vitro* experiments. Methyl jasmonate (JA-Me) was added to the autoclaved and cooled to 45 °C Potato-Dextrose-Agar (PDA) medium at final concentrations of 2, 20, 40, 100, 250 and 500 $\mu g \cdot cm^{-3}$. Additionally, to the part of the PDA medium containing 40 $\mu g \cdot cm^{-3}$ JA-Me the gums of cherry, plum, apricot and peach were added at a final concentration of 5 $mg \cdot cm^{-3}$. The gums were dissolved in 5 cm^3 of distilled and sterilized water and added to the medium before sterilization. The medium without JA-Me addition was used as a control. The prepared solutions were poured onto 90 mm Petri dishes. The disks of PDA medium, 5 mm in diameter, overgrown by 7-day-old cultures of *V. dahliae* and *A. alternata*, were transferred onto solidified medium in the middle part of the dishes. After 2, 4, 6 and 8 days of incubation at a temperature of 25 °C in the dark, the diameter of the mycelium colony was measured in two perpendicular directions and calculated as the mycelium surface area (cm^2). For each treatment, five Petri dishes, in two series, at a weekly interval were analysed.

For ethylene assays, 5 mm plugs taken from 7-day-old culture of *V. dahliae* and *A. alternata* were placed into 26 ml vials with 7 ml of mineral Czapek-Dox (CzD) liquid medium or PDA medium, respectively, without or with JA-Me at different concentrations. The gums formed in stone fruit trees were added to the part of CzD or PDA medium with 40 $\mu g \cdot cm^{-3}$ of JA-Me. The gums of cherry, plum, apricot and peach at final concentration of 5 $\mu g \cdot cm^{-3}$ were dissolved in 5 cm^3 of distilled and sterilized water and added to the medium before sterilization. The vials were tightly sealed with rubber caps and held for 3, 7, 10 and 17 days at room temperature. Ethylene production was measured using gas chromatograph HP 4890 D, equipped with a flame ionization detector and a glass column packed with chromosorb 102. After 3, 7, 10 and 17 days, the vials were opened, flushed with air and resealed for ethylene measurement. The experiment was carried out in two series. The results of the experiments were processed statistically, using the variance analysis. Duncan's multiple range t-test was used for assessment of differences between the means, adopting the significance level of $p = 0.05\%$.

RESULTS AND DISCUSSION

Methyl jasmonate (JA-Me) applied directly to the PDA medium exerted inhibitory effect on the growth of *V. dahliae* and *A. alternata*. JA-Me applied to PDA medium at concentrations of 2, 20 and 40 $\mu g \cdot cm^{-3}$ only slightly inhibited mycelium growth of *V. dahliae* and *A. alternata* (Figs. 1 & 2). However, the higher concentrations of 100, 250 and 500 $\mu g \cdot cm^{-3}$ JA-Me inhibited the mycelium growth of these pathogens, proportionally to the applied concentrations. After 8 days of incubation, JA-Me at a concentration of 500 $\mu g \cdot cm^{-3}$ limited the area of mycelium surface of the *V. dahliae* and *A. alternata* by 7-8 times, but did not inhibit the pathogens' growth completely (Figs. 1 & 2). These results confirmed previous studies of Jarecka Boncela & Saniewska (2011) that JA-Me inhibited partly *in vitro* mycelium growth of *Fusarium oxysporum* f. sp. *callistephi*, f. sp. *dainthi* and f. sp. *narcissi*.

The gums formed in stone fruit trees did not affect mycelium growth of *V. dahliae* (data not presented). Addition of gums of cherry and peach to the medium containing 40 µg·cm^{-3} JA-Me did not influence the mycelium growth of *V. dahliae*, but gums of plum and apricot slightly stimulated mycelium growth, in comparison to JA-Me alone (Fig. 3). In the case of *A. alternata*, the gums formed in stone fruit trees added to the medium with 40 µg·cm^{-3} JA-Me did not affect the mycelium growth, in comparison to JA-Me alone (Fig. 4). Saniewska (2002) showed that tulip gums greatly stimulated the mycelium growth of *F. oxysporum* f. sp. *tulipae in vitro*, when added to different media Czapek-Dox-Agar (CzDA), Malt-Extract-Agar (MEA) and Potato-Dextrose-Agar (PDA).

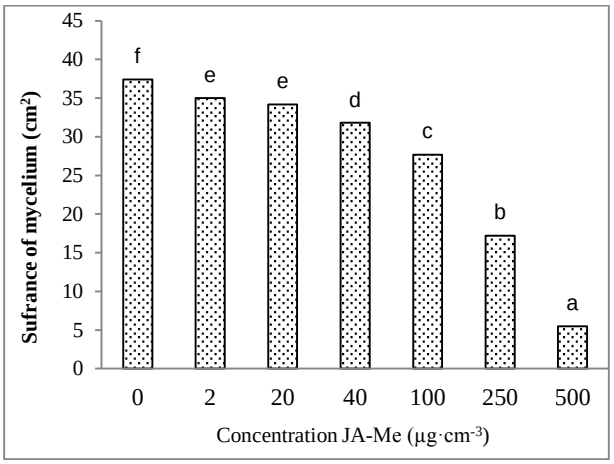

Fig. 1. The influence of methyl jasmonate (JA-Me) added to the PDA medium on *in vitro* growth of *Verticillium dahliae* after 8 days of incubation

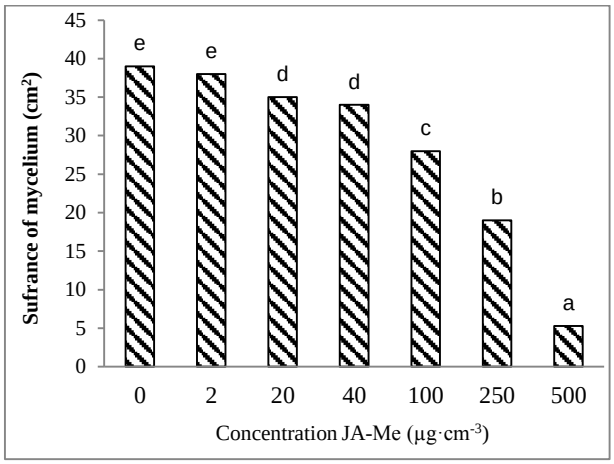

Fig. 2. The influence of methyl jasmonate (JA-Me) added to the PDA medium on *in vitro* growth of *Alternaria alternata* after 8 days of incubation

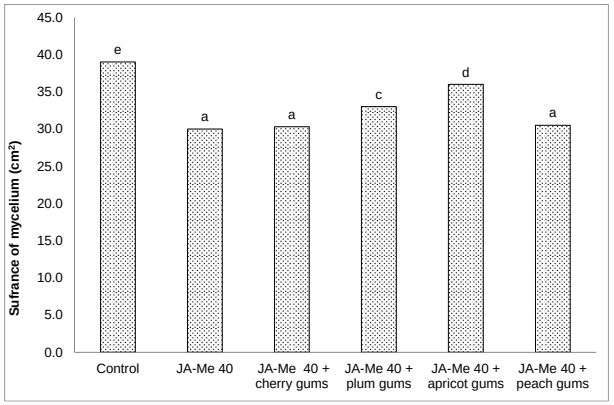

Fig. 3. The influence of JA-Me (40 µg·cm^{-3}) and gums formed in stone-fruit trees added to the PDA medium on *in vitro* growth of *Verticillium dahliae* after 8 days of incubation

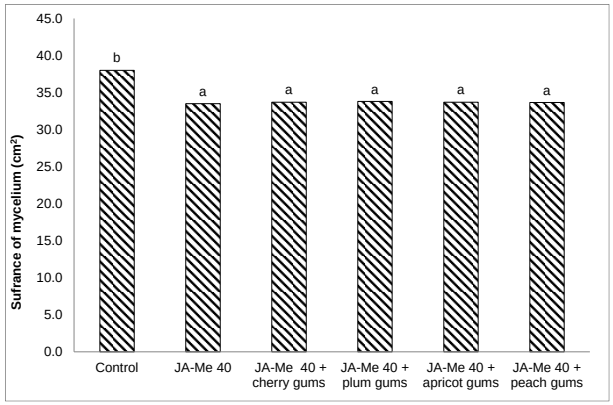

Fig. 4. The influence of JA-Me and gums formed in stone-fruit trees added to the PDA medium on *in vitro* growth of *Alternaria alternata* after 8 days of incubation

Our earlier studies have shown that *Verticillium* species were able to produce ethylene *in vitro*, when growing on CzD medium; the highest level of ethylene evolution was recorded after 10 days of incubation in case of *V. dahliae* cultures. When grown on PDA medium, the mycelium of *A. alternata* produced small amounts of ethylene (unpublished data). Low concentrations of methyl jasmonate, 2 and 40 $\mu g \cdot cm^{-3}$, clearly stimulated the ethylene production by mycelium of *V. dahliae* after 10 days of incubation (Fig. 5). *A. alternata* produced much less of ethylene than *V. dahliae* (Fig. 6). Ethylene biosynthesis in plants proceeds according to the Yang cycle, wherein methionine is converted to S-adenosylmethionine (SAM) by the enzyme SAM synthase. The conversion of SAM to 1-amino-cyclopropane-1-carboxylic acid (ACC) is then catalysed by the ACC synthase (ACS), and ACC is oxidized to ethylene by ACC oxidase (ACO) (Yang & Hoffman 1984). The ACC pathway for ethylene biosynthesis has not been identified in bacteria and fungi. It was found to exist only in the slime mold *Dictyostelium mucoroides* (Amagai & Maeda 1992) and in *Penicillium digitatum* (Jia et al. 1999). In microorganisms, ethylene can be produced either from methionine via 2-keto-4-methylobutyric acid (KMBA) or from glutamic acid via 2-oxoglutarate (Fukuda et al. 1993).

Some fungal pathogens produce high level of ethylene *in vitro*. For example, *F. oxysporum* f. sp. *tulipae*, pathogen of tulips, under *in vitro* conditions can produce significantly higher amounts (even several thousand-fold) of ethylene than other *formae speciales* of *F. oxysporum* and other *Fusarium* species (Swart & Kamerbeek 1976, 1977). ACC, the direct precursor of ethylene in plants, was not detected in mycelium extracts of *F. oxysporum* f. sp. *tulipae* cultured *in vitro*, indicating that the ethylene biosynthesis pathway in the pathogen differs from that in plants (Hottiger & Boller 1991). These authors suggest that ethylene in *F. oxysporum* f. sp. *tulipae* is derived from arginine and passes through glutamate/2-oxoglutarate.

The mechanism of the stimulatory effect of JA-Me on the ethylene production by mycelium of *V. dahliae* and *A. alternata in vitro* is unknown.

Different kinds of oligosaccharides can function in higher plants as molecular signals (elicitors) that regulate growth and development through elicitation of various physiological and biochemical processes, including ethylene biosynthesis (Ebel & Mithöfer 1998; Côte & Hahn 1994; Aldington et al. 1991; Darvill et al. 1992). Plant gums are composed of different substances, but the most important constituents are polysaccharides of highly individual structure (Boothby 1983). Jarecka Boncela (2009) showed that mycelium of *F. oxysporum* f. sp. *tulipae* cultured on Water Agar (WA) is able to produce ethylene. Methyl jasmonate in low concentration (2.5 and 5.0 $\mu g \cdot cm^{-3}$) applied simultaneously to the medium, evidently increased ethylene production, but higher concentrations of JA-Me inhibited the ethylene evolution in the culture grown on WA. It is interesting that although the growth of mycelium of *F. oxysporum* f. sp. *tulipae* was very poor on WA in comparison to PDA medium, the ethylene production was substantially higher. The growth of mycelium of *F. oxysporum* f. sp. *tulipae* on Gerlite (polysaccharide consisting of glucose, rhamnose and glucuronic acid) and tulip gums was poor and ethylene evolution by the pathogen was also low. Agar is a mixture of heterogeneous galactans, and many agarolytic microorganisms commonly produce agarases, which catalyse the hydrolysis of agar (Chi et al. 2012). It is possible that *F. oxysporum* f. sp. *tulipae* is also able to hydrolyse agar and some fragments of galactans (oligosaccharides) stimulate ethylene production. It is well known that some fungi contain different fungal enzyme sets that are responsible for plant polysaccharide degradation (Van den Brink & de Vries 2011).

The gum of apricot added to CzD medium jointly with JA-Me stimulated ethylene production by mycelium culture of *V. dahliae*. The other gums of stone fruit trees did not affect the ethylene production stimulated by methyl jasmonate in mycelium of *V. dahliae* (Fig. 7). The gums of plum, peach, apricot and cherry added to the PDA medium in conjuction with methyl jasmonate did not influence the ethylene production, in comparison to JA-Me treatment alone in culture of *A. alternata* (Fig. 8). In the case of *V. dahliae* and *A. alternata*, it is clear that only methyl jasmonate stimulated ethylene production when

these pathogens were cultured on CzD and PDA media, but simultaneous application of gums of stone fruit trees did not affect ethylene production. It is possible that JA-Me stimulated ethylene production in these pathogens through interaction with some fragments of galactans formed during hydrolysis of agar. The lack of interaction of JA-Me with polysaccharides of stone fruit trees gums concerning ethylene production may be associated with their specific structure, which disables stimulation of the ethylene biosynthesis.

Little is known about the structure and regulation of host genes involved in estabilishing *V. dahliae* and *A. alternata* interactions. In *Arabidopsis thaliana* infected by *A. alternata*, synergy between jasmonates and ethylene was documented for the induction of the plant defense gene *PDF1.2* (Penninckx et al. 1998). Zhang et al. (2011) proposed that both jasmonic acid and ethylene promote the *A. alternata* f. sp. *lycopersici* toxin-induced cell death. Thaler et al. (2004) concluded that whereas ethylene has been associated mainly with the development of disease symptoms in *V. dahliae* interactions with their host, jasmonic acid has been implicated in actual resistance, as JA-deficient tomato and JA-insensitive Arabidopsis plants were found to suffer more severely from *Verticillium* infections. Thus, early host responses after infection with *Verticillium* that result in resistance or susceptibility have been associated mainly with jasmonic acid (Thaler et al. 2004).

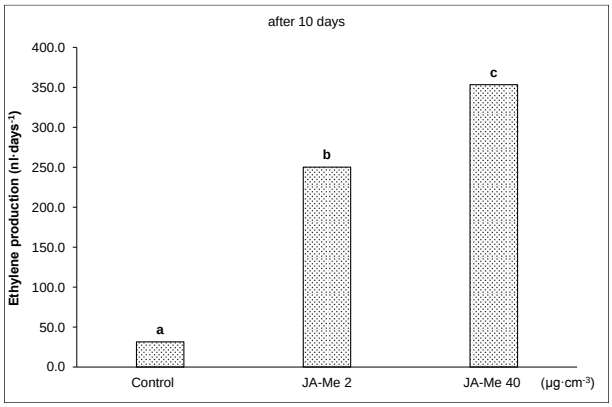

Fig. 5. The effect of methyl jasmonate (JA-Me) added to the CzDA medium on *in vitro* ethylene production by *Verticillium dahliae* cultures

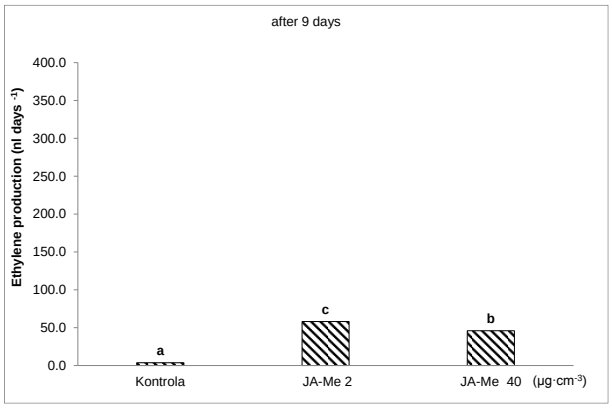

Fig. 6. The effect of methyl jasmonate (JA-Me) added to the PDA medium on *in vitro* ethylene production by *Alternaria alternata* cultures

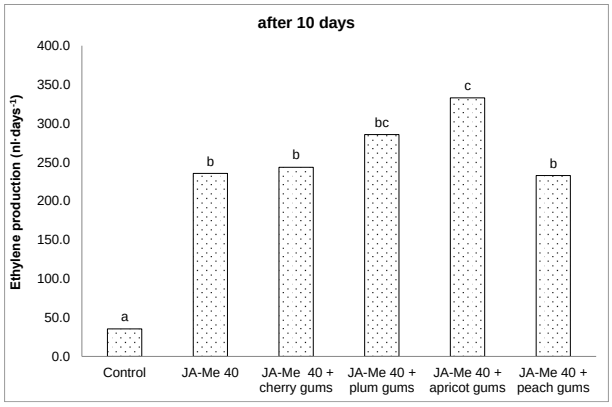

Fig. 7. The influence of JA-Me and gums formed in stone-fruit trees added to the CzDA medium on *in vitro* ethylene production by *Verticillium dahliae* cultures

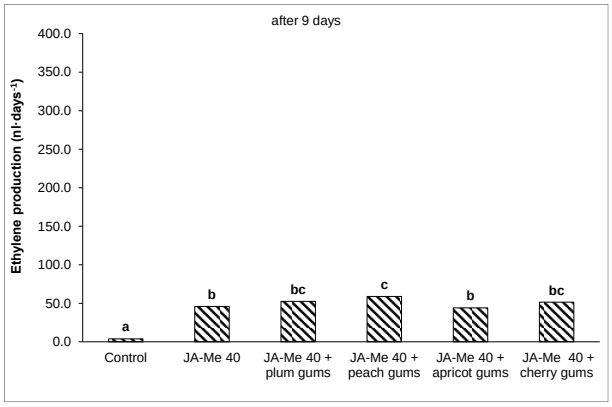

Fig. 8. The influence of JA-Me and gums formed in stone-fruit trees added to the PDA medium on *in vitro* ethylene production by *Alternaria alternata* cultures

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REFERENCES

Aldington S., McDougall J., Fry S.C. 1991. Structure-activity relationship of biologically active oligosaccharides. Plant. Cell Environ. 14: 625-636. DOI: 10.1111/j.1365-3040.1991.tb01535.x.

Amagai A., Maeda Y. 1992. The ethylene action in the development of cellular slime molds: an analogy to higher plants. Protoplasma 167: 159-168. DOI: 10.1007/BF01403379.

Bashan Y. 1994. Symptom expression and ethylene production in leaf blight of cotton caused by *Alternaria alternata* alone and in combination. Can. J. Botany 72: 1574-1579.

Boothby D. 1983. Gummosis of stone-fruit trees and their fruits. J. Sci. Food Agr. 34: 1-7. DOI: 10.1002/jsfa.2740340102.

Van den Brink J., de Vries R.P. 2011. Fungal enzyme sets for plant polysaccharide degradation. Appl. Microbiol. Biot. 91: 1477-1492.

Chi W.-J., Chang Y.-K., Hong S.-K. 2012. Agar degradation by microorganisms and agar-degrading enzymes. Appl. Microbiol. Biot. 94: 917-930.

Côte F., Hahn M.G. 1994. Oligosaccharins: structures and signal transduction. Plant Mol. Biol. 26: 1379-1411.

Darvill A., Augur C., Bergmann C., Carlson R.W., Cheong J.-J., Eberhard S., Hahn M.G., Lo V.-M., Marfè V., Meyer B., Mohnen D., O'Neill M.A., Spiro M.D., Van Halbeek H., York W.S., Albersheim P. 1992. Oligosaccharides that regulate development and defence responses in plants. Glycobiology 2: 181-198. DOI: 10.1093/glycob/2.3.181.

Davis D.A., Low P.S., Heinstein P. 1998. Purification of a glycoprotein elicitor of phytoalexin formation from *Verticillium dahliae*. Physiol. Mol. Plant P. 52: 259-273.

Ebel J., Mithöfer A. 1998. Early events in the elicitation of plant defence. Planta 206: 335-348. DOI: 10.1007/s004250050409.

Fradin E.F., Thomma B.P.H.J. 2006. Physiology and molecular aspects of *Verticillium* wilt diseases caused by *V. dahliae* and *V. albo-atrum*. Mol. Plant Pathol. 7: 71-86. DOI: 10.1111/j.1364-3703.2006.00323.x.

Fukuda H., Ogawa T., Tanase S. 1993. Ethylene production by micro-organisms. Adv. Microb. Physiol. 35: 275-306. DOI: 10.1016/S0065-2911(08)60101-0.

Hottiger T., Boller T. 1991. Ethylene biosynthesis in *Fusarium oxysporum* f. sp. *tulipae* proceeds from glutamate/2-oxoglutarate and requires oxygen and ferrous ions in vivo. Arch. Microbiol. 157: 18-22. DOI: 10.1007/BF00245329.

Jarecka Boncela A. 2009. Relationships between the development of *Fusarium oxysporum* f. sp. *tulipae*, ethylene production and gummosis in tulips (in Polish). Doctoral Thesis, Research Institute of Pomology and Floriculture, Skierniewice.

Jarecka Boncela A., Saniewska A. 2011. The influence methyl jasmonate on growth and development of three special forms of *Fusarium oxysporum*. Progress in Plant Protection/Postępy w Ochronie Roślin 51: 648-651.

Jia Y.J., Kakuta Y., Sugawara M., Igarashi T., Oki N., Kisaki M., Shoji T., Kanetuna Y., Horita T., Matsui H., Honma M. 1999. Synthesis and degradation of 1-aminocyclopropane-1-carboxylic acid by *Penicillium citrinum*. Biosci. Biotech. Bioch. 63: 542-549.

Kępczyńska E., Kępczyński J. 2005. Inhibitory effect of methyl jasmonate on development of phytopathogen *Alternaria alternata* (Fr.) Keissl. and its reversal by ethephon and ACC. Acta Physiol. Plant. 27: 491-496.

Koda Y. 1992. The role of jasmonic acid and related compounds in the regulation of plant defence. Int. Rev. Cytol. 135: 155-198.

Van Loon L.C., Rep M., Pieterse C.M.J. 2006. Significance of inducible defense-related proteins in infected plants. Annu. Rev. Phytopathol. 44: 135-162. DOI: 10.1146/annurev.phyto.44.070505.143425.

Miersch O., Bohlmann H., Wasternack C. 1999. Jasmonates and related compounds from *Fusarium oxysporum*. Phytochemistry 50: 517-523. DOI: 10.1016/S0031-9422(98)00596-2.

Penninckx I.A.M.A., Thomma B.P.H.J., Buchala A., Metraux J.-P., Broekaert W.F. 1998. Concomitant activation of jasmonic acid and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. Plant Cell 10: 2103-2113.

Robb J. 2007. *Verticillium* tolerance: resistance, susceptibility, or mutualism? Can. J. Botany 85: 903-910.

Saniewska A. 2002. The effect of gums induced by *Fusarium oxysporum* Schlecht. f. sp. *tulipae* Apt. in

tulip bulbs on the mycelium growth and development of the pathogen *in vitro*. Zesz. Probl. Post. Nauk Roln. 481: 577-583.

Saniewski M. 1997. The role of jasmonates in ethylene biosynthesis. In: Kanellis A.K., Chang C., Kende H., Grierson D. (Eds.), Biology and Biotechnology of the Plant Hormone Ethylene, Kluwer Academic Publishers, Dordrecht, pp. 39-45.

Saniewski M., Ueda J., Miyamoto K. 1999. Interaction of ethylene with jasmonates in the regulation of some physiological processes in plants. In: Kanellis A.K., Changh C., Klee H., Bleecker A.B., Pech J.C., Grierson D. (Eds.), Biology and Biotechnology of the Plant Hormone Ethylene II, Kluwer Academic Publishers, Dordrecht, pp. 173-180.

Sembdner G., Parthier B. 1993. The biochemistry and the physiological and molecular actions of jasmonates. Annu. Rev. Plant Phys. 44: 569-589. DOI: 10.1146/annurev.pp.44.060193.003033.

Swart A., Kamerbeek G.A. 1976. Different ethylene production *in vitro* by several species and *formae speciales* of *Fusarium*. Neth. J. Plant Pathol. 82: 81-84. DOI: 10.1007/BF01976953.

Swart A., Kamerbeek G.A. 1977. Ethylene production and mycelium growth of tulip strains of *Fusarium oxysporum* as influenced by shaking of oxygen supply to the culture medium. Physiol. Plantarum 39: 38-44. DOI: 10.1111/j.1399-3054.1977.tb09282.x.

Thaler S.J., Owen B., Higgins V.J. 2004. The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. Plant Physiol. 135: 530-538. DOI: 10.1104/pp.104.041566.

Tzeng D.D., DeVay J.E. 1984. Ethylene production and toxigenicity of methionine and its derivatives with riboflavin in cultures of *Verticillium*, *Fusarium* and *Colletotrichum* species exposed to light. Physiol. Plantarum 62: 545-552. DOI: 10.1111/j.1399-3054.1984.tb02797.x.

Ueda J., Kato J. 1980. Isolation and identification of a senescence-promoting substance from wormwood (*Artemisia absinthium* L.). Plant Physiol. 66: 246-249. DOI: 10.1104/pp.66.2.246.

Yang S.F., Hoffman N.E. 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol. 35: 155-189. DOI: 10.1146/annurev.pp.35.060184.001103.

Zhang L., Jia C., Liu L., Zhang Z., Li C., Wang Q. 2011. The involvement of jasmonates and ethylene in *Alternaria alternata* f. sp. *lycopersici* toxin-induced tomato cell death. J. Exp. Bot. 62: 5405-5418. DOI: 10.1093/jxb/err217.