

# Techniques for improving *Phytophthora* resistance in potential new floricultural crop *Newcastelia interrupta*

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## ***Table of Contents***

Abstract.....	1
Introduction .....	2
Research methodology .....	4
Plant material.....	4
Cutting propagation.....	4
Grafting .....	5
Mycorrhizal associations in the natural population .....	7
Induced mycorrhizal associations in pot culture .....	7
Results.....	9
Cutting propagation.....	9
Grafting .....	9
Mycorrhizal associations in the natural population .....	11
Induced mycorrhizal associations in pot culture .....	13
Discussion.....	16
Cutting propagation.....	16
Grafting .....	16
Mycorrhizal associations in the natural population .....	16
Induced mycorrhizal associations in pot culture .....	17
Conclusions .....	18
Acknowledgements.....	18
References .....	19

## **Abstract**

*Newcastelia interrupta* is an herbaceous perennial plant that produces attractive flowering stems well-suited to ornamental horticulture. Initial attempts to cultivate the species have revealed that it is susceptible to the fungal root pathogen *Phytophthora cinnamomi*. Several *N. interrupta* genotypes were collected from a natural population in southern Queensland and subjected to trials aimed at improving resistance to *P. cinnamomi* infection. One approach was to graft *N. interrupta* onto rootstock of coastal rosemary (*Westringia fruticosa*), a related species known to be *Phytophthora* resistant. It was demonstrated that grafted plants could be produced, however the grafting success rate was extremely low for both side (10%) and wedge (1%) grafted plants after 28 weeks. A second approach was to determine whether *N. interrupta* is capable of forming mycorrhizal associations (mutually beneficial relationships with certain species of soil fungi) and, if so, whether these associations could be used to protect the plant against *P. cinnamomi* infection. *N. interrupta* root samples from the natural population exhibited two types of mycorrhizal associations, arbuscular mycorrhizae and ectomycorrhizae. A subsequent pot trial found that a commercial inoculant containing both arbuscular mycorrhizal fungi and ectomycorrhizal fungi produced plants with a higher shoot biomass than non-mycorrhizal plants. However, mycorrhizal plants showed no difference in growth or survival compared with non-mycorrhizal plants when challenged by inoculation with *P. cinnamomi*. Genotype was shown to have an effect on the survival of plants inoculated with *P. cinnamomi*, with one genotype exhibiting a survival rate of 95% compared with <62% for all other genotypes. Therefore, improving *Phytophthora* resistance in *N. interrupta* might be achieved through selection of genotypes that exhibit natural resistance towards the fungal pathogen.

## Introduction

Australia has a diverse range of plant species, many of which provide the unique and attractive visual displays that are constantly being sought by horticulturalists worldwide. However, several species of Australian plants are susceptible to the soil-borne fungal pathogen *Phytophthora cinnamomi* and this poses a major barrier to their domestication.

One such species is *Newcastelia interrupta* (Family Lamiaceae). This herbaceous perennial plant endemic to southern Queensland produces attractive flowering stems over a long flowering season. Combined with a compact growth habit and soft grey-green foliage, *N. interrupta* is aesthetically well-suited to landscape plantings. The species also has potential as a cut-flower crop (Figure 1). Market research has found *N. interrupta* stems were well-received by florists in Japan (the largest importer of Australian native cut flowers/foilage)<sup>1</sup> and anecdotal evidence suggests that the stems ship well and exhibit a long vase life.



Figure 1. Flowering stems of *N. interrupta* being incorporated into a bridal bouquet by a florist in Japan (left; sourced from Lim-Camacho<sup>1</sup>), a 7-month-old cutting propagated potted plant (above right) and a close-up image of a flowering stem showing the unique cotton ball-like calyx (below right).

Past attempts to establish in-ground plantings of *N. interrupta* have resulted in widespread plant losses. Subsequent diagnostic tests attributed the losses to *Phytophthora cinnamomi* infection. Application of a broad-spectrum fungicide for root/stem rot diseases has been

effective in extending the lifespan of plants in potted culture. However, from an economic and environmental viewpoint, it is undesirable to rely on this as a routine measure for plant maintenance.

Grafting is one promising means of overcoming *Phytophthora* susceptibility. Previous work on native mint (*Prostanthera* spp.), also from the Lamiaceae family, showed that plants under cultivation are susceptible to *Phytophthora* infection and that it is possible to extend the garden life of these plants by grafting them onto coastal rosemary (*Westringia fruticosa*) rootstock<sup>2</sup>. Wrigley and Fagg<sup>3</sup> reported that these grafted plants continued to thrive over 20 years later in an area known to have *Phytophthora* present in the soil.

A second option is to induce mycorrhizal associations with *N. interrupta* roots. Arbuscular mycorrhizal (AM) fungi and their associated symbiotic interactions with plants can reduce damage caused by soil-borne pathogens, including *Phytophthora* species.<sup>4,5</sup> Not all plant species are capable of forming mycorrhizal associations. Whilst such associations have not previously been investigated for *Newcastelia*, other Lamiaceae species endemic to Australia have been shown to develop AM associations in the natural environment<sup>6,7</sup>.

The aim of this project was to develop a means of improving the *Phytophthora* resistance of *Newcastelia interrupta*, thus eliminating the main barrier to domestication for this species. A germplasm collection was established using cutting material collected from a natural population. Selected genotypes were investigated for grafting compatibility with *W. fruticosa* rootstock. Root samples from field-collected specimens were examined for the presence of mycorrhizal associations. A pot trial was conducted in which *N. interrupta* plants were inoculated with commercial formulations of mycorrhizal fungi and then challenged by exposure to *P. cinnamomi*.

## **Research methodology**

### ***Plant material***

Plant material was collected in March (early autumn) from a natural population of *N. interrupta* that occurs along a 20 km section of the Moonie Highway, approximately 200 km west of Dalby in southern Queensland. A collection permit was obtained from the Queensland Transport and Main Roads Department prior to this work. Plants predominantly occurred in exposed situations such as graded road shoulders (Figure 2) and sparsely vegetated areas. Material was harvested from mature plants that were at least 50 cm tall. Cuttings were collected from 50 specimens and root samples from 10 specimens.



**Figure 2.** Harvesting of cuttings from a natural population of *N. interrupta* in southern Queensland. Plants commonly occur in exposed sites such as this graded roadside.

### ***Cutting propagation***

Field-collected cutting material was disinfested in 0.2% sodium hypochlorite solution for 2 min. and rinsed in tap water. Thirty cuttings from each of the 50 genotypes were prepared using terminal shoots from which the soft tips had been removed. The cuttings were trimmed to 7 cm in length and three pairs of leaves at the distal end were retained. Cutting bases were dipped in 2 g.L<sup>-1</sup> indole-3-butyric acid solution for 5 s. Cuttings were placed in

trays of propagation medium comprising 6:3:1 perlite/vermiculite/peat amended with 15 g.L<sup>-1</sup> dolomite and 2 g.L<sup>-1</sup> Basacote Mini 3M (NPK 13-6-16; Compo, Brazil), and were maintained in a propagation house equipped with intermittent misting and under-bench heating until root development (4-6 weeks).

Subsequent propagation using cutting material from potted plants was conducted in August and September (late winter and early spring, respectively). Cuttings were prepared as outlined above with the exception that the propagation medium comprised equal parts perlite/vermiculite/peat which had been twice autoclaved at 121°C for 30 min and then amended with 2 g.L<sup>-1</sup> Basacote Mini 3M (NPK 13-6-16; Compo, Brazil). The number of cuttings obtained from each genotype varied from 2 to 19, according to the availability of material.

### ***Grafting***

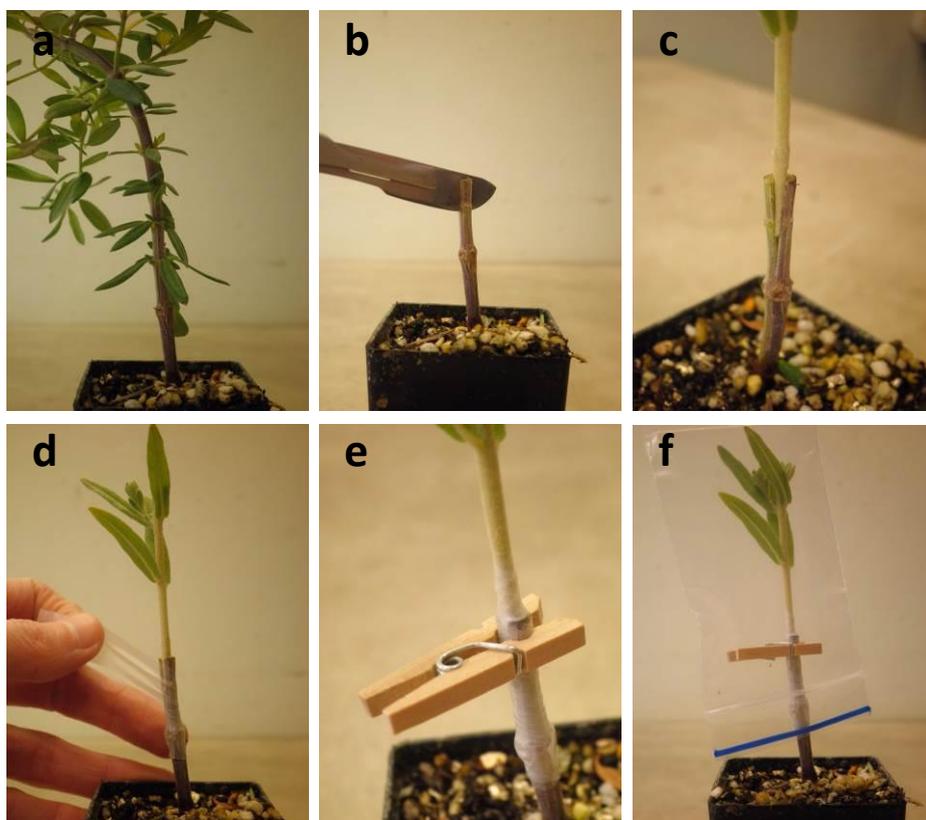
Five genotypes of *N. interrupta* were investigated for their amenability to grafting onto a *Phytophthora*-resistant species. Two grafting techniques (side and wedge) were trialled ( $n=18$ ) using cutting-propagated tubestock of *W. fruticosa* as the rootstock. *W. fruticosa* plants were maintained in a greenhouse and lateral shoots from the lower 7 cm of stem were regularly removed to aid development of a straight, upright primary stem for grafting.

Scion material was sourced from 1-year-old *N. interrupta* plants in 140 mm pots that were maintained in a greenhouse on drip irrigation. Semi-hardened shoots were tip pruned in late June (winter) and the remaining stem stripped of all but the top two pairs of leaves. After five weeks the stems were harvested from the mother plant and grafted onto the rootstock.

Wedge grafting (Figure 3) involved pruning the rootstock back to a single stem 3-4 cm tall and removing all leaves and side shoots. A scalpel was used to make a 1 cm deep vertical incision down the centre of the stem. Scion material comprising two pairs of leaves and 2-3 cm of bare stem was trimmed at the base with two angled cuts to produce a wedge. The scion was inserted into the incision on the rootstock to a depth where a small portion of the scion cambium was visible above the rootstock (to aid callus formation around the graft). Parafilm® was firmly wound around the graft site and a peg used to apply pressure to the graft. A zip-lock plastic bag was placed over the top of the grafted plant.

Side grafting (Figure 4) involved the removal of all leaves and lateral shoots from the basal 5 cm of the rootstock. A small horizontal incision into the cambium layer was made 1-2 cm from the base of the stem. Approximately 2 cm above this, a flat, shallow cut was made down the side of the rootstock to meet the first incision. Scion material comprised two pairs of leaves and 3 cm of bare stem. A long, sloping cut was made down one side of the scion stem and matched to the cut face of the rootstock. Parafilm® was firmly wound around both stems to hold the graft together. The rootstock was pruned to retain approximately 5

cm of top growth above the graft and a zip-lock plastic bag placed over the grafted plant.



**Figure 3. Wedge grafting of *N. interrupta* onto *W. fruticosa* rootstock showing (a) the rootstock prior to grafting, (b) the vertical incision through the centre of the rootstock stem, (c) the scion “wedge” fitted to the rootstock, (d) binding of the graft site with Parafilm®, (e) pegging the graft site and (f) placement of a plastic bag over the grafted plant.**

All grafted plants were placed in a propagation house equipped with intermittent misting and under-bench heating. Plastic bags were removed after 2 weeks and the plants transferred to a greenhouse after 4 weeks. Side-grafted plants were pruned to remove the rootstock top growth at this time. Pegs were removed from the wedge-grafted plants after 8 weeks. After 15 weeks, all plants were transferred to 100 mm pots of composted pine bark medium amended with 2 g.L<sup>-1</sup> Osmocote® Plus 8-9 month, 1 g.L<sup>-1</sup> Osmocote® Exact 3-4 month, 2 g.L<sup>-1</sup> Nutricote® 7 month, 1.3 g.L<sup>-1</sup> Osmoform® 4 month, 1.3 g.L<sup>-1</sup> Osmocote® Coated Iron, 1.2 g.L<sup>-1</sup> dolomite and 1.2 g.L<sup>-1</sup> SaturAid®. Plant height and survival was assessed at 4, 8, 12 and 28 weeks.

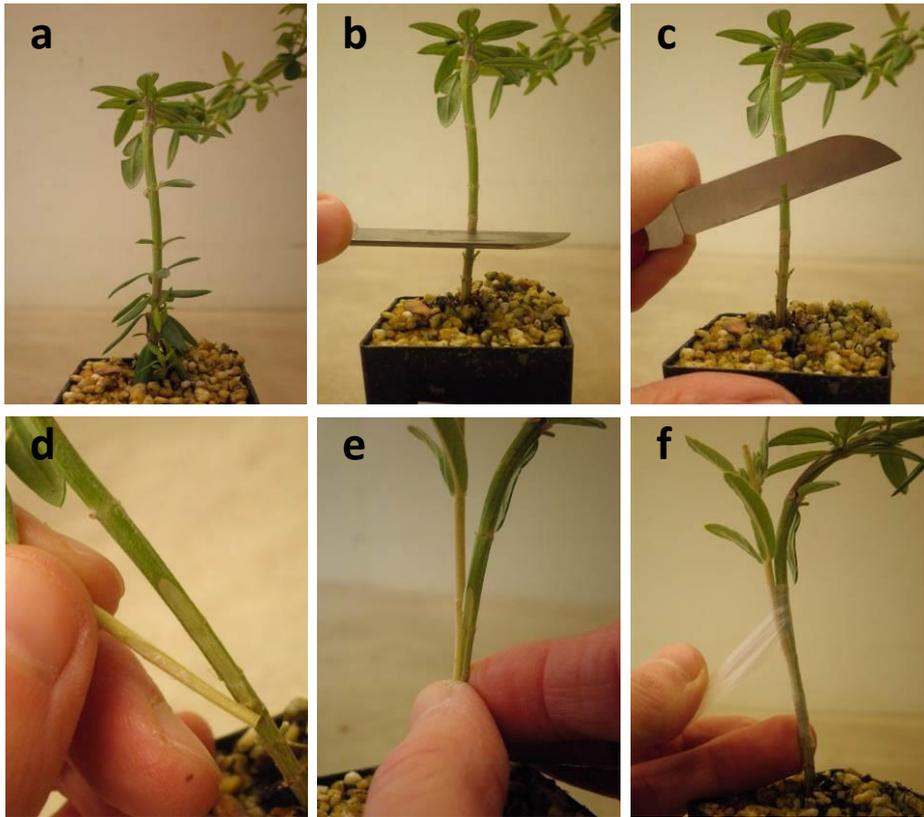


Figure 4. Side grafting of *N. interrupta* onto *W. fruticosa* rootstock showing (a) the rootstock prior to grafting, (b) the horizontal incision into the rootstock cambium, (c) the shallow vertical cut to create the grafting surface, (d) the scion with a sloping cut down one side, (e) matching of the cut surfaces of the scion and rootstock and (f) binding of the graft site with Parafilm®.

### ***Mycorrhizal associations in the natural population***

Field-collected roots samples from 10 plants were rinsed with tap water over a sieve to remove soil particles. The clean roots were then stored in 50% ethanol. Subsequent microscopic examination for the presence of mycorrhizal associations required clearing of the tissues with 10% KOH for 2 h at 80°C, followed by staining with 0.03% chlorazol black E in lactoglycerol for 5 h at 80°C.<sup>8</sup> The cleared and stained root samples were stored in 50% glycerol solution. Microscopic examination of roots under high magnification (x200) was used to confirm the presence of fungal structures characteristic to AM fungi (internal hyphae, coils, arbuscules and vesicles) and ECM fungi (Hartig net).

### ***Induced mycorrhizal associations in pot culture***

Mycorrhizal fungi were investigated for their ability to firstly enhance the growth of *N. interrupta* and, secondly, provide bio-protection against *P. cinnamomi*. Cuttings from five *N. interrupta* genotypes were propagated in a medium comprised of equal parts perlite/vermiculite/peat which had been twice autoclaved at 121°C for 30 min and amended with 2 g.L<sup>-1</sup> Basacote Mini 3M (NPK 13-6-16; Compo, Brazil). Rooted cuttings were

transferred to 50 mm squat square tubes of the same medium and maintained under greenhouse conditions. Five-month-old *N. interrupta* tubestock were transferred to 100 mm pots containing a 2:1 sand/peat medium which had been twice autoclaved at 121°C for 30 min and amended with 2 g.L<sup>-1</sup> Basacote 3-4 month, 2 g.L<sup>-1</sup> Basacote Plus 6 month and 10 g.L<sup>-1</sup> of granular mycorrhizal fungi inoculum. Two commercial formulations of mycorrhizal fungi were tested: MycoApply® Endo and MycoApply® Endo/Ecto (MAI Australia). Both formulations contained four species of AM fungi, with the latter also containing three species of ECM fungi. (The identity of the fungal species was not specified by the manufacturer.) Plants grown in a medium to which no inoculum was added served as the control.

The plants were grown under greenhouse conditions for four weeks and then half were inoculated with a *P. cinnamomi* isolate of known pathogenicity. Colonised French millet seed was used as the inoculum, with eight seeds evenly distributed around the base of each plant at a depth of 2 cm and distance of 2cm from the stem. Plants to which twice autoclaved un-inoculated millet seed was added served as the control.

Preparation of the inoculum involved soaking 10 g millet seed overnight in distilled water, decanting off the excess water, autoclaving twice at 121°C for 30 min and adding five 1 cm x 1 cm pieces of agar excised from the edge of a *P. cinnamomi* culture grown on clarified V8 juice agar with 10% calcium carbonate. The millet was then incubated in darkness at 25°C for 4 weeks. Colonisation of the inoculated millet and sterility of the un-inoculated millet was confirmed by plating 10 millet seeds on potato dextrose agar and incubating in darkness at 25°C for 24 h.

After addition of the millet, all pots were placed in round plastic disposable food containers (120 mm diameter, 60 mm depth, 440 mL capacity) and 100 mL of water applied to the surface of the potting medium. Plants were maintained in a glasshouse and manually sub-irrigated on a weekly cycle: every second day for a period of five days the containers around each pot were filled with 170 mL of water. After watering on the fifth day, the plants were left unwatered for 2 days, allowing a period of moisture stress to occur.

The experimental design was a randomised complete block design with five *N. interrupta* genotypes, six treatments (no mycorrhizal fungi, AM fungi or AM/ECM fungi; with or without *P. cinnamomi*) and seven replicates. Hence, a total of 210 plants were used in the trial. Plants were assessed three times per week for disease progression (permanent wilting and plant necrosis) and time to anthesis. Plant height was recorded at the beginning and end of the trial. The aboveground portion of the plants were harvested 12 weeks after *P. cinnimomi* inoculation or at plant death, whichever was sooner, and shoot biomass dry weights obtained. Note that shoot biomass data from plants that died before the end of the trial were excluded from statistical analyses.

## Results

### *Cutting propagation*

Propagation of cuttings from field-collected material resulted in severe losses within two weeks. Of the 1500 cuttings propagated from the field-collected material, only 18 developed roots and survived beyond the tubestock stage (a survival rate of 1.2%). Nine of the original 50 genotypes were successfully established as potted plants. Subsequent cuttings harvested from these plants exhibited substantially higher rooting rates than the field-collected material, with most genotypes achieving rates of 100% (Table 1). Genotype NI-25 exhibited consistently low rooting rates and was therefore excluded from the grafting and mycorrhizal trials.

**Table 1. Comparison of root development in *N. interrupta* cuttings derived from field-collected and container-grown material.**

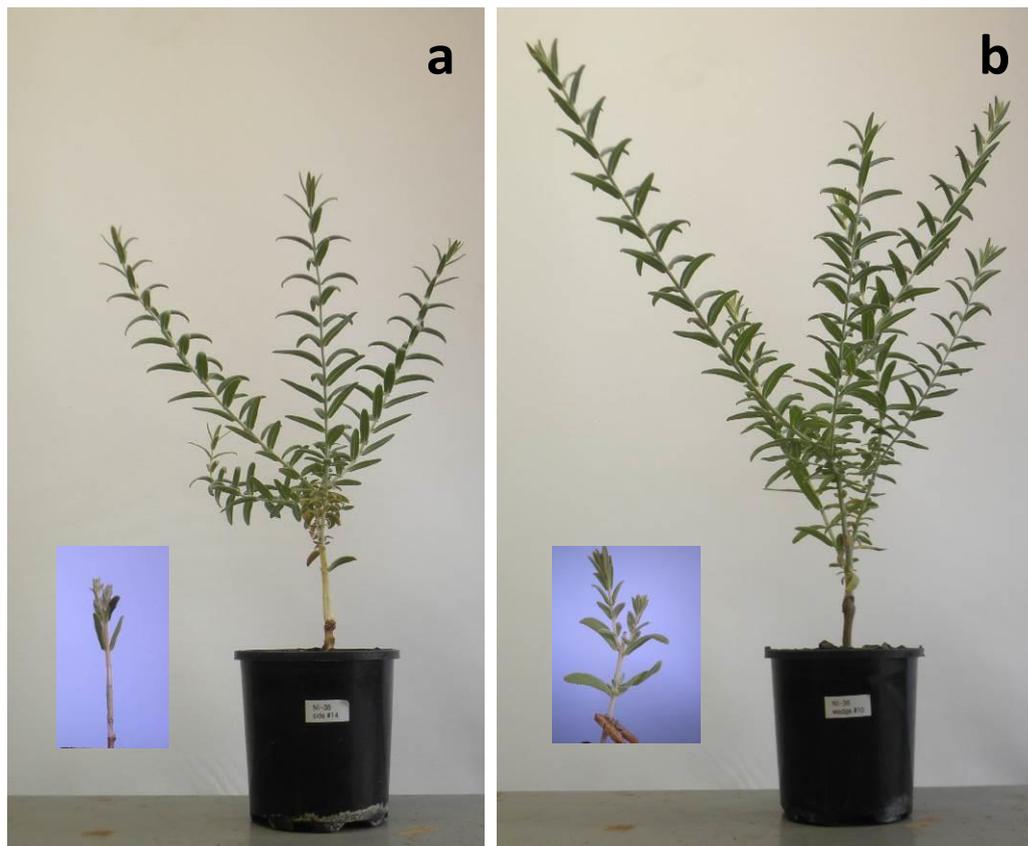
Genotype	Rooting rate (%)		
	Cuttings from field-collected material (March)	Cuttings from potted stock plants (August)	Cuttings from potted stock plants (September)
NI-09	3	100	100
NI-10	7	100	100
NI-13	3	100	100
NI-16	7	100	100
NI-20	27	88	-
NI-22	3	100	100
NI-25	3	33	25
NI-38	23	100	-
NI-48	10	80	100

### *Grafting*

The survival rate for the grafted plants after 4 weeks was greater than 90%. However by Week 12 many grafts had failed to take, with only 28% of side-grafted plants and 3% of wedge-grafted plants remaining. By Week 28, these figures had dropped to 10% and 1%, respectively (Table 2). No further losses were observed beyond this time. Active growth was observed in the surviving grafted plants: by Week 28, plant heights were between 2.6 and 6.2 times greater than those observed at Week 4 (Figure 5).

**Table 2. Survival of *N. interrupta* grafted onto *W. fruticosa* rootstock, 28 weeks after grafting.**

Genotype	Survival rate (%)	
	Side-grafted plants	Wedge-grafted plants
NI-10	0	0
NI-13	11	0
NI-20	17	0
NI-38	11	6
NI-48	11	0
All genotypes	10	1



**Figure 5. Side-grafted (a) and wedge-grafted (b) *N. interrupta* plants of genotype NI-38 at 4 weeks (inset) and 28 weeks after grafting.**

### ***Mycorrhizal associations in the natural population***

Fungal structures characteristic of AM associations were found in all specimens of field-collected *N. interrupta* roots (Table 3). Arbuscules were identified in seven of the ten genotypes (Figure 6). Short, lateral roots surrounded by a thick mantle of fungal hyphae were observed in some of the root samples (Figure 7). Such root morphology is typical of ectomycorrhizal (ECM) associations. Subsequent microscopic examination of root cross-sections revealed the presence of a Hartig net (i.e. a network of fungal hyphae penetrating between root epidermal cells) in five of the genotypes (Table 3; Figure 8), confirming that the altered root morphology is the result of ECM associations.

**Table 3. Mycorrhizal associations identified in field-collected root samples of *N. interrupta*, showing the presence (+) or absence (-) of characteristic fungal structures.**

Genotype	Arbuscular mycorrhizae				Ectomycorrhizae	
	Internal hyphae	Coils	Arbuscules	Vesicles	Mantle	Hartig net
NI-01	+	+	+	+	+	+
NI-04	+	+	+	+	+	+
NI-06	+	+	+	+	+	+
NI-09	+	+	+	+	+	+
NI-19	+	+	+	+	-	-
NI-26	+	+	-	+	-	-
NI-40	+	-	+	-	-	-
NI-41	+	+	+	+	-	-
NI-46	+	-	-	+	-	-
NI-48	+	+	-	+	+	+

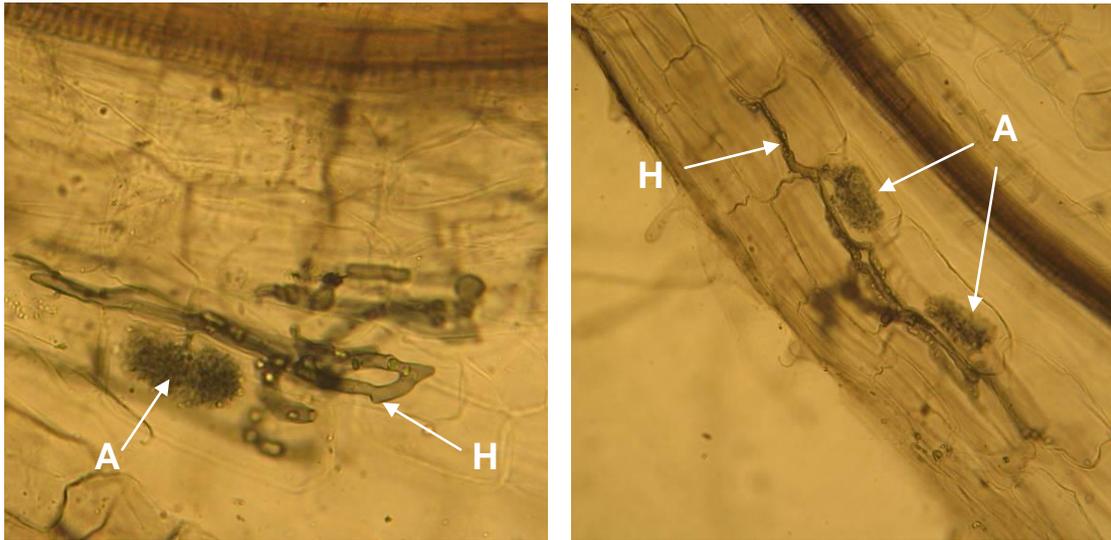


Figure 6. Arbuscular mycorrhiza observed in field-collected *N. interrupta* root samples of genotypes NI-19 (left) and NI-01 (right). Arrows indicate internal hyphae (H) and arbuscules (A). Magnification x 200.

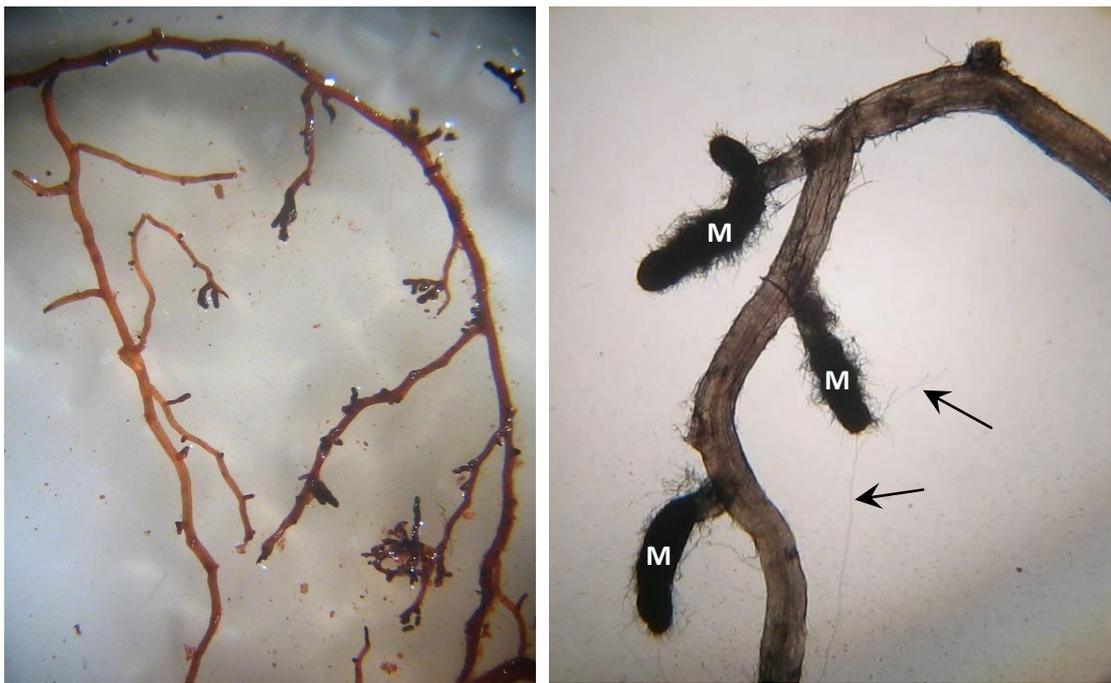


Figure 7. ECM roots of *N. interrupta* as observed in unstained specimens (left) and cleared specimens stained with Chlorazol black E (right), showing a dense mantle (M) and external hyphae (arrows). Magnification x 7 and x 40, respectively.

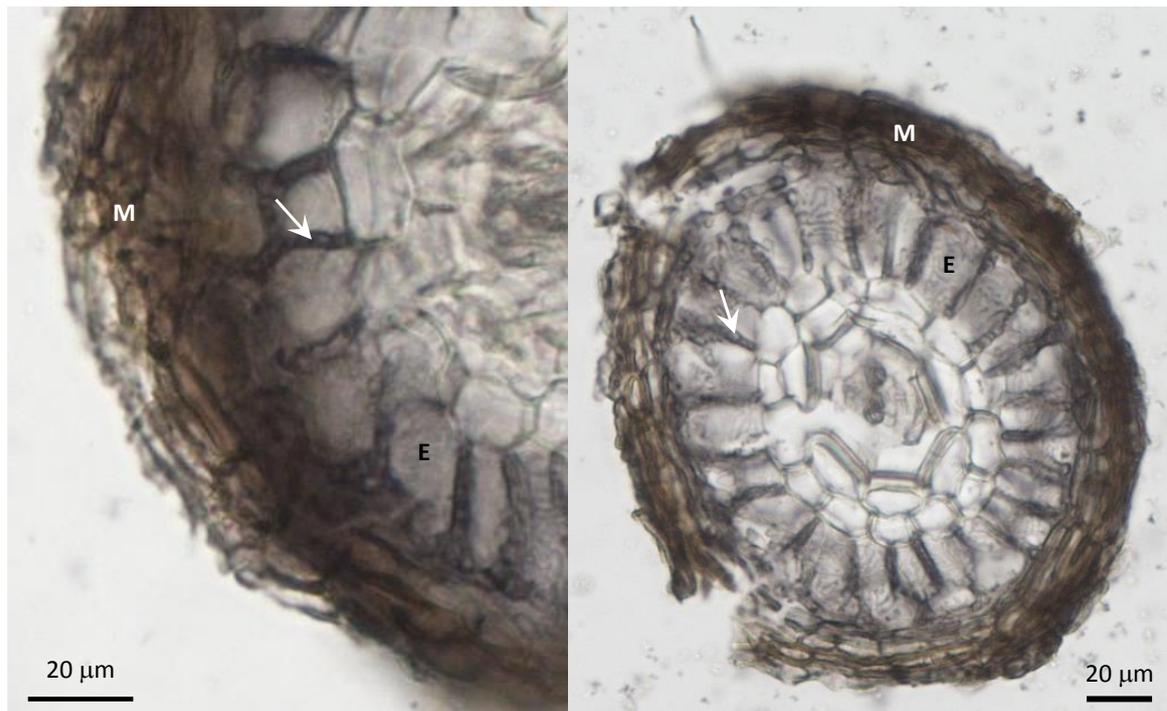


Figure 8. *N. interrupta* ECM root cross-sections prepared from cleared hand sections of field-collected root material stained with Chlorazol black E, showing the mantle (M) and Hartig net hyphae (arrows) extending between elongated epidermal cells (E).

### ***Induced mycorrhizal associations in pot culture***

The plants used in the trial were of uniform size and vigour at the time of *P. cinnamomi* inoculation (Week 0; Figure 9). Plants exposed to *P. cinnamomi* began to exhibit symptoms of infection (i.e. permanent wilting and lack of water uptake) as early as 8 d after inoculation. The first instances of plant death were recorded 24 d after inoculation. Logistic regression found that the inclusion of mycorrhizal fungi in the growing medium had no effect on plant survival (data not shown). Genotype was found to affect plant survival, with 95% of *Phytophthora*-inoculated NI-20 plants remaining alive at the end of the 12-week trial period, compared with <62% for the other genotypes (Table 4). Plants that were not exposed to *P. cinnamomi* exhibited a 100% survival rate.



**Figure 9.** Mycorrhizal pot trial of *N. interrupta* plants at 0 weeks (left) and 6 weeks (right) after inoculation with *P. cinnamomi*.

**Table 4.** Effect of genotype and *P. cinnamomi* inoculation on survival of *N. interrupta* potted plants after 12 weeks.

Genotype	Survival rate (%)	
	<i>P. cinnamomi</i> inoculated plants	Non-inoculated plants
NI-10	57.2 <sub>a</sub>	100.0 <sub>a</sub>
NI-16	61.9 <sub>a</sub>	100.0 <sub>a</sub>
NI-20	95.3 <sub>b</sub>	100.0 <sub>a</sub>
NI-38	38.1 <sub>a</sub>	100.0 <sub>a</sub>
NI-48	61.9 <sub>a</sub>	100.0 <sub>a</sub>

*Different letters within the same column denote significant difference (P<0.05).*

Plants that were not exposed to *P. cinnamomi* exhibited a positive growth response to mycorrhizal fungal inoculation. Greater shoot biomass was observed for plants grown in a medium containing a combined AM/ECM fungal inoculant compared with the non-mycorrhizal control plants (Table 5). No difference in shoot biomass was observed between the non-mycorrhizal plants and those inoculated with only AM fungi.

When plants were challenged by exposure to *P. cinnamomi*, neither the AM inoculant or the AM/ECM inoculant were shown to have any effect on shoot biomass compared with the non-mycorrhizal control (Table 5). However, shoot biomass was greater in AM-inoculated plants than in AM/ECM inoculated plants.

**Table 5. Effect of mycorrhizal fungi and *P. cinnamomi* inoculation on shoot biomass of *N. interrupta* potted plants after 12 weeks.**

Mycorrhizal treatment	Shoot biomass (g DW)	
	<i>P. cinnamomi</i> inoculated plants	Non-inoculated plants
Control	8.8 <sub>ab</sub>	8.3 <sub>a</sub>
AM	9.1 <sub>a</sub>	8.6 <sub>ab</sub>
AM/ECM	7.9 <sub>b</sub>	9.2 <sub>b</sub>

*Different letters within the same column denote significant difference (P<0.05).*

Mycorrhizal inoculation delayed flowering of *N. interrupta*, with the AM inoculated plants exhibiting a time to anthesis of 29.6 d compared with 22.4 d for the non-mycorrhizal control plants. The time to anthesis for AM/ECM inoculated plants was 26.4 d and was not significantly different to either the control or the AM inoculated plants.

## ***Discussion***

### ***Cutting propagation***

On several occasions prior to this study, *N. interrupta* was found to be amenable to cutting propagation, with rooting rates >80% typically achieved, irrespective of whether the material was sourced from the field or nursery stock plants. The high rate of cutting losses encountered in the current study presented an unforeseen challenge. The presence of pathogens in the propagation medium was considered a possible cause for this extensive loss of cuttings and therefore all subsequent propagation attempts employed a medium that had been heat sterilised. The use of sterilised medium resulted in a marked improvement in rooting rate for all genotypes. It is therefore recommended that sterilised medium be routinely used for cutting propagation of *N. interrupta*.

### ***Grafting***

Inter-generic grafting of Australian species of the Lamiaceae family was successfully demonstrated by Wrigley and Jauihainen<sup>2</sup>. Their attempts to side graft five *Prostanthera* species onto *W. fruticosa* rootstock resulted in survival rates ranging from 10 to 80%, depending on scion species. Some of these grafted plants were reported to be thriving 20 years later in an area known to have *Phytophthora* present in the soil.<sup>3</sup> Apart from imparting resistance to *Phytophthora*, *W. fruticosa* offers the added benefits of being easy to propagate from cuttings and tolerant of a range of soil types and climatic conditions.

In the current study, survival rates of side-grafted *Newcastelia* plants were low (10%) and appeared to result from incompatibility of the rootstock and scion species. *Newcastelia* is not as closely related to *Westringia* as is *Prostanthera*, and this is a likely cause of the grafting incompatibility. Whilst all three genera are in the same sub-family (Prostantheroideae), *Newcastelia* belongs to the Chloantheae tribe whereas *Prostanthera* and *Westringia* belong to the sister tribe Westringieae.<sup>9</sup>

At this stage, grafting does not offer a commercially viable solution to overcoming *Phytophthora* susceptibility in *N. interrupta*. However, the study has shown that grafting is possible and refinements to the technique and timing, as well as choice of scion/rootstock genotype, may greatly improve survival rates.

### ***Mycorrhizal associations in the natural population***

Several members of the Lamiaceae family are aromatic herbs of medicinal or culinary importance (e.g. basil, sage, oregano, mint, thyme) and their ability to produce AM associations has been well documented.<sup>10-17</sup> Members of the Lamiaceae family endemic to Australia have been investigated to a lesser degree. However, the three species that have been studied (*Prostanthera aspalathoides*<sup>6</sup>, *Westringia grevillina*<sup>6</sup> and *Hemigenia sericea*<sup>7</sup>)

were all identified as having the ability to form AM associations.

In the present study, the presence of AM structures, particularly arbuscules, in field-collected root samples showed that *N. interrupta* was typical of many members of the Lamiaceae family in its ability to form AM associations. This is the first known report of AM associations in the genus *Newcastelia*. The extensive AM fungal colonisation observed in the natural population of *N. interrupta* justified subsequent trialling of commercial AM fungi formulations to promote growth and survival of *N. interrupta* potted plants.

An unexpected finding was the occurrence of short, lateral roots surrounded by a thick mantle of fungal hyphae in half of the field-collected root samples. Such root morphology is typical of ECM associations. However, ECM associations have not previously been found to occur in members of the Lamiaceae family. Altered root morphology is insufficient evidence for identifying ECM associations. It is generally accepted that the presence of a Hartig net must be observed before an ECM association can be confirmed.<sup>8</sup> In the current study, a Hartig net was found and, hence, a commercial inoculant containing both AM and ECM fungi was included in the subsequent pot trial. (An inoculant containing ECM fungi only would have been preferable but was not available commercially.)

### ***Induced mycorrhizal associations in pot culture***

The 100% survival rate for plants not exposed to *P. cinnamomi* showed that successful pot culture of *N. interrupta* can be achieved when heat-sterilised growing medium and good nursery hygiene are employed. Differences in shoot biomass between mycorrhizal and non-mycorrhizal plants were small and observed only in the AM/ECM treatment. It is possible that the incorporation of a controlled release fertiliser in the growing medium confounded any plant growth response to the AM treatment. A previous study found no difference in shoot biomass between well-fertilised *Acacia mangium* seedlings grown with or without AM inoculant, even though a high rate of mycorrhizal colonisation (78%) was observed in the roots of the former.<sup>18</sup>

Neither mycorrhizal treatment was found to protect *N. interrupta* plants against *P. cinnamomi*. Previous research has demonstrated that time is a key factor when using mycorrhizal fungi to guard against root pathogens. In one study, AMF exhibited a protective effect against *P. cinnamomi* in conifer seedlings only when AMF inoculation occurred at least 6 months prior to pathogen exposure.<sup>19</sup> No protective effect was observed in seedlings receiving AMF inoculation simultaneously with or 2 months prior to *P. cinnamomi* inoculation. In the current study, the 1 month period between mycorrhizal fungi inoculation and pathogen inoculation may not have allowed sufficient time for mycorrhizal associations to develop to an extent capable of protecting the roots from *P. cinnamomi* infection.

ECM fungi appeared to suppress plant growth in the presence of *P. cinnamomi*, with shoot biomass for the AM/ECM inoculated plants being significantly lower than that for the AM inoculated plants. In the absence of the pathogen however, no difference in shoot biomass

between the two treatments was observed. Previous studies have shown that ECM associations can have either a positive or negative effect on plant growth.<sup>20-22</sup> In the case of *N. interrupta*, further investigation is needed to understand the interactive effect of ECM fungi and *P. cinnamomi* on growth.

The high survival rate observed for *P. cinnamomi* inoculated plants of genotype NI-20 suggested that a level of natural resistance to the pathogen may exist in particular individuals of the species. If this is the case, overcoming the problem of *Phytophthora* susceptibility may be as simple as screening the natural population for resistant genotypes and selecting from these for commercialisation.

## **Conclusions**

Improving *Phytophthora* resistance in *N. interrupta* may best be achieved through selection of genotypes that exhibit natural resistance towards the fungal pathogen and by pot culture in pathogen-free medium. Whilst induced mycorrhizal associations displayed no protective effect against *P. cinnamomi* infection, they were shown to be useful in promoting plant growth. Grafting onto *Phytophthora* resistant rootstock had limited success and is inherently labour-intensive and costly. In its current form, grafting is not a viable option for overcoming *Phytophthora* susceptibility in *N. interrupta*.

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