THE ROLE OF NITROGEN IN RELATION TO CONE PRODUCTION IN *PINUS RADIATA*

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ABSTRACT

Using nitrogen fertilisers on **Pinus radiata** grafts and cuttings, substantial increases were made in the concentrations of free arginine in buds with a potential for cone production.

The increases, when promoted in seed orchards, did not lead to increased cone production.

It is suggested that the major role of N, where this has increased cone production in **Pinus**, may have been to increase crown size and thus the number of sites in the crown where cones may be initiated. This would only happen on sites where growth rate and crown size were limited by the availability of N.

INTRODUCTION

The early literature on the effect of fertilisers on cone production in seed orchards was confused. With increasing research however, a general acceptance has developed that for optimal cone production all mineral nutrients in the seed orchard should be maintained at a level which is optimal for vegetative growth. In addition there is widespread belief that N of the correct form, applied at the right time, can substantially increase seed yields (see e.g., review by Sweet, 1975). Based on these assumptions, the use of fertilisers in pine seed orchard management has become common in many parts of the world.

Of the many seed orchard programmes using fertilisers routinely, one of the first was that of the N. Carolina State Cooperative Tree Improvement Programme. Early spring applications of NPK fertilisers are followed by a heavy application of ammonium nitrate (up to 600 kg/ha), made just prior to flower initiation. Annual reports of the Cooperative from 1971 onward indicate that applications such as this are beneficial in increasing seed yields of *Pinus taeda* and other southern pine species in SE U.S.A. It is equally clear, however, that not all attempts to increase seed production in *Pinus* with N fertilisers have been successful — see e.g. review by Puritich (1972).

While there is no clear understanding of how N may operate to increase cone and seed yields, there are indications that, for a number of species, treatments need to provide high N levels in the tree at a rather specific time: normally when initiation

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and/or differentiation of cones is taking place. Additionally, not all types of N fertiliser are equally effective. The available evidence suggests that the effectiveness of an N source may be linked to its ability to increase the proportion of arginine and other guanidines in the soluble-N pool of the shoot (Ebell and McMullan, 1970). Nitrogen treatments which have conjointly increased both sexual differentiation, and arginine levels ,in the plants concerned, have been reported for Douglas fir (Ebell and McMullan, 1970; Ebell, 1972), the southern pines (Barnes and Bengtston, 1968a, b; Stanley and Smith, 1970) and for apple (Grasmanis and Edwards, 1974; Grasmanis and Leeper, 1967). Treatments which have failed to increase cone production have generally not increased arginine levels when these have been examined (Ebell and McMullan, 1970; Ebell, 1972).

In Douglas fir, cone production is most readily increased with a nitrate fertiliser (Ebell and McMullan, 1970). With pine the position is less clearcut. Ammonium nitrate appears to be a particularly successful fertiliser (North Carolina State Cooperative Tree Improvement Programme; 1971, 1972, 1973; Barnes and Bengtston, 1968a; Schmidtling, 1974, 1975; Greenwood, 1977), but it is uncertain whether the NH_4^+ or the NO_3^- ions are important. Work by Pharis *et al.* (1974) and Barnes and Bengtston (1968b) would suggest that it is the NH_4^+ ion which promotes increased arginine levels in *P. taeda.* This is supported by Durzan and Steward (1967) for *P. banksiana* and Rothamsted Annual Report (1972) for *P. sylvestris.* The latter two studies show similar results for *Picea glauca* and *Picea sitchensis* respectively, and work by Carrow (1973) indicates a similar result in *Abies grandis.*

In New Zealand there is a need to increase the output from existing seed orchards to help meet the seed requirements for a greatly expanded planting programme in *P. radiata.* The question of N-treatments to seed orchards has thus been considered, and a research programme initiated. There was no information available as to whether N might promote flower production in *P. radiata,* and if so as to the form of N and timing of application which would be optimal.

Following the implications from the literature that the amino acid arginine may have a role in cone initiation, experiments were designed to examine this point in *P. radiata.* In an initial experiment, seasonal levels of bud arginine were examined to see whether they were high at the time of cone initiation and differentiation. Subsequently the response of bud arginine levels to N application was examined, as was the response of coning to N application generally and increased arginine levels specifically.

METHODS OF N EXTRACTION AND MEASUREMENT

Extractions were made only from the terminal bud of a tree or from buds of the major first order branches. Following a preliminary examination of the variability of free arginine within buds the following standard procedure was adopted for arginine determinations. At collection, buds were trimmed to a length of 3 cm, frozen in liquid nitrogen and (in all except one experiment) freeze dried. Extraction was by the method of Plaisted (1958), and Bieleski and Turner (1966). Buds were homogenised with 80% ethanol, centrifuged and loaded onto a Dowex 50W-X8, 200-400 mesh H^+ column. The column was eluted with 80% ethanol (discarded) and then with 0.4N NH_4OH, followed by 4N NH_4OH. The combined ammonium fractions were dried and then loaded onto a thin-layer silica gel Eastman chromatogram plate (No. 6061). The

chromatograms were developed with butanol : acetic acid : water (4 : 1 : 1 v/v), and the plates sprayed with ninhydrin. The compound formed from arginine present was eluted and its optical density at 510 nm read and compared with a standard curve.

Total amino acids were extracted and measured in a similar way to arginine, while total nitrogen determinations were made by Kjeldahl digestion followed by NH_4^+ estimation by an automated colorimetric procedure.

PART I: A ROLE FOR ARGININE?

1. *SEASONAL LEVELS OF ARGININE*

Introduction

If a positive causal relationship exists between bud arginine levels and cone differentiation in *P. radiata,* then it might be expected that arginine levels would be high at the time of cone differentiation. To examine this, bud samples were taken from 4 clones approximately every 2 weeks during a 13 month period, and their free arginine and total amino acid levels determined.

The clones were grafts of mature trees which, at the time of the experiment, had been grafted for 10 years. They were located at the Forest Research Institute, Rotorua. All buds samples were from the apex of first-order branches which had initiated cones the previous year. The clones were selected for variability in cone production, Clone 1 being the best cone producer, followed sequentially by Clones 2, 3 and 4. Additionally, the clone called No. 1 in this study had previously been the subject of considerable study as to the timing of flower differentiation on the leading shoot (Bollmann and Sweet, 1976).

Results

Bud arginine levels on all clones were lowest in the spring, increased generally until mid-summer, and then declined again gradually to a relatively low winter level (Fig. 1).

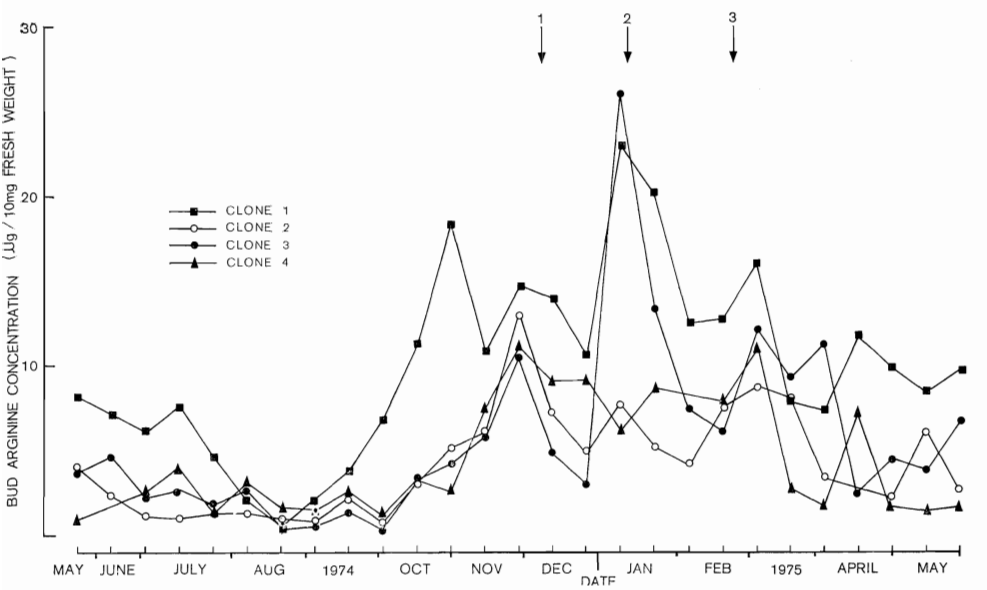

FIG. 1—Seasonal variation in bud arginine concentrations of 4 clones.

Clone 1 showed four peaks during the summer period while the other clones showed three peaks. Differences in arginine levels were reasonably consistent between clones, and there appeared to be a general relationship between bud summer arginine levels and the cone production capability of the clones.

The arrows at the top of Fig. 1 indicate the approximate dates at which long shoot buds were initiated on the terminal shoot of Clone 1 on the same site the previous year (Bollmann and Sweet, 1976). It can be seen that there is an association with high arginine concentrations.

2. *THE EFFICIENCY OF DIFFERENT N SOURCES AND APPLICATION METHODS IN INCREASING BUD ARGININE CONCENTRATION*

Introduction

The experiment reported here is representative of a number in which bud arginine levels were increased by N application. Urea and ammonium nitrate were applied to 50-cm-high rooted cuttings (potted into 9-litre buckets) in a glasshouse. The N was applied by 3 different methods:

1. A single foliar spray (to run-off) of a 2% N solution, thus applying approximately 0.4 g N.
2. Two successive foliar sprays of 2% N at a 3-day interval*.
3. A soil application of 2 g N in 200 ml solution.

Each plant required for treatment was carefully matched with an untreated control plant, and 4 clones × 2 ramets per clone were then treated during late winter. In a subsidiary experiment, the effect of the surfactant Multifilm X-77 (at 0.1% concentration) was tested on a further 3 clones.

Four weeks after treatment the terminal bud was removed from each cutting, thoroughly washed, freeze dried and its free arginine levels determined.

Results

Table 1 presents the ratio of arginine levels in treated buds to that in control buds.

In the four clones used in the main experiment, 2 successive foliar sprays were generally more successful than one; and urea as a spray was more effective than ammonium nitrate.

The subsidiary experiment demonstrates a clone × treatment interaction. One clone (No. 11) responded to the urea spray only in the presence of the surfactant, while the response of Clone 10 was not increased by a surfactant. Clone 9 did not respond at all. While all clones responded well to soil application of ammonium nitrate, cones 6 and 7 did not increase their bud arginine levels following soil application of urea, providing a further example of clone × treatment interaction.

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* The timing of the second spray was based on a study by Court (1974) which showed that all urea sprayed on to **P. radiata** seedling needles was taken into the foliage within 24 hours.

TABLE 1—Free arginine ratios of terminal buds: treated v. control plants

(a) Main experiment

		Clone no. 5	6	7	8	Mean*
Urea	1 foliar spray	2.6	0.5	1.2	1.3	1.4 a
	2 foliar sprays	14.6	2.7	2.7	4.9	6.2 b
	Soil application	3.9	0.7	0.8	3.0	2.1 a
Ammonium nitrate	1 foliar spray	1.6	1.8	1.6	1.5	1.6 a
	2 foliar sprays	4.6	1.6	3.7	3.2	3.3 a
	Soil application	14.6	4.9	13.8	22.5	14.0 c

* Values not sharing a common letter differ significantly at 5% level.

(b) Subsidiary experiment

Time from treatment to harvest (weeks)	Treatment	Clone 9	10	11
2	Urea	1.2	2.7	1.0
	Urea & Multifilm	1.0	2.5	4.1
4	Urea	0.5	2.4	0.8
	Urea & Multifilm	0.7	2.3	2.5

3. *THE RELATION BETWEEN INCREASED BUD ARGININE LEVELS AND CONE INITIATION*

Introduction

The previous experiment indicated the likelihood that no single fertiliser type and application method would be optimal for all clones, and this was confirmed in a series of experiments which in total investigated 20 clones. Large and repeatable clone × N-form × application method interactions were shown. Once the appropriate means of increasing bud arginine levels in a clone had been ascertained it was possible to examine the effect of such treatment on cone initiation. The following experiment is typical of a number carried out.

Three clones were used, all grafts from ortets presently aged about 50 years. The grafts were in a part of Kaingaroa seed orchard which, at the time of the experiment, was 4 years from planting. They averaged 4.6 m in height with a mean diameter of 7.1 cm. Stocking was 132 trees/ha.

Ten ramets were selected from each of the 3 clones, and carefully matched into 5 pairs. The grass around all trees was killed with a desiccant spray and, on 29 October one tree of each pair was treated with 1.5 kg of urea spread underneath the area of its crown. The other tree acted as a control On 17 dates during the next 4 months a bud from a potentially flowering branch was collected off a treated and a control tree from each clone (with collection spread equally over all ramets) and its free arginine levels analysed. In the spring following treatment, cone counts were made at anthesis.

Results

Bud arginine levels are presented in Fig. 2, and cone counts in Table 2.

The data in Fig. 2 indicate peaks of arginine concentration in treated trees more than 3 times greater than control levels in Clones 13 and 14, but no effective response to treatment in Clone 12. The highest bud arginine levels in the treated ramets were in mid-January, a period coinciding with the time of cone differentiation for the general area of the seed orchard (Bollmann and Sweet, 1976 and unpub. data) according to present knowledge.

Table 2 shows that the increased arginine levels were not accompanied by increased cone production in any of the 3 clones, either the following spring or one year later. Nor was cone loss during the 12 months following anthesis reduced in the clones with a high arginine level.

4. *THE RELATIONSHIP OF ARGININE TO TOTAL AMINO ACIDS AND TOTAL N*

Introduction

The literature suggests that N treatments capable of inducing coning are those that increase levels of arginine and guanidines in the plant preferentially over those of other forms of soluble N. The negative coning results from the last (and other similar)

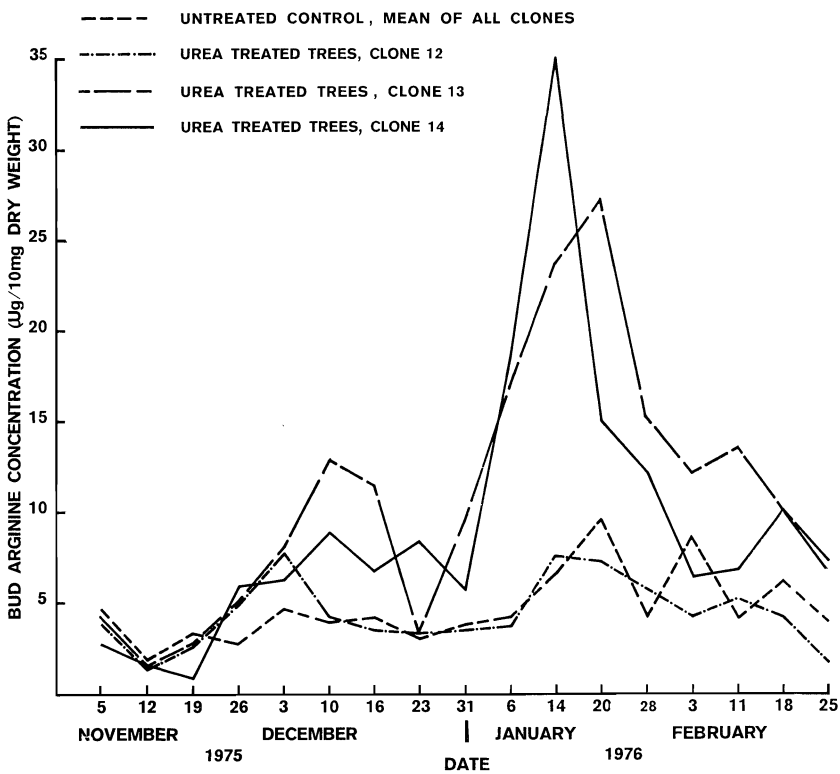

FIG. 2—Changes in bud arginine levels in seed orchard trees following soil applications of urea.

TABLE 2—Receptive cone counts made (a) in the spring following treatment, and (b) one year later. The figures under (c) represent the percentage loss over a 12 month period of the cones counted at (a)

		Clone no. 12			13			14		
		a	b	c	a	b	c	a	b	c
Mean no. cones per tree	Treated	27.0	58.5	28.9	15.0	36.8	72.1	14.2	5.0	15.5
	Control	30.2	63.4	11.3	16.8	43.3	36.4	13.0	6.8	13.8
Mean no. cones per cluster	Treated	3.86	2.71		2.78	3.07		2.15	1.56	
	Control	3.97	3.11		3.11	3.28		2.95	1.88	
Mean no. clusters per tree	Treated	7.0	21.6		5.4	12.0		6.6	3.2	
	Control	7.6	20.4		5.4	13.2		4.4	3.6	

Values are means of 5 replicates. No treatment means are significant.

experiments suggest a need to examine the relationship between arginine and other forms of N in *P. radiata.* Two sets of data are presented which provide information on this point.

Results

Figure 3 illustrates the relationship between free arginine concentration and total free amino acid concentration in the buds of the 4 clones whose arginine levels were

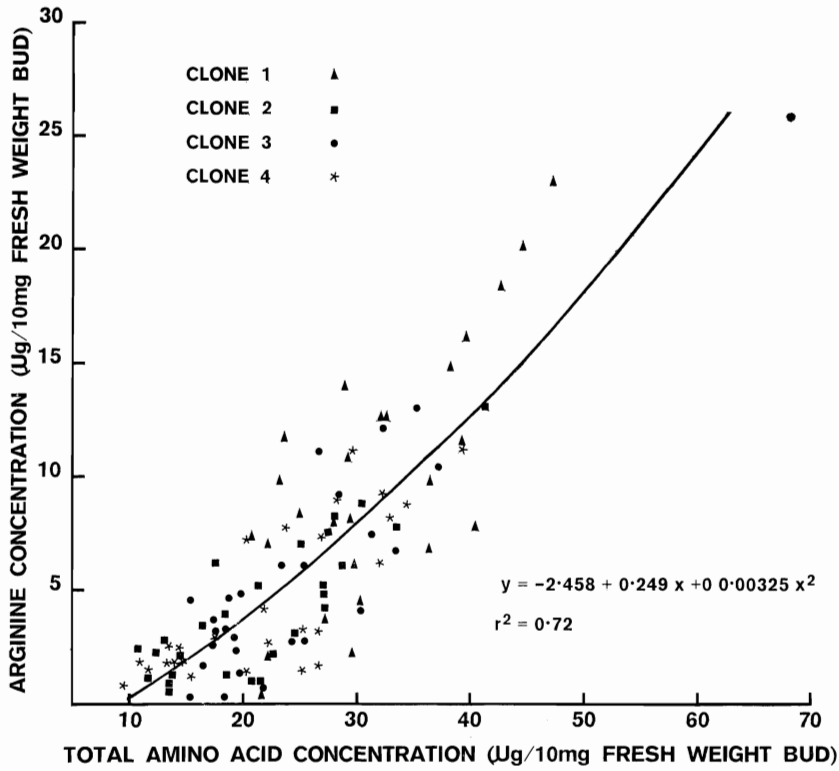

FIG. 3—Regression of arginine concentration against total amino acid concentration in buds for 4 clones over a 13-month period.

examined seasonally in Fig. 1. It is clear that the strong seasonal fluctuations in bud arginine concentration closely reflect seasonal variation in total amino acid concentration.

Figure 4 presents data from an experiment in which bud arginine levels were increased by N application.* The figure indicates that a very high proportion of the changes in bud arginine level resulting from the application of 3 different forms of N simply reflect changes in bud levels of total N. There is no indication in either figure that in *P. radiata* arginine varies in concentration independently of other amino acids or total N, and the implication exists from Fig. 4 that the clone × N-form interactions so widely demonstrated relate to N-uptake — not simply the conversion of N to arginine.

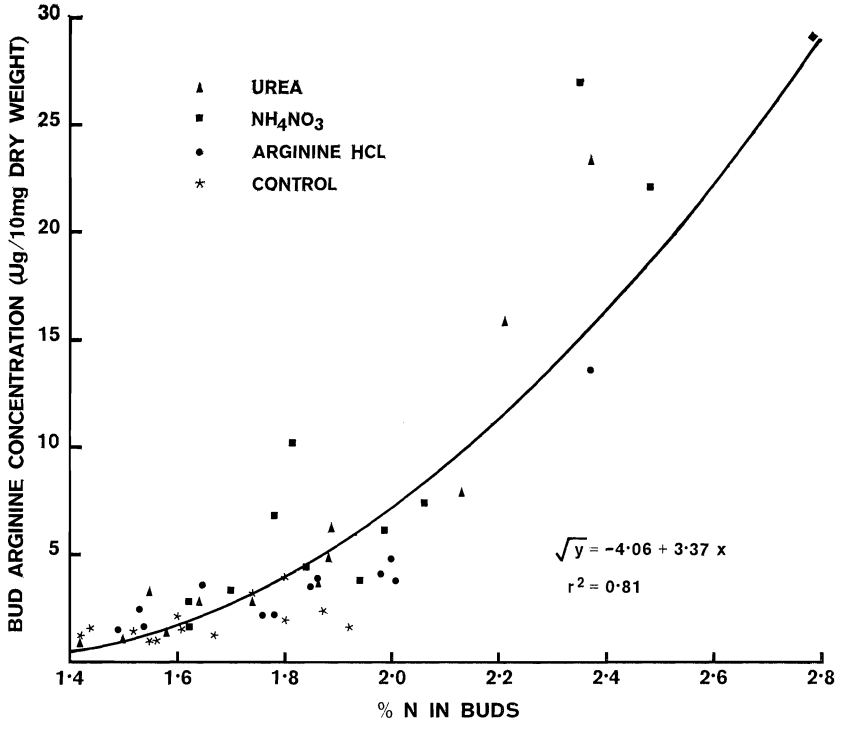

FIG. 4—Regression of arginine concentration against total N concentration in buds.

* In the experiment, 4 treatments (equivalent amounts of N applied as urea, ammonium nitrate, L-arginine hydrochloride; and a control treatment), each with 3 sub-treatments involving method and amount of application (soil and foliar applications ranging from 0.4 to 4 g N per tree), were applied to 4 clones of cuttings (rooted from mature ortets) in a glasshouse. The trees averaged 50 cm in height and each sub-treatment was applied to 2 ramets. Four weeks after treatment the terminal bud of each ramet was harvested, washed thoroughly and freeze dried for N and arginine analyses.

PART II: SEED ORCHARD APPLICATIONS OF N

INTRODUCTION

The studies reported in the previous section would indicate that if N has a role in increasing cone production in *P. radiata* seed orchards it is likely to be independent of arginine, and probably does not relate to a specific timing of application associated with cone initiation or differentiation. Those implications are supported by a number of large-scale studies not presented here which failed to increase cone production by applying N in a form and at a time which must have increased arginine levels in many of the seed orchard trees at the time of cone initiation/differentiation.

Thus, in a final study, the range in timing of N application and quantity of N applied was broadened.

STUDY 1

Methods

The trial was established in the same grafted seed orchard as the cone initiation experiment reported earlier in the paper. It was located as 2 separate sub-trials in different parts of the orchard.

The first sub-trial consisted of 8 plots, each containing one ramet of each of 30 clones, while the second sub-trial consisted of 12 plots each containing a single ramet of each of 28 clones. The grass around each tree in the trial was killed with a chemical spray and the plots were treated with soil applications of urea and ammonium nitrate as shown in Table 3.

Full counts of receptive cones were made at anthesis the following spring (October) and were repeated 5 months later to obtain information on loss of cones. Tree heights and basal diameters were measured in the spring, and stem volumes calculated from the values.

Results

In each sub-trial there was a significant plot correlation between cone count at anthesis and tree size, represented as stem volume. The regressions (Fig. 5) show that the relationship accounted for more than half the total variation in cone count. Table 3 presents cone counts adjusted by covariance on the basis of the regressions shown in Fig. 5. It also presents data on loss of cones during the first 5 months after anthesis. These latter values are not correlated with tree size ($r^2 = 0.02$), and thus have not been adjusted.

The data do not provide any definitive indication that the application of N fertiliser, at any time between spring and autumn, has led to an increase in cone numbers the following spring. There is no indication from Fig. 5 that tree size has increased following fertiliser application and, following adjustment for tree size, cone counts show neither a response to the time pattern of application of N, nor a clear-cut difference between N-treated plants and the untreated controls. The application of increased quantities of urea in Sub-trial 2 has been no more effective than the more standard application rate in Sub-trial 1. Neither apparently has the percentage loss of cones during the first 4 months after anthesis been influenced by N treatment.

TABLE 3—Details of treatments, adjusted mean cone counts per tree at anthesis following treatment, and cone losses during the first 4 months after anthesis

Sub-trial 1				Sub-trial 2			
Plot No.	1.5 kg (700g N) urea per tree applied on:	Cones*	% cone loss	Plot No.	2 kg (700g N) NH_4NO_3 per tree applied on:	Cones*	% cone loss
1	29 October	57.0	54.2	1	29 October	41.8	55.6
2	19 November	47.4	58.4	2	19 November	37.8	46.1
3	20 December	49.8	63.4	3	20 December	40.4	57.4
4	20 January	51.1	52.8	4	20 January	35.8	49.8
5	20 February	50.1	67.4	5	20 February	42.0	56.9
6	30 March	50.2	54.3	6	30 March	36.4	55.8
7	Control (no urea)	43.2	68.4	7	Control (no NH_4NO_3)	40.8	58.8
8	Control (no urea)	50.0	64.2	8	Control (no NH_4NO_3)	38.2	56.7
				9	Control (no NH_4NO_3)	38.9	52.8
				10	1.5 kg urea per tree applied repititively on each of the 6 dates	43.6	54.3
				11	6 kg urea per tree applied 29 October	39.9	58.1
				12	6 kg urea per tree applied 20 January	39.3	66.0
Coefficient of variation of the mean plot = 73%†				Coefficient of variation of the mean plot = 83%†			

* Mean number of cones per tree adjusted on the basis of the regression shown in Fig. 5.
† Of the total variance in the control plots, 64% was due to clonal variation within plots, with the remaining 36% due to variation between plots and clone × plot interaction.

STUDY 2

Introduction

Because the highest cone count in the previous trial followed a soil application of urea on 29 October, and it is not certain that there was no real correlation, the following experiment is briefly reported.

Nine plots of orchard, similar to those already described, each containing one ramet of each of 27 clones, were used to provide 3 replicates of 3 treatments as follows:

Treatment 1. 1.5 kg urea applied to bare soil under each tree on 29 October.
Treatment 2. 2% N solutions of urea, applied as foliar sprays to run off on 26 November and again on 1 December.
Treatment 3. Control — no N application.

Counts of receptive cones were made (a) at anthesis the following spring and (b) 12 months later, to establish the percentage loss.

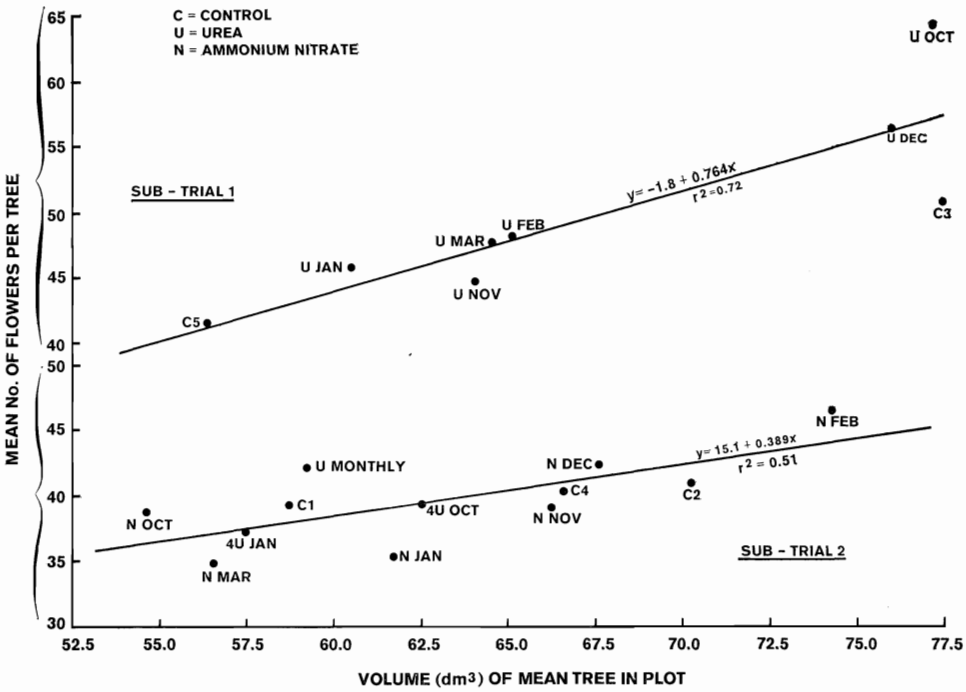

Fig. 5—Relationship between cone count and tree size.

Results

These (Table 4) make it clear that, following N application, there was no statistically significant increase in cone production either in the spring following treatment, or 12 months later. Nor did N applications affect the percentage loss of cones during the first 12 months after receptivity.

TABLE 4—Cone counts and cone losses following nitrogen treatments

	First Spring following treatment			Second Spring following treatment		
	Control	Urea soil application	Urea spray application	Control	Urea soil application	Urea spray application
Mean no. cones/tree	33.2	33.6	34.8	45.8	46.3	48.9
Mean no. cones/cluster	3.3	3.2	3.3	2.9	2.9	3.1
Mean no. clusters/tree	10.2	10.5	10.6	15.9	15.7	16.0
% cone loss in 1st 12 months	44.5	41.3	42.9			
Mean tree ht. (m)	5.4	5.2	5.5			
No. trees in sample	71	76	72	71	76	72

No treatment effects are statistically significant.

DISCUSSION

The experiments reported here would seem to indicate that neither the amino acid arginine nor high N levels in general have a specific role in cone production in *P. radiata* on the sites tested.

In the penultimate experiment reported, N was applied at a level sufficient to increase bud arginine and N levels 2- or 3-fold in a number of treated trees. This occurred over a development period which (allowing time for the N to be taken up and increase the concentration in the buds) began some 2 weeks prior to any initiation of 1st cycle long shoots, and terminated at a time when developing cones were well differentiated (Bollmann and Sweet in preparation). It is difficult to visualise N having a specific role in cone initiation or differentiation outside that time period. Nor were counts able to show an effect of N in reducing conelet drop subsequent to anthesis.

One thus needs to consider why *P. radiata,* on the sites examined, should differ from other conifer species in other countries where cone production *has* been increased by N generally, and apparently in specific association with high arginine levels. In terms of arginine, it is clear from the literature generally (e.g. Barnes and Bengtston, 1968b) that it is present in large quantities in response to any application of N which is successfully taken into the tree. Thus, at least on sites where N is not limiting to growth, no special significance should be attached in *P. radiata* to N applications which increase arginine levels.

In young seed orchards a major increase in coning comes with increasing age and thus tree size. *Pinus taeda* seed orchards in SE U.S.A. for example, increase cone yields per hectare some 6- or 7-fold between ages 5 and 10 (Porterfield, 1964), and *P. radiata* in Australia approximately doubles its seed yield per hectare every year from ages 5 to 8 (Pederick and Brown, 1976). It is thus pertinent to query whether the increase in coning resulting from N application to *Pinus* in SE U.S.A. may in fact not be a specific cone response, but rather resulted from an increase in tree crown size following application of N to soils which are basically N deficient (N.C. State Forest Fertilisation Co-op, 1976). If the response was due primarily to increased tree and crown size (a response well documented for N — Barker, 1978) one would not expect timing of application to be particularly critical, but one would expect the increased coning response to last for more than one year. Examination of the literature for loblolly pine in SE U.S.A. shows disagreement as to whether the response varies with time of N application (e.g. Greenwood, 1977; cf. Schmidtling, 1975), but there is evidence for an increase in tree size and for the response carrying over into the second year (Schmidtling, 1975; Greenwood, pers. comm.). In the New Zealand seed orchard used in the studies reported here, N levels are some 30% above those regarded as limiting for vegetative growth of *P. radiata* and the application of N to the orchards did not result in increased stem growth.

If such an interpretation is correct, and the role of N in *Pinus* is primarily to increase crown size at a time when there is a large coning response to this, it is important that seed orchardists be aware of the fact. In essence it would restrict the effectiveness of N in pines to situations where tree or crown size limits cone production, and to sites and soils where there is a growth response to N.

A further important factor emerging from these experiments is the distinctive

clone × fertiliser interaction demonstrated. There was no single fertiliser which raised arginine (and thus by implication N) levels in the buds of all clones of *P. radiata* tested, nor was there a single optimal method of application. Thus, even in a situation where N might be effective in increasing tree size and thus coning, it would not be possible to utilise a single N source and application method.

Interactions between clone and biochemical response to N have also been demonstrated for *P. elliottii* by Stanley and Smith (1970) and there are well-documented clone × fertiliser interactions in the literature in the flowering response of Southern pines (e.g. Schmidtling, 1975; Jahromi *et al.* 1976). It is likely that these reflect general clone × treatment interactions which will become important in many aspects of seed orchard management. Some of the management implications of such interactions have been examined elsewhere by Sweet and Krugman (1977).

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