Experimental seeding trials for the root parasite *Dactylanthus taylorii*

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ABSTRACT

A robust experimental method for sowing *Dactylanthus taylorii* seed was used to investigate host species preference, host age, and sowing density. The results from these trials, when analysed, are likely to assist future translocations, or restoration of sites with *Dactylanthus*.

Keywords: root parasite, *Dactylanthus taylori*, Balanophoraceae, cultivation, seeding trials, New Zealand

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1. Introduction

1.1 OBJECTIVE

To conduct a seeding trial at Waipapa Ecological Area, using a scientifically robust design to allow for comparison between different sowing rates and habitat types.

1.2 BACKGROUND

Dactylanthus taylorii (pua o te reinga, wae-wae-atua, dactylanthus) is New Zealand's only fully parasitic native flowering plant and the southernmost member of the mainly tropical family of root parasites Balanophoraceae. It lives as a subterranean tuber attached to the root of a number of native tree and shrub host species, which are mainly characteristic of secondary (regrowth) broad-leaved forest (Moore 1940; Ecroyd 1996; Holzapfel 2001).

Since work began on the recovery of dactylanthus, cultivation of the species has been one of the primary goals. Successful cultivation would allow for new populations to be established on sites with intensive control of browsing animals, as well as on offshore islands that are completely free of introduced browsing animals. Cultivation of plants would also facilitate future research, public education, and the transfer of established parasite / host associations (*Dactylanthus* Recovery Plan: Objectives 5 and 6 in Ecroyd 1995).

In 1989 and 1990, seeds of dactylanthus were sown by Chris Ecroyd close to a potential host tree (kohuhu: *Pittosporum tenuifolium*) in a private garden, and onto roots of broadleaf (*Griselinia littoralis*) in planter boxes. The latter plants were later transferred into a forest-like site on the grounds of Forest Research, Rotorua.

In 1998, more than eight years after the last sowing, inflorescences of dactylanthus were observed at both sites for the first time (Ecroyd, unpublished report to the *Dactylanthus* Recovery Group 1998). Although plants may have flowered unnoticed the year before, these results indicate the long time involved in any cultivation of dactylanthus.

Research on the germination process for the species suggests that after seeds become buried, every year a proportion of the seeds will germinate in suitable conditions (moist soil), regardless of the presence of a host root (Holzapfel 2001). Germinated seeds are able to survive for considerable time without attachment, during which time they attach to a young root growing nearby. A successful attachment to a host root and subsequent infection, therefore, seems to depend to a large extent on chance, and will thus in turn be dependent on the density of seeds in the soil.

No information is available about the number of seeds used in Ecroyd's cultivation experiment. A trial investigating the importance of sowing density therefore adds valuable insight into the optimal procedures for sowing.

Conducting the trial at sites with different habitat types will provide additional clues about the importance of habitat and host age.

Experimental seeding trials are currently underway in four conservancies (Tongariro/Taupo, Bay of Plenty, East Coast/Hawke's Bay, and Waikato). The technique described here was developed for trials at Pureora Forest Park (Waikato) in 1999, and adopted for trials at Mokoia Island (Bay of Plenty). The expected long time lag between sowing and flowering does not yet allow an assessment of these sowing trials. Yet the technique described here may assist in planning and carrying out further, similar or related experimental seeding trials for dactylanthus, or other species.

2. Methods

2.1 SEED SOURCE

Infructescences were collected on 6 January 1999 from different dactylanthus tuber clumps at one site ('Renee's patch', Plains Road) in Pikiariki Ecological Area, Pureora Forest Park. This site is about 6 km away (straight line) from the sowing trial area at Waipapa. Recent information on the genetic diversity of dactylanthus indicates that proximity is not necessarily a good indicator of population similarity (Faville et al. 2000; Holzapfel et al. 2002). Nevertheless, any seeds should be collected from the nearest suitable population, if the sowing site is very close to an existing dactylanthus population.

As seeds are known to remain viable for several years under moist conditions (Ecroyd 1996; Holzapfel 2001), infructescences mostly from the previous year's (1998) flowering season were collected. That is, the seeds were 9–10 months old, possibly of 1997 vintage, or even older.

A total of 37 infructescences were initially collected, of which 24 with high or medium fruit set (Barkla & Holzapfel 1997) were selected for the trial. Surplus infructescences were returned to the collection site on 13 January 1999.

2.2 SEED PREPARATION

Seeds are generally between 0.8 mm and 1.6 mm in length. They were gently removed from infructescences under running water, and caught in a soil sieve (0.5 mm mesh). Larger particles were removed by running the material through a larger sieve (3.0 mm mesh). Seeds were cleaned by agitating the sediment under water and floating off debris (intact, healthy dactylanthus seeds are heavy and aggregate in water with the sand fraction). The seeds from all 24 infructescences were then mixed, drained over a small tea-sieve, blotted dry through the sieve with paper tissue, and aliquoted into 24 equal samples by weight on a laboratory scale.

The number of seeds in one randomly chosen sample was counted as 1477 seeds. Ecroyd (1996) found the average number of flowers on a female inflorescence was 3660 where each flower potentially developed into one seed. Therefore, each aliquot sample represented just below half of the average number of seeds expected on a fully pollinated inflorescence. A similar amount could be expected on an infructescence with medium fruit set.

Seeds were prepared one day before sowing, and samples stored individually in plastic Petri dishes on moist filter paper. Storage for several weeks is possible in Eppendorff vials (250 mL volume, obtained from lab supplies) with seeds kept moist by adding a drop of water to the vial before closing.

2.3 SOWING SITES

Site selection should take into account that seeds (and probably also tubers) of dactylanthus prefer moist ground.

Four sites were chosen at Waipapa Ecological Area, Pureora Forest Park. All sites are within a radius of just above 1 km, and more or less on the same elevation.

Sites A and D are on a slight sloping face, therefore probably more exposed and prone to dryness than sites B and C.

- **Site A**—Open canopy, dominated by juvenile and adult lancewood (*Pseudopanax crassifolius*), with bracken (*Pteridium esculentum*) undergrowth. The site is somewhat exposed, on a slight slope (with a similar gradient and close to site D). It is probably drier than the other sites that have a closed canopy and/or are on level ground.
- **Site B**—Closed canopy, dominated by a mix of juvenile and adult lancewood, and open understorey. The sowing sites were as close to adult lancewood trees as possible.
- **Site C**—Closed canopy, dominated by adult kohuhu (*Pittosporum tenuifolium*), with numerous 5–10 cm-high kohuhu seedlings in the understorey.
- **Site D**—Closed canopy, dominated by up to 3 m-high, adult fivefinger (*Pseudopanax arboreus*). The site is on a slight slope, similar in gradient and close to site A.

Variables tested in regard to sites are, therefore:

- **Canopy/exposure**—Open canopy, more exposed (site A) versus closed canopy, less exposed (site B). Both sites are dominated by lancewood.
- **Dominant host species**—Lancewood (site B) versus kohuhu (site C) versus fivefinger (site D). All sites have a closed canopy.

At each site three patches suitable for the establishment of two sowing plots were chosen. Paired plots were as close to each other as possible, and similar in terms of slope, aspect, distance to the nearest host trees, and type of ground cover. In each patch, two sowing densities (see below) were used; the sowing density in one plot allocated by flipping a coin, the other plot in the same patch

receiving the alternative density. Thus, each patch contained one of three replicates for each sowing density per site.

2.4 PLOT PREPARATION

A rectangular area of 50 × 50 cm was marked on the ground with a template made from wire-mesh (5 cm mesh size, 10 mesh across each side). The mesh provided the grid used to sow at exact locations within the plot (Fig. 1).

The corners of the template were marked in the ground with permanent aluminium pegs (c. 20 cm long). The grid was laid out according to compass bearing, with the upper side (grid nos. I1 to I10: Fig. 1) facing south. A plot identification number was fixed onto the north-east corner of the grid (grid no. X1: Fig. 1.)

Plot and site localities were recorded as accurately as possible and transferred onto individual recording sheets for each plot, prior to the first monitoring. Appendix 1 shows a recording sheet (in Microsoft Excel).

2.5 SOWING RATES

Two sowing rates were applied, using the same number of seeds (Fig. 1):

- A single aliquot of seeds, placed in the central grid of the plot; this simulated burial of an isolated infructescence of medium fruit set
- A single aliquot of seeds, placed evenly over all squares within the grid; this simulated an even dispersal of individual seeds from one infructescence over a small area

Each sowing rate was replicated three times at each of the four sites, giving a total of 24 plots.

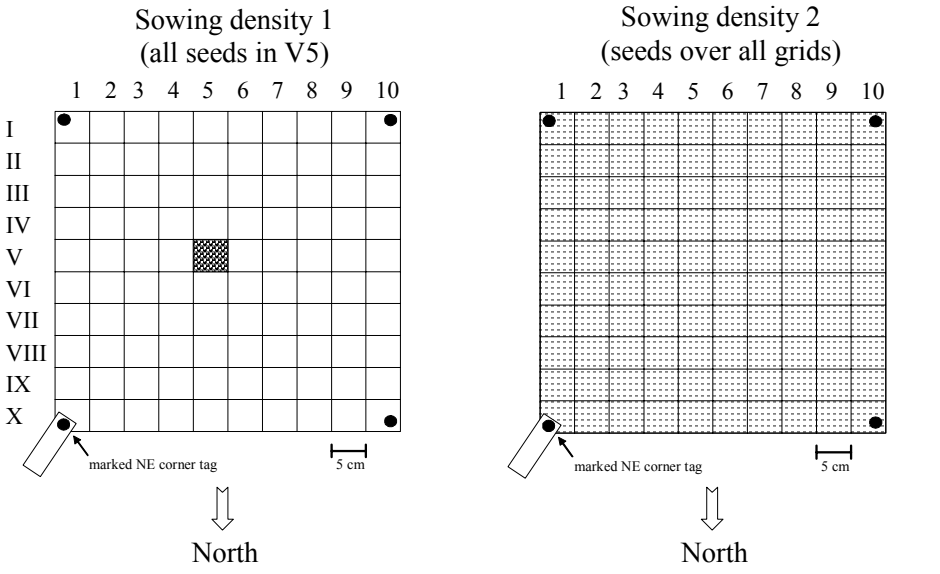

Figure 1. Layout of sowing grid and placement of seeds for sowing densities 1 and 2.

2.6 SOWING

Sowing was conducted on 12 January 1999. Each aliquot was mixed with fine dry sand (1 film container = 30 cm^3) to facilitate the seeds' spread and sown directly onto the ground after removal of any leaf litter. As the bare ground was tightly compacted at all plots, the soil was broken up to a depth of 5 cm before sowing to expose living fine roots. It is assumed that dactylanthus infects only young roots, probably at or just above the root-hair section (Moore 1940), therefore, seeds placed close to such roots optimise infection chances. Leaf litter was replaced after sowing, and the grid removed.

2.7 IDENTIFICATION OF PLOTS AND SITES

Three different identification markers were used:

- **Individual plot markers** for each sowing plot (on the NE corner of each sowing plot). Plot markers were small aluminium tags attached on the corner peg (Fig. 1). They were stamped with a code, such as **A1a**. The first letter identified the site (A–D), the digit identified the sowing density (1 or 2), and the third character identified the replicate number (a–c).
- **Replicate markers** for each pair of sowing densities (one marker for each replicate pair). Replicate markers were triangular plastic track markers, stamped with 'Plot', followed by the site code (A–D) in the second line, and the replicate code (a–c) in the third line. Replicate markers were attached to a tree near each plot pair, about 50 cm above ground level.
- **Site markers** for each site A–D. Site markers were triangular plastic track markers stamped with 'Site', followed by the site code (A–D) and the date (dd/mm/yy). Site markers were attached to prominent trees or wooden stakes.

The distance (metres) and bearing (degrees magnetic) from the site marker to replicate markers, and from replicate markers to the plot marker were recorded, as well as the exact locality of site markers (map reference or GPS reading). In our setup, all sites were along the same road, so distance (odometer reading) between each site was also noted. A diagram of each site was prepared from these measurements and taken into the field on each monitoring occasion to facilitate location of the plots, and to check for measurement errors. An example of a site diagram is given in Fig. 2.

2.8 MONITORING

All plots were monitored annually during the time when the nearest populations were flowering.

Yearly monitoring should continue for a period of at least 10 years, or until the emergence of dactylanthus occurs. Weekly monitoring of all plots over a period of 6 weeks is recommended, once the first emergence is noted.

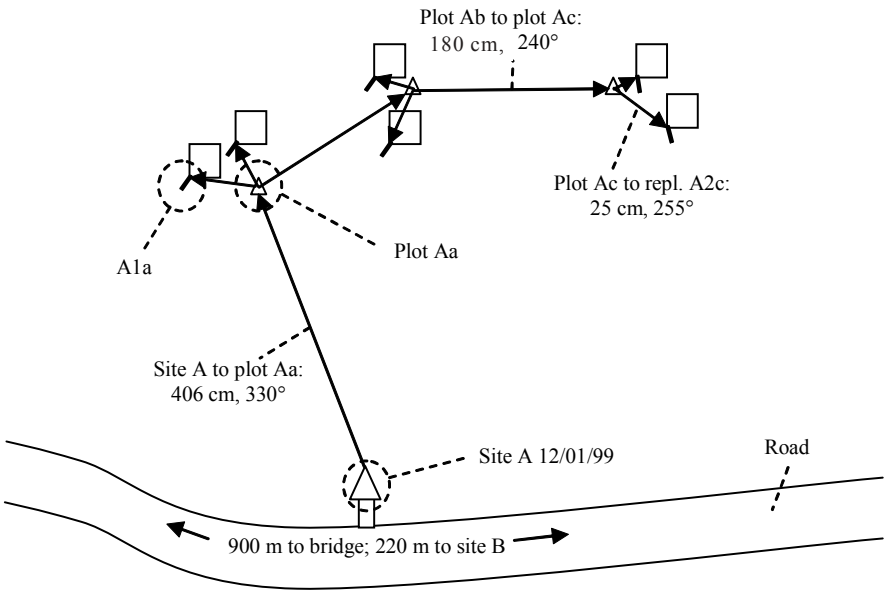

Figure 2. An example of a site diagram; only a selection of marker inscriptions, bearings and distances is given. All bearings and distances from marker to marker (solid arrows) would be recorded.

Since the trial was initiated, 3 years of monitoring have been undertaken. Sites, plots and the exact sowing grid position were located without difficulties where the original measurements were correct. Exact measurement, in particular of individual plots, proved to be important: leaf litter had built up at some sites to such an extent that the permanent corner pegs were completely covered.

When monitoring, the plot is carefully examined (visually) for signs of inflorescences or tubers. The original mesh grid is placed over the four permanent corner pegs to find the exact area of sowing. Disturbance of the soil layer is avoided as much as possible, but leaf litter is sometimes lifted for inspection (and later replaced). The attachment of young seedlings of dactylanthus is known to be extremely fragile, so great care must be exercised when leaf litter is moved.

Other data is collected on every visit, such as recording vegetation within the plot and within the site. False measurements are noted and corrected, and any missing corner pegs, tags or markers are replaced.

When an inflorescence or tuber is encountered in a plot, the exact position of the inflorescence or tuber is noted on the recording sheet. The grid will then be removed again.

To protect the emerging inflorescences, cages are placed over the plots as soon as emergence is observed, or when the soil becomes visibly scratched by browsers. Cages attract attention and may lead to theft and should, therefore, be camouflaged (spray painted brown and black). Where collection is less of a problem, caging the plots from the beginning of the trial will minimise loss of inflorescences before they can be detected.

2.9 DATA ANALYSIS

The data will be analysed in several ways to compare between sites and between sowing rates. For the analysis, results for all three replicates for each sowing rate on one site will be combined, and the mean result used in the analysis.

The following parameters are used in the analysis:

- Mean number of plants per plot per sowing rate
- Position of plants in the plot
- Size of the tubers (if feasible)
- Sex of the plants
- Time of first emergence
- Sequence of emergence (if more than one plant is found over the whole monitoring period)

Intensive monitoring will largely cease when no new plants are found, but general monitoring will be carried out annually to record changes in the number of inflorescences per plant, their sex, and the size and shape of the tubers.

3. Conclusions

The method described here provides a robust experimental design for sowing dactylanthus seeds, and tests the importance of three variables (host species, host age and sowing density). These variables can be exchanged for other variables (e.g. slope or aspect) in other experiments; however, if the total number of variables is to be increased, then the number of replicated plots must also be increased accordingly, so that a minimum of three replicates per variable combination is maintained. The present method facilitates long-term monitoring (10 years) of the exact area where seeds have been sown. This is of particular importance because of the cryptic habit of dactylanthus, which otherwise would make confirmation of plant establishment very difficult. Results from these trials should assist future translocations or restoration of sites with dactylanthus.

4. Acknowledgements

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APPENDIX 1. MONITORING SHEET FOR THE *Dactylanthus* SEEDING TRIAL

Note: Distances and bearings have been changed from the original, to protect site identity.

Dactylanthus taylorii SEEDING TRIAL, WAIPAPA ECOLOGICAL AREA, COMMENCED 12 JANUARY 1999. PLOT SHEETS.

SITE	SITE MARKER LOCALITY	SITE DESCRIPTION	PLOT REPLICATE	REPLICATE MARKER LOCALITY	REPLICATE DESCRIPTION	PLOT	PLOT MARKER LOCALITY	SOWING RATE	NOTES 2000
A	2990 m after crossing of bridge, 400 m past site C, 500 m before site A. Site marker on middle stem of triple-stemmed, mature lancewood, visible from the road. Distance from road 6 m, bearing 160°	Dominated by juvenile and adult lancewood, with bracken and *Blechnum* undergrowth. Open canopy. Site exposed on a slight slope (same slope as site D), therefore probably drier than remaining sites with closed canopy	Aa	170°, 2 m from site marker	Ground dry, only slightly scarred before sowing, covered back mainly with leaf litter	A1a	220° , 15 cm from Aa	1	Moist fern and toe-toe leaf-litter. Pegs OK. No sign of *Dactylanthus*
						A2a	140°, 190 cm from Aa	2	Moist fern and toe-toe leaf-litter. Pegs OK. No sign of *Dactylanthus*
	Overall for site A: **No disturbance noticeable during 2000 visit**		Ab	120° , 5 m from Aa to Ab. 250°, 720 cm from site marker A to Ab. Above plot marker A1b	Ground dry, some moisture (coolness), ground scarred to about 2.5 cm depth, covered with soil and leaf litter after sowing	A1b	50°, 5 cm from Ab	1	Not much leaf-litter apart from some lancewood and fern fronds. Pegs OK. No sign of *Dactylanthus*
						A2b	16°, 30 cm from Ab. 195°, 110 cm from plot marker A1b	2	More leaf-litter than A2a. Pegs OK. No sign of *Dactylanthus*
			Ac	120°, 80 cm from Ab.	Ground drier than in replicate b, scarred to about 3 cm depth, covered as in replicate b	A1c	220°, 20 cm from Ac. 325°, 310 cm from plot marker A1b	1	Some more leaf-litter than A2a (little *Blechnum*). Pegs OK. No sign of *Dactylanthus*
						A2c	40°, 140 cm from Ac. 230°, 200 cm from plot marker A1c	2	Very little leaf-litter. Pegs OK. No sign of *Dactylanthus*