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The role of specific plant organs and polar auxin transport in correlative inhibition of leafy spurge (*Euphorbia esula*) root buds[1]

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Abstract:

Localization of the source of the signal(s) controlling correlative inhibition of leafy spurge root buds (underground adventitious shoot buds located on the lateral roots) was studied by sequential removal of various plant organs. It was determined that full correlative inhibition of root buds was lost only after excision of all aerial tissue from the plant, or after excision of all aerial tissue except the stem. If mature leaves or growing axillary buds (or both) were left intact, no growth of root buds was observed. The synthetic auxin, (α-NAA, prevented release of apical dominance and subsequent outgrowth of stem and crown buds when applied to the cut end of the stem or crown. Exogenous application of NAA to either the stem or the crown had little effect on root bud growth. Application of the auxin transport inhibitor NPA around the base of the crown had no effect on root bud quiescence. These data are not consistent with the previous studies (Weed Sci. 35: 155-159 (1987)) that indicate a role for auxin in maintenance of correlative inhibition of root bud growth in leafy spurge. The results of auxin transport inhibitor studies presented here suggest that correlative inhibition of root bud growth does not rely on the classic polar auxin transport system.

Nomenclature:

Leafy spurge, *Euphorbia esula* L. #[3] EPHES; NAA, naphthalene acetic acid; NPA, *N*-1-naphthylphthalamic acid; TIBA, 2,3,5-triiodobenzoic acid.

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[3] Letters following this symbol are a Weed Science Society of America approved computer code from Composite List of Weeds, Revised 1989. Available from the Weed Science Society of America.

Keywords:

Root buds, apical dominance, auxin, NPA.

Introduction

Leafy spurge, *Euphorbia esula,* is a noxious perennial weed that primarily infests range and recreational lands throughout the northwestern United States and southern Canada (12). Clonal propagation of the plant through the growth of numerous underground adventitious shoot buds (on both the roots and crown) is the primary means of reproduction and maintenance of the perennial growth habit of this species (4). Under normal growing conditions, these buds develop but are maintained in a quiescent state until the aboveground portion of the plant is killed or the root system is disturbed and root sections containing buds are separated from the rest of the plant (4). When this occurs, these adventitious buds resume growth and form the active apical meristems of new shoots. The process by which the axillary and adventitious buds are maintained in a quiescent state is known as correlative inhibition (2).

Currently, the nature of the signal that induces or permits growth in the root bud is unknown. Evidence indicates that auxin may play a critical role in the control of axillary bud growth (2). However, studies on the effects of auxin on the growth of adventitious buds are limited. Two studies on isolated root buds and root sections of leafy spurge suggest that auxin is likely to play a role in this system as well (1, 11). Growth of root buds on isolated root sections is inhibited by high levels (10^{-5} M) of indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) (11). However, experiments on isolated buds in culture show that auxin (10^{-7} M) is required for bud growth (K.A. Biewer, unpublished observations). Other factors, such as nitrogen availability (6), competition for water (7), and photosynthates (8), also may play a role in controlling root bud growth.

To gain a better understanding of the mechanisms of the correlative inhibition of leafy spurge root buds, the source of the signal(s) involved in the control of root bud growth was examined. Additionally, the effect of a synthetic auxin and an auxin transport inhibitor on the quiescent state of the adventitious and axillary buds was studied.

Methods and Materials

Plant material

Plants used for these experiments were all started from shoot cuttings as a small group of plants that were originally isolated from a wild *E. esula* L. population in North Dakota and have been maintained by clonal propogation for more than 8 years. To reduce any possible seasonal effects, shoot cuttings from the green house culture were placed in

potting mix[4] and maintained in a greenhouse under an 18 h photoperiod at approximately 28 ± 4ºC for 2-3 months (throughout the duration of all experiments). Induction of root bud growth was rated 7 or 10 days after various treatments.

Plant treatments

In experiments designed to determine the source of the signal(s) that control correlative inhibition of the root buds, crown buds were removed from plants to prevent them from intercepting or generating signals that would interfere with root bud development. Plants were otherwise left intact (Control) or subjected to the following treatments (see Fig. 1): the upper 7-13 cm of the aerial portion of the plant was excised (Meristemless); the aerial portion of the plant was excised and the remaining leaves were removed (Meristemless/Leafless); the aerial portion of the plant was excised and the remaining stem buds were removed (Meristemless/Budless); the aerial portion of the plant was excised and both the remaining stem buds and leaves were removed (Meristemless/Leafless/Budless); the entire plant above the base of the crown was excised (Induced). One set of five plants each was used for each treatment in two separate experiments. Buds were rated as growing if they were both green and had elongated to at least 3 mm in length 7 days after treatment.

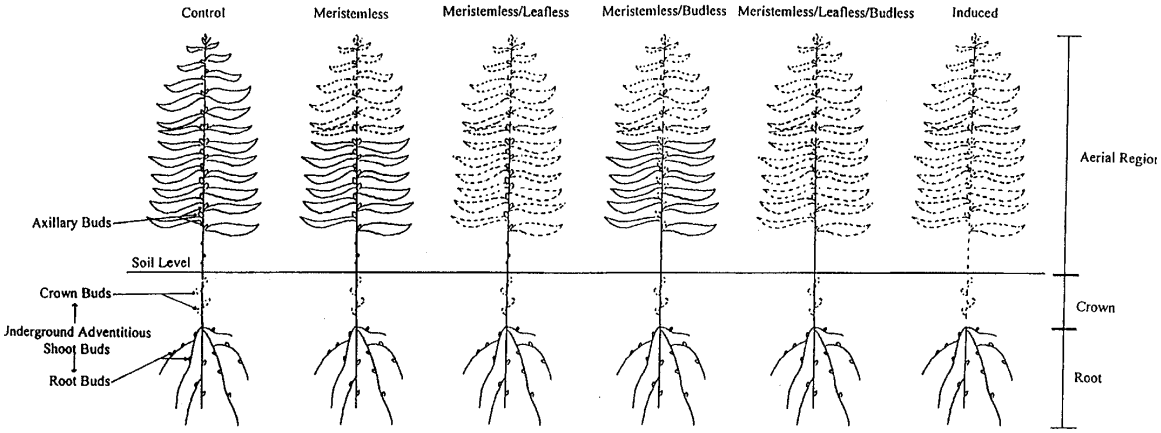

Fig. 1. Graphic representation of plant treatments and labeling of plant organs. Organs drawn with broken lines were excised.

Auxin and auxin transport inhibitor treatment

Lanolin mixtures containing 1% (w/w) β-NAA, α-NAA, or NPA were prepared as described previously (10). Briefly, 25 mg of β-NAA, α-NAA, or NPA were dissolved in 14 drops of Tween20 and mixed with 2.5 g of lanolin at 50ºC. The mixture solidified as it cooled to room temperature. Two- to 3-month-old plants were then prepared by abrading

[4] Sunshine I potting mix (Sun Gro Horticulture Inc., Bellevue, Wash.)

a 6 mm wide ring around the crown approximately 15 mm above the roots to enhance penetration of the compounds into the plant. Approximately 100 mg of lanolin paste containing either β-NAA, or α-NAA were applied evenly to an abraded region at the base of the stem. One set of 10 plants was tested for each treatment in two independent experiments. The top portion of a set of two additional sets of five plants were excised at a point approximately 7.5 cm above soil level, and approximately 100 mg of these lanolin pastes were then applied to the cut surfaces of the stems. Also, two sets of five plants were excised approximately 2.5 cm above the base of the crown, and 100 mg of the lanolin mixtures were applied directly to the cut end. Buds were scored as growing if they were both green and had elongated at least 3 mm 7 days after treatment.

To determine if α-NAA was toxic to stem and crown buds, two sets of five plants were treated as described above following excision 2.5 cm above the base of the crown or 7.5 cm above soil level. One week following treatment, the top 0.5 cm of the treated stump was excised. Bud growth was scored as described above 7 days after removal of the α-NAA treated section.

The effectiveness of the auxin transport inhibitor NPA was determined by preparing two sets of 12 plants (3 plants per treatment) by excising the aerial portion of the plant 4 cm above the base of the crown, and abrading each plant 1 cm below the cut end. Plants were left untreated, or had approximately 100 mg of a 1% lanolin paste of α-NAA applied to the cut end of the crown, and (or) a 1% mixture of NPA applied around an abraded section of the crown as described above. Sections above and below the abraded area were scored as growing if one or more crown buds had turned green and elongated 10+ mm after 7 days.

Results

Sequential removal of foliage and breaking of root bud quiescence

To determine which portions of the plant are responsible for maintaining the correlative inhibition of the root buds, various plant organs were removed from the plants, and the effects on root bud growth were observed (Table 1). The results from this experiment indicate that root buds were released from correlative inhibition if the entire aerial portion of the plant above the base of the crown was removed, or if only a naked stem was left intact (Table 1, Induced, Meristemless/Budless/Leafless). All of the root buds remained quiescent both in intact plants (Table 1, Control), and in plants where only the apical meristem was removed (Table 1, Meristemless). Additionally, correlative inhibition of the root buds was maintained in plants with only mature leaves (Table 1, Meristemless/Budless) or only growing axillary buds (Table 1, Meristemless/Leafless).

Effect of α-NAA on root bud quiescence

Reports in the literature suggest a role for auxin in the maintenance of correlative inhibition in the root buds of leafy spurge (1, 11). To gain a better understanding of the

Table 1. The effects of removal of various plant organs on correlative inhibition of root buds.

Control*	Induced	Meristemless	Meristemless/ Budless	Meristemless/ Leafless	Meristemless/ Leafless/Budless
0	24.3±2.3	0	0	0	34.1±6.1

Note: Combined data from two replicates of five plants for each treatment expressed as a percentage of green and growing root buds 7 days after treatment. See Fig. 1 for treatment details.*±95% confidence interval.
*±95% confidence interval.

transport and effect of auxin on root bud growth, experiments were undertaken in which 2-month-old plants were tested for their response to the synthetic auxin α-NAA. The inactive analogue, α-NAA, was used as a control for these experiments.

The results, summarized in Table 2, indicated that α-NAA had only a minor effect on the control of root bud quiescence in leafy spurge (13 ± 7.2%[5] growing buds compared with 17.2 ± 7.1%[4] in β-NAA treated plants, Table 2) when applied to the excised crown. However, it should be noted that plants treated with both α-NAA and β-NAA consistently (but not significant statistically) showed reduced root bud growth when compared with the excised controls (13 ± 7.1%[4] and 17.2 ± 7.2%[4] compared with 24.3 ± 2.3%[4], respectively; Table 2). There was no growth of any buds in the untreated controls (Table 2). However, α-NAA prevented growth of both crown and stem buds on all of the plants where it was applied distally to these organs (Table 2). Subsequently, growth was observed on both stem and crown buds following removal of the α-NAA by excising the top 0.5 cm of the treated stump. This indicated α-NAA was not simply killing the quiescent buds (Table 2). It should be noted that most of the growing buds were located within 1 cm of the excised stump following removal of the NAA-treated section. When α-NAA was applied to an abraded section of the crown (with the aerial portion of the plant left intact) it had no obvious effect on the buds above or below the treated region (Table 2). It did, however, tend to increase root growth (data not shown).

Table 2. Ability of NAA to substitute for plant-generated signal(s) in maintaining correlative inhibition of leafy spurge adventitious and axillary buds.

	Root buds	Crown buds	Stem buds
Application of NAA to abraded crown			
Control	0	0	0
Abraded	0	0	0
Abraded + β-NAA	0	0	0
Abraded + α-NAA	0	0	0
Application of NAA to proximal end of excised stem			
Control	0	0	0
Excised	0	0	27.5±7.9*
Excised + β-NAA	0	0	29.4±9.7
Excised + α-NAA	0	0	0
Excised + α-NAA > removal of α-NAA	0	0	7.2±0.4

[5] *±95% confidence interval.

	Root buds	Crown buds	Stem buds
Application of NAA to proximal end of crown			
Control	0	0	0
Excised	24.3±2.3	41.3±12.4	na†
Excised + β-NAA	17.2±7.1	35.6±2.0	na
Excised + α-NAA	13±7.2	1.7±2.3	na
Excised + α-NAA after removal of α-NAA	12.8±1.5	45.8±4.6	na

Note: Combined data from replicates of five plants for each treatment expressed as a percentage of the plants with growing buds of the indicated type 7 days after treatment.
*±95% confidence interval.
†na, not applicable because buds were excised by the treatment.

Effects of the auxin transport inhibitor NPA on root bud growth

The role of polar auxin transport on the growth of root buds was assessed by comparing root bud growth ± application of NPA with an abraded area of crown (Table 3). Also, to determine if NPA treatment inhibited root bud growth, bud growth was first induced by excision of the aerial portion of the plants, and plants were then treated with NPA (Table 3). None of the root buds from intact plants grew with or without the NPA treatment. In excised plants, 27.4 ± 6.1%[4] and 24.3 ± 2.3%[4] of the root buds grew with and without NPA treatment, respectively. These results indicated that NPA had neither a positive nor a negative effect on root bud quiescence.

Table 3. Effectiveness of NPA in blocking transport of signal(s) controlling correlative inhibition of root buds.

Control	Abraded	Abraded + NPA	Excised	Excised + NPA
0	0	0	24.3±2.3*	27.4±6.1

Note: Combined data from replicates of five plants for each treatment expressed as percentage of green and growing root buds 7 days after treatment.*±95% confidence interval.
*±95% confidence interval.

Since the NPA treatment appeared to be ineffective in blocking the signal(s) that maintained root bud quiescence, it was necessary to ascertain the effectiveness of NPA in blocking polar auxin transport. Previous studies indicated that NAA could substitute for the aerial portion of the plant in maintaining correlative inhibition of the crown buds (Table 2). This effect was utilized to determine if NPA could inhibit NAA from reaching crown buds distal to the NPA treated area. In these experiments, all of the sections below the NPA-treated area had at least two growing buds regardless of whether NAA was or was not added distally (Table 4). However, as was previously observed, NAA did effectively inhibit crown bud growth above the NPA treated section. This study demonstrated that NPA could block the effects of NAA on crown buds below the NPA-treated area, but had little effect on crown buds above it. Crown buds from control plants (excised and abraded, but with no NAA or no NPA) broke quiescence above and below the abraded area. Crown buds above and below the abraded area both remained quiescent when NAA

was applied to the cut end of the crown. When NPA alone was applied to the abraded area, it had no effect on crown bud quiescence. However, when both NAA and NPA were applied to the crown sections, all of the crown buds above the NPA treated area remained quiescent and bud crown bud growth was observed on all of the sections below the NPA-treated area.

Table 4. Effect of NPA on polar transport of α-NAA.

	Excised and Control	+α-NAA	+NPA	+α-NAA and +NPA
Above abraded area	100	0	100	0
Below abraded area	100	0	100	100

Note: Combined data from replicates of plants for each treatment expressed as a percentage of crown sections with growing buds.

Discussion

These experiments were designed to determine in which organs the signal(s) for correlative inhibition of root buds originate. Also, because auxin appeared to be involved in this process, it was of interest to determine if exogenous application of auxin or a polar auxin transport inhibitor could interfere with the signals controlling correlative inhibition of the root buds. Previous experiments on the growth and development of root buds in leafy spurge have also shown that root buds remain in a quiescent state until the root is separated from the aerial portion of the plant (4). Little is known about the signals that maintain correlative inhibition of root buds in leafy spurge, although auxin (1, 11), competition for nitrogen (6), water (7), carbohydrates (8), and phytochrome (5) have been suggested to play a role.

Consistent with a role for auxin in correlative inhibition of leafy spurge root buds is the finding that application of NAA to the cut end of root sections maintains the quiescence of the root buds (11), although complete inhibition of root bud growth following exogenous NAA treatment was not observed. This might be due to poor transport through the roots of leafy spurge. Indeed, in experiments where auxin was applied to the cut end of root sections, over 80% remained at the site of application (13).

In earlier studies, the auxin transport inhibitor, TIBA (2,3,5-triiodobenzoic acid), increased root bud growth (11). Interestingly, the auxin transport inhibitor NPA has little effect on the growth of the root buds even though it appeared to effectively block transport of NAA when applied to the crown. This apparent discrepancy could be explained by the differences in specificity of the two inhibitors, or by differences in the interpretation of root bud growth. In the present study, buds were only scored as growing if they had both elongated and turned green. In the earlier study, only bud elongation was required for a positive result. Also, it is possible that the additional plant manipulations in the earlier study, together with the TIBA treatment, induced root bud growth (plants were removed from the soil and the root buds were scored prior to the TIBA treatment). This possibility is suggested by the fact that there was an increase in the percentage of growing root buds in the control plants (13), a phenomenon not observed in the present study.

The findings with NPA-treated plants suggest that if auxin is the primary signal responsible for maintaining the correlative inhibition of the root buds, the primary mode of its translocation is not likely to be through the classic polar auxin transport system. Thus, the primary mode of its translocation is either by diffusion through the phloem, or by being directed down the plant in a conjugated form via a system that is not inhibited by NPA. Indeed, there is evidence for auxin being conjugated with other components or moving in the form of an auxin precursor (3, 9, 14). The application of α-NAA to the cut end of the crown did not inhibit root bud growth, but did inhibit growth of crown buds (Table 3). This suggests that the role of polar auxin transport in correlative inhibition may be limited to the main stem section in leafy spurge.

Auxin, however, is not likely to be the only signal that controls root bud quiescence. Leafless spurge plants with intact and growing stem buds are probably producing auxin in the growing buds and young leaves, although perhaps not at levels equivalent to those in intact plants. Also, in earlier experiments, it was noted that some root buds from leafless plants will still break quiescence if they are not covered by soil (D.P. Horvath, unpublished observations). Therefore, other factors appear to modify or compete with the primary signal that is responsible for maintaining the correlative inhibition of root buds.

Perhaps the most interesting result from these studies is that both leaves and stem buds are required to fully maintain correlative inhibition of the leafy spurge root buds. In most plant systems that have been studied, control of axillary bud growth is the result of apical dominance that is often presumed to occur via the effects of auxin produced in an active meristem (2). However, the observation that loss of all meristematic tissue did not result in root bud growth suggests that quiescence in root buds of leafy spurge is the result of at least one additional signal produced by mature leaves of the plant. One possibility is that this leaf-derived signal is a carbohydrate. Earlier work on this topic has indicated that sucrose may play a role in maintenance of root bud quiescence (8). Also, expression of cyclin D3 from *Arabidopsis,* a key cell division regulatory protein, is altered by both auxin and sucrose in cultured cells (15). Nonetheless, it is still possible that auxin from the leaves is sufficient for maintaining correlative inhibition of root buds, and that either leaves or stem buds produce enough auxin to prevent root bud growth. We are currently in the process of cloning genes that are induced in the root buds by the same conditions that induce root bud growth. Studying the expression of such genes may provide some answers to this question. Understanding the nature, source, and transport of the signal(s) that control correlative inhibition of the root buds of leafy spurge could lead to new measures to help control this weed. Such measures might include inducing root bud growth at times when such growth would be disadvantageous to the plant or to prevent root bud growth when it is needed.

Acknowledgments

I thank Dr. David Davis and Dr. Don Galitz for their critiques and discussions of the manuscript and data.

References

1. Budd, T.W. 1973. An excellent source of vegetative buds for use in plant hormone studies on apical dominance. Plant. Physiol. 52: 82-83.
2. Cline, M.G. 1991. Apical dominance. Bot. Rev. 57: 318-358.
3. Cohen, J.D., and Bandurski, R.S. 1982. Chemistry and physiology of the bound auxins. Annu. Rev. Plant. Physiol. 33: 403-430.
4. Coupland, R.T., Selleck, G.W., and Alex, J.F. 1955. Distribution of vegetative buds on the underground parts of leafy spurge *(Euphorbia esula* L.). Can. J. Agric. Sci. 34: 161-167.
5. Galitz, D.S. 1994. The biology of leafy spurge. Proceedings of the Leafy Spurge Strategic Planning Workshop, Dickinson, N. Dak., March 29-30, 1994. Agricultural Experiment Station, NDSU Extension Service, North Dakota State University, Fargo, N. Dak.
6. McIntyre, G.I. 1971. Developmental studies on *Euphorbia esula.* The influence of the nitrogen supply on correlative inhibition of root bud activity. Can. J. Bot. 50: 949-956.
7. McIntyre, G.I. 1979. Developmental studies on *Euphorbia esula.* Evidence of competition for water as a factor in the mechanism of root bud inhibition. Can. J. Bot. 57: 2572-2581.
8. Metzger, J.D. 1994. Evidence that sucrose is the shoot-derived signal responsible for the correlative inhibition of root bud growth in leafy spurge. Plant. Physiol. 105(Suppl.): 97.
9. Michalczuk, L., and Chisnell, J.R. 1982. Enzymatic synthesis of 5-^{3}H-indol-3-acetic acid and 5-^{3}H-indol-acetyl-myo-inositol from 5-^{3} H-L-tryptophan. J. Labelled Compd. Radiopharm. 19: 121-128.
10. Mitchell, J.W., and Livingston, G.A. 1986. Methods of studying plant hormones and growth-regulating substances. U.S. Dept. Agric. Agric. Handb. No. 336.
11. Nissen, S.J., and Foley, M.E. 1987. Correlative inhibition in root bud of leafy spurge. Weed Sci. 35: 155-159.
12. Noble, D.L., Dunn, P.H., and Andres, L.A. 1979. The leafy spurge problem. Proceedings of the Leafy Spurge Symposium, June 26-27, 1979, Bismark, N. Dak. North Dakota Coop. Ext. Serv., Fargo, N. Dak.
13. Thompson, W.M. 1995. Herbicide absorption and adventitious shoot bud growth in leafy spurge *(Euphorbia esula).* Masters Degree Thesis, Univ of Nebraska, Lincoln, Neb.
14. Skoog, F. 1937. A deseeded *Avena* test method for small amounts of auxin and auxin precursors. J. Gen. Physiol. 20: 311-334.
15. Soni, R., Carmichael, J.P., Shah, Z.H., and Murray, A.H. 1995. A family of cyclin D homologs from plants differentially controlled by growth regulators and containing the conserved retinoblastoma protein interaction motif. Plant Cell, 7: 85-103.