RESEARCH PAPER

Effects of brassinosteroid, auxin, and cytokinin on ethylene production in Arabidopsis thaliana plants

Richard N. Arteca* and Jeannette M. Arteca

Department of Horticulture, The Pennsylvania State University, University Park, PA 16802, USA

Received 15 February 2008; Revised 2 May 2008; Accepted 8 May 2008

Abstract

Inflorescence stalks produced the highest amount of ethylene in response to IAA as compared with other plant parts tested. Leaf age had an effect on IAA-induced ethylene with the youngest leaves showing the greatest stimulation. The highest amount of IAA-induced ethylene was produced in the root or inflorescence tip with regions below this producing less. Inflorescence stalks treated with IAA, 2,4-D, or NAA over a range of concentrations exhibited an increase in ethylene production starting at 1 μM with increasingly greater responses up to 100 μM, followed by a plateau at 500 μM and a significant decline at 1000 μM. Both 2,4-D and NAA elicited a greater response than IAA at all concentrations tested in inflorescence stalks. Inflorescence leaves treated with IAA, 2,4-D, or NAA exhibited the same trend as inflorescence stalks. However, they produced significantly less ethylene. Inflorescence stalks and leaves treated with 100 μM IAA exhibited a dramatic increase in ethylene production 2 h following treatment initiation. Inflorescence stalks showed a further increase 4 h following treatment initiation and no further increase at 6 h. However, there was a slight decline between 6 h and 24 h. Inflorescence leaves exhibited similar rates of IAA-induced ethylene between 2 h and 24 h. Light and high temperature caused a decrease in IAA-induced ethylene in both inflorescence stalks and leaves. Three auxin-insensitive mutants were evaluated for their inflorescence’s responsiveness to IAA. aux2 did not produce ethylene in response to 100 μM IAA, while axr1-3 and axr1-12 showed reduced levels of IAA-induced ethylene as compared with Columbia wild type. Inflorescences treated with brassinolide alone had no effect on ethylene production. However, when brassinolide was used in combination with IAA there was a dramatic increase in ethylene production above the induction promoted by IAA alone.

Key words: Arabidopsis, auxin, brassinolide, cytokinin, ethylene, indole-3-acetic acid.

Introduction

Ethylene is the simplest plant hormone known today. Even though the structure is very simple it participates in the regulation of a variety of developmental processes in plants, from seed germination through organ senescence and abscission (Abeles et al., 1992). In recent years there has been an explosion of ethylene research, thereby leading to enormous strides forward in this area. There are a number of ethylene-signalling and -response pathways, which are very complex. There are many factors that stimulate these pathways, some of which are as follows: ethylene, cytokinins, auxins, brassinosteroids, plant development, stress pathogens, sugars, jasmonic acid, gibberellins, abscisic acid, and a variety of others (Stepanova and Alonso, 2005). There are a number of reports in the literature showing that auxin stimulates ethylene production in a variety of plant tissues. In fact, many of the responses that were once attributed to auxins have now been shown to be due to ethylene (Abeles et al., 1992; Arteca and Arteca, 2001). More than 30 years ago brassinosteroid research began when John Mitchell and co-workers at the US Department of Agriculture began screening pollens in search of new plant hormones. Today brassinosteroid (BR) has been shown to be involved in a wide range of physiological processes in plants, which have been summarized in numerous review articles (Adam
and Marquardt, 1986; Mandava, 1988; Sakurai and Fujioka, 1993; Arteca, 1995; Chory et al., 1996; Yokota, 1997; Clouse and Sasse, 1998; Haubrick and Assmann, 2006). It was nearly a decade ago when BR-deficient Arabidopsis mutants were discovered, establishing BR as an essential plant hormone, thereby stimulating many studies on multiple mechanisms of BR signalling (Gendron and Wang, 2007). In addition to auxin, BR, a growth-promoting compound with a similar structure to animal steroid hormones, has been shown to induce ethylene production alone and, acts synergistically to stimulate ethylene production in etiolated mung-bean seedlings (Arteca et al., 1983; Yi et al., 1999; Swarup et al., 2002). More recently Joo et al. (2006) showed that epibrassinostoid induces AtACS4 an auxin-responsive ACC synthase gene in Arabidopsis. Although auxins and BRs have been shown to promote ethylene alone and, when applied in combination, act synergistically in the stimulation of ethylene production (Arteca et al., 1983, Swarup et al., 2002), most of the work has been performed in mung bean, which has a number of disadvantages associated with it, one of the main ones being that genetic analysis is difficult. To date there has been no detailed analysis of auxin and BR induction of ethylene alone or in combination in Arabidopsis, nor has a model experimental system been established as to what the optimal tissue, stage of development, or other factors are required for maximal hormonal stimulation of ethylene production. The purpose of this study was to evaluate which tissues were most sensitive to plant hormones, the optimal conditions for the induction of ethylene by different plant hormones, and the interactions between the different plant hormones on the induction of ethylene in Arabidopsis, thereby establishing a model experimental system. By establishing a model experimental system in Arabidopsis, utilizing genetic and molecular tools currently available when using Arabidopsis as an experimental organism, it will be possible to go into more depth as to how and why auxins and BR trigger ethylene production in green plants.

**Materials and methods**

**Conditions for plant growth**

Arabidopsis thaliana (L.) Heynh ecotype Columbia wild-type (WT) and the auxin-insensitive mutant (axr2, axr1-3, and axr1-12) plants were grown in soil or hydroponically. Plants grown in soil were as described in the Arabidopsis Biological Resource Center (ABRC) manual. Plants were grown hydroponically according to the procedure outlined by Arteca and Arteca (2000). The environmental conditions used to grow plants were the same for those grown in both soil or hydroponically. Temperatures were maintained at 22±2 °C for a 16 h day/8 h night period. The light levels were maintained between 100 and 120 μmol m⁻² s⁻¹ with cool white fluorescent lights. Plants grown in soil were watered by sub-irrigation with tap water twice a week.

**Treatments**

In order to evaluate IAA-induced ethylene production in different Arabidopsis plant parts, 35- to 45-d-old plants grown hydroponically were divided into different plant parts including roots, young rosette leaves, inflorescence leaves, terminal inflorescence bud, lateral inflorescence bud, axillary leaf bud, and primary, secondary, and lateral inflorescence stalk. The different plant parts were put into 12×75 mm test tubes containing 200 μl of 100 μM IAA or water, which served as a control, then sealed with serum caps and placed in the dark. The temperature was maintained at 22±2 °C and ethylene analysed after 24 h unless specified otherwise. In all cases values are expressed as the mean of four replications ±standard error unless specified otherwise.

To determine if leaf position had an effect on IAA-induced ethylene production in Arabidopsis plants, 24-d-old plants grown in soil were divided into four groups, based on their position in the rosette from the youngest to the oldest, as described by Arteca and Arteca (2001). Leaves from different positions were put into test tubes and treated as previously described.

Root systems from 24-d-old Arabidopsis plants grown hydroponically were divided into four 5 cm sections in order to determine if the location on the root had an effect on IAA-induced ethylene. The different sections were put into test tubes and treated as previously described.

To evaluate if the location on the inflorescence had an effect on IAA-induced ethylene production, 35-d-old Arabidopsis plants grown in soil, with inflorescences 10 cm long, were divided into five 2 cm sections. The 2 cm sections were cut into five sections 4 mm in length. The different sections were put into test tubes and treated as previously described.

Primary inflorescences, 4-6 cm in length with flowers partially opened, from 35- to 45-d-old Arabidopsis plants were used in order to determine the effects of different concentrations of indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), naphthaleneacetic acid (NAA), and tryptophan (TRP) on ethylene production in inflorescence stalks and leaves. The inflorescence tip containing the flower was removed and the top 2 cm from the inflorescence stalk and the top two leaves were used. The inflorescence stalk was cut into five 4 mm sections. The different plant parts were put into varying concentrations (0–1000 μM) of IAA, 2,4-D, NAA, or TRP in test tubes and treated as previously described. The tubes were then sealed with serum caps, placed in the dark at 22±2 °C and ethylene analysed after 24 h. In order to determine the kinetics of induction, the effects of 100 μM IAA on ethylene induction in inflorescences stalks and leaves were evaluated over a time course from 0 h to 26 h following treatment initiation under the conditions previously described.

To establish if light had any effect on IAA-induced ethylene production, inflorescences 4–6 cm in length with flowers partially open were used from 35- to 45-d-old Arabidopsis plants. The inflorescence tip containing the flower was removed and the top 2 cm from the inflorescence stalk and the top two leaves were used. The inflorescence stalk was cut into five 4 mm sections. The different plant parts were put into 12×75 mm test tubes containing 200 μl of 100 μM IAA or water, which served as a control, then sealed with serum caps, placed in the dark at 110 μmol m⁻² s⁻¹ with cool white fluorescent lights or in darkness. The temperature was maintained at 22±2 °C and ethylene analysed after 24 h.

To evaluate the effect of high temperature on IAA or wound-induced ethylene production, inflorescences 4–6 cm in length with flowers partially open were used from 35- to 45-d-old Arabidopsis plants. The inflorescence tip containing the flower was removed and the top 2 cm from the inflorescence stalk and the top two leaves were used. The inflorescence stalk was cut into five 4 mm sections. The different plant parts were put into 12×75 mm test tubes containing 200 μl of 100 μM IAA or water, which served as a control, then sealed with serum caps, placed in the dark at 22±2 °C and ethylene analysed after 24 h.
Different plant parts were put into 12 ml of 100 |M IAA or water, which served as a control. Samples were put at 29, 37, or 43 °C in the dark for 15, 30, or 60 min, while the control samples were put in the dark at 22±2 °C. At the designated time interval, test tubes were removed from the heat chamber, allowed to cool for 10 min in the dark, and the heat-treated sample with the appropriate control sealed with serum caps and put in the dark at 22±2 °C for a 24 h period prior to ethylene analysis.

The effects of varying concentrations of IAA alone or in combination with BL or BAP on ethylene production were evaluated in order to determine the relationship between IAA and BL or BAP. Inflorescences, 4–6 cm in length with flowers partially open, were used from 35- to 45-d-old Arabidopsis plants. The inflorescence tip containing the flower was removed and the top 2 cm from the inflorescence stalk and the top two leaves were used. The inflorescence stalk was cut into five 4 mm sections. The different plant parts were put into 12 ml of varying concentrations of IAA from 0 |M to 100 |M IAA alone or in combination with 2 |M BL or 10 |M BAP or water, which served as a control, then sealed with serum caps and placed in the dark. The temperature was maintained at 22±2 °C and ethylene analysed after 24 h.

Ethylene determinations
Ethylene was analysed with a Hewlett Packard 5890 gas chromatograph equipped with a 1.83 m×3.175 mm o.d.×1.2 m stainless steel Porapak Q column. Injector port, flame ionization detector, and column temperatures were 70, 200, and 70 °C, respectively. Headspace gases were evaluated at designated treatment times.

Results
Different plant parts varied in IAA-induced ethylene production, inflorescence stalks showed the greatest induction, while all other plant parts tested produced significantly less (Table 1). Leaf age had an effect on IAA-induced ethylene production with the youngest leaves showing the greatest stimulation, and, as the age of the leaf increased, there was a reduction in their ability to produce ethylene (Table 2). When inflorescence stalks from Arabidopsis Columbia WT, which served as a control, and three auxin-insensitive mutants were treated with 100 |M IAA, there was an increase in ethylene production in WT. However, reduced levels were produced in the auxin-insensitive mutants: axr1-12 produced slightly less IAA-induced ethylene than the WT control; axr1-3 produced lower levels of IAA-induced ethylene than axr1-12; while axr2 barely produced detectable levels of ethylene (Fig. 1).

The highest amount of IAA-induced ethylene production was produced in the root tip with regions below this producing less (Table 3). IAA-induced ethylene production was also greatest from the tip of the inflorescence stalk to 2 cm below the tip, and from this point down the length of the inflorescence there was a reduction in ethylene production (Table 4).

Table 1. IAA-induced ethylene production in different Arabidopsis plant parts including root, young rosette leaves, inflorescence leaves, terminal inflorescence bud, lateral inflorescence bud, axillary leaf bud, primary inflorescence stalk, secondary inflorescence stalk, and lateral inflorescence stalk

| Plant part                  | Ethylene production (nl g⁻¹ FW h⁻¹) |
|-----------------------------|-------------------------------------|
|                             | Water control | 100 |M IAA |
| Root                        | 0.9±0.3       | 2.8±1.1 |
| Young rosette leaf          | 1.2±0.4       | 18.6±3.2 |
| Inf. leaf                   | 0.8±0.3       | 16.2±2.0 |
| Terminal inf. bud           | 0.8±0.2       | 3.4±0.8 |
| Lateral inf. bud            | 0.8±0.2       | 3.4±0.9 |
| Axillary leaf bud           | 0.8±0.2       | 2.4±0.6 |
| Primary inf. stalk          | 4.6±0.8       | 213.3±8.9 |
| Secondary inf. stalk        | 3.2±0.7       | 201.4±9.3 |
| Lateral inf. stalk          | 3.8±0.4       | 207.1±7.6 |

Table 2. Effects of leaf position on IAA-induced ethylene production in 24-d-old Arabidopsis plants

| Leaf position | Ethylene production (nl g⁻¹ FW h⁻¹) | Water control | 100 |M IAA |
|---------------|-------------------------------------|---------------|------|
| 8–9 (youngest)| 1.3±0.3                             | 19.8±1.1      |
| 6–7           | 1.0±0.4                             | 17.6±1.2      |
| 4–5           | 0.8±0.3                             | 15.8±1.0      |
| 2–3 (oldest)  | 0.8±0.2                             | 12.7±0.6      |

Fig. 1. Effects of IAA on ethylene production in Arabidopsis Columbia WT and three auxin-insensitive mutants, axr1-12, axr1-3, and axr2. Inflorescences, 4–6 cm in length with flowers partially open, were taken from 35- to 45-d-old Arabidopsis plants. The inflorescence stalk was cut into five 4 mm sections and put in 12×75 mm test tubes containing 200 |l of 100 |M IAA (white columns) or water control (black columns). The tubes were then sealed, placed in the dark and ethylene analysed after 24 h. Values are expressed as the mean of four replications ±standard error.
When inflorescence stalks were treated with IAA, 2,4-D, or NAA over a range of concentrations from 0 \( \mu M \) to 1000 \( \mu M \) there was an increase in ethylene production starting at 1 \( \mu M \) with increasingly greater responses up to 100 \( \mu M \), followed by a plateau with the 500 \( \mu M \) treatment and a significant decline at 1000 \( \mu M \). Both 2,4-D and NAA elicited a greater response than IAA at all concentrations tested in inflorescence stalks. Inflorescence leaves treated with IAA, 2,4-D, and NAA also exhibited an increase in ethylene production at a 1 \( \mu M \) concentration, reaching a maximum at 500 \( \mu M \), and was followed by a significant decline at the 1000 \( \mu M \) concentration. Both 2,4-D and NAA elicited a greater response than IAA at all concentrations tested in inflorescence stalks. Inflorescence leaves treated with IAA, 2,4-D, and NAA also exhibited an increase in ethylene production at a 1 \( \mu M \) concentration, reaching a maximum at 500 \( \mu M \), and was followed by a significant decline at the 1000 \( \mu M \) concentration. Inflorescence leaves produced significantly less ethylene than stalks at all concentrations of auxins tested. TRP did not induce ethylene at concentrations from 1 \( \mu M \) to 1000 \( \mu M \) in inflorescence leaves or stalks (Fig. 2).

Inflorescence stalks and leaves treated with 100 \( \mu M \) IAA exhibited a dramatic increase in ethylene production 2 h following treatment initiation. Inflorescence stalks showed a further increase 4 h following treatment

Table 3. Ethylene production of roots of 24-d-old Arabidopsis plants grown hydroponically

| Location on the root | Ethylene production (nl g\(^{-1}\) FW h\(^{-1}\)) |
|----------------------|--------------------------------------|
|                      | Water control | 100 \( \mu M \) IAA |
| Tip to 5 cm          | 0.8±0.3     | 3.8±0.6 |
| 5–10 cm              | 0.7±0.4     | 2.7±0.2 |
| 10–15 cm             | 0.9±0.3     | 1.8±0.3 |
| 15–20 cm             | 0.7±0.4     | 0.8±0.1 |

Table 4. Ethylene production of inflorescences, 10 cm in length with flowers partially open, taken from 35- to 45-d-old Arabidopsis plants

The inflorescence tip containing the flower was removed and 2 cm sections were taken from the tip to the base of the inflorescence. The 2 cm sections were cut into five 4 mm sections. Five sections were put into 100 \( \mu M \) IAA or water as a control in 12×75 mm test tubes containing 200 \( \mu l \) of treatment solution. The tubes were then sealed, placed in the dark, and ethylene analysed after 24 h. Values are expressed as the mean of four replications ± standard error.

| Location on inflorescence stalk | Ethylene production (nl g\(^{-1}\) FW h\(^{-1}\)) |
|--------------------------------|--------------------------------------|
|                                | Water control | 100 \( \mu M \) IAA |
| Tip to 2 cm                    | 3.9±0.9     | 209.6±10.1 |
| 2–4 cm                         | 3.1±0.4     | 189.8±9.2  |
| 4–6 cm                         | 2.5±0.5     | 153.8±9.0  |
| 6–8 cm                         | 1.1±0.4     | 108.3±11.6 |
| 8–10 cm                        | 0.8±0.2     | 78.4±6.1   |

Fig. 2. Effects of different auxins on ethylene production. Inflorescences, 4–6 cm in length with flowers partially open, were taken from 35- to 45-d-old Arabidopsis plants. The inflorescence tip containing the flower was removed and the top 2 cm from the inflorescence stalk (white columns) or the top two leaves (black columns) were used. The inflorescence stalk was cut into five 4 mm sections. The plant parts were put into varying concentrations of (A) IAA, (B) NAA, (C) 2,4-D, or (D) TRP in 12×75 mm test tubes containing 200 \( \mu l \) of treatment solution. The tubes were then sealed, placed in the dark, and ethylene analysed after 24 h. Values are expressed as the mean of four replications ± standard error.
initiation with no further increase at 6 h. Between 6 h and 24 h there was a decline in the rate of ethylene production, while at the 26 h sampling the rates of ethylene produced were the same as the 24 h readings. Inflorescence leaves exhibited similar rates of IAA-induced ethylene production between 2 h and 26 h following treatment initiation (Table 5).

When inflorescence stalks and leaves were treated with IAA and kept in the light there was a 36% reduction in IAA-induced ethylene production in inflorescence stalks and a 28% reduction in the leaves as compared with plant parts kept in the dark (Table 6). When inflorescence stalks or leaves were treated at 43 °C for 30 min there was no effect on IAA-induced ethylene in the inflorescence stalk, while there was a 15% reduction in the leaves. After a 60 min treatment at 43 °C, IAA-induced ethylene production was reduced 41% in the inflorescence stalks and 42% in the leaves (Table 7). When inflorescence stalks or leaves were treated at 29 °C or 37 °C for 30–60 min, there was no effect on IAA-induced ethylene (data not shown).

When 2 μM brassinolide (BL) or 10 μM 6-benzylaminopurine (BAP) were used alone to treat inflorescence stalks there was no enhancement of ethylene production. However, when 2 μM BL was used in combination with 0.1, 1.0, or 10.0 μM IAA there was a dramatic increase in ethylene production above the induction promoted by the IAA treatment alone. At the highest concentration of IAA (100 μM) tested there was no further enhancement of IAA-induced ethylene production by BL. BAP was found to be ineffective in the enhancement of IAA-induced ethylene production (Table 8).

**Discussion**

The plant can regulate ethylene internally, by non-stressful environmental factors or a variety of stress factors. Internally controlled factors include the plants genetics, stage of development, aging and senescence, type of organ, feedback regulation, and/or hormones. Regulation by non-stressful environmental factors includes temperature, light, oxygen, carbon dioxide, nutrition (both organic and inorganic), touch, gravity, and circadian rhythm. Stress factors regulating ethylene production are chilling and freezing stress, high temperature stress, excessive water or flooding stress, drought stress, chemical stress, radiation stress, and/or mechanical stress. There are also a variety of biotic stresses including viral, bacterial, and fungal diseases that can induce ethylene production (Abeles et al., 1992). The purpose of this study was to evaluate which tissues were most sensitive to plant hormones, the optimal conditions for the induction of ethylene by different plant hormones, and the interactions between the different plant hormones on the induction of ethylene in Arabidopsis, thereby establishing a model experimental system. By establishing this new and innovative model experimental system in Arabidopsis, it will be possible to go into more depth as to how and why auxins and BR trigger ethylene production in green plants, utilizing genetic and molecular tools currently available when using Arabidopsis as an experimental organism.

The different plant hormones used to study ethylene production in light-grown Arabidopsis plants were IAA, 2,4-D, NAA, BL, and BAP, each of which had been previously shown to stimulate ethylene production in etiolated mung-bean segments (Abeles et al., 1992).

In this study, it has been shown that young leaves and the apical portions of inflorescences and roots are most responsive to auxins, with inflorescences being the most responsive of the three. These findings are in agreement with the literature which shows that high rates of ethylene production are associated with actively dividing cells, as is the case in younger leaves (Abeles et al., 1992). In addition, it was shown that inflorescence stalks from auxin-insensitive mutants produced less IAA-induced ethylene than the WT controls. This is the first report, as

| Treatment | Ethylene production (nl g⁻¹ FW h⁻¹) |
|-----------|-----------------------------------|
|           | 2  | 4  | 6  | 24 | 26  |
| Inflorescence stalk |     |     |     |     |     |
| Water control | 4.7±0.1 | 4.9±0.1 | 4.5±0.1 | 4.2±0.1 | 4.5±0.1 |
| 100 μM IAA | 172.8±12.2 | 280.1±25.4 | 253.4±14.1 | 200.2±12.5 | 207.2±8.1 |
| Inflorescence leaves |     |     |     |     |     |
| Water control | 0.8±0.1 | 0.9±0.1 | 0.7±0.2 | 0.9±0.1 | 0.8±0.1 |
| 100 μM IAA | 20.8±2.2 | 21.9±2.4 | 20.1±1.1 | 20.2±2.5 | 20.4±2.1 |
In previous work with etiolated mung-bean sprouts which did not promote any stimulation. These results differ from those tested, in both inflorescence stalks and leaves, while TRP insensitive mutants produced less IAA-induced ethylene production.

It has also been shown that light causes a dramatic reduction in auxin-enhanced ethylene production. This is the first report, as far as is known, on the light inhibition of IAA-induced ethylene production in Arabidopsis. However, there are reports in the literature in other experimental organisms showing that light inhibits normal, and stress- and hormone-induced ethylene production.

There are also reports which show that light increases ethylene production in melon petioles (Toppan and Chung, 1983), which is in agreement with the present findings. There are also reports which show that light increases ethylene production in melon petioles (Toppan and Chung, 1983), which is in agreement with the present findings.

Table 6. Effects of light versus darkness on IAA-induced ethylene production

| Treatment          | Ethylene production (nl g⁻¹ FW h⁻¹) |
|--------------------|-------------------------------------|
|                    | Light                              | Dark                              |
| Inflorescence stalk| 1.7±0.1                            | 3.9±0.3                           |
| 100 μM IAA         | 131.2±12.9                         | 206.5±13.8                        |
| Inflorescence leaves| Water control                       | 0.6±0.1                           |
|                    | 100 μM IAA                          | 14.9±0.8                          |

Table 7. Effects of short-term exposure to high temperature on IAA-induced ethylene production

| Treatment          | Ethylene production (nl g⁻¹ FW h⁻¹) |
|--------------------|-------------------------------------|
|                    | Time at 43.5 °C (min)               |
|                    | 0                                  | 15                                | 30                                | 60                                |
| Inflorescence stalk| 4.4±0.5                            | 4.2±0.4                           | 4.0±0.3                           | 2.4±0.5                           |
|                    | 209.8±12.2                         | 199.9±8.4                         | 203.4±7.0                         | 122.8±14.5                        |
| Inflorescence leaves| Water control                       | 0.9±0.1                           | 0.8±0.1                           | 0.6±0.1                           | 0.2±0.1                           |
|                    | 100 μM IAA                          | 21.6±1.5                          | 19.9±0.7                          | 18.4±0.7                          | 12.5±0.9                           |

Infloraces, 4–6 cm in length with flowers partially open, from 35- to 45-d-old Arabidopsis plants were used alone to treat Arabidopsis inflorescence stalks and leaves. The inflorescence stalk was cut into five 4 mm sections. The plant parts were put into 100 μl of treatment solution. The tubes were then sealed, placed in the dark or, and ethylene analysed after 24 h. Values are expressed as the mean of four replications ± standard error.

Infloraces, 4–6 cm in length with flowers partially open, were taken from 35- to 45-d-old Arabidopsis plants. The inflorescence stalk containing the flower was removed and the top 2 cm from the inflorescence stalk was used. The inflorescence stalk was cut into five 4 mm sections. The inflorescence stalk was cut into five 4 mm sections. The five sections were put into 12×75 mm test tubes containing 200 μl of varying concentrations of IAA alone or in combination with 2 μM BL or 10 μM BAP or water as a control. The tubes were then sealed, placed in the dark, and ethylene analysed after 24 h. Values are expressed as the mean of four replications ± standard error.

Table 8. The effects of varying concentrations of IAA alone or in combination with BR or BAP on ethylene production

| Treatment    | Ethylene production (nl g⁻¹ FW h⁻¹) |
|--------------|-------------------------------------|
|              | IAA (μM)                            |
|              | -BL       | +BL       | -BAP      | +BAP      |
| 0            | 4.0±0.3   | 3.8±0.3   | 3.8±0.2   | 4.1±0.1   |
| 0.01         | 4.2±0.3   | 4.7±0.2   | 4.1±0.3   | 4.0±0.2   |
| 0.1          | 6.5±1.2   | 23.6±2.4  | 5.1±1.1   | 5.6±1.2   |
| 1.0          | 22.9±6.4  | 79.4±8.3  | 23.6±5.6  | 28.5±4.2  |
| 10.0         | 67.6±7.4  | 159.9±12.3| 75.6±5.2  | 80.1±6.3  |
| 100          | 203.9±13.3| 234.4±14.3| 197.2±9.8 | 195.3±12.4|

far as is known, that Arabidopsis inflorescence stalks are much more responsive to auxin treatment than any other plant part, and that inflorescence stalks from auxin-insensitive mutants produced less IAA-induced ethylene than the WT controls.

It has also been shown that both 2,4-D and NAA induce higher levels of ethylene than IAA at all concentrations tested, in both inflorescence stalks and leaves, while TRP did not promote any stimulation. These results differ from previous work with etiolated mung-bean sprouts which showed that 100 μM IAA promoted higher levels of ethylene than either NAA or 2,4-D (Arteca et al., 1983). Also in this study it has been shown that light causes a dramatic reduction in auxin-enhanced ethylene production. This is the first report, as far as is known, that short-term heat treatments inhibit auxin-induced ethylene production. It has been suggested that the optimal temperature for ethylene production is near 30 °C and that the rate of ethylene production decreases beyond this point (Burg and Thimann, 1959); however, it has also been shown that heat treatment above 40 °C can stimulate ethylene production (Yang et al., 1990), which differs from the present results.

In this study, it has been shown that when BL or BAP were used alone to treat Arabidopsis inflorescence stalks there was no enhancement of ethylene production. This differs from previous work in etiolated mung beans showing that BL (Arteca et al., 1983) or BAP (Schlagnhauber et al., 1984) alone stimulated ethylene production.
production. In this study, it was also shown that when BL was used in combination with IAA there was a dramatic increase in ethylene production above the induction promoted by the IAA treatment alone, whereas BAP had no interactive effect. This is in agreement with the present findings that BL acts synergistically with IAA to stimulate ethylene production in etiolated mung-bean segments (Arteca et al., 1983). However, it is not in agreement with previous results which showed that BAP interacts to stimulate ethylene production in etiolated mung-bean segments (Schlagnhaufer et al., 1984).

In summary, both 2,4-D and NAA induce higher levels of ethylene than IAA at all concentrations tested in both inflorescence stalks and leaves, while TRP did not promote any stimulation. Inflorescence stalks, including primary, secondary, and lateral, produced the greatest amount of ethylene in response to IAA, whereas all other plant parts tested produced significantly lower levels of ethylene in response to the hormone treatment. The apical portion of the inflorescence produced the highest levels of IAA-induced ethylene with less ethylene produced as sections were taken farther away from the tip. The inflorescence stalks from three auxin-insensitive mutants produced less IAA-induced ethylene than their WT control. Short-term heat treatments with high temperatures inhibited IAA-induced ethylene production in both inflorescence stalks and leaves. Light also had an inhibitory effect on IAA-induced ethylene production. BL interacted with IAA to produce higher levels than either alone, while BAP had no interactive effect. Future studies are now in progress to look for changes in ethylene biosynthetic signalling genes in order to better understand how auxins and BRs are triggering ethylene production at the molecular level. In addition, various auxin-insensitive mutants will be studied in more depth and BR Arabidopsis mutants that are insensitive or do not produce these hormones will be evaluated for their ability to produce ethylene in response to IAA or BL. With the data presented in this paper there is now an excellent model system utilizing Arabidopsis inflorescences that will enable us to explore in more depth how and why auxins and BRs trigger ethylene production and the signalling pathways involved.

Acknowledgements

This is a contribution no. 463 of the Department of Horticulture, The Pennsylvania State University and was supported in part by the Pennsylvania Agricultural Experiment Station project number 4106.

References

Ables FB, Morgan PW, Salveit Jr MEJr. 1992. Ethylene in plant biology, 2nd edn. New York, NY: Academic Press.
Adam G, Marquardt V. 1986. brassinosteroids. Phytochemistry 25, 1787–1799.
Arteca JM, Arteca RN. 2001. brassinosteroid-induced exaggerated growth in hydroponically grown Arabidopsis plants. Physiologia Plantarum 112, 104–112.
Arteca RN. 1995. brassinosteroids. In: Davies PI, ed. Plant hormones: physiology, biochemistry and molecular biology. New York, NY: Kluwer Academic Publishers, 206–213.
Arteca RN, Arteca JM. 2000. A novel method for growing Arabidopsis thaliana plants hydroponically. Physiologia Plantarum 108, 188–193.
Arteca RN, Bachman JM. 1987. Light inhibition of brassinosteroid-induced ethylene production. Journal of Plant Physiology 129, 13–18.
Arteca RN, Tsai DS, Schlagnhaufer C, Mandava NB. 1983. The effects of brassinosteroid on auxin-induced ethylene production by etiolated mung bean segments. Physiologia Plantarum 59, 539–544.
Bassi PK, Spencer MS. 1983. Does light inhibit ethylene production in leaves? Plant Physiology 73, 758–760.
Burg SP, Thimann KV. 1959. The physiology of ethylene formation in apples. Proceedings of the National Academy of Science, USA 45, 335–344.
Chory J, Catterjee M, Cook RT, et al. 1996. From seed germination to flowering, light controls plant development via the pigment phytochrome. Proceedings of the National Academy of Science, USA 93, 12066–12077.
Clouse SD, Sasse JM. 1998. brassinosteroids: essential regulators of plant growth and development. Annual Review of Plant Physiology and Plant Molecular Biology 49, 427–451.
Craker LE, Standley LA, Starbuck JJ. 1971. Ethylene control of anthocyanin synthesis in sorghum. Plant Physiology 48, 349–352.
DeGreef JA, Van Dijick R, DeProft M, Mekers O. 1988. Activity of the flowering maturity and ethylene production capacity of Aechmea victoriana through ACC application. Acta Horticulturae 137, 211–216.
DeLaat AAM, Brandenburg DCC, van Loon LC. 1981. The modulation of the conversion of 1-amino-cyclcopropane-1-carboxylic acid to ethylene in the light. Planta 153, 193–200.
Genrom JM, Wang Z-Y. 2007. Multiple mechanisms modulate brassinosteroid signaling. Current Opinion in Plant Biology 10, 436–441.
Haubrick LL, Assmann SM. 2006. Brassinosteroids and plant function: some clues, more puzzles. Plant, Cell and Environment 29, 446–457.
Joo S, Seo YS, Kim SM, Hong DK, Young Park KY, Kim WT. 2006. brassinosteroid induction of AtACS4 encoding an auxin-responsive 1-amino-cyclcopropane-1-carboxylate synthase 4 in Arabidopsis seedlings. Physiologia Plantarum 126, 592–604.
Mandava NB. 1988. Plant growth-promoting brassinosteroids. Annual Review of Plant Physiology and Plant Molecular Biology 39, 23–52.
Porter AJR, Borlakoglu JT, John P. 1986. Activity of the ethylene-forming enzyme in relation to plant cell structure and organization. Journal of Plant Physiology 125, 207–216.
Rodecap KD, Tingey DT. 1983. The influence of light on ozone-induced 1-amino-cyclop propane-1-carboxylic acid and ethylene production from intact plants. Zeitschrift fur Pflanzenphysiologie 110, 419–427.
Sakurai A, Fujioka F. 1993. The current status of physiology and biochemistry of brassinosteroids. Plant Growth Regulation 13, 147–159.
Schlagnhaufer CD, Arteca RN, Yopp JH. 1984. A brassinosteroid-cytokinin interaction on ethylene production by etiolated mung bean segments. Physiologia Plantarum 60, 347–350.
Stepanova AN, Alonso JM. 2005. Ethylene signaling and response pathway: unique signaling cascade with a multitude of inputs and outputs. Physiologia Plantarum 123, 195–206.
Swarup R, Parry G, Graham N, Allen T, Bennett M. 2002. Auxin cross-talk: integration of signaling pathways to control plant development. *Plant Molecular Biology* 49, 411–426.

Toppan A, Esquerre-Tugaye MT. 1984. Cell surfaces in plant–microorganism interactions. IV. Fungal glycopeptides which elicit the synthesis of ethylene in plants. *Plant Physiology* 75, 1133–1138.

Wright STC. 1981. The effect of light and dark periods on the production of ethylene from water-stressed wheat leaves. *Planta* 153, 172–180.

Yang RF, Cheng TS, Shewfelt RL. 1990. The effect of high temperature and ethylene treatment on ripening of tomatoes. *Journal of Plant Physiology* 136, 368–372.

Yi CH, Joo S, Nam KH, Lee JS, Kang BG, Kim WT. 1999. Auxin and brassinosteroid differentially regulate the expression of three members of the 1-aminocyclopropane-1-carboxylate synthase gene family in mung bean (*Vigna radiata* L.). *Plant Molecular Biology* 41, 443–454.

Yokota T. 1997. The structure, biosynthesis and function of brassinosteroids. *Trends in Plant Science* 2, 137–143.