Effect of Abscisic Acid on Sucker Development and Callus Formation on Excised Roots of *Populus tremuloides*

By

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Abstract

Abscisic acid (ABA) inhibited sucker development and promoted callus formation from cambium exposed at cut ends of excised roots from two clones of trembling aspen, *Populus tremuloides* Michx. Clonal differences in response to ABA inhibition of sucker outgrowth appeared to be related to the developmental stage of the preexisting shoot primordia. When ABA treatment was followed about a month later by gibberellic acid, ABA inhibition was partially reversed. This is one of few investigations in which both promotive and inhibitory effects of ABA have been demonstrated simultaneously on the same plant part.

Introduction

Inhibition of stem growth by exogenous applications of abscisic acid (ABA) has been reported for a number of plant species (reviewed by Addicott and Lyon 1969). When applied to elongating shoots, ABA delays bud break in nondormant plants and induces the formation of resting buds (Eagles and Wareing 1964, El-Antably *et al.* 1967, Little and Eidt 1968). The growth-inhibiting effects of ABA can often be overcome by exogenous application of gibberellic acid (GA_3).

Adventitious shoots (suckers) on aspen roots result from the outgrowth of two kinds of primordia, early (EP), including currently initiated meristems, and later stages (LP) of development (Schier 1972 a). It is possible to segregate developmental stages for treatment since clones can be found whose roots have primarily one or the other of these general primordial types. Schier (1972 b) showed that GA_3 inhibits shoot outgrowth from EP roots and stimulates it from LP roots. Addicott and Lyon (1969) reviewed many cases where ABA has been shown to be antagonistic to the action of GA_3. Therefore, it was thought useful to test the effect of ABA on sucker outgrowth from root segments from several aspen clones. In the only previously demonstrated effect of exogenous ABA on adventitious shoot development, Heide (1968) found that ABA promoted bud initiation in detached leaves of Begonia.

During preliminary studies, observations indicated that ABA promoted callus development from cambium exposed at the cut ends of excised roots. This response was investigated more thoroughly in the present study. Although ABA is generally known as a growth inhibitor (Addicott and Lyon 1969), it has induced callus formation in citrus bud cultures (Altman and Goren 1971).

Abbreviations: (EP) early, (LP) later stages of primordia.

Methods

In June 1971, root material was collected from two clones of aspen, *Populus tremuloides* Michx., growing in the Wasatch Mountains east of Logan, Utah. EP roots were obtained from clone 1, LP roots from clone 2. Procedures for collecting and pretreating root cuttings (10 × 1 to 2 cm) and for culturing root suckers were generally the same as those described in an earlier report (Schier 1972 b). Cuttings from each clone were randomly divided into five treatment lots and immersed for 24 hours in aqueous solutions of *cis-trans* ABA (Hoffman-LaRoche & Co.) at concentrations of 1, 10, 25, 50, and 100 mg/l. Control cuttings were soaked for the same amount of time in distilled water. Each ABA experiment involved six treatments (including control). Unsealed cuttings in lots of five per treatment were randomized in a vermiculite medium in each of four plant trays that were then placed in a greenhouse (25°C, day, to 15°C,

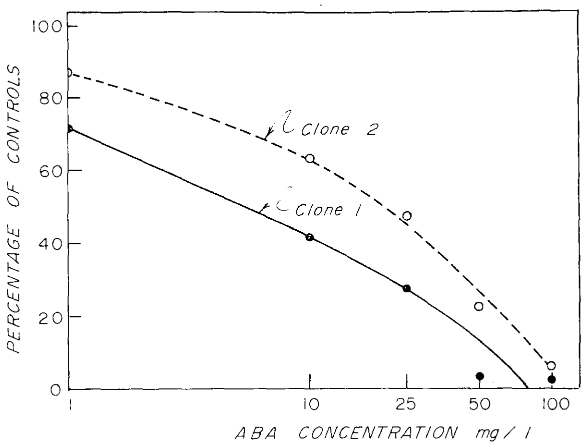

Figure 1. *Effects of ABA on sucker production from root cuttings of two aspen clones.* Average sucker number per cutting is expressed as the suckering percentage of control cuttings. Twenty cuttings were used per treatment.

night). Duplicate series of treatments were prepared for clone 1 roots. After 26 days, all roots from one of these duplicate series were removed from the growing medium, immersed for 24 hours in a 100 mg/l aqueous solution of GA_3 (90 % minimum, Sigma Chemical Co.) and then replanted. Forty days after initial ABA treatment, all cuttings were removed from the trays and the numbers of suckers more than 5 mm long recorded. The amount of callus growth on each cutting was also determined, except where specimens had been treated by ABA + GA_3.

Results

Analysis of variance, obtained by using the square-roots of the sucker counts, indicated that ABA significantly (1 % level) reduced the number of suckers produced from root cuttings (Figure 1). Retardation of shoot outgrowth was significantly greater in clone 1 (EP) than in clone 2 (LP) roots.

Gibberellin A_3 partially reversed ABA inhibition of shoot outgrowth from clone 1 roots (Table 1). The degree of reversal was dependent upon the ABA concentration. GA_3 was most effective when the treatment concentration of ABA was within the range of 10 to 50 mg/l and least effective at 100 mg/l ABA.

Chi-square contingency tests established that ABA significantly stimulated the development of callus at the cut ends of root segments (Table 2). Callus that did develop on controls was significantly smaller than that on ABA-treated cuttings. Optimum concentration for callus development on clone 2 roots was between 10 and 25 mg/l (Table 2). Callus formation on clone 1 roots increased to 50 mg/l ABA and then leveled off.

Table 1. *Effects of ABA and GA_3 on the average number of suckers produced from clone 1* (EP) *root cuttings.* ABA treatments were made at the start of the experiment, GA_3 treatments after 26 days, and all cuttings were examined at the end of 40 days. Twenty cuttings were used per treatment.

Treatments		Average number of suckers per cutting	Percentage inhibition	Percentage of inhibition reversal
ABA mg/l	GA_3 mg/l			
0	0	8.45 a*	—	—
0	100	8.05 a	—	—
1	0	6.10 b	28	—
1	100	5.85 b	26	—
10	0	3.50 c	59	—
10	100	5.55 b	30	49
25	0	2.30 d	73	—
25	100	4.40 c	43	41
50	0	0.30 e	96	—
50	100	4.35 c	44	55
100	0	0.20 e	98	—
100	100	1.45 d	78	20

* Treatments with no common letter are significantly different at 5 % level.

Discussion

This study has shown that abscisic acid inhibits sucker development and promotes callus formation on excised roots of aspen. ABA is known to cause promotive and inhibitory effects depending on the system used (Addicott and Lyon 1969), but this is one of the few investigations in which both effects have been demonstrated simultaneously on the same plant part.

ABA had a greater inhibitory effect on sucker production from clone 1 (EP) than from clone 2 (LP) roots. Differences in clonal response to ABA may reflect differences in sensitivity of primordia at early stages of development (clone 1 cuttings) and at later stages (clone 2 cuttings). However, physiological differences (caused by the growing site, genetic factors, *etc.*) in the root material from the clones may also affect inhibition by ABA, as was suggested by clonal differences in ABA-induced callus formation (Table 2). Because exogenous auxin and cytokinin interact with ABA in callus formation (Altman and Goren 1971), endogenous levels of these hormones in the aspen root cuttings may have affected their responses to ABA.

Table 2. *Effects of ABA on the percentage of root cuttings* (20 per treatment) *showing callus development at cut ends 40 days after treatments.*

Clone	ABA, mg/l					
	0	1	10	25	50	100
1	10	20	10	30	45	45
2	10	20	45	45	35	25

Studying adventitious shoot development in detached leaves of Begonia, Heide (1968) found that ABA promoted bud initiation. However, had he investigated later stages in shoot development, he might have found that ABA inhibits shoot outgrowth in Begonia as in aspen roots.

Partial reversal of ABA inhibition by GA_3 suggests that ABA may be a specific antagonist to gibberellic action or perhaps an inhibitor of gibberellin biosynthesis (Galston and Davies 1970). As previously reported, endogenous gibberellin plays an important role in sucker outgrowth (Schier 1972 b). Regardless of ABA concentration, a growth retardation of about 30 % remained that could not be reversed by exogenous application of GA_3 (Table 1). Residual retardation may result from the interaction of ABA and other hormones that have a role in shoot outgrowth (Leopold 1971). Or, since GA_3 is known to be inhibitory during the early stages of sucker development (Schier 1972 b), the 30 % retardation may represent the relative number of primordia that ABA prevented from developing to a stage at which they could be promoted rather than inhibited by GA_3. ABA + GA_3 inhibition did not change significantly as the ABA concentration increased from 1 to 50 mg/l. At 100 mg/l, the number of suckers per cutting was sharply reduced. This ABA concentration probably caused a large increase in the proportion of primordia not yet able to be stimulated by GA_3.

Studies correlating endogenous levels of growth inhibitors and development of root suckers (Eliasson 1971) provided no evidence that variations in the concentration of an inhibitor fraction (probably containing ABA) affected sucker production from root segments. However, these earlier results do not rule out the possibility that inhibitors affect sucker production in intact trees. Therefore, until specific methods are used to quantitatively measure ABA, the role of endogenous ABA in root sucker production will not be clarified.

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