



Interactive effect of cytokinin and potassium on sink-source relationships in *Lupinus angustifolius*

Fei-Hu Liu^{1,2,*} and Nancy Longnecker²

¹Life Science and Chemistry College, Yunnan University, 650091, Kunming, People's Republic of China;

²Centre for Legumes in Mediterranean Agriculture, The University of Western Australia, 6907, Nedlands, WA, Australia; *Author for correspondence (e-mail: pgbreed@public.km.yn.cn; phone: 0086-871-5035256 (Office), 0086-871-5031635 (Home); fax: 0086-871-5147702)

Received 9 January 2001; accepted in revised form 14 March 2001

Key words: Cytokinin, Harvest index, *Lupinus angustifolius*, Pod set, Potassium, Seed yield

Abstract

This study examined whether increased K supply in conjunction with BAP could increase lupin seed yield and harvest index by enlarging sink volume (pod number), increasing assimilate and improving assimilate partitioning to fill the additional pods induced by BAP treatment. Narrow-leaved lupin (*Lupinus angustifolius*, cv. Danja abs⁻ mutant) was grown in a glasshouse, in pots containing sandy soil with four K treatments (0, 15, 60 and 120 mg K/kg soil). BAP (2 mM) was applied daily to all main stem flowers throughout the life of each flower from opening to senesced. BAP application did not affect assimilate production (as measured by total above-ground biomass), but changed assimilate partitioning. On BAP-treated plants, there were greater proportions of seed to pod wall dry weight on the main stem but smaller proportions on the branches, and an increased weight ratio of seed to pod wall overall which meant more assimilate was used for seed growth rather than pod wall growth. BAP increased the number of pods per plant by 35% and this more than compensated for the decreases in seeds per pod and seed weight. Therefore, there was an increased harvest index (+11%) and seed yield per plant (+13%) in BAP-treated plants. BAP also increased the number of pods with filled seeds (146%) on the main stem and main stem seed K⁺ concentration (from 0.81% to 0.87%). Added K increased biomass but only slightly affected assimilate partitioning. As applied K increased, relatively more assimilate was used for pod wall growth rather than seed growth. Added K increased seed yield per plant by about 14% due to increases in seed weight and the number of pods on the main stem. Moreover, K⁺ concentration in seeds and shoots increased with increasing level of applied K. Seed yield was enhanced more by BAP when K was supplied at high levels. Increasing K supply interacted positively with added BAP by increasing narrow-leaf lupin seed yield and harvest index through increases in assimilate supply and its partitioning into seeds.

Abbreviations: BAP – N6-benzylaminopurine, K – potassium

Introduction

Lupin is an important crop in Australia, with the narrow-leaved lupin (*Lupinus angustifolius* L.) comprising 80% of the crop (Palta and Ludwig 1998). Narrow-leaved lupin can yield poorly and have a low and variable harvest index resulting from factors such as poor soil nutrition, excessively high temperature and soil drought during seed development (Biddiscombe

1975; Downes and Gladstones 1984a; Dowens and Gladstones 1984b; French and Turner 1991; Ma et al. 1998; Ozanne and Petch 1978; Palta and Dracup 1994; Reader et al. 1997). Its low and variable seed yield have been related to poor pod set and pod survival (Pigeaire et al. 1992). Since 40–93% of flowers and/or young pods may abscise (Pigeaire et al. 1992), it is critical to decrease flower and pod abortion and increase seed filling in order to enhance seed yield.

Exogenous application of cytokinin (N^6 -benzylaminopurine, BAP) to lupin flowers (pedicel and sepals) almost completely eliminated flower abortion on the main stem, but the seed yield and harvest index did not increase (Ma et al. 1998) or decreased (Atkins and Pigeaire 1993; Palta and Ludwig 1996) because seeds were smaller and there were fewer seeds per pod or because the additional pods produced unfilled seeds. This may have been the result of shortage of assimilates to fill the added pods (Atkins and Pigeaire 1993) or a blockage of assimilate translocation (Ma et al. 1998).

Potassium (K) improves plant photosynthetic capacity and translocation of photosynthates (Marchner 1986). In lupin, increased K application may increase the production of assimilate which could then fill the increased number of pods set by the application of BAP. Potassium deficiency occurs in some lupin production areas (Cox 1978), but the effect of K deficiency on narrow-leaved lupin has not been widely investigated.

In this study, we tested the hypothesis that increased K supply could allow extra pods in BAP treated plants to be filled and that seed yield and harvest index could increase with the interaction of these two treatments.

Material and methods

This experiment was carried out at The University of Western Australia, using a narrow-leaved lupin mutant-Danja abs^- (*Lupinus angustifolius* L. cv Danja). The abs^- mutant senesces normally, but no organs abscise, making it ideal for investigating harvest index (Clements 1996). A complete factorial design of four potassium levels (0, 15, 60 and 120 mg K/kg soil, termed K0, K15, K60 and K120), and two cytokinin treatments (0 and 2 mM BAP), was used with 5 replicates.

Plant culture

Each undrained pot contained 6 kg of Lancelin sandy soil (collected from a place in Western Australia called Lancelin, with <0.5% of K as received and 28 mg/kg of K by HCO_3^-) (Tang 1998), added with the appropriate K_2SO_4 solution and basal nutrients (mg/kg soil) $Ca(H_2PO_4)_2 \cdot H_2O$, 66.7; $MgSO_4 \cdot 7H_2O$, 19.9; $CuSO_4 \cdot 5H_2O$, 2.13; $ZnSO_4 \cdot 7H_2O$, 4.97; $MnSO_4 \cdot H_2O$, 9.94; $CoSO_4 \cdot 7H_2O$, 0.36;

$Na_2MoO_4 \cdot 2H_2O$, 0.18. Seven seeds per pot were sown in early Sep and inoculated with rhizobia (*Bradyrhizobium lupinii* strain WU425) suspension. Three seedlings were left per pot after thinning with a population density of 60 plants m^{-2} on the bench and alkanthene beads used as mulch. Plants were cultured in a glasshouse (temperature 30–33/22–23 °C day/night) under natural light with adequate water added every second day or daily (maintaining 75–100% of field water capacity) throughout the experiment. The growth season was from Sep to Dec.

Cytokinin application

Freshly prepared BAP was applied daily at a concentration of 2 mM solution to the pedicels and lower part of sepals of open flowers on the main stem using a tiny camel hair brush, from flower opening to senesced (BAP was dissolved with a small amount of 1 M KOH, diluted with dionized water, 0.002 g/kg of BS1000 added as wetting agent). The flowers of control plants were treated with the same quantity of solution without BAP.

Data collection

Measurements included total shoot dry biomass (including pods), seed yield, harvest index (dry weight ratio of seeds to above-ground biomass), the numbers of pods per plant and seeds per pod, 100-seed weight, total and effective podding percentage of the main stem (ratio of the number of total pods or pods with filled seed(s) to the number of flowers), ratio of seed weight to pod wall weight, K concentration in the whole shoot (sampled at the late flowering stage of the main inflorescence) and in the seed of the main stem (using $HNO_3/HClO_4$ digestion and atomic absorption spectrometry), and sucrose concentration in sap of young pods on the main stem (the sap was collected by cutting off the tip of young pods and measured with a 0–28% sugar refractometer). All data were analysed statistically by Statistica.

Results

Seed yield per plant

In this experiment, both K and BAP increased the yield of seed from the whole plant (Figure 1A) due to the increase of yield from the main stem, even

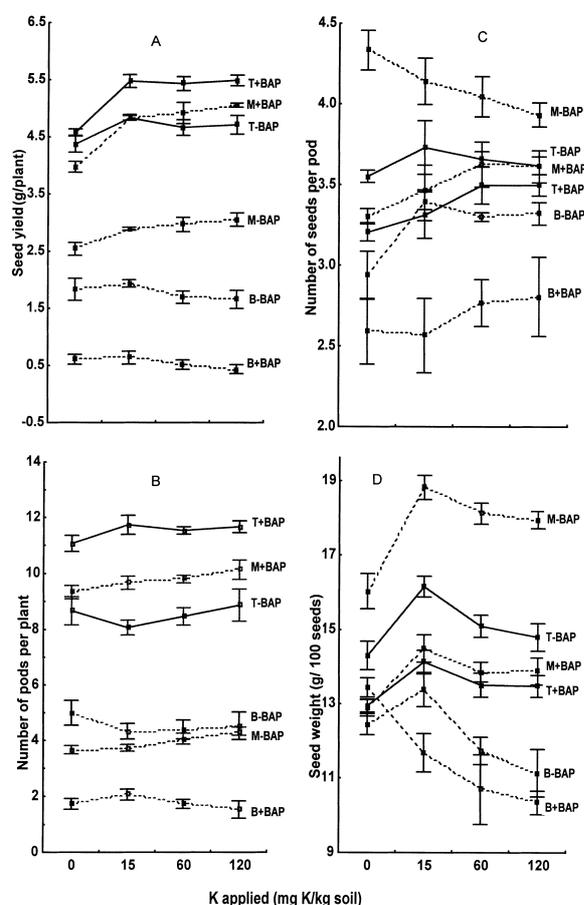


Figure 1. Effects of cytokinin (BAP applied to the main stem flowers) and K nutrition on seed yield (A), pods per plant (B), seeds per pod (C) and air-dried seed weight (D). (B + BAP) lateral branch with BAP; (B-BAP) lateral branch without BAP; (M + BAP) main stem with BAP; (M-BAP) main stem without BAP; (T + BAP) total plant with BAP; (T-BAP) total plant without BAP.

though BAP reduced branch seed yield. Added K increased yield by 13% to 15%, but there was no significant difference among the K15, K60 and K120 treatments. Cytokinin application increased whole plant seed yield by 13%. Moreover, there was a positive interactive effect of K and cytokinin on the seed yield of the whole plant or the main stem, which implied that BAP increased seed yield more when higher levels of K added.

Pods per plant

BAP increased the pod number per whole plant by 35% (Figure 1B), which resulted from the increase in the number of pods on the main stem (6 pods/plant) in spite of a reduction in pod number on the lateral

branches (3 pods /plant). Added K only increased the number of pods on the main stem.

Seeds per pod

There were fewer seeds per pod in the BAP-treated plants, but K did not affect this in general (Figure 1C). However, the number of seeds per pod on the main stem increased as K supply increased when BAP was added, but decreased when BAP was not added, resulting in a negative interactive effect (data not shown). Moreover, both K and BAP increased the total number of seeds per plant, when the number of pods per plant and seeds per pod on the main stem and the lateral branches were considered. A positive interaction of K and BAP was also observed for the number of seeds per plant on the main stem (data not shown).

Seed weight

BAP decreased the average individual seed weight overall (Figure 1D) by reducing it on the main stem, although BAP did not affect the seed weight on the lateral branches. In contrast, adding 15 mg K/kg soil increased the individual seed weight overall and from the main stem over the control (without K addition), while added K decreased the weight of individual seeds from lateral branches.

Biomass and partitioning

Added K increased the above-ground biomass i.e. assimilate production (Figure 2A). BAP had no effect on biomass but changed assimilate partitioning such that the proportion of seed on the main stem increased relative to above-ground biomass, while that of seed on the lateral branches decreased (Figure 2B). BAP increased the ratio of seed weight to pod wall weight suggesting that BAP further influenced the assimilate partitioning by attracting more assimilate to seeds rather than to pod wall growth. Higher levels of K supply had the opposite effect (Figure 2C).

Harvest index

BAP increased harvest index by 11% (Figure 3) ($P < 0.05$), but K had no significant effect. However, there was a trend towards a positive interactive effect of BAP and K on harvest index, although this was not significant ($P = 0.057$).

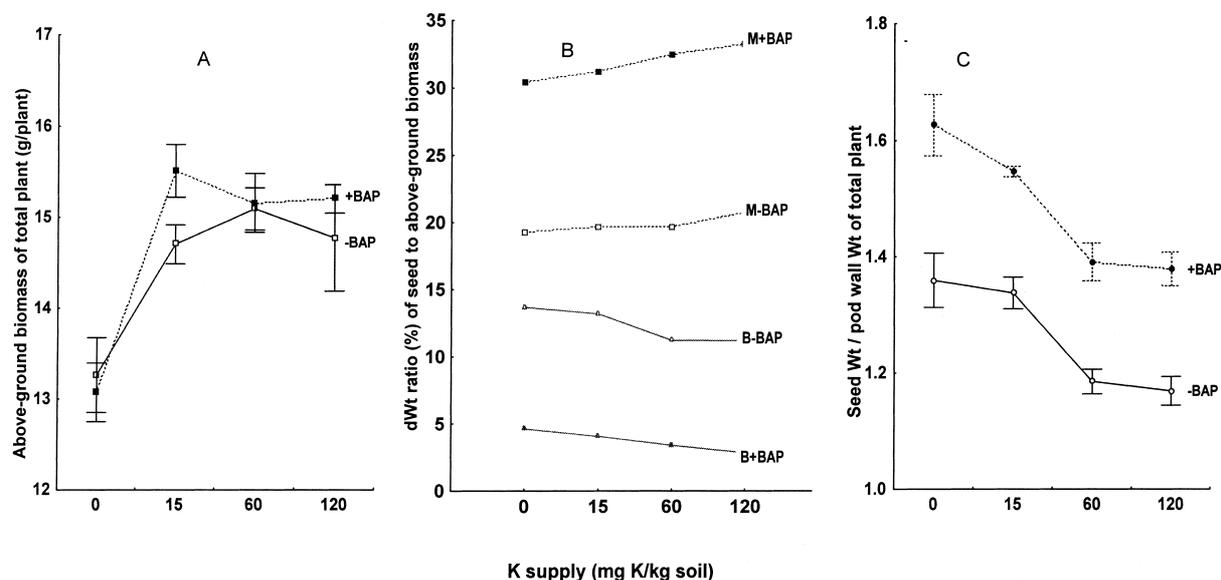


Figure 2. Biomass production of total plant (A), assimilate partitioning into seeds (B) and ratio of seed weight to pod wall weight for total plant (C) as affected by K and cytokinin (BAP applied to the main stem flowers). (M + BAP) main stem with BAP; (M - BAP) main stem without BAP; (B + BAP) branches with BAP; (B - BAP) branches without BAP.

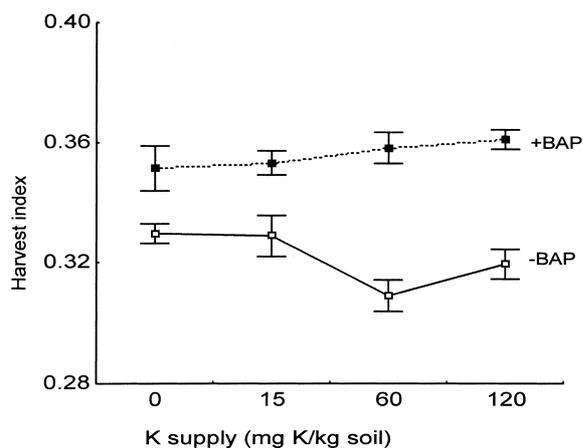


Figure 3. Effect of cytokinin application on harvest index (BAP applied to the main stem flowers). (+BAP) with BAP; (-BAP) without BAP.

Effective podding percentage on the main stem

Both BAP and K increased the total podding percentage (number of total mature pods/number of flowers) and the effective podding percentage (number of pods with filled seed(s)/number of flowers) on the main stem (Figure 4). A positive interaction of K and BAP was observed on the effective podding percentage. However, BAP, as well as higher levels of K, decreased the number of flowers on the main stem (data

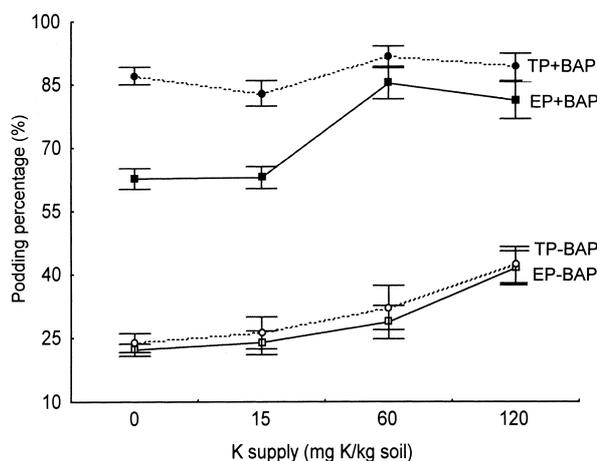


Figure 4. Podding percentage of main stem flowers as affected by cytokinin (BAP applied to the main stem flowers) and K. (+BAP) with BAP; (-BAP) without BAP. (EP) effective podding percentage = number of pods with filled seed(s)/number of flowers; (TP) total podding percentage = number of matured pods/number of flowers.

not shown), which is not consistent with the results of a previous study.

Other effects of BAP and K nutrition

The sucrose concentrations in sap of young pods on the main stem of BAP-treated plants were generally lower than sap from non-BAP-treated plants (Fig-

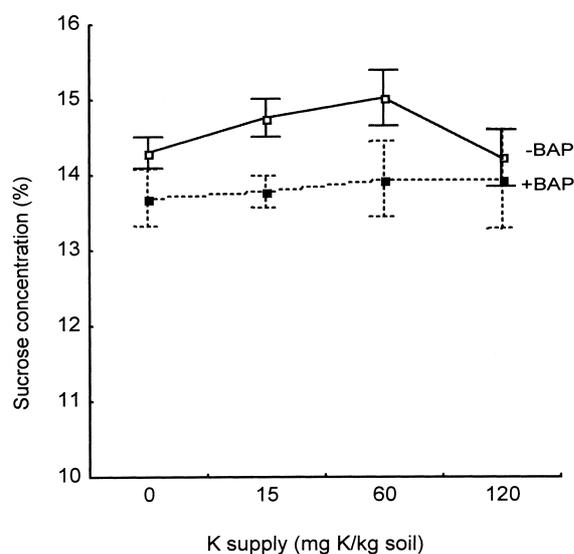


Figure 5. Sucrose concentration in sap of young pods on the main stem as affected by cytokinin (BAP applied to the main stem flowers). (+BAP) with BAP; (-BAP) without BAP.

ure 5), possibly because of the dilution effect of many more pods on BAP-treated plants.

BAP and K both increased K concentration in seeds borne on the main stem, which reached a plateau at K60 (Figure 6). A close positive correlation between seed K concentration and seed yield for the main stem was also observed. Shoot K concentration of the K15 treatment sampled at the late flowering stage of main inflorescence was 0.78%, lower than the critical K concentration of 1.2% for narrow-leaved lupin given by Reuter and Robinson (1997). While slight K deficiency symptoms were observed in this experiment, there was no decrease in biomass, seed yield or yield components.

Discussion

BAP increased seed yield

In this experiment, BAP application to the main stem flowers increased seed yield (13%) and harvest index (11%) of the whole plant. This differed from results of previous studies in which applied BAP had no effect on seed yield and harvest index (Ma et al. 1998) or decreased them (Atkins and Pigeaire 1993; Palta and Ludwig 1996). In this study, increasing the number of effective pods per plant compensated for the slight decrease in seed weight and number of seeds per pod. The different response of narrow-leaved lu-

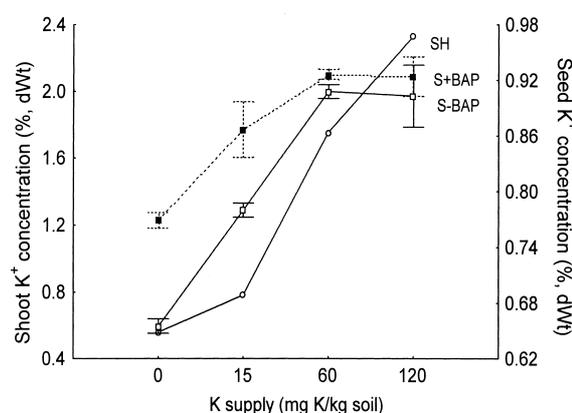


Figure 6. K concentration (% dWt) in shoots and seeds from the main stem as affected by K and cytokinin (BAP applied to the main stem flowers). (+BAP) with BAP; (-BAP) without BAP; (S) seeds; (SH) shoots sampled at flowering stage of the main inflorescence without BAP.

pin to BAP application in this study may be because there were fewer extra pods to be filled in this experiment (8–12 pods per plant) compared to previous experiments (20–30 pods per plant) (Ma et al. 1998; Palta and Ludwig 1996). The higher temperature during this experiment compared to that experienced by lupins during a normal growth season accelerated the development and shortened the period of flower bud differentiation. This supports the view that increasing pod set per plant would improve yield and harvest index in lupins.

The physiological effects of BAP and K and their interaction

Atkins and Pigeaire (1993) suggested that BAP application did not increase pod set through an effect on fertilisation, as the frequency of fertilisation was equally high in developing and abscised flowers. BAP application was unlikely to alter the flower abortion process itself either. It has been observed in *Lupinus angustifolius* that all fertilised flowers transiently lost their ability to attract photosynthetic assimilates and only those which formed pods regained their “sink strength” (Pate and Farrington 1981). BAP application might help those flowers which would normally be destined to stop developing regain and retain their “sink strength” and attract more assimilate to the pods thus ensuring their further development. This idea is supported by results from this experiment such as increased proportion of seed to pod wall on the main stem and pod numbers on the main stem, while these

parameters decreased on the lateral branches. This was also observed by Ma et al. (1998).

We postulated that the function of K in increasing the assimilate source and possibly increasing assimilate transportation to the sites of strongest sink strength which could have been induced by application of BAP, would result in a positive interaction of BAP and K in increasing seed yield. This was observed by the fact that in this experiment, added K increased the shoot biomass which apparently provided the assimilate supply for increased seed filling in the BAP-treated plants. Therefore, BAP and K appear to have a complementary action on “source-sink” relationships in *Lupinus angustifolius*.

Acknowledgements

The authors are grateful to the help from Dr Qifu Ma, Mrs Lorraine Osborne, Dr Tim Colmer, Dr Caixian Tang and Dr Melisa Narbey of in carrying out the experiment and analyses.

References

- Atkins C.A. and Pigeaire A. 1993. Application of cytokinins to flowers to increase pod set in *Lupinus angustifolius* L. Aust. J. Agric. Res. 44: 1799–1819.
- Biddiscombe F.F. 1975. Effect of moisture stress on flower drop and seed yield of narrow-leafed lupin (*Lupinus angustifolius* L. cv. Unicrop). J. Aust. Inst. Agric. Sci. 41: 70–72.
- Clements J.C. 1996. Physiological aspects of abscission in *Lupinus angustifolius*. PhD Diss., University of Western Australia, Perth, Australia.
- Cox W.J. 1978. Potassium deficiency in lupins—identification, rates, times and method of application. J. Agric. West. Aust. 4: 27–31.
- Downes R.W. and Gladstones J.S. 1984a. Physiology of growth and seed production in *Lupinus angustifolius* L. I. Effects on pod set of controlled short duration high temperatures at flowers. Aust. J. Agric. Res. 35: 493–499.
- Downes R.W. and Gladstones J.S. 1984b. Physiology of growth and seed production in *Lupinus angustifolius* L. II. Effect of temperature before and after flowering. Aust. J. Agric. Res. 35: 501–509.
- French R.J. and Turner N.C. 1991. Water deficits change dry matter partitioning and seed yield in narrow-leafed lupins (*Lupinus angustifolius* L.). Aust. J. Agric. Res. 42: 471–484.
- Ma Q., Longnecker N. and Atkins C. 1998. Exogenous cytokinin and nitrogen do not increase grain yield in narrow-leafed lupins. Crop. Sci. 38: 717–721.
- Marschner H. 1986. Mineral Nutrition of Higher Plants. Academic Press, Orlando, pp. 254–267.
- Ozanne P.G. and Petch A. 1978. The application of nutrients by foliar sprays to increase seed yields. In: Proceedings of the 7th International Plant Nutrition Colloquium. August–September 1978, Wellington, New Zealand., pp. 361–366.
- Palta J.A. and Dracup M. 1994. Water deficits in narrow-leafed lupin: Implications for seed yield. In: Dracup M. and Palta J.A. (eds), Proceedings of the First Australian Lupin Technical Symposium. Western Australian Department of Agriculture, Perth, WA, pp. 288–291.
- Palta J.A. and Ludwig C. 1996. Pod set and seed yield as affected by cytokinin application and terminal drought in narrow-leafed lupin. Aust. J. Plant Physiol. 48: 81–90.
- Palta J.A. and Ludwig C. 1998. Yield response of narrow-leafed lupin plants to variations in pod number. Aust. J. Agric. Res. 49: 63–68.
- Pate J.S. and Farrington P. 1981. Fruit set in *Lupinus angustifolius* cv. Unicrop. II. Assimilate flow during flowering and early fruiting. Aust. J. Plant Physiol. 8: 307–318.
- Pigeaire A., Delane R.J., Seymour M. and Atkins C.A. 1992. Pre-dominance of flowers and newly-formed pods in reproductive abscission of *Lupinus angustifolius* (L.). Aust. J. Agric. Res. 43: 1117–1129.
- Reader M.A., Dracup M. and Atkins C.A. 1997. Transient high temperatures and seed filling in narrow-leafed lupin (*Lupinus angustifolius* L.). I. High temperatures reduce seed weight. Aust. J. Agric. Res. 48: 1169–1178.
- Reuter D.J. and Robinson J.B. 1997. Plant Analysis: An Interpretation Manual. 2nd edition. CSIRO Publishing, Collingwood, VIC 3066, Australia, pp 125–163.
- Tang C. 1998. Factors affecting soil acidification under legumes I. Effect of potassium supply. Plant and Soil. 199: 275–282.