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Induction of soybean vegetative storage proteins and anthocyanins by low-level atmospheric methyl jasmonate

(signal transduction/hormones/intracellular messengers/nitrogen partitioning)

VINCENT R. FRANCESCHI AND HOWARD D. GRIMES*

Department of Botany, Washington State University, Pullman, WA 99164-4238

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ABSTRACT **Soybean seedlings were exposed to atmospheric methyl jasmonate (MJ) to determine if low levels of this compound could regulate the expression and accumulation of the vegetative storage proteins (VSPs) in soybeans. Low levels of atmospheric MJ induced the accumulation of three VSPs with molecular masses of 27 kDa, 29 kDa, and 94 kDa (vsp27, vsp29, and vsp94, respectively). Atmospheric MJ caused vsp94 to be accumulated in all above-ground organs of the seedling uniformly after just 3 days of exposure. vsp27 preferentially accumulated in shoot tips and primary leaves, whereas vsp29 preferentially accumulated in the cotyledons. In addition to these effects, MJ also induced the biosynthesis of anthocyanins in light-grown seedlings but inhibited anthocyanin biosynthesis in etiolated seedlings. It is concluded that low levels of atmospheric MJ regulate anthocyanin biosynthesis and the organ-specific accumulation of VSPs in developing soybean seedlings. The organ-specific differential accumulation may reflect changes in the pattern of nitrogen partitioning between various compounds and/or organs. These results lend substance to the hypothesis that volatile MJ may act as a gaseous messenger or growth regulator in plants.**

Jasmonic acid and its methyl ester (methyl jasmonate, MJ) are fatty acid-derived naturally occurring cyclopentanone-based compounds of wide distribution in the plant kingdom (1–4). They are probably the best characterized of the potential chemical messengers derived from the lipoxygenase-dependent oxidation of fatty acids in plants (1). MJ is a lipophilic compound that is volatile at room temperature. This volatility may be a factor in the physiological activity of this compound in that it may act as a gaseous hormone in plants, analagous to ethylene (1, 5).

These compounds exhibit a number of phytohormone-like effects in a range of plant species and systems (1). Several studies have demonstrated jasmonic acid or MJ can induce senescence effects such as degradation of chlorophyll (6, 7), ribulose-1,5-bisphosphate carboxylase/oxygenase (7, 8), and starch (9). In various systems, jasmonic acid or MJ application stimulates polyphenol oxidase activity (10, 11) and β-carotene accumulation (12), whereas it inhibits anthocyanin production (13) and polygalacturonase activity in tomato fruit (14). These effects are observed when jasmonic acid is applied directly, at relatively high concentrations, to the plant tissue (6, 7, 9).

Direct applications of aqueous jasmonic acid also selectively induce the accumulation of two vegetative storage proteins (VSPs) of 27 kDa and 29 kDa in soybean plants and suspension cultures (15, 16) and induce the expression of their respective mRNAs (17, 18). Soybeans are able to temporarily store nitrogen in the form of these VSPs. The VSPs are highly modulated during development and respond to the immediate need to store surplus nitrogen or amino acids (19, 20). vsp27 and vsp29 are glycosylated (20) and are prominent in leaves, where they are stored in a specialized tissue termed the paraveinal mesophyll (21). The VSPs, however, also occur in the epidermis (17) as well as in stems and petioles at fairly low levels under normal plant growth conditions (22, 23). The jasmonic acid-induced expression of vsp27 and vsp29 seems to be due to a repartitioning of nitrogen between different pools in soybean tissue. Hence, these effects are quite different from the senescence-type effects that have been observed.

Direct evidence for physiological or developmental effects due to atmospheric MJ is rare. Farmer and Ryan (5) demonstrated that airborne MJ induced the expression of proteinase inhibitor proteins in tomato leaves. They hypothesized that jasmonic acid or its methyl ester may be a key component of signaling in response to wounding or pathogenesis since exogenous application of MJ in the absence of wounding or pathogenesis was equally effective in proteinase inhibitor induction. Further substantiation of either physiological or developmental effects is needed before MJ can be established as another gaseous plant growth regulator or signaling substance.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Soybean (*Glycine max* Merr cv. Wye) seeds were planted in potting compost in 7.6-cm pots and grown in a controlled environment chamber with a photon flux density of 360–400 microeinsteins·m^{-2}·s^{-1} of photosynthetically active radiation at canopy height (1 einstein = 1 mol of photons), 16-hr photoperiod, and 24°C/18°C day/night temperatures.

MJ Treatments. Experiments were conducted in sealed Plexiglas boxes (11.3-liter volume) within the controlled-environment chamber. Plants were exposed to MJ vapor by placing MJ (which is a liquid at room temperature) dissolved in 100% ethanol (MJ/ethanol, 1:9) onto the tip of a cotton swab anchored in a central location in the pot but not directly contacting the plants. Controls contained 100% ethanol. MJ was added at a final concentration of 2.5 μl and 12 μl per liter (of box volume), with the controls receiving 12 μl of ethanol per liter. At the initiation of our experiments, the maximum amount of atmospheric MJ (assuming a fully saturated atmosphere and 24°C) has been calculated to be about 2.4×10^{-7} mol/liter of air (T. Farmer, personal communication). Exposure to MJ was for 1–5 days. All plants grew well under these conditions and were equal in size or slightly larger than plants grown outside the boxes. MJ was obtained from Bedoukian Research (Danbury, CT).

Plants of three different developmental stages were routinely used. To delineate between these treatments, each of

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Abbreviations: VSP, vegetative storage protein; MJ, methyl jasmonate; DAS, days after sowing.

*To whom reprint requests should be addressed.

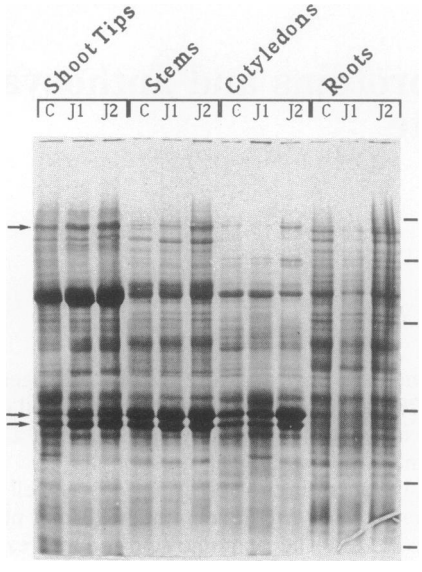

FIG. 1. Induction of vsp27, vsp29, and vsp94 in emerging soybean seedlings by atmospheric MJ. The exposure period was from 4.5 DAS to 9.5 DAS. C, control; J1, 2.5 μl of MJ per liter; J2, 12 μl of MJ per liter. Molecular mass standards indicated on the right are 97.4, 66.2, 45, 31, 21.5, and 14.4 kDa. Arrows on the left point to vsp27, vsp29, and vsp94.

these is described and all ages are referred to as days after sowing (DAS) of the seeds. (*i*) The exposure period was from 4.5 DAS to 9.5 DAS. Freshly germinated (the hypocotyl hook and cotyledons had just emerged above soil level) soybean seedlings were treated for 5 days. (*ii*) The exposure period was from 10 DAS to 15 DAS. Soybean seedlings with their primary leaves 50–80% expanded and first trifoliate still folded around the shoot apex were treated for 5 days. (*iii*) Older plants (first two trifoliolates expanded, third trifoliolate 80–100% expanded) were treated from 35 DAS to 40 DAS.

Protein Extraction and Electrophoresis. Total proteins from various organs were extracted as described (15). Prior to electrophoresis, protein samples were heated at 90°C for 5 min and then centrifuged at 13,000 × *g* for 3–5 min. Supernatant from this final spin was loaded onto the gels and resolved according to Laemmli (24) except that a 7.5–15% acrylamide gradient was used.

Extraction and Analysis of Anthocyanin Pigments. For anthocyanin determinations, freshly isolated hypocotyls were chopped into 1-cm pieces and anthocyanins were extracted according to Mancinelli (25). The quantity of anthocyanins was determined spectrophotometrically (A_{530} and A_{657}). The formula $A_{530} - A_{657}$ was used to compensate for the contribution of chlorophyll derivatives to absorption at 530 nm (25).

Ethylene Measurements. Plants were grown in chambers as described above. Air samples were taken in duplicate at the end of 3 days since an appreciable effect of MJ on the plants occurs by this period of exposure. Samples were analyzed with a Packard model 427 gas chromatograph with a Poropak QS column (30.5 cm) with a column temperature of 50°C and N_2 as the carrier gas. An ethylene standard of 10 ppm was used for calibration, and sample size was 1 ml.

RESULTS

Atmospheric MJ Induces the Accumulation of VSPs in Soybean Seedlings. The changes in soluble leaf protein composition in seedlings exposed to MJ for 5 days (from 4.5 DAS to 9.5 DAS) were analyzed by SDS/PAGE. Fig. 1 demonstrates that accumulation of vsp27 and vsp29 is induced by atmospheric MJ in all organs except the roots. MJ differentially induces vsp27 and vsp29 in different organs. In shoot tips (primary leaves and shoot apex), MJ preferentially induces accumulation of vsp27 over that of vsp29 (Fig. 1). In the cotyledonary tissue, vsp29 is preferentially accumulated in response to MJ (Fig. 1). vsp27 and vsp29 seem to be uniformly induced by MJ in stem tissue (Fig. 1). A third vsp, vsp94 (26), also is strongly induced by atmospheric MJ in shoot tips, stems, and cotyledons (Fig. 1). None of the VSPs was induced in roots. All three VSPs showed a dose–response to MJ since more VSPs had accumulated at the highest MJ level after the same time period (Fig. 1). Close examination of Fig. 1 indicates that other polypeptides are unaffected by the MJ treatment.

A time course experiment was performed to determine when the MJ induction was first observable. Soybean seedlings were placed into boxes 4.5 DAS and exposed to MJ (12 μl/liter). Plants were harvested from the chambers at 1, 2, 3, and 4 days after MJ addition (corresponds to 4.5–5.5 DAS, 4.5–6.5 DAS, 4.5–7.5 DAS, and 4.5–8.5 DAS exposure periods). Fig. 2 demonstrates that in the shoot tips, vsp94 is clearly induced after 3 days, and some increase is evident

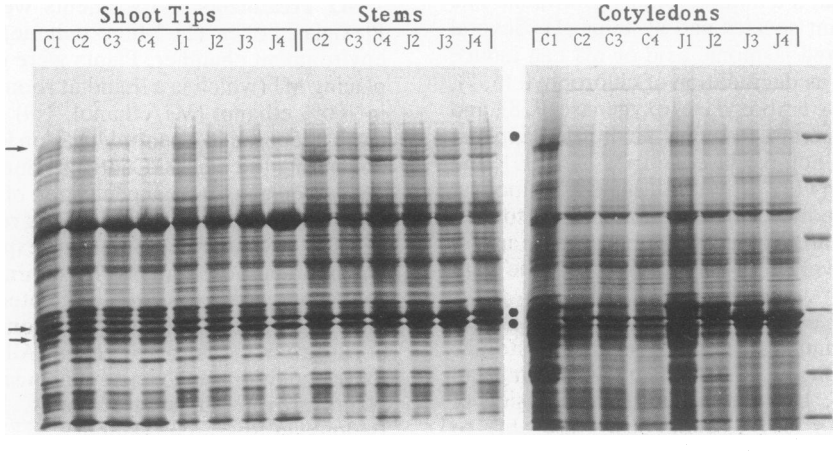

FIG. 2. Time course of vsp27, vsp29, and vsp94 induction by MJ in emerging seedlings. The experiment was performed as in Fig. 1, except only control and MJ (12 μl/liter) treatments were performed. Seedlings were removed from the chambers and sampled at 1, 2, 3, and 4 days after MJ addition. Lanes 1–8 are shoot tips (lanes 1–4, controls at 1, 2, 3, and 4 days; lanes 5–8, MJ at 1, 2, 3, and 4 days). Lanes 9–14 are stems (lanes 9–11, controls at 2, 3, and 4 days; lanes 12–14, MJ at 2, 3, and 4 days). Lanes 15–22 are cotyledons (lanes 15–18, controls at days 1, 2, 3, and 4 days; lanes 19–22, MJ at 1, 2, 3, and 4 days). Molecular mass standards are shown (97.4, 66.2, 45, 31, 21.5, and 14.4 kDa). Arrows and circles indicate the positions of vsp27, vsp29, and vsp94.

even at 1 and 2 days exposure to MJ. vsp27 and vsp29 levels increase after 3 days, again with vsp27 accumulating to a greater extent than vsp29. Stems show a rapid induction of vsp94 after 2 days in MJ (Fig. 2). vsp27 and vsp29 seemed to be equally induced in stems after 2 days. Because of the small size of the seedlings after only 1 day of growth, there was not enough stem protein to analyze the 1 day time point. In the cotyledons, induction of vsp94 is quite strong even after 1 day of exposure to MJ. In addition, vsp29 is induced after 3 days. A differential induction of vsp29 is easily observable over that of vsp27 in the cotyledons (Fig. 2), which is the reverse of that seen in shoot tips. In general, MJ-induced vsp94 accumulation was noticeable earlier than that of vsp27 or vsp29.

Since MJ induced VSP accumulation in germinating soybean seedlings, we also determined if older plants showed similar responses. Fig. 3 shows the results of an experiment performed with soybean seedlings that had their primary leaves fully expanded before they were exposed to MJ. These seedlings were exposed to MJ for 5 days (from 10 DAS to 15 DAS). Again, MJ induced the expression of vsp27, vsp29, and vsp94 in all parts of the shoot (Fig. 3) with similar patterns of expression as seen in the younger seedlings shown in Fig. 1. Several experiments with older plants showed only intermittent induction of vsp27, vsp29, or vsp94 by MJ treatment (data not shown). This may be due to the fact that chamber design was not optimum for these larger plants and other physiological effects may have interfered with the MJ treatments.

Atmospheric MJ Induces Anthocyanin Accumulation in Light-Grown Soybean Seedlings. The amount of anthocyanins present in the plants treated with MJ compared to control plants was quantitated. Fig. 4 indicates that atmospheric MJ induces a 5- to 7-fold increase in anthocyanin accumulation in light-grown seedlings after 5 days. The only other effect from the MJ was a very slight reduction in plant height in plants treated with 12 μl of MJ per liter.

Light quality and quantity are known to affect photoregulation of anthocyanin biosynthesis (27). Hence, the effect of MJ on anthocyanin biosynthesis was examined in etiolated seedlings. Seedlings were exposed to MJ from 4.5 DAS to 9.5 DAS in chambers wrapped in aluminum foil. Anthocyanin accumulation was inhibited by about 50% in etiolated seedlings (Fig. 4). It was also evident that the MJ had a stronger inhibitory effect on seedling growth in the dark than in the light. The average height (taken from the shoot/root interface to the top of the shoot tip) from the etiolated control plants was 24.2 ± 1.7 cm compared to 18.1 ± 1.1 cm in the etiolated MJ-treated plants.

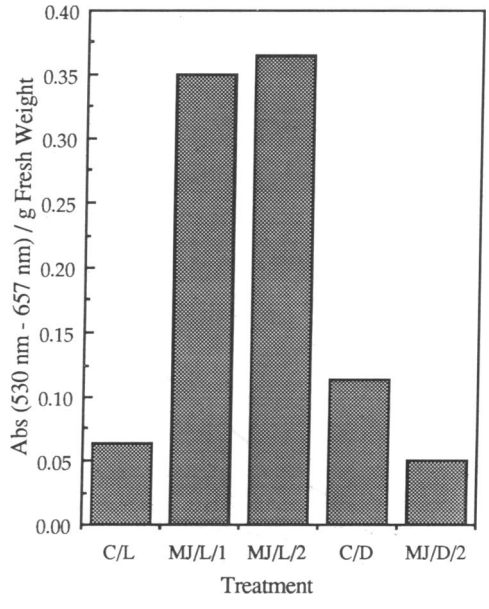

FIG. 4. Effect of MJ on anthocyanin accumulation in emerging soybean seedlings grown in either light or dark. The experiment was performed as in Fig. 1. When seedlings were harvested, the hypocotyl was excised and anthocyanins were extracted. In separate experiments, the anthocyanins were extracted from seedlings grown for either 5 days in the light (L) or 5 days in the dark (D). C, control; 1, 2.5 μl/liter; 2, 12 μl/liter.

Since MJ was shown to induce VSP accumulation and anthocyanin production in light-grown seedlings but had an inhibitory effect on anthocyanin production in etiolated seedlings, it was of interest to determine if MJ would induce the VSPs in etiolated seedlings. Fig. 5 demonstrates that all VSPs are induced by MJ treatment in etiolated seedlings and that the organ-specific expression of vsp27 and vsp29 is similar to seedlings treated in the light.

Because the MJ-induced increase in anthocyanins was so striking in light-grown seedlings after 5 days, a time course

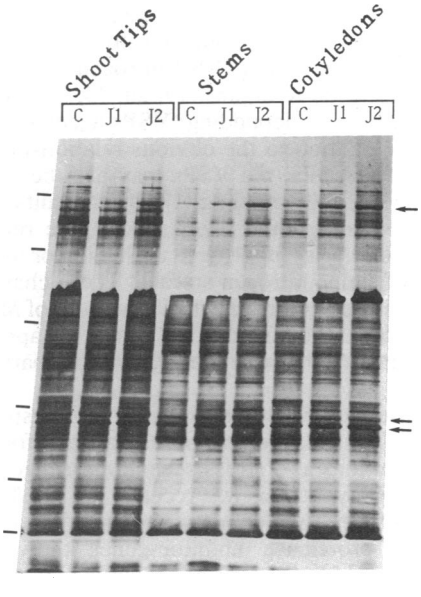

FIG. 3. Induction of vsp27, vsp29, and vsp94 by atmospheric MJ in young (with primary leaf expanded) soybean seedlings. The exposure period was from 10 DAS to 15 DAS. C, control; J1, 2.5 μl of MJ per liter; J2, 12 μl of MJ per liter. Markings are as in Fig. 1.

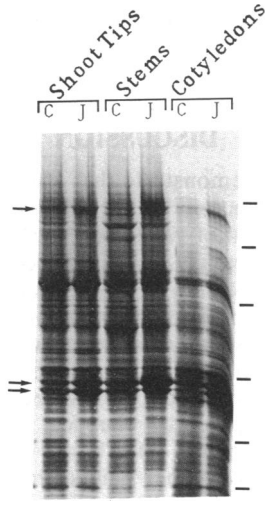

FIG. 5. Induction of vsp27, vsp29, and vsp94 by MJ in etiolated emerging soybean seedlings. C, control; J, 12 μl of MJ per liter. Markings are as in Fig. 1.

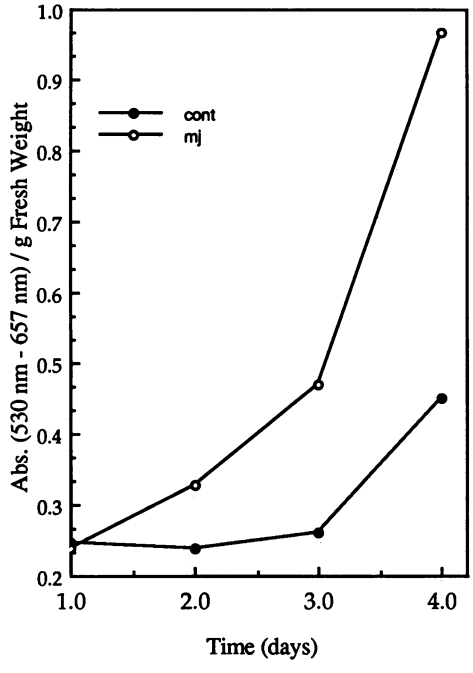

FIG. 6. Time course of MJ-induced anthocyanin accumulation in emerging soybean seedlings grown in the light. cont, Control.

experiment was performed to determine the kinetics of this inductive effect. Seedlings at 4.5 DAS were transferred to chambers where they were treated with ethanol (control) or MJ (12 μl/liter) and exposed for 1, 2, 3, and 4 days (corresponding to 4.5–5.5 DAS, 4.5–6.5 DAS, 4.5–7.5 DAS, and 4.5–8.5 DAS exposure periods). Fig. 6 shows that after just 2 days of MJ treatment, a large increase in anthocyanins (≈2-fold) could be detected. Anthocyanin production was stimulated even more with increasing time of exposure to the MJ (Fig. 6).

Atmospheric MJ Does Not Affect Ethylene Levels. Previous reports indicate that MJ may either inhibit or stimulate ethylene production (10, 11, 13). To ensure that ethylene was not the causative agent in changes seen in our MJ-treated plants, ethylene levels were assayed in chambers of control and MJ treatments. The ethylene level in both chambers was calculated to be 0.017 ppm, which is very close to background levels of ethylene. More important, the control and MJ-treated chambers had identical levels of ethylene. Thus, the changes in VSP accumulation and anthocyanin biosynthesis seen after exposure to MJ cannot be attributed to MJ-induced ethylene formation.

DISCUSSION

In this report, we demonstrate that atmospheric MJ at low concentrations is a potent inducer of (*i*) organ-specific preferential accumulation of vsp27 and vsp29, (*ii*) a rapid accumulation of anthocyanins in the hypocotyl of germinating soybean seedlings, and (*iii*) uniform accumulation of vsp94. These findings are particularly relevant to the question of whether MJ is a gaseous messenger or growth regulator in plants. Jasmonic acid and MJ are derived from the lipoxygenase-dependent oxidation of linolenic acid and are the best-characterized potential chemical messengers derived from fatty acids in plants (1). Their wide distribution in at least nine families of higher plants provides support for a physiological role of the jasmonates in plant tissues (2–4, 6, 28–30).

In mammals, eicosanoid synthesis is triggered by release of arachidonic acid from membranes into the cytoplasm, where it is metabolized into stress-related second messengers (31–33). Analogously, metabolites of linoleic or linolenic acid, which are similar to animal eicosanoids, are possible candidates for plant stress second messengers. In addition to this possibility, the jasmonates may function as plant growth regulators. Jasmonic acid and the plant growth regulator abscisic acid (ABA) have similar molecular masses, solubility properties, and pK values. At relatively high exogenous levels, jasmonic acid induces senescence phenomena similar to those produced by ABA. Thus far the mechanisms for the regulatory effects caused by the jasmonates remain unknown, but available data suggest that the mode of action is different than ABA and other plant growth regulators (34).

The VSPs, which were the focus of this study, were first identified by Wittenbach (19, 20) and later demonstrated to be localized in the paraveinal mesophyll of mature soybean leaves (21, 26, 35). Upon exposure to MJ, however, these VSPs accumulated in young soybean seedling organs that normally express them at very low levels if at all. Furthermore, vsp27 and vsp29 were differentially accumulated in different organs. There was a preferential expression of vsp27 in primary leaves and expanding first trifoliolates, whereas vsp29 was preferentially expressed in cotyledons. Both were expressed uniformly in stems after induction by MJ. A uniform accumulation of vsp94 was observed. Two studies indicate that mature leaves treated directly with MJ preferentially accumulate vsp29 (36, 37). In untreated developing and mature leaves of the soybean cultivar used here, vsp27 was found to accumulate to a greater amount than vsp29 (26).

Identification of the mechanism behind this organ-specific differential expression of vsp27 and vsp29 deserves further study. Staswick (38) and Mason and Mullet (18) have shown that the cDNA sequences of these two VSPs are ≈80% homologous and are encoded by only one or two gene copies. They have also shown that transcript accumulation in response to direct application of jasmonic acid is at least partially regulated at the transcriptional level (18, 38). These results, coupled with our own, raise the possibility that organ-specific cis- or trans-acting factors may be involved in the regulation of vsp gene expression.

A major question raised by our results is why does MJ induce the accumulation of the VSPs? VSP levels in soybean are undoubtedly dictated by nitrogen availability, which in turn is affected by a combination of developing sink-source relationships (19, 20, 35, 39) and exogenous nitrogen supply (17, 40). When there is a strong sink for nitrogen, such as developing pods and seeds, VSP nitrogen is mobilized to support growth of these organs (19, 20, 35, 39). If the sinks are removed (e.g., by depodding) VSPs accumulate to high levels (20). In addition to the obvious relationship between nitrogen and the VSPs, the VSPs may also be involved in water stress (18) and wounding (17). Our results, however, suggest that MJ may either alter sink-source relationships with respect to priority of nitrogen utilization or increase the overall rate of amino nitrogen storage. These changes occur without affecting plant growth when low levels of MJ are used under our experimental conditions. Thus, MJ appears to be able to regulate or otherwise modify nitrogen partitioning in soybean.

The increase in anthocyanin biosynthesis in response to MJ in the light is a puzzle. One possible explanation for this result is that MJ functions as a stress second messenger in plants. Support for this hypothesis was recently obtained by Farmer and Ryan (5) when they demonstrated that MJ induced the expression of proteinase inhibitor genes known to be involved in resistance to herbivory. Our model would imply that a stress, perhaps photooxidative, is perceived and that MJ, or in its demethylated form jasmonic acid, stimulates the stress response by a signaling mechanism. This model, however, does not address the observation that MJ inhibits

anthocyanin biosynthesis in the dark. We suspect that two distinct mechanisms operate, one in the light and one in the dark, but currently have no explanation for the dark mechanism.

In spite of the current uncertainty as to whether the jasmonates function as metabolic regulatory compounds (messengers) or growth regulators, our data demonstrate very specific effects in soybeans exposed to low levels of MJ. These results are supportive of the hypothesis that atmospheric MJ may function as either a gaseous messenger (5) or a growth regulator. The potency of this compound as a messenger or growth regulator is indicated by its effectiveness at very low levels (we estimate that at the start of exposure the concentration is ≈240 nM). In soybean seedlings, MJ may, in fact, have several roles. The possibilities include regulation of nitrogen assimilation and partitioning, signal transduction of stress, and modulation of photocontrol of anthocyanin biosynthesis.

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1. Anderson, J. M. (1989) in *Second Messengers in Plant Growth and Development*, eds. Boss, W. F. & Morre, D. J. (Liss, New York), pp. 181–212.
2. Meyer, A., Miersch, O., Buttner, C., Dathe, W. & Sembdner, G. (1984) *J. Plant Growth Regul.* **3**, 1–8.
3. Vick, B. A. & Zimmermann, D. C. (1984) *Plant Physiol.* **75**, 458–461.
4. Yamane, H., Sugawara, J., Suzuki, Y., Shimamura, E. & Takahashi, N. (1980) *Agric. Biol. Chem.* **44**, 2857–2864.
5. Farmer, E. E. & Ryan, C. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7713–7716.
6. Ueda, J. & Kato, J. (1980) *Plant Physiol.* **66**, 246–249.
7. Weidhase, R. A., Lehman, J., Kramell, H., Sembdner, G. & Parthier, B. (1987) *Physiol. Plant.* **69**, 161–166.
8. Popova, L. P. & Vaklinova, S. G. (1988) *J. Plant Physiol.* **133**, 210–215.
9. Saniewski, M. & Czapski, J. (1989) *Bull. Pol. Acad. Sci. Biol. Sci.* **37**, 49–53.
10. Czapski, J. & Saniewski, M. (1988) *Bull. Pol. Acad. Sci. Biol. Sci.* **36**, 127–132.
11. Czapski, J., Saniewski, M., Puchalski, J., Lange, E. & Nowacki, J. (1988) *Fruit Sci. Rep.* **15**, 103–110.
12. Saniewski, M. & Czapski, J. (1983) *Experimenta* **39**, 1371–1374.
13. Saniewski, M., Nowacki, J., Lange, E. & Czapski, J. (1988) *Fruit Sci. Rep.* **15**, 97–102.
14. Saniewski, M., Urbanek, H. & Czapski, J. (1987) *J. Plant Physiol.* **127**, 177–181.
15. Anderson, J. M., Spilatro, S. R., Klauer, S. F. & Franceschi, V. R. (1989) *Plant Sci.* **62**, 45–52.
16. Anderson, J. M. (1988) *J. Plant Growth Regul.* **7**, 203–211.
17. Staswick, P. E. (1990) *Plant Cell* **2**, 1–6.
18. Mason, H. S. & Mullet, J. E. (1990) *Plant Cell* **2**, 569–579.
19. Wittenbach, V. A. (1983) *Plant Physiol.* **73**, 121–124.
20. Wittenbach, V. A. (1983) *Plant Physiol.* **73**, 125–129.
21. Franceschi, V. R., Wittenbach, V. A. & Giaquinta, R. T. (1983) *Plant Physiol.* **72**, 586–589.
22. Wittenbach, V. A., Franceschi, V. R. & Giaquinta, R. T. (1984) *Curr. Top. Plant Biochem. Physiol.* **3**, 19–30.
23. Bantroch, D., Greenwood, J. S. & Staswick, P. E. (1989) *Plant Physiol.* **89S**, 88.
24. Laemmli, U. K. (1970) *Nature (London)* **277**, 680–684.
25. Mancinelli, A. L. (1984) *Plant Physiol.* **75**, 447–453.
26. Klauer, S. F., Franceschi, V. R. & Ku, M. S. B. (1991) *Plant Physiol.*, in press.
27. Mancinelli, A. L. (1990) *Plant Physiol.* **92**, 1191–1195.
28. Dathe, W., Ronsch, H., Preiss, A., Schade, W., Sembdner, G. & Schreiber, K. (1981) *Planta* **153**, 530–535.
29. Parthier, B. (1990) *J. Plant Growth Regul.* **9**, 57–63.
30. Herman, G., Lehmann, J., Peterson, A., Sembdner, G., Weidhase, R. A. & Parthier, B. (1989) *J. Plant Physiol.* **134**, 703–709.
31. Hostetler, K. Y. (1985) in *Phospholipids and Cellular Regulation*, ed. Kuo, J. F. (CRC, Boca Raton, FL), Vol. 1, pp. 181–206.
32. Samuelson, B. (1983) *Science* **220**, 568–575.
33. van Kuijk, F. J. G. M., Sevanian, A., Handelman, G. J. & Dratz, E. A. (1987) *Trends Biochem. Sci.* **12**, 31–34.
34. Curtis, R. W. (1984) *J. Plant Growth Regul.* **3**, 157–168.
35. Franceschi, V. R. & Giaquinta, R. T. (1983) *Planta* **157**, 411–421.
36. Klauer, S. F. (1989) Ph.D. dissertation (Washington State Univ., Pullman).
37. Anderson, J. M. (1991) *J. Plant Growth Regul.* **10**, 5–10.
38. Staswick, P. E. (1988) *Plant Physiol.* **87**, 250–254.
39. Franceschi, V. R. & Giaquinta, R. T. (1983) *Planta* **157**, 422–431.
40. deVeau, E. J., Robinson, J. M., Warmbrodt, R. D. & vanBerkum, P. (1990) *Plant Physiol.* **94**, 259–267.