J Plant Growth Regul (1999) 18:93–100

Journal of
Plant
Growth
Regulation

Effects of Exogenously Applied Jasmonates on Growth and Intracellular pH in Maize Coleoptile Segments

H. R. Irving,[1]* G. Dyson,[2] R. McConchie,[3]† R. W. Parish,[3] and C. A. Gehring[2]

Department of [1]Pharmaceutical Biology and Pharmacology, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville Campus, Melbourne, Victoria 3052, Australia; [2]School of Biological and Chemical Sciences, Deakin University, Geelong, Victoria, 3217; and [3]School of Botany, La Trobe University, Bundoora Campus, Melbourne, Victoria 3083 Australia

Received February 5, 1999; accepted August 25, 1999

Abstract. The effects of jasmonic acid (JA) on elongation growth of coleoptile segments from etiolated maize (*Zea mays* L.) were investigated in the presence and absence of auxin. When supplied alone, at physiological concentrations (10^{-9}, 10^{-8}, and 10^{-5} M), JA (or methyl-JA) inhibited growth. JA at a similar range of concentrations also inhibited auxin-induced elongation growth. To determine whether this effect on growth depended on endogenous abscisic acid (ABA), we grew maize coleoptiles in the presence of norflurazon (an inhibitor of carotenoid biosynthesis) that results in reduced endogenous ABA levels. Growth of etiolated coleoptile segments from these plants was inhibited by JA (or methyl-JA) in both the absence and presence of auxin. Previously, we have observed a correlation between elongation growth and cytosolic pH (pH_i), in which auxin lowers pH_i, and growth inhibitors such as ABA raise pH_i. We examined the effect of low concentrations of methyl-JA on pH_i with dual emission dye, carboxy seminaphthorhodafluor-1, and confocal microscopy. To confirm these studies, we also used in vivo ^{31}P NMR spectrometry to ascertain the changes in pH_i after addition of jasmonate to maize coleoptiles. Coleoptiles grown in either the absence or presence of norflurazon responded to methyl-JA or JA by increases in pH_i of approximately 0.2 pH unit. This response occurs over a period of 15–20 min and appears to be independent of endogenous ABA. This alkalization induced by JA is likely to form a permissive environment for JA signal transduction pathway(s).

Key Words. Absicic acid—Auxin—Cytosolic pH—Growth—Jasmonic acid—*Zea mays*

Jasmonates are linolenic acid (18:3)–derived plant growth regulators. The major natural jasmonates are (−)-jasmonic acid (JA), its stereo isomer, (+)-7-isoJA, and the most familiar is the volatile methyl ester of (−)-JA (JA-Me), which is a fragrant essential oil of *Jasminum* (Sembdner and Parthier 1993). Jasmonates induce a number of physiological responses, ranging from activation of defense mechanisms to promotion of senescence-related events (for reviews, see Creelman and Mullet 1997, Koiwa et al. 1997, Wasternack and Parthier 1997). Jasmonates have a major regulatory role in plant defense responses that involves a complex interplay with abscisic acid (ABA) and ethylene. ABA acts in a complementary fashion with the perception of the defense trigger to induce the JA production necessary for gene induction (Koiwa et al. 1997, Wasternack and Parthier 1997).

The role of jasmonates in growth is less well understood, although evidence exists that they could act in a complementary fashion with other hormones such as abscisic acid (ABA) or auxin (IAA) or gibberellic acid (GA). Jasmonic acid inhibits root growth in both di- and monocotyledonous plants (Berger et al. 1996, Sembdner and Parthier 1993, Staswick et al. 1992, Yamane et al. 1981) independently of ethylene (Berger et al. 1996). The effect on shoot growth is not so clear. For example, some early reports (see Sembdner and Parthier 1993) suggest that JA inhibits seedling growth. More recently

Abbreviations: ABA, abscisic acid; GA, gibberellic acid; IAA, indole-3-acetic acid; JA, jasmonic acid; JA-Me, methyl jasmonate; Mes, 2-[N-morpholino]ethanesulfonic acid; NMR, nuclear magnetic resonance; pH_i, intracellular pH; Pi, phosphate; Pipes, piperazine-N,N′-bis[2-ethanesulfonic acid]; Snarf-1, carboxy-semi-naphthorhodafluor-1

*Author for correspondence: E-mail: helen.irving@vcp.monash.edu.au
†Current address: Department of Crop Sciences, University of Sydney, Sydney, NSW 2006

Montague (1997) found that JA alone induces elongation growth in the GA-responsive oat internodal tissue. However, when GA was present, elongation growth was reduced by JA in a synergistic fashion with ABA (Montague 1997), suggesting that these two plant hormones act independently of each other. JA-Me was as effective as ABA at inhibiting radial and elongation growth promoted in *Rananculus sceleratus* petioles by auxin (indole-3-acetic acid; IAA) and ethylene, respectively (Smulders and Horton 1991). Moreover, JA inhibits IAA-induced elongation of oat coleoptiles after a lag of 2 h, which can be alleviated by the presence of sucrose (Miyamoto et al. 1997, Ueda et al. 1994). Because ABA inhibition of IAA-induced elongation was not affected by sucrose, Ueda et al. (1994) argue that the JA inhibition is different from that of ABA. In maize, IAA loosens the cell wall and promotes cell wall synthesis and ABA inhibits elongation by reducing cell wall extensibility (Kutschera and Schopfer 1986). ^{14}C-Glucose uptake studies indicated that JA appears to act by blocking glucose incorporation into the cell wall of oat coleoptiles (Miyamoto et al. 1997, Ueda et al. 1995). However, JA had no effect on IAA-induced elongation of epicotyl segments in the dicotyledons, pea, and azuki (Miyamoto et al. 1997), which could reflect differences in cell wall polysaccharide composition between di- and monocotyledonous plants (Miyamoto et al. 1997). Furthermore, different regions of the oat appear to have distinct responses to JA. The internode contains a GA-responsive region, where JA acts synergistically with ABA to inhibit GA-induced growth and a graviresponsive (or auxin responsive) region where JA inhibits growth more potently than ABA (Montague 1997). The effects of JA in this tissue were independent of glucose (Montague 1997), suggesting that mechanisms underlying growth inhibition differ in different tissues of the same species.

Weak acids also stimulate elongation growth in maize and the degree of elongation correlates with the extent to which they acidify the cytoplasm (Brummer et al. 1984). Exogenous IAA induces a decrease in cytosolic pH (pH_i) (Brummer et al. 1985, Felle et al. 1986, Felle 1988, Frohnmeyer et al. 1998, Gehring et al. 1990), whereas ABA induces an increase in pH_i in cereals (Gehring et al. 1990, van der Veen et al. 1992). We have suggested that these divergent pH changes could underlie the antagonistic effects of IAA and ABA on growth. ABA also raises pH_i in guard cells of the noncereals, *Paphiopedilum* and *Vicia* (Blatt and Armstrong 1993, Irving et al. 1992). We have shown that JA also raises pH_i in guard cells and stimulates stomatal closure (Gehring et al. 1997).

In this article, we describe effects of JA and JA-Me on elongation of maize coleoptiles grown in the absence or presence of norflurazon to test if the observed growth inhibition is mediated by endogenous ABA levels. Norflurazon prevents in vivo accumulation of unsaturated carotenoids (Bartels and McCullogh 1972) and reduces ABA levels (Feldman and Sun 1986). We also measured pH_i in coleoptiles and demonstrate that the effect of JA-Me on pH_i correlates with the observed growth inhibition. These responses are not dependent on normal endogenous ABA levels.

Materials and Methods

Plant Material

Seeds of maize (*Zea mays* L., var Terrific or Punchline) were imbibed for 8–18 h either in the presence or absence of 100 μM norflurazon (Sandoz 9789; 4-chloro-5(methylamino)-2-(α,α,α-trifluoro-m-tolyl)-3(2H)-pyridazinone). The imbibed seeds were germinated in moist vermiculite and grown in the dark for 5 to 7 days at 27 ± 1°C as previously described (Gehring et al. 1994). At this stage, coleoptiles were harvested for either elongation assays or pH_i measurements. The shoots were removed with a clean, sharp incision and the tip of the coleoptile discarded. The coleoptiles were cut to length and the primary leaves were removed, leaving coleoptile segments.

Elongation Growth Assays

For each sample, five to six excised coleoptile segments (from different plants) 5–6 mm long (unless otherwise stated) were threaded apical end up onto a "nichrome" wire (diameter, 0.8 mm) with every effort being made to keep the correct orientation of the segments. The segments were threaded onto the wire for ease of handling and to aid with individual segment identification. The segments were submerged in a test tube containing "incubation buffer" (8 mM KCl, 1 mM $MgCl_2$, and 10 mM Mes (Sigma), pH 6.3); washed for 30–60 min; measured; transferred to fresh buffer containing the treatment; and imbibed under vacuum for 1.5 min. The segments were grown at 28 ± 1°C in the dark, their length was measured after 5–6 h and growth rate was calculated. Individual coleoptile segments (and/or the total length of all six coleoptiles) were measured with a binocular dissecting microscope with a graduated ruler. All experiments were repeated with different batches of coleoptiles. Statistical analysis (ANOVA and Newman-Keuls multiple comparison test) was performed with GraphPad Prism.

Cytosolic pH Measurements Using a Fluorescent Dye, Snarf-1

Maize coleoptile segments (2–2.5 mm long) were loaded in "loading buffer" (10 mM Pipes (Sigma), pH 6.3 plus 1 mM $MgCl_2$ and either 8 or 50 mM KCl) with the acetoxymethyl esterified form of 20 μM carboxy-seminaphthorhodafluor-1 (Snarf-1), (Molecular Probes Inc., Eugene, Oregon) for 30 min at 20 to 25°C in the dark as previously described (Gehring et al. 1994). Routinely, 8 mM KCl was used, but to make valid comparisons with previous studies, 50 mM KCl was sometimes used. Neither the growth nor pH_i responses in control, IAA, or ABA treatments were affected by the different KCl levels (data not shown). Loading was terminated by rinsing the tissue several times in fresh buffer. Viability was assessed by staining tissue segments with either Neutral red or fluorescein diacetate at the end of some experiments. Most cells were alive as indicated by dye accumulation.

Images were acquired and processed with a CLSM-FLUOVERT System (Leica Lastechnik Gmbh, Heidelberg, Germany) as previously

described (Gehring et al. 1994). Intracellular fluorescence was excited at 514 nm and emitted fluorescence was collected on two channels to calculate an emission ratio. Emitted fluorescence was passed through a long-pass filter (610 nm cut-on) and emitted fluorescence less than 580 nm was deflected and passed through a long-pass filter (555 nm cut-on). The use of Snarf-1 emission ratioing alleviates the problems associated with uneven dye loading, dye heterogeneity, dye leakage, and dye photo bleaching (Gehring et al. 1994, Read et al. 1992, Roos 1992) because the ratio of the emission peaks contributes to the determination of pH_i. The signal above 610 nm increases with increasing pH, whereas that between 555–580 nm increases with decreasing pH. Snarf-1 emission (630 nm/580 nm) was calibrated in vitro in a fluorescence spectrophotometer (Hitachi 650-10S) and found to be linear over the pH range 6.8–7.8. For microscopic calibration, tissues were incubated in a range of buffers (and in nigericin for intracellular calibration), scanned, and emission signals greater than 610 nm and between 555 and 580 nm collected. For intracellular calibration of coleoptile cells, the extracellular fluorescence background value was subtracted and the ratio was determined to be linear over the pH range 6.8–7.8 (Gehring et al. 1994) although better fitted by a curve (see inset in Fig. 5). The extracellular calibration fell into a similar dynamic range (Gehring et al. 1994). Variability occurs in the ratio estimates, and changes of less than 0.1 pH unit cannot be readily quantified. The emission ratio was calculated for five coleoptile cells per segment after treatment. The emission ratio was analyzed by one way repeated measures ANOVA with SigmaStat.

Intracellular pH Measurements using ^{31}P NMR Spectrometry

^{31}P NMR was performed with a JEOL (GX270 FT) NMR spectrometer (JEOL, Tokyo, Japan). Excised coleoptiles (approximately 3 cm long) were superfused in 10-mm long glass tubes (Ø 8 mm) in a suspension medium (10 mM Pipes pH 6.2, 50 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, and 100 μM $CaCl_2$), and the sample temperature was maintained at 22°C. ^{31}P spectra represent an average of 2000 scans acquired at an operating frequency of 51.47 Hz. The pulse recycle time was 0.5 s, resulting in a 17-min acquisition time. The sample was not spun to avoid disturbances, and in some samples glucose 6-phosphate was included as an internal reference.

Results

Effect of JA on Coleoptile Elongation Growth

The effects of several concentrations of JA were tested on maize coleoptile elongation growth. Individual coleoptile segments were measured at zero time and again after 5–6 h to determine the growth of each coleoptile segment. The data obtained were pooled from several experiments and analyzed by ANOVA. JA significantly reduced maize coleoptile elongation growth at concentrations from 10^{-9} and 10^{-8} M (the lowest concentrations tested) and at 10^{-5} M (Fig. 1). Although JA also inhibited growth at 10^{-7} and 10^{-6} M, this effect was not significant.

It has been demonstrated previously that JA inhibits IAA-induced elongation in oat coleoptiles (Ueda et al. 1994, 1995). Fig. 2 shows that IAA-induced elongation growth of maize coleoptiles was inhibited by JA. This effect was potent as elongation growth was promoted by IAA at 10^{-5} M, and JA at levels of 10^{-7} and 10^{-5} M could significantly inhibit the growth. Although JA at 10^{-6} inhibited IAA-induced growth, this effect was not significant. The effect of 10^{-7} to 10^{-5} M levels of JA at

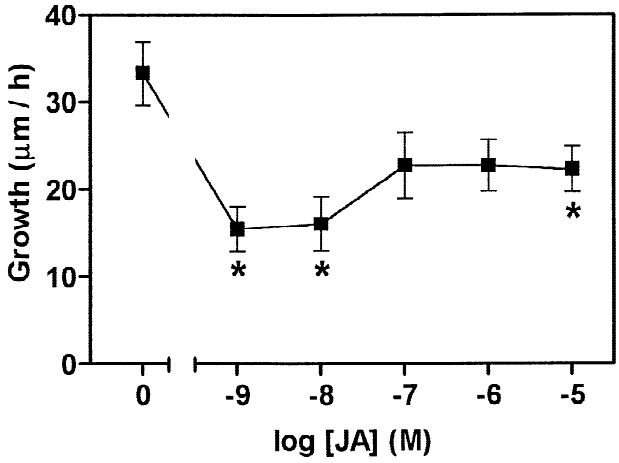

Fig. 1. Dose response of *Zea mays* etiolated coleoptiles to JA. Coleoptile segments (5–6 per wire) were threaded apical end upwards on nichrome wire and incubated in incubation buffer with different concentrations of JA. Control incubations contained 0.01% ethanol because the JA stock was dissolved in ethanol. Individual coleoptile segments were measured at zero time and again after 5–6 h to determine the growth of each coleoptile segment. Data are mean ± SE growth of the segments per wire (n = 12–15 wires), and asterisks indicate the values significantly different from the control ($p < 0.05$).

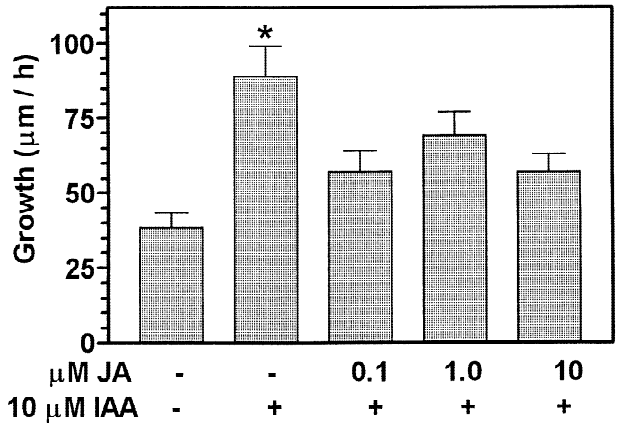

Fig. 2. Effect of JA on elongation of IAA-treated *Zea mays* etiolated coleoptiles. Coleoptile segments (5–6 per wire) were threaded apical end upwards on nichrome wire and incubated in incubation buffer with different concentrations of JA in the presence of 10^{-5} M IAA. Control incubations contained 0.02% ethanol because both JA and IAA stocks were dissolved in ethanol. Individual coleoptile segments were measured at zero time and again after 5–6 h to determine the growth of each coleoptile segment. Data are mean ± SE (n = 9 wires). The 10 μM IAA treatment is significantly different from the control and both the 0.1 μM and 10 μM JA plus IAA treatments ($p < 0.05$).

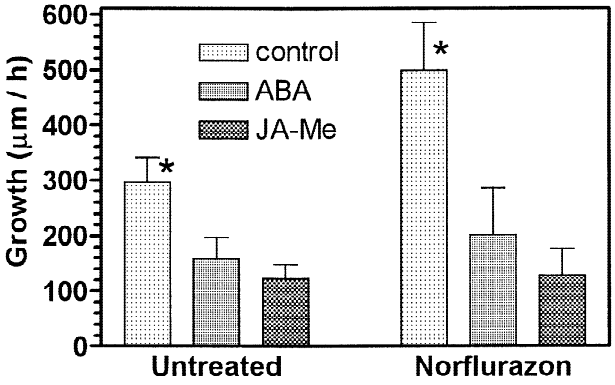

Fig. 3. Effect of JA-Me and ABA on elongation of *Zea mays* etiolated coleoptiles treated with norflurazon. Coleoptile segments (60–68 mm long) were obtained from untreated seedlings ($n = 6$) or 100 μM norflurazon-treated seedlings ($n = 5$) and exposed to 10 μM ABA or 100 nM JA-Me in incubation buffer. Data are mean ± SE, and the asterisk indicates that the treatments are significantly different from their respective controls ($p < 0.05$).

inhibiting growth was similar to that induced by 10^{-5} M ABA (data not shown). Previously, sucrose was shown to alleviate JA inhibition of IAA-induced growth in oat coleoptiles (Ueda et al. 1994, 1995) so we tested the effect of sucrose on maize coleoptile growth. In the presence of 0.1 M sucrose, JA at equimolar concentrations reduced IAA-induced growth of maize coleoptiles to control levels (data not shown).

Effect of Norflurazon on Coleoptile Elongation Growth

Norflurazon inhibits accumulation of unsaturated carotenoids (Bartels and McCullogh 1972). Maize grown in the presence of norflurazon is bleached in appearance and distinctly white compared with normal etiolated maize seedlings, which have a yellowish color presumably caused by endogenous carotenoids. Such maize shoots contained less than 10% of ABA levels found in normally grown shoots (R. Munns, personal communication), indicating that norflurazon treatment reduces ABA in maize shoots and in roots (Feldman and Sun 1986). Growth of coleoptile segments from seedlings grown in the presence of norflurazon was significantly inhibited by JA-Me or ABA (Fig. 3). Fig. 3 also demonstrates that the initial length of the coleoptile segments does not affect the trend of the response to JA-Me. JA-Me also effectively inhibited elongation growth induced by IAA in seedlings grown either in the presence or absence of norflurazon (Fig. 4). However, in this particular experiment, the basal growth rate was low so that JA-Me at 10^{-8} M by itself was not effective at inhibiting growth. Although ABA inhibited IAA-induced growth, it too was ineffective at significantly reducing basal growth in this experiment. JA also inhibits growth of norflurazon-treated maize seedlings in the presence or absence of IAA (data not shown).

Cytosolic pH Responses to JA in Maize Coleoptiles Measured by a Fluorescent Dye and Confocal Microscopy

Previously we showed that JA induces cytosolic alkalization and closure of guard cells of *Paphiopedilum* in a similar manner to ABA (Gehring et al. 1997). Because JA modulated coleoptile growth responses with some similarity to ABA and these responses occurred when ABA was maintained at very low levels, we speculated that JA might also raise pH_i in maize coleoptiles. To test this hypothesis, we ester loaded coleoptiles with Snarf-1 and followed fluorescence changes by confocal microscopy. After exposure to JA-Me (50 nM bathing solution), increases in emission ratios were observed in maize coleoptile cortical cells (Fig. 5). A range of emission ratio values is seen for both the control and treated cells, which reflects the heterogeneity between cells within the tissue. The cortical cells have basal oscillations in pH_i that are out of phase with each other while maintaining a resting pH_i of approximately 6.95–7.1. It should be noted that not all cells of the coleoptile segment responded identically to the presence of JA-Me; many increased their ratio value while some others oscillated their ratio values (data not shown). The data presented in Fig. 5 are the mean of cortical cells randomly selected within the image field and followed over the entire imaging period for the control and JA-Me–treated coleoptile segments. Cortical cells were selected because the number of cortical cells with a cytoplasmic area in focus is larger per section than for epidermal cells. Also there is conceivably less nonspecific dye binding to cortical cells than epidermal cells, although we observed a similar trend in the responses of both cortical and epidermal cells to JA-Me. The emission ratios from these cells were then analyzed by one way repeated measure ANOVA. After JA-Me treatment at 50 nM, a distinct alkalization trend sets in by 10 min and reaches approximately 0.4 pH unit after 15–20 min, which is significantly greater than the control coleoptile (Fig. 5). In the absence of JA-Me, no significant change in fluorescence ratio is observed over 15 min (Fig. 5).

Cytosolic pH responses Measured by in vivo ^{31}P NMR Spectroscopy

To validate the data obtained from the fluorescent dye studies, we also obtained ^{31}P NMR spectra of coleoptile segments before and after exposure to JA. A typical

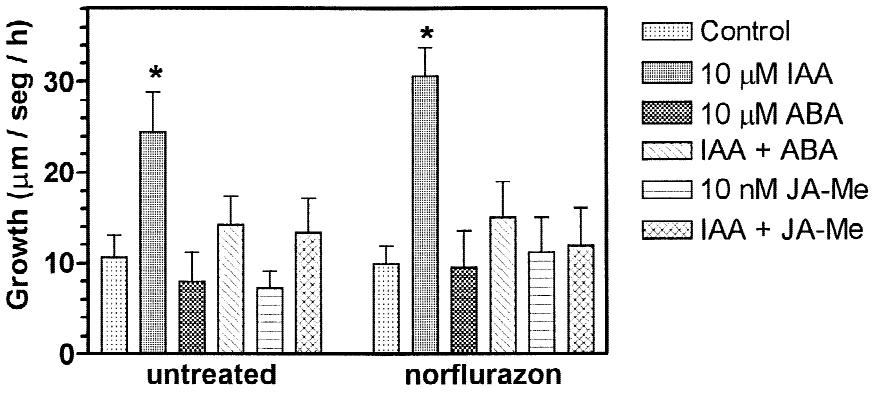

Fig. 4. Effect of JA-Me and ABA on elongation of IAA-treated *Zea mays* etiolated coleoptiles treated with norflurazon. Six excised coleoptile segments from either untreated or seedlings treated with 100 μM norflurazon were threaded apical end upwards on nichrome wire, incubated in incubation buffer with the appropriate treatment at 26°C, and the total length of the combined six segments measured. Control incubations contained 0.05% ethanol because IAA stock was dissolved in ethanol. Data are mean ± SE ($n = 9$ wires), and all treatments were significantly different from IAA alone ($p < 0.05$).

spectrum is presented in Fig. 6. It can be seen that a large peak corresponds to the vacuolar phosphate (Pi) and a relatively small peak corresponds to the cytoplasmic Pi in the untreated segments. After the addition of JA, the vacuolar peak is nearly unchanged, whereas the cytoplasmic peak decreases corresponding to a cytosolic alkalization. We observed such a shift in the minor cytoplasmic peak in two other experiments ($n = 4$), but at not time were shifts in the major vacuolar peak evident.

Cytosolic pH Responses to JA in Norflurazon Treated Coleoptiles

To determine whether pH_i would rise in the absence of possible increases in endogenous ABA levels, coleoptiles from maize grown in the presence of norflurazon were also loaded with Snarf-1. When JA-Me (45 nM bathing solution) is added to norflurazon-treated coleoptile segments ($n = 12$), an increase in fluorescence ratio is observed in cells from 67% of the coleoptile segments, and no change or a very slight increase was observed in cells from the remaining segments. Fig. 7 depicts the change observed in maize coleoptile cortical cells to JA-Me. In this particular example, the emission ratio of the cells in the treated coleoptile was significantly higher than their basal level and the control coleoptile within 5 min, and maximal alkalization was reached by 8 min (Fig. 7). Alternatively, in another type of response seen in other experiments, a significant increase in emission ratio was not observed until 10 or 15 min after exposure to JA-Me. No such changes were seen in the absence of JA-Me (Fig. 7), and these control values do not differ from those of maize germinated with water only (compare Figs. 5 and 7). Coleoptiles from maize grown in the presence of norflurazon also responded to ABA with a significant increase in emission ratio, corresponding to an increase in pH_i of 0.2 (Fig. 7). The time and magnitude of this response to 50 μM ABA is similar to that in the slower response to JA-Me (e.g., Fig. 5), although slower than the response to 45 nM JA-Me depicted in Fig. 7.

Discussion

Jasmonates (at μM levels) have been reported to directly inhibit growth of roots (other than tubers) and shoots (Sembdner and Parthier 1993, Smulders and Horton 1991, Yamane et al. 1981), although their effect on maize is not documented. However, more recent work suggests that JA alone does not inhibit growth (Montague 1997, Ueda et al. 1994, 1995) and may in some instances even promote growth as in oat internode tissue (Montague 1997). Our results indicate that JA at physiological concentrations of 10^{-9}–10^{-5} M can inhibit elongation growth in etiolated maize coleoptiles (Fig. 1). JA-Me at 10^{-7} M also inhibited growth in etiolated maize coleoptiles (Fig. 3), indicating that the effects of both JA and JA-Me on growth are similar. The shape of the growth inhibition curve (Fig. 1) is rather unusual, with the lower concentrations of JA being more effective. Whether this result is specific to maize coleoptiles remains to be determined. The concentrations of JA used in this study were generally lower (nM compared with μM levels) than those used in other studies, and this could indicate that maize coleoptiles are more responsive to JA. Alternatively, because the lower concentrations of JA were more effective at inhibiting growth, it is possible that such as response may also occur in other species (which has not been tested to our knowledge). Although we generally observed inhibition of growth in response to JA, this was dependent on the rate of basal control growth (see Fig. 4). These observations may explain some of the inconsistencies reported in the literature about the ability of JA alone to inhibit growth.

Although reports on the effect of JA alone on elongation growth appear inconsistent, several studies indicate

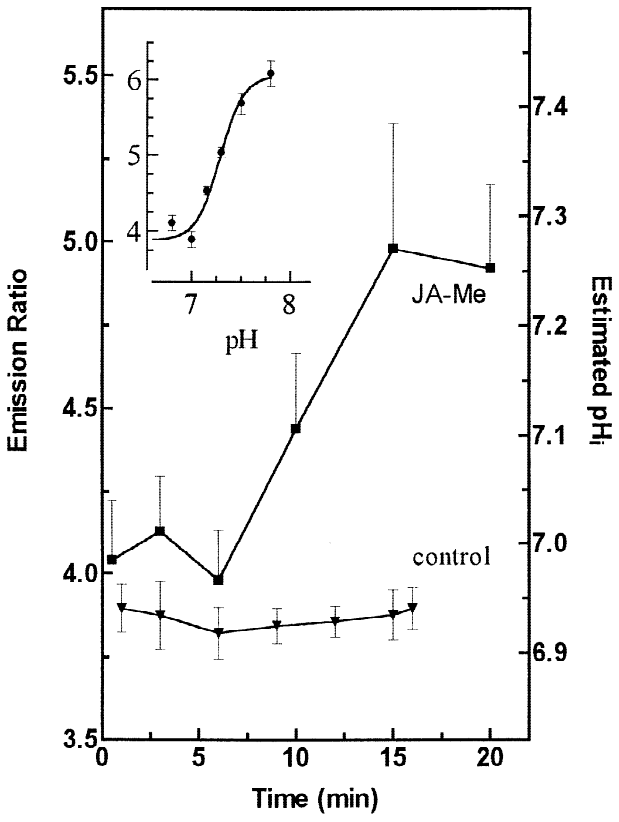

Fig. 5. Effect of JA-Me on intracellular pH of *Zea mays* etiolated coleoptile cells. Coleoptile segments were loaded with Snarf-1, excited at 514 nm, and emission signals greater than 610 nm and between 555 and 580 nm collected. The background was subtracted, and the resultant signal ratioed for five individual cells from each coleoptile segment. The mean ± SE of the control cells (▼) and 50 nM JA-Me treated cells (■). The inset shows the calibration curve determined for intracellular pH of cells (●) within coleoptile segments.

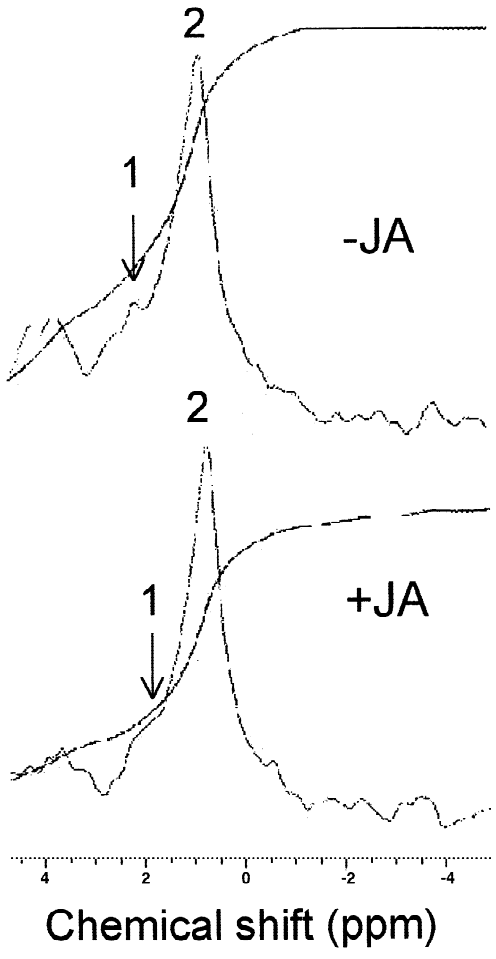

Fig. 6. Effect of JA on the in vivo ^{31}P NMR spectra of *Zea mays* coleoptiles. The upper spectrum was acquired during 17 min and in the absence of JA. The lower spectrum represents the identical sample after the addition of JA (final concentration 100 nM) and a further 17-min acquisition. Resonances are assigned to cytoplasmic Pi (peak 1) and vacuolar Pi (peak 2).

that JA antagonizes elongation-promoting signals such as IAA and GA. For example, elongation growth promoted by auxin in oat coleoptiles and pulvini was inhibited by JA (Montague 1997, Ueda et al. 1994, 1995) as was GA-promoted elongation in oat internodes (Montague 1997). In this work, we have found that JA or JA-Me at concentrations from 10^{-8}, 10^{-7}, and 10^{-5} M inhibited the IAA-induced growth of excised maize coleoptiles (Figs. 2 and 4). Some slight indication also exists of a biphasic response because JA at 10^{-6} M was less effective at inhibiting IAA-induced growth. The effective concentrations are two or more orders of magnitude lower than that described as inhibiting auxin-induced growth in other species. This again may indicate that maize is particularly sensitive to JA and confirms that in at least two cereals (oat and maize), JA and JA-Me inhibit IAA-induced elongation growth in etiolated coleoptiles.

Because many of the actions of ABA and jasmonates are similar (Sembdner and Parthier 1993), it is possible that the effect of JA or JA-Me is mediated by ABA. To minimize the effect of ABA in maize coleoptiles, we imbibed and grew the maize in norflurazon, which inhibits carotenoid production (Bartels and McCullough 1972) and reduces ABA levels (Feldman and Sun 1986). Coleoptiles from these seeds grew in response to auxin, and their growth was inhibited by either JA-Me or ABA (Fig. 3 and 4). In our hands, JA-Me (at 100 nM) was more potent than ABA (10 μM) at inhibiting elongation growth (Fig. 3). These results indicate that JA-Me and JA act independently of ABA to inhibit growth. The results of two other recent studies on oat also suggested that JA and ABA acted by different mechanisms to inhibit elongation growth. Sucrose or glucose alleviated JA inhibition of IAA-induced growth in oat coleoptiles, whereas

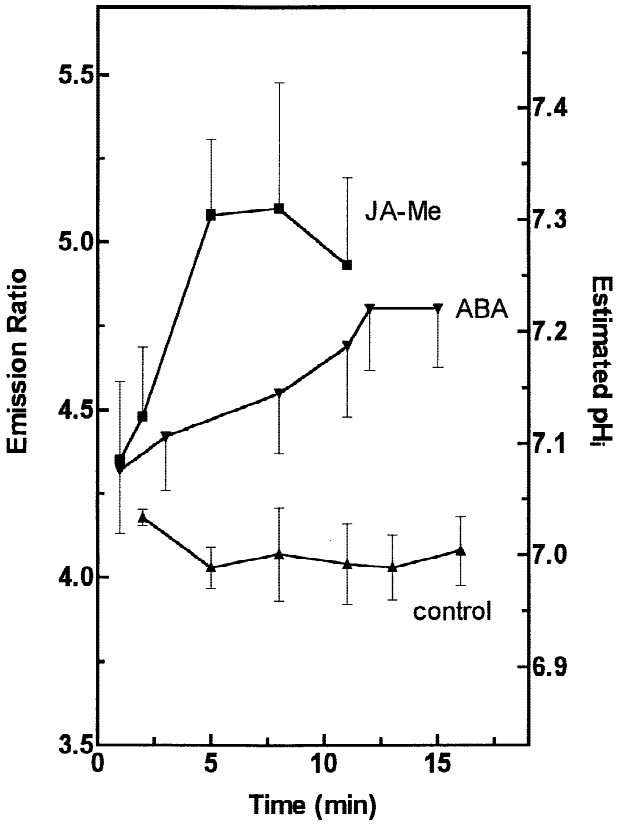

Fig. 7. Effect of JA-Me on intracellular pH of etiolated coleoptile cells from *Zea mays* grown in the presence norflurazon. Etiolated coleoptile segments from seedlings treated with 100 μM norflurazon were loaded with Snarf-1, excited at 514 nm, and emission signal greater than 610 nm and between 555 and 580 nm collected. The background was subtracted and the resultant signal ratioed. The mean ± SE of five cells from control (▲), 45 nM JA-Me (■), and 50 μM ABA (▼) treated coleoptile segments.

the response to ABA was unaffected by these treatments and occurred earlier than the response to JA (Ueda et al. 1994, 1995). However, we observed that sucrose had no effect on the response to JA in maize coleoptiles, indicating differences between maize and oat coleoptiles. ABA and JA act with different kinetics to synergistically inhibit growth in the GA-responsive oat internode region (Montague 1997).

The elongation growth of maize coleoptiles is also inhibited by ABA (Kutschera and Schopfer 1986) and procaine (Brummer et al. 1985). Both of these treatments raise cytosolic pH (Gehring et al. 1990). We previously observed that JA-Me raised pH_i in guard cells (Gehring et al. 1997), and in this study we also observed an increase in the pH_i of maize coleoptile cells in response to JA and JA-Me at nM concentrations (Fig. 5 and 6). These increases in pH_i were detected by two different methods (fluorescent dyes and NMR), hence we are confident that they represent a genuine physiological response to low concentrations of JA or JA-Me. The NMR recognizes the vacuole as the spatially dominant compartment of the cell (in which we incidentally see no change in pH) and the cytosol as a significantly smaller part where we observe an increase in pH. The signal that we observe from Snarf-1 theoretically represents the whole interior of the cell because some Snarf-1 may be present in the vacuole that we cannot detect as vacuolar pH is below the range of our detection methods (see Gehring et al. 1994). Increases in pH_i were also observed in coleoptile cells from norflurazon-treated maize in response to either JA-Me or ABA (Fig. 7), again implying an ABA-independent signal pathway for JA. This signal transduction pathway activates a downstream effector(s), resulting in increased pH_i that is associated with growth inhibition.

There appears to be a correlation with elongation growth and changes in pH_i. Auxin treatment of maize coleoptiles results in a decrease in pH_i (Brummer et al. 1985, Felle et al. 1986). Two different methods, namely pH microelectrodes and pH-indicator dyes, both detected equivalent decreases in pH_i after treatments with IAA or weak acids (Brummer et al. 1985, Felle et al. 1986, Gehring et al. 1990). However, ^{31}P NMR studies on cytoplasmic and vacuolar pH in pea internode sections failed to detect pH changes in response to IAA (Talbot et al. 1988). The reasons for this inconsistency are not clear. At any rate, substances that appear to acidify the cytosol of maize coleoptiles, namely GA (Gehring et al. 1994), IAA, and weak acids, all induce elongation growth. On the other hand, agents that alkalize the cytosol (e.g., ABA and procaine) interfere with auxin-induced growth. Because JA and JA-Me also raise pH_i and inhibit such growth in maize coleoptiles, the results further support a role for pH_i changes in the regulation of elongation growth. The mechanism is unknown. The acid growth theory postulates that auxin increases the outwardly directed electrogenic proton pump, the consequent acidification of the cell wall causing wall loosening and growth by activating expansins (Cosgrove 1998). However, small changes in pH_i are unlikely to directly affect H^+ secretion (Frohnmeyer et al. 1998).

A distinct pattern is emerging suggesting that changes in pH_i provide a permissive milieu for signal transduction events (i.e., by activating some proteins and inhibiting others). Small changes in pH_i may function as a means to integrate the signals from messenger molecules such as plant hormones. Protons can exert effects on responsive proteins without requiring specialized binding sites (Busa 1986). Such changes in pH_i in plants regulate the outward and inward K^+ channels in guard cells (Blatt and Armstrong 1993, Blatt and Thiel 1994, Frohnmeyer et al. 1998). When pH_i is buffered with the weak acid propionate, JA no longer induces stromatal closure (Gehring et al. 1997). Under such conditions ABA cannot activate the outward K^+ channel that is sensitive to pH_i (Blatt and Armstrong 1993). The alka-

lization induced by JA could form a permissive environment for the signal transduction pathway underlying many of the previously observed effects of jasmonates on growth.

Acknowledgments. This work was supported by the Australian Research Council and the Monash Research Fund. We thank Dr. C. A. Ryan (Washington State University, Pullman, Washington) and Dr. W. Taylor (CSIRO Plant Industry, Canberra ACT, Australia) for kindly supplying the methyl jasmonate and norflurazon, respectively. We also gratefully acknowledge Dr. R. Munns (CSIRO Plant Industry, Canberra ACT, Australia) for processing the ABA assay and Ms. C. Ainslie and Mr. R. Wallis who assisted with some of the growth experiments.

References

Bartels P, McCullogh C (1972) A new inhibitor of carotenoid synthesis in higher plants. Biochem Biophys Res Commun 48:16–22

Berger S, Bell E, Mullet JE (1996) Two methyl jasmonate-insensitive mutants show altered expression of *AtVsp* in reponse to methyl jasmonate and wounding. Plant Physiol 111:525–531

Blatt MR, Armstrong F (1993) K^+ channels of stomatal guard cells: Abscisic-acid-evoked control of the outward rectifier mediated by cytoplasmic pH. Planta 191:330–341

Blatt MR, Thiel G (1994) K^+ channels of stomatal guard cells: bimodal control of the K^+ inward-rectifier evoked by auxin. Plant J 5:33–68

Brummer B, Bertl A, Potrykus I, Felle H, Parish RW (1985) Evidence that fusicoccin and indole-3-acetic acid induce cytosolic acidification of *Zea mays* cells. FEBS Lett 189:109–113

Brummer B, Felle H, Parish RW (1984) Evidence that acid solutions induce plant cell elongation by acidifying the cytosol and stimulating the proton pump. FEBS Lett 174:223–227

Busa WB (1986) Mechanisms and consequences of pH-mediated cell regulation. Annu Rev Physiol 48:389–402

Cosgrove DJ (1998) Cell wall loosening by expansins. Plant Physiol 118:333–339

Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 48:355–381

Feldman LJ, Sun PS (1986) Effects of norflurazon, an inhibitor of carotenogenesis, on abscisic acid and xanthoxin in the caps of gravistimulated maize roots. Physiol Plant 67:472–476

Felle H (1988) Auxin causes oscillations of cytosolic free calcium and pH in *Zea mays* coleoptiles. Planta 174:495–499

Felle H, Brummer B, Bertl A, Parish RW (1986) Indole-3-acetic acid and fusicoccin cause cytosolic acidification of corn coleoptile cells. Proc Natl Acad Sci USA 83:8992–8995

Frohnmeyer H, Grabov A, Blatt MR (1998) A role for the vacuole in auxin-mediated control of cytosolic pH by *Vicia* mesophyll and guard cells. Plant J 13:109–116

Gehring CA, Irving HR, McConchie R, Parish RW (1997) Jasmonates induce intracellular alkalization and closure of *Paphiopedilum* guard cells. Ann Bot 80:485–489

Gehring CA, Irving HR, Parish RW (1990) Effects of auxin and abscisic acid on cytosolic calcium and pH in plants cells. Proc Natl Acad Sci USA 87:9645–9649

Gehring CA, Irving HR, Parish RW (1994) Gibberellic acid induces cytoplasmic acidification in maize coleoptiles. Planta 194:532–540

Irving HR, Gehring CA, Parish RW (1992) Changes in cytosolic pH and calcium of guard cells precede stomatal movements. Proc Natl Acad Sci USA 89:1790–1794

Koiwa H, Bressan R, Hasegawa P (1997) Regulation of protease inhibitor and plant defense. Trends Plant Sci 2:379–384

Kutschera U, Schopfer P (1986) Effect of auxin and abscisic acid on cell wall extensibility in maize coleoptiles. Planta 167:527–535

Miyamoto K, Oka M, Ueda J (1997) Update on the possible mode of action of the jasmonates: Focus on the metabolism of cell wall polysaccharides in relation to growth and development. Physiol Plant 100:631–638

Montague MJ (1997) Exogenous jasmonic and abscisic acids act differentially in elongating tissues from oat stem segments. J Plant Growth Regul 16:11–19

Read ND, Allan WTG, Knight H, Knight MR, Malho R, Russell A, Shacklock PS, Trewavas AJ (1992) Imaging and measurement of cytosolic free calcium in plant and fungal cells. J Microsc 166:57–86

Roos W (1992) Confocal pH topography in plant cells: acidic layers in the peripheral cytoplasm and the apoplast. Bot Acta 105:253–259

Sembdner G, Parthier B (1993) The biochemistry and physiological and molecular actions of jasmonates. Annu Rev Plant Physiol Plant Mol Biol 44:569–589

Smulders MJ, Horton RF (1991) Ethylene promotes elongation growth and auxin promotes radial growth in *Ranunculus sceleratus* petioles. Plant Physiol 96:806–811

Staswick PE, Su W, Howell SH (1992) Methyl jasmonate inhibition of root growth and induction of a leaf protein are decrease in an *Arabidopsis thaliana* mutant. Proc Natl Acad Sci USA 89: 6837–6840

Talbot LD, Ray PM, Roberts JKM (1988) Effect of indoleacetic acid- and fusicoccin-stimulated proton extrusion on internal pH of pea internode cells. Plant Physiol 87:211–216

Ueda J, Miyamoto K, Aoki M (1994) Jasmonic acid inhibits the IAA-induced elongation of oat coleoptile segments: a possible mechanism involving the metabolism of cell wall polysaccharides. Plant Cell Physiol 35:1065–1070

Ueda J, Miyamoto K, Kamisaka S (1995) Inhibition of the synthesis of cell wall polysaccharides in oat coleoptile segments by jasmonic acid: relevance to its growth inhibition. J Plant Growth Regul 14:69–76

van der Veen R, Heimovaara-Dijkstra S, Wang M (1992) Cytosolic alkalization mediated by abscisic acid in necessary, but not sufficient, for abscisic acid-induced gene expression in barley aleurone protoplasts. Plant Physiol 100:699–705

Wasternack C, Parthier B (1997) Jasmonate-signalled plant gene expression. Trends Plant Sci 2:302–307

Yamane H, Takagi H, Abe H, Yokota T, Takahashi N (1981) Identification of jasmonic acid in three species of higher plants and its biological activities. Plant Cell Physiol 22:689–697