

JASMONIC ACID ACCUMULATION IN BEAN HYPERSENSITIVELY RESISTANT TO *UROMYCES PHASEOLI*

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Dedicated to Prof. Antonio Graniti
on the occasion of his 75th birthday

SUMMARY

Jasmonic acid, in synergism with ethylene, seems to play key roles in activating multiple resistance in various host-parasite combinations. Utilising indirect ELISA and a monoclonal antibody, changes in jasmonic acid content were studied in bean-*Uromyces phaseoli* combination, both compatible and incompatible, at 0, 13, 20, 42, 62 and 92 h after inoculation, in three experiments. In the susceptible cultivar 'Bountiful', infection with *U. phaseoli* induced an early, slight, but statistically significant JA increase at 13 h after inoculation, but no further significant increase. In the hypersensitive cultivar 'Kentucky Wonder 765', after a slight but significant JA accumulation at 13 h, larger significant increases were detected at 42 h, and a peak at 62 h (673% of the control value), coincident with the appearance of the hypersensitive flecks; at 92 h accumulation was drastically reduced. Uredospores of the fungus contained only 0.95 ± 0.11 SE ng of JA g⁻¹ d.w. and germinated uredospores 0.46 ± 0.10 SE ng. The great JA increase detected in Kentucky Wonder 765, at the same time as appearance of the hypersensitive flecks, appear connected with a specific defensive response associated with the hypersensitive reaction.

Key words: jasmonic acid, compatible, incompatible, susceptible, rust.

INTRODUCTION

The hypersensitive reaction (HR) is associated with a co-ordinated induction of plant defence responses to pathogens, including an oxidative burst and accumulation of reactive oxygen species (ROS, see Morel and Dangl, 1997; Pieterse and van Loon, 1999), a two-phase phenomenon, showing an early, weak, aspecific phase I and a stronger, specific phase II (Lamb and Dixon, 1997).

The biosynthetic pathway of jasmonic acid (JA) starts from linolenic acid (released from the plant membrane by an activated intracellular lipase) which, through several steps mediated by lipoxygenase (LOX), allene oxide synthase (AOS) and allene oxide cyclase (AOC), is converted into 12-oxo-phytodienoic acid (12-oxo-PAD or PDA). The action of a reductase and three β -oxidation cycles lead from PDA to JA (see Creelman and Mullet 1997), both potent activators of a series of defence related genes (see Pieterse and van Loon, 1999).

Exogenous JA increases host resistance in the potato-*Phytophthora infestans* interaction (Cohen *et al.*, 1993), while methyl jasmonate (JAME) does the same in tomato-*P. infestans* (Cohen *et al.*, 1993), cotton-*Verticillium* and tomato-*Fusarium* (Li *et al.*, 1996) interactions. In a mutant of *Arabidopsis thaliana* (fad3-2 fad7-2 fad8), sensitive to JA but unable to accumulate it and extremely susceptible to *Pythium mastophorum*, JAME administration induced transcripts of three JA-responsive defence genes and protects the mutant from root rot, while it does not protect the JA-insensitive mutant coi1 (Vijayan *et al.*, 1998). In common bean, JA or a precursor, as well as *Pseudomonas syringae* pv. *tabaci* infection, mediate the induction of the multi-functional acetyl-CoA carboxylase, cytosol isoform (García-Ponce and Rocha-Sosa, 2000), whose function seems to be the synthesis of antimicrobial flavonoids (Ebel and Hahlbrock, 1977) and stilbenes (Schroder *et al.*, 1988).

In the network of interactions among the different defence signalling pathways (see Penninckx *et al.*, 1996; Pieterse and van Loon, 1999; Schenk *et al.*, 2000) JA and ethylene are emerging as important signalling molecules (Pieterse and van Loon, 1999). JA appears to act synergistically with ethylene in inducing defence genes (Xu *et al.*, 1994; Penninckx *et al.*, 1998). In spite of the above evidence, the mechanisms of JA action and its involvement in resistance are still not entirely clear (Pieterse and van Loon, 1999).

In the present work, using methyl jasmonate monoclonal antibody and indirect ELISA, jasmonic acid content was determined in both compatible and incompatible bean-*Uromyces phaseoli* interactions to find out if

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there are changes in concentration, and if such changes are related to any particular phase of the disease, particularly the hypersensitive reaction.

MATERIALS AND METHODS

Plant material. Cultivars 'Bountiful' and 'Kentucky Wonder 765' ('KW 765') bean (*Phaseolus vulgaris* L.) were sown in sterilised standard potting compost (sand and peat, 50/50) in a greenhouse at 20-24°C, 65-75% R.H. and natural lighting, supplemented with artificial light (400 watt, Philips HLRG, Belgium) for a total 14 h, with night conditions (10 h) of 14-16°C, 80-90% R.H. Four to five days after sowing, a batch of uniform plants was transferred to a growth chamber held at the following conditions: 24 ± 1°C, 60-65 % R.H. and 10 h illumination at 240 µmol m⁻² sec⁻¹ photosynthetic active radiation (PAR), produced by daylight lamps and fluorescent lamps (Powerstar HQI-T 400W/D day light lamp Osram, Germany and 58W/33 fluorescent lamp Philips TLD, Netherlands); dark conditions for 10 h at 20 ± 1°C, 75-86% R.H. and a 2 h transition, both before and after the full illumination period, at 22 ± 1°C, 60-65% R.H. and 120 µmol PAR. The plants were watered daily with nutrient solution and not manipulated before sampling, to avoid mechanical induction of JA.

Pathogen. The *U. phaseoli* (Pers.) Wint. isolate used is incompatible (hypersensitivity) in 'Kentucky Wonder 765' and compatible (susceptibility) in 'Bountiful' bean cultivar (Montalbini, 1989).

Inoculation. Inoculation was carried out after 4 days acclimatisation in the growth chamber, on almost fully expanded primary leaves (13 days after sowing), by spraying an uredospore suspension on both leaf surfaces. Inoculated and control (water sprayed) plants were incubated for 24 h in a shaded humid chamber.

Cyto-histological features and reactions. These were recorded for both cultivars by microscopy of decolourised tissue fragments, obtained by treatment with chloral hydrate-saturated aqueous solution. Some specimens were then mounted in glycerol-ethanol (1/1, v/v), and others, for better contrast, in glycerol-cotton blue (1:1, v/v). Observations were made at the most important phases of the disease: inoculation (0 h); penetration and formation of first haustoria (13 h); intermediate phases (20 and 42 h); development of hypersensitive lesions in the resistant 'KW 765' (62 h); post-necrotic phase in the resistant, corresponding to initiation of pustule formation or 'white fleck' stage in the suscepti-

ble variety (92 h).

Reactions of the two cultivars were evaluated according to Stavely (1984) and the intensity of infection using the modified Coob scale (Stavely, 1985).

Sampling for jasmonic acid analysis as cyta-histological observations. JA analyses were done at the same intervals. In the 1st and 2nd experiments 6 samples, while in the 3rd experiment 4 samples, were harvested from control and infected plants at each sampling. Each leaf was immediately wrapped in aluminium foil, dipped in liquid N and stored at -80°C, pending extraction.

Extraction. The leaves were ground in liquid N, immediately extracted in diethyl ether (60 min in 20 and 30 min in 15 ml, and the extracts combined; Müller and Brodschelm, 1994). The solid matter was dried at 85°C for dry weight determination. Uredospores were germinated as follows. One gramme of uredospores was washed for 2 h with 800 ml of water and left germinating at 20°C for 20 h in 8 l of distilled water, on a 700 cm² surface (see Marte, 1971); 0.73 g of ungerminated uredospores were ground in liquid N and extracted with diethyl ether.

Purification. Chlorophyll, flavonoids, alkaloids, lipids and JAME (which may interact with the antibody, Albrecht *et al.*, 1993) were eliminated utilising an activated solid phase aminopropyl column (Müller and Brodschelm, 1994). The eluate was quickly evaporated under vacuum then in N flux (Miersch *et al.*, 1986; Albrecht *et al.*, 1993), collected in 600 µl methanol, depleted from phenols (Luisoni, 1984), centrifuged (2 min at 13,000 rpm) and 450 µl collected in a vial and stored overnight at -20°C.

Methylation. The sample was concentrated to 150-200 µl under N flux, and two volumes (350-350 µl) of diazomethane (CH₂N₂) solution (30 ml diethyl ether, 1 ml of KOH 40%, 1 g of N-Nitroso-N-methylurea plus KOH pellets as needed to absorb water) added until a stable yellow colour developed and, after 1 min shaking, the reaction was interrupted with a slight N flux (Albrecht *et al.*, 1993). After evaporation the sample was recollected in 600 µl TBS methanol (80:20 v/v).

Quantification. The immobilised-antibody protocol of Weiler (1986), modified by Albrecht *et al.* (1993) was used. The reagent concentration (for volume and timing see Weiler, 1986) were as follows: rabbit serum against anti-mouse immunoglobulin (RAMIG) 12.5 µg ml⁻¹; monoclonal antibody against JA 25 µg ml⁻¹; alkaline phosphatase-conjugated JA (tracer) activity 25 pkat

per well (Albrecht *et al.*, 1993). Readings were taken at 405 nm (Titertek Multiskan Plus).

Calculation of JA concentration. Dose-response curves were calculated according to Weiler (1986); see also Albrecht *et al.* (1993). Recoveries increased gradually with increasing JA concentrations in the samples and the readings were adequately compensated.

Statistical analysis. The data of each sampling was submitted to analysis of variance. Standard errors and significant differences (Student's t-test), for inoculated versus non-inoculated leaves (controls), were calculated.

RESULTS

JA contents of the uninoculated controls were similar in the two cultivars and quite constant during the experiments (particularly in the resistant 'Kentucky Wonder 765'). Sham-inoculated control plants, *i.e.* plants sprayed with water only, did not show any variation in JA content (Fig. 1).

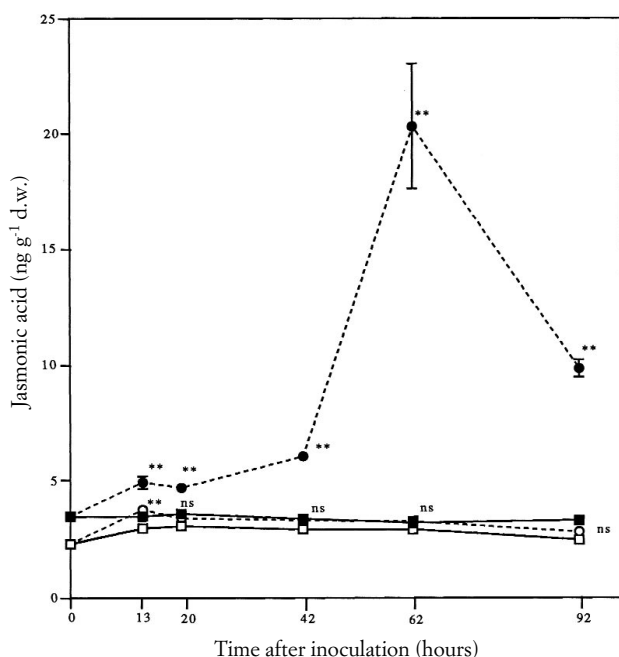


Fig. 1. Jasmonic acid content in bean leaves (ng g^{-1} d.w.) of the susceptible 'Bountiful' (infected ----○---- and control —□—) and resistant 'Kentucky Wonder 765' (infected ----●---- and control —■—) cultivars, at different time (hours) after inoculation with *U. phaseoli*. The data are the average of 3 independent experiments of 4 or 6 samples each. Asterisks indicate significant differences between infected and controls: **, significant at $P \leq 0.01$; ns, not significant. Vertical bars represent \pm SE.

In the susceptible cultivar 'Bountiful', the fungus produced 21-39 pustules per 1.5 cm^2 ($14-26 \text{ cm}^2$) in the three experiments, corresponding to intensity 4 of the modified Coob scale; there was abundant mycelium and no evidence of necrosis in the host tissues (data not shown). The fungus gave pustules of 0.8 mm diameter, corresponding to a degree 6 infection (data not shown), according to the uniform bean rust grading scale. In the susceptible cultivar 'Bountiful', JA increased slightly but significantly within 13 h after inoculation (Fig. 1). Though the content was slightly higher in infected leaves as compared to the controls through the phase of the fungal colonisation, the increases were not significant, even in pool confrontation analysis (*i.e.* pooling JA detected at 20, 42, 62 and 92 h after inoculation in the infected versus the control leaves).

In the hypersensitive cultivar 'Kentucky Wonder 765', the intensity of infection was 11-20 loci of infection per 1.5 cm^2 ($7-13 \text{ cm}^2$), corresponding to degree 3 of the modified Coob scale. Dead cells were detected at 60-62 h after inoculation in the three experiments (data not shown). The isolate used gave an infection grade of about 2^+-2^{++} , according to the uniform bean rust grading scale, corresponding to necrotic spots of 0.3-1 mm and of 1-3 mm on the upper and lower leaf surfaces respectively. In the hypersensitive cultivar 'KW 765' a modest though highly significant JA increase started at 13 h and remained detectable at 20 h post-inoculation (Fig. 1). A new higher increase started at 42 h, peaked at 62 h (673% of the control value), upon development of the hypersensitive reaction, and clearly declined at 92 h post inoculation.

JA content was 0.95 ± 0.11 SE in the ungerminated and 0.46 ± 0.10 SE ng g^{-1} d.w. in the germinated ure-dospores (mean of two determinations, data not shown).

DISCUSSION

JA levels of the controls were similar to those reported for bean cell cultures (Parchmann *et al.*, 1997) and for bean primary leaves (Clarke *et al.*, 2000). Sham inoculation did not induce any JA accumulation, indicating that no mechanical stimulation was applied; in contrast, JA accumulation was induced in bean 12 h after sham mechanical virus inoculation (Clarke *et al.*, 2000).

The time of expression of cell death and the grade of infection on the hypersensitively resistant 'KW 765', as well as the symptoms on the susceptible 'Bountiful' cultivar, were very similar to those described by Montalbini (1989). The fungus in itself does not seem to contribute directly to JA accumulation in the infected tis-

sues, because JA content of ungerminated and particularly of germinated uredospores was much lower than that detected in the host tissues, and no significant JA increase was found with increasing mycelium development in the susceptible cultivar.

In the susceptible 'Bountiful', the early increase of JA (13 h after inoculation) concurs with what was reported in tobacco infected with an hrpL mutant (unable to induce HR) of *P. syringae* pv. *phaseolicola* (Kenton *et al.*, 1999), apart from a delay attributable to differences between the two models (particularly in the time required by the fungus for infection, not needed by infiltrated bacteria). This early JA increase in 'Bountiful' coincides with a similar slight and transitory (though significant) ethylene increase in the susceptible Pinto 111 infected with the same *U. phaseoli* isolate (Montalbini and Elstner, 1977); early increases in both JA and ethylene coincide with stoma and cell wall penetration and initiation of haustorium formation by the fungus in the susceptible host. As hypothesised for the early, weak and transient oxidative burst and ROS accumulation (phase I see Lamb and Dixon, 1997), the early JA increase may be considered a biologically unspecific reaction of the compatible host. The lack of further JA increase in 'Bountiful' during fungal colonisation concurs with the lack of JA accumulation in tobacco infected with the hrpL mutant of *P. syringae* pv. *phaseolicola* (Kenton *et al.*, 1999) and the lack of specific oxidative burst and ROS accumulation (phase II) in various compatible host-pathogen combinations (see Lamb and Dixon, 1997). All this may be related to the activation, documented in the compatible barley-*Blumeria* (*Erysiphe*) *graminis* f.sp. *hordei*, of several antioxidative processes, which may lessen the damage of oxidative stress and inhibit lipid peroxidation (El-Zahaby *et al.*, 1995), the starting point of JA biosynthesis (see Creelman and Mullet 1997). In synthesis in the compatible Bountiful-*U. phaseoli* combination an initial aspecific host reaction may occur, subsequently inhibited by the pathogen; this hypothesis also agrees with cyto-histological observations on compatible maize-*Physopella zaeae* combination (Heath and Bonde, 1988) and particularly on bean-*U. phaseoli* (Heath, 1995).

In the hypersensitive 'KW 765', the early, transitory and slight JA increase appears unspecific for resistance, since it was also observed in the susceptible 'Bountiful'. The high JA accumulation documented during fungal colonisation, with a peak at the appearance of cell death, concurs with the increase observed in tobacco infected with HR inducing *P. syringae* pv. *phaseolicola* (Kenton *et al.*, 1999), though in bean the increase appeared later and was much less evident than in tobacco (this may be mainly because of the longer infection process and fewer

lesions respectively). JA accumulation in 'KW 765' also concurs with the specific oxidative burst and ROS accumulation (phase II) in various incompatible combinations (see Lamb and Dixon, 1997) and with lipoxygenase activation in incompatible bean-*P. syringae* pv. *phaseolicola* (Croft *et al.*, 1993), coffee-*Hemileia vastatrix* (Rojas *et al.*, 1993) and barley-*Blumeria* (*Erysiphe*) *graminis* f.sp. *hordei* (El-Zahaby *et al.*, 1995). The peak of JA accumulation in 'KW 765' coincides with that of ethylene (36 fold ethylene increase at 62 h after infection, Montalbini and Elstner, 1977), documented in the identical host-parasite combination. This appears in agreement with the hypothesised synergistic action of JA and ethylene in activating resistance mechanisms (see Xu *et al.*, 1994; Penninckx *et al.*, 1996; 1998) in our KW 765-*U. phaseoli* experiments.

In conclusion, presence of early, slight accumulation (phase I, aspecific for resistance) and lack of later JA accumulation (phase II, specific) in the compatible 'Bountiful' agrees with the observations on ethylene (Montalbini and Elstner, 1977) and ROS content, respiratory burst and LOX activity (Croft *et al.*, 1993; Rojas *et al.*, 1993; El-Zahaby *et al.*, 1995).

In the incompatible KW 765, JA accumulation follows the pattern of ethylene and ROS accumulation and concurs with LOX activation in various incompatible host-pathogen combinations (see above). This seems to indicate that JA accumulation in KW 765, at 62 h after inoculation (HR appearance), is a specific host reaction (synergistic with ethylene accumulation).

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REFERENCES

- Albrecht T., Kehlen A., Stahl K., Knöfel H.D., Sembdner G., Weiler E.W., 1993. Quantification of rapid, transient increases in jasmonic acid in wounded plants using a monoclonal antibody. *Planta* **191**: 86-94.
- Clarke S.F., Guy P.L., Jameson P.E., Schmierer D., Burritt D.J., 2000. Influence of white clover mosaic potyvirus infection on the endogenous levels of jasmonic acid and

- related compounds in *Phaseolus vulgaris* L. seedlings. *Journal of Plant Physiology* **156**: 433-437.
- Cohen Y., Gisi U., Niderman T., 1993. Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. *Phytopathology* **83**: 1054-1062.
- Creelman R.A., Mullet J.E., 1997. Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 355-381.
- Croft K.P.C., Jüttner F., Slusarenko A.J., 1993. Volatile products of the lipoxygenase pathway evolved from *Phaseolus vulgaris* (L.) leaves inoculated with *Pseudomonas syringae* pv. *phaseolicola*. *Plant Physiology* **101**: 13-24.
- Ebel K., Hahlbrock H., 1977. Enzymes of flavone and flavonolglycoside biosynthesis. Co-ordinated and selective induction in cell-suspension cultures of *Petroselinum hortense*. *European Journal of Biochemistry* **75**: 201-209.
- El zahaby H.M., Gullner G., Kiraly Z., 1995. Effects of powdery mildew infection of barley on the ascorbate-glutathione cycle and other antioxidants in different host-pathogen interactions. *Phytopathology* **85**: 1225-1230.
- García-Ponce B., Rocha-Sosa M., 2000. The octadecanoic pathway is required for pathogen-induced multi-functional acetyl-CoA carboxylase accumulation in common bean (*Phaseolus vulgaris* L.). *Plant Science* **157**: 181-190.
- Heath M.C., 1995. Signal exchange between higher plants and rust fungi. *Canadian Journal of Botany* **73**: 616-623. (Supplement 1)
- Heath M.C., Bonde M.R., 1988. The temporal relationship between the development of intracellular hyphae and haustoria by *Physopella zae* in *Zea mays*. *Canadian Journal of Botany* **66**: 742-744.
- Kenton P., Mur L.A.J., Atzorn R., Wasternack C., Draper J., 1999. (-)-Jasmonic acid accumulation in tobacco hypersensitive response lesions. *Molecular Plant-Microbe Interactions* **12**: 74-78.
- Lamb C., Dixon R.A., 1997. The oxidative burst in plant disease resistance. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 251-271.
- Li J., Zingen-sell I., Buchenauer H., 1996. Induction of resistance of cotton plants to *Verticillium* wilt and of tomato plants to *Fusarium* wilt by 3-aminobutyric acid and methyl jasmonate. *Journal of Plant Disease Protection* **103**: 288-299.
- Luisoni E., 1984. Diagnosi virologica, il metodo ELISA. *Informatore Fitopatologico* **1**: 9-16.
- Marte M., 1971. Studies on self-inhibition of *Uromyces fabae* (Pers.) De Bary. *Phytopathologische Zeitschrift* **72**: 335-343.
- Miersch O., Meyer A., Volkefeld S., Sembdner G., 1986. Occurrence of (+)-7-iso-jasmonic acid in *Vicia faba* L. and its biological activity. *Journal of Plant Growth Regulation* **5**: 91-100.
- Montalbini P., 1989. Effect of infection by *Uromyces phaseoli* (Pers.) Wint. on levels of uricase, allantoinase and ureides in susceptible and hypersensitive bean plants. *Annali della Facoltà di Agraria-Università degli Studi di Perugia*. XLI-II, 495-532.
- Montalbini P., Elstner E.F., 1977. Ethylene evolution by rust infected, detached bean (*Phaseolus vulgaris* L.) leaves susceptible and hypersensitive to *Uromyces phaseoli* (Pers.) Wint. *Planta* **135**: 301-306.
- Morel J.B., Dangl J.L., 1997. The hypersensitive response and the induction of cell death in plants. *Cell Death and Differentiation* **4**: 671-683.
- Müller M.J., Brodschelm W., 1994. Quantification of jasmonic acid by capillary gas chromatography-negative chemical ionization-mass spectrometry. *Analytical Biochemistry* **218**: 425-435.
- Parchmann S., Gundlach H., Müller M.J., 1997. Induction of 12-oxo-phytodienoic acid in wounded plants and elicited plant cell cultures. *Plant Physiology* **115**: 1057-1064.
- Penninckx I.A.M.A., Eggermont K., Terras F.R.G., Thomma B.P.H.J., De Samblanx G.W., Buchala A., Mettraux J., Manners J.M., Broekaert W.F., 1996. Pathogen-induced systemic activation of a plant defense gene in *Arabidopsis* follows a salicylic acid-independent pathway involving components of the ethylene and jasmonic acid responses. *Plant Cell* **8**: 2309-2323.
- Penninckx I.A.M.A., Thomma B.P.H.J., Buchala A., Métraux J.-P., Broekaert W.F., 1998. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. *Plant Cell* **10**: 2103-2113.
- Pieterse C.M.J., van Loon L.C., 1999. Salicylic acid-independent plant defence pathways. *Trends in Plant Science* **4**: 52-58.
- Rojas M.L., Montes-De Gomez V., Ocampo C.A., 1993. Stimulation of lipoxygenase activity in cotyledonary leaves of coffee reacting hypersensitively to the coffee leaf rust. *Physiological and Molecular Plant Pathology* **43**: 209-219.
- Schenk M.P., Kazan K., Wilson I., Anderson J.P., Richmond T., Somerville S.C., Manners J.M., 2000. Co-ordinate plant defence responses in *Arabidopsis* revealed by microarray analysis. *Proceedings of the National Academy of Sciences of the USA* **97**: 11655-11660.
- Schroder G., Brown J.W., Schroder J., 1988. Molecular analysis of resveratrol synthase. cDNA, genomic clones and relationship with chalcone synthase. *European Journal of Biochemistry* **172**: 161-169.
- Stavely G.R., 1984. Pathogenic specialization in *Uromyces phaseoli* in the United States and rust resistance in beans. *Plant Disease* **68**: 95-99.
- Stavely G.R., 1985. The modified Cobb scale for estimating bean rust intensity. *Annual Report Bean Improvement Cooperative* **28**: 31-32.

Vijayan P., Shockey J., Lévesque C.A., Cook R.J., Browse J., 1998. A role for jasmonate in pathogen defense of *Arabidopsis*. *Proceedings of the National Academy of Sciences of the USA* **95**: 7209-7214.

Weiler E.W., 1986. Plant hormone immunoassays based on monoclonal and polyclonal antibodies. In: Linskens, H.F., Jackson, J.F. (eds.). *Immunology in plant sciences* (Mod-

ern methods of plant analysis, vol. 4), pp. 1-17. Springer-Verlag, Berlin Heidelberg New York London Paris Tokyo.

Xu Y., Chang P.L., Liu D., Narasimhan M.L., Raghoutama K.G., Hasegawa P.M., Bressan R.A., 1994. Plant defence genes are synergistically induced by ethylene and methyl jasmonate. *Plant Cell* **6**: 1077-1085.

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