Fungus gnats and other Diptera in South African forestry nurseries and their possible association with the pitch canker fungus

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Fusarium circinatum **is the causal agent of a serious disease of seedlings in South African pine nurseries. Insects, especially fungus gnats (Diptera: Sciaridae, Mycetophlidae), are suspected of transmitting this fungus in nurseries. The aim of this study was to ascertain which species of gnats are present in South African pine nurseries, and to consider whether these and other Diptera carry** ***F. circinatum.*** **Dipteran fauna were surveyed in four major forestry nurseries between 2000 and 2001. Fungi were isolated from these flies and the resulting** ***Fusarium*** **species were identified.** ***Bradysia difformis*** **was the only fungus gnat species found and it occurred in all nurseries. Other Dipteran families collected included Agromyzidae, Cecidomyiidae, Chironomidae, Ephydridae, Muscidae, Simulidae and Tachinidae. This is the first report of** ***B. difformis*** **in South Africa.** ***Fusarium circinatum*** **was not isolated from any of the Diptera collected.** ***Fusarium oxysporum*** **and** ***F. stilboides*** **were isolated from Chironomidae, but these fungi are not considered important pathogens in the nurseries surveyed.**

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Introduction

Nematoceran Diptera belonging to the families Sciaridae and Mycetophilidae have a broad distribution and a wide range of habitats. These insects, known as fungus gnats, are found in rotten wood, under the bark of fallen trees, associated with wild fungi as well as in leaf mould and manure piles.[1,2] They are also found in nurseries of various crops, including legumes,[3,4] mushrooms,[5] fuchsias,[6] cucumbers,[7] alfalfa,[8] tomatoes,[3] forestry plants,[9,10] cloves[11] and other ornamentals.[12]

Fungus gnat larvae feed on animal excrement,[2] decaying and living plant tissues,[2,4] and fungal structures,[13] including cultivated mushrooms.[14] The diet of the adult flies is not certainly known, although they have been fed on sucrose solutions in captivity.[14] In nurseries the larvae also feed on decaying and healthy plant roots as well as fungi.[15,16]

The feeding of fungus gnat larvae on healthy roots causes a reduction in plant vigour[4,16,17] and provides infection sites for various pathogenic fungi.[17] The larvae come into contact with fungi while moving in the soil, feeding on infected plant roots or directly on the fungi. Ingested fungal spores can remain intact and viable inside the digestive tract of the larvae, and may be dispersed via the faeces, conveyed to the adult via trans-stadial transmission, and spread via adult cadavers.[18,19] Alternatively, the adult insects can acquire and distribute fungal pathogens by moving from infected to non-infected plants.[19,20] Fungus gnats therefore can act directly or indirectly as vectors of fungal pathogens. Pathogens reported to be transmitted by these flies include *Botrytis cinerea* Pers.: Nocca. and Balb on conifer seedlings,[9] *Verticillium albo-atrum* Reinke and Berthold on alfalfa

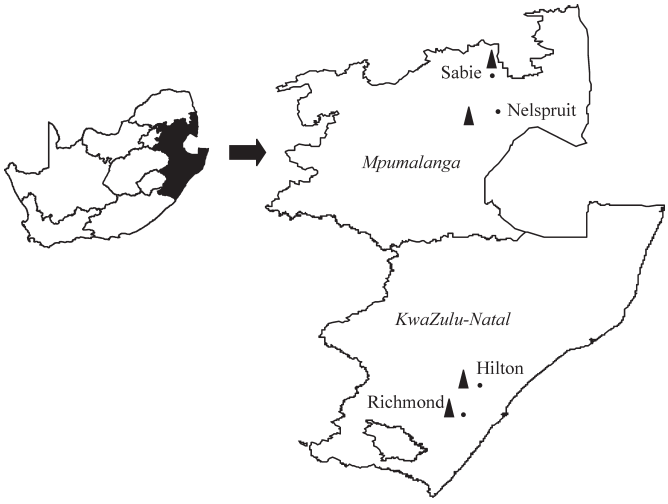

Fig. 1. Locations of the four main pine-growing nurseries, in Mpumalanga and KwaZulu-Natal, sampled between 2001 and 2002.

plants[20] and *Fusarium oxysporum* Schlecht f. sp. *radicis-lycopersici* Jarvis and Shoemaker on bean and tomato plants.[3]

In South African forestry nurseries, the pitch canker fungus, *Fusarium circinatum* Nirenberg et O'Donnell [formerly *Fusarium subglutinans* (Wollenw. et Reinking) Nelson *et al.* f. sp. *pini*)], is a serious pathogen of pine seedlings.[21,22] The fungus causes lesions at the root collars and the cotyledon node regions of seedlings.[21,23] Symptoms of diseased seedlings include tip dieback, damping-off, chlorotic or reddish-brown needle discoloration and wilting.[23,24]

The association between fungus gnats and fungal pathogens in other crops has led to the suggestion that these insects may be vectors of *F. circinatum* in South African forestry nurseries, although no surveys of these insects have ever been undertaken. These nurseries commonly apply insecticides for the control of fungus gnats, hoping to reduce the impact of *F. circinatum.* There is, however, no experimental evidence showing an interaction between fungus gnats and *F. circinatum,* and chemical control of the flies may represent an unnecessary expense and threat to non-target, possibly beneficial insects. Conversely, greater knowledge of fungus gnat biology and control is required if an interaction between these insects and *F. circinatum* does exist. The aim of the study reported here was to determine whether Sciaridae or Mycetophilidae are present in selected South African forestry nurseries. Furthermore, we considered whether these Diptera, or any other Diptera in local forestry nurseries, might be involved in transmitting *F. circinatum.*

Materials and methods

Collection sites

Diptera were collected in four of the main pine-growing nurseries in South Africa, over a two-year period, from 2001 to 2002. Two of the nurseries were in Mpumalanga province, near Nelspruit (*c.* 25°34′S, 30°41′E) and Sabie (*c.* 25°06′28″S, 30°47′05″E). The other two nurseries were in KwaZulu-Natal, near Richmond (*c.* 29°51′54″S, 30°15′50″E) and Hilton (*c.* 29°33′50″S, 30°18′24″E) (www.gpswaypoints.co.za) (Fig. 1). These nurseries were selected specifically because serious losses due to *Fusarium circinatum* have been reported in them.

Collection of insects

Because Diptera keys rely primarily on adult stages (and because sufficient numbers of the larvae proved difficult to gather at the nurseries), only adult Diptera were collected for identification. Initially, yellow sticky traps were used to capture the adults.[9] These traps consisted of yellow sheets of plastic (14.0 cm × 7.5 cm) covered with insect glue (Flytac). The traps were placed randomly within nurseries, among the pine seedlings. Some of the traps were suspended just above the seedlings, while others were placed upright on the seedling trays, at the level of the seedlings. The traps succeeded in catching the adult fungus gnats and other Diptera, but because of the sticky nature of the traps, the specimens could not be removed intact for effective identification. Paraffin and similar liquids could not be used to remove the insects from the traps, as the fungi would have been killed.

As an alternative to sticky traps, aspirators were used to collect specimens from around the nurseries.[18,25] Sweep nets were used when swarms of Diptera were observed and for foliage sweeping. The insects were then removed from the net using an aspirator. Although this method was time-consuming, the specimens remained in good condition for later identification and fungal isolations.

Identification of Diptera

Dipteran families were identified using taxonomic keys.[26–28] Mervin Mansell (USDA-APHIS, Pretoria) assisted in confirming some of the identifications. Specimens belonging to the Sciaridae and Mycetophilidae were divided into morphospecies and later identified by Hans-Georg Rudzinski (Entomographisches Studio, Schwanewede, Germany).

Isolation and identification of fungi

For primary isolations of fungi, Dipteran specimens were crushed and placed on *Fusarium*-selective medium.[29] To purify fungi, isolates were transferred to 2% malt extract (15 g malt extract, Merck, plus 20 g agar). *Fusarium* species were placed on Synthetic Low-Nutrient Agar (SNA) medium[30] to promote the formation of the microconidia for species identification. Single conidial isolates were made from cultures of *Fusarium* spp. Each of these isolates was transferred to half-strength potato dextrose agar (Difco, South Africa) and Carnation Leaf Agar. *Fusarium* species were identified using taxonomic keys.[31] Identifications of the *Fusarium* spp. were confirmed by W.F.O. Marasas (PROMEC, Medical Research Council, South Africa).

Results

Identification of insects

Only a single fungus gnat species—*Bradysia difformis* Frey [= *Bradysia paupera* (Tuomikoski) (Diptera: Sciaridae)]—was collected in this study (Fig. 2), but in all four nurseries (Table 1). Approximately 300 *B. difformis* were captured, in the ratio of 24 males to one female. No Mycetophilidae were collected.

Other Dipteran families found in the nurseries included Agromyzidae (leaf miners), Cecidomyiidae (gall midges), Chironomidae (midges), Ephydridae (shore flies), Muscidae (muscids), Simulidae (black flies) and Tachinidae (tachinids) (Table 1). Of these, only Ephydridae were collected in all four nurseries. Chironomidae were present in three of the four nurseries and were observed swarming in large numbers. These swarms were often confused with fungus gnats by nursery employees. Muscidae and Agromyzidae were collected in two of the four nurseries. Cecidomyiidae, Simulidae and Tachinidae were captured in only one nursery (Table 1).

Isolation and identification of fungi

Fusarium circinatum was not isolated from any of the Dipteran specimens collected. Two other *Fusarium* spp. (*F. oxysporum,*

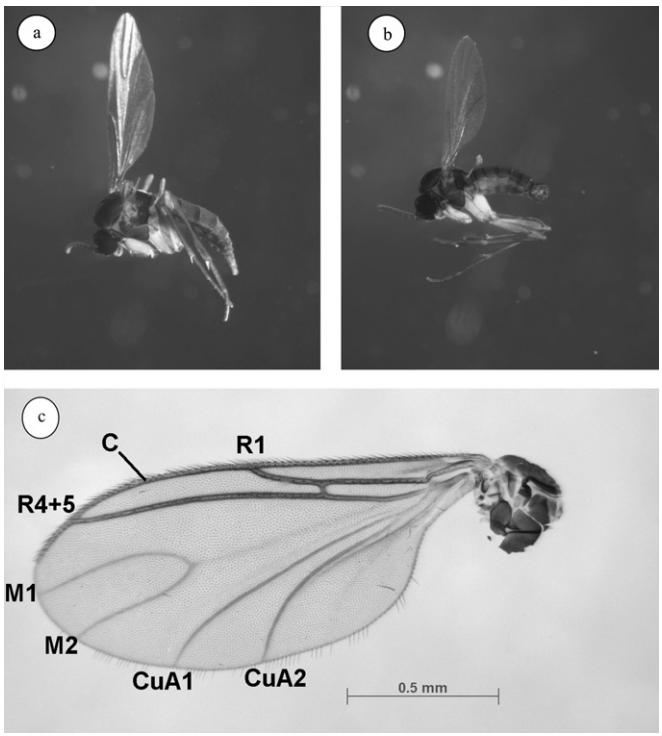

Fig. 2. *Bradysia difformis.* **a**, Female; **b**, male; **c**, wing venation, showing symmetrical fork of media veins (M) starting distal to the point where radius vein R1 joins the costa (C)(CuA, cubitus anterior veins; M, media veins; R, radius veins).

F. stilboides) were isolated from chironomids. *Fusarium oxysporum* was isolated from these insects at the Nelspruit and Hilton nurseries. *Fusarium stilboides* was isolated only from chironomids collected at the Nelspruit nursery.

Discussion

This study confirms that fungus gnats were present in South African forestry nurseries suffering from pitch canker fungus infections, albeit in small numbers. However, only a single species of fungus gnat—*Bradysia difformis*—was collected, though it was well distributed geographically. This study also represents the first report of this species in Africa, although other *Bradysia* species have previously been recorded in South Africa.[32–34]

The genus *Bradysia* has a worldwide distribution,[35] but *B. difformis* has been recorded only in Britain,[36] Norway and Sweden,[37] the United States and Brazil[35] in the past. The insect appears to have been introduced into South Africa from the northern hemisphere. It is not clear how this might have happened, although imported plants, growth medium or logs are possible sources.

Table 1. Diptera recorded in four of the principal pine-growing nurseries in South Africa during 2001 and 2002.

Suborder	Family	Nursery*			
		S	H	N	R
Cyclorrhapha	Agromyzidae	✓	✓		
Cyclorrhapha	Ephydridae	✓	✓	✓	✓
Cyclorrhapha	Muscidae		✓		✓
Cyclorrhapha	Tachinidae		✓		
Nematocera	Cecidomyiidae				✓
Nematocera	Chironomidae		✓	✓	✓
Nematocera	Sciaridae†	✓	✓	✓	✓
Nematocera	Simulidae		✓		

*Nursery locations: S, Sabie; H, Hilton; N, Nelspruit; R, Richmond.
†Sciaridae was the only family of fungus gnats recorded.

Bradysia difformis is known as a pest in European nurseries. In Britain, it damages ornamentals[6] and is a minor pest of mushrooms.[14,36] *Bradysia difformis* is the dominant sciarid in Norwegian greenhouses and possibly the most common pest in Swedish ones.[37] In these greenhouses, *B. difformis* larvae feed on the roots and root collar region of the plants. It is possible that its larvae in South Africa feed on pine seedlings, but this still has to be tested experimentally.

No association between *B. difformis* and fungal pathogens has been recorded. However, various other *Bradysia* species have been found to transmit fungal pathogens (including *Fusarium* spp. in two cases).[3,20,38] It is, therefore, surprising that *B. difformis* that are clearly living in a close association with *F. circinatum* were not found to carry the fungus. It is possible that larger numbers of insects would have yielded cultures of the fungus, but these adults do not appear to be important vectors of *F. circinatum* in South African nurseries.

Although a high male to female ratio was observed for *B. difformis,* this may not be representative of the actual sex ratio in the nurseries. Males congregate at the surface of the growing medium to mate with emerging females and engage in a zigzag running motion when attracted by the female's sex pheromone.[14,39] Females are less conspicuous or active than males, and generally remain on the underside of leaves.[39] In our study, flies were collected from the seedlings, the seedling beds and when they were observed flying in the vicinity of seedlings. The more conspicuous flies would, therefore, have been collected more often than those under the leaves of the seedlings, resulting in a biased sex ratio.

Besides fungus gnats, ephydrids were the only other Dipteran family collected in all four nurseries. Ephydrids occur in a variety of habitats, from oil pools to saline marshes and they exploit a diverse range of food resources, including algae, bacteria, plants, fungi and decaying carcasses.[40,41] Most species are not economically important, but certain species have been recorded as pests of crops[40,41] and plants in greenhouses.[42,43] In greenhouses, the ephydrid *Scatella stagnalis* has been shown to acquire and transmit the pathogen *Thielaviopsis basicola* from infected and healthy corn-salad plants[43] and *F. oxysporum* f. sp. *lycopersici* from infected and healthy tomato plants.[42] *Thielaviopsis basicola* was isolated from the larvae, pupae, adults and faeces of *S. stagnalis*.[43] None of the other Dipteran families collected from the nurseries is reported to be associated with plant pathogenic fungi or with pine seedlings.

Isolations in this study were only from adult Diptera and it is possible that the larvae carry the pathogen. Sciarid larvae feed on fungi and plant roots,[2,4,13,18] a habit not shared by the adults. Thus the larvae may come into contact with fungal pathogens while moving in the soil or during feeding. It is also possible that sciarid larvae wound roots, allowing infection by *F. circinatum* to occur, which may explain why nursery managers have reported a reduction in the incidence of pitch canker fungus infections when insecticide treatments are used (unpublished observations).

Fusarium oxysporum and *F. stilboides* were the only *Fusarium* species isolated from the Dipteran specimens. Both *F. oxysporum* and *F. stilboides* were isolated from chironomid specimens. Chironomids have been recorded to carry Trichomycetes (Zygomycota).[44] These fungi live obligately within the gut of their hosts and are not plant pathogens. There are no reports of chironomids transmitting plant pathogenic fungi or of having any association with such fungi.[2]

Neither *F. oxysporum* nor *F. stilboides* are considered important pathogens of pines in South African forestry nurseries.

F. oxysporum has been isolated from the necrotic roots of *Pinus patula,*[21] *P. strobus*[45] and other conifer seedlings such as those of *Pseudostuga menziesii* (Douglas fir).[46,47] However, the pathogenicity of *F. oxysporum* on *P. patula* is significantly lower than that of *F. circinatum,*[21] which is considered the primary cause of seedling mortality in South African pine nurseries. *Fusarium stilboides* is not pathogenic to pine, but causes disease symptoms on other crops, including coffee,[48] passion fruit[49] and bamboo.[50]

Fungus gnats and other Diptera are widespread throughout South African forestry nurseries. Although these flies are often suspected of transmitting *F. circinatum* and other fungal diseases, this study showed that the insects in the nurseries investigated do not play a significant role in transmitting diseases to pine seedlings. However, further studies are needed to determine the role of the larvae, specifically fungus gnat larvae, in facilitating infection of *F. circinatum* and other diseases of pine seedlings.

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