

## Signals controlling root suckering and adventitious shoot formation in aspen (*Populus tremuloides*)

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**Summary** We determined the effects of removal of leaves, stem axillary buds, or the entire shoot on root suckering (adventitious shoot formation by roots) and basal stem sprouts in 3- and 4-year-old potted seedlings of aspen (*Populus tremuloides* Michx.). The greatest number of root suckers ( $67.9 \pm 8.5$  per plant) emerged after excision of the entire shoot. Defoliated and debudded stems were the major source of inhibitory agents for root suckering, although axillary buds and developing new leaves also exerted a significant inhibitory effect. Removal of mature leaves had only a minor effect on root suckering. Removal of a continuous band of bark (girdling) at the base of the stem consistently stimulated growth of adventitious shoots from the stem below the girdle and occasionally promoted root suckering. Exogenous application of indole-3-acetic acid to excised stumps inhibited root suckering and basal stem sprouting. Naphthylphthalamic acid (NPA), an auxin polar transport inhibitor, had no effect on root suckering or stem sprouting when it was applied to the bark of the basal stem. However, NPA significantly increased root suckering when it was applied to the exposed surface of xylem after girdling. These results suggest that polar transport of auxin in the xylem parenchyma is an important inhibitor of root suckering. On decapitated stems, vacuum extraction of xylem sap from the root system lowered the frequency of root suckering compared with decapitation alone, indicating that substance(s) originating in the root system also play a significant role in controlling root suckering.

**Keywords:** indole-3-acetic acid (IAA), naphthylphthalamic acid (NPA), vegetative reproduction.

### Introduction

Aspen (*Populus tremuloides* Michx.) regenerates vegetatively by root suckering, i.e., the sprouting of shoots developed from preformed or adventitious shoot primordia (hereafter called sucker buds) formed on roots. Without aboveground disturbance, the roots of a healthy mother tree rarely develop suckers. It is thought that the sucker buds are held in a state of paradormancy by signaling agents from outside of the dor-

mant tissue (Rohde et al. 2000, Anderson and Chao 2001). When the aboveground part of the plant is removed or damaged, the suppressed sucker bud primordia develop or adventitious sucker buds are formed. Consequently, root suckering is thought to be related to apical dominance and auxins and cytokinins are believed to play prominent roles in these processes (Cline 1991). Although there is much literature on the development and control of lateral buds on stems (Cline 1991, Sachs 1991), the mechanism controlling the formation and growth of sucker buds in roots has rarely been studied (Horvath et al. 2003).

Auxins (notably indole-3-acetic acid (IAA)) are thought to be synthesized primarily in young leaves and shoot apices and transported basipetally through the stem to inhibit the growth of lateral buds (Cline 1991). Application of IAA to cut stumps inhibits suckering in aspen (Farmer 1962). Although Schier (1978) was unable to verify this finding in intact plants, he found that auxins inhibit suckering of aspen root segments (Schier 1981). These studies did not consider the roles of lateral buds and stems in root suckering of aspen. Lateral buds are an important source of IAA in many plant species (Cline 1991, Sachs 1991, Horvath 1999). High concentrations of IAA are also found in the vascular cambium and xylem of stems in hybrid aspen (*Populus tremula* L. × *Populus tremuloides*) (Tuominen et al. 1997). Although removal of the aboveground part of the plant at the ground level is widely reported to promote root suckering (Farmer 1962, Schier 1978), it is unclear which aboveground organs are the primary causes of inhibition of root suckering in aspen. In soybean, stems are reported to be the source of lateral bud growth inhibition (Peterson and Fletcher 1975).

Long-distance transport of IAA occurs in both mature phloem elements (Baker 2000) and through other living cells in stems (Lomax et al. 1995). Transport through living cells is unidirectional (Friml and Palme 2002) and is thought to occur mainly in the vascular cambium, the expansion zone of the xylem and in parenchyma cells (Morris and Thomas 1978, Tuominen et al. 1997). Therefore, disrupting the continuity of the phloem by girdling should not be expected to block polar auxin transport completely, as has sometimes been suggested

(Farmer 1962, Schier 1972). Polar auxin transport that occurs from cell-to-cell is specifically inhibited by auxin transport inhibitors, such as naphthylphthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA) (Goldsmith 1977, Lomax et al. 1995). Naphthylphthalamic acid inhibited endogenous IAA polar transport in the cambial region of a 1-year-old *Pinus sylvestris* L. shoot (Sundberg et al. 1994).

Cytokinins are another group of growth regulators implicated in organogenesis. Cytokinins are principally produced in the root system and are transported to shoots in the transpiration stream (Letham 1994). High concentrations of cytokinins inhibit primary and lateral root elongation (Hopkins 1999), but promote development of stem buds and shoots (Schmuelling 2002). Accumulation of nitrate in roots can also act as a signaling substance; nitrate promotes the synthesis of cytokinins and concomitantly inhibits root growth and formation of lateral roots (Crawford 1995, Scheible et al. 1997, Takei et al. 2001, Werner et al. 2001, Forde 2002a, 2002b, Schmuelling 2002). The concept of nitrate-mediated root growth via regulating cytokinin concentration could also be applied to shoot growth in response to nitrate availability. It has long been speculated that substances (probably cytokinins and inorganic nitrogenous compounds) that accumulate in the stumps and roots after removal of the aboveground stem, help induce root suckering in aspens (Farmer 1962, Eliasson 1971b). The ratio of auxins to cytokinins is thought to be crucial in mediating organ regeneration in plant tissue culture (Sachs 1991). A high ratio induces root initiation, whereas a low ratio promotes shoot regeneration from cultured callus (Skoog and Miller 1957). Accumulation of cytokinins and a reduction in auxin in decapitated plants are thought to be the main reasons for the production and growth of sucker buds on roots.

We conducted a series of experiments to investigate various internal factors that affect root suckering in aspen. Objectives were to determine: (1) which organs of the aboveground stem are the major sources of inhibition of root suckers; (2) whether IAA applied to cut stems and an IAA transport inhibitor applied to girdled stems affect suckering; and (3) whether the transpiration stream is important in the removal of compounds that control shoot bud development on roots.

## Materials and methods

### Plant material

Experiments were conducted with open-pollinated aspen (*Populus tremuloides* Michx) seedlings produced by the Peace River Tree Nursery (Peace River, AB) from local seed sources (Fickle Lake, 53°27' N, 116°45' W and Soldar Creek, 57°02' N, 117°42' W). The seedlings were grown for one year in 5 × 12 cm containers and transplanted to a nursery bed in the second year. In May 2003, the 2-year-old seedlings were potted in 25-cm diameter, 6.5-l containers filled with a 2:1 (v/v) mix of peat moss and sand. The seedlings were grown outside at the University of Alberta, Edmonton, AB. During the growth periods, the seedlings were regularly fertilized with a 20,20,20 N,P,K commercial fertilizer and watered when nec-

essary. The plants were hardened and overwintered outside in the same location. During the winter, pots were buried in snow to protect the root systems from frost. Seedlings were about 100-cm tall in 2004. In Experiment 3 (see below), seedlings grown from Fickle Lake seeds were used. For the other experiments, we used seedlings from the Soldar Creek seed source.

In all experiments, a fully randomized design was employed with 10 seedlings per treatment. The seedlings were numbered and the numbers randomly drawn to assign the seedlings to specific treatments. The pots were arranged in five rows, each with 20 seedlings. Treatments lasted four weeks. Root-borne shoots that were longer than 3 mm were scored as suckers and the smaller preformed or adventitious shoot primordia on the roots were classified as sucker buds. If adventitious buds emerged and grew from the stem or the root collar, the new shoot was classified as a basal stem sprout.

### Experiment 1: Removal of shoot parts and stem girdling

To determine the aerial sources of signal(s) that inhibit aspen root suckering, 4-year-old seedlings were subjected to seven treatments (Figure 1) in June 2004. The treatments were: (1) removal of the entire shoot 2 cm aboveground (decapitation); (2) removal of all the leaves, shoot apices and lateral buds from stems (debudding + defoliation); (3) removal of all shoot apices and lateral buds from stems (debudding); (4) removal of all of the leaves including the leaf primordia (defoliation-once); (5) removal of all of the leaves followed by the removal of the young leaves that flushed from lateral buds about 10 days later (defoliation-twice); (6) removal of a 10-mm-wide ring of bark 2 cm above the root collar, scraping the vascular cambium off with a razor blade and covering the exposed area with Parafilm and aluminum foil (girdled) and (7) intact seedlings (control).

### Experiment 2: Full versus partial shoot removal

To investigate the role of the stem in aspen root suckering, 4-year-old seedlings were subjected to the following treatments in July–August 2004. The treatments were: (1) excision of the whole stem and any visible buds from the stem base (decapitation); (2) excision of the upper half of the stem and removal of all the remaining lateral buds and foliage (half-stemmed); and (3) removal of all the leaves, shoot apices and lateral buds from stems (debudding + defoliation).

### Experiment 3: Stem girdling and application of IAA and NPA

We investigated the roles of IAA (Sigma, St. Louis, MO, USA), NPA (TCI, Tokyo Kasei Kogyo, Tokyo, Japan) and girdling in the control of suckering of 3-year old seedlings treated in July–August 2004. One-hundred mg of IAA and NPA were dissolved in about 50 drops of Tween 20 and mixed with 10 g of lanolin at 50 °C. Plain lanolin was used in the control treatments. In seedlings in the NPA treatment, a 10-mm wide ring of the periderm was abraded 2 cm above the root collar to remove the wax layer and thus allow the NPA-lanolin mixture to penetrate the bark tissues. The abraded area was covered with the lanolin mixture and wrapped with parafilm and then aluminum foil. The control treatment was similar except NPA was not mixed in the lanolin. The girdling treatment was as de-

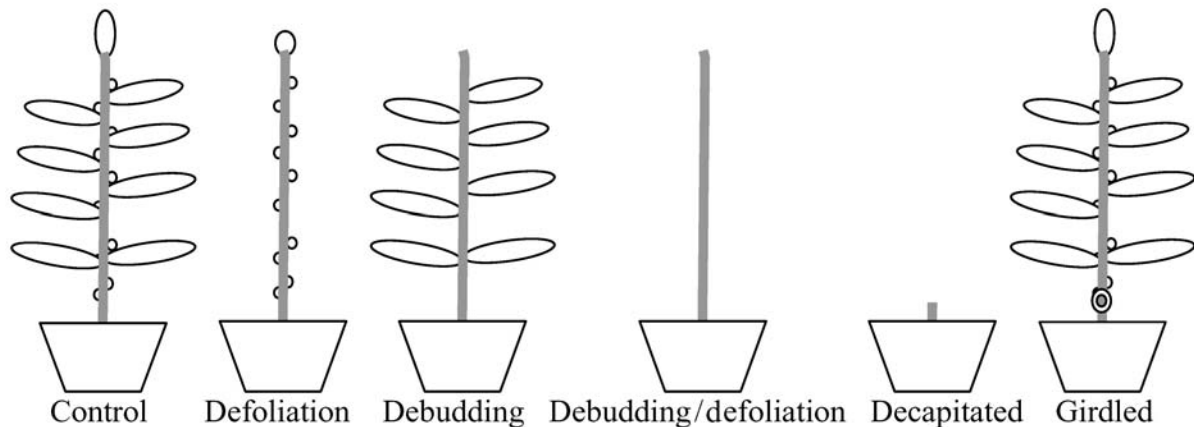


Figure 1. Diagrammatic representation of the treatments applied in Experiment 1 to study the effects on root suckering and stem sprouting in aspen (*Populus tremuloides*) of removing various aboveground organs.

scribed above (Experiment 1). The girdled + NPA treatment was similar except NPA was added to the lanolin covering the xylem exposed after removal of the bark. In the decapitation treatment, the stem was cut off 2 cm above the root collar and the surface of the excised stem was covered with lanolin, Parafilm and then aluminum foil. The decapitated + IAA treatment was similar except IAA was added to the lanolin. About 0.3 g of the lanolin mixture (with or without other chemicals) was used for each application and it was replaced each week. The top of the stump in the last treatments was scraped with a razor before each application of the lanolin mixture.

#### Experiment 4: Removal of xylem sap

To investigate if daytime withdrawal of xylem sap from the roots affected suckering, 4-year-old seedlings were decapitated 2 cm above the root collar. A vacuum line was attached to the decapitated stem of half of the decapitated plants. Thick-walled rubber tubing connected the stem to a collecting flask which, in turn, was connected to a vacuum pump which maintained a pressure of  $-0.08$  MPa during the daytime ( $18$  h  $\text{day}^{-1}$ ). Sap (exudate from root pressure) from the other half of seedlings was similarly collected but without applied vacuum. The volume of sap collected was measured every second day.

#### Statistical analysis

Values presented are the means of 10 replicated seedlings. The data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple comparison using the mixed procedure of the SAS 8.0 software package (SAS Institute Inc., Cary, NC). The  $t$  tests were performed with Excel (Microsoft, Office XP). All statistically significant differences were tested at the  $\alpha = 0.05$  level.

## Results

#### Removal of shoot parts and stem girdling

Decapitation of seedlings induced the highest numbers of root

suckers per plant (68), followed by the debudding + defoliation (23), defoliation-twice (7), and debudding (6) treatments (Table 1). Although seedlings in the girdled and defoliation-once treatments occasionally produced a few root suckers, there were no significant differences between these treatments and the control (Table 1). Roots from control seedlings had an average of five sucker buds, but did not develop any root suckers. Among the treated seedlings, roots of decapitated seedlings had the greatest number of sucker buds per plant (73) (Table 1). Roots of seedlings in the debudding and debudding + defoliation treatments had on average 25 sucker buds, followed by seedlings that had been defoliated twice (15). Roots of seedlings that were girdled and those that were defoliated once had similar numbers of sucker buds as control roots (Table 1). Only decapitated and girdled seedlings produced significant amounts of basal stem sprouts (Table 1).

#### Full versus partial shoot removal

The decapitation treatment resulted in the largest number of root suckers (49.3), followed by the half-stemmed (24.6) and the debudding + defoliation treatment (11.7) (Figure 2). Similarly the number of sucker buds on the roots was highest for seedlings in the decapitation treatment followed by seedlings in the half-stemmed and debudding + defoliation treatments (Figure 2).

#### Stem girdling and application of IAA and NPA

Auxin (IAA) applied to the proximal end of the excised stem completely inhibited root suckering (Figure 3A), basal stem sprouting and development of sucker buds (Figure 3B). Application of NPA to the abraded periderm of the stem did not affect the number of root suckers or basal stem sprouts. However, application of NPA to the exposed xylem promoted the development of sucker buds and growth of root suckers, but did not influence basal stem sprouting when compared with the girdled only treatment (Figure 3C). Girdling slightly increased the numbers of root suckers relative to the control and the decapitation + IAA treatment (Figure 3A). With and with-

Table 1. Effects of removing various organs on inhibition of root suckers, sucker buds and basal stem sprouts (Experiment 1). Defoliation II removed leaves twice in 10 days, whereas Defoliation I removed leaves only once at the beginning of treatment. The mean  $\pm$  SE numbers of root suckers, sucker buds or basal stem sprout per plant are shown. Means followed by different letters in the column refer to significant difference at  $\alpha = 0.05$  level ( $n = 10$ ).

Treatment	Suckers	Sucker buds	Basal stem sprout
Control	0.0 d	5.3 $\pm$ 1.6 e	0.0 c
Decapitated	67.9 $\pm$ 8.5 a	73.2 $\pm$ 4.3 a	3.4 $\pm$ 1.0 b
Debudding/defoliation	23.1 $\pm$ 4.9 b	21.0 $\pm$ 3.3 bc	0.0 c
Defoliation II	6.9 $\pm$ 3.0 c	15.0 $\pm$ 3.7 cd	0.0 c
Debudded	5.6 $\pm$ 2.4 c	29.5 $\pm$ 5.1 b	0.0 c
Girdled	2.1 $\pm$ 1.3 cd	4.8 $\pm$ 1.1 e	11.9 $\pm$ 2.2 a
Defoliation I	2.0 $\pm$ 0.6 cd	12.5 $\pm$ 5.1 de	0.0 c

out the NPA treatment, girdling induced more basal stem sprouts compared with the decapitation treatment (Figure 3C).

#### Removal of xylem sap

Vacuum extraction of xylem sap from the roots of decapitated seedlings removed more than 3 times (39.4 versus 13.1 ml per seedling) the amount of sap that exuded as a result of root pressure alone (Figure 4). Sap flow under vacuum approached zero by Day 16, whereas root pressure-driven exudation almost stopped by the Day 10. Xylem sap extraction under vacuum significantly reduced the number of root suckers, but did not affect the number of basal stem sprouts (Figure 5).

#### Discussion

We demonstrated that several organs in the aboveground aspen plant are sources of signals inhibiting root suckering and basal stem sprouting. The debudding and defoliation treatments pro-

moted a few root suckers, the combined debudding + defoliation treatment caused a moderate increase in root suckering and complete decapitation resulted in a large increase in root suckering. The organ with the largest influence on root suckering appeared to be the stem. Thus, in seedlings in the debudding + defoliation treatment, nearly 2/3 of the suckers were suppressed compared with decapitated seedlings (Table 1). Similarly, removing half of the completely defoliated and debudded stem promoted suckering more than leaving the entire debudded and defoliated stem (Figure 2). Tuominen et al. (1995) reported that the free IAA concentration in mature stems of hybrid aspen (*Populus tremula*  $\times$  *Populus tremuloides*) is twice that of the terminal buds and young leaves and about 30 times that of mature leaves. Peterson and Fletcher (1975) found that the presence of a long single internode above a lateral bud of a soybean stem was enough to prevent its outgrowth, presumably because the stem was a significant source of auxin. In contrast, Horvath (1998) suggested that the buds and leaves of the non-woody plant, leafy spurge (*Euphorbia esula* L.), are the sole shoot source of inhibitory agents. The occurrence of secondary radial growth and the large amount of living cells in the stems of woody plants compared with herbaceous plants might explain the difference in the ability of the stems of aspen and leafy spurge to act as a source of inhibitory agents.

We found that polar transport of IAA played an important role in the inhibition of root suckering. Application of IAA to a cut stem inhibiting root suckering and stem sprouting (Figure 3), indicating that it had replaced the effect of the intact shoot. Previous research on IAA application to cut stumps has yielded conflicting results. Farmer (1962) observed that IAA applied to stumps effectively inhibited both stem sprouting and root suckering in aspen. In contrast, Schier (1978) found that IAA inhibited stem sprouting but not root suckering; however, Schier applied IAA only for a short time after decapitation. Because auxins are reported to have a rapid turnover in detached root systems of European trembling aspen (*P. tremula*) (Eliasson 1971a), a continuous supply of exogenous auxin may be required to inhibit root suckering in decapitated plants.

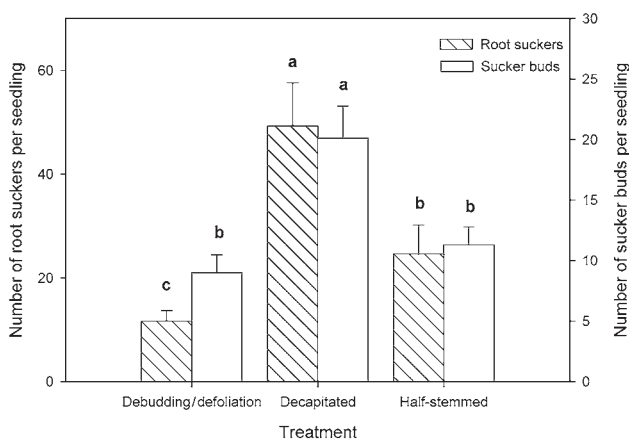


Figure 2. Effects of defoliation and bud removal (debudding/defoliation), removal of half the debudded and defoliated stem (half-stemmed) and removal of the complete stem (decapitated) on the numbers of root suckers and sucker buds (Experiment 2). The stems were completely debudded and defoliated in each treatment. Different letters indicate significant differences among treatment means for each variable ( $P \leq 0.05$ ). Values are means  $\pm$  SE ( $n = 10$ ).

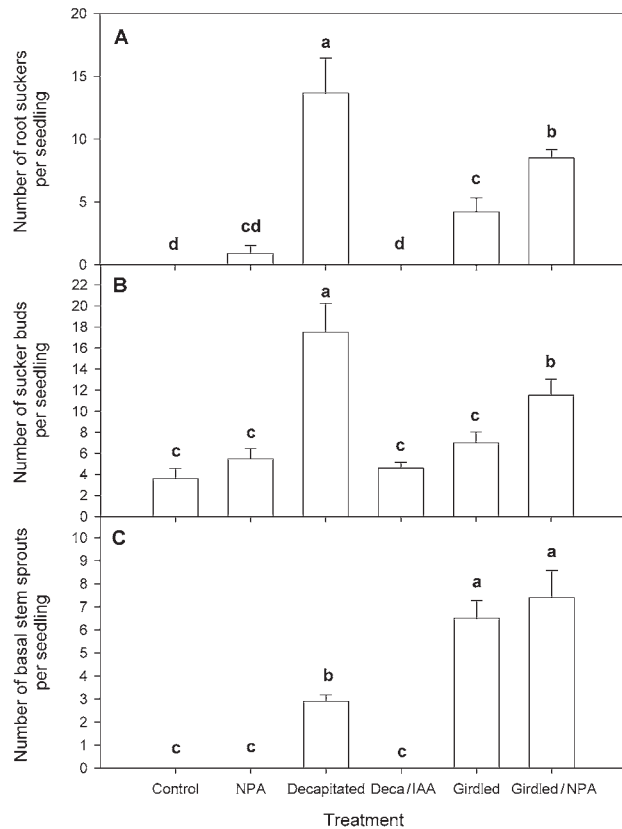


Figure 3. Effects of indole-3-acetic acid (IAA) and naphthylphthalamic acid (NPA) on the numbers of (A) root suckers, (B) sucker buds and (C) basal stem sprouts. Treatments: NPA = NPA in lanolin was applied to a 10-mm wide ring of the periderm abraded 2 cm above the root collar; Decapitated = removal of entire stem; Deca/IAA = after decapitation, IAA in lanolin was applied to the cut surface of the stump; Girdled = removal of a 10-mm wide ring of bark, phloem and vascular cambium 2 cm above the root collar; Girdled/NPA = after girdling, NPA in lanolin was applied to the exposed surface of xylem; and Control = lanolin was applied to a 10-mm wide ring of the periderm abraded 2 cm above the root collar. Different letters indicate significant differences between treatment means ( $P \leq 0.05$ ). Values are means  $\pm$  SE ( $n = 10$ ).

Application of NPA to the bark did not affect root suckering relative to the control, but NPA promoted root suckering and primordia initiation when applied to the exposed wood surface (Figure 3). The direction of auxin transport in phloem depends on source–sink relationships, therefore auxin transport in phloem is not susceptible to inhibition by NPA or TIBA (Goldsmith 1977), which could explain why application of NPA to the bark had little influence on root suckering and stem sprouting. Despite the abrasion of the periderm, NPA presumably failed to pass through the bark layer to affect polar auxin transport in the cambial and xylem elements (Saltveit and Fonteno 1983). Polar transport of auxin is believed to occur primarily in the vascular cambium and its immediate derivatives (Morris and Thomas 1978). Although girdling removed the vascular cambium and part of the developing wood tissues, auxins can still move through the living cells in the xylem,

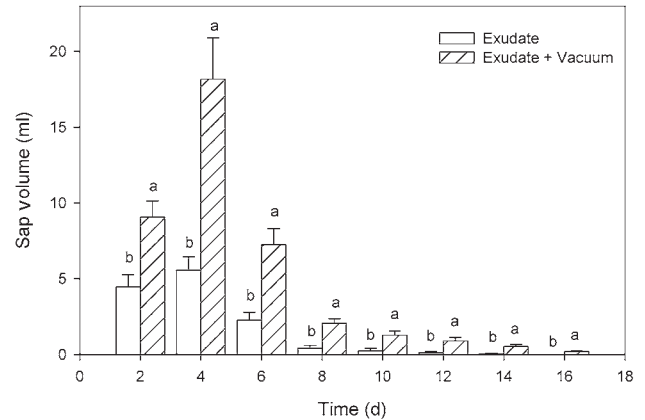


Figure 4. Volume of xylem sap collected from decapitated root systems by exudation and under vacuum ( $-0.08$  MPa). Exudate refers to exudation by root pressure and Exudate + Vacuum refers to sap volume obtained by applied vacuum plus root pressure. Within a collection date, different letters indicate significant differences between treatment means ( $P \leq 0.05$ ). Values are means  $\pm$  SE ( $n = 10$ ).

which could explain why girdling did not consistently promote root suckering in aspen (Farmer 1962, Schier 1978). Girdled seedlings in Experiment 1 produced virtually no root suckers, whereas the girdled seedlings in Experiment 3 produced moderate numbers of root suckers. The timing of girdling might also be important because of seasonal changes in the amount of undifferentiated living xylem cells present in the stem available for auxin transport. Experiment 1, with the highest suppression of suckering by the intact shoot was conducted in June when diameter growth had just commenced. As a result, stems likely contained more undifferentiated living xylem cells compared with plants in Experiment 3, which was conducted in August when diameter growth and tissue devel-

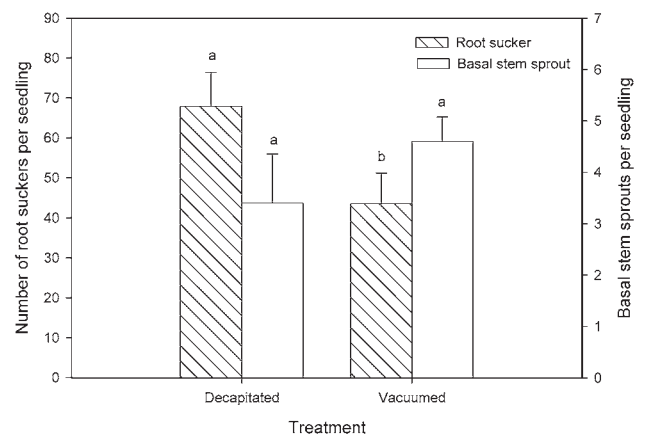


Figure 5. Numbers of root suckers and basal stem sprouts produced on aspen seedlings that were decapitated (Decapitated) or decapitated and the stump subjected to a vacuum of  $-0.08$  MPa (Vacuumed). Within a variable, different letters indicate significant differences between treatment means ( $P \leq 0.05$ ). Values are means  $\pm$  SE ( $n = 10$ ).

opment were nearly completed. However, genotypic differences cannot be ruled out because the seed sources used in Experiments 1 and 3 were 500 km apart. Girdling promoted more basal stem sprouts than root suckers in both Experiments 1 and 3 (Table 1 and Figure 3). The girdling treatment induced more basal stem sprouts than the decapitation treatment. We have no explanation for this response.

The finding that defoliation increased the number of sucker buds relative to the control (Table 1) indicates that other shoot-derived signals play a role in the control of root suckering. Horvath et al. (2002) reported that sugars, derived from mature leaves, inhibited root suckering of leafy spurge; therefore, a decline in sugar concentration as a result of the defoliation treatment might account for the promotion of the sucker buds.

Our results lend support to the hypothesis that substances (probably cytokinins or inorganic nitrogenous compounds, or both) that accumulate in the stumps and roots after removal of the aboveground stem help induce root suckering in aspen (Farmer 1962, Eliasson 1971b). Roots are major sites of synthesis of cytokinins that are translocated in the xylem to the aboveground parts of plants (Van Staden and Davey 1979). Decapitation causes an accumulation of root-derived cytokinins in the residual stem (Mader et al. 2003). Vacuuming the stump of the decapitated root system reduced the promotion of aspen root suckering that occurred following decapitation (Figure 5). This reduction occurred even though the vacuum treatment produced a tension of only  $-0.08$  MPa, which is far less than the natural tension found at midday in aspen saplings (as low as  $-0.9$  MPa in our study) and that the flow of xylem sap declined over time (Figure 4), likely as a result of a wound-healing response.

In conclusion, we found that the debudded and defoliated stems of aspen seedlings were the major sources of inhibitory agents controlling root suckering. Terminal and lateral buds, as well as young leaves, were important sources of inhibitory agents, but a single defoliation had a much smaller effect on the control of root suckering than decapitation. Based on the inhibition of aspen root suckering and basal stem sprouting observed following application of IAA to cut stumps, we conclude that IAA, which can be transported to the roots through the living cells of the xylem, has an important role in controlling root suckering and basal stem sprouting in aspen. Further, removal of root xylem sap (presumably containing the cytokinins and other compounds) resulted in a reduction of root suckering compared to decapitation.

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