

Is domatia production in *Coprosma rotundifolia* (Rubiaceae) induced by mites or foliar pathogens?

Dean M. O'Connell^{1*}, Adrian Monks², Katharine J. M. Dickinson³ and William G. Lee²

¹Library, Teaching and Learning, PO Box 84, Lincoln University, Lincoln 7647, New Zealand

²Landcare Research, Private Bag 1930, Dunedin 9054, New Zealand

³Department of Botany, University of Otago, PO Box 56, Dunedin 9054, New Zealand

Author for correspondence (Email: dean.oconnell@lincoln.ac.nz)

Published online: 26 March 2015

Abstract: Plant–invertebrate mutualisms involve the production of food and/or shelter by plants to co-opt invertebrate groups in order to either prevent herbivore or pathogen damage or facilitate seed dispersal. Plant structures and the provision of food are relatively expensive, and a reactive plant response to attack may reduce those costs provided the fitness benefit of the mutualism is maintained. We investigated whether foliar domatia in the New Zealand shrub *Coprosma rotundifolia* (Rubiaceae) were an induced mutualism, whose density is dependent on the abundance of foliar mites and/or foliar fungi. Alternatively, domatia may be a defence that is always present irrespective of local conditions, i.e. a constitutive mutualism. Beneficial mites inhabit the domatia (mite houses), feeding on leaf fungi and small herbivorous arthropods from the leaf surface. We attempted to manipulate mite and fungal densities to test (1) whether the density of foliar mites on shrubs stimulated increased domatia production and the domatia opening size of new leaves, and (2) whether the density of foliar fungi on old leaves influenced domatia production in new leaves. Under experimental treatments (with or without miticide and different levels of foliar fungal detritus) *C. rotundifolia* shrubs showed no significant differences in the mean relative change in domatia production in new season's leaves compared with old leaves. We propose that domatia on *C. rotundifolia* are potentially part of a defence that is always present irrespective of local conditions, i.e. a constitutive mutualism, as plants produce many domatia, apparently in excess of requirements. A constitutive mutualism suggests that plants have a consistent fitness advantage by maintaining these structures every year, presumably because of constant pressure from foliar invertebrate herbivores and pathogens.

Keywords: beneficial; constitutive mutualism; fungi; invertebrates

Introduction

Mutualisms are interspecific interactions involving net individual fitness benefits to both species that are greater than when they occur alone (Bronstein 1998). In terrestrial communities, mutualisms may involve functions that enable plant survival, reproduction, and protection from attack (Howe 1984; Herms & Mattson 1992; Heil 2008). Plant–invertebrate mutualisms are widespread and based on the provision of protection and/or food by the plant to the invertebrate partner (Sabelis et al. 1999). To facilitate invertebrate involvement, the plants provide food and shelter in the form of extrafloral nectaries, fruiting bodies, or hollow structures (e.g. O'Dowd & Willson 1989; Walter & O'Dowd 1995; Linsenmair et al. 2001).

In the majority of plant–invertebrate mutualisms, plant structures or resources that encourage partners are invariably provided, irrespective of the density of the invertebrate partner. These cases are termed constitutive mutualisms (e.g. Beattie 1985; Jolivet 1996) and are common where the advantages of the mutualism for the plant are consistently high. In contrast, induced mutualisms refer to cases where the costs of the mutualism to the plant may be high, but the benefits only intermittent. For example, induced mutualisms are evident when plants reduce or stop the production of rewards in the absence of a mutualist partner (e.g. Risch & Rickson 1981; Bronstein 1998; Bluthgen & Wesenberg 2001; Heil 2008). This flexibility involved with induction decreases the 'spending' on the mutualism, yet maximises the benefits to

the plant without an overinvestment of resources. In ant–plant mutualisms evidence suggests that induced mutualisms are frequent in temperate and some tropical systems (Bixenmann et al. 2011), but evidence of induced mutualisms in plant–mite systems is lacking.

Plant–mite mutualisms can be facilitated by domatia (O'Dowd & Willson, 1989). Foliar domatia are minute enclosed spaces usually located at the junction of primary and secondary veins on the underside of leaves (O'Dowd & Willson 1989; Pemberton & Turner 1989). Domatia formation has been proposed to occur during early leaf ontogeny prior to the emergence of the leaf (e.g. O'Dowd & Willson 1989; Leroy et al. 2010). Plants that possess domatia support abundant and persistent numbers of predaceous and microbivorous mites, resulting in greater consumption of damaging phytophagous insects, pollen, fungal epiphytes and pathogens (Janzen 1966; O'Dowd & Willson 1989; Walter 1996; Bronstein 1998; Heil & McKey 2003; Monks et al. 2007). Furthermore, some predatory mites may supplement their diet with leaf detritus such as pollen (McMurtry & Croft 1997; Van Rijn et al. 2002).

Monks et al. (2007) found that while the presence of domatia did not necessarily benefit the plant by allowing mites to reach a high enough density to control fungi, they suggested that the presence of domatia played a role in ensuring some resident mites occur on new leaves. Furthermore, because investment in foliar domatia appears sensitive to carbon availability and leaf area (Monks et al. 2007; O'Connell et al. 2010b), there would be a selective advantage, when pathogens

were rare, for plants to reduce the number and possibly the size of domatia. Hence, this would result in a reduction in relative carbon costs, if domatia production were primarily a result of an induced mutualism.

We are aware of only one example of variable production of domatia in relation to inter-annual shifts in the benefits derived from hosting invertebrates (*Pseudomyrmex* ants and stem domatia on *Vochysia vismiaefolia* (Vochysiaceae); Bluthgen & Wesenberg 2001). The occurrence of plant pathogens may also trigger a response by the plant by activating or increasing food rewards or the production of plant structures (Karban & Myers 1989; Agrawal 1999). However, fungal infections on plants are ubiquitous (Pegg & Ayres 1990) and delays could result in an increase in vulnerability for the time the plant takes to implement the induced mutualism (e.g. Karban & Myers 1989; Baldwin & Preston 1999). Therefore, domatia may function most effectively as a constitutive mutualism independent of invertebrate density (Hamilton 1896; Greensill 1902; Shirley & Lambert 1922; Beattie 1985; Walter 1996).

To date, there have been no experimental tests of whether the domatia–mite mutualism is a constitutive or an inducible defence strategy. The mechanisms that induce domatia production could be associated with the mites or the fungi which, depending on mite activity levels, could trigger shifts in the number of domatia produced in new leaves. Concurrently, a constitutive mechanism could occur at a finer scale where changes occur at the earliest stages of formation of the domatia, influencing their opening size and thus their resulting number. We investigated whether foliar domatia in the New Zealand shrub *Coprosma rotundifolia* A. Cunn. (Rubiaceae) were an induced or constitutive plant–mite mutualism. We manipulated mite and fungal densities to test (1) whether foliar mite density on experimental shrubs stimulated increased domatia production and opening size in new leaves; and (2) whether the density of foliar fungi on old leaves influenced domatia production and opening size in new leaves.

Methods and materials

Study site and species

The study was conducted along the margins of secondary forest dominated by *Fuchsia excorticata* (J.R.Forst. & G.Forst.) L.f. (Onagraceae) and *Meliclytus ramiflorus* (J.R.Forst. & G.Forst.) (Violaceae), in the Leith Valley, Dunedin, South Island, New Zealand (45°50'S, 170°29'E; c. 100 m a.s.l.). Over the 152-day experiment (December 2007 to April 2008), the minimum/maximum daily temperature at the site ranged from 8.6/20.4°C in December to 4.0/13.8°C in April, with the monthly rainfall ranging from 45 mm in December to 78 mm in April (NIWA, 2008).

Coprosma rotundifolia occurs naturally in the understorey, along with several other *Coprosma* species and various ferns. *Coprosma rotundifolia* is a shrub 2–4 m tall, found throughout New Zealand in low to montane forests. Leaves are orbicular (10–25 mm long), often covered with fine trichomes (Poole & Adams 1990), and have between zero and seven pouch-type domatia (median: 4; DMO'C unpubl. data).

Experimental design

To test for an induced or constitutive plant–mite mutualism in new leaves on the experimental plants, we attempted to manipulate foliar mite and fungi densities for their influence

on domatia production. We used 80 individual 2-year-old potted *C. rotundifolia* shrubs (c. 50 cm tall) sourced from a commercial nursery. The experimental shrubs were initially dipped into a 1% solution of miticide (Maverick, Yates New Zealand; active ingredient 9.6 g L⁻¹ tau-fluvalinate). After 4 weeks no mites were located on the *C. rotundifolia* shrubs via an extensive survey ($n = 240$ leaves sampled).

The experimental shrubs were then randomly allocated to one of four treatments – control; miticide applied; fungicide applied; miticide and fungicide applied – then allocated to one of five blocks. The miticide treatment would allow us to assess domatia production with very low (or nil) mite densities (i.e. loss of the induced mutualism) but not fungi, while the fungicide treatment would allow us to test whether domatia production was influenced by mites at very low (or nil) fungi densities (the potential antagonist). The combined application of miticide and fungicide would allow the experiment to assess whether domatia production was affected when either a potential antagonist (i.e. the fungi), or the mites were significantly reduced in intensity (the induced mutualist). Each block contained 16 shrubs, with four replicates per treatment per block. Replicates within blocks were placed into four rows with each row containing one of four treatments. All shrubs were positioned to ensure no contact occurred between the foliage of adjacent shrubs. Mite colonisation was allowed to occur naturally once the experimental shrubs were placed at the study site.

To reduce the potential effects of chemical applications on plant physiological processes, we used a minimalist application approach when spraying the experimental shrubs (lowest recommended application/volume (10 ml L⁻¹ water) and longest recommended interval between applications (one month)). Research has indicated conflicting evidence that the chemical compounds used in controlling pests/pathogens affect plant gas exchange (e.g. Abdel-Reheem et al. 1991; Haile et al. 2000). However, because the plant carbon economy and domatia production are positively associated in *C. rotundifolia* (O'Connell et al. 2010b), we attempted to account for any potential effect of chemical interference to the photosynthetic processes by reducing the frequency of application minimising a potential chemical effect.

The first application of miticide occurred immediately after field placement. Fungicide treatments received foliar applications of 5 g L⁻¹ copper oxychloride (Yates, New Zealand). Control shrubs were sprayed with tap water only. To avoid spray drift onto adjacent shrubs, plants were moved to a nearby area (c. 10 m away) for treatment applications.

Mite counts

Mite communities were sampled at the end of the experiment. Three fully expanded leaves from the current and previous season were randomly selected and removed from each experimental shrub to determine differences in mite diversity and density between old and new leaves. Mites from both leaf surfaces and within domatia were counted and identified to the lowest possible taxonomic level.

Fungi and pollen loads

To assess leaf fungi and pollen densities (mm⁻²) at the end of the experiment, two old leaves per shrub were surveyed. Pollen density was assessed because evidence shows that predatory mites may supplement their diet with leaf detritus such as pollen (McMurtry & Croft 1997; Van Rijn et al. 2002).

A 0.5-cm² leaf peel was obtained from the upper and lower surface of each leaf by pressing and removing clear sticky tape, then staining with lactophenol blue for 24 h. Leaf fungi (hyphae and spores) and pollen densities were estimated on a divided 10 × 10 mm ocular quadrat (400× magnification) for five random fields for each leaf peel.

Domatia production, foliar carbon and nitrogen

To determine the effect of the treatments on the number and entrance size of domatia produced we sampled three leaves, both old and new, from each shrub at the end of the experiment. For each leaf, the number of domatia was counted and leaf area measured using a digital scanner and the WinFOLIA software package (Régent Instruments Inc., Switzerland). Leaf area was measured because the maximum number of domatia per leaf in *Coprosma* is strongly constrained by leaf size (O'Connell et al. 2010b). Leaves were dried at 70°C for 3 days and then weighed. Relative leaf investment in domatia (D_{MASS} : number of domatia per milligram of dry weight) was calculated along with the number of domatia per area (D_{AREA} : number of domatia per square centimetre). Leaf samples were pooled per shrub and assayed for total nitrogen and carbon on mature new season's leaves at the end of the experiment. For nitrogen, each sample was heated in a stream of high purity oxygen in a Leco furnace (Laboratory Equipment Corp., St Joseph, MI, USA). A subsample of the combustion gas was passed through a heated copper catalyst and the N₂ in the sample was measured by thermal conductivity. Foliar carbon was obtained by measuring the CO₂ from the combusted sample, using an infrared detector and calculated as a percentage of the dry weight (Chiariello et al. 1989).

Statistical analyses

We sought to determine whether selected variables describing mites, phylloplane fungal hyphae, and foliar carbon and nitrogen explained domatia expression in new leaves. Where possible we analysed the data using mixed-effect models to investigate differences in morphology, nutritional parameters and mite numbers. Sidak's multiple comparisons were used to investigate where significant differences occurred within the model. However, because of the relatively low mite number ($n = 60$), resulting in few assumptions of parametric tests being met, we used the non-parametric Kruskal–Wallis test on

mite densities and pooled functional groups. When analysing domatia investment, leaf area, and domatia opening size, we calculated the mean relative change between new leaves and old leaves, with treatment as a fixed effect and block as a random effect. When examining the relationships between foliar detritus and mite densities, we analysed the data using regression. All analyses were performed using Minitab (version 14.1, Minitab, Coventry, UK). Data were log-transformed where necessary, to remove heteroscedasticity and normalise residuals. Response and predictor values were averaged across leaves within shrubs prior to analysis.

Results

Mites

Fungivores and organic scavenging mites dominated mature leaves of the control (c. 62% of observations), consisting of *Orthotydeus californicus* (51.6%; Tydeidae), Winterschmidtidae sp. 1 (8.3%) and Oribatida (1.7%). Predatory mites (c. 35% of observations) comprised *Mullederia* sp. 1 (21.6%; Stigmaeidae) and *Amblyseius* sp. 1 (13.3%; Phytoseiidae). Herbivorous mites from the family Eriophyiidae were also recorded (Eriophyiidae sp. 1: 3.3%).

The total density of mites that successfully colonised the experimental shrubs was relatively low (Table 1). Overall, mite densities showed significant treatment effects (Table 1). Mite densities were 3 to 35 times higher on the control shrubs compared with the remaining treatments (Table 1). However, treatment effects on mite densities varied depending on the functional mite group. Fungivorous mites showed a significant difference across treatments (Table 1), with mite densities on the control shrubs about five times higher compared with the fungicide treatment. This result appears driven by the higher number of *Orthotydeus californicus* that occurred on the control. There were no fungivorous or herbivorous mites on the miticide and miticide + fungicide treatments. Predatory mite densities showed no significant differences across treatments although densities were greater on the miticide treatment compared with the control (Table 1), driven by *Mullederia* sp. 1. The herbivorous mite group occurred only on the fungicide treatment. Mite densities between old and new leaves showed no significant differences ($F_{1, 149} = 0.18$, $P = 0.670$).

Table 1. Mite densities (cm⁻²; mean ± SE) for functional groups on experimental *Coprosma rotundifolia* shrubs (Kruskal–Wallis non-parametric test). Different letters (a, b) and bold indicate significant differences among treatments. Number of leaves sampled = 480. ‘-’ indicates where no mites occurred.

	Mite species	Control	Miticide	Fungicide	Miticide + Fungicide	H-value	Kruskal–Wallis test d.f.	P-value
Predator	<i>Mullederia</i> sp. 1	0.005 ± 0.005	0.06 ± 0.06	0.003 ± 0.003	0.006 ± 0.006	3.07	3	0.381
	<i>Amblyseius</i> sp. 1	0.02 ± 0.01	-	0.03 ± 0.01	-	1.01	3	0.798
	Predator density	0.025 ± 0.01	0.06 ± 0.06	0.033 ± 0.03	0.006 ± 0.006	2.05	3	0.561
Fungivore	<i>Orthotydeus californicus</i>	0.15 ± 0.03 ^a	-	0.03 ± 0.02 ^b	-	18.00	3	<0.001
	Winterschmidtidae sp. 1	0.04 ± 0.02	-	-	-	-	-	-
	Oribatida sp. 1	-	-	0.007 ± 0.007	-	-	-	-
	Fungivore density	0.19 ± 0.04 ^a	-	0.037 ± 0.02 ^b	-	20.95	3	<0.001
Herbivore	Eriophyiidae sp. 1	-	-	0.007 ± 0.007	-	-	-	-
	Herbivore density	-	-	0.007 ± 0.007	-	-	-	-
	Total mite density	0.21 ± 0.05 ^a	0.06 ± 0.03 ^b	0.07 ± 0.03 ^b	0.006 ± 0.006 ^b	15.61	3	<0.001

Fungal and pollen loads

The application of fungicide and miticide had varied effects on densities of leaf fungi and detritus on old leaves (Table 2). We used hyphae as an indicator of the severity of fungal infection. Shrubs in the miticide-only treatment showed significantly higher levels of hyphae on the upper and lower surfaces (control and miticide + fungicide treatments respectively; Table 2). However, the fungicide treatment had no significant effect on foliar fungal growth (Table 2). Fungal hyphae on the upper and lower leaf surfaces were not significantly associated with the number of mites per leaf (Fig. 1a; upper surface: $r^2 = 0.03$, $F_{1,76} = 2.22$, $P = 0.140$; lower surface: $r^2 = 0.02$, $F_{1,76} = 1.68$, $P = 0.198$).

Fungal spore density on the upper leaf surface in the fungicide treatment was significantly higher compared with the control (Table 2). There was no significant association with fungal spores (upper surface: $r^2 = 0.05$, $F_{1,76} = 0.35$, $P = 0.553$; lower surface: $r^2 = 0.06$, $F_{1,76} = 0.43$, $P = 0.516$) or pollen (upper surface: $r^2 = 0.03$, $F_{1,76} = 0.20$, $P = 0.652$; lower surface: $r^2 = 0.02$, $F_{1,76} = 0.07$, $P = 0.959$) and mites ($r^2 = 0.05$, $F_{1,76} = 0.17$, $P = 0.865$).

Domatia production

The application of fungicide and miticide had no significant effect on the mean relative change or association with domatia production (D_{MASS}) in new season leaves compared with older leaves (total mean Δ in domatia production 0.51 ± 0.02

domatia mg^{-1} ; Table 3). We found no significant differences in the mean relative change in leaf area or domatia entrance size between old and new leaves (Table 3). There were no significant differences across treatments in new leaves in foliar carbon or nitrogen content (Table 3). We found no significant association (Fig. 1b–e) between domatia investment and mite numbers ($r^2 = 0.04$, $F_{1,76} = 0.13$, $P = 0.732$), hyphae ($r^2 = 0.02$, $F_{1,76} = 0.81$, $P = 0.679$), foliar carbon ($r^2 = 0.29$, $F_{1,76} = 2.26$, $P = 0.136$) or foliar nitrogen ($r^2 = 0.12$, $F_{1,76} = 0.92$, $P = 0.340$). There was a significant negative association between domatia investment and leaf area ($r^2 = 0.35$, $F_{1,76} = 41.18$, $P < 0.001$) (Fig. 1f).

Discussion

Domatia induction

The production of foliar domatia in the new leaves of *C. rotundifolia* was similar across treatments (Fig. 1b). The relatively low mite densities during the experiment (≤ 0.21 mites cm^{-2} , Table 1) may not have been enough to elicit an induced mutualism in our system. The induction of domatia could instead be linked to mite activity rather than their density, which was not measured in this experiment. For example, in an ant–plant system, Bluthgen and Wesenberg (2001) proposed that constant activity from *Pseudomyrmex*

Table 2. Mean densities (\pm SE; mm^{-2}) of foliar fungi (hyphae), spores, and pollen loads on the old leaves of *Coprosma rotundifolia*. Superscript letters indicate significant differences across rows (Sidak's multiple comparison, ANOVA: $P < 0.05$). Total leaves sampled = 160. Leaf fungi hyphal density (mm^{-2}) was estimated using the number of divided squares occupied in a 10×10 mm ocular quadrat ($400\times$ magnification).

	Treatment				ANOVA		
	Control	Miticide	Fungicide	Miticide + Fungicide	F-value	d.f.	P-value
Hyphae							
Lower	$0.59 \pm 0.15^{\text{ab}}$	$0.80 \pm 0.10^{\text{a}}$	$0.51 \pm 0.07^{\text{ab}}$	$0.35 \pm 0.06^{\text{b}}$	4.77	3, 72	$P = 0.004$
Upper	$0.98 \pm 0.13^{\text{b}}$	$1.91 \pm 0.19^{\text{a}}$	$1.02 \pm 0.11^{\text{b}}$	$1.05 \pm 0.09^{\text{b}}$	9.34	3, 58	$P < 0.001$
Fungal spores							
Lower	0.05 ± 0.03	0.05 ± 0.03	0.04 ± 0.01	0.11 ± 0.11	0.11	3, 72	$P = 0.956$
Upper	$0.03 \pm 0.01^{\text{b}}$	$0.05 \pm 0.01^{\text{ab}}$	$0.09 \pm 0.02^{\text{a}}$	$0.05 \pm 0.01^{\text{ab}}$	3.32	3, 58	$P = 0.026$
Pollen							
Lower	0.003 ± 0.006	0.002 ± 0.001	0.006 ± 0.003	0.005 ± 0.005	1.83	3, 72	$P = 0.150$
Upper	0.006 ± 0.002	0.003 ± 0.002	0.009 ± 0.005	0.004 ± 0.002	0.69	3, 58	$P = 0.559$

Table 3. Measured morphological and nutritional parameters of *Coprosma rotundifolia* leaves (mean \pm SE) by treatment. Morphological features: ΔD_{MASS} , the mean relative change in D_{MASS} between old and new leaves (domatia mg^{-1}); ΔLA , the mean relative change in leaf area between old and new season's leaves (cm^{-2}); $\Delta \text{Domatia size}$, opening of domatia (μm). Nutritional parameters: foliar carbon (C) and nitrogen (N) percentage of dry weight. Number of leaves sampled = 240. The ANOVA tested the mean relative change between old and new leaves for morphological leaf traits only. The ANOVA test for foliar nutrients was on new leaves only.

	Control	Fungicide	Miticide	Miticide + Fungicide	ANOVA		
					F-value	d.f.	P-value
ΔD_{Mass}	0.46 ± 0.23	0.49 ± 0.12	0.57 ± 0.12	0.52 ± 0.12	0.85	3,71	0.467
ΔLA	-0.11 ± 0.07	0.006 ± 0.08	-0.04 ± 0.09	-0.03 ± 0.10	0.23	3,71	0.878
$\Delta \text{Domatia size}$	-0.03 ± 0.08	-0.02 ± 0.05	-0.02 ± 0.1	-0.09 ± 0.09	0.49	3,70	0.689
C	44.7 ± 0.2	44.8 ± 0.2	44.2 ± 0.2	44.8 ± 0.2	2.27	3,70	0.088
N	2.01 ± 0.1	3.2 ± 0.2	2.7 ± 0.2	3.2 ± 0.1	2.62	3,70	0.058

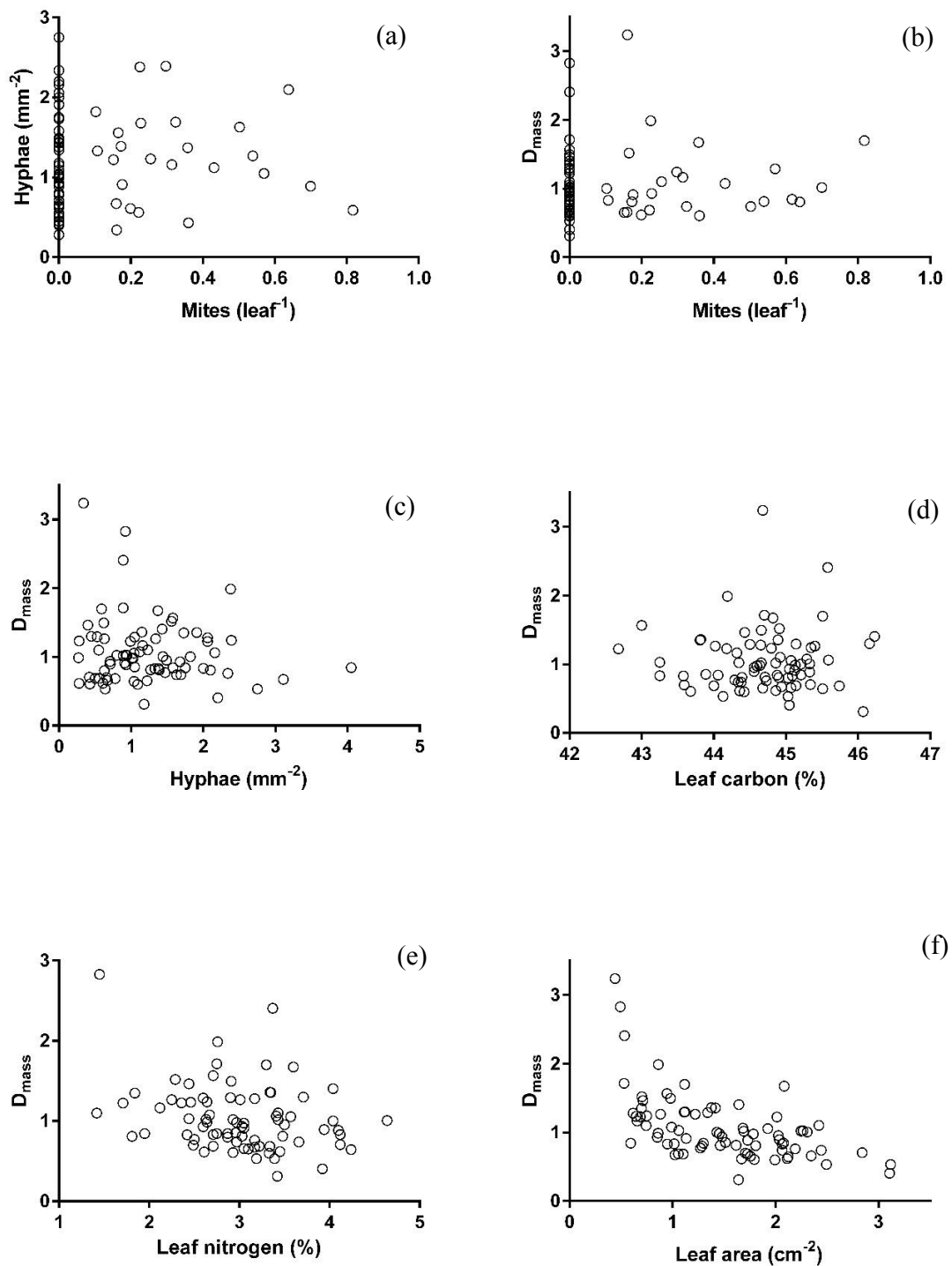


Figure 1. Relationships on experimental *Coprosma rotundifolia* shrubs between mites per leaf and (a) hyphal density (upper surface) and (b) domatia per leaf mass, D_{mass} (number of domatia per milligram of dry weight). Relationships between D_{mass} and (c) hyphal density, upper surface; (d) leaf carbon; (e) leaf nitrogen; and (f) leaf area.

ants was required and could even accelerate domatia growth. The miticide treatment was relatively effective, because overall mite densities were lower on the miticide (and fungicide) treatments compared with the control. The presence of a predatory *Mullederia* species on the miticide-treated shrubs (Table 1) is consistent with previous observations, where this species has been observed in experimental field trials on *Coprosma* shrubs treated with miticide + domatia removed (DMO'C pers. obs., 2005; O'Connell et al. 2010a) at similar

densities to this experiment. However, a caveat exists in our findings in that the density of mites may not have been high enough to elicit a plant response.

We found no significant association between foliar fungi and domatia production on *C. rotundifolia* (Fig. 1c), contrasting with the negative relationship in new season's leaves found by Monks et al. (2007) on *C. lucida*. They suggested that the carbon economy of *C. lucida* was negatively affected by the high number of fungal hyphae in the absence of fungivorous

mites, resulting in reduced foliar carbon levels and fewer domatia in new leaves. Foliar fungal infection can be associated with reduced stomatal conductance and photosynthesis (Luque et al. 1999; El Omari et al. 2001; Aldea et al. 2006). The link between the carbon economy and domatia was subsequently supported by O'Connell et al. (2010b), who found a positive relationship between primary productivity, leaf carbon and domatia production in *C. ciliata*, *C. foetidissima*, and *C. rotundifolia*. Hyphal densities were relatively low in the present study ($0.4\text{--}2$ hyphae mm^{-2}) compared with Monks et al. (2007; $c. 12\text{--}30$ hyphae mm^{-2}) and may have been insufficient to significantly impair photosynthetic performance. In addition, our experiment did not identify whether the fungi present on the leaf surfaces were saprophytic or pathogenic. Many New Zealand plant species host a large variety of fungi many of which are both pathogenic and saprotrophic (Gadgil et al. 2005), but little is known about the types of fungi that occur on *C. rotundifolia* (P. Gadgil, pers. comm., 2008).

We found that the density of fungal hyphae (upper leaf surface) was similar between the miticide treatment and the control (Table 2). This suggests that fungivorous mites, mediated by domatia, were as effective as the fungicide treatment at reducing density of fungal hyphae. This finding partially supports English-Loeb et al. (2007) who found that fungivorous mites were as effective as or better than fungicide in controlling powdery mildew. Fungivorous mites significantly reduced hyphal density on the upper leaf surface compared with where miticide was applied (Table 2). The application of miticide resulted in no fungivorous mites on the experimental shrubs, allowing an increase in hyphae densities (Table 2). However, while our results indicated no effect of fungal hyphae on domatia production, the limiting factor in this experiment was that overall fungal densities were relatively low compared with other studies (e.g. ~ 200 mm^{-2} (Melidossian et al. 2005); ~ 21.3 mm^{-2} (Monks et al. 2007)). Therefore we were unable to compare across a wide range of fungal densities. Certainly, evidence shows that fungi inhibit domatia production by lowering carbon production on *C. lucida* (Monks et al. 2007).

Implications of non-inducibility

Our results suggest that domatia are not inducible structures on *C. rotundifolia*, at least at the low levels of mite and fungal densities in this study; the production of foliar domatia was similar across all treatments. We suggest that the continuous presence and threat of fungal infection may require the plant to maintain 'constant vigilance'. Thus, maintaining structural refugia such as domatia, trichomes, thickened cuticle or some combination of these may be a more efficient strategy against general attack (e.g. Heath 1996; Kole et al. 1996). The additional production cost to plants of a constitutive mutualism, such as domatia, may be less than the fitness cost of a delayed response to a fungal or invertebrate threat of induced domatia, particularly when fungal pathogens appear to play such an important role in reducing plant fitness (Luque et al. 1999; El Omari et al. 2001; Aldea et al. 2006; English-Loeb et al. 2007). Induced mutualisms have a potential time lag that can range from hours to weeks (Baldwin & Preston 1999; Bluthgen & Wesenberg 2001). However, this period would need to be even longer for domatia because the process is probably ontogenetically determined in new leaves (O'Dowd & Willson 1989; Walter 1996). If the production of domatia was elicited via an induction pathway, the plant would gain little benefit for older leaves, which may remain on the plant for several years. Given that the New Zealand flora is predominantly

evergreen (McGlone et al. 2004), foliar domatia are unlikely to be induced except in a few deciduous species. Evergreen leaves potentially result in the accumulation of fungal hyphae, which could negatively impact the photosynthetic capabilities of the leaf and clog stomata. The induction of domatia would render the protection of the mutualism largely ineffectual as older leaves would potentially lose all, or a substantial part of, their functionality. Furthermore, as relatively low densities of mites are required to control fungi (Monks et al. 2007), perhaps the domatia function just to keep the mites in the system. Therefore, the plant needs to produce some domatia annually to maintain a viable mite population.

The negative association between domatia investment (D_{MASS}) and leaf area (Fig. 1f) suggests that investment in foliar domatia may be sensitive to leaf area (O'Dowd & Willson 1989; O'Connell et al. 2010b). This association suggests that the relative cost of domatia production is greater for small leaves compared with large leaves, not only within a single *Coprosma* species (Fig. 1a), but across different *Coprosma* species (O'Connell 2009; O'Connell et al. 2010b). Our findings suggest that domatia on *C. rotundifolia* are potentially part of a constitutive mutualism, as plants produce many domatia, apparently in excess of requirements.

Acknowledgements

We thank Timm Döbert for technical assistance in the field; University of Otago colleagues Brian Niven, Department of Mathematics and Statistics, for his statistical advice; Julie Clark, Department of Geography, for her assistance in foliar assays; and Robert Poulin, Department of Zoology, for his advice and ideas. Landcare Research and the Department of Botany, University of Otago, provided logistical support and funding through a University of Otago PhD scholarship and a Manaaki Whenua Landcare Research grant-in-aid, with further support from a University of Otago Postgraduate Publishing grant. We are grateful to Mike Hutchings, Sarah Richardson, Jo Monks and two anonymous reviewers for comments on earlier versions of this manuscript.

References

- Abdel-Reheem S, Belal MH, Gupta G 1991. Photosynthesis inhibition of soybean leaves by insecticides. *Environmental Pollution* 74: 245–250.
- Agrawal AA 1999. Induced plant defense: Evolution of induction and adaptive phenotypic plasticity. In: Agrawal AA, Tuzun S, Bent E eds *Induced plant defenses against pathogens and herbivores*. St Paul, MN, APS Press. Pp. 251–268.
- Aldea M, Hamilton JG, Resti JP, Zangerl AR, Berenbaum MR, Frank TD, DeLucia EH 2006. Comparisons of photosynthetic damage from arthropod herbivory and pathogen infection in understory hardwood saplings. *Oecologia* 149: 221–232.
- Baldwin IT, Preston CA 1999. The eco-physiological complexity of plant responses to insect herbivores. *Planta* 208: 137–145.
- Beattie AJ 1985. *The evolutionary ecology of ant-plant mutualisms*. Cambridge Studies in Ecology. Cambridge University Press.

- Bixenmann RJ, Coley PD, Kursar TA 2011. Is extrafloral nectar production induced by herbivores or ants in a tropical facultative ant-plant mutualism? *Oecologia* 165: 417–425.
- Blüthgen N, Wesenberg J 2001. Ants induce domatia in a rain forest tree (*Vochysia vismiaefolia*). *Biotropica* 33: 637–642.
- Bronstein JL 1998. The contribution of ant-plant protection studies to our understanding of mutualism. *Biotropica* 30: 150–161.
- Chiariello NR, Mooney HA, Williams K 1989. Growth, carbon allocation and cost of plant tissues. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW eds *Plant physiological ecology: field methods and instrumentation*. London, Chapman and Hall. Pp. 327–365.
- El Omari B, Fleck I, Aranda X, Moret A, Nadal M 2001. Effect of fungal infection on leaf gas-exchange and chlorophyll fluorescence in *Quercus ilex*. *Annals of Forestry Science* 58: 165–174.
- English-Loeb G, Norton AP, Gadoury D, Seem R, Wilcox W 2007. Biological control of grape powdery mildew using mycophagous mites. *Plant Disease* 91: 421–429.
- Gadgil PD, Dick MA, Hood IA, Pennycook SR 2005. Fungi on trees and shrubs in New Zealand. *Fungal Diversity Research Series* 16. Hong Kong, Fungal Diversity Press. 437 p.
- Greensill NAR 1902. Structure of leaf of certain species of *Coprosma*. *Transactions and Proceedings of the New Zealand Institute* 35: 342–355.
- Haile FJ, Kerns DL, Richardson JM, Higley LG 2000. Impact of insecticides and surfactant on lettuce physiology and yield. *Journal of Economic Entomology* 93: 788–794.
- Hamilton AG 1896. On domatia in certain Australian and other plants. *Proceedings of the Linnean Society of New South Wales* 21: 758–792.
- Heath MC 1996. Plant resistance to fungi. *Canadian Journal of Plant Pathology* 18: 469–475.
- Heil M 2008. Indirect defence via tritrophic interactions. *New Phytologist* 178: 41–61.
- Heil M, McKey D 2003. Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution and Systematics* 34: 425–453.
- Herms DA, Mattson WJ 1992. The dilemma of plants: To grow or defend. *The Quarterly Review of Biology* 67: 283–335.
- Howe HF 1984. Constraints on the evolution of mutualisms. *The American Naturalist* 123: 764–777.
- Janzen DH 1966. Coevolution of mutualism between ants and acacias in Central America. *Evolution* 20: 249–275.
- Jolivet P 1996. Ants and plants, an example of coevolution. Leiden, The Netherlands, Backhuys. 85 p.
- Karban R, Myers JH 1989. Induced plant responses to herbivory. *Annual Review of Ecology and Systematics* 20: 331–348.
- Kole C, Teutonico R, Mengistu A, Willaims PH, Osborn TC 1996. Molecular mapping of a locus controlling resistance to *Albuga candida* in *Brassica rapa*. *Phytopathology* 86: 367–369.
- Leroy C, Jauneau A, Quilichini A, Dejean A, Orivel J 2010. Comparative structure and ontogeny of the foliar domatia in three neotropical myrmecophytes. *American Journal of Botany* 97: 557–565.
- Linsenmair KE, Heil M, Kaiser WM, Fiala B, Koch T, Boland W 2001. Adaptations to biotic and abiotic stress: *Macaranga*-ant plants optimize investment in biotic defence. *Journal of Experimental Botany* 52: 2057–2065.
- Luque J, Cohen M, Savé R, Biel C, Álvarez IF 1999. Effects of three fungal pathogens on water relations, chlorophyll fluorescence and growth of *Quercus suber* L. *Annals of Forestry Science* 56: 19–26.
- McGlone MS, Dungan RJ, Hall GMJ, Allen RB 2004. Winter leaf loss in the New Zealand woody flora. *New Zealand Journal of Botany* 42: 1–19.
- McMurtry JA, Croft BA 1997. Life-styles of phytoseiid mites and their roles in biological control. *Annual Review of Entomology* 42: 291–321.
- Melidossian HS, Seem RC, English-Loeb G, Wilcox WF, Gadoury DM 2005. Suppression of grapevine powdery mildew by a mycophagous mite. *Plant Disease* 89: 1331–1338.
- Monks A, O'Connell DM, Lee WG, Bannister JM, Dickinson KJM 2007. Benefits associated with the domatia mediated tritrophic mutualism in the shrub *Coprosma lucida*. *Oikos* 116: 873–881.
- O'Connell DM 2009. Plant-arthropod interactions: Domatia and mites in the genus *Coprosma* (Rubiaceae). PhD thesis, University of Otago, Department of Botany, Dunedin. 190 p.
- O'Connell DM, Lee WG, Monks A, Dickinson KJM 2010a. Does microhabitat structure affect foliar mite assemblages? *Ecological Entomology* 35: 317–328.
- O'Connell DM, Monks A, Lee WG, Downs TM, Dickinson KJM 2010b. Leaf domatia: carbon-limited indirect defence? *Oikos* 119: 1591–1600.
- O'Dowd DJ, Willson MF 1989. Leaf domatia and mites on Australasian plants: ecological and evolutionary implications. *Biological Journal of the Linnean Society* 37: 191–236.
- Pegg GF, Ayres PG 1990. Preface: Fungal infection of plants. In: Pegg GF, Ayres PG eds *Fungal infection of plants*. 2nd edn. Cambridge, Cambridge University Press.
- Pemberton RW, Turner CE 1989. Occurrence of predatory and fungivorous mites in leaf domatia. *American Journal of Botany* 76: 105–112.
- Poole AL, Adams NM 1990. *Trees and shrubs of New Zealand*. Rev. edn. Wellington, DSIR. 256 p.
- Risch SJ, Rickson FR 1981. Mutualism in which ants must be present before plants produce food bodies. *Nature* 291: 149–150.
- Sabelis MW, Janssen A, Bruin J, Bakker FM, Drukker B, Scutareanu P, Van Rijn PCJ 1999. Interactions between arthropod predators and plants: a conspiracy against herbivorous arthropods? In: Bruin J, van der Geest LPS, Sabelis MW eds *Ecology and evolution of the Acari*. Dordrecht, Kluwer. Pp. 207–229.
- Shirley J, Lambert CA 1922. On *Coprosma Baueri*, Endlicher. *Proceedings of the Royal Society of Victoria* 35: 21–23.
- van Rijn PCJ, van Houten YM, Sabelis MW 2002. How plants benefit from providing food to predators even when it is also edible to herbivores. *Ecology* 83: 2664–2679.
- Walter DE 1996. Living on leaves: mites, tomenta, and leaf domatia. *Annual Review of Entomology* 41: 101–114.
- Walter DE, O'Dowd DJ 1995. Life on the forest phylloplane: hairs, little houses, and myriad mites. In: Lowman MD, Nadkarni NM eds *Forest canopies*. London, Academic Press. Pp. 325–351.