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. They are also found in nurseries of various crops, including legumes, mushrooms, fuchsias, cucumbers, alfalfa, tomatoes, forestry plants, cloves and other ornamentals.

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

Fusarium circinatum is the causal agent of a serious disease of seedlings in South African pine nurseries. Insects, especially fungus gnats (Diptera: Sciaridae, Mycetophilidae), 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.
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* and chemical control of *F. oxysporum* has been reported in them.25-27 The fungus causes lesions at the root collars and the cotyledon node regions of seedlings.25-27 Symptoms of diseased seedlings include tip dieback, damping-off, chlorotic or reddish-brown needle discoloration and wilting.25-28

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 these 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.16,22 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, Schwanevede, 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) medium30 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.E.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*, *Fusarium subglutinans*).21,22 The fungus causes lesions on South African Journal of Science 103, January/February 2007

Fig. 1. Locations of the four main pine-growing nurseries, in Mpumalanga and KwaZulu-Natal, sampled between 2001 and 2002.
Bradyisia difformis is known as a pest in European nurseries. In Britain, it damages ornamentals and is a minor pest of mushrooms.\textsuperscript{3,4,13,19} Bradyisia difformis is the dominant sciarid in Norwegian greenhouses and possibly the most common pest in Swedish ones.\textsuperscript{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 Bradyisia species have been found to transmit fungal pathogens (including \textit{Fusarium} spp. in two cases).\textsuperscript{5,20,19} It is, therefore, surprising that \textit{B. difformis} that are clearly living in a close association with \textit{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 \textit{F. circinatum} in South African nurseries.

Although a high male to female ratio was observed for \textit{B. difformis}, it was not 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.\textsuperscript{4,20} Females are less conspicuous or active than males, and generally remain on the underside of leaves.\textsuperscript{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.\textsuperscript{40,41} Most species are not economically important, but certain species have been recorded as pests of crops and plants in greenhouses.\textsuperscript{42,43} In greenhouses, the ephydrid \textit{Scatella stagnalis} has been shown to acquire and transmit the pathogen \textit{Thielaviopsis basicalca} from infected and healthy corn-salad plants\textsuperscript{44} and \textit{Fusarium oxysporum f. sp. lycopersici} from infected and healthy tomato plants.\textsuperscript{45} \textit{Thielaviopsis basicalca} was isolated from the larvae, pupae, adults and faeces of \textit{S. stagnalis}.\textsuperscript{45} 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,\textsuperscript{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 \textit{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).

\textit{Fusarium oxysporum} and \textit{F. stiboides} were the only \textit{Fusarium} species isolated from the Dipteran specimens. Both \textit{F. oxysporum} and \textit{F. stiboides} were isolated from chironomid specimens. Chironomids have been recorded to carry \textit{Trichomycetes} (Zygomycota).\textsuperscript{46} 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.\textsuperscript{14}

Neither \textit{F. oxysporum} nor \textit{F. stiboides} are considered important pathogens of pines in South African forestry nurseries.
F. oxysporum has been isolated from the necrotic roots of Pinus patula, P. strobus and other conifer seedlings such as those of Pseudotsuga menziesii (Douglas fir). However, the pathogenicity of F. oxysporum on P. patula is significantly lower than that of F. circinatum, which is considered the primary cause of seedling mortality in South African pine nurseries. Fusarium stilboioides is not pathogenic to pine, but causes disease symptoms on other crops, including coffee, passion fruit and bamboo.

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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