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Short communication

Arginase from kiwifruit: properties and seasonal variation

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Abstract The in vitro activity of arginase (EC 3.5.3.1) was investigated in youngest-mature leaves and roots (1–3 mm diameter) of kiwifruit vines (*Actinidia deliciosa* var. *deliciosa*) during an annual growth cycle, and enzyme from root material partially purified. No seasonal trend in the specific activity of arginase was observed in roots. Measurements in leaves, however, rose gradually during early growth and plateaued c. 17 weeks after budbreak. Changes in arginase activity were not correlated with changes in the concentration of arginine (substrate) or glutamine (likely end-product of arginine catabolism) in either tissue during the growth cycle. Purification was by $(NH_4)_2SO_4$ precipitation and DEAE-cellulose chromatography. The kinetic properties of the enzyme, purified 60-fold over that in crude extracts, indicated a pH optimum of 8.8, and a K_m (L-arginine) of 7.85 m*M*. Partially-purified enzyme was deactivated by dialysis against EDTA, and reactivated in the presence of Mn^{2+}, Co^{2+}, and Ni^{2+}.

Keywords *Actinidia deliciosa*; arginase; arginine; kiwifruit; nitrogen metabolism; seasonal variation

INTRODUCTION

Fundamental reasons exist for wanting to understand how nitrogen (N) is assimilated, stored, remobilised, and ultimately utilised in kiwifruit vines (*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa*). In the first place, the kiwifruit of commerce is essentially a wild plant which has evolved under conditions quite different from those in which it is now cultivated (Smith et al. 1988). In the acidic forest litters of its native habitat, ammonium might be expected to be the predominant external N source. In commercial situations on mineral soils however, nitrate would be expected to be the dominant source, with nitrate being supplied either by direct application of salts, or indirectly from the rapid nitrification of ammonium or urea fertilisers. Vines seem equally-well adapted to either conditions for reasons unknown. Second, in a temporal study of nitrogenous compounds in components of whole kiwifruit vines destructively harvested over a growing season, free arginine (Arg) was identified as the predominant amino acid in root tissues and contributed c. 50% of the total root-N present at all times throughout the year (Clark & Smith 1991, 1992). During dormancy, roots contained c. 50% of the total N in the vine's entire perennial framework, whereas woody components above-ground contained sufficient total N to satisfy only 25% of the N requirements of vegetative growth at budbreak. Reserve forms of N, including Arg, might therefore be expected to support new season's growth until such time as exogenous N became available (Clark & Ledgard 1993). And third, there have been unsubstantiated reports of N adversely affecting keeping quality of fruit during storage

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(Prasad & Spiers 1991). This brings into question just how the plant distributes and utilises its nitrogenous resources, and its impact on fruit quality. Apart from report of an unusual thermostable nitrate reductase extracted from the roots of kiwifruit (Godlewska & Clark 1996), the biochemistry of the enzymes associated with N assimilation and utilisation processes in kiwifruit have not been investigated.

Although Arg is widely regarded as being an important low molecular weight N-storage species in many plants (Pietilä et al. 1989), the compound has limited xylem mobility (Hill-Cottingham & Lloyd-Jones 1968). There are, however, substantial concentrations of glutamine (Gln), an ultimate end-product of Arg catabolism and a readily-transported form of N, in xylem sap of kiwifruit around budbreak (Ferguson et al. 1983; Shargool et al. 1988; Clark & Smith 1991). Hence exploring the factors which influence the storage and breakdown of Arg would seem to be essential in understanding N utilisation in this crop, and especially those events contributing to vegetative growth in early spring. Accordingly, this paper reports on the characteristics of arginase (L-arginine amidinohydrolase, EC 3.5.3.1) in kiwifruit, a metallo-enzyme catalysing hydrolysis of L-arginine to L-ornithine as the first step in Arg breakdown in higher plants. In vitro assays were used to investigate the seasonal activity of arginase in leaves and roots, and we report the properties of partially-purified enzyme extracted from root material.

MATERIALS AND METHODS

Plant material

A row of 11-year-old vines (cv. 'Hayward'), trained on a T-bar trellis, and located in a kiwifruit research orchard near Hamilton, New Zealand, was used as the source of plant material. Vines were managed according to standard commercial practice and received 1000 kg lime, 250 kg $(NH_4)_2HPO_4$, 500 kg urea, and 400 kg KCl/ha on 3 September 1993. To reduce the intensity of sampling on single vines, 10 vines were grouped into five replicates of two vines. Bulk samples (n = 5) of roots (c. 60 g, 1–3 mm diam.) and leaves (20 youngest-fully-mature leaves from non-fruiting shoots—minus their petioles) were collected at 2-week intervals from 15 September 1993 to 14 September 1994 inclusive. Tissues were stored on ice for transportation to the laboratory. There, tissues were washed with deionised water and divided into two samples for analysis. Both were frozen in liquid N_2. One sample was stored at –80°C for extraction and determination of protein concentration and enzyme activity, whereas the other was lyophilised shortly after collection, ground to a powder and stored at –20°C for determination of amino acids.

A bulk sample (5 kg) of roots for purification of arginase was collected from a number of vines within the orchard block over a 10-day period (15–24 September 1993), a time during which an earlier study (Clark & Smith 1991) suggested activity might be greatest.

Enzyme extraction, partial purification, and characterisation

All extraction procedures were carried out at 4°C and used Sigma chemicals unless stated otherwise. Samples in the seasonal study were extracted by the procedure of Roubelakis & Kliewer (1978b) at a sample/solution ratio of 10 g fresh weight to 100 ml extraction buffer. The final extracts, which were unstable at 4°C, were either assayed immediately, or else frozen at –80°C until later.

The purification procedure developed for kiwifruit arginase is an amalgam of the extraction method described for grape (Roubelakis & Kliewer 1978b), plus further steps routinely used in protein purification. Roots (100 g fresh weight) were macerated for 2–3 min in 1 litre of extraction buffer containing: 500 m*M* Tris.HCl (pH 8.7, 4°C), 7 m*M* Diethyl-dithiocarbamic acid (trihydrate Na salt), 6 m*M* L-cysteine, 2.5 m*M* EDTA, 1 m*M* DTT, and 7% (w/v) polyethylene glycol (PEG 4000). The homogenate was filtered through Mira cloth (CalBiochem) and the filtrate made up to 40% (w/v) by addition of further PEG 4000. This extract was stirred for 1 h and centrifuged (30 000*g*, 25 min). Supernatant was discarded and the pellet suspended in 50 ml of homogenising buffer comprising 10 m*M* Tris.HCl (pH 8.0), 1% (v/v) Nonidet P–40 detergent, 1 m*M* $MnCl_2$, and 1 m*M* DTT, then centrifuged (19 000*g*, 15 min). The supernatant was brought to 30% saturation with solid $(NH_4)_2SO_4$, stirred for 1 h at 4°C, and centrifuged (23 000*g*, 30 min). Because of the presence of the detergent, a distinct pellet did not form, but precipitate did aggregate sufficiently to be separated out on filter paper and discarded. Ammonium sulphate was removed from solution by dialysis against several changes of homogenising buffer, in which Nonidet had been omitted. Dialysed extract was applied to a DEAE cellulose column (18 × 2.5 cm) previously

equilibrated with 500 m*M* Tris.HCl (pH 8.0). The column was washed with starting buffer containing 10 m*M* Tris.HCl (pH 8.0) and 1 m*M* DTT. Enzyme was eluted at a flow rate of 1.5 ml/min with a linear gradient of 0–500 m*M* KCl (over a volume of 200 ml) made up in the starting buffer. Six µl of 1*M* $MnCl_2$ was subsequently added to each of the fraction tubes (7.5 ml). The active fractions, i.e., fractions 22–24 (1.65–1.80 bed volumes containing KCl concentrations c. 400–450 m*M*) were stored at –80°C for characterisation studies.

The pH dependence of the enzymatic activity was determined by adjusting the pH of the buffer used in the assay reaction mixture (100 m*M* Tris.HCl) for the range between 7.5 and 9.5, or replacing it with 100 m*M* CAPS for values between 9.5 and 10.7.

Ability of other metal ions to substitute for Mn was determined by dialysing partially-purified enzyme against EDTA buffer (pH 8.0), then using the dialysed enzyme in the assay reaction with addition of 1 m*M* solutions containing $MgCl_2$, $CaCl_2$, $MnCl_2$ (routinely added to the standard reaction mixture), $FeSO_4$, $FeCl_3$, $CoCl_2$, $NiCl_2$, $CuCl_2$, or $ZnCl_2$.

Analytical procedures

The assay for arginase in crude and partly-purified extracts was based on colorimetric detection of ornithine after Roubelakis & Kliewer (1978b). One unit of activity is expressed as 1 µmol L-ornithine produced/g fresh weight per min.

Protein content was determined using Bio-Rad Protein Assay Dye Reagent Concentrate (Bradford 1976), with bovine serum albumin as the standard.

Free amino acids were extracted from 0.5 g of lyophilised sample with a monophasic methanol/chloroform/water solution, followed by hot water extraction of the residue. Quantification was by separation with Li citrate buffers and ninhydrin detection, using an Alpha Plus Amino Acid Analyser (LKB Biochrom, Cambridge, England). Full details of the extraction and detection procedures are described in Walton et al. (1991).

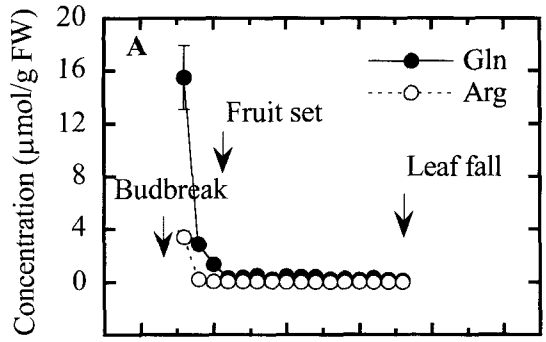
RESULTS

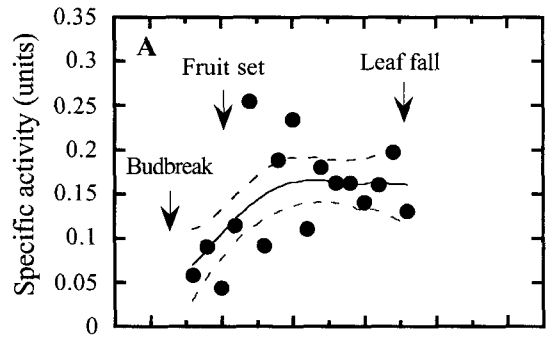
Preliminary experiments demonstrated arginase activity in a number of kiwifruit tissues including: whole seedlings macerated at the 2–3 leaf stage (0.09 units); the flesh (0.03), core (0.05), and seeds (0.06) of eating-ripe fruit; the cortical tissue of structural roots (0.11); as well as leaves (0.40) and fine roots (0.12). Compared with grape leaves (the only other vine in which arginase activity has been investigated—Roubelakis & Kliewer (1978b)), crude extracts of mature kiwifruit leaves contained c. 5 times more arginase activity and 25% less protein. Crude extracts of kiwifruit leaves were considerably less stable though, losing 25% of their initial activity overnight, and 60% after 6 days, compared with product from grape leaves that was stable within experimental error over 6 days. Activity could be stabilised for at least 4 weeks, however, if kiwifruit extracts were stored at –80°C.

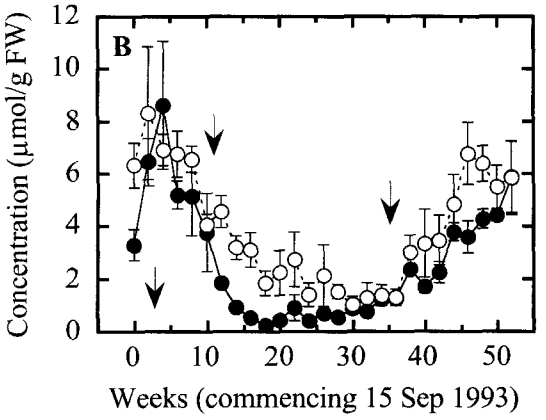
Seasonal variation of substrate, end-product, and enzyme activity

Concentrations of Arg and Gln in new season's leaves declined sharply after budbreak, so that by fruit set, 8 weeks after budbreak, concentrations had reached values which remained constant for the rest of the season (Gln, 1–2 µmol/g fresh weight; Arg < 0.2 µmol/g fresh weight) (Fig. 1A). The decline is attributed to growth dilution: the actual content or amount of Arg or Gln on a per leaf basis increased during the season, but not at a rate commensurate with accumulation of dry matter. In roots, concentrations of Arg and Gln displayed seasonal trends similar to one another, Arg concentrations generally exceeding those of Gln at all times during the year (Fig. 1B). Both were greatest around the time of budbreak, and at a minimum some 25 weeks later at commercial harvest. Concentrations gradually returned to their early-season maximum during dormancy. The trends here are consistent with the temporal patterns suggested by the restricted measurements of Clark & Smith (1991).

The seasonal activity of arginase and the concentration of protein in leaves ranged between 0.14 and 0.52 units, and 1.08 and 3.95 mg/g fresh tissue, respectively. Corresponding measurements in roots were 0.03–0.11 units, and 0.15–0.82 mg/g fresh tissue. Specific activity in leaves was modelled using a Bayesian smoothing technique (Upsdell 1994) to cope with perturbations in the data. The analysis implied specific activity rose gradually during the season, and plateaued at a maximum (0.15 units/mg protein) c. 17 weeks after budbreak (Fig. 2A). By contrast, changes in specific activity in roots were non-systematic and fluctuated about an average of 0.15 units/mg protein throughout the season (Fig. 2B). The arginase activity and protein concentration in leaves was consistently higher than that in fine roots at all harvests. However, the specific activity was within a similar range,







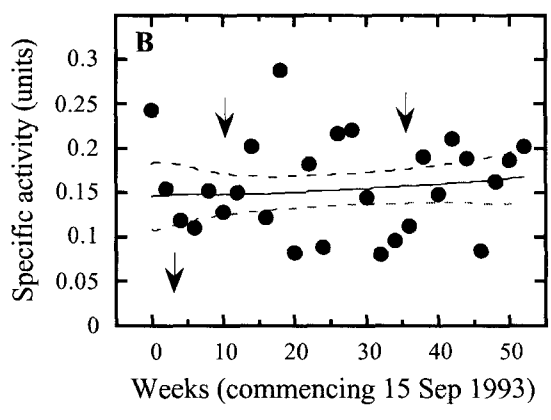


Fig. 1 Seasonal changes in the concentration of arginine and glutamine in **A**, leaves; and **B**, roots (1–3 mm diam.) of kiwifruit (*Actinidia deliciosa*). Each datum point is the average of five replicates. Error bars are 1 standard error either side of the mean and are absent where the value is smaller than the symbol size. (FW = fresh weight.)

Fig. 2 Seasonal changes in the specific activity of arginase in **A**, leaves; and **B**, roots (1–3 mm diameter) of kiwifruit (*Actinidia deliciosa*). Each datum point is the average of five replicates. Solid line represents the fitted curve, and the dotted lines a 95% confidence interval. Data are consistent with any continuous curve that lies within these bands (Upsdell 1994).

indicating the proportion of arginase to other protein species was similar in both tissues.

Purification and properties of partially-purified enzyme

Procedures involving $(NH_4)_2SO_4$ precipitation, dialysis, and DEAE-cellulose chromatography resulted in a 60-fold purification of arginase extracted from roots (Table 1). This specific activity is similar to that found for other plant arginases, and if arginase is a major protein in kiwifruit, then this level of purification is about all that can be expected. The apparent rise in yield in the final step (Table 1) is almost certainly the result of the presence in the plant extract of compounds interfering with protein determination and/or enzymes assays.

Attempts were made to assess the progress of purification, and to determine the molecular weight of root arginase using native and conventional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and starch gels. However, interpretation of SDS-PAGE gels stained with Coomassie Blue or silver (Wray et al. 1981) was confounded by the presence of residual contaminants, and no bands were observed on either native gels, or catalytically-stained activity gels using rabbit liver extract as a comparison (Farron 1973). The low concentration in roots coupled with the enzyme's inherent instability were contributing

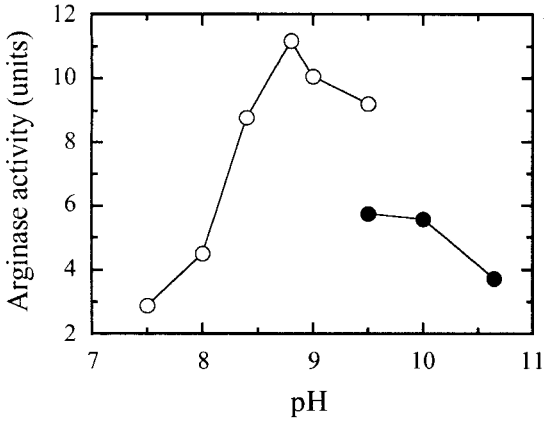


Fig. 3 Determination of the optimum pH for arginase from kiwifruit (*Actinidia deliciosa*) roots. pH dependence was determined by replacing the buffer used in the enzyme assay with 100 m*M* Tris.HCl (o) for the range 7.5–9.5, and 100 m*M* CAPS (•) between 9.5 and 10.7.

factors when deciding to terminate the purification process after DEAE-cellulose chromatography.

Partially-purified arginase from roots had a pH optimum of 8.8 (Fig. 3) and a K_m (L-arginine) of 7.85 m*M*. The K_m value, determined by least squares analysis of a Lineweaver-Burke plot, has a standard error of 0.57 m*M*, and a 95% confidence interval of 6.5–9.2 m*M*.

Removal of the metal cofactor from the partially-purified kiwifruit arginase by dialysis against 5 m*M* EDTA resulted in complete loss of activity. However when the dialysis buffer contained 1 m*M* DTT, as well as EDTA, only 50% of the activity was lost. In both instances, activity was completely recovered when the reaction mixture contained Mn^{2+}. No appreciable activity was detected when Ca^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, or Zn^{2+} were added (in place of Mn^{2+}) to reaction mixture containing arginase dialysed against EDTA. Apart from Mn^{2+} (100% of original activity restored), Co^{2+} (90%) and Ni^{2+} (130%) were the only alternative metal ions identified which successfully reactivated kiwifruit arginase (Table 2).

A number of compounds including both isomers of citrulline, lysine, and cysteine inhibit arginase activity in other species. However, interference from these compounds in the ornithine assay prevented collection of reliable data for kiwifruit. A reaction mixture containing guanidinoacetic acid (5 m*M*) in the presence of Arg substrate (50 m*M*) was found to inhibit kiwifruit arginase activity by 40%.

DISCUSSION

Partially purified arginase from kiwifruit exhibits properties in common with that extracted from other plants. The pH optimum of most arginases, for example, is at least 8.8 (as was found for kiwifruit) and the range of optima extends to at least 10, as observed for *Pheretima communissima* (Iino & Shimadate 1986). The ratio of charged to neutral forms of the arginine side chain may be important for reactivity and thus dictate the high pH optimum observed for this enzyme (Pace et al. 1980). Complexation between Mn^{2+} and the enzyme is an effective means of sequestering the metal ion in an available form under pH conditions where the divalent hexaquo-ion would normally be rendered unavailable by hydrolysis and/or precipitation. At alkaline pH, Mn (II) is oxidised to the Mn (IV) state and precipitates as MnO_2. Under these circumstances Mn is not available from the aqueous solution.

Kiwifruit arginase activity is highly dependent upon the presence of Mn^{2+}. This has been observed for arginase derived from other sources such as yeast (Green et al. 1991), but the Mn^{2+} dependence may be much less, as in species like *Lathyrus sativus* (Cheema et al. 1969). The variation between

Table 1 Partial purification of arginase from roots (1–3 mm diam.) of kiwifruit (*Actinidia deliciosa*).

Step	Volume (ml)	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Purification (-fold)	Yield (%)
Crude extract	1000	5.5	448	0.012		100
$(NH_4)_2SO_4$ (30%)	50	4.8	56.4	0.085	7	88
Dialysis	66	3.8	29.4	0.13	11	70
DEAE-cellulose	70	7.0	9.6	0.73	60	130

different arginases suggests that the divalent ion is bound with different affinities and may imply the presence of variable Mn^{2+} binding sites. The alternative metals, Co^{2+} and Ni^{2+}, are common successful substitutions in arginase from a wide variety of sources (Table 2).

Although variation in Arg concentration and the dominant position Arg occupies as a proportion of the soluble-N and total-N pools in the kiwifruit vine suggest importance as a N storage metabolite (Clark & Smith 1991, 1992), changes in arginase activity did not correlate well with changes in Arg concentration in either roots or leaves. There are reports of arginase activity in a number of plant species, but mainly the seeds and seedlings of annual plants. For example, in the cotyledons of developing pumpkin seedlings (Splittstoesser 1969) and developing grape seedlings (Robelakis & Kliewer 1978a), the concentration of Arg and arginase activity showed parallel patterns of change during the different stages of development. Namely, there was an increase to a maximum, followed by a decline in both the concentration of the substrate and the activity of the enzyme. There are fewer reports involving temporal studies using field-grown plants. The seasonal activity of arginase in Jerusalem artichoke tubers was shown to increase during autumn and winter (April–September), whereas free Arg was maximal in April–May and low in late August when enzyme activity was greatest (Wright et al. 1981). With examples such as these, it is implied from the close relationship between appearance and decline of free Arg and arginase activity, that arginase is intimately associated with Arg degradation and indicative of inducibility at high substrate levels (Splittstoesser 1969). This is not always the situation though, and in instances like kiwifruit, pea, and pine where arginase and free Arg co-exist (and even accumulate) without degradation, some form of intracellular regulation must be employed (de Ruiter & Kollöffel 1983; Pietilä et al. 1989). This can be achieved by compartmentation of enzyme and substrate, association of Arg with anionic compounds, or enzyme inhibition (de Ruiter & Kollöffel 1983; Shargool et al. 1988). In higher plants, arginase has been shown to be associated with the mitochondria (Kollöffel & van Dijke 1975; Taylor & Stewart 1981; Wright et al. 1981), whereas Arg is primarily located within the vacuoles (Yamaki 1982). Intracellular localisation within kiwifruit too would allow arginase activity in roots to remain constant throughout the year, while the concentration of Arg fluctuated. Other alternatives can not presently be excluded however, in light of the limited information we have about specific biochemical transformations in this plant and the factors that affect them.

Table 2 Alternative metal ion studies for arginase from a variety of sources.

Arginase source	Alternative metal ion	Activity relative to Mn^{2+} (%)
Pumpkin cotyledons[1]	Fe^{2+}	<10
	Co^{2+}	<10
	Cu^{2+}	<10
Lathyrus sativus[2]	Fe^{3+}	~200
	Co^{2+}	100
Bitter lupin[3]	Co^{2+}	~40
	Ni^{2+}	~50
Bacillus caldovelox[4]	Co^{2+}	100
	Ni^{2+}	200
	Cd^{2+}	60
Kiwifruit[5]	Fe^{2+}	0
	Co^{2+}	90
	Ni^{2+}	130

[1]Splittstoesser (1969).
[2]Cheema et al. (1969).
[3]Muszynska & Reifer (1968).
[4]Patchett et al. (1991).
[5]This study.

REFERENCES

Bradford, M. 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Analytical biochemistry 72*: 248–254.

Cheema, P. S.; Padmanaban, G.; Sarma, P. S. 1969: Arginase from *Lathyrus sativus*. *Phytochemistry 8*: 409–411.

Clark, C. J.; Ledgard, S. F. 1993: Uptake of ^{15}N by kiwifruit vines from applications of N fertiliser prior to budbreak. *Annals of botany 71*: 311–316.

Clark, C. J.; Smith, G. S. 1991: Seasonal variation of nitrogenous compounds in components of the kiwifruit vine. *Annals of botany 68*: 441–450.

Clark, C. J.; Smith, G. S. 1992: Seasonal dynamics of biomass and mineral nutrient partitioning in mature kiwifruit vines. *Annals of botany 70*: 229–237.

De Ruiter, H.; Kollöffel, C. 1983: Arginine catabolism in the cotyledons of developing and germinating pea seeds. *Plant physiology 73*: 525–528.

Farron, F. 1973: Arginase isozymes and their detection by catalytic staining in starch gel. *Analytical biochemistry 53*: 264–268.

Ferguson, A. R.; Eiseman, J. A.; Leonard, J. A. 1983: Xylem sap from *Actinidia chinensis*: seasonal changes in composition. *Annals of botany 51*: 823–833.

Godlewska, E. A.; Clark, C. J. 1996: Occurrence of a stable nitrate reductase in *Actinidia* spp. *New Zealand journal of crop and horticultural science 24*: 89–94.

Green, S. M.; Ginsburg, A.; Lewis, M. S.; Hensley, P. 1991: Roles of metal ions in the maintenance of the tertiary and quaternary structure of arginase from *Saccharomyces cerevisiae*. *Journal of biological chemistry 266*: 21474–21481.

Hill-Cottingham, D. G.; Lloyd-Jones, C. P. 1968: Relative mobility of some organic nitrogenous compounds in the xylem sap of apple shoots. *Nature 220*: 389–390.

Iino, T.; Shimadate, T. 1986: Purification and properties of gut arginase from earthworm *Pheretima communissima*. *Comparative biochemistry and physiology 83B*: 79–84.

Kollöffel, C.; van Dijke, H. D. 1975: Mitochondrial arginase activity from cotyledons of developing and germinating seeds of *Vicia faba* L. *Plant physiology 55*: 507–510.

Muszynska, G.; Reifer, I. 1968: Purification, properties and inhibition of plant arginase. *Acta biochimica Polonica 15*: 55–66.

Pace, C. N.; Buonanno, A.; Simmons-Hansen, J. 1980: Steady-state kinetic studies of arginase with an improved direct spectrophotometric assay. *Analytical biochemistry 109*: 261–265.

Patchett, M. L.; Daniel, R. M.; Morgan, H. W. 1991: Characterisation of arginase from the extreme thermophile *Bacillus caldovelox*. *Biochimica et biophysica acta 1077*: 291–298.

Pietilä, M.; Kuusipuro, P.; Pietiläinen, P.; Lähdesmäki, P. 1989: Specificity and seasonal variation of arginase, glutamate synthase and nitrate reductase activities in Scots pine needles. *Plant science 64*: 153–160.

Prasad, M.; Spiers, T. M. 1991: The effect of nutrition on the storage quality of kiwifruit (a review). *Acta horticulturae 297*: 579–584.

Roubelakis, K. A.; Kliewer, W. M. 1978a: Changes in the activities of ornithine transcarbamylase and arginase, and the concentration of nitrogenous substances during germination and seedling development of *Vitis vinifera* L. *Vitis 17*: 377–385.

Roubelakis, K. A.; Kliewer, W. M. 1978b: Enzymes of Krebs-Henseleit cycle in *Vitis vinifera* L. III. *In vivo* and *in vitro* studies of arginase. *Plant physiology 62*: 344–347.

Shargool, P. D.; Jain, J. C.; McKay, G. 1988: Ornithine biosynthesis, and arginine biosynthesis and degradation in plant cells. *Phytochemistry 27*: 1571–1574.

Smith, G. S.; Clark, C. J.; Buwalda, J. G.; Gravett, I. M. 1988: Influence of light and form of nitrogen on chlorine requirement of kiwifruit. *New phytologist 110*: 5–12.

Splittstoesser, W. E. 1969: The appearance of arginine and arginase in pumpkin cotyledons. Characterisation of arginase. *Phytochemistry 8*: 753–758.

Taylor, A. A.; Stewart, G. R. 1981: Tissue and subcellular localisation of enzymes of arginine metabolism in *Pisum sativum*. *Biochemistry and biophysics research communications 101*: 1281–1289.

Upsdell, M. P. 1994: Bayesian smoothers as an extension of nonlinear regression. *New Zealand statistician 29*: 66–81.

Walton, E. F.; Clark C. J.; Boldingh, H. L. 1991: Effect of hydrogen cyanamide on amino acid profiles in kiwifruit buds during budbreak. *Plant physiology 97*: 1256–1259.

Yamaki, S. 1982: Distribution of sorbitol, neutral sugars, free amino acids, malic acid and some hydrolytic enzymes in vacuoles of apple cotyledons. *Plant cell physiology 23*: 991–889.

Wray, W.; Boulikas, T.; Wray, V. P.; Hancock, R. 1981: Silver staining of proteins in polyacrylamide gels. *Analytical biochemistry 118*: 197–203.

Wright, L. C.; Brady, C. J.; Hinde, R. W. 1981: Purification and properties of the arginase from Jerusalem artichoke tubers. *Phytochemistry 20*: 2641–2645.