The Role of Gravity in Apical Dominance

EFFECTS OF CLINOSTATING ON SHOOT INVERSION-INDUCED ETHYLENE PRODUCTION, SHOOT ELONGATION AND LATERAL BUD GROWTH

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ABSTRACT

Shoot inversion-induced release of apical dominance in Pharbitis nil is inhibited by rotating the plant at 0.42 revolutions per minute in a vertical plane perpendicular to the axis of rotation of a horizontal clinostat. Clinostating prevented lateral bud outgrowth, apparently by negating the restriction of the shoot elongation via reduction of ethylene production in the inverted shoot. Radial stem expansion was also decreased. Data from experiments with intact tissue and isolated segments indicated that shoot-inversion stimulates ethylene production by increasing the activity of 1-aminocyclopropane-1-carboxylic acid synthase. The results support the hypothesis that shoot inversion-induced release of apical dominance in Pharbitis nil is due to gravity stress and is mediated by ethylene-induced retardation of the elongation of the inverted shoot.

That apical dominance is sensitive to gravity can be easily demonstrated in Pharbitis nil by observing the altered patterns of lateral bud outgrowth which result from horizontal or inverted orientation of the plant. In the horizontally placed plant, two or three lateral buds in random locations on the main shoot will grow out, while in the inverted plant, several buds near the base of the shoot will elongate. In both cases, there is a concomitant reduction of elongation in the shoot apex. In a recent review, Hillman (15) described a number of gravity effects on lateral bud growth. He suggested that gravity-induced ethylene could affect bud outgrowth.

In woody plants, it has been shown by Wareing and Nasr (33) and Smith and Wareing (32) that inactive lateral buds can be caused to grow out by bending down branches so that the buds are positioned on the stem's upper side at the top of the arch.

Robitaille and Leopold (30) found that the bending down of apple shoots is followed by an increase in ethylene production beginning after 16 h, an inhibition of growth in the lowered terminal shoot by d 14 and the outgrowth of lateral buds by d 21. They postulated that if stress-induced ethylene resulting from stem bending played a regulatory role in lateral bud outgrowth and the slowdown of growth in the lowered terminal shoot, the responsible mechanism probably involved more than a simple increase in ethylene levels.

Physical restriction of apical growth of an upright Phaseolus plant induces ethylene production in the restricted region and outgrowth in the next lateral bud basipetal to the restricted region (16). Treatment of the apical portion of the shoot with ethephon also retards apical growth and stimulates outgrowth of the lateral

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2 Abbreviations: HLB, highest lateral bud; ACC, 1-aminocyclopropane-1-carboxylic acid; PLP, pyridoxal phosphate; SAM, S-adenosylmethionine.
of the shoot, the effects of clinostating have been investigated. The use of clinostating, which involves a continued change in the direction of the gravistimulus in relation to the plant, is of particular interest in this study on the role of gravity in apical dominance. It is demonstrated that when a *Pharbitis nil* plant is clinostated with its upper shoot bent down in an inverted position, ethylene production and retardation of extension growth in this inverted shoot are significantly reduced, and the release of apical dominance is inhibited. A preliminary report of these data has recently appeared (9).

**MATERIALS AND METHODS**

**Plant Material.** Seeds of the violet strain of Japanese morning glory (*Pharbitis nil* [L.] or more correctly, *Ipomoea nil* [L.] Roth) from Marutane Co. Ltd. Kyoto, Japan were germinated in Petri dishes and transferred to sandy loam soil at 25 ± 2°C. The plants were grown under continuous light (cool-white fluorescent and incandescent lamps; General Electric; 54 to 126 μmol m⁻² s⁻¹, the range of photosynthetic active radiation from the minimum to the maximum plant height) as measured with a Li-Cor 185 B Photosynthometer. A fertilizer treatment (NPK: 20:20:20) was given during the second week. Plants, 19- to 22-d-old, were used.

**Growth Studies of Intact Buds and Shoots.** The effects of clinostating and shoot orientation on the growth of buds and shoots were analyzed by measuring bud and shoot extension growth, shoot fresh and dry weights, and internode diameter growth in both upright (straight) and inverted (bent) plants. Upper shoots were inverted by bending down the shoot at a point just above the fourth node and tying the inverted shoot apex to the lower region of the stake with a string to prevent the shoot from growing upwards. For clinostat experiments, this plant with its bent shoot (referred to as "inverted"), was clamped to the horizontal clinostat (Henry Troemmer, Philadelphia) and rotated at 0.42 rpm (Fig. 1). Upright (straight) plants were also clinostated. Measurements of bud and/or shoot lengths were made with a ruler. Shoot length was measured as that distance between the fourth node and the shoot tip. This portion of the stem includes all of the elongating region which extends 13 cm behind the tip. Fresh and dry weights of the growing region (beginning at 13 cm behind the tip on d 0) were determined for different treatments over a 7-d period. The effect of clinostating and shoot orientation on stem radial expansion was determined by measuring the diameter of the seventh internode over a 4-d period with callipers.

**Ethylene and ACC Determinations.** Ethylene production was determined by enclosing the sample tissue (seven 2.5-cm segments taken in sequence from each upright or inverted shoot beginning from 1 cm behind tip) in a 10-ml vial sealed with a rubber serum cap. Moist filter paper was enclosed in the vial to prevent drying. Ethylene released from the tissue was allowed to accumulate in the vial for 1 h; thereafter a single 1-ml sample of air was drawn from each vial and assayed for ethylene with a Hewlett-Packard gas chromatograph fitted with a flame ionization detector (24). Wound-induced ethylene production in *Pharbitis* has been determined to extend from about 1 to 7 h (with a peak of 1.5 ng g⁻¹ h⁻¹ at 3 h) following excision (data not shown). ACC content was determined according to the method of Miller and Pengelly (21) which is a modified method of Lizada and Yang (20). The tissue (300–400 mg) which was collected from 20-cm upper stem region from 3-cm behind the shoot apex, was homogenized in 2 ml of 5% (w/v) 5-sulfosalicylic acid (pH 1.8) and the homogenate was centrifuged at 30,000 g for 30 min at 4°C. The reaction mixture contained 0.2 ml of the supernatant, 0.6 ml of 5% 5-sulfosalicylic acid, and 0.1 ml of 50 mM HgCl₂. The vials were sealed and then injected with 0.1 ml NaOCl reagent (Clorox and 10 n NaOH at 1:1 ratio). The vials were incubated in an ice bath for 15 min after initial shaking, and 1-ml of gaseous sample was drawn and assayed for ethylene. The efficiency of ACC oxidation, which averaged 62%, was estimated by analyzing replicate samples having ACC internal standards.

**Assay of ACC Synthase.** ACC synthase activity was assayed according to the method of Kang et al. (17) which is a modified method of Boller et al. (4) and Yoshii and Imaseki (36). One g of tissue was homogenized with 3 ml of 100 mM Tris-HCl (pH 8.0), containing 10 mM EDTA, 2 mM MgCl₂, 4 mM DTT, 10 μM PLP, and 30% (v/v) glycerol. The homogenate was centrifuged at 25,000 g for 15 min, and the supernatant was passed through a Sephadex G-50 column which had been neutralized with 10 mM Tris-HCl (pH 8.0), containing 1 mM EDTA, 10 μM PLP, 2 mM MgCl₂, 1 mM DTT, and 30% (v/v) glycerol. The protein fraction was collected and used for assaying ACC synthase activity. The reaction mixture contained 0.4 ml of enzyme preparation (1.1–1.4 mg protein), 200 μM SAM, 10 μM PLP, and 100 mM Hepes (pH 8.5), in a total volume of 0.6 ml and incubated for 30 min at 30°C. To terminate the reaction, 0.1 ml of 50 mM HgCl₂ was added, and the amount of ACC formed was determined as described above.

**Protein determination in enzyme preparations was made according to the method of Bradford (5).**

**Stem Segment Studies.** For studies on the effect of clinostating and orientation on ethylene production, ACC content and ACC synthase activity in stem segments, seven segments (2.5 cm) of fifth and sixth internodes of an upright plant were collected from the upper 20-cm stem region 3-cm behind the shoot apex. The segments were oriented in upright and inverted positions on a
horizontal clinostat (0.42 rpm) in a 10-ml vial which was sealed with a rubber serum cap. The inner base of the vial was lined with 1% agar on which the stem segments were oriented vertically. This agar lining helped to prevent the displacement of segments against the vial wall during clinostat rotation. Appropriate chemical determinations were carried out over a 6 h period.

**Chemicals.** All the chemicals used were from Sigma Chemical Co.

**RESULTS**

Rotating the *Pharbitis nil* plant at 0.42 rpm on a clinostat with the upper portion of its shoot bent down in an inverted position (Fig. 1) prevented the release of correlative inhibition, i.e. the HLB did not grow out as was the case with the stationary plant with the inverted upper shoot (Fig. 2). Furthermore, the inverted upper shoot of the clinostated plant continued vigorous elongation at a rate only somewhat less than that of the stationary upright control plant but significantly more than that of the inverted stationary plant (Fig. 2). The growth rate of the shoot of the clinostated upright plant was about the same as that of the clinostated plant with the inverted shoot. Hence, clinostating significantly inhibited the retarding effect of shoot inversion on elongation in the inverted shoot and the preventive effect of shoot inversion on HLB outgrowth.

Increased radial expansion was also observed in stems of inverted upper shoots (Table I). Clinostating reduced this stem thickening to some extent. On both a fresh-weight and dry-weight basis, shoot inversion reduced growth by 43% over a 7-d period (Table II). Clinostating significantly negated this reduction effect by shoot inversion.

Significantly increased levels of ACC synthase activity, ACC content and ethylene production were present in the stationary inverted upper shoot as compared with those of the stationary upright shoot (Fig. 3). Clinostating increased ethylene production in the upright shoot 2-3-fold and decreased it in the inverted shoot by about half. The ethylene production levels in the clinostated upright and the clinostated inverted shoots were very nearly the same (Fig. 3). Clinostating also negated the reducing effect of shoot inversion on ACC synthase activity and ACC content.

When isolated 2.5-cm stem segments from the fifth and sixth internodes were clinostated in vials in either upright or in inverted position, the effects were equivalent to those obtained with the similarly clinostated intact stems. Clinostating decreased ACC synthase activity, ACC content, and ethylene production compared with those values obtained for the inverted segments (Table III). Differences in ethylene production were greater at 4 h than either 2 or 6 h (data not given).

**DISCUSSION**

The most significant result of the present study was that clinostating prevented the release of apical dominance caused by inversion (bending) of the upper shoot. The fact that clinostating significantly counteracted both the promotive effect of shoot inversion on ethylene production in the inverted shoot and the inhibitory effect of shoot inversion on elongation of the inverted shoot are consistent with the hypothesis that inversion of the upper shoot causes the release of apical dominance by ethylene-induced restriction of elongation of the inverted shoot.

That clinostating negated the gravity stress effect of shoot inversion was evident when it was observed that precisely the same responses in the shoot (i.e. in regard to ethylene production,
Elongation, and release of apical dominance) were found to occur during clinostating regardless of whether the upper shoot was inverted or not. Ethylene production was low. Shoot elongation was high and the release of apical dominance was prevented. Clinostating was not employed here only because it was another method for decreasing ethylene synthesis. It was also used because of its presumed effects on gravitational stress in the inverted shoot. This is of particular significance in a study which attempts to elucidate the role of gravity in apical dominance.

If gravity stress resulting from inverted orientation of the shoot enhances ethylene production as has been previously demonstrated, and if clinostating has a negating effect on this gravity stress, then one would expect, as has been found here, that clinostating would reduce ethylene production in the inverted shoot. However, there are many reports (14, 19, 23, 31) which indicate that clinostating increases ethylene production. These apparently conflicting data are resolved when the distinction between the effect of clinostating on ethylene production in an upright (straight) shoot and on that in an inverted (bent) shoot is taken into account.

Robitaille (29) found that clinostating of detached bent and unbent apple shoots not only reduced ethylene production below the levels of the stationary bent and horizontally oriented unbent shoots but also below the level of the stationary upright unbent controls. Although the latter result is puzzling, he found, as we did, that clinostating negated the enhancing effect of gravity stress by shoot bending on ethylene production.

For comparisons of ethylene production, ACC content and ACC synthase activity in equal-aged tissues in upright, inverted, and clinostated intact shoots, the stems to be segmented should have been marked before treatment rather than after, as was done (Fig. 3). Because of the slower growth of the inverted stems, segments from inverted shoots included more mature tissue than those from upright shoots. Since mature *Pharbitis* stem tissue evolves less ethylene than the young stem tissue (26, 27), the general effect was to underestimate inversion-induced ethylene production. This same qualification probably would also apply to effects on ACC production and ACC synthase activity. Inasmuch as much ethylene production occurs within 6 or 7 cm of the tip (27), it is unlikely that the additional mature tissue in the segments caused a significant decrease in ethylene evolution. The major conclusions of this study should not be affected. This supposition is further supported by the existence of a correlation between the growth responses to the inversion and clinostat treatments (Fig. 2) and the ethylene production from these treatments (Fig. 3).

It is also noted (Table I) that clinostating reduces shoot-inversion induced radial growth (of the inverted shoot) which, in addition to elongation restriction, is symptomatic of increased ethylene production.

The simultaneous inhibition of cell elongation and the promotion of lateral cell expansion are well known responses to ethylene (2, 11, 18). We have found in *Pharbitis* that the rate of shoot elongation is decreased by 30 to 40% within the first 24 h following shoot inversion (7, 27) and essentially ceases within about 2 weeks (7). Seven d after upper shoot inversion in the present study, total fresh and dry weight of the shoot decreased by 43% below that of the upright control (Table II). Clinostating negated the inhibitory effect of inversion on the growth of the inverted shoot to a large extent. Hence, it appears that shoot inversion in *Pharbitis* inhibits not only extension growth but also total growth during this 7-d period.

It is possible that the gravity stress of shoot inversion may induce the production of ethylene via accumulation of auxin in the inverted shoot due to inhibition of auxin transport. It is also possible that gravity stress may directly stimulate ethylene production without the mediating effect of auxin. In any event our studies indicate that inversion of the upper shoot probably induces ethylene production via enhancement of ACC synthase activity.

To answer the question as to whether shoot inversion release of apical dominance is due to a change in the direction of the gravity vector in relation to the orientation of the shoot or due to the change in the influence of some other factor associated with shoot inversion, the fact that clinostating has been shown here to dramatically alter this apical dominance response, provides strong evidence for gravity involvement. This *Pharbitis* shoot inversion system appears not only to provide a promising approach for investigating the interaction between gravity and apical dominance but also may possibly aid in the elucidation of the underlying control mechanisms of apical dominance.

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**Fig. 3.** The effect of clinostating on ethylene production, ACC content, and ACC synthase activity in intact stems. Upright plants had straight stems. The shoots of the inverted plants were bent down at the fourth node. For chemical determinations, seven 2.5 cm segments were taken in sequence from each shoot beginning 1 cm behind the tip. Stationary upright plant (O), clinostated upright plant (△), clinostated with inverted upper shoot (▲), stationary with inverted upper shoot (♦). Each point represents the mean value for at least three determinations except for the ACC synthase curve in clinostat inverted treatment where each point represents the mean value for two determinations. Vertical lines ± SD.

**Table III.** Effect of Clinostating on Ethylene Production, ACC Content, and ACC Synthase Activity in 2.5-cm Stem Segments from the Fifth and Sixth Internodes after 4 Hour Mean values for 3 determinations ± SD.

<table>
<thead>
<tr>
<th>Shoot Orientation</th>
<th>Ethylene</th>
<th>ACC</th>
<th>ACC Synthase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nl g⁻¹ h⁻¹</td>
<td>nmol g⁻¹</td>
<td>nmol h⁻¹ mg⁻¹ protein</td>
</tr>
<tr>
<td>Stationary upright</td>
<td>1.8 ± 0.2</td>
<td>16.3 ± 2.1</td>
<td>0.588 ± 0.12</td>
</tr>
<tr>
<td>Stationary inverted</td>
<td>2.0 ± 0.2</td>
<td>27.3 ± 2.9</td>
<td>1.357 ± 0.15</td>
</tr>
<tr>
<td>Clinostat inverted</td>
<td>2.0 ± 0.1</td>
<td>18.4 ± 2.7</td>
<td>0.772 ± 0.13</td>
</tr>
</tbody>
</table>
clinstats and to Dr. T. W. Tibbits for his helpful suggestions concerning their operation. Discussions with Dr. F. B. Salisbury have also been helpful.

LITERATURE CITED