Host specificity studies on *Gynaikothrips* (Thysanoptera: Phlaeothripidae) associated with leaf galls of cultivated *Ficus* (Rosales: Moraceae) trees

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Abstract

Host specificity tests on *Gynaikothrips ficorum* (Marchal) and *Gynaikothrips uzeli* (Zimmerman) (Thysanoptera: Phlaeothripidae) have shown that under experimental conditions, *G. ficorum* will induce leaf galls on both *Ficus benjamina* L. and *Ficus microcarpa* L. f. (Rosales: Moraceae), but *G. uzeli* will induce galls only on *F. benjamina*. A further interesting aspect of the results is that gall induction by *G. uzeli* on *F. benjamina* appears to have been suppressed in the presence of *F. microcarpa* plants in the same cage. *Liothrips takahashii* (Moulton) (Thysanoptera: Phlaeothripidae), an inquiline in the galls of these *Gynaikothrips*, is reported for the first time from Australia, mainland China, Malaysia, Costa Rica, and western USA.

Key Words: *Gynaikothrips uzeli*; *Gynaikothrips ficorum*; *Ficus benjamina*; *Ficus microcarpa*; gall-inducing thrips; *Liothrips takahashii*

Resumen

Las pruebas de especificidad de plantas hospederas de *Gynaikothrips ficorum* (Marchal) y *Gynaikothrips uzeli* (Zimmerman) (Thysanoptera: Phlaeothripidae) han demostrado que en condiciones experimentales, *G. ficorum* inducirá agallas tanto en *Ficus benjamina* L. y *Ficus microcarpa* L. f. (Rosales: Moraceae), pero *G. uzeli* inducirá agallas solamente en *F. benjamina*. Un aspecto adicional interesante de los resultados es que la inducción de agallas por parte de *G. uzeli* en *F. benjamina* parece haber sido suprimida por la presencia de plantas de *F. microcarpa* en la misma jaula. Se reporta por primera vez *Liothrips takahashii* (Moulton) (Thysanoptera: Phlaeothripidae), un inquilino en las agallas de estos *Gynaikothrips*, de Australia, China continental, Malasia, Costa Rica, y el oeste de EE.UU.

Palabras Clave: *Gynaikothrips uzeli*; *Gynaikothrips ficorum*; *Ficus benjamina*; *Ficus microcarpa*; trips que inducen agallas; *Liothrips takahashii*

Forty species are currently listed under the generic name *Gynaikothrips* Zimmerman (Thysanoptera: Phlaeothripidae) (ThripsWiki 2014). Some of these are known to induce galls on young developing leaves of their hosts, and 12 of the species are recorded from various species of *Ficus* (Rosales: Moraceae) in the Old World tropics (Mound 1994; Tree & Walter 2009). The 2 most widely cultivated ornamental figs *Ficus benjamina* L. and *Ficus microcarpa* L. f. commonly bear leaves that are distorted due to the feeding of either *Gynaikothrips uzeli* (Zimmerman) or *Gynaikothrips ficorum* (Marchal) (Mound et al. 1995; Held et al. 2005; Tree 2012; Melo et al 2013). These distortions are induced by the feeding of 1 or more adults, and this causes a young leaf to fold and/or to curl. Eggs are laid on the surface of the leaf, where the resultant larvae and adults continue to feed within this enclosure (Mound & Morris 2005; Tree & Walter 2009).

Gynaikothrips uzeli is very similar in structure to *G. ficorum.* The only obvious structural difference between them is that females of *G. uzeli* usually have the pronotal posteroangular pair of setae at least 0.7 times as long as the pronotal epimeral setae, whereas *G. ficorum* females have the posteroangular setae usually no longer than the pronotal discal setae and never more than 0.5 times as long as the pronotal epimeral setae (Mound et al 1995; Tree 2012). However, the length of the posteroangular setae (Fig. 1) can vary in both species, causing identification problems, particularly when identification of a single individual is requested (Mound & Marullo 1996; Goldarazena et al. 2008). Moreover, bilateral asymmetry has been observed in the lengths of

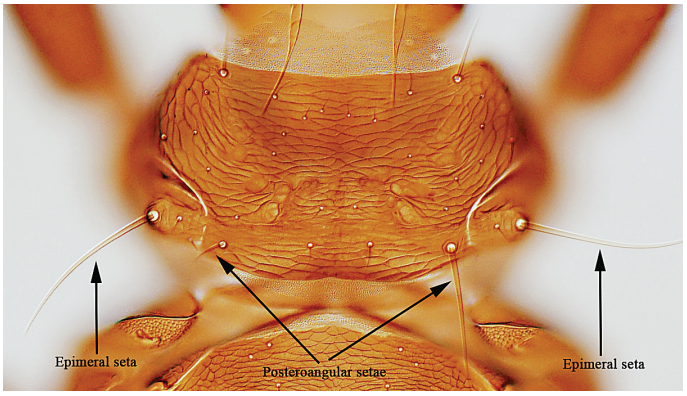

Fig. 1. Pronotal posteroangular setae showing variation in length.

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these setae on some individuals (Mound & Tree, personal observations 2014) (Fig. 1). Despite these limited morphological differences, *G. uzeli* and *G. ficorum* are still considered 2 distinct species (Mound et al. 1995; Mound & Marullo 1996). The 2 thrips species usually are associated with 2 different *Ficus* species, *G. uzeli* with *F. benjamina* and *G. ficorum with F. microcarpa*, but identification of these 2 plant species by entomologists may not be entirely reliable. Generally, the leaves of *F. microcarpa* are wider in the apical than the basal half and have an acute apex, whereas leaves of *F. benjamina* are widest in the basal half and have an acuminate apex. However, published host data for thrips may still reflect incorrect identifications of the host species.

There are no published experimental studies on the apparent host specificity of *G. uzeli* and *G. ficorum*, and the host specificity tests reported here were designed to examine 2 questions. Can *G. uzeli* induce leaf galls only on *F. benjamina* and not on *F. microcarpa*? And conversely, can *G. ficorum* induce leaf galls only on *F. microcarpa* and not on *F. benjamina*?

Materials and Methods

Two host specificity experiments were conducted in a greenhouse at James Cook University, Cairns, North Queensland, Australia, in late 2013. The 1st experiment tested the host preferences of *G. ficorum* on *F. microcarpa* and *F. benjamina*, and the 2nd experiment tested the host preferences of *G. uzeli* on *F. microcarpa* and *F. benjamina.*

Fifteen rearing cages, 61 × 61 × 91.5 cm (24 × 24 × 36 inch), were used to house young fig trees in a greenhouse, with 4 fig trees per cage (Fig. 2). The collapsible white mesh (90 µm) had 3 mesh sides for ventilation and 1 clear vinyl side for easy viewing (Bioquip, California, USA). Thirty young plants 30 to 40 cm (12 to 16 inch) in height of *F. benjamina* (unregistered cultivar 'Cairns *benjamina*' voucher: Cairns ex. cult. Dec 2013 *Field A.R. ARF4051* CNS) and 30 young plants of *F. microcarpa* (unregistered cultivar 'Cairns *hillii'* voucher: details Cairns ex cult. Dec 2014 *Field A.R. 4055* CNS) were purchased from Limberlost Nursery, Freshwater, Cairns, Australia, and care was taken to ensure that the plants were free from all invertebrates and insecticides. The plants were foliar sprayed with a non-residual pyrethroid insecticide and washed 28 d before the start of the experiments. Five cages were set up with 4 *F. microcarpa* plants in each cage (no-choice), 5 cages with 2 *F. microcarpa* plants and 2 *F. benjamina* plants in each cage (choice) and 5 cages with 4 *F. benjamina* plants in each cage (no-choice). The cages were arranged randomly in the greenhouse. The plants were foliar and pot fertilized on alternating fortnights with spray drench of Seasol (www.seasol.com.au) at 4 mL/L, GrowForce 9 (www.growforce.com.au; N:PK 14:3.6:23.2) applied at 5g/L, calcium nitrate applied at 1g/L, and magnesium sulphate applied at 1g/L. This regimen encouraged the generation of new leaves for the thrips to feed on and induce galls. Shoot tips were pruned once every 6 wk to maintain plant size and to stimulate and even out new leaf production. As the experiments were conducted during summer and the plants were well watered, this pruning ensured that the plants continually produced new leaves throughout the duration of the experiments.

Fig. 2. Four *Ficus benjamina* plants inside a collapsible cage.

GYNAIKOTHRIPS FICORUM HOST SPECIFICITY TEST

Approximately 80 mature, infested leaf galls of *G. ficorum* were collected from a local *F. microcarpa* tree (parent plant) (unregistered cultivar Cairns '*hillii*'). The fig species was confirmed by A. Field (Senior Botanist, Queensland Herbarium, Australia). The galls were kept on ice in a cooler while the identity of the thrips species in the leaf galls was confirmed. The cooler temperatures helped prevent thrips moving out of the galls until they were required to inoculate the fig plants. Twenty of the leaf galls were sampled destructively to confirm the identification of the *Gynaikothrips* species. Four mature *Gynaikothrips* were slide mounted from each of these 20 galls (i.e., 80 adults) and confirmed as *G. ficorum* according to Mound et al. (1995).

The remaining 60 galls were placed inside the 15 cages, 1 gall per plant, and placed on top of the soil. The plants were not watered for the first 2 d, to prevent drowning of the thrips and to allow the thrips to move out of the galls. The plants then were watered continuously from the base of the pot when the base trays dried out, generally every 3 d.

Forty-two days after the commencement of the test, all the galls were removed from the plants and counted (Table 1). Total numbers of galls were recorded per fig species per cage and the thrips removed from all galls and stored in 95% ethanol. Representative samples of the thrips were slide mounted from 8 of the 15 cages and identified. The remaining thrips from the galls were identified with a stereo micro-

Table 1. *Gynaikothrips ficorum* host specificity test. Number of galls and thrips species per cage per plant species.

Cage number – plant species	Number of thrips galls	Number of thrips adults
Cage 1 – *F. microcarpa* × 4	3	9 *G. ficorum*
Cage 2 – *F. microcarpa* × 4	2	35 *G. ficorum*
Cage 3 – *F. microcarpa* × 4	20	73 *G. ficorum*
Cage 4 – *F. microcarpa* × 4	4	41 *G. ficorum*
Cage 5 – *F. microcarpa* × 4	28	95 *G. ficorum*
Cage 6 – *F. microcarpa* × 2	19	31 *G. ficorum*
Cage 6 – *F. benjamina* × 2	6	98 *G. ficorum*
Cage 7 – *F. microcarpa* × 2	2	2 *G. ficorum*
Cage 7 – *F. benjamina* × 2	14	57 *G. ficorum,* 6 *G. uzeli*
Cage 8 – *F. microcarpa* × 2	4	9 *G. ficorum*
Cage 8 – *F. benjamina* × 2	0	0 thrips
Cage 9 – *F. microcarpa* × 2	1	2 *G. ficorum*
Cage 9 – *F. benjamina* × 2	5	10 *G. ficorum*
Cage 10 – *F. microcarpa* × 2	0	0 thrips
Cage 10 – *F. benjamina* × 2	16	90 *G. ficorum*
Cage 11 – *F. benjamina* × 4	5	14 *G. ficorum*, 1 *G. uzeli*
Cage 12 – *F. benjamina* × 4	3	18 *G. ficorum*
Cage 13 – *F. benjamina* × 4	9	18 *G. ficorum,* 1 *G. uzeli*
Cage 14 – *F. benjamina* × 4	3	5 *G. ficorum*
Cage 15 – *F. benjamina* × 4	2	13 *G. ficorum*

scope by determining the length of the pronotal posteroangular setae. Total adult (but not larval) thrips numbers were counted (Table 1). Larvae were not counted as their identification could not be confirmed.

GYNAIKOTHRIPS UZELI HOST SPECIFICITY TEST

The plants used in the above *G. ficorum* host specificity test were subsequently used in this *G. uzeli* host specificity test. After the 1st experiment, all galls were removed, branches were tip pruned, and the plants rested for 43 d. During this time, the plants were sprayed and then washed with non-systemic and non-residual pyrethroid insecticide to ensure no invertebrates were carried over from the 1st to the 2nd experiment. The plants were fertilized to encourage generation of new leaves. For at least 1 wk prior to setting up the 2nd experiment, no thrips leaf galls were observed on these plants.

Approximately 80 mature, infested leaf galls of *G. uzeli* were collected from a local *F. benjamina* tree. The fig species was confirmed by A. Field (Senior Botanist, Queensland Herbarium, Australia). Twenty of these galls were sampled destructively to confirm the identification of the *Gynaikothrips* species. Four mature *Gynaikothrips* were slide mounted from each of these 20 galls and confirmed as *G. uzeli* according to Mound et al. (1995).

The remaining 60 galls were placed inside the 15 cages, 1 gall per plant, and placed on top of the soil. The plants were not watered for the first 2 d, to prevent drowning of the thrips and to allow the thrips to move out of the galls. The plants then were watered continuously from the base of the pot when the base trays dried out, generally every 3 d.

Forty-five days after the commencement of the test, all the galls were removed from the plants and counted (Table 2). Total numbers of galls were recorded per fig species per cage and the thrips removed from all galls and stored in 95% ethanol. Representative samples of the thrips were slide mounted from 6 of the 15 cages and identified. The remaining thrips from the galls were identified with a stereo microscope by determining the length of the pronotal posteroangular setae. Total adult (but not larval) thrips numbers were counted (Table 2). Larvae were not counted as their identification could not be confirmed.

Table 2. *Gynaikothrips uzeli* host specificity test. Number of galls and thrips species per cage per plant species.

Cage number – plant species	Number of thrips galls	Number of thrips adults
Cage 1 – *F. microcarpa* × 4	0	0 thrips
Cage 2 – *F. microcarpa* × 4	0	0 thrips
Cage 3 – *F. microcarpa* × 4	0	0 thrips
Cage 4 – *F. microcarpa* × 4	0	0 thrips
Cage 5 – *F. microcarpa* × 4	0	0 thrips
Cage 6 – *F. microcarpa* × 2	0	0 thrips
Cage 6 – *F. benjamina* × 2	0	0 thrips
Cage 7 – *F. microcarpa* × 2	0	0 thrips
Cage 7 – *F. benjamina* × 2	15	32 *G. uzeli*
Cage 8 – *F. microcarpa* × 2	0	0 thrips
Cage 8 – *F. benjamina* × 2	0	0 thrips
Cage 9 – *F. microcarpa* × 2	0	0 thrips
Cage 9 – *F. benjamina* × 2	0	0 thrips
Cage 10 – *F. microcarpa* × 2	0	0 thrips
Cage 10 – *F. benjamina* × 2	0	0 thrips
Cage 11 – *F. benjamina* × 4	28	74 *G. uzeli*
Cage 12 – *F. benjamina* × 4	9	10 *G. uzeli*
Cage 13 – *F. benjamina* × 4	38	60 *G. uzeli*
Cage 14 – *F. benjamina* × 4	25	79 *G. uzeli*
Cage 15 – *F. benjamina* × 4	23	161 *G. uzeli*

Results

GYNAIKOTHRIPS FICORUM HOST SPECIFICITY TEST

The 20 plants held in the 5 cages that housed only *F. microcarpa* plants produced altogether 57 leaf galls, and these galls contained 253 *G. ficorum.* The 20 plants held in the 5 cages that housed both *F. microcarpa* (10) and *F. benjamina* (10) plants produced 67 leaf galls, and these galls contained 299 *G. ficorum* and 6 *G. uzeli*. The 26 leaf galls on *F. microcarpa* contained 44 *G. ficorum*, and the 41 leaf galls on *F. benjamina* contained 255 *G. ficorum* and 6 *G. uzeli*. The 20 plants held in the 5 cages that housed only *F. benjamina* produced 22 galls and these galls contained 68 *G. ficorum* and 2 *G. uzeli*. The 8 *G. uzeli* specimens listed above were all found inside galls together with *G. ficorum*.

In summary, 146 leaf galls were produced on both *F. microcarpa* and *F. benjamina* plants. From these galls, 620 *G. ficorum* were recovered together with 8 *G. uzeli*. The 83 leaf galls on *F. microcarpa* contained 297 *G. ficorum*, and the 63 leaf galls on *F. benjamina* contained 323 *G. ficorum* and 8 *G. uzeli*.

GYNAIKOTHRIPS UZELI HOST SPECIFICITY TEST — SECOND EXPERIMENT

The 20 plants held in the 5 cages that housed only *F. microcarpa* plants produced no leaf galls. The 20 plants held in the 5 cages that housed both *F. microcarpa* (10) and *F. benjamina* (10) plants produced 15 leaf galls. These leaf galls were found on 2 *F. benjamina* plants in 1 cage and contained 32 *G. uzeli*. The 20 plants held in the 5 cages that housed only *F. benjamina* produced 123 galls, and these galls contained 384 *G. uzeli*.

In summary, 138 leaf galls were produced on *F. benjamina* plants, and these contained 416 *G. uzeli*. There were no leaf galls induced on any *F. microcarpa* plants, and no *G. ficorum* were found in any of the leaf galls on *F. benjamina* in this 2nd experiment.

Discussion

Under these experimental conditions, the results indicate that *G. ficorum* can readily induce leaf galls on both *F. microcarpa* and *F. benjamina* (Table 1), whereas *G. uzeli* will induce leaf galls only on *F. benjamina* (Tables 1 and 2). Curiously, when given a choice between *F. microcarpa* and *F. benjamina*, *G. ficorum* not only produced more galls on *F. benjamina* than on *F. microcarpa* (41 vs. 26), but also considerably more adults (255 vs. 44). This apparent preference by *G. ficorum*, under experimental conditions, for a plant species on which it is not commonly found under field conditions, will require further study. One possible explanation for this preference by *G. ficorum* is that in the field, *G. uzeli* "outcompetes" *G. ficorum* when inducing galls on *F. benjamina*. It would be useful to include in further studies the testing of both *Gynaikothrips* species together in the same cages within the same experimental design. The cultivated *F. benjamina* and *F. microcarpa* used were both chosen because they were typical of wild populations and were observed to be susceptible to leaf galls in nursery culture.

Thrips are well known to be thigmotactic in behavior, that is, they like to seek shelter in tiny cracks and crevices on the bases of leaves or stems/branches, making them sometimes impossible to detect. This could have been the reason why low numbers of *G. uzeli* were detected in the 1st experiment. Also, *G. uzeli* could have been present in low numbers in the *G. ficorum* leaf galls collected from the parent plant at the beginning of the experiment. This would not be surprising, because Tree & Walter (2009) found that *Gynaikothrips* adults can move

freely between galls, generally during the day. Moreover, over the last 10 yr, the first author has collected *Gynaikothrips* species widely from fig trees across Australia and has observed on several occasions mixed species within the same leaf gall on both *F. benjamina* and *F. microcarpa*. Therefore, the method of using the host species identification as an aid in the identification of the *Gynaikothrips* species cannot be relied upon.

There were limitations to the experimental design involved. The plants were not standardized, either in their selection or in their arrangement during the experiments. Some plants received more light or more heat than others, and similarly, some plants suffered more water lack than others as a result of their position in the available greenhouse. As a result, generation of new leaves was not consistent, both within and between cages. Because of these design limitations, no attempt has been made to apply any statistical analyses to the results. Similar experiments are required, with a more rigorous protocol and with other thrips populations, to further evaluate the host specificity of these thrips.

This study also revealed the presence in small numbers of an unrelated inquiline thrips species in some of the galls on *F. microcarpa*. This species was *Liothrips takahashii* (Moulton) (Thysanoptera: Phlaeothripidae), the identity of which was confirmed with the diagnostic key to *Liothrips* of Japan (Okajima 2006), and also by comparison with specimens of this species from Taiwan in the Australian National Insect Collection. Originally described from Taiwan, this species is recorded by Okajima (2006) from southern Japan (Ryukyu Islands) and Indonesia (Java and Sumatra). During our studies on *Gynaikothrips*, we have seen specimens of *L. takahashii* from the following localities, all of which constitute new locality records: Australia (Queensland, Cairns), Malaysia (Selangor), China (Sichuan), western USA (Los Angeles, California), and Costa Rica (Heredia, Santo Domingo).

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