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Haustorial Development and Growth Benefit to Seedlings of the Root Hemiparasitic Tree *Nuytsia floribunda* (Labill.) R.Br. in Association with Various Hosts

AINSLEY CALLADINE*†, JOHN S. PATE† and KINGSLEY W. DIXON‡

†Department of Botany, The University of Western Australia, Nedlands 6907, Western Australia and
‡Kings Park and Botanic Gardens, West Perth 6005, Western Australia

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Dry matter gains and haustorial production of pot-cultured seedlings of *Nuytsia floribunda* were assessed after a 12 month period of association singly with each of a range of potential woody host species. One species, *Adenanthos cygnorum*, of similar size to most parasitized hosts, served as measure of response of *Nuytsia* in a non-benefiting situation. Rated on this basis, all 23 parasitized hosts elicited greater mean dry weights of *Nuytsia* than when on *Adenanthos*, and seven of these instances were highly significant. Numbers and weights of penetrating and presumably functional haustoria formed on a host were broadly correlated with growth benefit to *Nuytsia*, but there were notable instances of unusually poor or great benefit from a host relative to the complement of haustoria involved. Experiments in which haustoria-bearing associations of *Nuytsia* partnered with nodulated *Acacia* hosts (*Acacia acuminata* and *A. cyclops*) were fed $^{15}N_2$ showed significant transfer of ^{15}N to the parasite, but failed to determine whether the label had been acquired through haustoria or directly by *Nuytsia* roots following turnover of nodule and root residues of the host in the rooting medium. A parallel study using the unusual non-protein amino acid, djenkolic acid, as a marker of benefit from the djenkolic acid-containing host *A. cyclops*, showed appearance and progressive build-up of the compound in foliage of *Nuytsia* over a 6 month period after partnering the species in pot culture. Presence of the compound at final harvest in xylem sap of both partners but not in soil solution of the cultures strongly indicated xylem transfer via haustoria as the principal avenue for N benefit to the parasite. Results are discussed in relation to a recent evaluation of haustorial structure and functioning of *N. floribunda*. © 2000 Annals of Botany Company

Key words: Root hemiparasite, *Nuytsia*, Loranthaceae, growth benefit, haustorial production, nitrogen transfer from hosts.

INTRODUCTION

Nuytsia floribunda (Labill.) R.Br., the Western Australian Christmas Tree, is one of three monospecific genera of root hemiparasites in the Loranthaceae, a family otherwise comprised of canopy-based mistletoes (see Kuijt, 1969; Fineran and Hocking, 1983). Ever since its systematic status was determined, *Nuytsia* was suspected to be a root parasite, but this was not substantiated until Herbert (1919) showed haustoria linking its roots to those of neighbouring plants of other species. Since then parasitism has been confirmed in a series of studies on the species by Main (1947), Grieve (1975), Lamont (1977), Hocking (1980), Hocking *et al.* (1980), Fineran and Hocking (1983), and Hocking and Fineran (1983).

Mature trees of *Nuytsia* are rhizomatous, up to 10–15 m tall, and may achieve statures in heathland and open woodland habitats greatly exceeding those of most partner species from which they are supposedly deriving benefit. As described by Hocking (1980), adult specimens possess large, deeply penetrating tap roots and haustoria-bearing roots on rhizome systems which radiate some 150 m or more outwards from a principal root stock. Sucker shoots arising from these underground rhizomes lead to formation of clones which may eventually occupy very extensive tracts of habitat (Lamont, 1977; Pate and Calladine, unpubl. res.). The species produces abundant fertile seed which germinates readily in its natural habitat, although survival of resulting seedlings beyond a first or second season is rare (Main, 1947; Hocking, 1980; Pate, unpubl. res.). Studies on potted seedlings of *Nuytsia* indicate good growth and survival for up to 18 months (Main, 1947; Grieve, 1975) and to 4 years (Calladine, unpubl. res.) without a host, indicating no obligate requirement for association with other species during juvenile development.

In this series of papers we examine the early stages of seedling growth of *Nuytsia floribunda* in pot culture when accompanied singly by a range of potential host species. We investigated whether growth benefit to seedlings from parasitism can be demonstrated, whether such benefit is correlated with the extent of haustorial contact made with a host, and whether abstraction of solutes from a host can be demonstrated experimentally using $^{15}N_2$ feeding studies on nodulated legume hosts and tracer analyses of transfer of host-specific non-protein amino acids from host to partner parasite.

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* For correspondence at: TESAG, James Cook University, Townsville 4811, Queensland, Australia. Fax +61 7 47814020, e-mail Ainsley.Calladine@jcu.edu.au

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MATERIALS AND METHODS

Plant culture

Fully ripe seed of *Nuytsia floribunda* was collected in late summer and germinated the following autumn in trays of sand from the natural habitat with a covering with 1–2 mm of sand. Emerging seedlings with radicals less than 1 cm in length were transplanted into 50 mm plastic pots of the same sand and were fed with a balanced slow release fertilizer (including trace elements) to promote early seedling growth. The following summer, associations were established between the then 9 month old seedlings of *Nuytsia* and a range of 6–12 month old seedling host species. The ten replicate pots of each association consisted of one seedling of a selected host and a single *Nuytsia* seedling potted together in a 5 l capacity pot containing a soil and sand mix, with a balanced slow release fertilizer added as nutrient resource. Ten replicate pots containing pairs of *Nuytsia* seedlings were also included in the trial. All pots were cultured outdoors in Perth, Western Australia (32°00′S, 115°50′E) and watered daily to avoid water stress during growth. The 24 native species chosen as hosts for our studies spanned a wide range of plant growth forms and root morphologies, mostly representing species common to the native sandplain habitats in which *Nuytsia* regularly occurs. With the notable exception of *Adenanthos cygnorum*, all were previously known from pot culture studies and excavations in the native habitat to be extensively parasitized by *Nuytsia* (Pate, Calladine and Dixon, unpubl. res.).

Harvesting for study of comparative growth and haustorial development of Nuytsia *on different hosts*

Harvesting commenced in January, 12 months after the parasite and hosts were planted together. Five pots from each association were randomly selected, while the remaining five pots were left for tracer studies and for examinations of haustorial structure and functioning (Calladine and Pate, 2000). Successive washings progressively removed soil from the entangled root systems, leading to essentially full recovery of root systems of parasite and host, with haustorial connections to host root fragments mostly intact. Thorough examinations of the root mass from each pot were then undertaken to separate root biomass of each species. The white colour and fleshy brittle nature of roots of *Nuytsia* enabled them to be readily distinguished from roots of any of the chosen hosts.

Haustoria of *Nuytsia* recovered from each pot were separated into three categories and counted:

(1) Pre-penetration stage. A haustorium had begun or completed development of its encircling cortical folds of tissue (haustorial collar), but had yet to sever the host root [see Calladine and Pate (2000) for details of haustorial development].

(2) Fully penetrated stage. A haustorium had successfully cut through the root and had developed vascular tissue facing the proximal and distal cut faces of the severed host root. Haustoria in this condition were considered to be potentially functional in abstraction of water and solutes from a host (see Calladine and Pate, 2000).

(3) Intraspecific parasitism. This included instances where haustorial-like graft unions had formed from one *Nuytsia* root back onto its own root or where one of the two *Nuytsia* seedlings in pots containing *Nuytsia* only had formed unions with the partner seedling.

Shoots, separated root biomass of host and parasite, and haustoria, separated into the categories described above were individually bagged and oven-dried at 80°C to a constant weight to determine total plant weight of shoot and root for each species. Dry weights and numbers of supposedly functional haustoria (category 2 above) for each association were used in comparisons of extent of contact with a host and measured growth benefit to the parasite from that host. In all cases except that involving *Nuytsia* seedlings grown together, category 2 represented 80% or more of the total complement of haustoria recovered from a pot.

$^{15}N_2$ tracer studies

The first study of this kind used pot-grown associations established between effectively nodulated 6 month old seedlings of *Acacia cyclops* and 9 month old seedlings of *Nuytsia*. As in other pot cultures, the plants had been fed with balanced slow release fertilizer. Harvesting of replicate pots prior to feeding showed that a substantial number of penetrating haustoria had developed on the host, while luxuriant growth of the parasite in comparison with *Nuytsia* seedlings grown without a host suggested that benefit had commenced within 3 months of partnering with a host. $^{15}N_2$ feeding experiments were carried out when the *Nuytsia* seedlings were 14 months old, i.e. 5 months after transplanting with the host and 2 to 3 months after visible benefit to the parasite was first evident.

Two pots each containing single intact plants of host and parasite were placed into closely-fitting plastic buckets and a split plastic lid fitted to the top of each bucket to fit around the lower shoots of host and parasite. Terostat VII (Terosan, Germany) was used to seal the lids around the rim of buckets and onto the bases of the shoots, thus creating a hermetically-sealed gas space around each enclosed root system. Each lid carried a small hole through which a plastic tube extended to the bottom of the pot; this was used to administer $^{15}N_2$ gas. A second hole with a plastic tube terminating near the top of the pot allowed the unlabelled gas displaced during $^{15}N_2$ feeding to be vented.

After checking for gas leaks by submerging the assembly into a tank of water, a mixture of 20% oxygen and 80% argon was flushed through the system for 2 h to expel unlabelled nitrogen from the root gas space, while maintaining the equivalent of the atmospheric concentration of oxygen to permit normal root functioning. A sample of 250 ml of gas, roughly equivalent to 90% of the gas space volume of the rooting medium, was withdrawn from the top of the container while synchronously administering 200 ml of $^{15}N_2$ (98·6 atom % excess $^{15}N_2$) and 50 ml O_2 through the lower tube. The fed plants with root systems still enclosed were incubated in a naturally lit glasshouse for 24 h, after which the seals were broken and the plants left for a further 48 h before being harvested. After harvesting of shoots, pot contents were washed out under running water to facilitate separation of roots of host and parasite and nodules of the *Acacia* host. Samples of roots, nodules and shoots were dried in an oven at 80°C for 48 h and then fine ground in a micro-hammer mill prior to mass spectrometric analysis. Two unlabelled associations were harvested at the same time to assess natural abundance of $^{15}N_2$ in above- and below-ground parts of host and parasite.

A second $^{15}N_2$ labelling experiment conducted and harvested as described above, involved feeding $^{15}N_2$ for 24 h to the rooting environment of two 12 month old associations between a single 30 cm high *Nuytsia* seedling and a similarly aged, well nodulated 1 m tall plant of *Acacia acuminata*. The four replicate associations used in the study (two labelled and two unlabelled controls) had been raised on a N-free mineral supplement, thus enforcing long term dependence of *Nuytsia* on symbiotically-fixed N from *Acacia*. Harvests of plants were conducted 20 d after the end of feeding period. It was found that over 100 penetrating haustoria had developed on roots of each *Acacia* host. Vigorous growth of *Nuytsia* indicated continued benefit from host-derived fixed N and this was confirmed in parallel studies showing mean net gains per plant of 61 mg N and 15 g dry mater in comparably aged and treated *Nuytsia* during an 8 month period of association with the *Acacia* host.

Finely ground dry matter samples of nodules, roots and shoots from the $^{15}N_2$ labelling studies were subjected to ^{15}N analysis using a VG Isogas SIRA 9 high resolution mass spectrometer and employing the digestion, distillation and mass spectrometric assay procedures detailed by Unkovich *et al.* (1993, 1994). Errors of measurement applied as indicated in these publications.

Use of the non-protein amino acid, djenkolic acid, as a tracer of xylem-mediated benefit to Nuytsia

This investigation utilized *Acacia cyclops* partnered singly with *Nuytsia* in minus N pot cultures. Studies prior to the experiment had shown that the unusual S-containing amino acid djenkolic acid was present in a relatively large amount in xylem sap and leaf tissues of *A. cyclops* (e.g. Pate *et al.*, 1991), but was not detectable in non-parasitic seedlings of *Nuytsia* or in *Nuytsia* associated with any of the non-*Acacia* hosts used in the pot cultures (Table 1). Djenkolic acid was also not detected in foliage of *Nuytsia* in habitats from

TABLE 1. *Species used singly as potential hosts in pot culture with seedlings of the parasite* Nuytsia floribunda

Species	Family	Growth form
Acacia acuminata Benth.	Mimosaceae	Tree[a,e]
Acacia cyclops Sweet ex G.Don	Mimosaceae	Tree[a,b,f]
Adenanthos cygnorum Diels	Proteaceae	Tall shrub[c]
Banksia prionotes Lindl.	Proteaceae	Tree[f]
Boronia purdieana Diels	Rutaceae	Small shrub
Callistemon glaucus (Bonpl.) Sweet	Myrtaceae	Tall shrub
Calothamnus quadrifidus R.Br.	Myrtaceae	Tall shrub
Calothamnus rupestris Schauer	Myrtaceae	Tall shrub
Calytrix strigosa A. Cunn.	Myrtaceae	Small shrub
Casuarina obesa Miq.	Casuarinaceae	Tree[f]
Chamelaucium uncinatum Schauer	Myrtaceae	Shrub
Conospermum stoechadis Endl.	Proteaceae	Shrub
Eucalyptus erythrocorys F. Muell.	Myrtaceae	Tree[f]
Eucalyptus lesouefii Maiden	Myrtaceae	Tree
Eucalyptus wandoo Blakely	Myrtaceae	Tree
Helichrysum cordatum A. Cunn. ex DC.	Asteraceae	Semi-woody shrub[g]
Hypocalymma angustifolium Endl.	Myrtaceae	Shrub
Hypocalymma robustum (Endl.) Lindl.	Myrtaceae	Shrub
Kunzea baxteri (Klotzsch) Schauer	Myrtaceae	Shrub
Melaleuca preissiana Schauer	Myrtaceae	Tree[f]
Nuytsia floribunda (Labill.) R.Br.	Loranthaceae	Tree, root hemi-parasite[d]
Phebalium anceps A. Cunn. ex DC.	Rutaceae	Shrub
Regelia inops (Schauer) Schauer	Myrtaceae	Shrub
Thysanotus multiflorus R.Br.	Anthericaceae	Perennial herb
Xanthorrhoea preissii Endl.	Xanthorrhoeaceae	Arborescent monocotyledon
Xylomelum occidentale R.Br.	Proteaceae	Tree[f]

[a] Used in $^{15}N_2$ feeding tracer study.
[b] Used in study of djenkolic acid transfer from host to parasite.
[c] Not parasitized by *Nuytsia* and used as control in evaluations of growth benefit to *Nuytsia*.
[d] 'Autoparasitism' of *Nuytsia* on *Nuytsia*.
[e] Used for deuterium-enriched water feeding (Calladine and Pate, 2000).
[f] Used for comparative studies of xylem water potentials of parasite and host (Calladine and Pate, 2000).
[g] Used for xylem feeding of dye (Calladine and Pate, 2000).

All species except *A. acuminata* used in analyses of growth benefit and in a companion study (Calladine and Pate, 2000) on haustorial development, structure and functioning.

which djenkolic acid-producing *Acacia* were absent (Pate, unpubl. res.).

Time courses of possible transfer of the compound from host to parasite were followed by sampling replicate 3 × 3 mm samples of leaf material from *Nuytsia* and host at the time of partnering of host and parasite and at monthly intervals thereafter over the next 6 months. Ethanol extracts of the foliage samples of host and parasite were assayed for free amino acids using the HPLC system described by Pate *et al.* (1985), and djenkolic acid separated and identified as described by Pate *et al.* (1991, 1994). After the study was completed, xylem sap samples were collected by mild vacuum extraction of shoots of host and parasite (see Pate *et al*., 1994) and rhizosphere water samples simultaneously collected from the potting medium. These samples were assayed for djenkolic acid and other amino acids.

RESULTS

Ranking of performances of Nuytsia *seedlings when associated with different hosts in pot culture*

Mean plant dry weights of *Nuytsia* grown in partnership with different host species were found to differ significantly ($F = 1{\cdot}896$, $P = 0{\cdot}013$), ranging from only 2·2 g per plant in partnership with the non-parasitized *Adenanthos cygnorum* to a massive 13·4 g per plant with the ‘best’ host, *Calothamnus rupestris* (Fig. 1). The mean dry weight per plant of the pair of *Nuytsia* seedlings grown without a host was 9·4 g, ranking it fifth in *Nuytsia* yield performance amongst the 25 partnerships tested.

When the mean dry weight of penetrating haustoria per plant on each host was plotted against corresponding mean total dry weights of *Nuytsia* on the same host (Fig. 2), a significant correlation was apparent between variables. However, a number of associations emerged as distinct outliers. Most noticeable among these was the partnership of *Nuytsia* and *Xanthorrhoea preissii*, which showed an unusually low mean ratio of haustorial weight to parasite dry weight, and the associations of *Nuytsia* with *Eucalyptus lesoufii* or *Helichrysum cordatum*, for both of which the above ratio was particularly high. No associations between *Adenanthos cygnorum* and *Nuytsia* showed penetrating haustoria, although one pot carried eight non-penetrating haustoria with a combined dry weight of only 3·6 mg. As expected, *Nuytsia* plants grown with *Adenanthos* were small and chlorotic with mean dry weights less than that of all other associations (Fig. 1).

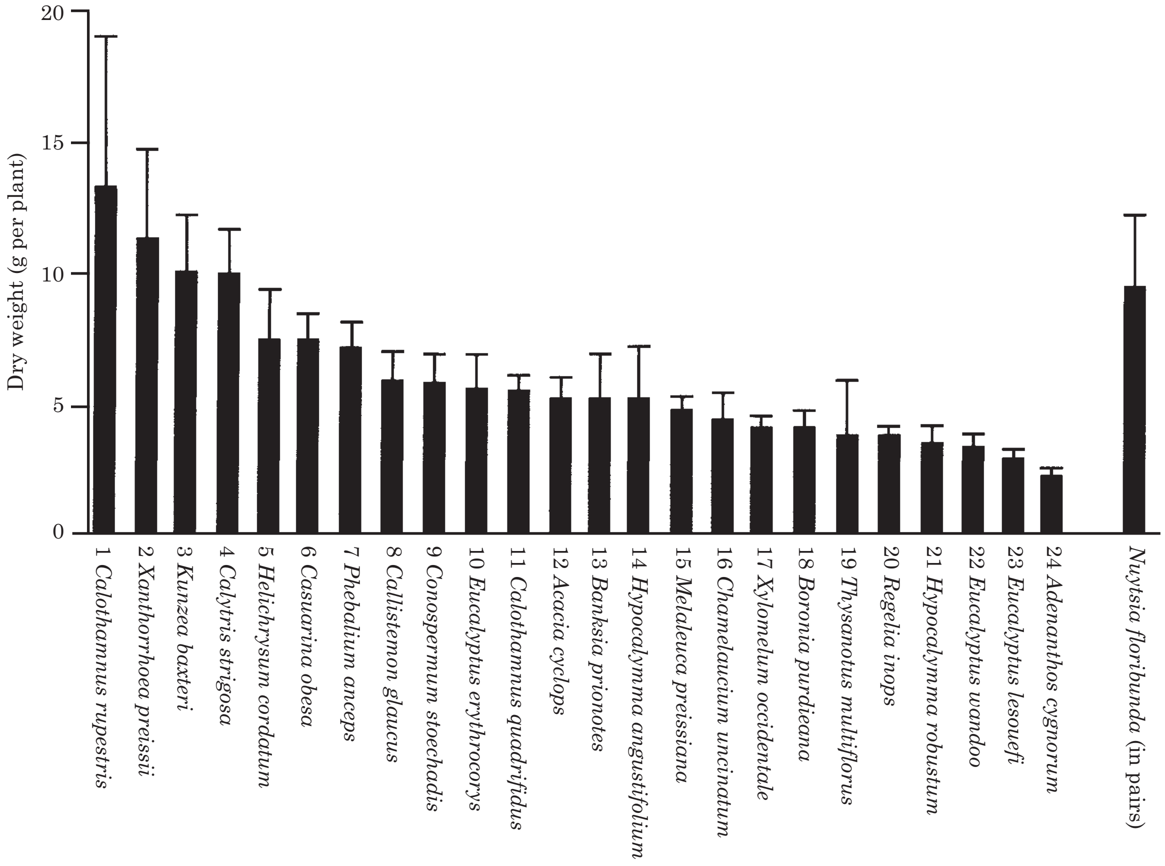

FIG. 1. Total dry weight (mean ± s.e., $n = 5$) of single *Nuytsia floribunda* in association with a single specified host after 12 months of pot culture. The 24 associations are ranked in order of decreasing final dry weight of the parasite. Data show dry matter gains in *Nuytsia* to be significantly different when grown with different hosts ($F = 1{\cdot}896$, $P = 0{\cdot}013$). *Nuytsia* failed to establish haustoria in only one association, *Adenanthos cygnorum*, its performance on this host ranked least. Performance of *Nuytsia* grown in pairs without a non-parasite host is shown to the right (mean dry weight per plant). See Table 1 for growth and life forms and taxonomic position of hosts.

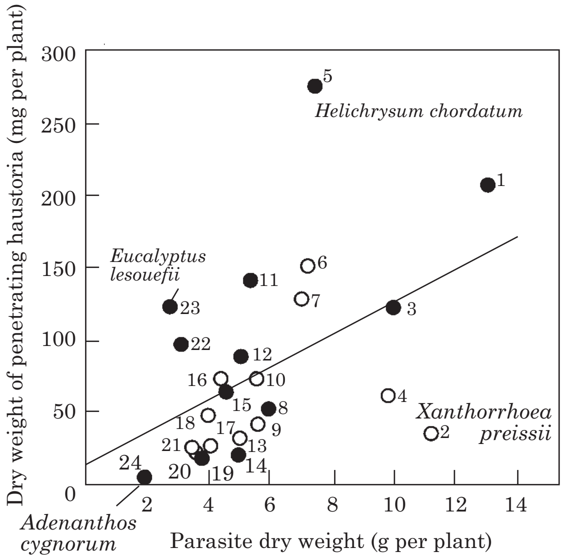

FIG. 2. Plot of dry weight of penetrating and presumably functional haustoria against final dry weight (mean, $n = 5$) of *Nuytsia floribunda* when associated for 12 months in pot culture in single association with a range of hosts ($r = 0{\cdot}43$, $P < 0{\cdot}05$, $n = 24$). No penetrating haustoria formed on *Adenanthos cygnorum*; *Nuytsia* performed better on all other hosts than in this abortive association. (●), Associations with similar host dry weights to that of *Adenanthos*. Associations with three other species (named) comprise notable outliers for which haustorial dry weights were disproportionately great or small relative to the dry weight increase for the parasite above that of the non-parasitized host *A. cygnorum*. The rank of *Nuytsia* performance (Fig. 1) indicates the numerical coding for this plot.

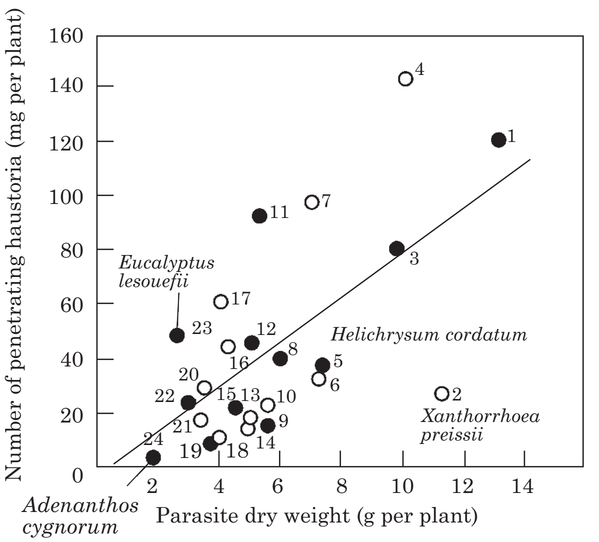

FIG. 3. Plot of mean numbers of penetrating and presumably functional haustoria against final dry weight (mean, $n = 5$) of *Nuytsia floribunda* when associated for 12 months in pot culture in single association with a range of hosts ($r = 0{\cdot}61$, $P < 0{\cdot}01$, $n = 24$). No penetrating haustoria formed on *Adenanthos cygnorum*; *Nuytsia* performed better on all other hosts than in this abortive association. (●), Associations with similar host dry weights to that of *Adenanthos*. Outliers designated in Fig. 2 are recorded in this plot. The rank of *Nuytsia* performance (Fig. 1) indicates the numerical coding for this plot.

Plots of mean numbers of penetrating haustoria against parasite dry weight (Fig. 3) gave results similar to those described above for haustorial weight, with the *Nuytsia*:*Xanthorrhoea* association again recording fewer functional haustorial numbers per unit parasite dry weight than most other associations. *Nuytsia* on *Helichrysum* developed unusually few haustoria compared with most associations studied, while the *Nuytsia*:*E. lesoufii* association showed a large mean number of haustoria proportional to *Nuytsia* dry weight.

Since the *Nuytsia*:*Adenanthos* association was essentially a non-parasitic one and the host concerned was of a size similar to that of many other hosts, this partnership was considered to represent a better 'control' for non-parasitic performance of *Nuytsia* than the pairs of *Nuytsia* grown alone. Our reasoning was that the relatively large *Adenanthos* would have provided levels of competition for water and nutrients similar to those experienced when *Nuytsia* plants were grown in association with comparably sized beneficial hosts. Conversely, inter-plant competition would obviously have been minimal in the treatments involving interaction of a diminutive pair of *Nuytsia* or in the *Nuytsia*:*Xanthorrhoea* association with a mean host dry weight of only 1·7 g. Both of these treatments almost equalled the largest *Nuytsia* dry weight mean measured. Using this approach, ten associations with similar host dry weight to that of *Nuytsia*:*Adenanthos* were further compared. The seven associations listed in Table 2 were found to have significantly higher total dry weights for their *Nuytsia* component than did non-parasitic *Nuytsia* partnered with *Adenanthos*.

TABLE 2. *Host species which promoted mean dry weights of* Nuytsia *seedlings that were significantly greater than those in the non-benefiting association with* Adenanthos cygnorum. *Only associations which shared similar host mean dry weights were compared*

Associations with:	Assumed benefit* (g per plant)	Significance ($n = 5$)†
Calothamnus rupestris	11·2	<0·01
Kunzea baxteri	8·2	<0·01
Callistemon speciosus	4·1	<0·01
Calothamnus quadrifidus	3·5	<0·01
Acacia cyclops	3·2	<0·01
Hypocalymma angustifolium	3·1	<0·01
Melaleuca preissiana	2·7	0·05

* Estimated as difference in mean dry weight of *Nuytsia* seedlings on the stated host and those with *Adenanthos cygnorum* (2·2 g).

† Derived from a multiple comparison *t*-test.

Tracer studies indicating benefit in $^{15}N_2$ *fed* Acacia : Nuytsia *association*

In the first experiment using relatively young associations, enrichment values for plant parts harvested a relatively short time (48 h) after $^{15}N_2$ labelling were generally low (Table 3). However, the data showed significant

TABLE 3. *Enrichment of pot cultured seedlings of* Nuytsia *and effectively nodulated* Acacia *hosts, when the enclosed root gas space of the associations were exposed to* $^{15}N_2$

Species	Plant part	$\delta^{15}N$ natural abundance of unlabelled controls (‰)	$\delta^{15}N$ of $^{15}N_2$ fed plants (‰)
Experiment 1. Plants harvested 48 h after end of 24 h feeding of $^{15}N_2$			
Acacia cyclops	Nodules	(not analysed)	+1295
	Roots	−1·8	+560
	Shoots	−1·4	+200
Nuytsia floribunda	Roots	−3·7	+21
	Shoots	−0·5	+6
Experiment 2. Plants harvested 20 d after end of 24 h feeding of $^{15}N_2$			
Acacia acuminata	Nodules	+10·1	+3150
	Roots	−2·3	+2141
	Shoots	−2·0	+2973
Nuytsia floribunda	Roots	−2·4	+441
	Shoots	−1·1	+1536

Data are compared to natural abundance levels of ^{15}N in similar unlabelled associations. $\delta^{15}N$ values refer to material pooled from the two replicate pots used for $^{15}N_2$ feeding and control plants used for assessment of $\delta^{15}N$ natural abundance.

enrichment for all parts analysed above the ^{15}N natural abundance levels recorded for unlabelled plants. Highest values were recorded for N_2 fixing nodules, followed by roots and shoots of the parent *Acacia*, and then by much lower enrichments for shoots and roots of the associated *Nuytsia*. Thus, while ^{15}N had subsequently been transferred to *Nuytsia*, the amounts involved were small relative to that fixed by *Acacia*.

The second $^{15}N_2$ labelling study involved older plants, minus nitrogen culture conditions enforcing dependency of *Nuytsia* on fixed N from *Acacia* and a longer (20 d) interval between feeding of label and harvest. The host species was *A. acuminata* not *A. cyclops*. As expected, $\delta^{15}N$ enrichment (Table 3) was generally greater than in the first experiment, with *Nuytsia* shoots achieving labelling intensities almost as great as those recorded for the donor host. Effective transfer of fixed N to the parasite was clearly indicated but, in view of the long lapse between labelling and harvest, appreciable amounts of the ^{15}N recovered in the parasite might have been obtained directly from the rooting medium following decomposition and mineralization of senesced root and nodule material of the host.

Evidence of djenkolic acid transfer from Acacia cyclops *to* Nuytsia

Analyses of small leaf samples clipped at monthly intervals from the host *Acacia* showed significant levels of djenkolic acid at all times of sampling, with the compound accounting for some 3–5% of the total ethanol-soluble amino acid-N of foliage of the species [see similar data of Pate *et al.* (1991)]. Corresponding analyses of leaf material from *Nuytsia* failed to show detectable levels of the compound at the time of transplanting or in the subsequent 3 months during which parasitism was being established. The compound appeared in trace quantities in *Nuytsia* after 4 months and increased over the next 2 months to reach levels equivalent to 0·3% of the total ethanol-soluble N fractions of the foliage. Xylem sap collected from shoots of both species at the completion of the study showed djenkolic acid in amounts equivalent to 12% of the xylem sap N fraction of *Acacia* and at a lesser, but still easily detectable proportion (0·8%) in xylem sap N of *Nuytsia*. Since aqueous eluates of the rooting medium failed to show djenkolic acid and, indeed contained only barely detectable levels of major *Acacia* amino acids such as asparagine, we concluded that uptake from host xylem via haustoria was the most likely, if not only, avenue for intake of djenkolic acid and other forms of organic N by the parasite from its host.

DISCUSSION

Although the list of native host species on which *Nuytsia* will form haustoria is very substantial (Herbert, 1919; Main, 1947; Grieve, 1975; Lamont, 1977; Hocking *et al.*, 1980; Pate, unpubl. res.) most reports on host range failed to note whether the haustoria concerned had cut through the host roots to which they were attached. One cannot, therefore, be certain whether the haustoria in question were suitably equipped to gain water and nutrients from a host as discussed by Calladine and Pate (2000).

In view of the obvious non-comparability between a potted pair of small *Nuytsia* and a similarly resourced and aged pot partnership between a single *Nuytsia* and a large competing host, differences in *Nuytsia* dry weight in these two situations provided a dubious index of the extent to which benefit had accrued from parasitism. Rated on this basis, only four associations showed greater *Nuytsia* dry weight than for *Nuytsia* grown with another *Nuytsia*, presumably since benefits generated through haustorial contact with a large host species were partly outweighed by the competitive influence which that host would have imposed on the parasite for space, light, water and nutrients.

Bearing in mind complications of this kind, we included *Adenanthos cygnorum* amongst our selection of partner species, since it was of a size similar to that of most other woody hosts used in the study but was virtually immune to attack by *Nuytsia*. Whatever the reason for failure in establishment of parasitism by *Nuytsia* on *Adenanthos*, we accordingly regard this association as our most definitive 'control' for rating how *Nuytsia* would behave when competing with, but unable to derive benefit from, its host. Rated on this basis (see Table 2), seven of the successfully parasitized hosts supported *Nuytsia* plants with mean dry weights significantly greater than that of *Nuytsia* in non-benefiting association with *Adenanthos*.

Using the above evidence we then analysed whether numbers and weights of mature penetrating haustoria forming on a host were positively correlated with the extent of apparent benefit to the parasite. The correlations (Figs 2 and 3) turned out to be poor, largely because several associations elicited unusually sparse or very intense benefits relative to the mass or number of haustoria involved. One must therefore conclude that certain, as yet to be defined, qualitative aspects of haustorial performance exercise an important moderating effect in determining the extent of benefit from a host. Features of an anatomical nature facilitating or detracting from effective exchange with a host might be involved in this connection or, as suggested in parallel studies on root hemiparasites other than *Nuytsia*, amounts and relative proportions of different classes of organic solutes in host xylem might be of major relevance. The latter explanation has been used to explain the superlative growth of parasites on leguminous hosts whose xylem sap proves to be unusually rich in nitrogenous solutes [e.g. Pate *et al.* (1994) on *Olax phyllanthi*; Tennakoon *et al.* (1997) on *Santalum acuminatum*; and Radomiljac *et al.* (1998) on *Santalum album*].

As far as we are aware, our present demonstration of uptake of ^{15}N by the parasite after feeding ^{15}N to a nodulated *Acacia* host offers the first conclusive evidence of chemically-specific benefit to *Nuytsia* from a given host. However, the studies fail to prove whether the label accumulating in the parasite had actually passed through haustoria or merely been taken up by *Nuytsia* roots after decomposition of fixed N in the nodule and root residues of the host. We accordingly regarded our parallel study using djenkolic acid as a marker as providing more definitive evidence of direct xylem benefit via haustoria, since the marker compound in question was shown to be consistently present in xylem of the host, was absent from *Nuytsia* when grown without a host, and built up progressively in foliage of the parasite once parasitism had been properly established. At this latter stage, djenkolic acid can be recovered from xylem sap of the parasite but not from the rhizosphere of the pot cultures, thereby refuting the possibility of direct uptake by *Nuytsia* from the rooting medium.

The substantial net gain of total N which can be made by *Nuytsia* when fully dependent on a legume host for nitrogen strongly supports the extremely effective abstraction of host N resources which can be accomplished by root hemiparasites when parasitizing N_2 fixing hosts. Our study is thus in full agreement with recent demonstrations of a monopolization of host N resources when the root hemiparasite *Olax phyllanthi* is partnered with nodulated *Acacia littorea* (Pate *et al*., 1994), or with the behaviour of plantation-cultured *Santalum* spp. on various tree legume hosts *vs.* a much less effective eucalypt host (Tennakoon *et al*., 1997; Radomiljac *et al*., 1999). Our companion paper in the series (Calladine and Pate, 2000), dealing with water relations of *Nuytsia* and associated hosts and anatomical features of the haustorial connection with hosts provides further information on likely transfer processes for solutes and water, particularly in relation to the unique mode of initiation and final structural features of the *Nuytsia* haustorium in comparison with that of other xylem-tapping parasites.

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