The plant growth regulator methyl jasmonate inhibits aflatoxin production by *Aspergillus flavus*

Marta Goodrich-Tanrikulu, Noreen E. Mahoney and Susan B. Rodriguez†

Author for correspondence: Marta Goodrich-Tanrikulu. Tel: +1 510 559 5627. Fax: +1 510 559 5777. e-mail: martat@pw.usda.gov

US Department of Agriculture, Agricultural Research Service, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710, USA

Aflatoxins are highly toxic and carcinogenic compounds produced by certain *Aspergillus* species on agricultural commodities. The presence of fatty acid hydroperoxides, which can form in plant material either preharvest under stress or postharvest under improper storage conditions, correlates with high levels of aflatoxin production. Effects on fungal growth and aflatoxin production are known for only a few of the numerous plant metabolites of fatty acid hydroperoxides. Jasmonic acid (JA), a plant growth regulator, is a metabolite of 13-hydroperoxylinolenic acid, derived from α -linolenic acid. The volatile methyl ester of JA, methyl jasmonate (MeJA), is also a plant growth regulator. In this study we report the effect of MeJA on aflatoxin production and growth of *Aspergillus flavus*. MeJA at concentrations of 10^{-3} – 10^{-8} M in the growth medium inhibited aflatoxin production, by as much as 96%. Exposure of cultures to MeJA vapour similarly inhibited aflatoxin production. The amount of aflatoxin produced depended on the timing of the exposure. MeJA treatment also delayed spore germination and inhibited the production of a mycelial pigment. These fungal responses resemble plant jasmonate responses.

Keywords: aflatoxin, *Aspergillus flavus*, *Aspergillus parasiticus*, lipoxygenase, methyl jasmonate

INTRODUCTION

Aflatoxins are highly toxic and carcinogenic secondary metabolites produced by the fungi *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. Aflatoxin contamination of agricultural commodities, including corn, cottonseed, peanuts, and many other oil-rich crops such as tree nuts, can occur following *Aspergillus* spp. infection (Payne, 1992), and is therefore a public health concern. Aflatoxin levels in crops often rise following exposure to certain stresses such as high temperature and drought (Payne, 1992; Sanders *et al.*, 1993). One factor influencing the production of aflatoxin is the presence of high levels of oxidized fatty acids such as fatty acid hydroperoxides (Fanelli & Fabbri, 1989), often found under stress (Merzlyak, 1990). Fatty acid hydroperoxides can be formed by autooxidation, or enzymically by lipoxygenases

acting on α -linoleic and α -linolenic acids (Vick, 1993). These hydroperoxides stimulate the formation of aflatoxin by *A. flavus* and *A. parasiticus* (Fabbri *et al.*, 1983; Fanelli & Fabbri, 1989). Degradation of the hydroperoxides by later steps in the plant lipoxygenase pathway leads to multiple byproducts, depending on the polyunsaturated fatty acid substrate, the positional specificity of the lipoxygenase, and the activities of enzymes catalysing the subsequent steps. The aldehydes hexanal, *trans*-2-hexenal, and *trans*-2-nonenal, which are hydroperoxide metabolites via hydroperoxide lyase (Vick, 1993), generally inhibit spore germination (Zeringue & McCormick, 1990) and aflatoxin production (Zeringue & McCormick, 1990; Zeringue, 1991; Zeringue & Bhatnagar, 1994), although stimulation of aflatoxin production has been observed under some conditions (Fabbri *et al.*, 1983; Zeringue, 1991).

Another α -linolenic acid metabolite, via lipoxygenase and hydroperoxide dehydratase, is jasmonic acid (JA) (Vick, 1993). JA and closely related compounds, such as its methyl ester, MeJA, are endogenous plant growth

†Present address: Department of Biological and Physical Sciences, Indiana University, Kokomo, IN 46904, USA.

Abbreviations: JA, jasmonic acid; MeJA, methyl jasmonate.

regulators. Jasmonates appear to be ubiquitous in both higher and lower plants (Staswick, 1992; Sembdner & Parthier, 1993). The jasmonates regulate a diverse set of responses, including inhibition of pollen and seed germination, altered synthesis of secondary metabolites, suppression of protein synthesis, and the activation of defence responses, in part by altering gene expression (reviewed by Parthier, 1991; Staswick, 1992; Sembdner & Parthier, 1993). MeJA is more volatile than JA, so exposure to it either in solution or in the gaseous phase can elicit plant responses. In addition, it is used as a fragrance in the perfume industry (Staswick, 1992).

JA was first identified as a plant growth inhibitor when it was isolated from culture filtrate of the fungus *Lasiodiplodia* (*Botryodiplodia*) *theobromae*, a plant pathogen (Aldridge *et al.*, 1971). Reports of direct effects of jasmonates on fungi are limited, and include the inhibition of mycorrhizal fungal growth (Gogala, 1991) and the inhibition of appressoria formation or spore germination in three plant pathogens (Neto *et al.*, 1991; Schweizer *et al.*, 1993, Cohen *et al.*, 1993).

Exposure of aflatoxigenic fungi to jasmonates is likely, since the fungi grow on plant material. Thus, we began this study by examining the effects of MeJA on a toxigenic strain of *A. flavus* growing *in vitro*. In this experimental system, effects of MeJA can be studied in a defined medium, and are therefore separable from effects of a plant/fungal interaction.

METHODS

Cultures, media and chemicals. MeJA was a gift from Bedoukian Research. JA, and standards of aflatoxins B₁, B₂ and G₁, sterigmatocystin, O-methylsterigmatocystin and cyclopiazonic acid were purchased from Sigma.

Spores (approximately 6000 in 5 µl suspension) of *A. flavus* strain WRR 3-90-42-12 (NRRC no. 25347) were inoculated as detailed by Rodriguez & Mahoney (1994) onto the centre of 60 mm Petri dishes. Plates contained Vogel's medium N (VMN) (Davis & deSerres, 1970) or modified CYA (Rodriguez & Mahoney, 1994) agar, with or without MeJA or JA added after autoclaving. For exposure to levels less than 10⁻⁴ M, dilutions of the MeJA stock were made in ethanol. Cultures were incubated at 25 °C.

Spore germination time was determined in both liquid and solid VMN in the presence or absence of 10⁻⁴ M MeJA or JA. The percentage of spores with germ tubes was counted microscopically at various times after inoculation. Regression lines were fitted to a plot of the percentage of germinated spores versus time after inoculation. The time required for 50% germination was used to compare mean germination times among treatments.

For exposure of cultures to airborne MeJA, the MeJA and the fungal inoculum were physically separated by removing a strip of agar (3 mm wide) between them (see Fig. 1). Some experiments tested the effects of shorter exposures to MeJA. In one set of these studies, the MeJA (0.2 µl) was added across the gap from the growing culture at defined intervals after inoculation. In a complementary set of studies, MeJA was added at the time of inoculation, but exposure was ended at defined intervals by removing from the plate the entire piece of agar where the MeJA had been applied (on the right in Fig. 1).

Fig. 1. Method of exposure of *A. flavus* cultures to airborne MeJA. A 3 mm (± 1 mm) strip of agar was removed from the plate with a sterile scalpel blade, baring the bottom surface of the plate, and leaving a gap in the medium which extended the entire length (off-centre) of the plate, as shown. *A. flavus* spores were inoculated on the wider section of agar, and MeJA (0.2 µl) was applied on the narrower section opposite the gap, as illustrated.

To examine the effect of MeJA treatment on aflatoxin levels in stored nuts, freshly field-harvested unshelled pistachio nuts were partially dried (15% residual water by weight) and kept in containers at 25 °C, allowing growth of the natural microflora. Each container (30.9 l) held 55 kg pistachios, incubated in either the presence or absence of MeJA vapour. For exposure to the vapour, the pistachios were equilibrated with a 100 µl pool of MeJA in a watchglass placed above the nuts. The containers were covered but not airtight. After 13 d, 20 randomly chosen samples of 100 nuts from both treatments were removed from the containers for aflatoxin assays. At this time, the pistachios from both containers showed extensive fungal contamination (mostly *A. niger*, with lesser contamination by *A. flavus*).

Aflatoxin and other biochemical assays. For assay of aflatoxins in pistachio nuts, each 100-nut sample was weighed and ground in a 1 l blender. One-tenth of the ground material (about 15 g) from each sample was removed for aflatoxin analysis. This material was blended for 1 min with 3 g NaCl in 75 ml methanol/water (60:40, v/v) and then centrifuged at 5000 g for 10 min. A 5 ml aliquot of the supernatant was serially diluted to a final dilution of 1:5000. Ten millilitres of this dilution was applied to an aflatest-P (VICAM) immunoaffinity column. The column was washed with 2 column vols water, and aflatoxins were then eluted with 2 ml acetonitrile. The acetonitrile was taken to dryness at 40 °C with N₂. The residue was derivatized with 200 µl each of trifluoroacetic acid and hexane for 10 min at room temperature, then again taken to dryness at 40 °C with N₂, and dissolved in 200 µl acetonitrile/water (1:9, v/v). The extract was syringe-filtered and analysed by reverse-phase HPLC (Rodriguez & Mahoney, 1994).

For *in vitro* assays, aflatoxins were extracted from cultures (entire plate, containing fungus and agar) and quantified by HPLC as detailed by Rodriguez & Mahoney (1994).

The aflatoxin precursors sterigmatocystin and O-methylsterigmatocystin, and the *A. flavus* secondary metabolite cyclopiazonic acid, were identified by comparison of migration and

staining properties following TLC of extracts and standards (Rodriguez & Mahoney, 1994).

Fatty acid composition of *A. flavus* was determined on liquid-grown cultures and on spores by lipid extraction and GC analysis of methyl esters of total fatty acids (Goodrich-Tanrikulu *et al.*, 1994).

RESULTS

Effects of jasmonates on growth and aflatoxin production of *A. flavus* in vitro

The aflatoxin which is usually produced at the highest levels by toxigenic strains of *A. flavus* is aflatoxin B₁, which is also the most potent aflatoxin (Payne, 1992). We first detected aflatoxin B₁ 3 d after *A. flavus* inoculation onto VMN. Aflatoxin B₁ production peaked at about 9 d, falling off gradually thereafter (Fig. 2). Aflatoxin B₂ was present only at trace levels. In cultures treated with 10⁻⁴ M MeJA, similar kinetics of aflatoxin production occurred, but the production of aflatoxin was inhibited approximately 80% throughout the time course (Fig. 2). Colony growth was essentially unaffected, aside from a slightly smaller (95% of untreated control) colony diameter through day 7 (data not shown). At day 8, the mean colony diameter was 45 mm for both cultures.

Germination of spores was slightly delayed by MeJA, enough to account for the slight early difference in colony size: on average, 50% of untreated spores had germ tubes 9.1 h after inoculation. MeJA-treated spores germinated at the same rate, but with a 1.6 h delay. Spore viability was not affected.

Consequently, MeJA inhibited aflatoxin production *per se*, and unlike many other inhibitors (Zaika & Buchanan, 1987; Rodriguez & Mahoney, 1994), with no apparent

Fig. 2. Effect of MeJA on aflatoxin production over time by *A. flavus*. Δ , MeJA-treated cultures; $\bullet$, untreated control cultures. Results presented are means $\pm$ SE of three or four experiments using triplicate plates. Spores of *A. flavus* strain 42-12 were inoculated onto the centre of VMN agar with or without 10⁻⁴ M MeJA, and harvested for aflatoxin analysis at the specified times. Aflatoxin B₁ was quantified by HPLC.

Fig. 3. Effect of various concentrations of MeJA in minimal medium on the amount of aflatoxin B₁ produced (culture plus medium) 8 d after inoculation. Results are means $\pm$ SE of two experiments using triplicate plates inoculated and assayed as described in Fig. 2, except that the MeJA concentration in the medium varied as indicated.

effect on mycelial growth or colony appearance. In contrast with the lack of observed effect on growth, the yellow mycelial pigmentation found in untreated cultures was absent at all concentrations of MeJA tested. Additionally, droplets of undiluted MeJA applied near colonies caused local growth avoidance, and spores in the vicinity of this zone produced a yellow-orange rather than green pigment.

Aflatoxin production was inhibited at all levels of MeJA tested, ranging from 10⁻³ to 10⁻⁸ M (Fig. 3); at the highest concentration tested, MeJA treatment reduced aflatoxin production by 96% 8 d after inoculation.

MeJA also inhibited aflatoxin production by *A. flavus* growing on other media, such as CYA medium, and on pistachios in storage (described later). Aflatoxin production in cultures treated with 10⁻⁴ M MeJA was 52% that of untreated cultures (which contained a mean of 31 µg aflatoxin B₁ per plate at day 8) in experiments using CYA, a complex medium (Rodriguez & Mahoney, 1994), compared to 17% of untreated cultures at day 8 on VMN, a defined minimal medium (Fig. 2). Substrate composition thus influences the degree of inhibition.

Each of the responses which we observed was more pronounced for MeJA than for JA: JA at equivalent concentrations was less effective at inhibiting aflatoxin production (35% inhibition at 10⁻⁴ M in VMN), delaying spore germination, and suppressing mycelial pigmentation. The lower effectiveness of JA compared to MeJA could be due to the unesterified compound (JA) having either a lower biochemical specificity, or an ionic character, which would be expected to lower membrane permeability.

Fig. 4. Effect of duration of exposure to airborne MeJA on aflatoxin production by *A. flavus*. Data are means $\pm$ SE of two experiments using triplicate plates, expressed relative to untreated controls (31 μ g aflatoxin B₁ per plate in these experiments). *A. flavus* 42-12 spores were inoculated onto VMN agar on day 0 and were exposed to MeJA as described in Fig. 1 and Methods. (a) Production of aflatoxin B₁ on day 8 when exposure to MeJA began at the time of inoculation and ended at specified times after inoculation. (b) Production of aflatoxin B₁ on day 8 when exposure to MeJA began at specified times after inoculation, and ended at time of harvest on day 8.

MeJA treatment apparently inhibited the biosynthesis of aflatoxin B₁. The immediate biochemical precursors of aflatoxin B₁, sterigmatocystin and *O*-methylsterigmatocystin (Payne, 1992), did not accumulate in cultures treated with 10^{-4} M MeJA (TLC plate not shown). The inhibition is thus likely to be prior to the formation of these compounds in the aflatoxin biosynthetic pathway. Inhibition may occur prior to the formation of the coloured anthraquinone intermediates early in the pathway, although the identity of the pigment(s) absent in mycelia of MeJA-treated cultures is not known. Levels of another secondary metabolite

produced by *A. flavus*, cyclopiazonic acid (Luk *et al.*, 1977), were unaffected by treatment (TLC plate not shown), demonstrating that the inhibition of aflatoxin production could be a specific effect, rather than a general one on secondary metabolism.

Effect of exposure to gaseous MeJA on aflatoxin production *in vitro* and on pistachio nuts

Inhibition of aflatoxin production occurred whether colonies were exposed to MeJA incorporated into the solid medium, or exposed to it in the gaseous phase. In experiments where MeJA was applied opposite a gap in the agar medium from the colony, it was similarly effective at reducing aflatoxin levels (Fig. 4). Thus, a source of MeJA need not necessarily be at the site of fungal growth to inhibit aflatoxin production.

A limited exposure to airborne MeJA was sufficient to reduce the production of aflatoxin. Exposure for only 6 h after inoculation was sufficient to inhibit total aflatoxin production by 39% at day 8 (Fig. 4a). This is remarkable, because 6 h after inoculation is prior to germ tube emergence (see earlier): exposure of single-celled germinating spores to MeJA inhibited aflatoxin production of the large (45 mm) multicellular colonies developing from them. Exposure for either 2 or 8 d after inoculation resulted in maximum inhibition, despite the increase in colony diameter from 20 to 45 mm between 2 and 8 d (an increase in area of nearly threefold). These results suggest that new fungal mycelia growing after MeJA exposure has ended retain MeJA-induced inhibition of aflatoxin production. Complicating this interpretation is the observation that part of the total inhibition at 8 d was potentially due to residual MeJA vapour in the Petri dishes after removal of the source of MeJA. Twelve percent inhibition was found in plates inoculated after pretreatment with MeJA (plates incubated for 6 h with MeJA as illustrated in Fig. 1, followed by removal of source, then inoculation). Thus, the net inhibition after a limited-duration exposure appears to be due to a combination of continuing inhibition after removal of MeJA, and traces of residual MeJA, which are less effective, but still potent.

Exposure to MeJA was required early, when conditions are favourable for spore germination and growth, to achieve inhibition of aflatoxin biosynthesis *in vitro*. Airborne MeJA applied 6 h after inoculation onto a favourable growth medium was partially effective at reducing later aflatoxin production, but application 3 d or more after inoculation was completely ineffective (Fig. 4b). Pretreatment of spores with MeJA prior to inoculation was also ineffective (data not shown). The inhibition of aflatoxin production thus requires exposure within an early phase of growth.

We also tested the effectiveness of airborne MeJA treatment on field strains of aflatoxin-producing fungi growing on a natural substrate, pistachio nuts. The natural microflora was allowed to grow on incompletely dried pistachios (15% residual moisture) for 13 d in the

Fig. 5. Aflatoxin content of pistachio nuts incubated with airborne MeJA. Partially dried pistachios containing natural microflora were stored for 13 d in the presence or absence of MeJA as described in Methods. For both treatments, 20 individual samples (each containing 100 nuts) were assayed for aflatoxin content as described in Methods. Vertical bars show aflatoxin levels for each of the 20 samples, which have been ranked from the highest to lowest levels. Solid bars, untreated nuts; shaded bars, MeJA-treated nuts. (a) Profile of aflatoxin B_1 levels detected per 100-nut sample. (b) Profile of aflatoxin G_1 levels detected per 100-nut sample; note difference in scale from (a).

presence or absence of MeJA vapour, prior to assaying aflatoxin levels. At this time, no difference in extent of fungal contamination was noted between treatments, and some nuts in both treatments were visibly infected by *A. flavus*. Both aflatoxins B_1 and G_1 were detected in random samples of nuts, although the maximum levels of aflatoxin B_1 formed were higher than those of G_1 (Fig. 5). Aflatoxin B_1 is produced by both *A. flavus* and *A. parasiticus*, whereas aflatoxin G_1 is produced by *A. parasiticus* (Payne, 1992). The natural microflora thus included both *A. flavus* and *A. parasiticus*.

The aflatoxin levels assayed in the nuts showed characteristic variability, with skewed (non-normal) distributions (Fig. 5). MeJA-treated nuts had lower levels of both aflatoxins B_1 (Fig. 5a) and G_1 (Fig. 5b) than untreated nuts; the reduction in sampled aflatoxin levels is significant (99%, sign test). Based on these samples, MeJA treatment may be more effective against aflatoxin B_1 production than against G_1 production (compare Fig. 5a, b). Six out of 20 of the untreated samples exceeded the highest levels of aflatoxin B_1 formed in the presence of

MeJA (Fig. 5a); the MeJA-treated nuts also had more samples with trace or no detectable aflatoxin (Fig. 5). Overall, nuts incubated with MeJA vapour had mean aflatoxin B_1 levels 17% and aflatoxin G_1 levels 56% of those of untreated nuts. Total aflatoxins in the MeJA-treated nuts were thus 20% of untreated nuts (799 µg and 3997 µg, respectively). These data demonstrate a broad effectiveness of MeJA on inhibition of production of aflatoxins by field strains of *A. flavus* and *A. parasiticus* growing on plant material, at least in pistachios.

DISCUSSION

Jasmonate production by fungi

MeJA inhibited aflatoxin production at levels lower than those reported for other plant-derived compounds inhibiting aflatoxin biosynthesis (reviewed by Zaika & Buchanan, 1987), including compounds likewise derived from fatty acid hydroperoxides (Fabbri *et al.*, 1983; Zeringue & McCormick, 1990; Zeringue & Bhatnagar, 1994). For example, in the presence of 2 µM hexanal, *A. parasiticus* shows radial growth 76% and aflatoxin B_1 production 56% that of untreated cultures (Zeringue & Bhatnagar, 1994); lower levels may be effective, but were apparently not tested.

MeJA was effective at reducing aflatoxin B_1 production at concentrations at least as low as 10^{-8} M (Fig. 3). Because of its effectiveness at submicromolar concentrations, and its residual effectiveness after exposure has ended (Fig. 4a), it is unlikely to directly affect enzymic activity. Instead, this high potency, and the observation of additional effects on pigmentation and spore germination, suggest a regulatory response mediated by a receptor. The observed level of effectiveness is probably an underestimate for two reasons. First, commercial preparations of jasmonates contain two enantiomers, only one of which may be physiologically active in plants (Vick, 1993). Second, since it is volatile, some MeJA originally in the medium will be lost over time. A high specificity for MeJA is also indicated by the lesser effectiveness of the structurally related compound JA, as noted above.

One of the *A. flavus* responses triggered by this putative receptor is an apparent inhibition of the biosynthesis or (indirectly) the activity of an enzyme or enzymes in the aflatoxin biosynthetic pathway. This and the other responses we observed to MeJA have parallels in plant systems, in which MeJA acts as a growth regulator: jasmonates affect the production of many secondary metabolites (Parthier, 1991; Sembdner & Parthier, 1993; Aerts *et al.*, 1994) and pigments, and delay pollen germination (Parthier, 1991; Sembdner & Parthier, 1993). The range of effective concentrations and the kinetics of MeJA responses in plants are remarkably similar to those observed for *A. flavus*. The similarity of the *A. flavus* responses to MeJA, and their pleiotropic nature, suggests that MeJA may act as a growth regulator in this fungus. At least two species of fungi, both plant pathogens, produce jasmonates (Aldridge *et al.*, 1971; Miersch *et al.*, 1992), and many others produce the fatty acid precursor α -linolenate (Staswick, 1992).

We do not know whether *A. flavus* produces jasmonates endogenously; α -linolenate has not been detected in lipid extracts of *A. flavus* (Fernando & Bean, 1986). We also could not detect this fatty acid in *A. flavus* extracts under our *in vitro* growth conditions. However, assuming this fungus, like other fungi including *A. niger* (Chattopadhyay *et al.*, 1985), incorporates fatty acids from its substrate, these fatty acids are capable of entering the lipoxygenase pathway, if present. Thus, *A. flavus* may be capable of forming jasmonates under conditions where α -linolenate is supplied in its substrate.

Inhibition of fungal spore germination by jasmonates has been previously reported for two other fungi, both plant pathogens (Neto *et al.*, 1991; Cohen *et al.*, 1993). In addition, growth of other structures of plant symbiotic or pathogenic fungi is inhibited (Gogala, 1991; Neto *et al.*, 1991; Schweizer *et al.*, 1993) or stimulated (Cohen *et al.*, 1993) by jasmonates. Other metabolites of the polyunsaturated fatty acids α -linolenate and α -linoleate also affect fungal growth (Zeringue & McCormick, 1990; Gogala, 1991; Neto *et al.*, 1991). Metabolites of arachidonic acid, a polyunsaturated fatty acid synthesized by animals and many fungi (but not found in *A. flavus* or higher plants), can elicit plant defence responses (Choi *et al.*, 1994), and are important endogenous regulatory molecules in animals and some fungi (Sembdner & Parthier, 1993; Choi *et al.*, 1994). Thus, the potential importance of jasmonates and structurally related compounds as fungal regulatory molecules is significant.

MeJA and aflatoxin control

Fatty acid hydroperoxides stimulate aflatoxin production (Fabbri *et al.*, 1983; Fanelli & Fabbri, 1989), whereas several of their metabolites, including various aldehydes (Zeringue & McCormick, 1990; Zeringue, 1991) and jasmonates (noted here), generally inhibit aflatoxin production. The net result of simultaneous exposure of *A. flavus* cultures to fatty acid hydroperoxides and their metabolites is not known. Effects in different systems may depend on many factors, such as the fatty acid substrate, lipoxygenase specificity, and the levels of various other enzymes involved in hydroperoxide metabolism.

Much interest has been focused on factors providing crop resistance to aflatoxin contamination. Soybeans, unlike many other oilseeds, are resistant to aflatoxin contamination (Doehlert *et al.*, 1993). Soybean oil is high in α -linoleic and α -linolenic acids, and soybeans contain an active lipoxygenase capable of forming the 13(*S*)-hydroperoxides from these fatty acids; the lipoxygenase pathway has been implicated as one important factor in the resistance of soybeans (Doehlert *et al.*, 1993). Hexanals and hexenals, which have antifungal properties, are suspected to be involved, although detected levels of hexenals derived from α -linolenate are lower than the levels anticipated to inhibit aflatoxin production (Doehlert *et al.*, 1993). This suggests that resistance could be due at least in part to the conversion of 13-hydroperoxylinolenate by either the fungus or soybeans to alternative products, e.g. jasmonates.

MeJA could be a useful tool for elucidating the control of the aflatoxin biosynthetic pathway. Our observation that the timing of MeJA exposure was critical to its effectiveness in *A. flavus* (Fig. 4) is consistent with studies of the suppression of aflatoxin biosynthetic capability by protein and RNA synthesis inhibitors in *A. parasiticus* (Buchanan *et al.*, 1987; Cleveland & Bhatnagar, 1989). Both types of studies have demonstrated that commitment to aflatoxin production occurs early in the growth of these fungi.

The effectiveness of MeJA further suggests a potential use in control of the production of aflatoxin in susceptible commodities. Since MeJA is a plant growth regulator, use in the field for aflatoxin control is not advisable. However, aflatoxin contamination can also occur postharvest, if the crop is not properly dried and stored (Payne, 1992). Based on our observations with pistachios, exposure to MeJA is likely to reduce levels of aflatoxin contamination which would otherwise occur in cases where the crop has been incompletely dried (Fig. 5), or accidentally or deliberately wetted. Our *in vitro* studies suggest that a brief exposure (i.e. 2 d; Fig. 4a) at this stage may be sufficient to minimize contamination. Including a source of MeJA in crop storage areas could be an effective, simple and safe means of reducing postharvest aflatoxin production.

ACKNOWLEDGEMENTS

We thank Bedoukian Research Incorporated, Danbury, CT, USA for their gift of methyl jasmonate.

REFERENCES

- Aerts, R. J., Gisi, D., De Carolis, E., De Luca, V. & Baumann, T. W. (1994). Methyl jasmonate vapor increases the developmentally controlled synthesis of alkaloids in *Catharanthus* and *Cinchona* seedlings. *Plant J* 5, 635–643.
- Aldridge, D. C., Galt, S., Giles, D. & Turner, W. B. (1971). Metabolites of *Lasiodiplodia theobromae*. *J Chem Soc Sect C* 1971, 1623–1627.
- Buchanan, R. L., Jones, S. V., Gerasimowicz, W. V. & Zaika, L. L. (1987). Regulation of aflatoxin biosynthesis: assessment of the role of cellular energy status as a regulator of the induction of aflatoxin production. *Appl Environ Microbiol* 53, 1224–1231.
- Chattopadhyay, P., Banerjee, S. K., Sen, K. & Chakrabarti, P. (1985). An unsaturated fatty acid mutant of *Aspergillus niger* with partially defective $\Delta 9$ -desaturase. *Can J Microbiol* 31, 346–351.
- Choi, D., Bostock, R. M., Avdiushko, S. & Hildebrand, D. F. (1994). Lipid-derived signals that discriminate wound- and pathogen-responsive isoprenoid pathways in plants: methyl jasmonate and the fungal elicitor arachidonic acid induce different 3-hydroxy-3-methylglutaryl-coenzyme A reductase genes and antimicrobial isoprenoids in *Solanum tuberosum* L. *Proc Natl Acad Sci USA* 91, 2329–2333.
- Cleveland, T. E. & Bhatnagar, D. (1989). Evidence for *de novo* synthesis of an aflatoxin pathway methyltransferase near the cessation of active growth and the onset of aflatoxin biosynthesis in *Aspergillus parasiticus* mycelia. *Can J Microbiol* 36, 1–5.
- 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.
- Davis, R. H. & deSerres, F. J. (1970). Genetic and microbiological

- research techniques for *Neurospora crassa*. *Methods Enzymol* **17A**, 79–143.
- Doehliert, D. C., Wicklow, D. T. & Gardner, H. W. (1993). Evidence implicating the lipoxygenase pathway in providing resistance to soybeans against *Aspergillus flavus*. *Phytopathology* **83**, 1473–1477.
- Fabbri, A. A., Fanelli, C., Panfili, G., Passi, S. & Fasella, P. (1983). Lipoperoxidation and aflatoxin biosynthesis by *Aspergillus parasiticus* and *A. flavus*. *J Gen Microbiol* **129**, 3447–3452.
- Fanelli, C. & Fabbri, A. A. (1989). Relationship between lipids and aflatoxin biosynthesis. *Mycopathologia* **107**, 115–120.
- Fernando, T. & Bean, G. (1986). Effects of tricyclazole on growth, release of aflatoxin, and sterol and fatty acid content by *Aspergillus* isolates. *Trans Br Mycol Soc* **87**, 445–449.
- Gogala, N. (1991). Regulation of mycorrhizal infection by hormonal factors produced by hosts and fungi. *Experientia* **47**, 331–340.
- Goodrich-Tanrikulu, M., Stafford, A. E., Lin, J.-T., Makapugay, M. I., Fuller, G. & McKeon, T. A. (1994). Fatty acid biosynthesis in novel *ufa* mutants of *Neurospora crassa*. *Microbiology* **140**, 2683–2690.
- Luk, K. C., Kobbe, B. & Townsend, J. M. (1977). Production of cyclopiazonic acid by *Aspergillus flavus* Link. *Appl Environ Microbiol* **33**, 211–212.
- Merzlyak, M. N. (1990). Syndrome of lipid peroxidation in plants. In *Plant Lipid Biochemistry, Structure and Utilization. Proceedings of the Ninth International Symposium on Plant Lipids*, pp. 281–288. Edited by P. J. Quinn & J. L. Harwood. London: Portland Press.
- Miersch, O., Brückner, B., Schmidt, J. & Sembdner, G. (1992). Cyclopentane fatty acids from *Gibberella fujikuroi*. *Phytochemistry* **31**, 3835–3837.
- Neto, G. C., Kono, Y., Hyakutake, H., Watanabe, M., Suzuki, Y. & Sakurai, A. (1991). Isolation and identification of (–)-jasmonic acid from wild rice, *Oryza officinalis*, as an antifungal substance. *Agric Biol Chem* **55**, 3097–3098.
- Parthier, B. (1991). Jasmonates, new regulators of plant growth and development: many facts and few hypotheses on their actions. *Bot Acta* **104**, 446–454.
- Payne, G. A. (1992). Aflatoxin in maize. *Crit Rev Plant Sci* **10**, 423–440.
- Rodriguez, S. B. & Mahoney, N. E. (1994). Inhibition of aflatoxin production by surfactants. *Appl Environ Microbiol* **60**, 106–110.
- Sanders, T. H., Cole, R. J., Blankenship, P. D. & Dörner, J. W. (1993). Aflatoxin contamination of peanuts from plants drought stressed in pod or root zones. *Peanut Sci* **20**, 5–8.
- Schweizer, P., Gees, R. & Mössinger, E. (1993). Effect of jasmonic acid on the interaction of barley (*Hordeum vulgare* L.) with the powdery mildew *Erysiphe graminis* f. sp. *hordei*. *Plant Physiol* **102**, 503–511.
- Sembdner, G. & Parthier, B. (1993). The biochemistry and the physiological and molecular actions of jasmonates. *Annu Rev Plant Physiol Plant Mol Biol* **44**, 569–589.
- Staswick, P. E. (1992). Jasmonate, genes, and fragrant signals. *Plant Physiol* **99**, 804–807.
- Vick, B. A. (1993). Oxygenated fatty acids of the lipoxygenase pathway. In *Lipid Metabolism in Plants*, pp. 167–191. Edited by T. S. Moore, Jr. Boca Raton, FL: CRC Press.
- Zaika, L. L. & Buchanan, R. L. (1987). Review of compounds affecting the biosynthesis or bioregulation of aflatoxins. *J Food Prot* **50**, 691–708.
- Zeringue, H. J., Jr (1991). Effect of C6 to C9 alkenals on aflatoxin production in corn, cottonseed, and peanuts. *Appl Environ Microbiol* **57**, 2433–2434.
- Zeringue, H. J., Jr & Bhatnagar, D. (1994). Effects of neem leaf volatiles on submerged cultures of aflatoxigenic *Aspergillus parasiticus*. *Appl Environ Microbiol* **60**, 3543–3547.
- Zeringue, H. J., Jr & McCormick, S. P. (1990). Aflatoxin production in cultures of *Aspergillus flavus* incubated in atmospheres containing selected cotton leaf-derived volatiles. *Toxicon* **28**, 445–448.

Received 12 May 1995; revised 25 July 1995; accepted 26 July 1995.