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# Exogenous Jasmonic and Abscisic Acids Act Differentially in Elongating Tissues from Oat Stem Segments

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**Abstract.** Segments can be cut from the peducular-1 internode of oat (*Avena sativa* L.) shoots so as to contain the graviresponsive, auxin-sensitive leaf sheath pulvinus, and the gibberellin-sensitive internodal tissue. These two growth-capable tissues were used to study the effects and interactions of jasmonic acid (JA) and abscisic acid (ABA) in regulating cell elongation. When supplied alone at physiologic concentrations ($10^{-5}$, $10^{-4}$ M), JA promoted growth and cell wall synthesis in the internodal tissue, whereas by itself, ABA inhibited internodal elongation and even inhibited JA-promoted growth. When gibberellic acid ($GA_3$) was used to stimulate internodal elongation, JA and ABA caused similar levels of inhibition and, at certain concentrations, were synergistic. Inhibition by ABA was initiated several hours earlier than inhibition by JA, and only the ABA effect could be partially overcome by $10^{-3}$ M aminoethoxyvinylglycine. Both JA and ABA inhibited elongation of pulvinar tissue that was induced to grow by gravistimulus or auxin, although here JA was more potent than ABA at equimolar concentrations. When $10^{-5}$ M fusicoccin was used as a general nonphysiologic growth stimulus, JA had no effect on the internode but inhibited the pulvinus, whereas ABA had no effect on the pulvinus but inhibited the internode. These results provide strong physiologic evidence that JA and ABA act by different mechanisms in the regulation of elongation, at least in this representative grass.

**Key Words.** Abscissic acid—*Avena*—Growth—Internode—Jasmonic acid—Pulvinus

**Abbreviations:** ABA, *cis*-abscisic acid; AVG, aminoethoxyvinylglycine; FC, fusicoccin; $GA_3$, gibberellic acid; Glc, glucose; IAA, indole-3-acetic acid; JA, jasmonic acid; MJ, methyl jasmonate; TES, *N*-tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid.

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Jasmonic acid (JA) and its derivatives such as methyl jasmonate (MJ) are found ubiquitously in plants and appear to have widely varying effects in different species (for reviews, see Parthier 1990, van den Berg and Ewing 1991, Staswick 1992, Sembdner and Parthier 1993, Reinbothe et al. 1994). Relatively little attention has been given to the regulation of elongation by JA, and some results have been contradictory. In early work, Yamane et al. (1981) reported that JA inhibited the elongation of gibberellic acid ($GA_3$)-sensitive rice seedling leaf sheaths and lettuce hypocotyls but failed to find an effect on indole-3-acetic acid (IAA)-stimulated *Avena* coleoptile growth. By using *Avena* coleoptile segments, however, Ueda et al. (1994, 1995) were able to show that JA inhibited IAA-induced elongation and, based principally on measurements of cell wall synthesis and on the finding that pretreatment with $3 \times 10^{-2}$ M sucrose overcame the inhibitory effect, concluded that JA may act by blocking the synthesis of cell wall polysaccharides.

Although ABA is also known to inhibit auxin-induced coleoptile growth (Kutschera and Schopfer 1986), no direct comparisons have been drawn between ABA and JA with regard to their effects and interactions in any auxin- or gibberellin-sensitive elongating plant tissue. A direct comparison was made, however, with regard to cytokinin-promoted growth in radish and cucumber cotyledons (Ueda and Kato 1982, Fletcher et al. 1983), where ABA was found to be a more potent inhibitor than MJ. On the other hand, several studies at the molecular level have demonstrated that many of the effects of JA are similar to those elicited by ABA, including the induction of the synthesis of similar patterns of specific mRNAs and proteins in barley leaves (Weidhase et al. 1987, Reinbothe et al. 1992, Lehmann et al. 1995). This type of evidence has been used to support a putative role for JA as an intermediate in the signaling pathway for the wounding response in potato leading from ABA to gene activation (Hildmann et al. 1992).

Segments cut from the next-to-last (peduncular-1) in-

ternode of 45-day-old *Avena sativa* L. (oat) plants (so as to contain the internodal intercalary meristem, the node below it, the encircling leaf sheath, and the leaf sheath pulvinus) constitute one of the most useful systems available for the study of GA-induced elongation (for a photograph of this system, see Montague 1995a). The internodal tissue within these 10-mm segments can elongate for at least 50 h, with a maximum growth rate of more than 1.5 mm/h, when $GA_3$ is supplied along with exogenous carbohydrate (Montague 1993). The graviresponsive, auxin-sensitive leaf sheath pulvinus, an anatomic feature common to several important cereals (e.g. oat, barley, wheat, rice), functions to reorient lodged grass shoots to a more upright position through differential growth. Auxin promotes both cell elongation and cell wall synthesis in this tissue and probably mediates the gravitropic response (Montague 1995b and references therein).

As defined above, the oat stem segment therefore possesses two distinct hormonally sensitive tissues. Each tissue responds to its own appropriate stimulus (the internodal tissue to GA, the pulvinar tissue to auxin and gravistimulus) with enhanced cell elongation, accompanied by cell wall synthesis, but without the complication of concomitant cell division (Kaufman et al. 1969, Dayanandan et al. 1976, Montague 1995b). Because the two tissues are closely connected via anastomosing vasculature (Montague et al. 1973, Montague 1995b), they are exposed simultaneously to any exogenous substance supplied through the node. Given these anatomic characteristics, the system provides a unique opportunity to compare the possible roles of JA and ABA in the regulation of cellular elongation in mature grass shoots by investigating two different growth-capable tissues, each with its own specific hormonal responsiveness, in the same isolated plant part. One aim of the comparison conducted in the present study was to obtain physiologic information bearing on the issue of whether JA and ABA are part of the same signaling pathway in grasses. Ultimately, of course, the goal of this ongoing work is to understand more completely the interaction of various signals in controlling the developmental physiology of agronomically important cereals.

## Materials and Methods

### *Plants*

Oat plants (*A. sativa* L. cv Victory; seed graciously supplied by Svalöf AB International, Svalöv, Sweden) were grown in 15-cm pots (200 seeds/pot) in a growth chamber (16 h light/8 h dark; 18°C/16°C) with a light intensity of 800 μE. Shoots were selected after about 45 days of growth so that the internode immediately below the peduncular node (p-1 internode) had reached a length between approximately 10 and 40 mm, thereby ensuring maximum growth response to $GA_3$ (Adams et al. 1973) as well as adequate gravitropic response (Montague 1995b). (For convenience, collected shoots were routinely held at 4°C for several days until further preparation. There was no appreciable decline over this time period in their response.)

### *Tissue Preparation and Growth Conditions*

Ten-mm segments containing the nongrowing node and leaf sheath, together with the growth-capable internodal tissue and leaf sheath pulvinus, were prepared with a razor blade cutting device. In experiments designed to study internodal elongation, the segments were placed upright in perforated plastic frames on filter paper in the lids of 55-mm plastic Petri dishes. Each dish contained 3 mL of treatment solution, which was taken up from the base (through the node) of each segment. Thus, the existing vasculature delivered nutrient and hormone to the segment. One dish was used per treatment, and the figure legends indicate the number of segments employed in each experiment. $GA_3$ was supplied at 100 μM, a concentration well in excess of the minimum necessary for maximum growth, to ensure that this hormone was never rate limiting (Montague et al. 1973). Glc was supplied at the maximally effective concentration of 0.1 M (Adams et al. 1973). Neither buffer nor other added ions were included in the medium because results from previous work (Montague 1993, 1995a, 1995b, and unpublished data) consistently showed that they had no effect on the maximum growth response in this system. (At the concentrations tested, the hormones did not appreciably affect the initial pH of the growth medium, which was about 5.5.) Segments were grown at 30°C in the dark enclosed in plastic containers at 100% relative humidity with provision for some air exchange. The lengths of the internodes were measured to the nearest 0.5 mm with a ruler using a magnifying lens under a dim green safelight.

In experiments designed to study the elongation of the leaf sheath pulvinus, segments with 2-mm pulvini were selected carefully and placed individually in wells of a 96-well microtiter plate. The node of each segment was in contact with a small piece of U.S.P. grade cotton (packed in the bottom of each well) saturated with 50 or 80 μL of 0.1 M glucose (Glc) with or without other compounds as described for each experiment. The segments were allowed to grow upright (vertical segments), or the 96-well plate was tilted 90° to provide the gravistimulus to the pulvini. Segments were incubated at 30°C in the dark as described above. The lengths of the downward facing sides of the pulvini (in the case of gravistimulus) or of the longest sides (in the case of treatment with IAA or fusicoccin [FC]) were measured to the nearest 0.5 mm with a ruler under a dim green safelight using a magnifying lens.

All key findings were repeated in alternate experimental contexts and with unrelated batches of segments. Statistical analyses were performed as appropriate, and details are given in the figure legends.

### *Cell Wall Preparation*

Cell wall material was prepared using a method adapted from Baker and Ray (1965) and Takahashi et al. (1995). Oat internodal tissue, dissected from three segments as described before (Montague 1995a, 1995b), was placed in 1.25-mL capped plastic tubes and frozen immediately on dry ice. The tissue was extracted with 1.0 mL of 80% (v/v) ethanol for at least 24 h at room temperature, the ethanol was removed, and the tissue was extracted once more with 1 mL of fresh 80% (v/v) ethanol for at least an additional 24 h. The tissue was then crushed between two glass plates, replaced in the capped tube with 1 mL of 0.1 M sodium acetate buffer (pH 6.5), and autoclaved at 121°C for 0.5 h. After the acetate buffer was removed, 1 mL of porcine pancreatic α-amylase (58 units/mL in 10 mM TESNaOH buffer (pH 7.0) was

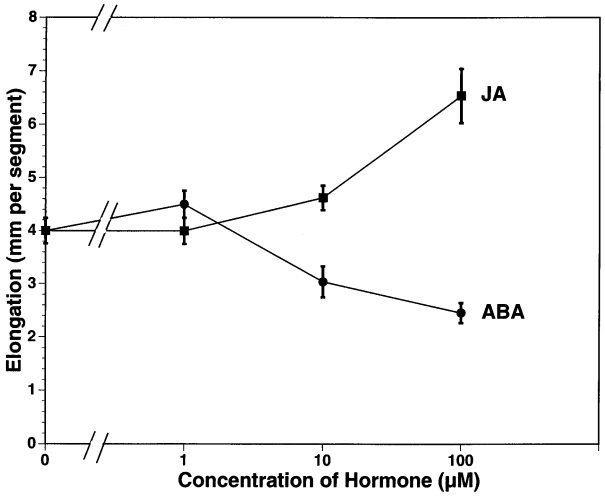


**Fig. 1.** Dose-response of *Avena* internodal tissue to JA and ABA. Samples of 12 stem segments were grown on 0.1 M Glc in the presence or absence of varying concentrations of JA or ABA for 71 h. Means for internodal elongation are presented with S.E.

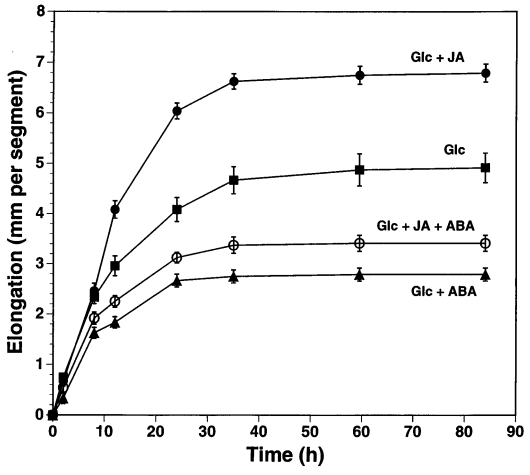


**Fig. 2.** Time course of the effect of JA and ABA on elongation of *Avena* internodal tissue. Samples of 11 stem segments were grown on 0.1 M Glc in the presence or absence of $10^{-4}$ M JA or ABA, alone or combined. Means for internodal elongation are presented with S.E.

added and held overnight at room temperature to digest any starch that might be present. After the α-amylase was removed, a pepsin (porcine stomach mucosa) solution (1,040 units/mL in 30 mM potassium phosphate buffer, pH 2) was added and held overnight at room temperature to digest protein. The pepsin was then removed, the material was washed with 1 mL of water, and the residue (considered to be total cell wall material) was dried over Drierite® for at least 3 days prior to determination of dry weight. Cellulosic and noncellulosic cell wall components, based upon differential solubilization in hot TFA and synthesized from [$^{14}$C]Glc, were determined as before (Montague 1995a, 1995b) following extraction of the internodal tissue with 80% (v/v) ethanol, pepsin, and α-amylase.

### *Chemicals*

±JA, ±ABA, IAA, FC, BA, $GA_3$, pepsin, α-amylase, aminoethoxyvinylglycine (AVG) and Glc were obtained from Sigma. JA, IAA, BA, FC, and ABA were dissolved in methanol before addition to the growth medium. The methanol concentration never exceeded 2%, and an identical concentration was supplied to control tissue. All other chemicals were of reagent grade.

## Results

### *Dose Response of Internodal Elongation to JA and ABA*

The effects of JA and ABA were determined first on internodal tissue in segments receiving no treatment other than Glc and the growth regulator. The results of a dose-response determination (0 to $10^{-4}$ M) are presented in Fig. 1. JA and ABA produced opposite effects in the internodal tissue over this physiologic concentration range. JA promoted elongation to a small extent at $10^{-5}$ M and by 164% of the control elongation at $10^{-4}$ M. In contrast, ABA inhibited internodal elongation, with the internodes growing to only 62% of the control elongation at $10^{-4}$ M. Thus, elongation in JA-treated internodal tissue was about 2.6-fold greater than in ABA-treated tissue when the hormones were each supplied at $10^{-4}$ M.

In a separate experiment designed to test the requirement for exogenous Glc, treatment with $10^{-4}$ M JA produced elongation to 154% of the control in the absence of Glc, whereas $10^{-4}$ M ABA resulted in elongation to 74% of the control. When 0.1 M Glc was supplied in the same experiment, $10^{-4}$ M JA produced elongation to 171% of the control, whereas $10^{-4}$ M ABA resulted in elongation to 50% of the control. Therefore, although exogenous Glc was routinely supplied, neither the stimulatory effects of JA nor the inhibitory effects of ABA required its presence.

### *Time Course of Internodal Elongation in Response to JA and ABA*

The time course of elongation of internodal tissue in segments treated with $10^{-4}$ M JA or ABA, separately and together, is shown in Fig. 2. As in Fig. 1, JA promoted and ABA inhibited elongation. The stimulatory effect of JA (compared with the Glc control) was initiated between 8 and 12 h, whereas the inhibitory effect of ABA was apparent by 2 h, which was the earliest time point taken. Also, ABA overcame much of the stimulatory effect of JA when the two hormones were supplied together. The final growth measurement after 84 h showed that JA produced 138% of the Glc control growth, whereas ABA-treated internodal tissue grew to only 57% of the control. Taken together with the results of Fig. 1,

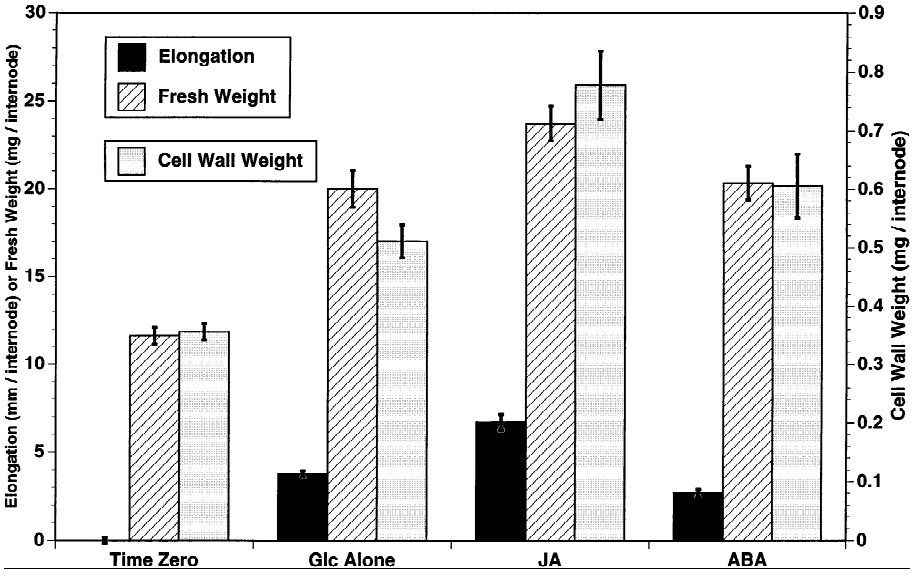


**Fig. 3.** Effects of JA and ABA or internodal elongation, fresh weight, and cell wall dry weight. Samples of 18 stem segments were grown on 0.1 M Glc in the presence or absence of $10^{-4}$ M JA or ABA for 24 h. At that time, elongation was measured, and the internodal tissue was dissected from the rest of the segment. The internodes were divided into six lots of three for fresh weight determination, cell wall isolation, and wall weight determination. Elongation is presented as the mean of 18 segments with S.E. Mean fresh weights and cell wall dry weights were calculated per segment and are presented with S.E. for the six lots.

these data indicate that JA and ABA act oppositely in regulating the elongation of oat internodal tissue when supplied without other hormones. Also, they show that ABA overcomes most of the promotional effect of JA when the two growth regulators are supplied together at equimolar concentrations.

### *Effects of JA and ABA on Cell Wall Synthesis in Internodal Tissue*

To determine whether the enhancement of elongation by JA was accompanied by enhanced cell wall synthesis, as occurs with $GA_3$ (Montague 1995a, 1995b), stem segments were grown in the presence of 0.1 M Glc alone or along with $10^{-4}$ M JA or ABA for 24 h. The results obtained from measurement of elongation, fresh weight, and cell wall dry weight are shown in Fig. 3. As expected, Glc-treated segments grew somewhat, as reflected in increased length, fresh weight, and cell wall weight compared with time zero. JA stimulated all three parameters compared with the Glc control (by 178, 119, and 152%, respectively). ABA-treated segments elongated to only 78% of the control level. Both fresh weight increase and cell wall increase with ABA treatment were similar to the Glc control. When cell wall synthesized from [$^{14}$C]Glc was studied under similar conditions, JA stimulated incorporation of radioactivity into both the cellulosic and noncellulosic components of the cell wall by 242 and 179% of the Glc control, respectively (data not shown).

### *Effects of JA and ABA on $GA_3$-promoted Internodal Elongation*

Of the substances known to promote the growth of oat internodal tissue, GA produces by far the largest response (Montague 1995b and references therein). $GA_3$-treated internodal tissue typically elongates eight- to tenfold more than tissue treated with Glc alone (Montague 1993). It was therefore of obvious interest to compare the interactions of JA and ABA with $GA_3$ when the hormones were supplied simultaneously to this tissue. Fig. 4 presents the results of treatment with a 4 × 4 matrix of concentrations where JA and ABA were supplied alone and together at 0, $10^{-6}$, $10^{-5}$, and $10^{-4}$ M. $GA_3$ was present in all treatments at a concentration of $10^{-4}$ M.

Both JA and ABA inhibited $GA_3$-dependent internodal elongation in a concentration-dependent manner. The dose-response relationship for ABA was similar to that reported previously by Kaufman and Jones (1974). As in Figs. 1 and 2, JA was active at physiologic levels ($10^{-6}$ to $10^{-4}$ M). At $10^{-4}$ M, JA and ABA inhibited $GA_3$-induced elongation to about the same extent (by 60–65% of the control growth). In addition, JA and ABA interacted with each other. At several concentrations, the inhibitory effect of one of the hormones was enhanced by the presence of the other. For example, when JA was supplied alone at $10^{-6}$ M, it inhibited growth by 23% of the control. When ABA was supplied alone at $10^{-4}$ M, it inhibited growth by 65% of the control. When $10^{-6}$ M JA was combined with $10^{-4}$ M ABA, however, growth was inhibited by about 78% of the control value, substantially more than with ABA alone. Such interactions were apparent with several other combinations, in particular at higher ABA levels.

These results show that JA and ABA have similar capacities to inhibit $GA_3$-induced growth in oat internodal tissue, even acting over the same concentration range. The two hormones probably do not act by precisely the same mechanism to accomplish this effect, however, as evidenced by the synergy observed when combinations of the two hormones were applied. At the concentrations studied, JA, supplied by itself or with

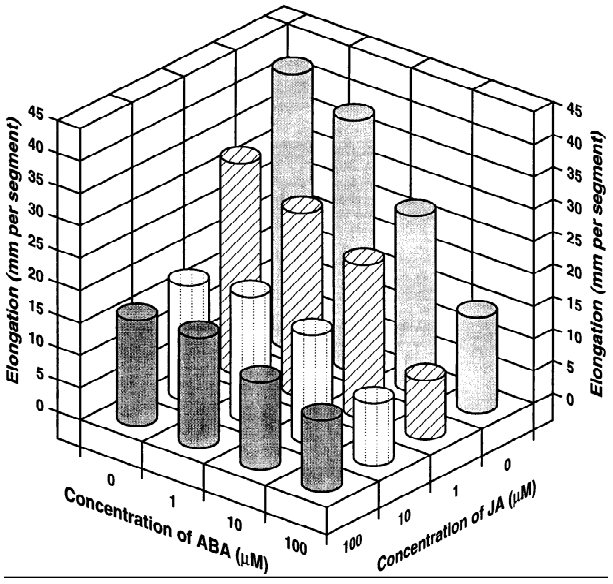


**Fig. 4.** Effects of JA and ABA on the elongation of $GA_3$-treated *Avena* internodal tissue. Samples of 11 stem segments were grown on 0.1 M Glc and $10^{-4}$ M $Ga_3$ in the presence or absence of the indicated concentrations of JA or ABA for 58 h. Means for internodal elongation are presented with S.E.

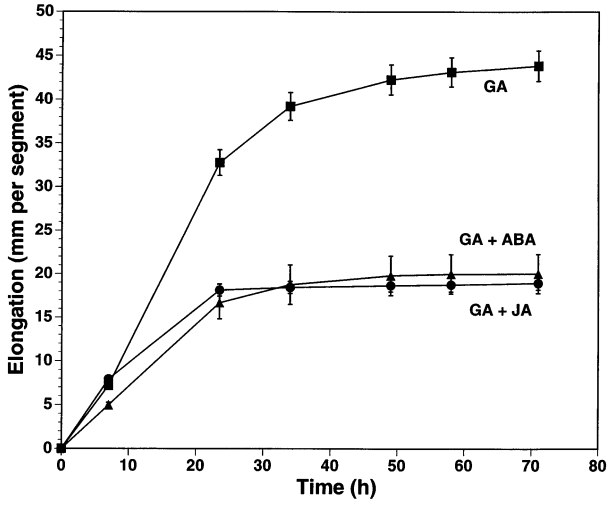


**Fig. 5.** Effects of JA and ABA on the kinetics of elongation of $GA_3$-treated *Avena* internodal tissue. Samples of 11 stem segments were grown on 0.1 M Glc and $10^{-4}$ M $Ga_3$ in the presence or absence of $10^{-4}$ M JA or ABA. Means for internodal elongation are presented with S.E.

$GA_3$, did not cause any apparent necrosis or loss of turgor even after 70 or 80 h of exposure, indicating limited general toxicity to the tissue. In fact, based strictly on the appearance of the tissue, JA was less toxic than ABA. Microscopic examination of epidermal peels revealed no obvious abnormalities in either ABA- or JA-treated internodal tissue. Attempts to reverse the inhibition caused by JA or ABA by supplying BA (which itself inhibited $GA_3$-promoted internodal elongation) were not successful (data not shown). Moreover, treatment with JA failed to alter the $GA_3$ dose-response relationship of the internodal tissue (data not shown), indicating that JA does not act by decreasing the sensitivity of the tissue to exogenous $GA_3$.

### *Kinetics of Elongation of $GA_3$-treated Internodal Tissue in Response to JA and ABA*

The time course of elongation of internodal tissue from stem segments supplied with Glc and $GA_3$, with or without $10^{-4}$ M JA or ABA, is shown in Fig. 5. As in Fig. 4, $10^{-4}$ M JA and ABA inhibited elongation to about the same extent (by about 55% of the control). The kinetics, however, were different for the two hormones. Inhibition by JA was not observed until after 7 h of growth, whereas the inhibition by ABA was observed even at this early time point, consistent with results obtained by Kaufman and Jones (1974). This difference in time of onset of the effects of JA and ABA was observed repeatedly and consistently in many different experiments. Although the final magnitudes of the inhibitory effects of JA and ABA were similar at similar concentrations, the times of onset of the inhibition were different, with ABA acting earlier.

### *Effect of AVG on Internodal Elongation*

AVG is a potent, relatively specific inhibitor of ethylene biosynthesis in plants (Guzman and Ecker 1990). To determine whether ethylene might be involved in the inhibition of $GA_3$-induced elongation of oat internodal tissue by either JA or ABA, $10^{-3}$ M AVG was supplied in combination with $GA_3$, JA, or ABA, each at $10^{-4}$ M. The results in Fig. 6 show first that AVG had no effect on $GA_3$-promoted elongation. Given alone, both JA and ABA inhibited elongation substantially (by about 68% of the $GA_3$ control), as before. Although AVG had no statistically significant effect on inhibition of growth by JA, it did substantially (though not entirely) overcome the inhibitory effect of ABA. Elongation obtained with $GA_3$ + ABA + AVG was nearly twofold greater than elongation obtained with $GA_3$ + ABA alone.

In other experiments, JA- or ABA-treated segments (also supplied with $GA_3$) were allowed to grow in the same enclosed vessel with reporter segments (treated with Glc alone or with Glc + $GA_3$). When compared with appropriate controls, no evidence was obtained that either JA- or ABA-treated segments produced a gas that could influence the growth of the reporter segments (data not shown). Nonetheless, AVG could still act by inhib-

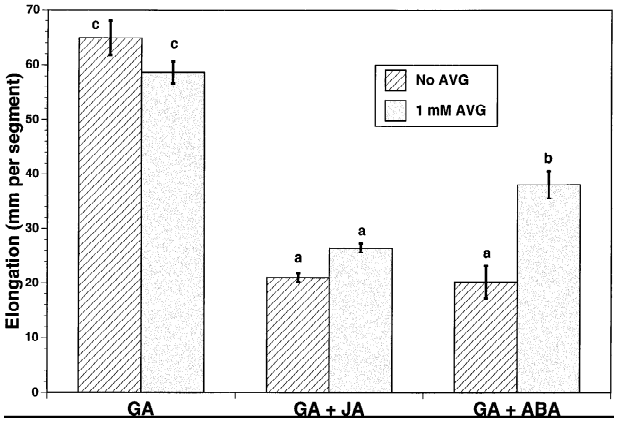


**Fig. 6.** Effects of AVG on the inhibition of elongation of $GA_3$-treated *Avena* internodal tissue by JA and ABA. Samples of 11 stem segments were grown on 0.1 M Glc and $10^{-4}$ M $GA_3$ in the presence or absence of $10^{-4}$ M JA or ABA, with or without $10^{-3}$ M AVG. Elongation was measured after 81.5 h, and means are presented with S.E. Treatments identified with the same lowercase letter are not significantly different ($p < 0.05$) by the Studentized range procedure.

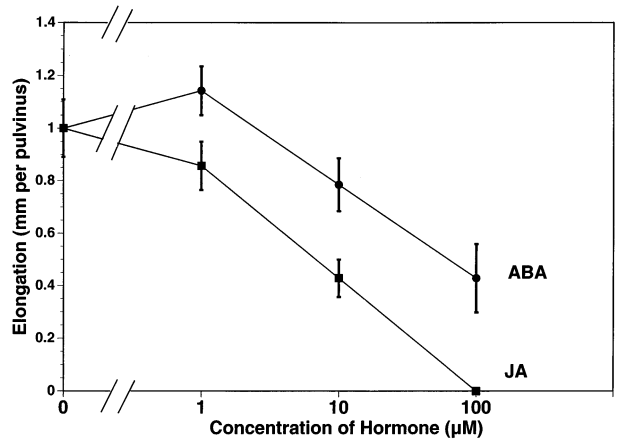


**Fig. 7.** Dose response of gravistimulated *Avena* pulvinar tissue to JA and ABA. Samples of seven stem segments were grown on 0.1 M Glc with gravistimulus in the presence or absence of varying concentrations of JA or ABA for 24.5 h. Means for pulvinar elongation are presented with S.E.

iting the biosynthesis of ethylene, which may display only a local effect in oat stem tissue. Whether or not ethylene is involved in the inhibitory response to ABA, treatment with AVG certainly revealed an additional difference between JA and ABA, again indicating that the two growth regulators inhibit $GA_3$-induced elongation of oat internodal tissue by different mechanisms.

### *Dose Response of Pulvinar Tissue to JA and ABA*

Turning to the pulvinus, the results of a dose-reponse comparison of the effects of JA and ABA (0–$10^{-4}$ M) on gravistimulated stem segments are presented in Fig. 7. Both JA and ABA inhibited elongation of the pulvinus, although JA was considerably more effective at all concentrations tested. At $10^{-4}$ M, JA consistently blocked all elongation in gravistimulated pulvini. As with the vertical segments of Fig. 1, JA promoted internodal elongation. In the present case, internodal elongation in gravistimulated, Glc-treated segments was 2.9 ± 0.2 mm compared with 5.8 ± 0.3 mm for segments additionally treated with $10^{-4}$ M JA, or 2.1 ± 0.1 mm for segments treated with $10^{-4}$ M ABA. Therefore, JA produced opposite effects on internodal and pulvinar tissue in the presence of gravistimulus as well as in vertical segments. (Note that pulvini from vertical segments do not elongate unless supplied with exogenous auxin or FC.) In contrast, ABA inhibited the growth of both internodal and pulvinar tissue. Unlike the result with $GA_3$-treated internodal tissue (Fig. 6), treatment with $10^{-3}$ M AVG failed to reverse the inhibitory effects of either ABA or JA in gravistimulated pulvinar tissue (data not shown). No evidence was found for synergy between JA and ABA when they were applied together to gravistimulated pulvini (data not shown). Finally, simultaneous treatment with $10^{-4}$ M BA failed to reverse the inhibition of growth in gravistimulated pulvini caused by JA (data not shown).

### *Kinetics of the Effects of JA and ABA on IAA-treated Pulvini*

Exogenous IAA, supplied through the node, also promotes the growth of pulvini, measured as elongation or fresh weight increase (Montague 1995b). Fig. 8 shows the time course of the effects of $10^{-4}$ M JA or ABA supplied with $10^{-4}$ M IAA. The IAA-promoted elongation of the pulvinar tissue was inhibited slightly by ABA and inhibited significantly by JA. Although $10^{-4}$ M ABA and JA inhibited the growth of IAA-treated pulvini less than they inhibited the growth of gravistimulated pulvini (compare with Fig. 7), in both cases JA was a more potent inhibitor than ABA. It is interesting to note that JA did not completely block IAA-induced promotion of pulvinar elongation, even at the relatively high concentration of $10^{-4}$ M, whereas it was able repeatedly to block all growth resulting from gravistimulus (Fig. 7). When the segments were pretreated with $10^{-4}$ M JA for 9 h prior to transfer to $10^{-4}$ M IAA (with JA), however, the stimulatory effect of IAA on pulvinar elongation was blocked completely (data not shown), indicating that JA is capable of entirely inhibiting IAA-promoted elongation.

### *Effects of JA and ABA on FC-induced Internodal and Pulvinar Elongation*

As a nonphysiologic stimulus, FC promotes elongation and cell wall synthesis in both internodal and pulvinar tissues (Montague 1995b). To compare the effects of JA

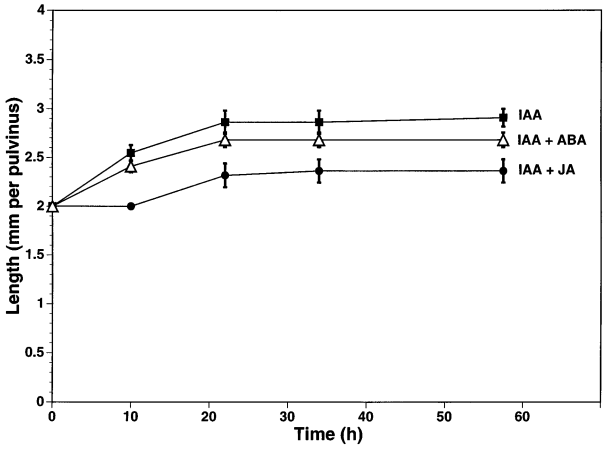


**Fig. 8.** Effects of ABA and JA on the kinetics of auxin-induced pulvinar elongation in oat stem segments. Samples of 11 stem segments were incubated with 0.1 M Glc, $10^{-4}$ M IAA, and with or without $10^{-4}$ M JA or ABA. Means for pulvinar elongation at the indicated times are presented with S.E.

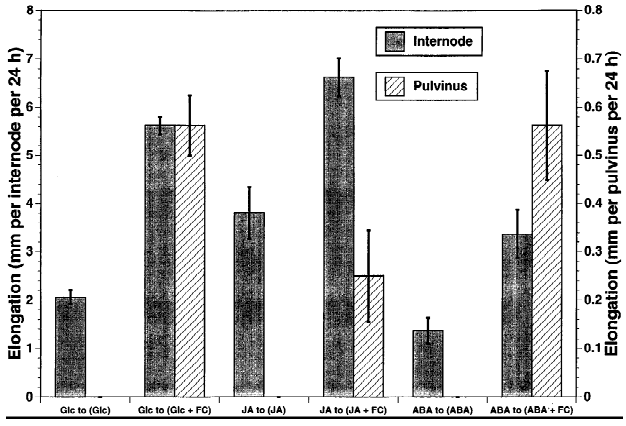


**Fig. 9.** Effects of ABA and JA on FC-induced elongation of *Avena* internodal and pulvinar tissue. Samples of eight stem segments were incubated with 0.1 M Glc with or without $10^{-4}$ M JA or ABA for 9 h. At that time internodal elongation was measured individually for each segment (the pulvini did not grow), and the segments were transferred to fresh medium containing 0.1 M Glc, with or without $10^{-4}$ M JA or ABA, and with or without $10^{-5}$ M FC, as indicated. At the time of transfer, the mean elongation for pooled internodes was 2.3 ± 0.1, 2.8 ± 0.1, and 1.5 ± 0.1 mm for Glc-, JA-, and ABA-treated segments, respectively. The elongation of individual internodes and pulvini which occurred during the 24-h treatment period was determined by difference (from the 9-h point), and means are presented with S.E.

and ABA on FC-induced pulvinar and internodal elongation, segments were pretreated with 0.1 M Glc in the presence or absence of $10^{-4}$ M JA or ABA for 9 h before transfer to fresh treatment solutions of the same compounds in the presence or absence of $10^{-5}$ M FC. Elongation was measured at 9 h and after an additional 24 h so that net growth following during the second treatment could be determined. The results are shown in Fig. 9. With regard to the internodal tissue, both FC and JA promoted elongation compared with the Glc control, whereas ABA inhibited elongation, consistent with the results of previous experiments (Figs. 1, 2, and Montague 1995b). Whereas JA had no effect on FC-induced elongation of the internode, ABA was decidedly inhibitory, reducing elongation to 60% of the Glc + FC control value. For the pulvinus, elongation was entirely dependent upon the supply of FC, since vertical pulvini do not elongate. In this case, JA reduced elongation to 44% of the Glc + FC control value, whereas ABA had no effect. These results provide further evidence that JA and ABA act differentially in these two tissues, even when they are stimulated to elongate by supplying a nonphysiologic compound.

## Discussion

Most reports on the effects of JA on growth in plant parts other than tubers demonstrate inhibitory activity (Sembdner and Parthier 1993). For example, Ueda et al. (1994, 1995) showed conclusively that JA inhibits both IAA-induced elongation and cell wall synthesis in oat coleoptile segments. Given this background information, the finding that JA *promotes* growth and cell wall biosynthesis in oat internodal tissue is at least somewhat unexpected (Figs. 1–3 and 9). It also stands in sharp contrast to the growth-inhibitory effect of ABA over the same range of physiologic concentrations. The observation that ABA actually inhibits the promotion of growth by JA (Fig. 2) is especially noteworthy because ABA and JA are commonly thought to have similar effects. The promotion of cell wall synthesis by JA (Fig. 3), especially when contrasted with the inhibition of wall synthesis in oat coleoptiles, shows that JA does not have universal effects on elongating tissues in grasses. Rather its effects apparently depend upon the specific tissue type and perhaps developmental stage.

The effect of JA on oat internodes is similar, at least superficially, to its effect on potato tuber cells (Takahashi et al. 1995). JA promotes cell expansion and the biosynthesis of cell wall polysaccharides in potato tuber slices, as it does in oat internodes (Fig. 3). The initiation of growth in oat internodal tissue was somewhat faster, with the elongation response noted within 8–12 h (Fig. 2), whereas the effect on tuber growth (measured as fresh weight increase) did not begin until 24 h after exposure of the slices to $3 \times 10^{-5}$ M JA. Note that there is some mention in the literature (presented without data) that JA promotes the growth of sugarcane internodes as well (Sembdner and Gross 1986).

The effects of JA on $GA_3$-treated oat internodal tissue and on IAA-treated or gravistimulated pulvinar tissue were consistent with the concept of JA as an inhibitor of

cell elongation. For such growth-induced internodal and pulvinar tissues, JA acted as a potent inhibitory compound at physiologic concentrations (Figs. 4–8). Unlike the situation with auxin-sensitive oat coleoptiles (Ueda et al. 1994, 1995), however, exogenous Glc clearly failed to prevent inhibition. Although the final growth attained with $10^{-4}$ M JA or ABA was similar in oat internodes also treated with $GA_3$, ABA acted earlier than JA (Fig. 5). In addition, the two hormones were strongly synergistic (Fig. 4). Finally, the ethylene biosynthesis inhibitor AVG was able partially to reverse the inhibition caused by ABA but had no effect on the inhibition caused by JA (Fig. 6). These three findings, combined with the opposing effects of JA and ABA in internodal tissue not stimulated to grow by exogenous $GA_3$ (Figs. 1 and 2), provide compelling evidence that the two growth regulators act by different mechanisms. It seems unlikely that the inhibitory effect of JA was due to general toxicity because no necrosis or loss of turgor was found even with long term application at $10^{-4}$ M JA, whereas such loss of turgor can be observed with long term application of $10^{-5}$ M FC (Montague 1995b).

As with oat coleoptiles (Ueda et al. 1994, 1995), JA and ABA inhibited the auxin-mediated graviresponsive elongation of the leaf sheath pulvinus (Figs. 7 and 8). This inhibition was more pronounced against growth resulting from gravistimulus than against growth resulting from IAA application. At $10^{-4}$ M, JA blocked gravistimulated pulvinar elongation entirely, but only partially inhibited IAA-induced elongation when supplied at the same time as $10^{-4}$ M auxin (Figs. 7 and 8). The difference in the level of inhibition of gravistimulated vs auxin-induced growth may result simply from the ultimate accumulation of more JA in the pulvinus, which might change the final JA/auxin ratio. Consistent with this explanation was the finding that pretreatment with $10^{-4}$ M JA for 9 h resulted in complete inhibition of auxin-promoted growth as well. The difference in potency between JA and ABA in affecting pulvinar growth is in contrast to $GA_3$-treated internodal tissue, where they appeared approximately equipotent (Fig. 4). Also, it should be noted that 0.1 M Glc did not overcome the inhibitory effects of either JA or ABA in auxin-treated or gravistimulated pulvinar tissue, which is in contrast to auxin-responsive oat coleoptile tissue (Ueda et al. 1994, 1995). Finally, JA and ABA showed opposite activities when FC was used as the growth stimulus. JA inhibited FC-induced growth of the pulvinus, without an effect on the internode; ABA inhibited FC-induced growth of the internode, without an effect on the pulvinus (Fig. 9).

Much recent molecular evidence points to remarkable similarities between the actions of ABA and JA (Weidhase et al. 1987, Hildmann et al. 1992, Reinbothe et al. 1992, Lehmann et al. 1995). Other molecular studies, however, portray species-dependent and other differences between JA and ABA. For example, Wasternack et al. (1996) studied gene expression in tobacco and tomato leaves as influenced by JA, ABA, osmotic stress, and desiccation. Based on differences between tobacco and tomato as well as differences between the mRNAs induced by JA and ABA in tobacco leaves, they proposed different signaling pathways for the two hormones.

The present work provides conclusive physiologic evidence that JA and ABA act by different mechanisms in oat stem segments. The promotion of elongation of the ordinarily GA-sensitive internodal tissue by JA is especially noteworthy and stands in sharp contrast to the inhibitory effect of ABA in the same tissue. Perhaps surprisingly, ABA actually inhibits this promotional effect of JA (Fig. 2). In $GA_3$-treated internodal tissue, the different kinetics of action of JA and ABA (Fig. 5) the synergy between them (Fig. 4), and the reversal of only the ABA effect by AVG (Fig. 6) also demonstrate differences. Finally, the effects of ABA and JA are apparently tissue specific when FC is used as the growth stimulus (Fig. 9). All of these results stand as counterpoint to any conclusion that JA and ABA are universally or fundamentally similar in their actions, at least in grasses. In addition, evidence presented herein indicates that a previously proposed mechanism of JA action involving a block in cell wall synthesis (Ueda et al. 1994, 1995) appears not to have universal applicability in elongating tissues.

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