

# **Position, position, position: Mites occupying leaf domatia are not uniformly distributed in the tree canopy**

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## **Highlights**

- Some woody species of plant possess modified leaf structures termed domatia.
- Mites are known to occur in these.
- A mutualistic relationship between plants and mites has been hypothesized.
- The distribution and species composition of mites within the tree canopy is demonstrated.
- Micro-climate data suggests that mites prefer cooler and more humid canopy sites.

## **Abstract**

Leaf domatia are plant-produced cavities usually found in the axils of major veins on the abaxial side of leaves. These structures are found in many woody dicotyledonous plants and mediate a mutualistic relationships between predacious and Fungivorous mites and the host plants they protect. Mites inhabit leaf domatia for shelter and to reproduce and develop. In turn, the plants are hypothesized to benefit from increased defense against pathogens and small arthropod herbivores. Here we assess the distribution of mites throughout the tree canopy to determine if certain regions of the canopy are preferred. Our results suggests that mites prefer leaves found in the lower regions of the tree canopy and avoid leaves at the top, where they may be exposed to

harsher climatic conditions. This study is one of the first to document aspects of the plant – mite mutualism from African species.

**Key words:** leaf domatia, *Gardenia thunbergia*, mites, mutualism, *Ocotea bullata*, Tree canopy, diurnal migration.

## 1. Introduction

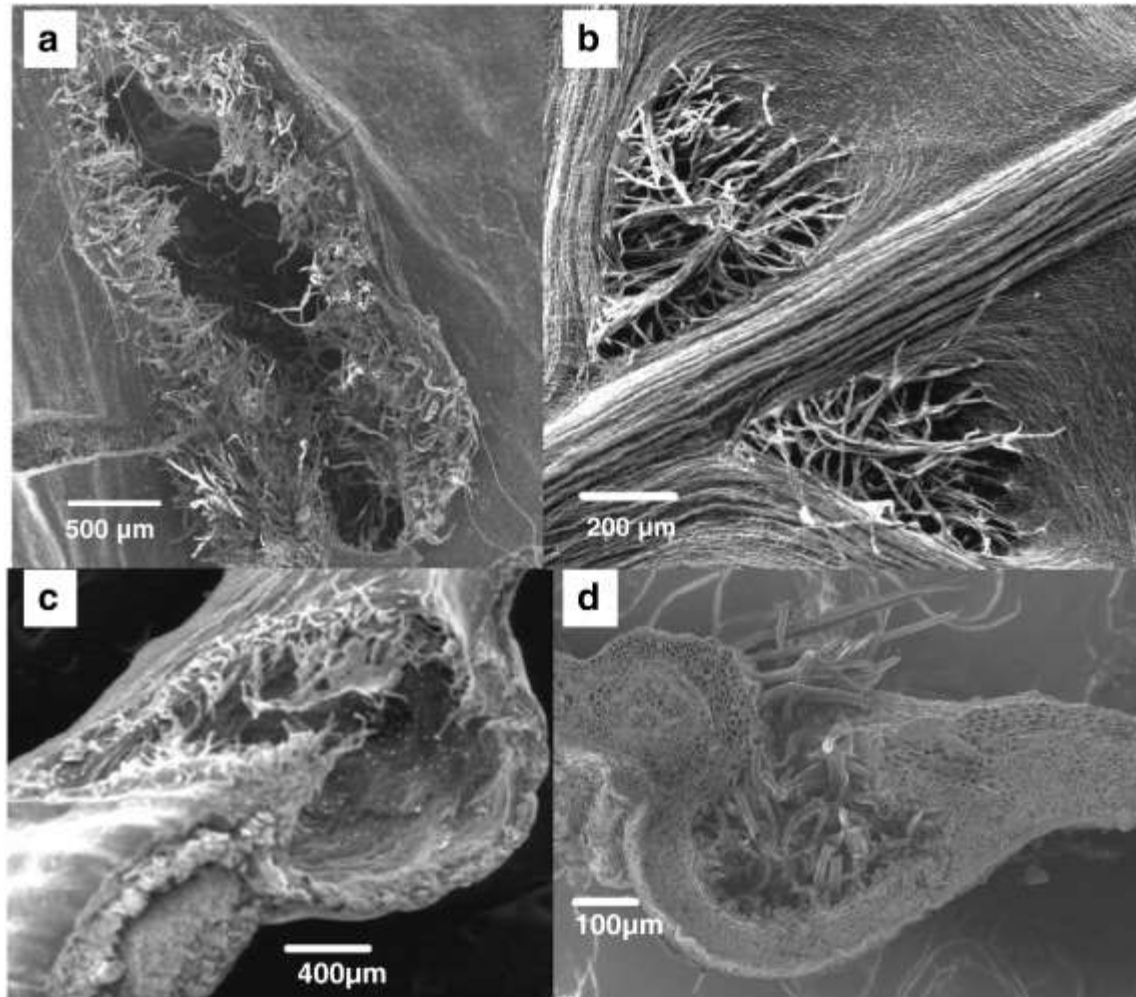
Forest canopies support a diverse range of arthropod assemblages and these are usually distinct from those of the forest floor (Erwin, 2013; Arroyo et al., 2010; Nadkarni, 1994). These organisms are an important component of forests, as they carry out a range of vital ecosystem services including decomposition and nutrient cycling in above ground deposits of litter and soils (Walter and Behen-Pelletier, 1999; Dial et al., 2006). Inventories of arthropods have shown that forest canopies contain a high abundance and diversity of arthropods and that these animals may respond to environmental gradients within the forest from the top of canopy to forest floor (Dial et al., 2006).

Forests canopies alter environmental and climatic variables such as solar radiation, air and soil temperature, rainfall, air humidity and wind, and thus create a micro-climate inside forests (Parker, 1995). The greatest changes to the micro-climate (patterns of temperature, moisture, wind and light) are brought about by adult stands with closed canopies and high leaf area indices (Aussenac, 2000). Forest trees may also modify their canopy micro-climate along a vertical gradient, and air temperature generally declines with canopy depth due to within-crown shading (e.g. Zweifel et al., 2002; Harley et al., 1996). Canopy structure therefore has a direct effect on

the climate surrounding individual leaves and on the large-scale environment of forest regions. These changes in micro-climate play an important role in determining the diversity of micro-organisms, insects, birds, and vascular epiphytes found in forest canopies (Nadkarni, 1994).

Within the tree crown, arthropods are associated with or graze on leaves. The phylloplane of leaves may provide a wide range of animals with suitable micro-habitat, and the surfaces of leaves are an important environment due to their diversity of anatomical, morphological and physiological properties (Pereira et al. 2002). These structures may support a rich arthropod fauna which is usually dominated by mites. Within forest canopies, mites exceed all other arthropods in species abundance (Beaulieu et al. 2010; Walter and Behen-Pelletier 1999), and mite assemblages comprise multiple families of predators, scavengers, grazers, and plant parasites. Most of the mites encountered on leaves graze on phylloplane fungi or are predatory to other mites (Pemberton and turner, 1989; O'Dowd and Willson, 1991; Walter and O'Dowd, 1992; O'Dowd and Pemberton 1998; Matos et al., 2006). However, harmful phytophagous mites may also be found (Pemberton and Turner, 1989; Walter and O'Dowd, 1995; Agrawel, 1997; Norton et al., 2000).

Some plant species possess leaves which bear structures known as leaf domatia (Figure 1). These often house large numbers of predatory mites, perhaps because this micro-habitat provides higher relative humidity than surrounding air (Walter and Behen-Pelletier, 1999; Pemberton and Turner, 1989; O'Dowd and Wilson, 1991; Walter and O'Dowd, 1992; O'Dowd and Pemberton, 1998; Matos et al., 2006). These structures influence the distribution and diversity of mites found on leaves.



**Fig. 1.** Scanning electron micrographs of leaf domatia of the study species: (a) external view of domatium of *Ocotea bullata*; (b) external view of domatium of *Gardenia thunbergia*; (c) SEM of transverse section through the domatium of *O. bullata*; (d) SEM of transverse section through the domatium of *G. thunbergia*.

Studies assessing the abundance of mites within forests have focused mainly at comparing the mite biota of the tree canopy and the forest floor (Lindo and Winchester, 2006; Arroyo et al., 2010; Beaulieu et al., 2010). A few studies (Onzo et al., 2003; Magalhães et al., 2012; Pickett et al., 1988; Wilson et al., 1983) that look at mite distribution within the tree canopy only focus on vertical movement of mites in response to predator-prey relationship. This study thus has the following aims:

- 1) To assess whether mite diversity and abundance is uniformly distributed throughout the canopy, irrespective of tree species.
- 2) To measure changes in temperature and humidity at different positions in the canopy and to determine if these might influence mite diversity and abundance.

## **2. Methods**

Two species found in forests in southern Africa were selected. *Ocotea bullata* (Lauraceae) and *Gardenia thunbergia* (Rubiaceae) both have pit-type domatia, surrounded by trichomes. (Figure 1). *O. bullata* (commonly known as Stinkwood) is restricted almost entirely to South Africa, where it occurs from Table Mountain (Cape Town) to the northern regions of the country (Boon, 2010). Field sampling of *Ocotea bullata* was conducted in the Tsitsikama forests (33°57'24.7"S, 23°54'33.0"E) near Storms River in the Eastern Cape, South Africa, on the 9<sup>th</sup> of September 2013 where a stand of these trees were felled as part of another research program on this species. Because of this species rarity, it was sampled only once.

*Gardenia thunbergia* is an evergreen shrub or small tree that grows up to 6 m in height, being found in both Afromontane and coastal forests of the Eastern Cape and KwaZulu-Natal regions of South Africa (Boon, 2010). The trees sampled here are located in the Grahamstown Botanical Gardens (33°19'09.4"S, 26°31'18.1"E), in the Eastern Cape. This population was sampled on two different occasions.

Five individual trees for each species were selected for sampling. Before sampling the north facing side of each tree was marked and then 10 leaves each were sampled from the North, South, East and West outside points, as well as the bottom, inside and top of the canopy. *O. bullata* trees were between 15 to 19.10 meters in height and the *G. thunbergia* trees were over

3.5 to 5 meters. In the case of *O. bullata*, the leaves were sampled immediately after the tree was felled, and a ladder was used to access the leaves of *G. thunbergia*. Only mature but not senescent leaves were sampled. All 10 leaves from each sampling position were placed in a labeled zip lock bag and kept cool. As soon after collection as possible, all the domatia present in each leaf were viewed under a dissecting microscope and the mites found on and inside the leaf domatia were counted. The number of mites found in each leaf was recorded from each sampling position and the mean number of mites per leaf at each canopy location was calculated. A non-parametric Kruskal-Wallis ANOVA was performed on STATISTICA version 2010 to compare mite abundance between all the canopy locations for each species.

Sampling of leaves of *G. thunbergia* occurred on 2 different occasions. On the first occasion, five individual trees were sampled in the early austral spring on the 4th of September 2014. On the second occasion in summer on the 26th of February 2015, prior to the second sampling period, seven iButton data loggers were placed in one of the trees at the positions of North, West, South, East, Inside, Top and Bottom of the canopy. The iButtons were glued in perforated plastic containers, and were then loosely covered in two layers of white cloth to minimize the effects of direct sunlight on the device. These were hung within the canopy at the sampling points. The iButtons were programmed to record temperature and relative humidity every 15 minutes and left in situ for 14 days. They were removed immediately after the leaves were sampled at the end of this period. Unfortunately budgetary constraints restricted the use of this technology to a single tree.

The different morpho-species of mites observed inside domatia were noted and a representative sample of these were collected and sent away for identification. A sub-set was prepared for Scanning Electron Microscopy (SEM) analysis. Mites specimens selected to be viewed under the

SEM were first dehydrated using 90% alcohol and then after being air dried, they were mounted into a stub using graphite tape. The stub was then sputter coated with gold and observed under the TESCAN Vega TS 5136LM. Photographs were taken and image analysis was done using Scandium software. The photographs taken were then used for mite identification.

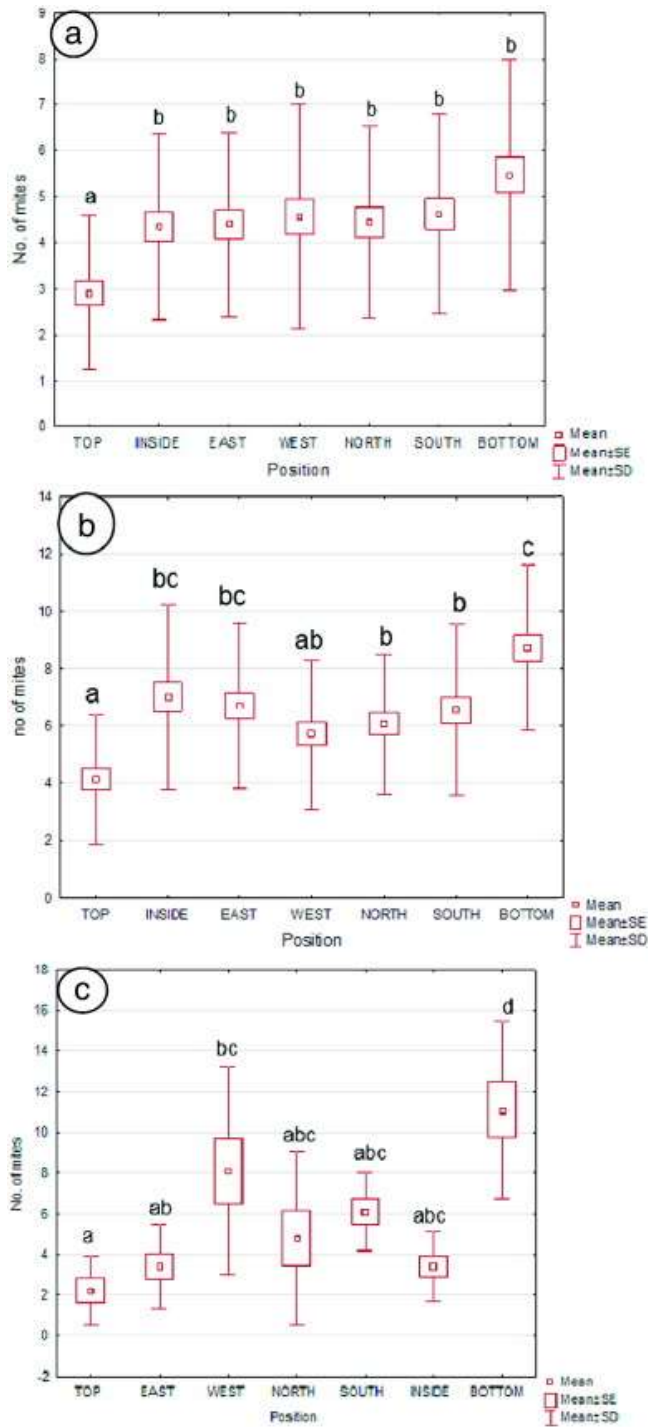
Another selected sub-set of the mites was collected for Light microscopy analysis. These mites were preserved in ethanol and later mounted on a slide using a Polyvinyl Alcohol (PVA) mounting medium and viewed under a light microscope (Olympus BX) for identification purposes. The PVA preparation process was adapted and modified from Krantz and Walter (2009).

### **3. Results**

#### *3.1 Mite abundance in relation to canopy position in *O. bullata* and *G. thunbergia**

*O. bullata* produce big domatia that protrude to the other side of the leaf and they can be up to 5 mm in length. On the other hand the domatia of *G. thunbergia* are very small (up to 1mm). While both these species have pit type domatia, the domatium of *G. thunbergia* is entirely lined internally with trichomes. In comparison the domatium of *O. bullata* lacks trichomes in the inside and these structures are only present in the rim of the entrance (Figure 1c and 1d).

Figure 2 shows mite abundance from the different canopy locations of both *O. bullata* and *G. thunbergia*. For *O. bullata*, no significant difference was found in mite abundance in terms of direction (east, west, north, south),  $p > 0.05$  (Figure 2a). However, differences were found in the vertical distribution of mites in the tree canopy, with the canopy top having significantly fewer mites ( $p < 0.001$ ). Furthermore the mite abundance at the top of canopy also differed from that of the east, west, north and south direction ( $p \leq 0.02$ ).



**Fig. 2.** Box and whisker plots showing the average number of mites found in the leaves of *Ocotea bullata* and *Gardenia thunbergia* at each tree canopy sampling location, i.e. north, south, west and east aspects and from the top, inside and bottom of the canopy. Figure (a) shows mite counts for *O. bullata*, (b and c) represent mite counts for *G. thunbergia* for the first and second sampling occasions, respectively.



A similar result was found for *G. thunbergia* which had a significantly lower abundance of mites at the top of the canopy (p value <0.001) on both sampling occasions (Figure 2b & c). In addition, the bottom leaves of the tree canopy had significantly higher mite numbers (p value <0.0001). The west side of the canopy was found to also contain fewer mites on the first sampling occasion and was not significantly different from the top position. However, on the second sampling occasion, the west side was significantly different from the top position (p value = 0.04).

### *3.2 Mite diversity in relation to canopy position*

Mite diversity (species composition) also varied within the canopy (Tables 1 and 2). Some taxa were found in all canopy sampling sites, while others were found at only some sites within the canopy. Only one taxon, *Bunaxella zebedielensis* was rare, and appeared to be restricted to the “inside” canopy position in September (Table 1) and the “south” position February (Table 2).

### *3.3. Environmental variability within the canopy of Gardenia thunbergia*

The first few days after the iButtons were placed in the tree, the weather was cold and wet. Thereafter the weather improved, and data from a period of 10 successive days was used in subsequent analyses. Unfortunately, the data logger on the west side of the tree was stolen or removed, so no data for this position is available. Table 1 gives a summary of the climate variables. The top, east and north facing aspect were the warmest, while south, inside and bottom were coolest positions (Table 3). The top and east and inside position had the lowest mite numbers, while the cooler sites (bottom and south) had more mites and greater diversity. Mite

**Table 1:** Sum of individuals of each mite species encountered inside domatia of *Gardenia thunbergia* obtained from 10 leaves sampled at each of the different canopy positions.

Family ( <i>species name</i> )	North	South	East	West	Top	Bottom	Inside
Eriophyoidea ( <i>Acalus comatus</i> )	124	64	70	108	85	133	54
Phytoseiidae ( <i>Euseius addoensis</i> )	16	20	27	8	13	17	22
Stigmaeidae ( <i>Agistemus tranatalensis</i> )	10	20	6	14	3	18	5
Phytoseiidae ( <i>Amblyseius anomalus</i> )	33	48	42	32	26	79	32
Anystidae ( <i>Anystis baccarum</i> (Linnaeus))	25	23	21	26	35	33	25
Iolinidae ( <i>Lourus citricolus</i> )	71	131	56	62	79	67	78
Cunaxidae ( <i>Bunaxella zebedielensis</i> )	4	4		7	2	2	2
Oribatidae (Oribatula sp)							3
Acaridae - nymph		1		1	2		3

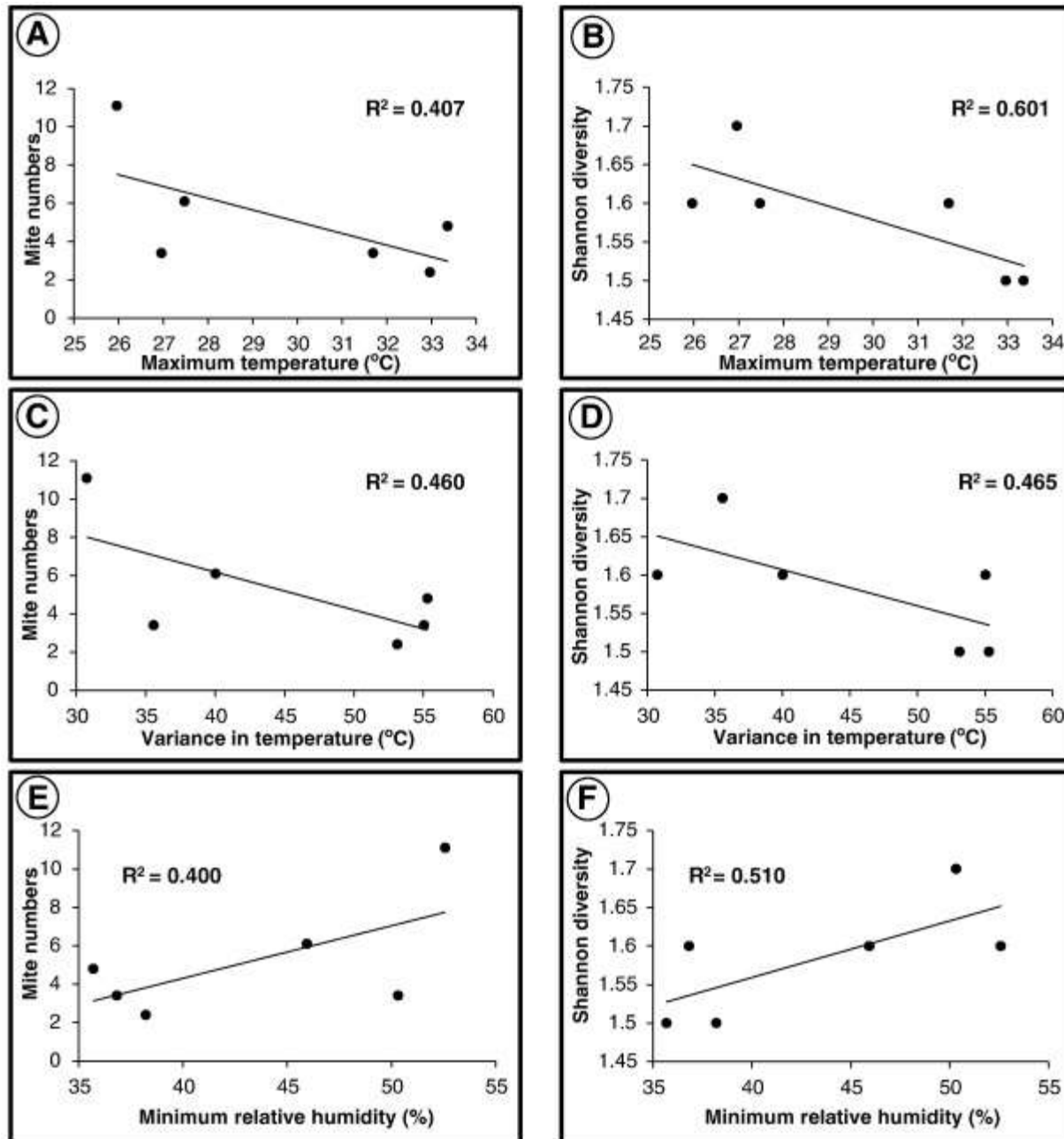
**Table 2:** Numbers of each mite species found on 10 leaves from each of the different canopy positions of the tree of *Gardenia thunbergia* fitted iButtons.

Family ( <i>species name</i> )	North	South	East	West	Top	Bottom	Inside
Eriophyoidea ( <i>Acalus comatus</i> )	14	15	8	43	6	74	
Phytoseiidae ( <i>Euseius addoensis</i> )	2	4	11	4	7		6
Stigmaeidae ( <i>Agistemus tranatalensis</i> )	5	2	2	10	2		
Phytoseiidae ( <i>Amblyseius anomalus</i> )	11	5	11	7	5	23	4
Anystidae ( <i>Anystis baccarum</i> (Linnaeus))	5	14	2	1		13	7
Iolinidae ( <i>Lourus citricolus</i> )	11	18		2		1	14
Cunaxidae ( <i>Bunaxella zebedielensis</i> )		3					
Oribatidae (Oribatula sp)							3

**Table 3:** Climatic data obtained from I-buttons placed in different canopy positions of *Gardenia thunbergia* .

	North	South	East	Top	Bottom	Inside
Average Max Temp	33°C (±7.04)	27°C (±4.86)	32°C (±5.62)	33°C (±7.20)	26 °C (±5.04)	27°C (±5.20)
Average min temp	13°C (±4.36)	13°C (±4.46)	12°C (±2.84)	14°C (± 4.36)	14°C (±4.20)	14°C (±4.24)
Average Max Re Humidity	98 (± 2,42)	102 (±1.96)	100 (± 1.80)	95 (±1.22)	99 (±1.94)	98 (±2.02)
Average Min Re Humidity	36 (±18.42)	45 (±11.55)	37 (±9.66)	38 (±12.45)	52 (±13.38)	50 (± 12.79)
Var. Temp	55.30	40.03	55.05	53.12	30.75	35.57
Var. Re Humidity	397.37	440.31	530.85	5359.07	307.81	328.83

number and mite diversity was found to be most strongly correlated with maximum temperature, variance in temperature and minimum relative humidity (Figure 3).



**Fig. 3.** Correlation plots showing relationships between mite abundance, Shannon diversity index and temperature and relative humidity for *Gardenia thunbergia*.

#### 4. Discussion

Little is known about the mite biota found within forest habitats (Arroyo et al. 2010) especially the forests of Southern Africa. An improved understanding of where mites are found within the

canopy of trees is important as it may enable a better understanding of how the protective mutualism between plants and mites might work and how mites provide protection to host plants. Such knowledge is also of value when planning future sampling strategies for studies on mites, as diversity and even species composition may vary depending on the canopy position sampled. Our results show that mites are more abundant and diverse at lower and internal parts of the tree canopy where it is cooler and more humid. We propose two not mutually exclusive explanations for this result:

Firstly, this might be a factor of leaf age; more mites may accumulate on older leaves that are found at lower levels and at the bottom of the canopy. The longer the leaf is there on the tree the more likely it is that that mite will colonize and that populations will establish. Parolin et al. (2011) found a positive relationship between the leaf maturity and the presence of the predatory mite *Amblyseius californicus* on *Viburnum tinus*; the mite was more frequent on old and mature leaves which possessed more domatia than young leaves. Moreover, mites may prefer older leaves because the domatia of older leaves are more developed than domatia on newly formed leaves. However, as an effort was made to sample mature leaves, this may not be an adequate explanation for the observed differences.

A second explanation is that mites may reside at lower levels of tree canopy because the micro-climate is more favorable (e.g. cooler, more humid and shaded; Aussenac 2000). Forest micro-climate (patterns of temperature, moisture, wind and light) plays an important role in influencing insects and arthropods habitat selections (Chen et al. 1999; Fukui 2001). Onzo et al. (2003) observed high densities of herbivorous mites on lower and older leaves of Cassava plants. They suggest that these mites migrated to older leaves in the presence of higher densities of predatory mites. However contrary to our findings they observed high densities of predatory mites in the

plant apex during the day and these predatory mites migrated down to forage at night. They suggest that these mites preferred to reside in the closed leaves of the apex during the day because they provide a safe refuge from harsh environmental conditions.

Desiccation is a major environmental hurdle for invertebrates especially during molting and relative humidity inside shelters is significantly higher than that of the leaf surface. The domatia of both species sampled had trichomes, the role of which is not understood, but they may aid in retaining moisture and high levels of humidity within domatia. It has been reported that higher relative humidity allows insect larvae to survive and grow better than at low humidity (Fukui, 2001). At the top of the canopy, the environmental gradients may be stronger, thus reducing humidity and increasing light levels such that mites avoid these parts of the tree, as evidenced by our iButton data which indicates that at the exposed top and northern sites, temperatures are higher, and humidity levels can decrease considerably.

## **5. Conclusion**

To the best of our knowledge, this study is the first to document mite distribution within the canopy of domatia-bearing tree species, and to correlate diversity and abundance to environmental variables. Our results show that mites may respond to intra-canopy micro-climate, avoiding canopy positions of greater temperature, and lower humidity. However, this result must be viewed as preliminary, as similar studies on additional tree species are required to confirm these findings. In addition, studies to document diurnal movements of mites and correlate these to canopy micro-climate variables would prove insightful. However, based on these results, we suggest that future studies on mites and leaf domatia should employ a comprehensive sampling

approach, and avoid the sampling of exposed and environmentally variable portions of the canopy.

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## **References**

- Agrawal, A., 1997. Do leaf domatia mediate a plant–mite mutualism? An experimental test of the effects on predators and herbivores. *Ecological Entomology* 22, 371–376.
- Arroyo, J., Moraza, M.L., Bolger, T., 2010. The Mesostigmatid mite (Acari, Mesostigmata) community in canopies of Sitka spruce in Ireland and a comparison with ground moss habitats. *Graellsia* 66(1), 29-37.
- Aussenac, G., 2000. Interactions between forest stands and climate: Ecophysiological aspects and consequences for silviculture. *Annals of Forest Science* 5, 287–301.

- Beaulieu, F., Walte,r D.E., Proctor, H.C., Kitching, R.L., 2010. The Canopy Starts at 0.5m: Predatory Mites (Acari: Mesostigmata) Differ between Rain Forest Floor Soil and Suspended Soil at any Height. *Biotropica* 42, 704-709.
- Boon, R., Pooley, E., 2010. Pooley's Trees of Eastern South Africa; A Complete Guide. Flora and Fauna Publications Trust, Durban.
- Chen, J., Saunders, S.C., Crow, T.R., Naiman, R.J., Brosofske, K.D., Mroz, G.D., Brookshire, B.L., Franklin, J.F., 1999. Microclimate in Forest Ecosystem and Landscape Ecology. *BioScience* 49, 288-297.
- Dial, R.J., Ellwood, M.D.F., Turner, E.C., Foste, W.A., 2006. Arthropod Abundance, Canopy Structure, and Microclimate in a Bornean Lowland Tropical Rain Forest. *Biotropica* 38, 643-652.
- Erwin, T.L., 2013. Forest Canopies, Animal Diversity. In: Levin, S.A., (ed.) *Encyclopedia of Biodiversity*, second edition, Volume 3, pp. 511-515. Waltham, MA: Academic Press.
- Fukui, A., 2001. Indirect interactions mediated by leaf shelters in animal–plant communities. *Population Ecology* 43, 31–40.
- Harley, P., Guenther, A., Zimmerman, P., 1996. Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (*Liquidambar styraciflua*) leaves. *Tree Physiology* 16, 25-32.
- Krantz, G.W., Evans, D. and Walter, D.E., 2009. *A manual of acarology* 3<sup>rd</sup> Edn. Texas University Press, Texas.



- Lindo, Z., Winchester, N.N., 2006. A comparison of microarthropod assemblages with emphasis on oribatid mites in canopy suspended soils and forest floors associated with ancient western redcedar trees. *Pedobiologia* 50, 31–41.
- Magalhães, S., Janssen, A., Sabelis, R.H.M.W., 2012. Flexible antipredator behavior in herbivorous mites through vertical migration in a plant. *Oecologia* 132, 143–149.
- Matos, C.H.C., Pallini, A., Chaves, F.F., Schoereder, J.S., Janssen, A., 2006. Do domatia mediate mutualistic interactions between coffee plants and predatory mites? *Entomologia Experimentalis et Applicata* 118, 185–192.
- Nadkarni, N.M., 1994. Diversity of Species and Interactions in the Upper Tree Canopy of Forest Ecosystems. *American Zoology* 34, 70–78.
- Norton, A.P., English-Loeb, G., Gadoury, D., and Seem, R.C., 2000. Mycophagous mites and foliar pathogens: leaf domatia mediate tritrophic interactions in grapes. *Ecology* 81, 490–499.
- O'Dowd, D.J., Pemberton, R., 1998. Leaf domatia and foliar mite abundance in broadleaf deciduous forest of north Asia. *American Journal of Botany* 85(1), 70–78.
- O'Dowd, D.J., Willson, M.F., 1991. Associations between mites and leaf domatia. *Trends in Ecology and Evolution* 6, 179–182.
- Onzo, A., Hanna, R., Zannou, I., Sabelis, M.W., Yaninek, J.S., 2003. Dynamics of refuge use: diurnal, vertical migration by predatory and herbivorous mites within cassava plants. *Oikos* 101, 59–69.
- Parker, G.G., 1995. The Structure and Microclimate of Forest Canopies In: Lowman, M.D., and Nadkarni, N.M., (Eds.), *Forest Canopies*, Academic press Inc., pp 73–106.

- Parolin, P., Bresch, C., Muller, M.M., Errard, A., Poncet, C, 2011. Distribution of acarodomatia and predatory mites on *Viburnum tinus*. *Journal of Mediterranean Ecology* 11, 41-48.
- Pemberton, R.W., Turner, C.E., 1989. Occurrence of predatory and fungivorous mites in leaf domatia. *American Journal of Botany* 76, 105-112.
- Pereira, P.T., de Carvalho, M.M., Gírio, F.M., Roseiro, J.C., Amaral-Collaco, M.T., 2002. Diversity of microfungi in the phylloplane of plants growing in a Mediterranean ecosystem. *Journal of Basic Microbiology* 42, 396- 407.
- Pickett, C.H., Wilson, L.T., Gonzalez D., 1988. Population Dynamics and Within-Plant Distribution of the Western Flower Thrips (Thysanoptera: Thripidae), an Early-Season Predator of Spider Mites Infesting Cotton. *Environmental Entomology* 17(3), 551-55.
- Walter, D.E., Behan-Pelletier, V., 1999. Mites in forest canopies: Filling the Size Distribution Shortfall? *Annual Review of Entomology* 44, 1–19.
- Walter, D.E., O'Dowd, D.J., 1992. Leaves with domatia have more mites. *Ecology* 73, 1514-1518.
- Walter, D.E., and O'Dowd, D.J., 1995. Beneath biodiversity: factors influencing the diversity and abundance of canopy mites. *Selbyana*, 16, 12–20.
- Wilson, L.T., Gonzalez, D., Leigh, T.F., Maggi, V., Foristiere, C., Goodell P., 1983. Within-Plant Distribution of Spider Mites (Acari: Tetranychidae) on Cotton: A Developing Implementable Monitoring Program. *Environmental Entomology* ,) 128-134.
- Zweifel, R., Bohm, J.P., Hasler, R., 2002. Midday stomatal closure in Norway spruce-reactions in the upper and lower crown. *Tree Physiology* 22, 1125–1136.