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SURFACTANTS AID UPTAKE OF CYTOKININ AND UREA INTO JUVENILE *PINUS RADIATA* PLANTLETS

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Abstract. The effects of surfactants Tween 80, Citowett, and Silwet L-77 on foliar absorption of cytokinin and urea on juvenile *Pinus radiata* plants were examined. BAP in the presence of either 0.2% Silwet L-77 or Citowett gave a 9 and 5-fold increase, respectively, in axillary shoots at the top of the plant as well as a marked increase in stem shoot number after 18 weeks. Tween 80 had little effect.

A 1% ^{15}N-urea + 0.1% Silwet L-77 foliar application doubled the amount of nitrogen absorbed. In the absence of L-77, seedlings tolerated 5% urea, whereas in the presence of 0.1% L-77 and urea concentrations higher than 2%, needle burning occurred.

Silwet L-77 ("Pulse", Monsanto) may be a useful surfactant for foliar applications of growth regulators, growth retardants, and nutrients to other plants.

REVIEW OF LITERATURE

Foliar applications of growth regulators, growth retardants, and nutrients are frequently used in horticulture and forestry. Absorption of nutrients through foliage can alleviate nutrient deficiencies more rapidly than soil application whenever nutrient uptake through roots is restricted. Growth regulators can be applied to leaves and growing tips to alter plant form for vegetative propagation or for commercial reasons and to promote flowering and senescence. Dixon *et al.* (2) found that oak (*Quercus alba*) seedling root and shoot growth was promoted with foliar mist applications of plant growth regulators in combination with foliar fertiliser solutions. Foliar applied cytokinins have been used to induce axillary branching and basal sprouts (5, 6) while gibberellins promote flowering (4). Ineffective spray applications may be due to poor foliar absorption caused in the main by epicuticular waxes impeding penetration.

Wetting agents or surfactants are often used to improve the performance from foliar applications of plant growth regulators and nutrients. Some of the more common ones reported include Tween 20, Tween 80, Buffer X (a proprietary surfactant) and Aromox C/12 w[3] with the concentration of surfactant used frequently left uncited (8). Surfactants enhance penetration and reduce the surface tension of water-based solutions thereby aiding wetting of tissues (8). However, other more subtle effects exist which may result in growth inhibition, stimulation, or phytotoxicity (8). At the Forest Research Institute, Rotorua, New Zealand. Dr. J. A. Zabkiewicz and R. E. Gaskin have evaluated herbicide-surfactant formulations for more effective gorse (*Ulex europaeus*) control. Silwet L-77 and Citowett were the surfactants under investigation.

This present study aims to test the effectiveness of these surfactants with cytokinin for promoting axillary bud development and with urea for increasing quantities of nitrogen absorbed in a single application.

MATERIALS AND METHODS

Experiment 1: Cytokinin spraying. Two clones of 9-month-old micropropagated *Pinus radiata* plantlets were topped and sprayed weekly (to runoff) in the glasshouse for 9 weeks with 50 mg/l benzylaminopurine (BAP) containing different surfactants. A Cambrian CSP/15 compressed air sprayer was used for spraying. The treatments were:

1. BAP plus 0.2% (v/v) Silwet L-77 (Union Carbide product supplied by Monsanto, also marketed as 'Pulse' in New Zealand).
2. BAP plus 0.2% (v/v) Citowett (York and Co. Ltd, USA).
3. BAP plus 0.2% (v/v) Tween 80 (Atlas Chem. Industries Inc., USA).
4. BAP only (BAP control).
5. Distilled water only (topped control).
6. Distilled water plus Citowett (surfactant control).

Each treatment had 6 plantlets, 3 from each clone. Axillary shoot numbers were counted after 18 weeks.

Experiment 2: ^{15}N-urea spraying. Nine month old *P. radiata* seedlings were sprayed to runoff in the nursery with 1% (w/v) ^{15}N-urea (95 atom % excess) containing either the surfactants Silwet L-77 or Tween 80, at concentrations of 0.1% and 0.5%, respectively. The control was sprayed with urea but without surfactant. Spray was delivered from a Pierce Quixspray Instant Aerosol unit and the surface of the plot was covered.

After 24 hours 5 seedlings from each treatment were washed, dried, and analysed for isotopic enrichment. Nitrogen was determined by the Kjeldahl method.

Experiment 3: Electrolyte conductivity measurements. In order to evaluate the degree of tissue damage caused by the urea-surfactant application, electrolyte conductivity of the needle surface was measured. The electrolyte conductivity measurement includes the electrolyte contribution from unabsorbed urea as well as electrolyte leakage from the cell arising from increased plant membrane permeability. Seedlings were sprayed with concentrations of urea ranging from 1 to 7% w/v in the presence or absence of 0.1% Silwet L-77. Before and after spraying, 18 needles were harvested per treatment and placed into 15 ml of double distilled water in test tubes at 25°C for 24 hours after which electrolyte conductivity was measured using a Metrohm 660 conductivity meter. Samples were placed into a boiling water bath for 20 minutes, cooled and total electrolyte leakage from the needles was measured. Replication was three-fold. Results were subtracted from a water/blank and the percentage conductivity on the surface of the needle was calculated from: (electrolyte conductivity before boiling/total electrolyte conductivity after boiling) × 100.

RESULTS

Experiment 1: Cytokinin spraying. Table 1 shows that only the Silwet L-77 and Citowett surfactants with BAP stimulated large numbers of axillary buds after 18 weeks. However, BAP plus Silwet L-77 gave the best results. Tween 80 was surprisingly ineffective. All controls produced between 4 and 6 shoots per plantlet from the top of the plantlet (Figure 1, left). Both Silwet L-77 and Citowett produced axillary shoots at the top and on the stem of the plantlet (Table 1). In the Silwet L-77 and Citowett treated clones the bud form was also tighter and more compact than for controls (compare Figure 1, left and right).

Figure 1. *Left.* Appearance after 18 weeks of topped control plantlet sprayed with BAP and no wetting agent. *Right.* Appearance after 18 weeks of topped plantlet sprayed with BAP plus Silwet L-17.

Table 1. Effect of BAP and surfactants on axillary shoot development in topped *Pinus radiata* plantlets after 18 weeks

Treatment	Average number of shoots per plantlet at the top	Average number of stem shoots per plantlet
BAP + Silwet L-77	37.0	32.5
BAP + Citowett	20.3	8.5
BAP + Tween 80	5.3	0
BAP control	4.0	0
Topped control	4.0	0
Wetting agent control	6.0	0

Experiment 2: ^{15}N-urea spraying. More isotopically labelled nitrogen was taken up by needles when surfactant was present (Table 2). In particular, almost twice as much urea-nitrogen was absorbed in the presence of 0.1% Silwet L-77 than without.

Table 2. Effect of surfactants on ^{15}N-urea nitrogen contribution in *Pinus radiata* seedlings

Treatment	^{15}N absorbed (mg)
Urea	1.3
Urea + 0.5% Tween 80	1.8
Urea + 0.1% Silwet L-77	2.2

Experiment 3: Electrolyte conductivity. The percentage of electrolytes on the needle surface 24 hrs after spraying was highest when Silwet L-77 was present (Table 3).

Table 3. Percentage of electrolytes on *Pinus radiata* needles sprayed with urea in the presence and absence of 0.1% Silwet L-77.

Percent urea (w/v)	Percent electrolytes	
	Urea	Urea + L-77
1	—	3
2	—	3
3	2	5*
4	2	7*
5	2	14*
6	5*	17*

*Needle burning

A combination Silwet L-77 and 3% (w/v) urea application was phytotoxic. A similar result was obtained with 6% (w/v) urea in the absence of surfactant. Increases in electrolyte accretion of 5% or more were associated with visible burning symptoms.

DISCUSSION

Our results demonstrate that the presence of surfactant enhanced the BAP effect of stimulating axillary bud development and enhanced urea absorption in *Pinus radiata* seedlings and clones. Furthermore, Silwet L-77 was more effective than Citowett and Tween 80. Silwet L-77 has also been successfully used with the herbicide, glyphosate (Roundup, Monsanto) for mature gorse control (12), in $FeSO_4$ sprays applied to lemon trees (7), and in KNO_3 spray to prune trees (9).

In some instances lack of response from growth regulator and nutrient applicators may be due to incorrect choice and concentration of surfactant for a particular plant species, and inappropriate droplet size during spray delivery, resulting in poor solution retention. Organosilicone-based surfactants (Silwet L-77) were reported to give a better spreading effect on waxy surfaces than hydrocarbon based ones (e.g. the Tween surfactants and Citowett) (7). Our results confirm this.

The large numbers of axillary shoots produced in our experiment by the BAP plus Silwet L-77 spray could be useful as explants for micropropagation or for rooting. This has already been done with shoots formed after cytokinin spraying in Douglas fir (1). The shape or form of the shoot after BAP application could also be important for micropropagation. It is difficult to sterilize field-grown juvenile *Pinus radiata* shoots for micropropagation (Aitken-Christie and Steele, unpublished) because of the open form of the bud (cf mature buds). The more compact type of bud formed by the Silwet L-77 and Citowett treatments may be easier for sterilisation if spraying was done in the field.

A major consideration during spraying is the problem of leaf burn which is related to the spray solution, nutritional status of the plant, and the environmental conditions at the time of application (3). On hot days when water from the spray can be easily evaporated, unabsorbed salts may accummulate on the leaf surface and cause leaf scorching. Attempts to supply major nutrients such as nitrogen by foliar application are often unsuccessful due to repeated applications which again can cause foliar scorching. Despite these problems, yield increases following foliar urea spraying have been reported for a variety of crops including wheat, potatoes, tomatoes, fruit trees, nut trees, and soybeans (3). The absorption of urea is rapid and differs from that of most other substances (11). This feature has been used to advantage with combination macro and micronutrient applications. e.g. phosphate and iron (10). Results presented here show that less urea is required if surfactant is added, thereby making an application more economical, although multiple applications may still be necessary. Electrolyte conductivity measurements may be useful for monitoring the degree of tissue damage and for optimising concentrations for foliar applications in order to avoid problems.

Silwet L-77 ("Pulse") could be useful for the foliar application of growth regulators, nutrients, and growth retardants to other plants. A combination spray of BAP and urea could also be beneficial to increase both growth and axillary branching. Previously unsuccessful attempts to induce flowering in *Pinus radiata*, may also be improved if gibberellin $A_{4/7}$ was applied with Silwet L-77.

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