Respiratory and carbohydrate changes during ethylene-mediated flower induction in Dutch iris

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Abstract

The promotion of flowering in small Dutch iris \((Iris \times hollandaica\) Hoog., cv. ‘Ideal’) bulbs by treatment for 24 h with 10 \(\mu l l^{-1}\) ethylene was associated with enhanced respiration, which continued long after the end of ethylene treatment. Shorter ethylene treatments, which were partially effective in inducing flowering, resulted in lower and shorter bursts of respiration. Changes in the soluble carbohydrate contents of the ethylene-treated bulbs were not detected until following the cold treatment. The possible mechanisms by which ethylene stimulates flowering are discussed.

Keywords: Bulbs; Carbohydrates; Dutch iris; Ethylene; Flowering; Respiration

1. Introduction

The remarkable effects of ethylene in stimulating sprouting \((Vacha and Harvey, 1927)\) and flowering \((Stuart et al., 1966)\) of geophytes are now used in commercial production of freesia, Dutch iris and tazetta narcissus. There have been many studies of the practical aspects of this effect; optimum concentrations \((De Munk, 1984; Imanishi and Berghoef, 1986; Imanishi and Yue, 1986)\), length of application \((Uyemura and Imanishi, 1984; Imanishi and Yue, 1986)\), use of ethephon \((Halevy et al., 1970; Swart and Schipper, 1982)\), treatment temperature \((Masuda and Asahira, 1981; Imanishi and Yue, 1986)\), and time of application \((Im-

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anishi and Berghoef, 1986; Yue and Imanishi, 1990) have all been explored. The system which has been most studied is the Dutch iris (Iris × hollandica Hoog.). Depending on cultivar and growing environment, under early forcing conditions all large (9–10 cm circumference) bulbs of this plant will flower after proper temperature pre-treatment. In smaller bulbs, flowering percentage falls until below a minimum critical size (5–7 cm circumference), the bulbs produce only vegetative plants (Rees, 1985). Treatment of bulbs above the critical size for as little as 3 h with 10 μl l⁻¹ ethylene increases flowering to nearly 100% (Stuart et al., 1966).

Despite the importance of these effects on flowering of geophytes, there have been few studies of the physiological or biochemical basis for the stimulation of sprouting and flowering by ethylene. Huelin and Barker (1939) demonstrated that the respiration of potato tubers, whose sprouting is stimulated by ethylene (Vacha and Harvey, 1927), rose substantially during ethylene treatment. Kamerbeek and Verlind (1972) showed that ethylene treatment of iris bulbs caused a similar increase in respiration. Sugar content of potato tubers treated with ethylene rose markedly (Huelin and Barker, 1939). Sachs (1977) suggested that changes in source/sink relationships, specifically increased mobilization of carbohydrates to the apex, might explain the chemical control of flowering. We hypothesized that ethylene treatment of iris bulbs might result in increased carbohydrate mobilization, which might favor conversion of a vegetative meristem to the reproductive state. The study reported here was carried out to test this hypothesis by measuring changes in flowering response, respiration, and soluble carbohydrate content of bulb scales and apices of different-sized iris bulbs treated with ethylene.

2. Materials and methods

2.1. Plant material

Commercially produced bulbs of Dutch iris (Iris × hollandica Hoog., cv. ‘Ideal’) were obtained shortly after harvest from the Washington Bulb Company, Mount Vernon, WA. Bulbs were kept at 20°C prior to treatment. They had been graded commercially for size, and a closer selection of bulb sizes was made by weighing individual bulbs, and selecting those within a specific weight range for each size class: 5–7 g for size 6 (6 cm circumference); 7–9 g for size 7 (7 cm circumference); and 14–16 g for size 9 (9 cm circumference).

2.2. Ethylene treatment

Bulbs were placed at 20°C in 30 l tanks (30 bulbs per tank) ventilated with a flowing stream (30 l h⁻¹) containing 10 μl l⁻¹ ethylene gas (or air) for different lengths of time. After treatment, bulbs were stored at 10°C for 5 weeks before being planted in wooden trays containing U.C. mix (1:1:1 v/v
peat:perlite:redwood sawdust) for greenhouse forcing (20/15 °C day/night temperature). The flowering response to each treatment was determined on three to five replicates of ten bulbs each. For flowering plants, the time to flowering was recorded, and the lengths of flower stalks and leaves were measured. The numbers of blind or blasted flowers also were recorded.

2.3. Determination of changes in soluble carbohydrate content

For carbohydrate analysis, five bulbs were sampled at the start of the experiment (initial), after the 1-day ethylene (or air) exposure, after storage for 5 weeks at 10 °C (precooled), and after a further 10 days in air at 20 °C. The bulbs were dissected into outer scales, inner scales, and the apical bud. Because of the small size of the leaves + meristem in the apical bud, these were combined for analysis. The bulb portions were frozen in liquid nitrogen, freeze-dried, stored at 20 °C, ground to a fine powder, and extracted for at least 6 h with ethanol in a Soxhlet extraction apparatus. The ethanolic extract, containing the soluble sugars, was vacuum dried using a rotary film evaporator, then redissolved in sufficient water to give a sugar concentration adequate for high performance liquid chromatographic (HPLC) analysis. The individual sugars were separated and quantified by HPLC, using an ion exchange column (BioRad Aminex HPX-87C) and refractive index monitoring of the column effluent.

2.4. Measurement of respiration

Bulbs were placed at 20 °C in 30 l tanks (30 bulbs per tank) ventilated with a flowing stream (30 l h⁻¹) containing 10 μl l⁻¹ ethylene gas (or air) for different lengths of time. Respiration was determined by measuring the CO₂ in the air leaving the treatment chambers, using an infrared CO₂ analyzer (ADC, Hoddlesdon, UK).

2.5. Statistical analysis

Percent data were transformed to arcsin √x and one dependent variable (number of leaves) was transformed to √x prior to analysis by the ANOVA procedure (Statistical Analysis Systems Institute Inc., 1990). Regression analyses and paired comparisons were used to test for differences among treatments. Orthogonal polynomial coefficients used in the regression analyses were corrected for the unequal intervals between treatment levels (Damon and Harvey, 1987).

3. Results

3.1. Effects of ethylene treatments on flowering of Dutch iris cv. 'Ideal'

Periodic dissection of 7 cm bulbs from the various treatments revealed no obvious effect of ethylene on meristem differentiation until the bulbs had been
transferred to 20°C after precooling. Immediately after precooling, all meristems were still vegetative, regardless of bulb size or treatment. After a further 10 days at 20°C, the apices of control bulbs were still vegetative, but meristems of the ethylene-treated bulbs had commenced floral differentiation (from broadening of the apical dome to differentiation of the floral organ primordia).

Increases in bulb size and extending ethylene exposure increased flowering (Fig. 1). Small bulbs (6 cm) held in air failed to flower, but a small percentage did flower in response to a 1-day treatment with ethylene. In intermediate bulbs (7 cm), treatment with ethylene for only 1 h tripled the percentage of bulbs that flowered and a 1-day ethylene treatment dramatically increased flowering, from 4 to 84%. Flowering was induced in 96% of the bulbs treated with ethylene for 1 day, but 10% of the flowers failed to develop normally ('blasting') (Table 1). The significant increase in flowering of ethylene-treated bulbs was the result of a reduction in the percentage of blind bulbs and not of blasted flowers. Controls in the larger-sized bulbs (9 cm) had a high percentage of flowering and ethylene treatment had no effect on the flowering of these large-sized bulbs.

3.2. Effects of length of ethylene exposure on flowering parameters

For all parameters recorded, except for percent flowering, treatment of 7 cm bulbs with ethylene for 3 h was as effective as treatment for 24 h (Table 2). Treatment for 1 h gave an intermediate effect for most parameters. Ethylene treatment significantly reduced days to sprouting (emergence of the first leaf), days to flowering, number of leaves and mean leaf length. Concomitantly, percentage flowering and flower stalk length increased.

Fig. 1. Effects of different durations of ethylene treatment on percentage flowering of different sized bulbs of Dutch iris cv. 'Ideal'. Bulbs were treated for different lengths of time with 10 μl l⁻¹ prior to precooling and forcing.
Table 1
Effect of ethylene treatment on flowering performance of different sized bulbs of iris (cv. Ideal). Bulbs were treated for 24 h with air or 10 μl l⁻¹ ethylene, and held for 5 weeks at 10°C, then for 1 week at 20°C prior to forcing in the greenhouse. Data are means of five replicates with ten bulbs per replicate.

<table>
<thead>
<tr>
<th>Bulb size (cm)</th>
<th>Treatment</th>
<th>Flowering (%)</th>
<th>Blasting (%)</th>
<th>Blindness (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Air</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Air Ethylene</td>
<td>14</td>
<td>0</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>7 Air</td>
<td>4</td>
<td>2</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Ethylene</td>
<td>86</td>
<td>10</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>9 Air</td>
<td>74</td>
<td>10</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Ethylene</td>
<td>92</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

**Paired comparisons**
Air vs. C₂H₄ in 6 cm **
Air vs. C₂H₄ in 7 cm ***
Air vs. C₂H₄ in 9 cm NS

* Plants with flowers that failed to develop normally.

<table>
<thead>
<tr>
<th>Bulb size (cm)</th>
<th>Treatment</th>
<th>Flowering (%)</th>
<th>Blasting (%)</th>
<th>Blindness (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Air</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Ethylene</td>
<td>14</td>
<td>0</td>
<td>86</td>
<td></td>
</tr>
</tbody>
</table>

**Significance** *0.01 < P ≤ 0.05; **0.001 < P ≤ 0.01; ***P ≤ 0.001. Numbers within parentheses indicate the P values.

Table 2
Effects of ethylene treatment on flowering parameters of 7 cm bulbs of Dutch iris cv. ‘Ideal’. Dormant bulbs were treated for different lengths of time with 10 μl l⁻¹ prior to bulb precooling and forcing in the greenhouse. Data are means ± SE of five replicates with ten bulbs per replicate.

<table>
<thead>
<tr>
<th>Ethylene treatment (h)</th>
<th>Days to sprouting (h)</th>
<th>Days to flowering (%)</th>
<th>Flowering (%)</th>
<th>Leaves (no.)</th>
<th>Leaf length (cm)</th>
<th>Flower stalk length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>24.5 ± 0.5</td>
<td>127.3 ± 1.3</td>
<td>6.0 ± 2.4</td>
<td>6.2 ± 0.3</td>
<td>80.3 ± 0.9</td>
<td>36.3 ± 2.3</td>
</tr>
<tr>
<td>1</td>
<td>17.7 ± 0.8</td>
<td>108.4 ± 3.9</td>
<td>30.0 ± 5.8</td>
<td>6.0 ± 0.3</td>
<td>67.5 ± 2.8</td>
<td>43.9 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>16.2 ± 0.5</td>
<td>94.6 ± 6.2</td>
<td>60.0 ± 11.5</td>
<td>5.1 ± 0.4</td>
<td>57.7 ± 5.1</td>
<td>46.0 ± 1.8</td>
</tr>
<tr>
<td>24</td>
<td>14.9 ± 0.3</td>
<td>89.8 ± 2.4</td>
<td>84.0 ± 4.0</td>
<td>4.6 ± 0.2</td>
<td>54.4 ± 1.8</td>
<td>43.1 ± 1.0</td>
</tr>
</tbody>
</table>

**Significance**
L Q Q Q Q Q Q Q Q

**Paired comparisons**
0 vs. 1 **
1 vs. 3 NS
3 vs. 24 NS

* L, linear; Q, quadratic. Polynomial coefficients used in the contrasts have been corrected for the unequal spacing between treatment levels.

**Significance** *0.01 < P ≤ 0.05; **0.001 < P ≤ 0.01; ***P ≤ 0.001. Numbers within parentheses indicate the P values.
3.3. Effects of ethylene treatment on iris bulb respiration

The respiration of iris bulbs treated with ethylene rose rapidly during the treatment period, then fell gradually after the ethylene was removed (Fig. 2). For a week after the treatment, the respiration of bulbs treated for 24 h with ethylene remained higher than that of control bulbs.

3.4. Effect of ethylene treatment on soluble carbohydrate content of the bulbs

The major soluble carbohydrates of iris bulbs were sucrose, glucose, and fructose. The total soluble carbohydrate content of the bulb scales at the start of the experiment was 70 mg g⁻¹ dry weight (Table 3). Sucrose comprised 75% of the soluble carbohydrates. The dissected apices (including the primordial leaves) of the bulbs initially contained 200 mg soluble sugars g⁻¹ dry weight, again with a high proportion of sucrose (Table 4). No changes in the carbohydrate content of the scales were detected after the 1 day treatment with ethylene. During preplant cool storage of the bulbs, there were only minor changes in the carbohydrate composition of the scales, but the total soluble carbohydrate content of the scales of control bulbs increased significantly. The content in the scales of ethylene-treated bulbs did not change and was significantly less than that in the controls after cool storage (Table 3). In the apical buds of control bulbs, sugar content did not change during the cold treatment but there were marked changes in the ratio of sucrose to the other sugars (Table 4). Total sugar content of apices from the ethylene-
Table 3
Soluble carbohydrate contents of bulb scales of 7 cm bulbs of Dutch iris cv. 'Ideal' in response to ethylene treatment and normal preplant conditions. Bulbs were treated for 24 h with air or 10 μl l⁻¹ ethylene and held for 5 weeks at 10°C, then for 10 days at 20°C. The soluble carbohydrate content of bulb scales from six replicate bulbs sampled at various times was analyzed by HPLC.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Treatment</th>
<th>Sucrose (mg g⁻¹ dry wt.)</th>
<th>Glucose (mg g⁻¹ dry wt.)</th>
<th>Fructose (mg g⁻¹ dry wt.)</th>
<th>Total soluble sugars (mg g⁻¹ dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td></td>
<td>52.1</td>
<td>6.1</td>
<td>14.7</td>
<td>71.4</td>
</tr>
<tr>
<td>After the treatment (1 day)</td>
<td>Air</td>
<td>50.8</td>
<td>3.3</td>
<td>13.5</td>
<td>67.7</td>
</tr>
<tr>
<td></td>
<td>Ethylene</td>
<td>61.0</td>
<td>2.6</td>
<td>10.9</td>
<td>74.5</td>
</tr>
<tr>
<td>After 5 weeks at 10°C (10°C)</td>
<td>Air</td>
<td>63.6</td>
<td>13.9</td>
<td>19.6</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>Ethylene</td>
<td>42.3</td>
<td>10.5</td>
<td>14.8</td>
<td>67.7</td>
</tr>
<tr>
<td>After 10 days at 20°C (20°C)</td>
<td>Air</td>
<td>61.4</td>
<td>6.3</td>
<td>14.5</td>
<td>82.2</td>
</tr>
<tr>
<td></td>
<td>Ethylene</td>
<td>56.6</td>
<td>3.6</td>
<td>11.4</td>
<td>70.4</td>
</tr>
</tbody>
</table>

Paired comparisons
- Air vs. C₂H₄ after the 1-day treatment: NS NS NS NS NS
- Air vs. C₂H₄ after 5 weeks at 10°C: NS ** NS ***
- Air vs. C₂H₄ after 10 days at 20°C: NS NS NS NS NS
- Initial vs. 1 day of control bulbs: NS *** NS NS NS
- 1 day vs. 10°C of control bulbs: * *** * **
- 10°C vs. 20°C of control bulbs: NS *** * NS
- Initial vs. 1 day of ethylene-treated bulbs: NS NS NS NS NS
- 1 day vs. 10°C of ethylene-treated bulbs: ** NS NS NS NS
- 10°C vs. 20°C of ethylene-treated bulbs: * NS NS NS NS

Single degree of freedom orthogonal contrast not significant (NS) or significant at: *0.01 < P ≤ 0.05; **0.001 < P ≤ 0.01; ***P ≤ 0.001.

Table 4
Soluble carbohydrate contents of apices of 7 cm bulbs of Dutch iris cv. 'Ideal' in response to ethylene treatment and normal preplant conditions. Bulbs were treated for 24 h with air or 10 μl l⁻¹ ethylene and held for 5 weeks at 10°C, then for 10 days at 20°C. The soluble carbohydrate content of apices from six composite samples collected at various times was analyzed by HPLC.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Treatment</th>
<th>Sucrose (mg g⁻¹ dry wt.)</th>
<th>Glucose (mg g⁻¹ dry wt.)</th>
<th>Fructose (mg g⁻¹ dry wt.)</th>
<th>Total soluble sugars (mg g⁻¹ dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td></td>
<td>161.3</td>
<td>6.2</td>
<td>31.5</td>
<td>197.5</td>
</tr>
<tr>
<td>After 5 weeks at 10°C (10°C)</td>
<td>Air</td>
<td>111.8</td>
<td>43.7</td>
<td>63.8</td>
<td>219.3</td>
</tr>
<tr>
<td></td>
<td>Ethylene</td>
<td>130.6</td>
<td>64.3</td>
<td>70.2</td>
<td>265.1</td>
</tr>
<tr>
<td>After 10 days at 20°C (20°C)</td>
<td>Air</td>
<td>214.6</td>
<td>11.0</td>
<td>48.2</td>
<td>275.0</td>
</tr>
<tr>
<td></td>
<td>Ethylene</td>
<td>283.7</td>
<td>16.6</td>
<td>43.4</td>
<td>343.7</td>
</tr>
</tbody>
</table>

Paired comparisons
- Air vs. C₂H₄ after 5 weeks at 10°C: NS ** NS *
- Air vs. C₂H₄ after 10 days at 20°C: *** NS NS NS
- Initial vs. 10°C of control bulbs: ** *** *** NS
- 10°C vs. 20°C of control bulbs: *** *** * **
- Initial vs. 10°C of ethylene-treated bulbs: * *** *** *
- 10°C vs. 20°C of ethylene-treated bulbs: *** *** ***

Single degree of freedom orthogonal contrast not significant (NS) or significant at: *0.01 < P ≤ 0.05; **0.001 < P ≤ 0.01; ***P ≤ 0.001.
treated bulbs increased significantly during the cold treatment, and a reduction in sucrose content was complemented by a marked increase in reducing sugars (glucose + fructose). Differences in sugar content between apical buds from ethylene-treated and control bulbs were evident after the cold treatment and the differences increased further after the bulbs had been transferred to warm temperatures. After 10 days at 20°C, the apical buds of the ethylene-treated bulbs contained 30% more sucrose than that of control bulbs.

4. Discussion

As reported previously (Imanishi and Yue, 1986), ethylene treatment, even of quite small iris bulbs, resulted in increased flowering percentage and earlier flowering (Fig. 1 and Table 2). The treatment therefore induced flowering in bulbs that might otherwise have remained vegetative. Examination of dissected apical buds of the bulbs at different times after ethylene treatment indicated that no microscopic changes in differentiation occurred until they had been precooled and returned to warm temperatures. Nevertheless, the relatively short ethylene treatment at the start of the experiment induced floral differentiation in smaller bulbs. This result suggests the need for further investigation of the initial biochemical and physiological responses of bulbs to ethylene.

In potatoes, where ethylene breaks dormancy of the buds (Rylski et al., 1974), the ethylene response is associated with increased respiration (Reid and Pratt, 1972), similar to that found in iris (Fig. 2; Kamerbeek and Verlind, 1972), brodiaea (Han et al., 1990), and freesia (Masuda and Asahira, 1981). These latter workers studied the effects on respiration of four compounds which are known to break dormancy in freesia: C$_2$H$_4$, CO, KCN, and CaCN$_2$. Ethylene and CO stimulated respiration; the other compounds inhibited it. These authors therefore concluded that the release from dormancy is not necessarily by stimulation of respiration. Since they measured respiration only in the first 5 h following treatment, this conclusion is open to the criticism that respiration may have risen later in response to CN$^-$ as has been reported in other tissues (Solomos and Laties, 1976). In our studies, we found the stimulation of respiration in ethylene-treated bulbs to be correlated with the flower-inducing effect of the treatment. This respiratory rise, which has been found in many ethylene-treated storage organs, may reflect a general response to ethylene (Reid, 1987). It is likely that the induction of flowering is only one of many events initiated by the ethylene treatment.

Our study explored a hypothesis based on Sachs' 'nutrient diversion' theory, which proposes that chemical induction of flowering may be the result of greater source or sink activity, leading to increased carbohydrate status of the apex (Sachs, 1977). Recent data obtained in freesia, which showed higher soluble carbohydrates in the second leaf of plants grown in temperature regimes that accelerated flowering (Berghoef and Zevenbergen, 1990), were suggested to be consistent with this hypothesis. Our data, in contrast, do not indicate a role for changing carbohydrate status of the apex in ethylene-induced flowering of iris. The apical
bud of iris bulbs already was richly supplied with soluble sugars shortly after harvest, and ethylene treatment (which greatly increased percentage flowering in medium-sized bulbs) had no immediate effect on the carbohydrate content of the apical bud. The differences apparent between ethylene-treated and control bulbs after 5 weeks in the cold should probably be interpreted as a correlate of the onset of flowering. Admittedly, the apical tissues that we analyzed comprised rudimentary leaves plus apical meristems, so it is possible that a change in mobilization might have occurred within the tissues of the apical bud.

An earlier study (Halevy et al., 1963) indicated a much higher carbohydrate content of iris bulb scales than that found here. These workers determined soluble solids content by measurement of refractive index of expressed juice. It appears likely that other soluble materials (e.g. oligosaccharides or fructans) may have been responsible for the high refractive index in these storage organs. The changes in proportion of the component sugars following precooling (Tables 3 and 4), which were similar in ethylene-treated and control bulbs, suggest an effect of temperature itself, rather than any particular association with the induction of flowering.

We have also studied the mechanism of flower induction in the cormous geophyte, *Triteleia laxa* (Han et al., 1990). In this species, too, there was little detectable change in carbohydrate partitioning following ethylene treatment. Electron microscopic examination of the meristem from ethylene-treated corms suggested that an early response to ethylene might be accelerated growth of the meristematic dome. The fact that ethylene-treated iris bulbs produced plants with fewer and shorter leaves suggests that in this species, too, ethylene may exert its flower-inducing effects through changes in the microscopic architecture of the meristem. Flower induction in iris is associated with meristems of greater than a minimal diameter (Doss and Christian, 1979). Rapid enlargement of the meristematic dome due to ethylene might reduce the number of cells devoted to each leaf primordium (and hence leaf size) as well as the time to differentiation of the floral meristem (and hence leaf number).

Uhring (1973) in a microscopic study of flower differentiation in iris, noted "the development of an advanced stage of floral bud from a single treatment of ethylene gas in the shed prior to heat curing". Unfortunately, his ethylene-treated samples were dissected subsequent to the events which would be critical in evaluating the early effects of ethylene treatment on meristem growth. Early and detailed microscopic examination of the development of the meristem in ethylene-treated iris bulbs will be required to test our hypothesis that changes in meristem architecture is a common mechanism in ethylene-mediated induction of flowering and stimulation of flower development.

References


