Infestation of *Mangifera indica* by the mango gall fly, *Procontarinia matteiana*, (Kieffer & Cecconi) (Diptera: Cecidomyiidae)

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Mango gall fly (*Procontarinia matteiana* Kieffer & Cecconi, 1906) is an orchard pest that infests flush leaves of mango, forming wart-like structures on the leaves. Serious outbreaks may result in reduced fruit yield. A natural parasite (*Chrysonotomyia pulcherimma* Kerrich, 1970) of the gall fly lays its eggs inside the gall and the larvae feed on the gall fly. Mango cultivars present varying susceptibilities to gall fly infestation, with cultivars ranging from completely resistant, highly susceptible to intermediate stages where pseudo-galls are formed. The latter cultivars are ovipositioned by the gall fly, but secondary metabolites within the leaves possibly halt the development, thereby preventing the development of true galls. Microscopy was used to identify characteristic features of the gall fly and its parasite inside the gall, to study the development of the insects and to distinguish them. Evidence was obtained that the use of insecticides curbs the development of the larvae. Tissue development within true and pseudo-galls was studied to provide insights into the role of secondary plant metabolites in arresting true gall formation. This study will contribute to a more holistic approach to pest management of mango.

Key words: mango gall fly, scanning electron microscopy, gall structures, systemic insecticide, parasite.

INTRODUCTION

Mangifera indica (mango) is often referred to as the king of fruit, because of the succulent, exotic flavour and delicious taste. Over 25 million tonnes of mangoes are produced annually by 87 countries with Africa contributing 9 % of world production (Saūco 2004). Mango is a profitable source of revenue for South Africa; however, there is a growing threat that fruit yields may be reduced as a result of mango gall fly infestations. Numerous species of mango gall fly have been identified worldwide (Raman *et al.* 2009). The predominant species occurring in South Africa is *Procontarinia matteiana* (Schoeman *et al.* 1996). Almost all of the *Procontarinia* midges induce galls on leaves, but did not evolve to parasitize other plant organs or taxa. Galls were discovered on fossil leaves of an ancestor of *M. indica* from Upper Palaeocene sediments of northeastern India. This indicates that the feeding behaviour of species of *Procontarinia* has not significantly changed over time. An explanation for this apparent feeding specialization may be low selection pressure due to the abundance and distribution of populations of *M. indica* (Raman *et al.* 2009).

The female gall fly oviposits on flush leaves, maggots hatch from the eggs and tunnel into the leaf tissue where the insects develop into mature gall flies. Tumour-like growths develop on the host plants as a result of chemical stimuli from the galling insects. These stimuli can be maternal secretions injected during ovipositioning or stimuli produced by larvae developing within the plant tissue (Pascual-Alvarado *et al.* 2008; Stone & Schönrogge 2003). Colonized plants provide the insect with food and shelter to the detriment of the host (Tooker & De Moraes 2008). In this study it was confirmed that a group of volatile compounds, produced by the plant is associated with gall fly infestation (Augustyn *et al.* 2010a).

Differential susceptibility of mango cultivars to gall fly infestation is a worldwide occurrence. Githure *et al.* (1998) classified 11 South African mango cultivars into categories of susceptibility to *P. matteiana*. Cultivars that are highly susceptible to gall fly infestation exhibit large numbers of galls

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per leaf, while other cultivars display signs of apparently unsuccessful gall development in the form of so-called pseudo-galls (Githure *et al.* 1998; Schoeman *et al.* 1996)

Resistant cultivars were found to be generally free of galls. This observed resistance has been attributed to antixenosis properties of the cultivar, rendering it unsuitable for feeding, shelter or ovipositioning by insects.

The mango gall fly is not a serious economic problem in India to which they are indigenous, because parasitoids are able to control their numbers (Sankaran 1988). Although the insect was not of concern in the past, areas such as Oman, Mauritius, Kenya, Réunion, Italy and recently South Africa, have experienced serious outbreaks. This is largely due to a lack of natural enemies, combined with the favourable ecological conditions prevailing in these regions. In the past, gall fly has been of little consequence to producers as only flush leaves were attacked, leaving the fruit unharmed (Sankaran 1988). However, in 2004 a newly identified species, *Procontarinia frugivora,* that attacks only fruit, was reported (Gagné & Medina 2004). Though this species is currently thought to be restricted to the Luzon Island of the Philippines, its emergence has placed mango gall fly in the spotlight as a potential threat to global mango production.

Mango cultivation is a lucrative industry and globally, growers constantly strive to improve production. To sustain yield and quality, insect pests are managed by the application of insecticides. Environmentally sustainable chemical control of the gall fly is only successful if the active substance applied is a systemic insecticide, such as thiamethoxam WG 250 g/kg (Daneel *et al.* 2000). Sprayed insecticides are only effective in the case of young flush leaves due to the immature nature of the epicuticular layers.

In this study, microscopy was employed to investigate the development and presence of the gall fly and its parasite inside the gall, as part of a more comprehensive approach to management of this pest. Tissue development within true and pseudo-galls was studied to provide insight into the role of secondary plant metabolites in arresting true gall formation.

MATERIAL AND METHODS

Scanning electron microscopy

Mango leaf sections with occupied galls and pseudo-galls were fixed in 10 % phosphate-buffered formalin and the relevant areas dissected for further processing. The fixative was replaced by 0.13 M Millonig's phosphate buffer (pH 7.2) for 30 min, whereafter the samples were rinsed in distilled water for 30 min. This was followed by dehydration in a graded absolute ethanol series up to 100 % absolute ethanol, critical-point drying (Polaron E3100, West Sussex, U.K.), mounting onto aluminium stubs and sputter-coating (Polaron E5100, Watford, U.K.) with palladium. Samples were examined with a JEOL 840 scanning electron microscope (JEOL, Tokyo, Japan) operated at 8 kV.

Light microscopy

Leaf sections displaying galls or pseudo-galls were fixed in 4 % formaldehyde in 0.075 M phosphate buffer (pH 7.4) for 1 h. Samples were first rinsed three times for 10 min, in 0.075 M phosphate buffer and were followed by an ascending series of ethanol solutions from 50 % to 100 % (15 min, in each solution) and twice more in additional fresh 100 % ethanol. The plant material was first infiltrated with 50 % LR White resin (SPI Supplies, West Chester, PA, U.S.A.) in ethanol for 1 h, and finally in 100 % LR White in ethanol for 4 h before polymerization at 60 °C for 24 h. Thin sections, 0.5–1.0 μm, were cut with an ultra-microtome (Reichert Ultracut E, Vienna, Austria), transferred onto droplets of water on a specimen slide, stained with Toluidine Blue (O'Brien & McCully 1981). Images were captured using a transmittance light microscope coupled to a DXM 1200 camera (Nikon Optiphod, Nikon Instech Co., Kanagawa, Japan).

Fluorescence microscopy

Leaf sections were stained with natural product (NP) reagent prior to viewing with a fluorescence microscope (Zeiss Axiovert 200, Carl Zeiss Werke, Göttingen, Germany). The NP reagent was prepared by dissolving 0.05 g of diphenylboric acid-β-ethylaminoester in 10 ml methanol. To this solution, 90 ml of a 5 % $AlCl_3$ aqueous solution was added, resulting in a 0.05 % NP reagent in $AlCl_3$ (Heinrich *et al.* 2002). One centimetre strips of fresh leaves were cut and soaked in the NP solution for 10 min. The leaf sections were dried on absorbent paper and mounted on a glass plate, without a cover glass, before viewing the fluorescence with a blue filter (excitation λ = 386 nm,

Table 1. Brief descriptions of morphological features used for distinguishing *Procontarinia matteiana* from *Chrysonotomyia pulcherimma.*

Part of organism	*P. matteiana*	*C. pulcherimma*	Figure reference
Antenna	Clear rounded modular segments	Segments flat and narrow	Figs 2B and 3B
Eyes	Stacked and slightly elongated eyes	Protrusion extends from each eye	Figs 2C and 3C
Wings	Rounded with a sharp tip	Round wings displaying fine hairs on the edges	Figs 2D and 3D
Legs	No obvious differences and not useful for identification purposes	Figs 2E and 3E	
Larva	Rounded in shape	Elongated	Figs 2F and 3F

emission λ = 490 nm). Digital images were captured using the DXM 1200 camera.

RESULTS AND DISCUSSION

Mangifera indica is utilized by over 250 insect species of which about 25 species are gall-inducing species, with *Procontarinia* spp. the most prevalent (Raman *et al.* 2009). In countries where mango is indigenous, gall fly numbers are kept under control by natural enemies. The most prevalent parasite (*C. pulcherimma*) in South Africa is found on all infested cultivars throughout the season, but is unable to suppress the gall fly population below the economic threshold (Grové *et al.* 2003). These parasites lay their eggs inside the gall and the larvae feed on the gall fly while it is still inside the structure. To investigate the life cycle of the gall fly and the effect of insecticides on this cycle, it is necessary to distinguish the gall fly from other insects inhabiting the gall structure such as *C. pulcherimma*. In this study a holistic view of both the gall fly and the parasite was adopted. Images of the male and female gall fly, as well as the parasite, are depicted in Fig. 1. Female gall flies have shorter antennae and the genitalia are less pronounced than those of males (Fig. 1A,B). The gall fly has a rounded head and a round abdomen. In contrast, the parasite (Fig. 1C) is characterized by more angular features that include a sharp triangular head and pointed abdomen.

In many cases, the insect emerging from the gall is the parasite, rather than the mature gall fly. Scanning electron micrographs of emerged insects simplified subsequent identifications. Figs 2A and 3A are micrographs of the intact insects, while Figs 2B–F and 3B–F represent those of the enlarged antennae, eyes, wings and legs of the gall fly and parasite, respectively. The eyes were found to be the most useful for identification purposes. A summary of the distinguishing features used for identification is provided in Table 1. Based on these morphological characteristics and the eye structure, the insect displayed in Fig. 4D was therefore identified as the parasite and not the gall fly.

Fig. 1. Light microscope photographs of adult flies and parasite. **A**, The female fly has a full abdomen and rounded posterior with no visible genitalia and short antennae; **B**, the male fly has a slender abdomen with visible genitalia and long antennae; **C**, the parasite of the gall fly. Photographs courtesy of E. Louw, Westfalia.

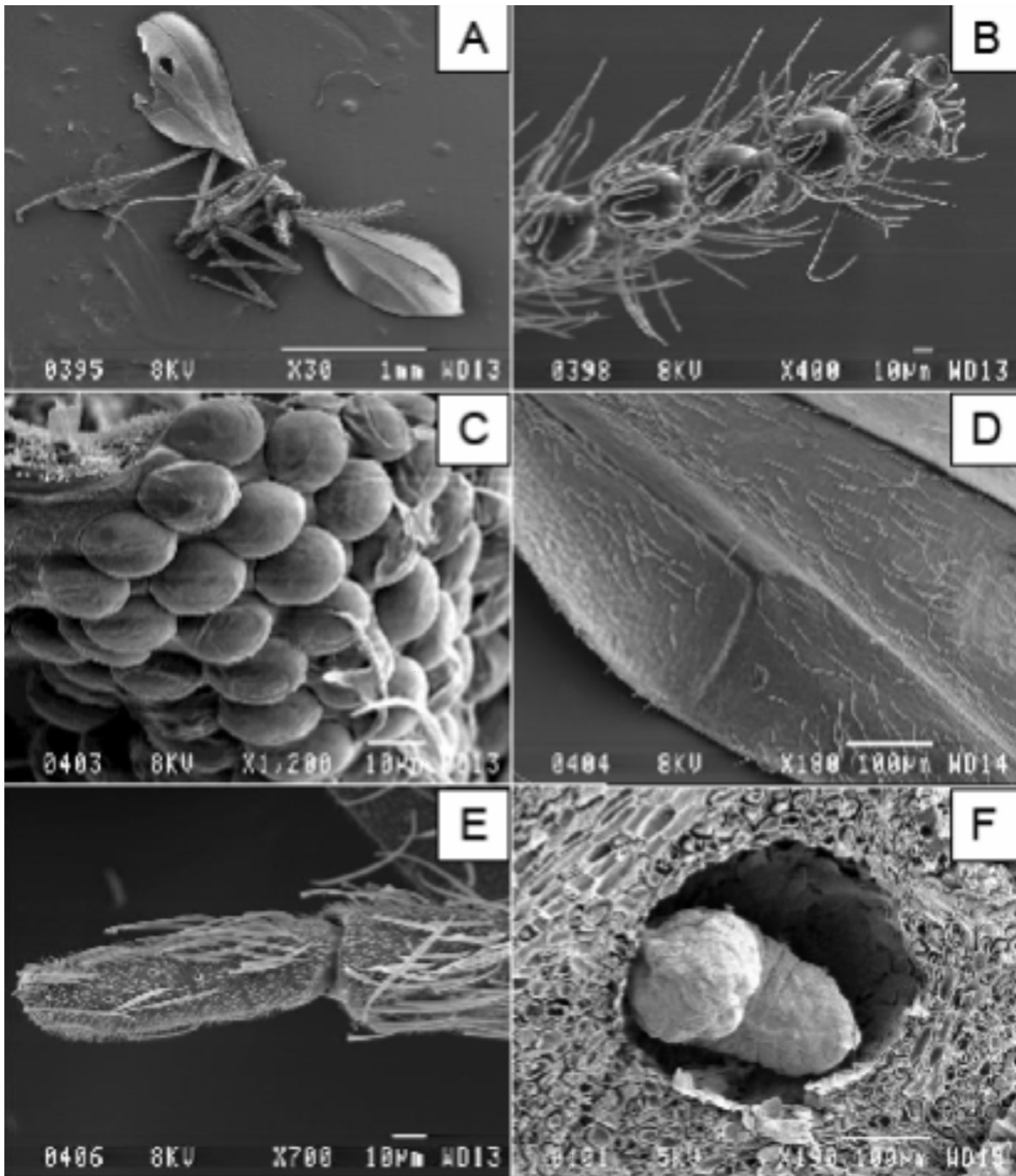

Fig. 2. Scanning electron micrographs of the mango gall fly and various parts of the insect. **A**, Intact fly; **B**, antennae; **C**, eye; **D**, wing; **E**, leg; **F**, larva.

Initially, Schreiner (1990) proposed that the gall fly develops only up to the larval stage within the leaf, after which it emerges, drops to the ground and the remainder of the life cycle is completed in the soil. Some species, such as *P. pustulata,* have been shown to pupate in the soil (Kolesik *et al.* 2009). The current SEM studies confirmed that *P. mateiena* completes its life cycle within the gall. Longitudinal and perpendicular sections of galls are presented in Figs 4A and 4B, illustrating the development of insects inside individual galls. The fully developed insects depicted in Figs 4C and 4D

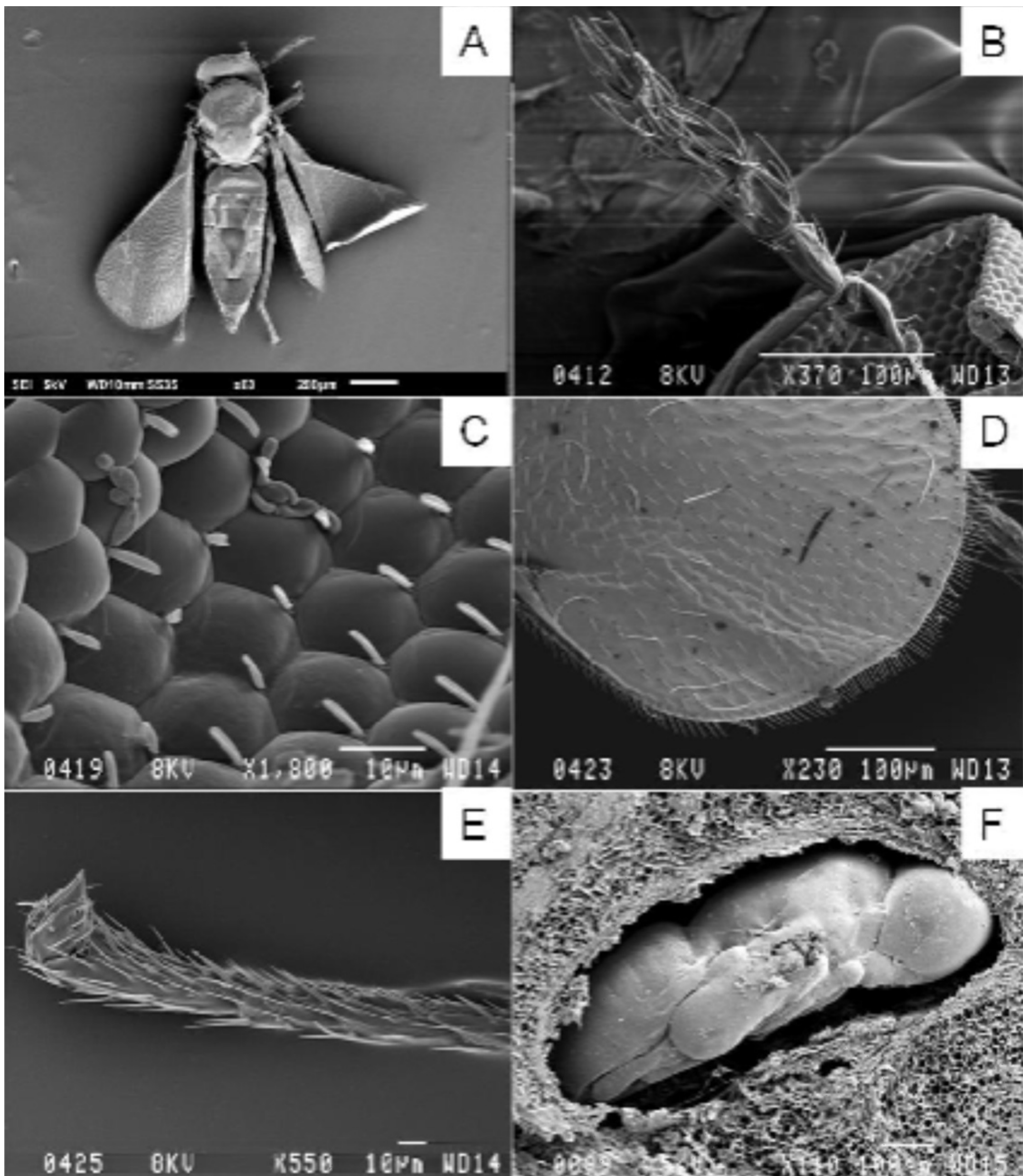

Fig. 3. Scanning electron micrographs of the parasite of the mango gall fly and various parts of the parasite. **A**, Intact insect; **B**, antennae; **C**, eye; **D**, wing; **E**, leg; **F**, pupa.

were identified as the gall fly and parasite, respectively, while the larvae of the gall fly and parasite are represented in Figs 4E and 4F, respectively. Once again, the rounded shape of the gall fly larva and the sharp abdomen of the parasite larva are evident.

The influence of the systemic insecticide thiamethoxam on gall and insect development is apparent in Fig. 5. In Fig. 5A, true galls containing insects are clearly visible. These galls are of the same age as the structures depicted in Fig. 5B, a micrograph of plant material treated with thia-

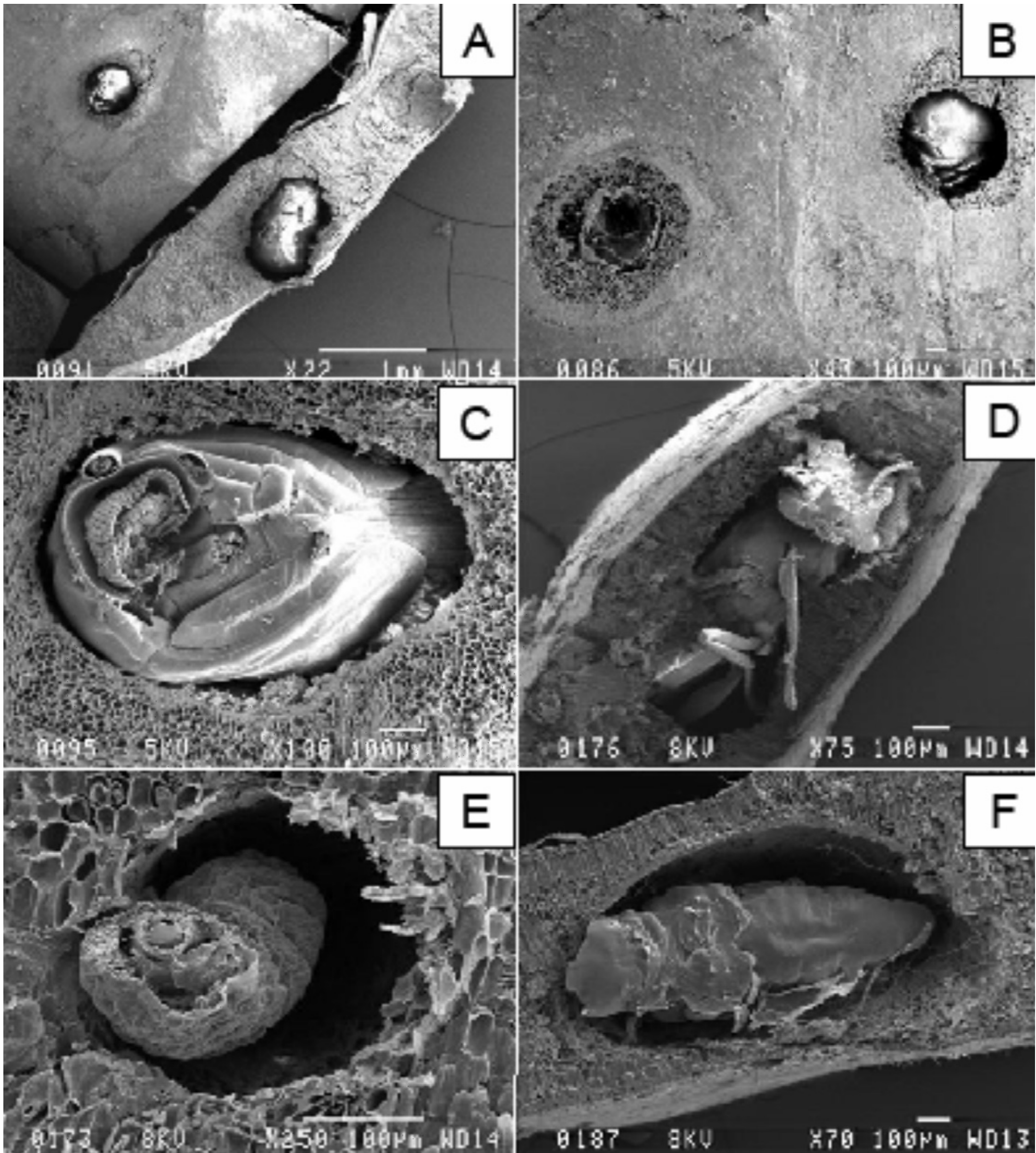

Fig. 4. Scanning electron micrographs illustrating the development of a mango gall fly and parasite within the gall. **A**, Longitudinal and perpendicular section of galls with insect larvae visible; **B**, longitudinal section of galls with larva in one gall, while the structure of a vacated gall is visible to the left; **C**, section through a gall fly with eye, antennae and extremities visible; **D**, parasite inside a gall; **E**, gall fly larva in gall; **F**, parasite larva in gall.

methoxam. Although the insecticide clearly prevented larval development, the gall-like structure was still consistent with that of true galls, with randomly ordered parenchyma cells. Thickening of the tissue still occurs due to a plant response following stimulation by the parasitizing insect. The visible damage observed after insecticide application can be attributed to earlier infestation while the leaves were very young and soft. Micrographs thus confirmed the efficacy of thiamethoxam as an insecticide to curb gall fly infestation in mango orchards. Systemic insecticides are

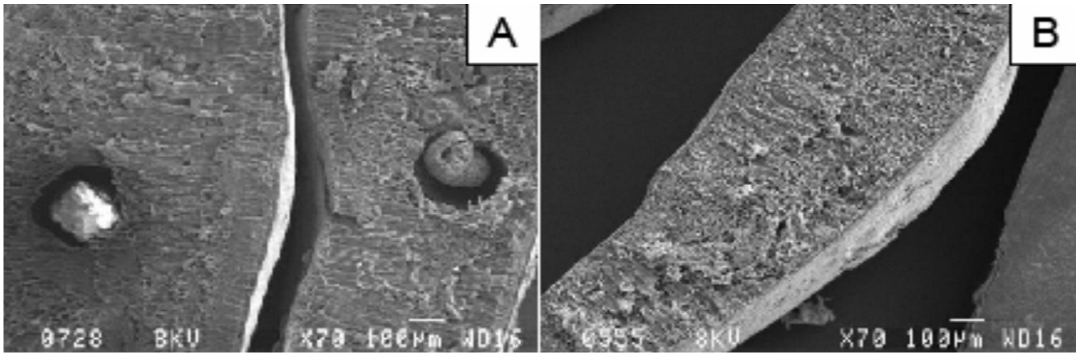

Fig. 5. Scanning electron micrographs of sections through galls to verify differences between leaves that were exposed or not exposed to commercial insecticides. **A**, Mango (cv. Tommy Atkins) leaves in an organic orchard with insect development in galls; **B**, mango (cv. Tommy Atkins) leaves exposed to insecticide – no insects visible.

therefore an effective means of pest management.

Although chemical control offers some relief, non-target organisms, including gall fly parasites, are also killed using this method, thereby eroding natural control mechanisms. Organophosphates, for example, have been used to combat gall fly, but are not recommended as these pesticides are even more detrimental to the natural enemy complexes associated with the mango and mango insect pests (Githure *et al.* 1998). Alternative natural compound-based solutions that reduce gall fly infestation, while maintaining parasite populations, are currently sought by the industry.

Mango gall flies oviposit on *M. indica* during the spike stage of flush leaves (encircled in Fig. 6A), resulting in the development of galls on leaf

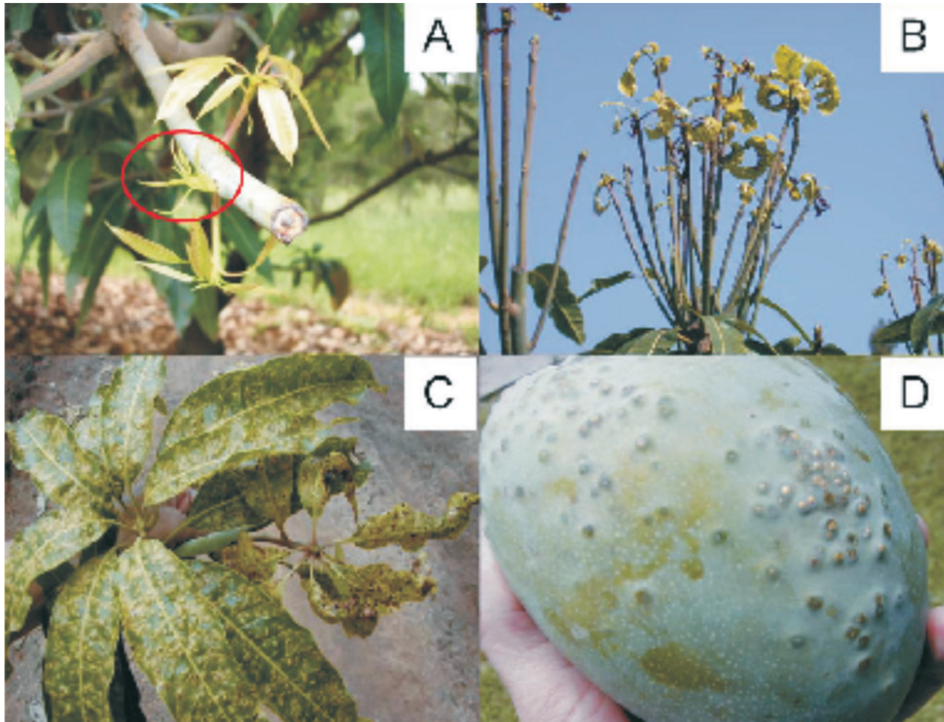

Fig. 6. Photographs of gall fly infestation on leaves and fruit of *Mangifera indica*. **A**, flush leaves with the spike stage circled; **B**, defoliated branches as a result of severe infestation; **C**, deformed mature leaves; **D**, infested fruit. Photographs courtesy of E. Louw, Westfalia, and D. Le Lagadec, Agri-Science Queensland, Australia.

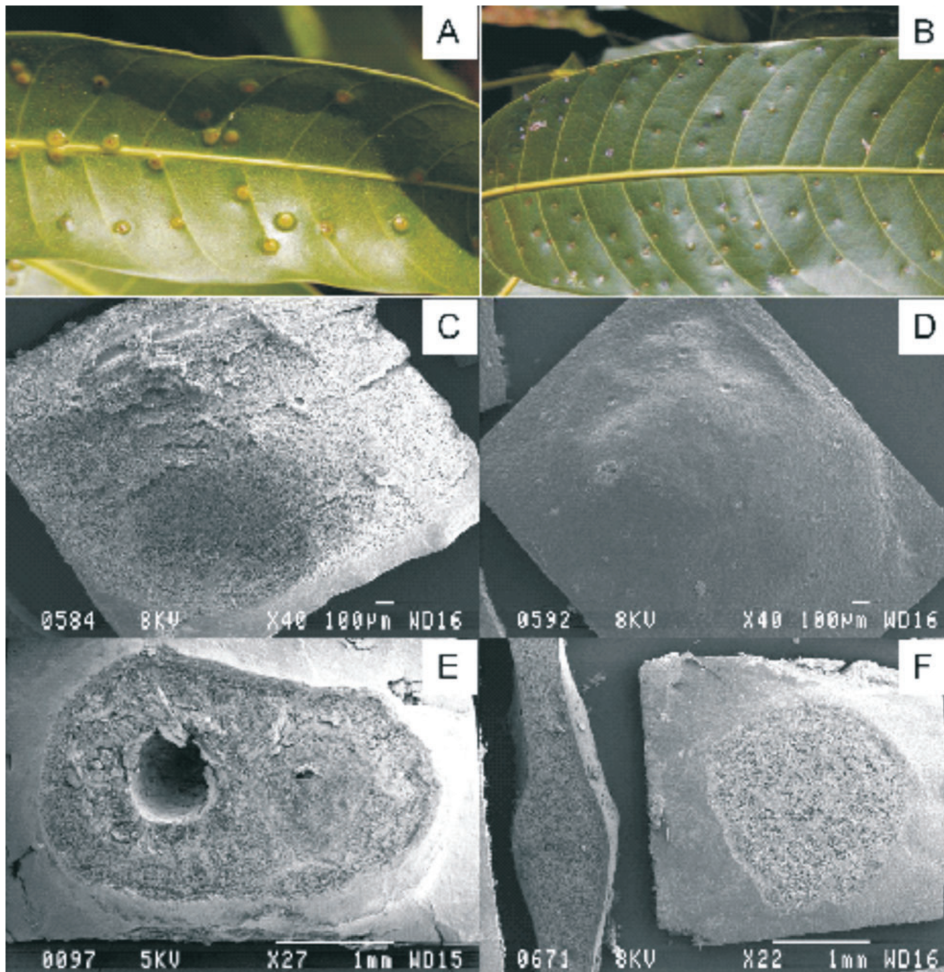

Fig. 7. Images of true galls and pseudo-galls. **A**, Photograph of a true gall; **B**, photograph of a pseudo-gall; **C**, scanning electron micrograph of a true gall; **D**, a longitudinal section through a pseudo-gall; **E**, scanning electron micrograph of a pseudo-gall; **F**, longitudinal section through a true gall.

surfaces. As shown, complete metamorphosis of the gall fly takes place within these structures. Colonized leaves appear deformed (Fig. 6C) and are likely to drop. Severe infestations may result in the defoliation of branches (Fig. 6B) thereby impacting seriously on fruit production and photosynthetic abilities of infected trees. In South Africa, the incidence of gall fly infestation on fruit is very low, but has been occasionally observed (Fig. 6D).

Larval mortality occurs in pseudo-gall-forming cultivars, as observed using microscopy. It is thought that secondary host-plant metabolites may contribute to this phenomenon (Augustyn *et al.* 2010b). The cultivars, 'Heidi', 'Haden', 'Peach', 'Zill', 'Kensington', 'Tommy Atkins' and 'Sabre' develop true galls (Augustyn *et al.* 2010b); Fig. 7A). In cultivars that present pseudo-galls, such as 'Keitt', 'Kent' and 'Irwin', the presence of compounds with antibiosis properties deter the development of the pest following oviposition. Typical shot-hole damage is evident on the leaves of these cultivars (Githure *et al.* 1998); Fig. 7B). These marks may be sites where oviposition did take place, but the young larvae failed to develop. Morphological differences were evident between true galls and pseudo-galls as illustrated in the micrographs in Fig. 7C–F. True galls (Fig. 7C) have a rounded, inflated shape, while pseudo-galls (Fig. 7D) appear deflated. Exudates are emitted by

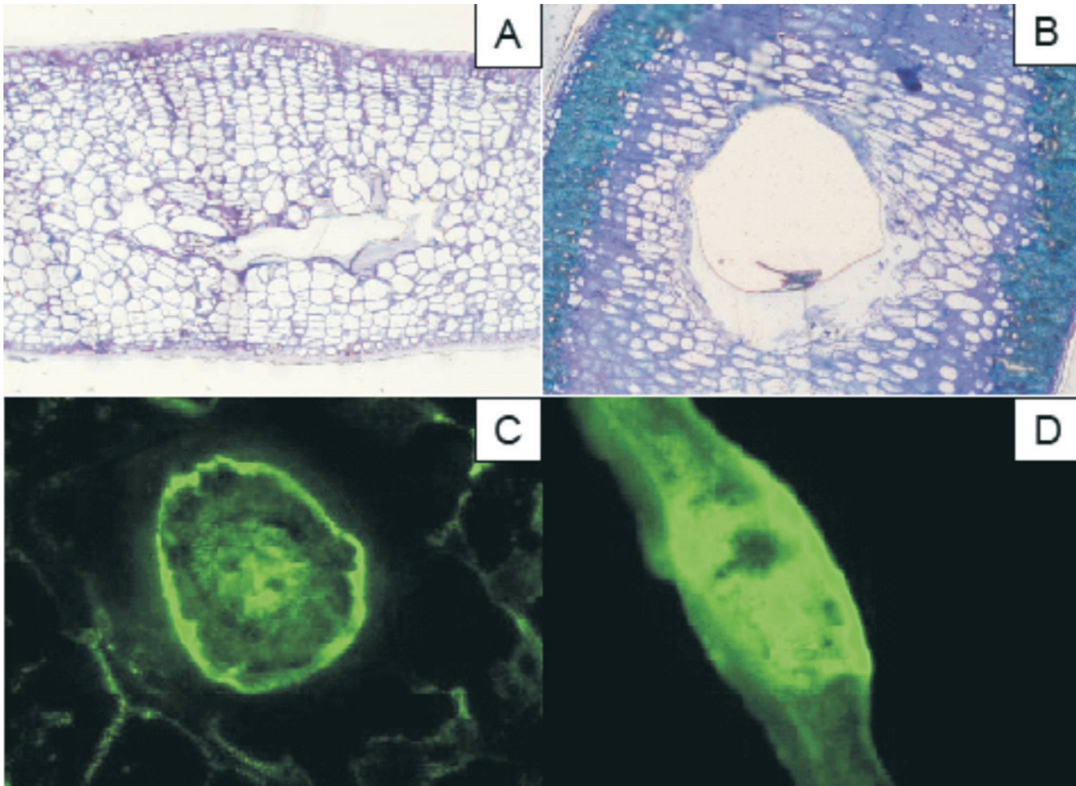

Fig. 8. Light microscope images comparing pseudo-galls (**A**) to galls (**B**). The fluorescence images at the bottom are longitudinal (**C**) and perpendicular (**D**) cuts through galls stained with natural product.

galls as observed in Fig. 7C; however, no exudates are present on leaves displaying pseudo-galls. A longitudinal section of a true gall (Fig. 7E) revealed the gall structure, as well as the cavity in which the insect developed. In contrast, the pseudo-gall (Fig. 7F) contains no insect, no cavity is visible and only disorganized plant parenchyma cells can be observed.

From the light microscopy of a semi-thin section of a pseudo-gall (Fig. 8A) the small size of the cavity can be seen. In true galls (Fig. 8B), the cavity and the parenchyma cells surrounding the gall are visible and correspond to the description of gall development by Lalonde & Shorthouse (1985). According to these authors, gall development in the Canada thistle (*Cirsium arvense*) consists of three phases after infestation with the tephritid fly *Urophora cardui*. In the initiation phase, the insect takes control of tissue development, whereafter parenchyma cells multiply rapidly, surrounding the larvae with a thick layer of cells, and primary nutritive cells appear. This layer is stimulated by larval feeding and separated from the rest of the gall by a thin wall of sclerenchyma (Stone & Schönrogge 2003) (Fig. 8B). In the maturation phase, secondary nutritive cells are formed and the gall parenchyma cells are lignified. These secondary cells are the main food source of the larvae (Lalonde & Shorthouse 1985). In Figs 8C and 8D, the green fluorescence indicates the presence of phenolic compounds after staining with natural product reagent. Excessive phenolic compounds are produced as a protection mechanism in response to infestation (Du Plooy *et al.* 2009). Galling insects may award some benefits to their hosts as a result of the induction of foliar phenolic defence compounds in the leaves that may indirectly protect against feeding by other herbivores (Pascual-Alvarado *et al.* 2008).

CONCLUSION

Scanning electron microscopy proved that the entire larval development of both the mango gall

fly and that of its parasite takes place within the gall cavity. These findings have contributed to the entomological data available for *P. matteiana,* as well as that of the parasite, *C. pulcherimma.* Parasites of the gall fly play a role in curbing gall fly numbers; however, they never occur in sufficient numbers to eradicate the gall fly problem. Electron microscopy confirmed that the application of the systemic insecticide thiamethoxam WG 250 g/kg indeed halted the development of the gall fly. This finding verified that the use of systemic insecticides should currently be retained as an integral part of pest management in the orchard. In addition, micrographs elucidated the structural differences between true and pseudo-galls, thereby verifying that larval development is terminated in the case of pseudo-gall-bearing cultivars. Orchard application of secondary metabolites found to be active in larval mortality may be a viable option for gall fly control. This study has provided the necessary tools to investigate the effect of natural compounds on larval development and true gall formation. Alternatively, cultivars that are good producers of antibiotic compounds should be selected and cultivated to obviate the need for insecticide application.

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