Effect of Oxygen Tension on the Course of Ethylene- & Gibberellin-Induced Foliar Abscission

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Introduction

As early as 1886, Molisch (6) reported an increase of abscission rate with increasing oxygen tension. This correlation has been confirmed in recent years by Carns et al. (4) for bean explants kept in atmospheres of oxygen content ranging from 0 to 55%. In a study of the toxicity of elevated oxygen tension on plants, Siegel and Gerschman demonstrated oxygen-induced abscission in Begonia and Euphorbia (8).

Furthermore, the effects of N₂, CO₂, ethylene, and gas mixtures have been examined in relation to constitutional changes, respiration, and hormone levels associated with abscission without yielding a satisfactory exposition of its mechanism (2, 3, 4, 7, 11).

The present investigation stems from work on the comparative physiological behavior of plants at sub-atmospheric O₂ levels which suggested that the responses to abscission agents other than oxygen might be altered by a change in the level of O₂ itself.

Materials & Methods

Explants of Coleus Blumei, Benth. were used. They were taken from plants maintained under the natural photo period of about eight to nine hours during the winter of 1961-62. The explant technique was a modification of that described by Addicott et al. (1). The explants consisted of a node with adjacent internode (ca. 10 mm) and a pair of attached petioles, each cut to 5 mm in length. In order to minimize fungus growth, the cuttings were immersed in E. R. Squibb and Sons Mycostatin solution (200 units Mycostatin per ml H₂O) for 5 minutes prior to use, and then blotted dry. Explants were then laid upon degassed 4% agar in petri dishes so that the node and petioles projected over an open space. The dishes were stacked in anaerobic jars of the type used in previous experiments (10). In some experiments the jars were evacuated and then refilled with desired mixtures of oxygen and argon to a partial pressure of 635 mm mercury, the remain-

ing 125 mm being made up with either argon or ethylene. The following gas mixtures were used: air, 10% O₂ + 90% A, 7.5% O₂ + 92.5% A, 5% O₂ + 95% A.

In other experiments the partial pressure of ethylene in the jars was varied from 0 to 253 mm Hg, while the O₂ level was maintained at 5% and total pressure adjusted to 760 mm with argon.

For comparative purposes, the effects of gibberellin A₃ (GA₃) on abscission in air and in 5% O₂ was investigated. Following the technique described by Carns et al. (5), GA₃ in agar was applied distally to the petiole (cut surface) at 20 μg/petiole.

Abscission was measured by examination of the total numbers both of explants and petioles which had undergone abscission. Sample size was five explants (10 petioles) and the number of replicates varied from two to six. The results are expressed as number of explants and petioles abscissed per five explants after 2 to 4 days exposure to the test atmospheres.

Results

The number of petioles which have abscised by the 4th day is reduced as the oxygen content is lowered. In 10% O₂ or less, (table I), abscission was totally inhibited (table I). Abscission was also counted after application of pressure to the free end of the explant, but gave more variable results from replicate to replicate. hence was rejected as a quanti-

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of petioles abscised per 5 explants after 4 days</td>
<td>Air</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>10% O₂ + 90% A</td>
<td>0</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>7.5% O₂ + 92.5% A</td>
<td>0</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0</td>
</tr>
<tr>
<td>5% O₂ + 95% A</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table I

Oxygen Dependency of Abscission in Coleus Blumei

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1 Received Sept. 27, 1962.
tative method. It was clear, in these trials too, however, that the number of abscised petioles was maximal in air, and declined in lesser oxygen levels.

Introduction of ethylene into the air doubles the number of abscised petioles, but has only a marginal effect on the number of petioles lost in 5% O₂ (table II).

### Table II

Protective Effect of 5% O₂ against Ethylene-Induced Abscission of Coleus Blumei

<table>
<thead>
<tr>
<th>Atmosphere</th>
<th>Experiment No.</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6</td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>2 3 8 3 5 2</td>
<td>3.8</td>
</tr>
<tr>
<td>Air &amp; Ethylene</td>
<td>9 6 8 10</td>
<td>8.3</td>
</tr>
<tr>
<td>5% O₂</td>
<td>0 0 0 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>5% O₂ &amp; Ethylene</td>
<td>0 3 1 0  . . .</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*P_Ethylene = 125 mm Hg in all cases; higher concentrations were not more effective.

Variations in the partial pressure of ethylene up to 253 mm in 5% O₂ did not alter appreciably the degree of abscission.

Application of 40 µg (2 × 20 µg) of gibberellic acid to the explants kept in air results in a fourfold enhancement of abscission (table III), a magnitude of response consistent with that reported by Carns et al. (5). In 5% O₂, however, the extent of abscission in explants treated with GA₃ attained only a marginal level.

In order to determine whether or not the experimental atmosphere would support active processes in Coleus (as opposed to a more or less dormant state), seeds were incubated in sterile potting soil at 25°C under 5% O₂ to 95% argon. Germination was slower than in air but attained the same maximum figure, 87%: 2 to 4 cm seedlings of normal appearance were produced in about six weeks.

### Discussion

These results confirm previous reports that abscission is an oxygen-dependent process. In addition, they show strikingly the effect of low atmospheric oxygen content against both ethylene and GA₃.

Enhancement of abscission in air by gibberellic acid has been demonstrated, confirming previous reports by Carns et al. (5). Modification of these effects in 5% O₂ has been established.

Siegel and Porto (9) have proposed that the onset of senescence is controlled by an unfavorable pile-up of oxidants, upsetting an endogenous antioxidant-antioxidant balance. It may be inferred that abscission, a manifestation of leaf senescence, may be retarded by conditions which tend to favor the preservation of a more juvenile and less oxidized state. It has been shown that bean seedlings grown in 5% O₂ are richer in substances which reduce molybdate and silver ions (phenols, particularly) than are bean seedlings of the same age grown in air (10). These seedlings were also distinguished from air-grown plants of the same chronological age (2 weeks) by retention of their cotyledons and by lower lignin content.

In the course of the present experiments it was noted repeatedly that abscission was preceded by the appearance of a brown zone of discoloration spanning the abscission region. Such discolorations are commonly associated with phenol oxidations. In 5% O₂, the discolored zone does not appear, even in the presence of ethylene or GA₃.

Although these observations do not constitute sufficient evidence to establish the operation of an antioxidant-antioxidant balance in abscission, further tests of the antioxidant-antioxidant hypothesis seem warranted.

### Summary

The retardation of abscission in atmospheres low in oxygen content has been demonstrated. The results of Carns and other workers have been confirmed.

The results provide evidence that oxygen is a limiting factor in ethylene and gibberellic acid-induced abscission.

The results are discussed in relation to the antioxidant-antioxidant balance hypothesis for the control of developmental processes.

### Literature Cited


Stem Pigmentation in Lowbush Blueberry 1, 2

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Introduction

The sweet lowbush blueberry, Vaccinium angustifolium Ait., comprises a large part of the commercially harvested stand in northeastern North America. It is represented by a heterogenous population of clones that vary widely morphologically (2), physiologically (3), and in biochemical content. These clonal differences are so striking that research conducted on a small number of clones can have but limited applicability when extrapolated to the general population. In consequence, one of us (W.G.B.) has embarked on a series of studies of these clonal characteristics to understand the nature and the degree of the differences that occur among clones.

One of the most striking observations concerns variation in the pigmentation of the blueberry stem. The color ranges from yellow-greens through intermediate tans and light red-browns to deep browns.

This present report is concerned with the pigments that produce these colors and with the reasons for the variations.

Materials & Methods

The study was made using shoots of Vaccinium angustifolium Ait. harvested in early spring (May), before bud break. These had made their growth the previous season. There was no attempt to determine color shifts of shoots within a clone as the season advanced. For practical reasons, all tissue external to the cambial region was used to recover the pigment responsible for the stem color although this is localized in the bark. Similarly, the entire tissue central to the cambial region was used to recover the leucoanthocyanins, although these only appear in the secondary xylem region, and not in the medulla.

Anthocyanins of the Bark: Five gram samples of bark, freshly stripped from live blueberry shoots, were extracted by homogenizing with 25 ml methanol in a Waring blender for 5 minutes. Each extract was filtered, concentrated to 1 ml, and made up to 2.5 ml with 1% HCl in methanol. One ml samples of this solution were applied to Whatman No. 3 MM chromatographic paper, each as a streak 13 cm long and chromatographed with the organic phase of butanol: acetic acid: water (4:1:5). The anthocyanins separated into two bands, Rf's 0.32(A) and 0.38(B). Each of these was eluted (when the paper was just dry) with 25 ml of 1% HCl in methanol. The eluate was concentrated to 10 ml and the optical density measured with a Beckman D.U. spectrophotometer at 536 nm.

Leucoanthocyanins from the Wood: Following removal of the bark the central regions (wood & medulla) of blueberry shoots were air dried and ground in a Wiley mill to pass through a number 60 sieve. Samples (0.8g) of this material were steeped in 25 ml hot 1% HCl for 1 hour. An equal

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1 Received Nov. 26, 1962.

2 Contribution No. 114 from the Research Station, Canada Department of Agriculture, Fredericton, New Brunswick.