INSECT-FUNGAL ECOLOGY ON SELECTED NEW CROPS IN SOUTH AFRICA

By

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The scientist does not study nature because it is useful. He studies it because he delights in it and he delights in it because it is beautiful.

Jules Henri Poincare (1845 – 1912)

Quoted in The Quest for Life in Amber, George and Roberta Poinar

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CHAPTER 1

The role of insects in the transmission of plant diseases caused by fungi, with specific reference to the orders Coleoptera, Diptera and Hemiptera

1.0 Introduction

Fungal pathogens cause more plant diseases than diseases caused by all other pathogens combined (Agrios, 1980). Albeit that wind, water, man and animals all effectively disperse propagules of fungal pathogens, insects are considered to be one of the most important agents of fungal dispersal. Over time, some fungal pathogens have evolved total dependence on certain insects to disseminate reproductive propagules, such as spores (Kevan, Chaloner and Savile, 1975 in Kevan and Baker, 1983). The best example of this is the close ecological association that exists between Ips bark beetles and fungi belonging to the genus *Ceratocystis* (Connor and Wilkinson, 1998), associated with Dutch Elm disease. A close association between insects and the transmission of fungal plant pathogens has however, been unequivocally established in very few cases (Agrios, 1980). Most associations between insects and plant pathogens have dealt with bacterial, viral and mycoplasmal diseases of plants. In other cases the relationship between the insect and associated fungus is more incidental or of secondary nature and bears no relation to the ecology of the insect or fungus. The dissemination of fungal diseases by insects is usually inferred from limited observations and experimental proof is often lacking as to the kind of relationship between insect and pathogen.

Carter (1973) classified insect-fungal relationships according to the fungus-insect relationship and transmission via: (i) pollination; (ii) external contamination; (iii) internal contamination; (iv) feeding wounding; (v) oviposition wounding; (vi) symbiotic associations between insects and fungi, and (vii) fungi feeding on insect exudates. The main problem with this classification is the lack of phylogenetic significance. In a more recent review, Agrios (1980) grouped plant diseases according to the plant parts or organs affected by

insects. This is a very convenient classification since it calls attention to common features of certain diseases and some common feeding or breeding habits of the insects involved.

Early reports state that 45 different fungal diseases of 34 plant species involving 100 species of insects and 66 species of fungi of all classes were found in close association with each other (Austwick, 1958). Since then several additional insect-fungal associations have become evident on different economically important plant species. Recognition of a particular insect-fungal interaction usually occurs after an insect pest of a particular crop is controlled by chemicals or other means and the consequent decrease in insect numbers correlates with a decrease in disease incidence in the same field. It can be assumed that the kind of interaction that exists between the insect and fungal pathogen determines whether the insect is a primary vector or secondary disseminator of the pathogen. A primary insect vector implies that a close ecological association exists between the insect and certain fungus, as the case is between Ips bark beetles (Coleoptera: Scolytidae) and *Ceratocystis ips*. On the other hand a secondary vector has no close ecological association with the fungus.

There are very few reviews of plant diseases associated with insect transmission of pathogen inoculum, especially as far as agricultural crops are concerned. The role of insects in the dissemination of phytopathogenic fungi has probably been underestimated because the dissemination of fungal spores by wind and rain is widely regarded as the primary agent of dispersal. Lack (1989) compared the spread of apple brown rot (*Monilinia fructigena*) by free aerial transmission of conidia and insect dissemination. It was shown that significantly more brown rot developed on fruit visited by insects, than fruit tissues that were not visited. Results of other workers indicate that insect spread of *M. fructigena* may be more important

than previously thought (Wormald, 1954; Willison and Dustan, 1956; Ogawa, 1957; Kable, 1969; Tate and Ogawa, 1975).

Insects have definite advantages over wind currents as dispersal mechanisms in that they can act selectively both in picking up inoculum and in depositing it at appropriate sites, by reducing the time gap from source to new substrate and by offering physical protection to the spores being carried (Lack, 1989). Any insect visiting both rotting and healthy fruit will be prone to act as a casual carrier of fungal propagules. Lack (1989) stresses the point that this happens with such frequency that insects must be regarded as important in the spread of disease.

Despite numerous examples where insects are implicated as the prime means of fungal dispersal, there are also examples where insects facilitate plant diseases by making oviposition or feeding wounds on plants which subsequently act as portals of infection (Jones, 1953; Daugherty, 1967).

The main focus of the present review will be on insects that are secondary disseminators of fungal inoculum to agricultural or horticultural crops. In other words, insects which are phytophages of crops, but which harbour fungal propagules on their exoskeleton or mouth parts which have the potential to infect the host plant and cause disease. The main purpose will be to provide a knowledge base from which to study insect-fungal associations that may possibly affect the cultivation of three new crops in South Africa, *viz.* pigeonpea (*Cajanus cajan* L. Millspaugh - Fabaceae), cactus pear (*Opuntia ficus-indica* L. Miller - Cactaceae) and pistachio (*Pistacia vera* L. - Anacardiaceae). Specific attention will therefore be given to the insect orders that are most prominent on the

aforementioned crops, since these insects will be the subject of further investigation in the current dissertation.

2.0 Insect orders associated with fungal transmission

This section has been categorised according to insect orders that are applicable and orders that have no relation to this study. Orders of insects differ dramatically in many ways. Only differences that are applicable to fungal dissemination by insects are taken into consideration. For instance, the type of mouthparts of an insect plays a major role in the dissemination of fungi and the density or number of setae on the exoskeleton could determine the number of fungal propagules harboured by the insect. This topic will be dealt with and focused on in Chapter 5.

2.1 Order: Hemiptera (true bugs)

The insects in the order Hemiptera are extremely diverse in size, shape and colour. There are at least 80 000 named species and probably many more unnamed ones. About 11 000 named species occur in North America, 5 600 in Australia and 1 600 in the United Kingdom (Dolling, 1991). This order includes the bugs, aphids, cicadas, leafhoppers and scale insects. Traditionally it was divided into two suborders the Heteroptera and the Homoptera (including the Sternorrhyncha, and Auchenorrhyncha), based basically on wing structure. Presently the order is divided into three suborders, *viz.* Sternorrhyncha, Auchenorrhyncha and Heteroptera, with the Auchenorrhyncha now believed to be more closely related to the Heteroptera than the Sternorrhyncha (Dolling, 1991).

A common morphological characteristic within the order is their sucking mouthparts (stylets) with which they suck plant sap and body juices from plants or other insects, respectively (Scholtz and Holm, 1985). Members of this family have an incomplete metamorphosis and their immatures (nymphs), look much the same as the adults, but are smaller and wingless. They may also have different colouring to the adult form. Some species aggregate in both the adult and nymphal stages (Dolling, 1991).

2.1.1 Association with fungal transmission

Lee, Tugwell, Fannah and Weidemann (1993) investigated the role of *Oebalus pugnax* (rice stink-bug, Hemiptera: Pentatomidae) in transmitting several fungal pathogens, *viz. Fusarium oxysporum*, *Cochliobolus miyabinus*, *Curvularia lunata*, *Alternaria alternata* and *A. padwickii* to rice. The transmission ability of *O. pugnax* was tested in a glasshouse and in the field. The bugs were captured and were not artificially infested with any fungi. Evidence found to support the vector relationship between the rice stink-bug and the significant pathogens, include the caryopsis which only became discoloured when wounded during inoculation, conducting several isolations of kernel discolouration micro-organisms from the saliva and stylets of the rice stink bug, substantial kernel discolourations on insect infested panicles in contrast to no effect on kernels without insects, and a lower incidence of discoloured kernels in insecticide treated plots in contrast to higher incidence of both classical symptoms occurring in fungicide treated and control plots.

Michailides and Morgan (1996) assessed pistachio fruit and noticed fruit with punctures and/or epicarp lesions that were caused by hemipterans. Isolations were made from the fruit and *Botryosphaeria dothidea* was eventually found to be associated with the

punctures and the lesions. One of the objectives of their investigation was to determine the involvement of hemipteran insects in transmitting *B. dothidea*. Four species of adult insects were used in the transmission experiments, *viz. Thyanta pallidovirens* (Hemiptera: Pentatomidae), *Chlorocroa uhleri* (Hemiptera: Pentatomidae), *Leptoglossus clypealis* (Hemiptera: Coreidae), and *Liorhyssus hyalinus* (Hemiptera: Rhopalidae). Specimens from each species were reared in the laboratory and then caged with fruit clusters that had been sprayed with *B. dothidea*. All the insect species were able to transmit *B. dothidea* to fruit clusters, but more fruit became infected with *B. dothidea* in fruit clusters caged with *L. clypealis* and *L. hyalinusi*. Hemipterans therefore all play an important role in spreading *B. dothidea* between pistachio orchards.

2.2 Order: Coleoptera

2.2.1 Nitidulidae (sap beetles)

Sap beetles have been found in various habitats feeding on flowers, fruits, sap, fungi, decaying and fermenting plant tissues or dead animal tissue (Parsons, 1943). Most species of sap beetles are attracted to the wounds of trees where they feed on sap. Although there are many species of sap beetles, only a few species are known agricultural pests of field and stored products. Sap beetles are often considered minor pests, but the presence of large numbers of sap beetles on a host plant can prove economic in terms of crop damage caused by the feeding beetles. Their real impact on crop value, however, is primarily the contamination of products ready for sale by both adults and larvae. In addition to damage caused by feeding, sap beetles have also been recognized as vectors of fungi (Dowd, 2000).

Adult sap beetles vary in size from 0.9 to 15 mm in length. The antennae are usually eleven segmented with the distal three segments forming a club that makes them easily recognizable. The clubbed portion, however, is quite variable within species, being quite distinct or only slightly developed (Parsons, 1943). Antennal grooves are usually present. The elytra are often shortened to expose two or three abdominal segments. The dorsal body surface usually has uniform punctation, but often punctures differ in diameter. The tarsal formula is usually 5-5-5. Sap beetles are characterized by a rather short larval development and comparatively long lived adults. This allows them to adapt to different environmental conditions. In temperate regions most of the species hibernate beneath logs, while in the tropics multiple generations may occur. especially if there is an available food source throughout the year.

Sap beetles occur widely and more than half the genera are cosmopolitan or nearly so (Parsons, 1943). In the United States the new world and two of the tropicopolitan genera appear to be relatively recent arrivals from the tropics. Many South African species feed on decaying and fermenting fruit. Species of the genera *Lasiodactylus*, *Haptoncus*, *Carpophilus*, *Pallodes* and *Pocadius* live in fungi (Scholtz and Holm, 1985).

2.2.1.1 Association with fungal transmission

Tate and Ogawa (1975) found that peach and nectarine decay caused by *Rhizopus stolonifer* and *Monilinia fructicola* respectively were associated with increased activity of nitidulid beetles. Moller and DeVay (1968) found that insects were often associated with bark injuries on stone fruit trees and several were proven vectors of *Ceratocystis fimbriata* in California. The nitidulid beetles, *Carpophilus freemani* and *Carpophilus hemipterus*.

were directly implicated. In stone fruit orchards affected by *Ceratocystis* canker, air and soil samples showed that no spores were trapped in air samples and soil was also found to serve as a limited source of inoculum. However, *C. freemani* was found to dominantly transmit the fungus to healthy trees.

Sap-feeding nitidulids have also been shown to vector oak wilt fungus (*Ceratocystis fagacearum*) (Appel, Kurdyla and Lewis, 1990). Spore viability was not affected by passage through the intestinal tract of the insects and inoculation of trees with insect excrement developed *Ceratocystis* cankers. After 8 days spores of *C. fimbriata* were still found on the insect's exoskeleton and after larvae had undergone pupation in sterile soil they were still able to transmit *C. fimbriata.* The fungus was also isolated from adults in the winter months, indicating a possible means of pathogen overwintering and a possible source of primary inoculum.

2.2.2 Scolytidae (bark beetles)

Scolytidae are a family of small (6-8mm) bark beetles of cylindrical form, dark in colour, with heads obscured by the pronotum (Borror, Triplehorn and Johnson, 1989). The clubbed and elbowed antennae indicate a close relationship with the weevils (Curculionidae). They tunnel under the bark of trees and each species produces a characteristic pattern, which may be the best means of identification (Borror, Triplehorn and Johnson, 1989). Heavy infestations kill trees, with the bark dropping off to reveal the galleries. Both sexes co-operate in tunnelling through the bark, where the male excavates a mating chamber, from which the female tunnels out, ovipositing at intervals. When the larvae hatch they tunnel out in various directions to form the characteristic gallery patterns,

pupating under the bark and in due course emerging through their own exit holes. *Scolytus scolytus*, one of the best known examples of bark beetles and referred to as the elm bark beetle, is responsible for the death of thousands of elm trees in Europe, through the spread of Dutch elm disease (Carter, 1973).

2.2.2.1 Association with fungal transmission

Paine, Raffa and Harrington (1997) studied Scolytidae beetles that colonise living conifers and simultaneously transmit specific fungal pathogens that are harboured in specialised morphological structures (mycangia) of the insect or are present on its body surface. Fungal pathogens are introduced into the conifers during attack by the insects. The frequent association indicates that there might possibly be a mutual benefit to the fitness of both the beetles and the fungal pathogen. It has been well documented that mycangial fungi benefit from the association with bark beetle vectors by being transferred between individual trees and the beetles therefore serving as a dependant vector. The specific development of mycangia, strongly suggests that the beetles also benefit from the insect-fungal association. The beetles may gain an advantage from the association with fungi by feeding on the fungi. Furthermore, fungi contribute to the degeneration of the host tree through; mycelial penetration of host tissue, toxin release, interactions with pre-formed and induced conifer defences, and the mutual action of both beetles and fungi during colonisation. The mycangial fungi's potential part in reducing tree resistance during colonisation in association with the beetle vector cannot be ignored. Overall it thus seems important to understand how the fungal species are transferred, since this may provide insight into the nature of their relationship with the insect.

2.2.3 Chrysomelidae (Leaf beetles, flea beetles)

This family is commonly referred to as leaf beetles. Most species of this family, in both adult and larval stages, feed on leaves of plants and some are considered pests. Leaf beetles adults usually range from 5 to 15 mm in size and are brightly coloured. They have different body shapes from elongate or flattened to globular. Some may even be mistaken for ladybird beetles (Coccinellidae) due to their oval shape. Their antennae are usually less than half the length of their bodies. Pupae are rarely seen. since the larvae drop from plants and pupate in the litter and soil below. Close to 1 500 species have been recorded from Southern Africa (Scholtz and Holm, 1985).

2.2.3.1 Association with fungal transmission

Shortt, Sinclair, Helm, Jeffords and Kogan (1981) conducted various experiments using bean leaf beetles (*Cerotoma trifurcata*) and *Alternaria tenuissima* on soybean plants (*Glycine max*). These tests included sampling of pathogens, pod and seed evaluation to quantify beetle and pathogen damage, pathogenicity tests of relevant fungi and isolations from the heads and abdomens of beetles. In total nine species of soybean pathogens were isolated of which *A. tenuissima* was predominant. Other species were *A. alternata*, *Epicoccum* sp., *Nematospora* sp., *Phoma* sp., *Fusarium equiseti*, *F. graminearum*, *F. tricinctum* and *Gliocladium roseum*. The authors state that because the mycoflora of insects commonly reflects the environment, the association of these fungi with the beetles only suggests that transmission to plants is possible. Conclusively, their data indicated that beetle injury is not required for any of the fungi to infect the soybean seeds. Even so, beetle feeding could increase the dispersion and inoculation of fungal pathogens to favourable sites

such as wounds and facultative transmission of fungal pathogens may compound the effect of feeding injury.

Dillard, Cobb and Lamboy (1998) investigated whether there was any association between *Phyllotreta cruciferae*, flea beetle infestations and incidence of alternaria leaf spot, and whether flea beetles can vector *Alternaria brassicicola* to cabbage. Flea beetles were isolated and *A. brassicicola* was isolated from several of the specimens. It was also noted that the isolation frequency increased as the season progressed. Three treatments were made on cabbage plants, *i.e.* non-inoculated plants, inoculated and flea beetle transmitted. The latter treatments yielded substantial lesions on the cabbage plants, but those, inoculated with the pathogen, were much more severe. Faecal matter was also examined for fungal propagules of *A. brassicicola* by microscopy and by isolation and conidia of *A. brassicicola* were observed. Scanning electron microscopy (SEM) was used to examine the flea beetle externally for any propagules and once again conidia of *A. brassicicola* were observed on all body parts of the flea beetle. It was also established that feeding injuries along leaf margins were often the site of entry for the fungus and eventually the development of alternaria leaf spot. The salivary and digestive fluids in which the conidia were deposited and the fluid exuded from wounded parts on the leaves were likely sources of moisture and nutrients for the conidia to germinate. The authors proposed that transmission could occur in four ways, *i.e.* passive deposition on stem and leaf surfaces, externally from the body, feeding sites that serve as entry for passive deposition of conidia, deposition of viable conidia within the faeces and deposition by mouthparts after flea beetles passed antennae through mouthparts.

2.2.4 Curculionidae (weevils)

The Curculionidae constitutes the largest insect family in the world and presently consists of about 45 000 described species (Scholtz and Holm, 1985). They vary from 1 to 60 mm in size and show enormous diversity in habits, shape and colour. Characteristically they all have a snout, ranging from short and broad to long and thin with mandibles at the end. All Curculionidae are primarily phytophagous as adults and larvae, feeding on all parts of plants and many species are pests of crops and stored products (Picker, Griffiths and Weaving, 2002). Some species are known to feed on fungi growing on dead or decaying wood. Often fungus-feeding weevils are present in leaf litter, humus or even deep soil and these species are characterised by rudimentary eyes and stout, elongate bodies (Scholtz and Holm, 1985).

2.2.4.1 Association with fungal transmission

Kadlec, Stary and Zumr (1992) analysed adult weevils (*Hylobius abietis*) and their excreta and showed that the weevils are vectors of *H. annosum*. They concluded that the feeding scars inflicted by the weevils in the bark of conifers may also assist in the process of infection of the trees by fungi, such as *H. annosum*. *H. abietis*, as a vector of *H. annosum*, may be significant in comparison with other modes of dispersal.

Leath and Hower's (1993) studied insect-vector relationships in alfalfa to determine both, the potential for *Sitona hispidulus* (Clover root curculio) to vector *Fusarium oxysporum* f. sp. *medicaginis* and fungal colonisation from larval feeding sites. Roots with feeding sites of the weevils were collected and three isolations were made, centripetally from the feeding site. Sequentially, root tissue from the feeding site yielded 75%, 30% and

5% fungal colonisation. Larvae head capsules were isolated and pathogenicity tests were conducted with two Fusarium isolates, consequently causing vascular necrosis and wilting in alfalfa plants (*Medicago sativa*). *S. hispidulus* eggs were sterilised, and deposited onto the surface of the soil of alfalfa plants treated with *F. o. medicaginis*,. The eggs hatched over 77 days, burrowed beneath the soil and fed on the roots. As a result *F. o. medicaginis* was isolated from all the symptomatic plants. From this it was concluded that increased feeding of the weevil larvae increased Fusarium wilt.

Hill, Newton, Zeiders and Elgin (1968), similarly to Leath and Byers (1977), exposed alfalfa plants (*Medicago sativa*) to larvae of the clover root curculio (*Sitona hispidulus*), *Fusarium* wilt fungus (*Fusarium oxysporum*), and bacterial wilt (*Corynebacterium insidiosum*) in factorial combinations in two separate experiments. The most significant damage was evident with *F. oxysporum* and *S. hispidulus* in combination, confirming that the presence of *S. hispidula* will increase the damage caused by *F. oxysporum*.

2.3 Order: Diptera

2.3.1 Drosophilidae (vinegar flies)

Drosophilidae are one of the largest families of the acalypterate Diptera (almost 3000 described species in over 60 genera) and are found worldwide. By 1984, 62 genera and 2822 described species of this family were known, and at least 4000 species were predicted to exist (Wheeler, 1986).

Adults of most species of Drosophilidae are attracted to and feed upon a great variety of fermenting substances, hence the common name of vinegar -, pomace -, or fruit flies (the

latter not to be confused with the true fruit flies of the family Tephritidae). Oviposition and larval development are, however, highly specialised and more readily in fruits or flowers, slime fluxes and decaying leaf matter (Wheeler, 1986). Two subfamilies are known from southern Africa, *i.e.* Steganinae and Drosophilinae. The genera *Leucophenga* and particularly *Drosophila* are well represented in southern Africa, representing about three quarters of the drosophilid fauna (Scholtz and Holm, 1985).

2.3.1.1 Association with fungal transmission

Drosophilidae have been shown to disseminate *Mucor piriformis* in peach and nectarine fruit (Michailides and Spotts, 1990). *Drosophila melanogaster* was shown to transmit propagules of *M. piriformis* from infected peaches to 75-100% of injured fruit. Viable fungal propagules of *M. piriformis* persisted for 15 days on *D. melanogaster.*

Louis, Girard, Kuhl and Lopez-Ferber (1996) studied the transmission mechanisms of the main grape fungus, *Botrytis cinerea*, by *D. melanogaster*, validating external (cuticular) transport of *B. cinerea* conidia, in addition to ingestion and conidial transit through the digestive tract. *Drosophila melanogaster* were first allowed to feed on a sporulating culture, and then given a chance to clean themselves. *B. cinerea* conidia were observed, adhering to the insect cuticle, by using scanning electron microscopy (SEM). Two separate techniques were used to determine whether conidia remained virulent at the end of intestinal transit, *i.e.* faeces cultivation and direct observation of *D. melanogaster* rectums. *Botrytis cinerea* development resulted from the culture of fly faeces. Dissection and microscopic examination of *D. melanogaster* that had fed on sporulating fungal cultures confirmed that the rectums were filled with non-germinated conidia. *In situ* germination of

spores occurred from each of the examined rectums. Females were noted to contain more propagules than males, possibly due to the fact that females consume more in order to realise oogenesis. The authors came to the conclusion that *Drosophila* spp., in general, appear to be effective vectors of *B. cinerea* by actively dispersing spores and that these flies are agents of non-persistent transmission because of their capability to harbour spores externally. They are also agents of semi-persistent transmission, because they can release viable spores after passage through the digestive tract. They can even be considered as potential agents of persistent transmission, due to the fungus developing in the fly crop and generating resistant forms. Thus, once harbouring fungal propagules, *Drosophila* spp. become a potential reservoir of rot in three ways, namely spores, mycelium and microsclerotia (Louis, Girard, Kuhl and Lopez-Ferber, 1995).

Butler (1961) investigated whether the vinegar fly, *D. melanogaster*, was responsible for transmission of Geotrichum rot. This was suggested after detecting the scattered pattern of rot distribution and the large numbers of vinegar flies in tomato fields when fruit were numerous. It was shown that *D. melanogaster* harbours propagules of *Geotrichum candidum* and that these insects transmit propagules to a relevant substrate. Since it had been observed that the flies visit decaying fruit, it seemed likely that *G. candidum* could be deposited on healthy tissue by flies that had previously visited fruit containing *G. candidum*. Results also showed that both the proportion of the flies harbouring propagules of *G. candidum* and the number of fruit with Geotrichum rot increased as the season advanced. It was found that *D. melanogaster* harbours the pathogen and can deposit it by coming into contact with a moist surface. Furthermore, it was also shown that air currents carry the spores, albeit in low numbers. Other fungi, such as *Rhizopus* spp. and *Mucor hiemalis*, were

also isolated from the flies. It was also found that *Drosophila* spp. feed on or ingest hyphae of *G. candidum*, since it was isolated from the digestive tract of wild flies (Phaff, Miller and Shifrine, 1956; Phaff, Miller, Recca and Shifrine, 1956 and Shehata, El-Tabey Awab and Mrak, 1951 in Butler, 1961). The tendency of adult *Drosophila* spp. to visit and oviposit in healthy fruit and also feed on the microorganisms in decaying tissues ensures the spread of Geotrichum rot in tomato fields. Other species of insects were also found to harbour the pathogen, but they do not visit growth cracks or other sites suitable for inoculation on the tomatoes.

Butler and Bracker (1963) conducted additional studies on the relationship between *D. melanogaster* and *G. candidum* relation to fruit rot, the potential role of *D. melanogaster* as a vector of fungi pathogenic to ripe tomato fruit and the traits of the flies relevant to fruit rot epiphytology. Once again isolations were made from the flies obtained from different fruit orchards (*i.e.* tomato, peach and melon) and the following pathogenic fungi were isolated: *G. candidum* (60%); *R. stolonifer* (35%); *Mucor* sp. (14%); and *Gilbertella persicaria* (4%). *G. candidum* and *R. stolonifer* was isolated from flies at all the collection sites. *Mucor* sp. was isolated from flies in tomato fields and it was shown to be virulent on ripe tomatoes. *G. persicaria* was isolated from flies in tomato fields and although it does not play a role in tomato rot, it is an important decay organism on peach fruit and its presence on flies in tomato fields located close to peach orchards indicates that flies migrate to tomato fields following peach harvest. *D. melanogaster* is therefore a factor in the epiphytology of tomato fruit rot caused by *G. candidum, G. persicaria* and *Mucor* sp. Both field and laboratory experiments concluded that *R. stolonifer* and *G. candidum* are transmitted by these flies and, importantly, it was shown that a single contaminated fly from

the field could transmit the *Rhizopus* rot and *Geotrichum* rot to several fruits. Furthermore, by individual isolation of body parts (head, thorax, abdomen, wings and legs), it was observed that *R. stolonifer* and *G. candidum* spores were present on all body parts. Since the flies were captured and placed together in a single container, the chances of cross contamination would have been high. In general flies are attracted to fruit at various stages of decay and these visitations ensure that a significant percentage of flies are able to acquire numerous spores on external body parts. Growth crack wounds that are fresh and moist, apparently emit volatile substances that attract *D. melanogaster.* In this regard ovipositing females thus have a greater chance of depositing spores on moist tissue or wounds, while larvae presumably spread the pathogen further by probing into the wounds. It is therefore clear that the transmission of rot by the flies is purely mechanical. Overall, the close integration of behavioural and ecological characteristics of the fly, the host, and the pathogen in the epiphytology of *Geotrichum* rot was verified.

Chymomyza procnemoides and to a lesser degree *D. melanogaster* have also been associated with the transmission of *Ceratocystis fimbriata* (Moller and DeVay, 1968).

2.3.2 Ephydridae (shore flies)

Four subfamilies of shore flies, *i.e.* Psilopinae, Notiphilinae, Parydrinae and Ephydrinae and 70 species occur in southern Africa (Scholtz and Holm, 1985). Most species of shore flies occur naturally on muddy or marshy lakeshores and intertidal zones of beaches. Shore flies in the genus *Scatella* are small (2 mm) black flies with reddish eyes and gray wings with clear spots. Shore flies resemble eye gnats, fruit flies, or vinegar flies

in general shape and are sometimes confused with dark winged fungus gnats that are about the same size and colour.

Adult and immature shore flies feed on microscopic algae, dinoflagellates, bacteria, cyanobacteria, and other unicellular forms. *Scatella* shore flies are commonly found in greenhouses where they breed on algae growing on the potting mix, pots, benches and floors. Females scatter eggs directly on the surface of the potting mix and these hatch within 2 - 3 days. The larvae are found within the crust of algae and very top layer of potting mix, feeding on bacteria and yeasts, as well as diatoms and flagellates growing on the surface of the potting mix. The larva mature in 3 - 6 days and pupates into a puparium. The puparium affords the relatively tender and completely helpless pupa protection from environmental hazards (including insecticides). Some of the puparia are found on top of the potting mix or very close to the surface. A new generation of adult flies emerges 4 - 5 days later. The adults crawl about on the surface of the potting mix, on the plants or they fly amongst the pots and plants. The flies are rapid fliers, but generally stay close to their breeding sites. The adults primarily feed on diatoms and flagellates on the surface of the potting mats.

2.3.2.1 Association with fungal transmission

Scatella stagnalis has been found to be a potential aerial vector of *Pythium aphanidermatum*, a detrimental root pathogen of hydroponically grown cucumbers in California (Goldberg and Stanghellini, 1990). Stanghellini, Rasmussen and Kim (1999) investigated the possibility of adult shore flies transmitting the pathogen *Thielaviopsis basicola*, the causal agent of root and stem rot of corn-salad plants (*Valerianella locusta*) in California. Their main objective was to determine whether the insects transmitted the

pathogen internally. The larva, pupae and adults of the adult shore flies were collected and examined for chlamydospores. Frass deposits isolated onto agar were also examined for chlamydospores. Larva, adults and the frass deposits, which were microscopically examined, had the majority of fungal propagules present. Remarkably, 14% of the pupae were also internally infested with chlamydospores. Twenty pathogen-free adult shore flies were placed in small chambers with naturally infected corn salad seedlings. Sequentially, ten of the flies were placed in Petri dishes and the frass was examined for the presence of the pathogen. The other ten were placed in a chamber with ten healthy corn salad seedlings. All the frass that was excreted by the adults was infested with chlamydospores. Several of the infested adult shore flies that were caged with healthy plants caused disease symptoms of *T. basicola*, 14 - 21 days later.

2.3.3 Sciaridae (fungus gnats)

Most species of dark winged fungus gnats feed on fungi and decaying organic matter and are not considered economically important (Osborne, 2000). A few species, however, attack healthy tissue of economic plants such as potatoes, wheat, red clover, alfalfa, cultivated mushrooms, pine seedlings, and various ornamentals, including tulip bulbs, ferns, begonias, coleus, geraniums, cacti, young orchids, areca palm, and dracaenas (Osborne, 2000). Sciarids are a problem in greenhouses, mostly concerning injury to plants, but also on account of large numbers of flying gnats being an occasional nuisance to workers. The immature stages are usually found in decaying plant material and some feed on fungi or animal excrement. The southern Africa fauna is poorly known and five genera and ten

species have been recorded of which most of the species belong to the genus *Sciara* (Scholtz and Holm, 1985).

2.3.3.1 Association with fungal transmission

Adult fungus gnats (*Bradysia impatiens*) are potential vectors of *Fusarium oxysporum* f. sp. *radicis-lycopersici* of tomato and hydroponically grown crops (Gillespie and Menzies, 1993). These authors do not report the method of acquirement. Jarvis, Shipp and Gardiner (1993) report that larvae of fungus gnats ingested and excreted viable oospores of *Pythium aphanidermatum* and ultimately could transmit the pathogen to healthy cucumber plants, which became infected. However, adult fungus gnats did not ingest the fungus and were not considered as a potential aerial vector of *P. aphanidermatum*.

2.3.4 Tephritidae (fruit flies)

Fruit flies are agriculturally the most important family of flies. Worldwide about 70 species of fruit flies are considered important agricultural pests, and many others are minor or potential pests (White and Elson-Harris, 1992). Fruit of citrus, mango, apples and many others are attacked, while some seed crops such as sunflower and safflower are also affected. Tephritidae are among the most attractive and biologically interesting Diptera, having patterned wings and often brightly coloured and/or patterned bodies, which may be used to mimic jumping spiders or wasps, or for elaborate courtship rituals and other behaviours. Most fruit flies breed within living plant tissues. Exceptions include the Tachiniscinae (the only reared species being a moth parasitoid), most Phytalmiinae (generally saprophages, although many species breed in damaged or recently dead tissues of

a limited range of plants), and several species that are predaceous within galls. The larvae of the phytophagous species may feed inside fruits, seeds, galls, leaf or stem mines, or flowers. Adult fruit flies usually only feed on liquids (sap, honeydew, droppings) or microorganisms, although adults of *Blepharoneura* and related genera have spines on their labella and are able to rasp and feed on plant tissues. Tephritids attack a broad range of plant families, although the host specificity of individual species vary greatly, and most are restricted to a few related or even single species of hosts.

2.3.4.1 Association with fungal transmission

Ito, Kunimoto and Ko (1979) investigated whether three species of fruit flies were able to transmit spores of the pathogen *Mucor hiemalis* which causes Mucor rot on guava fruit. The three species included *Dacus dorsalis* (Oriental fly), *Dacus cucurbitae* (Melon Fly) and *Ceratitis capitata* (Mediterranean fly). The first experiment proved that all three species of fruit flies were capable of transmitting Mucor rot from diseased to healthy fruits. In a separate experiment it was shown that young flies were not as capable in transmitting Mucor rot as older flies, probably because of less oviposition activity. Importantly, it was concluded that orchard sanitation reduced the percentage of fruits infected with Mucor rot.

2.3.5 Agromyzidae (leaf miner flies)

The Agromyzidae are small to minute flies (wingspan: 4mm). Larvae are leaf or stem miners of flowering plants, mostly within the daisy and carrot family. The yellow larvae usually mine the upper leaf epidermis and leave a black trail of faeces in the meandering mine. In South Africa, the larvae of the potato leaf miner, *Liriomyza*

huidobrensis, causes severe damage to potato crops, tomato and melons by mining leaves and consequently blemishing foliage and retarding growth (Picker, Griffiths and Weaving, 2002).

2.3.5.1 Association with fungal transmission

Letourneau and Msuku (1992) investigated the role of *Ophiomyia* sp. (Agromyzidae) as a vector of *Fusarium solani* f.sp. *phaseoli* on beans. The main objective of this study was to determine if the bean fly facilitates infection of beans by *F. solani* f.sp. *phaseoli* and if indeed so, how significant the interaction was. Initially several field surveys suggested the existence of an insect-fungal interaction. In a glasshouse trial the soil was inoculated with *F. solani* f.sp. *phaseoli*, bean seeds were sown, and stems infested with bean fly maggots were placed within the enclosure. An increase in the level of infection with *F. solani* f.sp. *phaseoli* in bean plants exposed to the bean fly was recorded. Furthermore, it was detected that a large number of flies could enhance infection rates without actually causing a lesion at the base of the stem. In the field experiment certain bean plants were covered with mesh and the rest were left open to bean fly exposure. Here, also, bean fly incidence had a dramatic effect on the incidence of infection with *F. solani* f.sp. *phaseoli*.

2.4 Other insect orders associated with fungal transmission

2.4.1 Order: Orthoptera

2.4.1.1 Acrididae and Tettigonidae

Two species of grasshopper have been shown to serve as a dispersal agent for the fungal genus *Colletotrichum (*Yang and TeBeest, 1994). A species of longhorn grasshopper (*Melanoplus differentialis*) and shorthorn grasshopper (*Conocephalus fasciatus*) were found feeding on the northern jointvetch weed that competes with rice. Both species of grasshopper were found to transmit *Colletotrichum gloeosporoides* f. sp. *aeschynomene* to healthy plants. Subsequent to pathogen-free grasshoppers feeding on anthracnose lesions, 65-75% of them transmitted the pathogen to healthy plants. It was also observed that lesions formed where no insect feeding had been noted, indicating that the pathogen is harboured externally and can be spread by contact.

2.4.2 Hymenoptera

2.4.2.1 Agaonidae

Michailides and Morgan (1998) investigated the spread of *Fusarium verticilloides* by a small symbiotic wasp (Agaonidae: *Blastophaga psenes*), which causes endosepsis, while pollinating Calimyrna figs in California. It was revealed that there was no significant difference in incidence of *F. verticilloides* in figs harvested from various positions (height, outer or inner tree canopy, sun or shade) within each tree. Moreover, it was revealed by the disease-spread experiments in both commercial and experimental orchards that endosepsis disease follows a pattern similar to distinctive spread for airborne diseases from the inoculum source (Jeger, 1990 in Michailides and Morgan, 1998). Although airborne

inoculum *per se* does not exist, the microconidia of the pathogen become airborne *via* the wasps. Convincingly, the behavioural patterns and the movement of the wasps were shown to directly affect the disease gradients. As expected the number of wasps was positively related to the incidence of endosepsis and negatively to the distance from the source.

2.4.3 Lepidoptera

2.4.3.1. Tortricidae (grape berry moth)

Fermaud and Le Menn (1992) investigated the larvae of *Lobesia botrana* (Tortricidae) and its potential to increase the severity of gray mould (*Botrytis cinerea*) on grapes. Second and third larval generations were found to transmit viable conidia to feeding sites on the grapes. In field experiments the second and third generation larvae were artificially infested with viable conidia of *B. cinerea*, which caused a significant increase in the level of larval injuries infested with *B. cinerea*. These findings suggest that *L.botrana* can vector *B. cinerea* from infected to healthy grape berries. Sanitation is thus important to reduce gray mould in vineyards.

2.4.4 Homoptera

2.4.4.1 Aphididae

Leath and Byers (1977) evaluated the interaction between *Fusarium* species that cause root rot, the pea aphid (*Acyrthosiphon pisum*) and the potato leafhopper (Cicadellidae: *Empoasca fabae*) on the leaves and stems of red clover, white clover and alfalfa plants. Separate groups of plants were exposed to *Fusarium*, aphids or leafhoppers, *Fusarium* and aphids, or *Fusarium* and leafhoppers, in factorial combinations. From this it was evident

that the most severe damage on plants were those inoculated with *Fusarium* and subjected to aphid feeding. The interaction of insect feeding with the development of rots is important since it points out the shortcoming of considering insect infestation and disease incidence as separate, discrete problems. It is thus important to bear in mind that biotic and abiotic stress may occur simultaneously, or sequentially and that they are always on the increase within the system being studied.

3.0 Mites (Acarina) as vectors of fungal pathogens

Kemp, Pretorius and Wingfield (1996) investigated the association of the mycophagous mite (*Siteroptes avenae*), which was frequently observed on diseased spikes of wheat, with the causal agent of wheat glume spot. Mites were placed in *Fusarium* cultures to infest them, whereafter the cultured petri dishes including the mites were placed within a canopy of healthy plants in a greenhouse. Symptoms characteristic of those in the field were produced after three weeks and *Fusarium poae* was isolated from symptomatic tissue. *S. avenae* has been reported to possess sporothecae behind the propodosomal plate. These consist of two elongate, sleeve-like sacs closed at one end. Regularly these sporothecae are withdrawn inside the body. According to Suski (1973), microconidia are collected into the sporothecae when the mites crawl over mycelium and are discharged from the sporothecae by changes in the internal body pressure.

Batra and Stavely (1994) observed that significantly more mites occurred on bean plants *Phaseolus vulgaris*, bearing uredinia of the rust fungus *Uromyces appendiculatus*, than on rust free bean plants. Their main objectives were to determine whether bean rust attracts mites, whether spores adhere passively or due to the mite behaviour and whether

mites vector rust spores. Mite populations were higher on rust infested than on rust free leaves. It was also observed that mites could disseminate urediniospores. Conclusively, it is doubtful whether mites are major vectors because of their limited capacity to migrate and they would therefore perform weakly when compared with the wind-dissemination of spores. However, when mites are associated with the rust fungus, overall damage to the crop could be enhanced.

4.0 Conclusion

The basic morphology of an insect is important when assuming that it is capable of fungal transmission. For instance, insects with piercing sucking mouthparts (Hemiptera), damage plant tissues while feeding, which results in the insect not only having the potential to transmit a pathogen, but it also leaves an opening for pathogens disseminated by other means, *e.g.* rain or wind, to infect the plant.

It is important to consider insects as disseminators, or vectors, of fungal pathogens if proper crop management is to be achieved. Most of the investigations into disseminators or vectors involved insects that cause extensive damage to the specific crops. However, insects that aren't recognised as pests but are numerous and come into extensive contact with the crop, should also be considered potential disseminators. Since isolations were made from most of the insects in the review, it could be important to consider insects as indicators of fungal species in crop cultivation.

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CHAPTER 2

Drosophilidae as disseminators of fungal phytopathogens to *Opuntia ficus-indica* (Cactaceae)

Abstract

Cactus pear (*Opuntia ficus-indica*) is an important alternative crop in the semi-arid regions of southern Africa upon which numerous insect pests and diseases have recently become a problem. Vinegar flies (Drosophilidae), are very common in cactus pear orchards where they feed and breed in mature fruit. *Drosophila* spp. are known vectors of fungi and the objective of this study was to investigate whether *D. melanogaster* and *D. hydei* harbour and transmit fungal pathogens of cactus pear. The ecological succession and fungal transmission potential of *Drosophila* spp. (Diptera: Drosophilidae) on the fruit of cactus pear was also studied. Specimens (n = 100) of each species were individually captured on *O. ficus-indica* plants and plated onto agar media. Twelve genera of mycelial fungi were identified from both species. Pathogenicity tests with isolated fungi on mature *O. ficus-indica* cladodes and fruits were positive for several of the fungal genera and Koch's postulates were confirmed. The most pathogenic fungus on cladodes and fruit was a species of *Michrodochium*. Scanning electron microscopy confirmed the presence of fungal propagules on the exoskeleton of both *Drosophila* species.

1.0 Introduction

The spineless cactus, *Opuntia ficus-indica* (L.) Miller, fits most requirements of a crop that can be used to contain desertification, while simultaneously providing food for humans, livestock and wildlife living in arid environments. This extremely hardy desert plant requires very little agronomic input, while providing many valuable products and by-products (Barbera, Ingelse and Pimienta-Barrios, 1995). The plant was first introduced into South Africa in 1652 from Mexico via Europe (Annecke and Moran, 1978). The introduction of several spineless cactus cultivars in 1914 (including various Burbank spineless cactus varieties) forms the basis of the fast growing cactus pear industry in South Africa (Brutsch and Zimmermann, 1993). Cactus pear plantations cover more than 2000 ha and the objective of local producers is to reach northern hemisphere markets during winter (Barbera, Ingelse and Pimienta-Barrios, 1995).

The most important constraint faced by the emerging cactus pear industry in South Africa, is the threat from insect pests and diseases. In Mexico, the world's largest producer of cactus pear, intensive monoculture of cactus pear has resulted in the development of numerous pest and disease problems. Approximately 122 species of insects are presently known to be associated with *Opuntia* spp. (Longo and Rapisarda 1995), while numerous species of pathogenic fungi and bacteria have also been recorded (Farr, Bills, Chamuris, and Rossman 1989; Granata, 1995).

Many insect taxa are vectors of phytopathogenic micro-organisms (Carter, 1962; Colby, Windham and Grant 1996; Harris and Maramorosch, 1980; Lucas, 1998; Stanghellini, Rasmussen and Kim, 1999). A large number of dipterous species, especially vinegar flies (*Drosophila* spp.), are vectors of fungal pathogens that cause fruit

diseases on various plants (Table 1). *Drosophila* spp. also play other roles in the Cactaceae, including the vectoring of yeasts (Do Carmo-Sousa, 1969; Barker and Starmer, 1982). The bacterium *Erwinia carotovora* subsp. *carotovora*, which may cause extensive damage on *O. ficus-indica* (Varvaro, Granata and Balestra,. 1993), is also the causal agent of potato blackleg. The pathogen is vectored internally and externally by *D. melanogaster* Meigen and *D. buskii* Coquillett (Brewer, Harrison and Winston, 1981). The possibility therefore exists that *D. melanogaster* can also vector *E. carotovora* subsp. *carotovora* to *O. ficus-indica.*

Drosophila larvae and adults feed on microorganisms present in decaying cactus pear fruits which serve as a rich substrate for fungi, yeasts and bacteria. Drosophilidae are commonly found around fallen fruit in cactus pear orchards, and their potential to disseminate pathogenic fungal propagules to healthy cactus pear plants is therefore obvious. The objectives of the present study were, firstly, to determine whether *D. melanogaster* Meigen (Fig. 1) and *D. hydei* Sturtevant (Fig. 2) occurring in cactus pear orchards in South Africa harbour external and/or internal fungi that are possibly pathogenic to cactus pear fruits and cladodes. A second objective was to study the ecology of *Drosophila* spp. in cactus pear orchards in terms of their interactions with other insects associated with cactus pear and their environment. A third objective was to ascertain whether vinegar flies are able to disseminate pathogenic fungi to fruit.

2.0 Materials and Methods

Collection of insects. Sampling of Drosophilidae was conducted weekly over a two month period during early autumn (1 March to 30 April 2000) in a small experimental orchard of *O. ficus-indica*, on the campus of the University of the Free State, Bloemfontein, South Africa. The orchard consisted of 30 4-year-old plants and included the cultivars Morado, Direkteur and Gymno Carpo. The plants were planted in three parallel rows, 3 m apart. An aspirator (pooter) was used to capture the flies and each captured fly was subsequently expressed into a sterile Eppendorf tube. To prevent cross contamination from fungi associated with flies, the aspirator was dipped into 70% ethanol for 30 seconds and allowed to air dry before capturing the next insect. Eppendorf tubes containing live flies were taken to the lab and placed in a freezer at a temperature of -75°C for 5 minutes in order to kill flies without harming fungal propagules that they might harbour. In total, approximately 100 flies of each species were captured and screened for fungi.

Isolation of fungi. Flies were aseptically transferred to individual Petri-plates (65 mm diameter) containing potato dextrose agar (PDA) (Difco) amended with streptomycin sulphate (0.33 g/1000 ml water) and incubated at 25°C in a light-dark cycle of 12 hours each. When fungal colonies became visible with the naked eye they were transferred to corn meal agar (CMA) (Difco) for the purpose of identification and quantification.

Artificial inoculation of fruit. The pathogenicity of the above-mentioned 14 fungal isolates was also tested by artificially inoculating semi-ripe fruit of *O. ficus-indica* (cultivar Algeria). PDA plates containing steam sterilised toothpick tips (1.5 mm x 15.0 mm) were inoculated with the respective fungal isolates and incubated for 10 days at

25°C. Cactus pear fruit were surface sterilised by wiping them with a cloth soaked in 95% ethyl alcohol. Each fruit was artificially inoculated by inserting the colonised toothpick tip into the fruit to a depth of 10 mm (Fig. 3). Artificially inoculated fruit were incubated at a mean daily temperature of 22°C for 14 days before the resulting lesions were measured. Each treatment (fungal isolate) was replicated 10 times using 5 fruit, with 2 inoculation sites per fruit. The control treatment comprised of sterile toothpick tips inserted into the surface of sterilised fruit.

Artificial inoculation of cladodes. The pathogenicity of 14 potentially phytopathogenic fungal isolates obtained from Drosophilidae was investigated by artificially inoculating detached 1-yr-old *O. ficus-indica* cladodes (cultivar Morado) in the glasshouse. Using a cork borer (9.5 mm diameter) an agar plug was removed from the advancing edge of an actively growing fungal culture and inserted into a wound made on a cladode by removing a small cylinder of tissue (9.5 x 10 mm) after the surface had been sterilised by wiping it with a cloth soaked in 95% ethyl alcohol. After inserting the inoculum plug, the wound was closed with the original tissue cylinder and a piece of moist filter paper was placed over the wound to prevent desiccation of the inoculum. Control treatments consisted of sterile agar plugs (Fig. 4). Each treatment was replicated twice on three separate cladodes and the experiment was conducted twice.

Cladodes were incubated in a glasshouse for 21 days at an average day temperature of 25°C for 12 hours and night temperature of 15°C for 12 hours. The diameter of each lesion that had formed around the inoculation site was measured along two perpendicular axes using a digital calliper. A piece of plant tissue was extracted

from the perimeter of each lesion, using a tweezer, incubated on PDA. Then emerging fungal colony was identified to confirm Koch's postulates.

Scanning electron microscopy. To confirm whether fungal propagules were harboured by the captured vinegar flies scanning electron microscopy (SEM) was conducted. The flies were placed in a freezer at -10°C for an hour and thereafter freeze-dried for 2 hours. Specimens were fixed onto SEM stubs by means of a plastic adhesive, sputter coated with gold and observed. All body parts were examined for signs of fungal propagules.

Ecological succession. The experiment was conducted twice on two separate occasions- the first from 12 February to 15 March and the second from 22 March to 20 May 2002. Ten small (height 25cm, diameter 20cm) buckets containing three fruits each were placed in a small experimental orchard of *O. ficus-indica*. Five buckets were placed in a shaded area and 5 were in a sunny area. Holes were pierced at the base of each bucket to allow drainage. The trial was inspected 2 - 3 times per week. Drosophilidae were counted by covering the bucket opening with a glass dish, shaking the bucket and waiting for flies to accumulate on the inner surface of the dish. After this was completed the dish was removed and insect species interacting with the fruit were counted.

Efficacy of Sporekill™ as a fungal propagule eliminator on Drosophila. Sporekill™ is commonly used to sterilize seeds of plants. It was sprayed on *Drosophilidae* specimens to determine whether the spores could be killed on the flies. Twenty flies were artificially infested with a *Penicllium* sp. and a *Mucor* sp.. A 3% solution of sporekill (a 5% solution is usually used to treat seeds) was made up and applied using a spray can. The flies were given a chance to recover and dry before being

plated on PDA agar (Difco) amended with streptomycin sulphate (0.33 g/1000 ml water). Developing fungi were then matched with the original fungi used to infest the flies.

Dissemination potential experiment. An experiment was conducted to determine whether flies are able to disseminate pathogenic fungi to cactus pear fruit and cause disease of the fruit. Flies were bred in the lab on cactus pear fruit and superficially sterilised by spraying them with a 3% Sporekill™ solution. Flies were also allowed to feed on autoclaved fruit for two days so as to remain sterile. They were then artificially infested with *Aspergillus niger* and *Fusarium* sp. 1 by placing them in Petri-plates containing cultures of the specific isolates for two hours. Cactus pears were treated by spraying them with a 5% Sporekill™ solution and sequentially rinsing them in 70 % ethanol and sterile water. Three fruits were then placed in a plastic container covered by gauze netting. The fruit were punctured on one half using a needle to provide wounds for possible fungal infection. The other half of the fruit was not wounded. Two replicates of each treatment and two control treatments were set up. The latter consisted of unsterilised fruit with no flies and one sterilised fruit with no flies. Another two control treatments consisted of two punctured and two non-punctured fruits per container. One treatment consisted of flies treated with Sporekill™ and fruit superficially sterilised, the other of flies not treated with Sporekill™ with fruit superficially sterilized. Two replicates were made of each. Punctured fruits were each pierced ten times with a dissection needle that had been dipped in 70% ethanol and flamed to ensure sterility before and after use on a fruit. All treatments were left in the laboratory at 25°C for 25 days and by using a rot rating scale (Fig. 5) diseased fruit were assessed. Isolations were

conducted from diseased tissue to confirm that fungi with which flies had been infested had caused the rot.

3.0 Results and Discussion

The results of the present study confirm that *D. melanogaster* and *D. hydei* harbour fungal propagules externally (and probably internally too). This finding is consistent with numerous reports that Drosophilidae are known vectors of various fungal pathogens and play an important role in the epidemiology of many plant diseases. Lack (1989) showed that significantly more brown rot was caused by *Monilinia fructigena* on injured apples that had been visited by *D. subobscura* than on unvisited ones. According to Michailides and Spotts (1990), *D. melanogaster* acquired propagules of *Mucor piriformis*, which persisted for at least 15 days on *D. melanogaster*, and transferred them to 75% - 100% of injured peach fruit. These insects were also contaminated with *Rhizopius stolonifer, Monilinia fructicola*, *Cladosporium* spp., *Penicillium* spp., and other species of *Mucor*. Louis, Girard, Kuhl, and Lopez-Ferber (1996) reported that conidia of *Botrytis cinerea*, the causal agent of bunch rot of grape, are carried externally on the cuticle of *D. melanogaster*, which is considered to be a plurimodal vector of *B. cinerea*, supporting non-persistent, semi-persistent, and possibly persistent transmission of the fungus.

Isolation of fungi. Fungi were consistently isolated from *Drosophila melanogaster* and *Drosophila hydei*. Eight genera of mycelial fungi were isolated from *D. melanogaster,* of which *Mucor* spp. and *Fusarium* spp. were most prominent (Table 2). In total twelve genera of mycelial fungi, of which *Mucor* spp. were most prominent,

were isolated from *D. hydei* and *D. melanogaster*. Five genera, *i.e. Alternaria*, *Ascochyta*, *Aureobasidium*, *Paecilomyces* and *Trichoderma* were each represented by a single species and were unique to *D. hydei*, while only *Cladosporium* sp. was unique to *D. melanogaster*.

These results indicate some degree of fungal host specificity on the *Drosophila* spp.. The significance of this finding can probably be attributed to interspecific interaction between the two species related to non-synchronous utilisation of resources and temporal or spatial priority (Hodge, Arthur and Mitchell, 1997). It has, for example, been shown that temperature tolerance may influence the structure of mycophagous fly communities (Worthen and Haney, 1999). Consequently, flies will be exposed to niches where the resident fungal species differ owing to the different types of substrate they utilize.

Yeasts comprised 20.4% and 22.0% of isolations made from *D. hydei* and *D. melanogaster* respectively. It is unfortunate that they could not be identified to species level due to time and resources, as many new and interesting species of yeasts have been previously discovered in the Cactaceae. Yeasts are a major source of food for both the larval and adult stages of numerous insect species and *Drosophila* spp., being mycophagous, are especially closely associated with yeasts (Do Carmo-Sousa, 1969; Spencer and Spencer, 1997). Cactophilic yeasts invade the rotting tissue of numerous species of the cacti, where they metabolise sugars and related compounds released by pectolytic bacterial action. *Drosophila* spp. feed on yeasts and lay their eggs in rotting cactus tissue. When the host plant dies and becomes desiccated, the adults move on to other rotting cacti, carrying the yeasts with them, and the cycle is repeated. A similar

scenario probably applies to the transmission of fungal spores by adult flies, the only difference probably being that the fungal propagules that were isolated are far less persistent than yeasts. Most are probably non-persistent or semi-persistent, such as the spores of *B. cinerea* (Louis *et al.*, 1996).

Artificial inoculation of fruit: The rate of lesion development was generally much greater on fruit than on cladodes, despite the diameter of the toothpicks being 1.5 mm against the 9.5 mm of the cork-borer and a 7-day shorter incubation period. *Michrodochium* sp. 2 formed the largest lesions (25.6 mm), followed by *Aspergillus* sp. (18.4 mm) and *Mucor* sp.3 (12.8 mm) (Fig. 6). The overall mean of lesions for all 14 isolates was 8.45 mm and the standard deviation 6.82. In most cases the full extent of rot was greater internally than reflected by the size of the exterior lesion on the peel of the fruit. After 21 days, most of the inoculated fruit had become very soft due to internal fungal colonisation, whereas fruit from the control treatment were still firm. Koch's postulates were confirmed by the re-isolation and identification of all artificially inoculated fungi.

The ability of mycelial fungi harboured by *D. melanogaster* and *D. hydei* to cause tissue necrosis and rot of *O. ficus-indica* cladodes and fruit was clearly demonstrated. As far as could be ascertained, no studies involving the artificial inoculation of *O. ficus–indica* with fungi isolated from *Drosophila* spp. have been conducted on cladodes and fruit elsewhere.

There was a significant positive correlation ($r = 0.76$) ($P < 0.05$) between cladodes and fruit with regard to the relative pathogenicity of the 14 isolates used for inoculation. *Microdochium* sp.2 caused the largest lesions on both cladodes and fruit, while *A.*

alternata was also able to cause significant necrosis on both organs. *Aspergillus* sp. and *Mucor* sp.3 were, however, markedly more pathogenic on fruit than on cladodes. The pathogenicity of the other *Mucor* spp. and the three *Phoma* spp. was considerably less than the mean overall lesion length for either fruit or cladodes. Although the relative pathogenicity of the isolates on cladodes or fruit may have no significance in terms of their disease-causing potential, what is important is that at least 6 new potential pathogens of *O. ficus-indica* were isolated from the two *Drosophila* spp. These findings are significant, not only in terms of elucidating potential fungal pathogens of cactus pear in South Africa, but also in understanding their epidemiology. Knowledge of the latter is imperative if integrated pest management strategies in cactus pear orchards are to be developed.

Artificial inoculation of cladodes: Twenty-one days after artificial inoculation, all isolates had formed lesions ranging from 12.34 mm (*Mucor* sp.1) to 19.9 mm (*Microdochium* sp.2) (overall mean = 13.46 mm). *Microdochium* sp.2, as well as *Alternaria alternata* (15.22 mm), formed significantly ($P < 0.05$) larger lesions than the other 12 inoculated fungi (Fig.7). No definite lesions formed around the control treatment. Re-isolating and identifying all fungi that had been artificially inoculated confirmed Koch's postulates.

Scanning electron microscopy. Fungal propagules were observed all over the exoskeleton of the flies. Most of these were observed to occur on the legs of the flies (Fig. 8.1). Identification of the fungi *in situ* is very difficult and mostly unachievable. Fungal propagules were observed in groups on some areas of the exoskeleton (Fig. 8.2).

Fruiting bodies, that are essentially containers for spores, were also observed (Fig. 8.3), whilst even setae on the legs often had fungal propagules (Fig. 8.4).

Mid-Devonian spores with complex ornamentation suggest animal dispersal, since the spines and retrorse hooks may have functioned as mechanical attachment to the setae of arthropods (Kevan, Chaloner and Savile, 1975 in Kevan and Baker 1983). In the present study it was important to confirm that fungal propagules were present on the exoskeleton of the insects. Louis *et al.* (1996) also used SEM to confirm fungal propagules present on the exoskeleton of *D. melanogaster*. Butler and Bracker (1963) isolated fungi from different body parts of Drosophilidae and found that all body parts yielded the same amount of fungi. However, their technique may have a drawback in terms of cross contamination, since all the flies were placed together in plastic bags. In this study, each fly was placed in a separate Eppendorf tube to prevent cross contamination. Most of the fungal propagules in this study were observed on the legs of flies, probably because legs are more often in contact with rotting fruit substrates, which may more readily result in the attachment of fungal propagules. The fact that fruiting bodies were observed on the exoskeleton of the insect may increase the chances of survival and spread of the inoculum. Repeated observation of fungal propagules on the insect body gave rise to the assumption that the acquisition and spread of these propagules is indeed effortless.

Ecological succession. Several patterns can be observed in terms of insect species visiting the cactus pear fruit and several assumptions can be made concerning the insects interacting with each other. First of all, there is an overall decreasing trend in most insect numbers as the fruit becomes riper and ages (Figs. 9 – 14). *Drosophila* spp

and Nitidulidae spp. (Coleoptera) were prominent numbers during the first half of all the trials and to a certain extent they also occurred concurrently (Figs. 9 – 14). *Drosophila* spp. showed preference for the shady areas (Figs. 11 and 14) and nitidulid spp. for the sunny areas (Figs. 9 and 13). In both trials (Fig. 15) a definite difference can be seen in terms of the average numbers per trial for both *Drosophila* spp. and nitidulid spp. thereby confirming that nitidulids seem to prefer the sunny microenvironment, while the *Drosophila* spp. seem to prefer the shady microenvironment. In the second trial in the sun (Fig. 13) a species of Formicidae was also numerically dominant. Then, for only a short period, a single species of Eucoilidae (Hymenoptera: Cynipoidea) (Figs. 10 and 12) was observed towards the end of the FM (February-March) trial in both sunny and shady areas. Two species of maggots (Figs. 10 and 12) were concurrently present and were as prevalent as the Drosophilidae adults in terms of population size. Results indicate that shady adapted larva seem to have been affected. A few random samples of these maggots were reared through to adult stage, and the resultant adults were the same species as those already sampled in the trials. There was a strong positive correlation ($r = 0.835$ for the sunny area) and ($r = 0.920$ for the shady area) between the Eucoilidae and the *Drosophila* maggots towards the end the first trials (Figs. 10 and 12). No Staphylinidae (Coleoptera) and Eucoilidae (Hymenoptera: Cynipoidea) were present in the second trial from March to May (Fig. 15) whilst maggots were only present towards the end of all trial (Figs. 10, 12, 13 and 14). Formicidae sp.1, a larger species than Formicidae sp. 2, were only present for a few days at the beginning and the end of the trials (Figs. 9, 13 and 14; and Figs. 11 and 13 respectively). It was evident that Formicidae sp. 1 preferred the sunny areas although they only reached a significant peak

in the second trial (Fig. 13). Formicidae sp.2 occurred constantly throughout the trials, reaching peaks just after rainy periods.

In this study there was an overall decreasing trend in the insect populations as the fruit became riper and more rotten. According to Price (1996) this trend is due to the decrease in the biomass of the trophic resource and is a usual scenario in ecological succession. As the fruit decayed, the chemical environment also changed and different species of insects were attracted. *Drosophila* spp. and Nitidulidae spp. were prominent on the fruit for the first half of the study. Nunney (1996) states that if the larval resource of the trophic guild is decaying fruit, then the species show minimum specialisation and can coexist on a single resource, which, in this study, is cactus pear fruit. This is also attributed to the high sugar and nutrient content of the fruit in the early stages of the aging process that ensures resource availability. According to Brown (1984) cited by (Nunney, 1996) insect species are commonly short lived, generalised feeders, with pronounced population fluctuations in the early stages. Furthermore, it is also expected that early colonisers are highly mobile for the rapid invasion of a new exposed substrate. Since *Drosophila* spp. numbers decrease as the fruit ages and other insect species start to colonise the fruit, a possible case of competitive displacement can be observed. The fact that it is occurring concurrently (Figs. 9, 11, 13 and 14) may be due to their broad niche specialisation. The *Drosophila* spp. showed preference for the shady areas (Figs. 11 and 14) and Nitidulidae spp. for the sunny areas (Fig. 9 and 13). Nitidulids have hardened, thickened elytra that buffer moisture loss in warmer and drier microenvironments. It is thus easier for them to regulate normal body functions in direct sunlight. On the other hand, *Drosophila* has a thinner integument and are therefore able to lose moisture much

faster than the nitidulids. In both trials (Fig. 15) a definite difference can be detected in terms of the average numbers per trial for both *Drosophila* spp. and nitidulid species, thereby confirming that nitidulids seemed to prefer a sunny microenvironment, while the *Drosophila* spp. prefer a shady microenvironment. According to Michailides, Morgan and Spotts (1992) some species of nitidulids are also capable of disseminating propagules of *Mucor piriformis*. Albeit that the *Drosophila* spp. and nitidulid spp. have a preference for different microenvironments, competition might still be possible when they colonise a similar niche.

A single species of Eucoilidae (Hymenoptera: Cynipoidea) was observed towards the end of the FM (February-March) trial in both sunny and shady areas. Simultaneously, the previously mentioned two species of *Drosophila* larvae were present. One potentially important influence on *Drosophila* community structure is the non-resource based influence of species-specific parasitoids (Nunney, 1996). In a study conducted by Pérez-Maluf, Kaiser, Wajnberg, Carton, and Pham-Delegue, (1998) it is mentioned that *Leptopilina boulardi* (Eucoilidae) is a parasitoid of *D. melanogaster*. Furthermore, one strain of *L. boulardi* was observed to search for the larval *D. melanogaster* host to parasitise in response to fruit aromas. This therefore explains the strong positive correlation between the Eucoilidae and *Drosophila* larvae. The fact that no Staphylinidae (Coleoptera) and Eucoilidae (Hymenoptera: Cynipoidea) were present in the second trial from March to May may be due to seasonality constraints (*i.e.* autumn, winter), in spite of the presence of *Drosophila* larval hosts. Grimaldi and Jaenike (1984) established that larval competition exists for *Drosophila* flies, suggesting that resource accessibility is important. Later successional species can only become established after habitat

modification by the previous colonizers. This phenomenon is heterotrophic succession and fits the facilitation model, which relates to the succession of consumers on carrion, logs, or dung, where some species rely on the partial breakdown of resource material before they can colonise it (Brown, 1984). *Drosophila* larvae prefer moist, warm surroundings, *e.g.* watery fruit rot caused by yeasts and other micoorganisms that were disseminated by the adult *Drosophila* flies.

The difference between the two Formicidae species in terms of colonisation and environmental preference can probably be attributed to their different habits. It is, however, important to note that ants are generalist predators and thus feed on a variety of resources and therefore cannot be seriously affected by any competitive displacement. Changes in density of one prey species may therefore bring a shift in the percentage composition in the diet of the predator, in this case the ants (Brown, 1984).

According to Brown (1984) succession in unstable habitats proceeds faster than in stable habitats. Since this experiment was conducted on aging rotting fruit it is suggested that this represents an unstable habitat. No stable community development is therefore possible since the fruit only has a limited duration.

Efficacy of Sporekill™ as a fungal propagule eliminator on Drosophila. Only 2 out of the twenty flies that were treated with Sporekill™ yielded fungi, *i.e.* a *Penicillium* sp. and an *Aspergillus* sp.. Five of the twenty flies yielded yeasts. The control treatment consisted of flies captured on site at the cactus pear orchard and plated out on PDA agar (Difco). All these flies yielded a variety of fungi. Since only 2 out of the twenty flies yielded a *Penicillium* sp. and an *Aspergillus* sp. it is evident that Sporekill™ can be used as an external sterilising technique, but it may not be fool proof.

Dissemination potential experiment. Of all the fruit treatments, those that were in contact with flies artificially treated with fungal pathogens (previously shown to cause severe lesions) showed most damage, whilst naturally occurring flies also seemed to have some capability of fungal dissemination (Fig. 16). The rot developed with these natural flies were obviously due to fungal propagules that were naturally acquired. Overall, fruits that were punctured and exposed to flies developed more rot than those that weren't punctured (Fig. 16). In Table 3, the first control consisting of fruit that was not sterilised and with no flies, had lesion formation in both repetitions. Fruit with punctures had a total of 4 fruits with lesions over the two experiments, while fruits without punctures had a total of 2 fruits with lesions over the two experiments. All these fruits only had a score of 1 on the rot rating scale. The second control, consisting of sterilised fruit with no flies, only had a total of 2 fruits (one punctured and the other not punctured) with lesion formation in experiment 2. The third control, consisting of sterilised fruit with sterilised flies had lesion formation in both repetitions. More lesions formed on the fruits that were punctured, than on the fruits that were non-punctured. Only a few fruits that were punctured had a score of 2 on the rot rating scale. The fourth control consisting of sterilised fruits and natural flies captured in the orchards overall had more severe lesion formation than the previous controls. Fruits that were exposed to flies artificially infested with fungal species and that were previously shown to cause lesion formation all developed more rot. The fungal species that were used to infest the flies was positively matched up (* in Table 3) with the isolations made from the fruit after their exposure to the flies.

Fruits allowed to come into contact with flies artificially treated with fungal pathogens (previously shown to cause severe lesions) yielded the most damage. Gravid female Drosophilidae can theoretically be seen as transmitting more fungi than males because they feed more on the fruits and they also oviposit on the fruit (Michelbacher and Middlekauff, 1954). In this study no differentiation was made between male and female Drosophilidae, since Butler and Bracker (1963) found that both sexes are capable of transmitting fungi. Louis *et al.* (1996) came to the conclusion that females transmitted more fungal propagules due to the fact that females ate far more to realize oogenesis and thus the number of spores that served as inoculum for the digestive proliferation was likely greater and the intestinal transit was more important. Another important observation by Louis, *et al.* (1996) was that spores are often specifically accumulated in the crop of the flies. In this study *Drosophila* spp. were shown to disseminate fungal propagules to cactus pear fruit after isolations were made from the fruit and matched up with the original fungal species that the flies were infested with. No trials showed whether it was externally or internally harboured (except for the SEM which confirmed externally harbouring). Natural flies also seemed to have a certain capability of dissemination and it was shown that several fungal species are naturally harboured by *Drosophila* species. The rot that developed on fruit exposed to natural flies was caused by fungal species that were not identified in this experiment.

Fruits that were punctured generally developed more rot than those that were not punctured. Carson (1951) remarks that yeasts or other microorganisms are required to attract *Drosophila* species. Butler and Bracker (1963), on the other hand, observed that wounded healthy tomato fruit are superior in attracting *D. melanogaster* than fruit

infected with fungi and yeasts. This suggests that *D. melanogaster* are more easily attracted to wounds on fruit. Additionally, the enlargement of natural wounds on fruit is not due to the flies *per se*, but to the activities of microorganisms. The fungus enters the fruit through wounds and growth cracks (Butler, 1960) or the mycelium is able to penetrate fruit tissues adjacent to the wound (Butler and Bracker, 1963).

Fruit with no flies also had lesions in both experiments, which may be due to contamination (Table 3). All these fruits only had an insignificant score of 1 on the rot rating scale. Sterilised fruit with sterilised flies had lesion formation in both repetitions. This lesion formation may be due to yeasts harboured by the *Drosophila* spp., which was previously shown in this study. Since only a mild fungicide was used for sterilisation, the yeasts were not controlled. The fruits that were punctured had a higher rot average than fruits that weren't punctured and had more severe lesion formation than the previous controls. This can once again be attributed to the activities of micoorganisms that are naturally harboured by the flies.

The fruits that were exposed to flies artificially infested with fungal species that were previously shown to cause lesion formation all developed more rot. The fungal species that were used to infest flies correlated (Table 3) with isolations made from fruit after exposure.

Since these trials were conducted in the laboratory the number of flies remained constant. Butler (1960) found that as the proportion of *D. melanogaster* harbouring *Geotrichum* increased under field conditions, the amount of Geotrichum rot on tomatoes also increased, in turn increasing the chances of dissemination. Additionally, as the growing season advances, the fly population is more efficient in transmitting fungi due to

a greater number of flies. Besides ovipositing in fruit, it has also been shown that some fungal propagules that are consumed by the flies are excreted and still serve as viable inoculum (Michelbacher and Middlekauff, 1954). Warner (1959) in Butler (1960) emphasises that vinegar flies are able to migrate for at least 2 miles, therefore increasing the chance that flies may disseminate potentially harmful fungi from one orchard to another. Once infected *Drosophila* flies become a potential reservoir for *B. cinerea* in three ways namely by spores, mycelium and microsclerotia. They may therefore not only play a part in the dissemination, but also in the overwintering or preservation of rots (Louis, *et al.* 1996)

4.0 Conclusion

Further investigations into interactions between *Drosophila* spp. and cactus pear are required in order to devise measures to control post-harvest diseases of cactus pear fruit. Since no systematic studies of yeast species interacting with *Drosophila* spp. and *Opuntia* spp. have been conducted in South Africa, many of the yeast species isolated in this study are probably unknown. This, together with the fact that certain entomopathogenic fungi were isolated from vinegar flies, implies that considerable potential for innovative integrated pest control measures exists within the fungal-insect complex associated with cactus pear.

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Table 1. Diseases associated with *Drosophila* spp.

Disease and/or Pathogen	**Fruit or Crop**	**Reference**
Colletotrichum gloeosporioides (Postbloom fruit drop)	Lime	Pena and Duncan (1990)
Monilinia fructigena (apple brown rot)	Apples	Lack (1989)
Mucor piriformis	Peaches	Michailides and Spotts (1990)
Mucor sp. *Aspergillus* sp. *Penicillium* sp.	Banana	Hodge, Arthur and Mitchell (1997)
Rhizopus nigricans (Rhizopus rot)	Peaches Fig	Carter (1962) Nitta, Furui and Ito (1997)
Aspergillus niger	Tomatoes	Purnima, Saxena and Sinha (1989)
Botrytis cinerea (Grape bunch rot)	Grapes	Louis, Girard, Kuhl and Lopez-Ferber (1996)

Table 2. Fungal taxa isolated from *Drosophila melanogaster* (D.m.) and *Drosophila hydei* (D.h.).

Fungi	Percentage recovery from insects	
	D. m.	*D. h.*
Arthrographis. sp.	1.7	6.8
Alternaria sp.	0.0	4.9
Aspergillus niger Tiegh	0.9	2.9
Aschochyta sp.	0.0	1.0
Aureobasidium sp.	0.0	1.0
Cladosporium sp.	4.2	0.0
Fusarium spp.	12.9	5.0
Michrodochium spp.	3.2	2.7
Mucor spp.	43.3	32.0
Paecilomyces sp.	0.0	2.9
Penicillium spp.	3.4	9.7
Phoma spp.	2.5	6.8
Trichoderma sp.	0.0	1.0
Yeasts	22.0	20.4
Unidentified fungi	5.9	2.9

Table 3. Dissemination potential experiment. Rot rating scale: 1=0-20%; 2=20-40%; 3=40-60%; 4=60-80%; 5=80-100%.

* = Positive match of fungi isolated from rotting fruit

EXPERIMENT 1 (06/03/02 - 26/03/02) 27 days										
	Fruits punctured			Mean		Fruits not punctured				Mean
Control - Non-treated fruit; no flies	0	0	1			0	0	0		
	1	0	0	0.3333		0	0	0	0	
Control - No flies	0	0	0			0	0	0		
	0	0	0	0		0	0	0	0	
Control - Treated flies	2	1	1			0	0	0		
	1	2	1	1.3333		0	1	0	0.1667	
Control - Natural flies	2	2	3			2	1	0		
	4	2	2	2.5		1	2	0	1	
					Match					Match
Fusarium sp.1 treated flies	3	4	2			2	2	1		
	3	2	4	3	*	1	2	2	1.6667	*
Aspergillus niger treated flies	5	4	5			3	3	2		
	4	5	3	4.3333	*	4	2	3	2.8333	*
EXPERIMENT 2 (11/04/02 - 13/05/02) 32 days										
	Fruits punctured			Mean		Fruits not punctured				Mean
Control - Non-treated fruit; no flies	0	0	1			1	1	0		
	0	1	0	0.3333		0	0	0	0.3333	
Control - No flies	1	0	0			0	0	0		
	0	0	0	0.1667		0	0	1	0.1667	
Control - Treated flies	2	2	1			0	2	0		
	0	1	0	1		0	0	0	0.3333	
Control - Natural flies	2	3	3			1	2	2		
	5	2	2	2.8333		1	1	2	1.5	
					Match					Match
Fusarium sp. 1 treated flies	5	5	4			3	1	3		
	4	5	5	4.6667	*	2	4	1	2.3333	*
Aspergillus niger treated flies	5	4	5			1	4	2		
	3	5	5	4.5	*	4	4	3	3	*

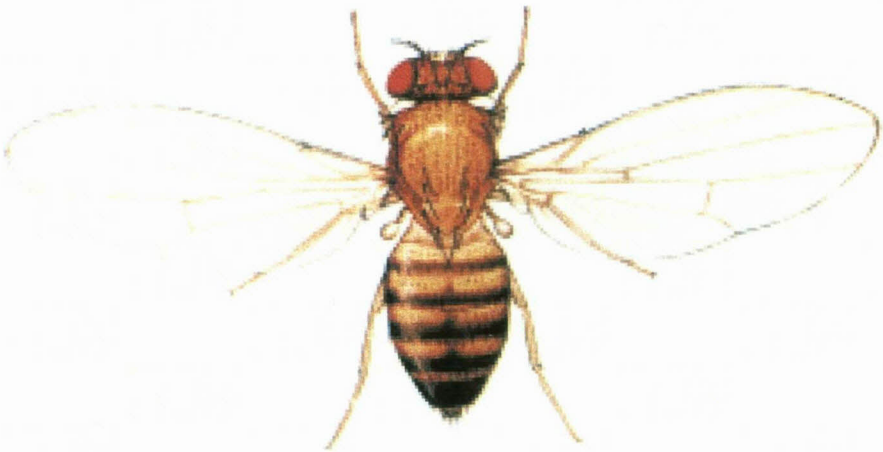

Figure 1. *Drosophila melanogaster*. (Length 2-3 mm) (Taken from Shorrocks, 1972)

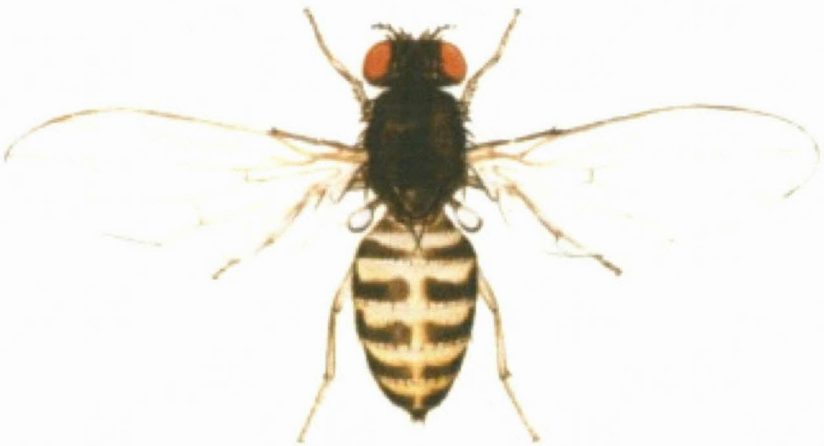

Figure 2. *Drosophila hydei,* (Length 3-4 mm) (Taken from Shorrocks, 1972)

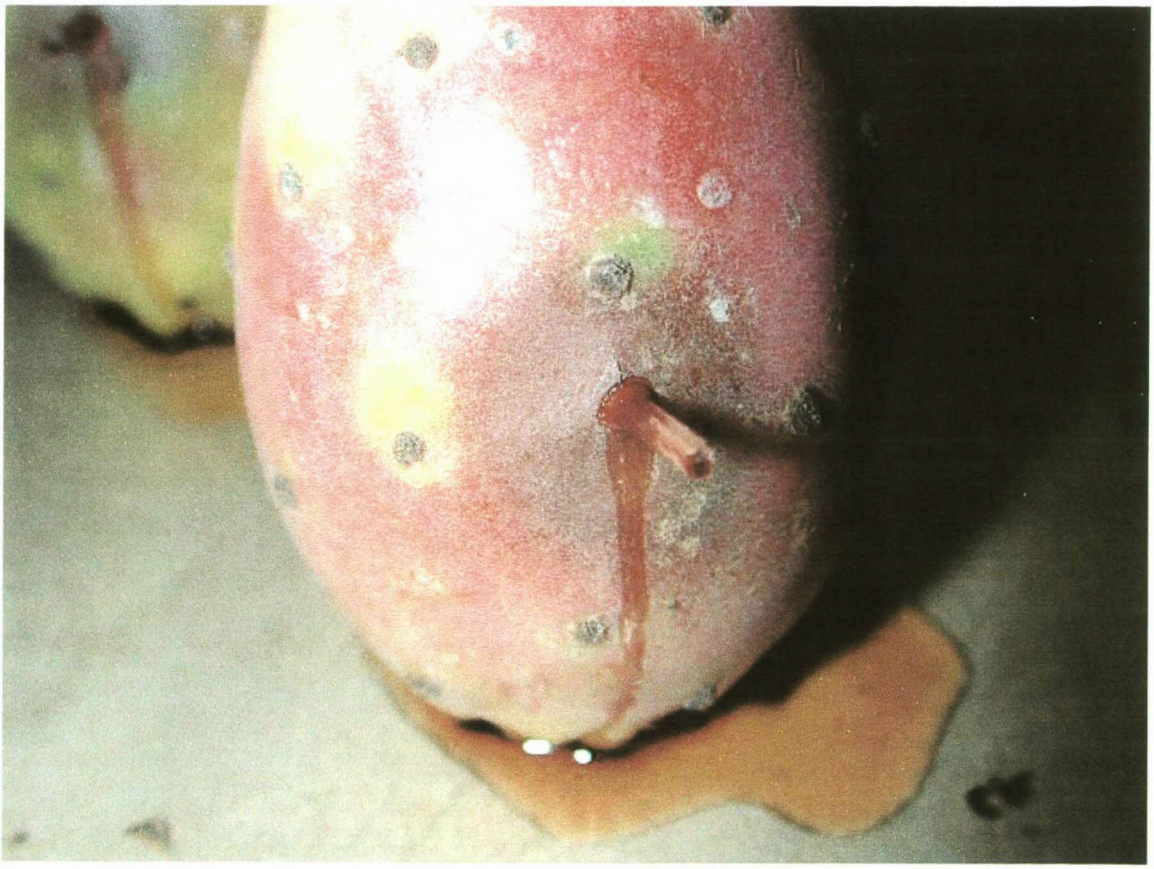

Figure 3. Inoculations of cactus pear fruit with toothpick with acquired pathogen.

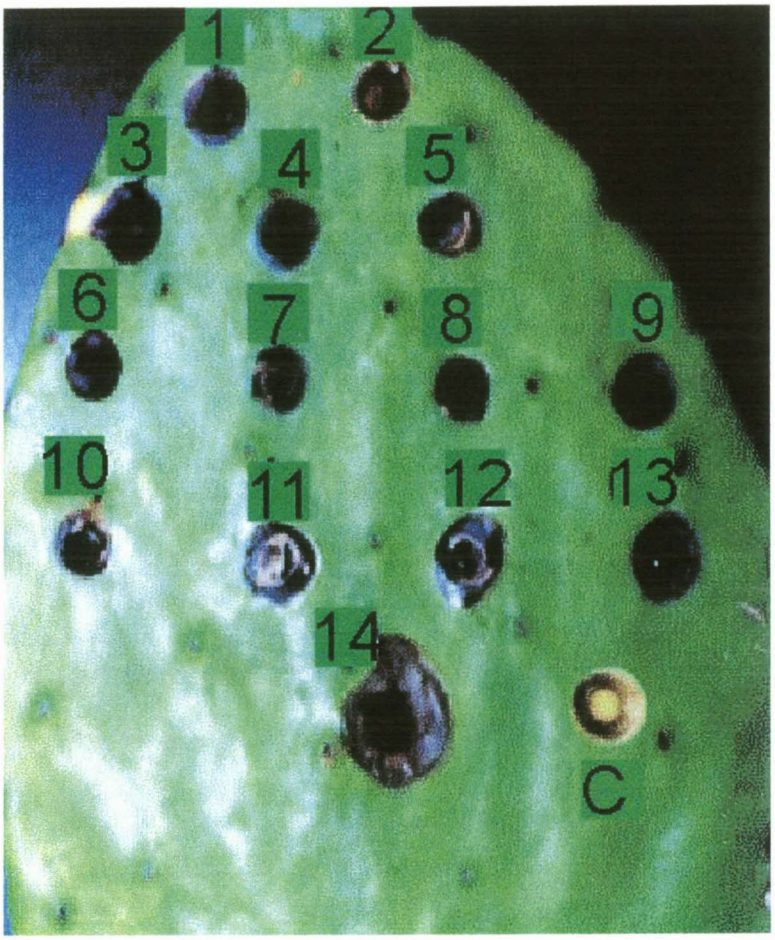

Figure 4. Inocultion of cladodes with 14 fungi isolated from *Drosophila* spp.. Lesion caused by: (1) *Ascochyta* sp. (2) *Aspergillus* sp. (3) *Alternaria* sp. (4) *Phoma* sp. 1 (5) *Phoma* sp. 2 (6) *Phoma* sp. 3 (7) *Mucor* sp. 1 (8) *Mucor* sp. 2 (9) *Mucor* sp. 3 (10) *Mucor* sp. 4 (11) *Fusarium* sp. 1 (12) *Fusarium* sp. 2 (13) *Michrodochium* sp. 1 (14) *Michrodochium* sp. 2 (C) Control.

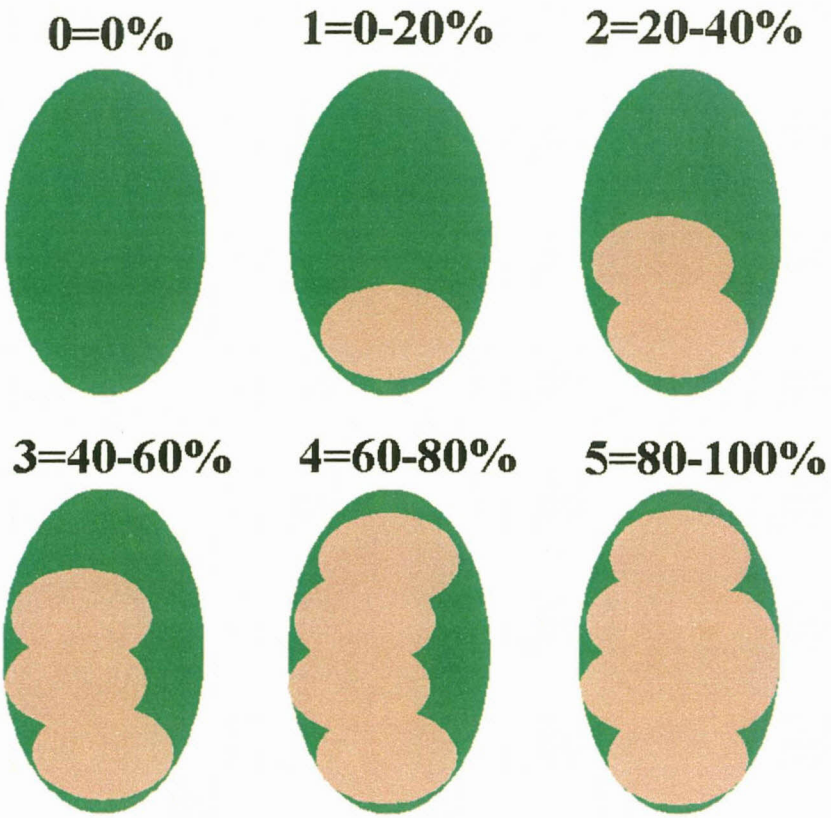

Figure. 5. Schematic representation of the calibration for the rot rating scale (Brown shaded areas reflect degree of rot).

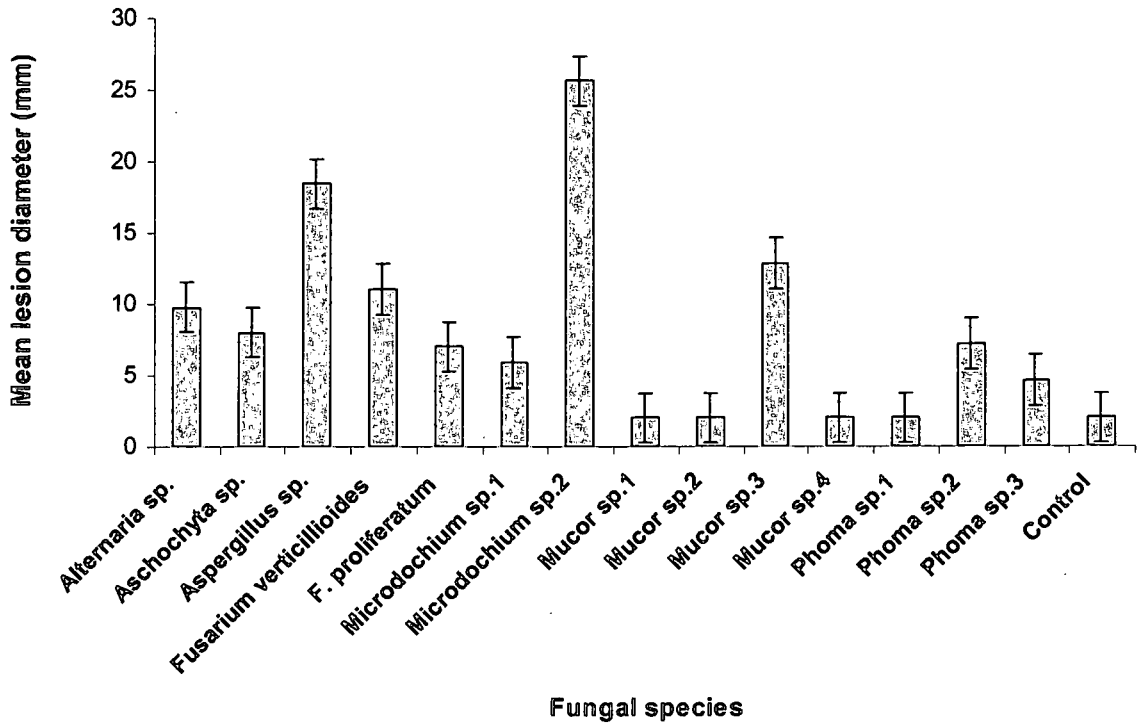

Figure 6. Mean lesion diameters caused by 14 fungal isolates and control treatment on fruit of *Opuntia ficus-indica* (cultivar Algeria). Lines on bars indicate standard error.

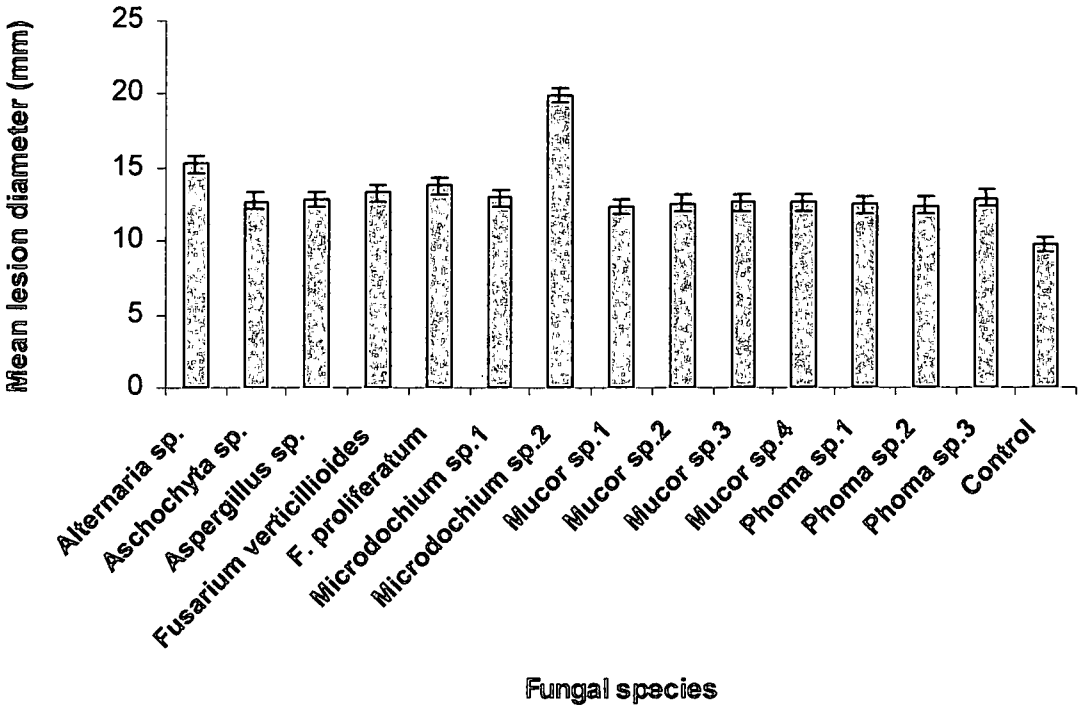

Figure 7. Mean lesion diameters caused by 14 fungal isolates and control treatment on cladodes of *Opuntia ficus-indica* (cultivar Morado). Lines on bars indicate standard error.

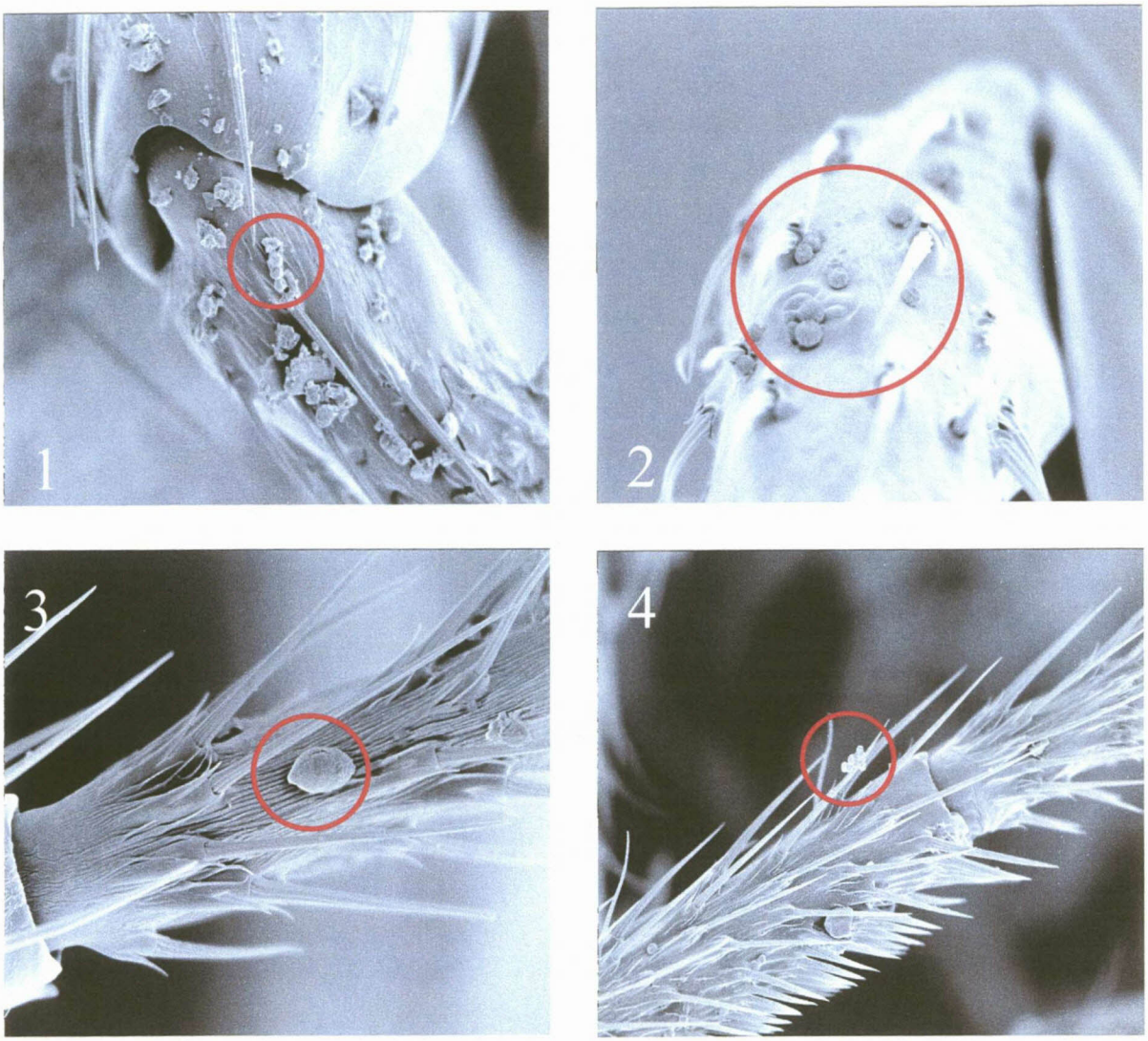

Figure 8 (1-4). Scanning electron microscopy (SEM) of fungal propagules on the exoskeleton of *Drosophila melanogaster.*

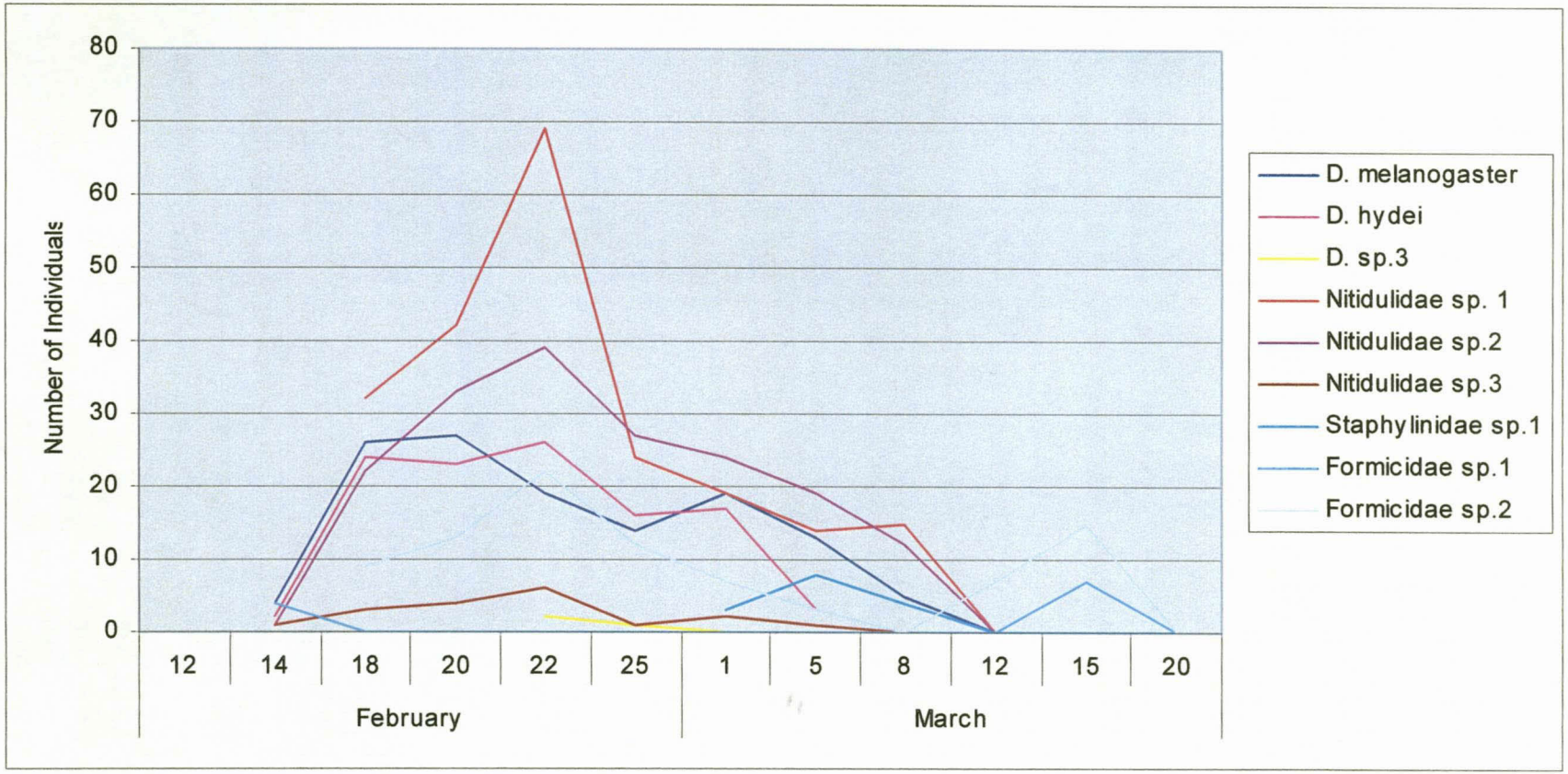

Figure 9. The succession of insect species over time on ripe cactus pear fruit in a sunny microenvironment.

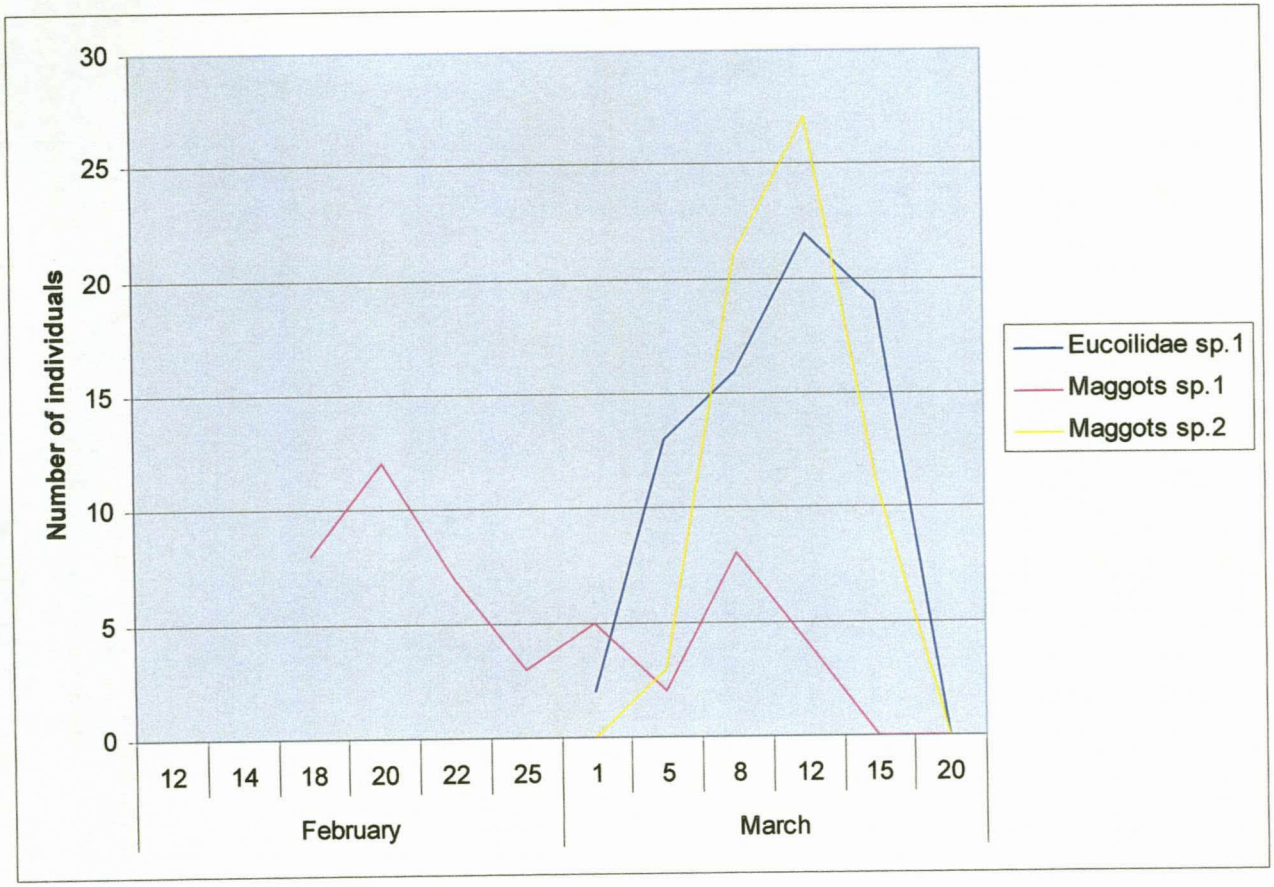

Figure 10. The succession of Eucoilidae and larva of Drosophilidae spp. over time on ripe cactus pear fruit in a sunny microenvironment.

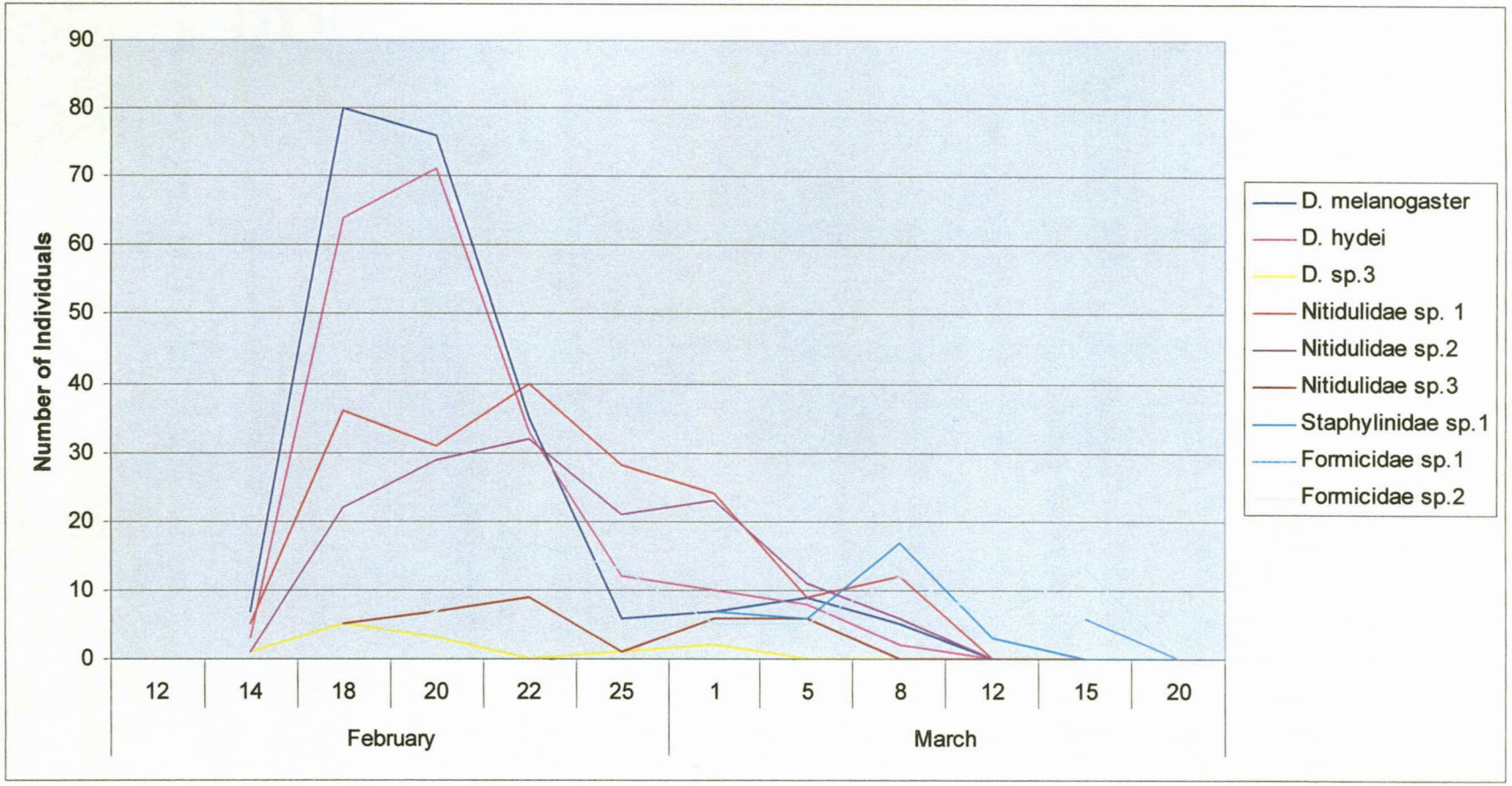

Figure 11. The succession of insect species over time on ripe cactus pear fruit in a shady microenvironment.

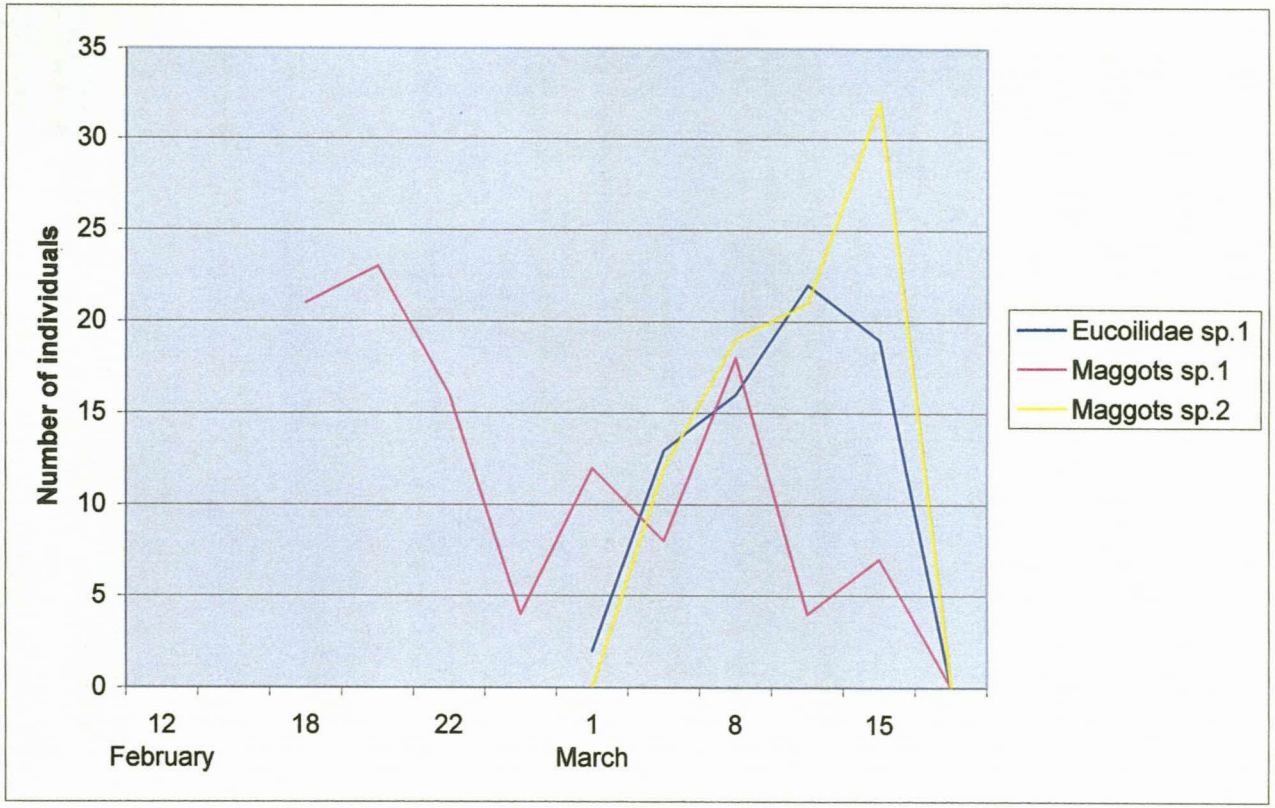

Figure 12. The succession Eucoilidae and larva of Drosophilidae spp. over time on ripe fruit in a shady microenvironment.

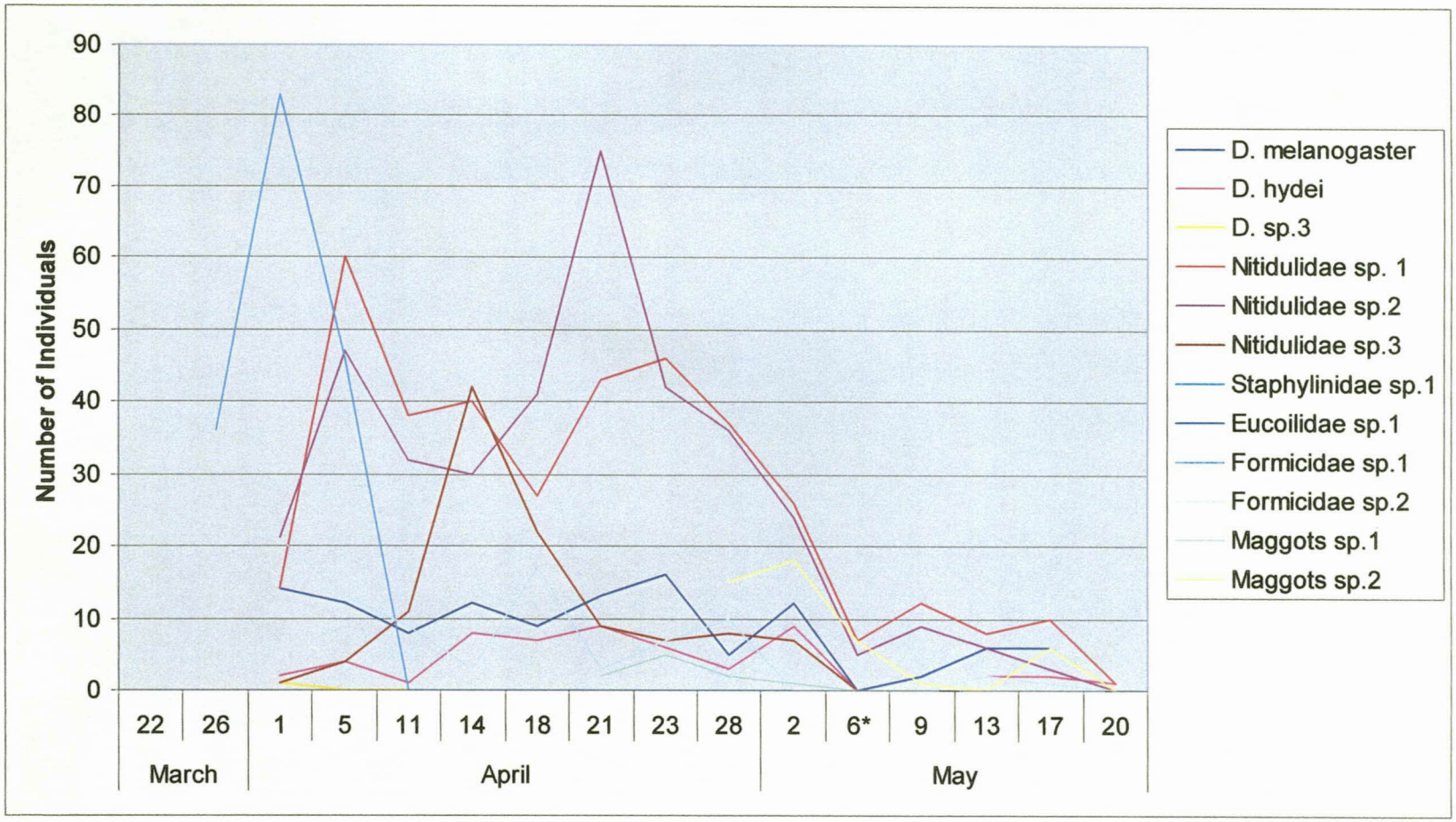

Figure 13. The succession of insect species over time on ripe cactus pear fruit in a sunny microenvironment.

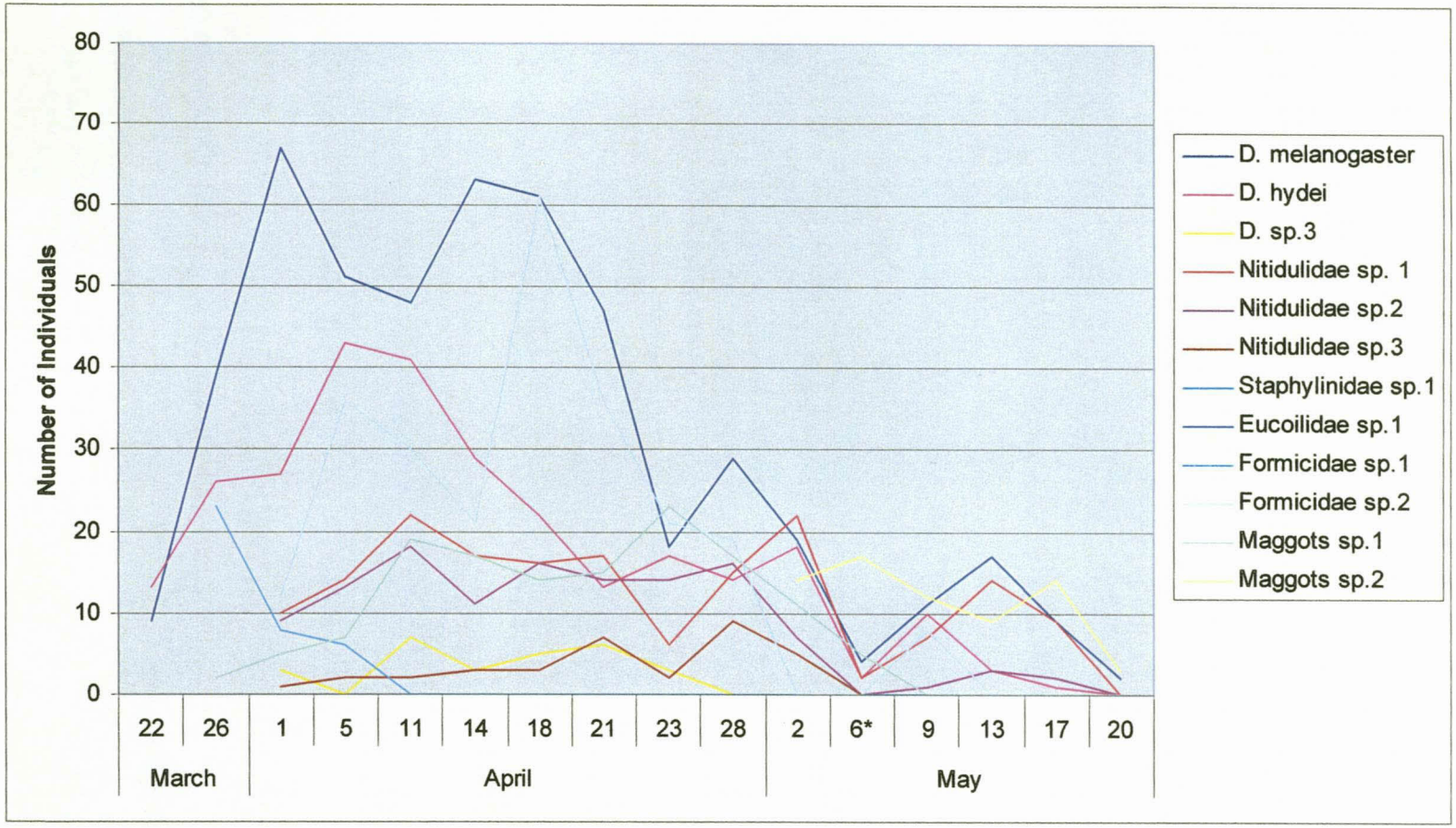

Figure 14. The succession of insect species over time on ripe cactus pear fruit in a shady microenvironment.

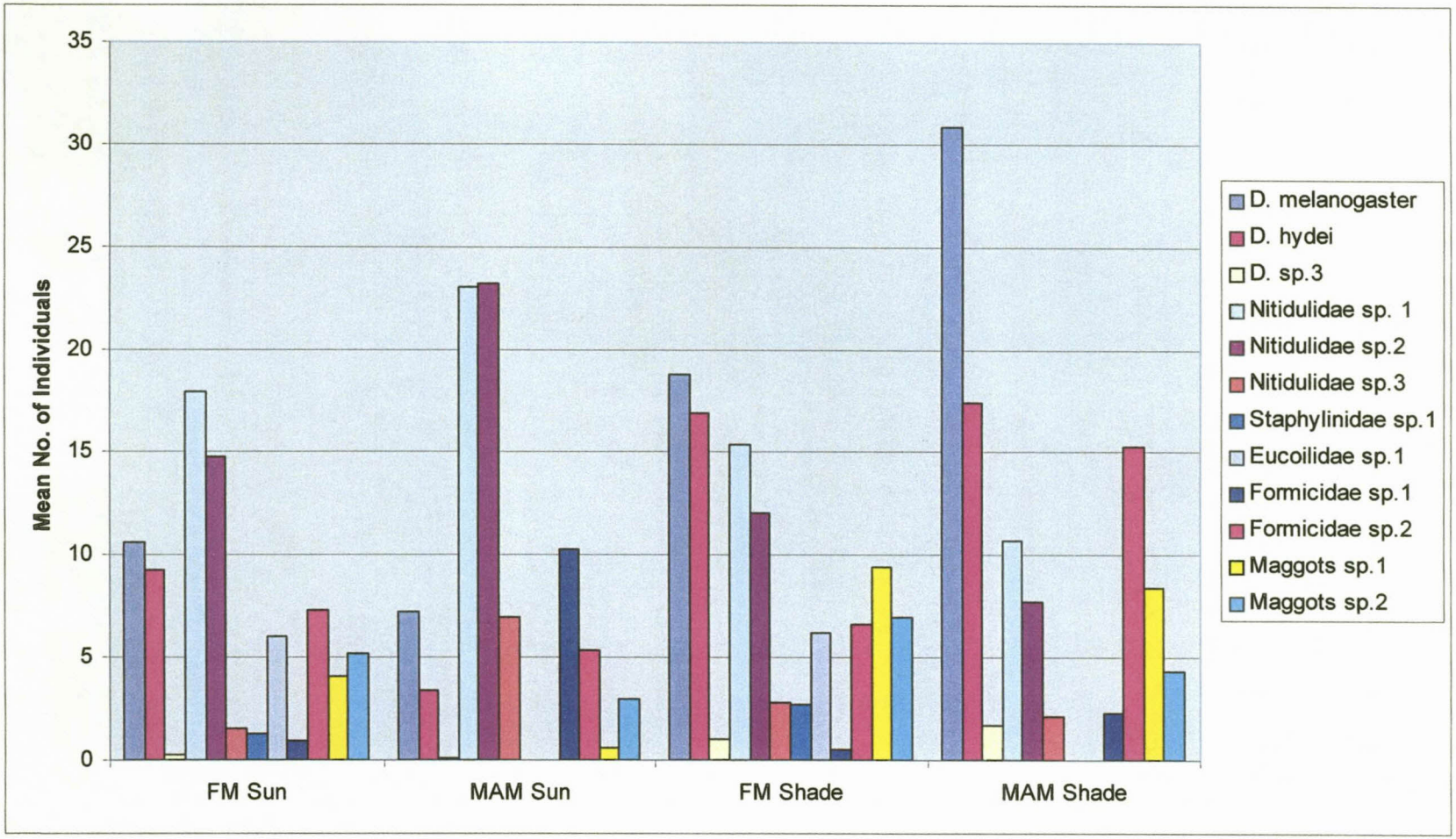

Figure. 15. Average number of insects that visited the fruit per day (FM: Febraury-March; MAM: March-April-May).

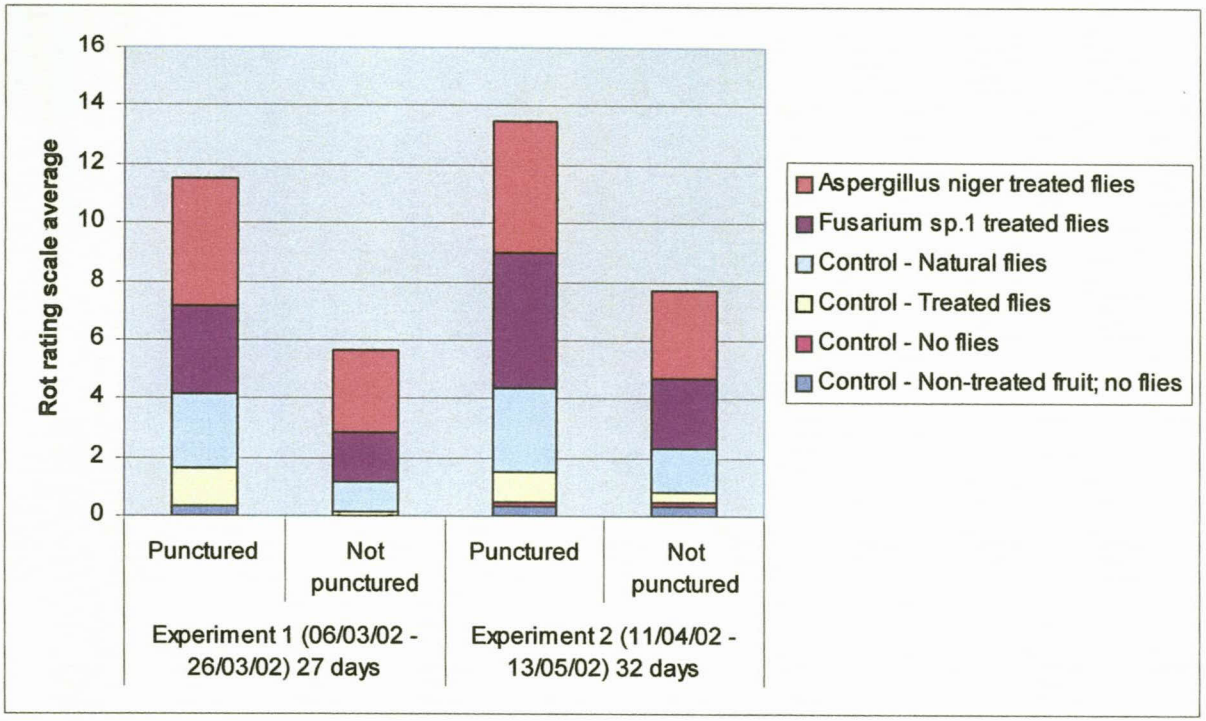

Figure 16. Average rot rating scale of cactus pear fruit exposed to *Drosophila* spp. under different circumstances.

CHAPTER 3

Scarabaeidae and Lygaeidae as disseminators of fungal phytopathogens to *Pistacia vera* (Anacardiaceae)

Abstract

Several insect pests and fungal diseases have been observed in orchards of pistachio (*Pistacia vera*) established near Prieska, South Africa. *Nysius natalensis* (Lygaeidae: Orsillinae) and *Sparrmannia flava* (Coleoptera: Scarabaeidae: Melolonthinae) are the most important insect pests, causing severe damage to young shoots and nuts, respectively. Two fungal species, *Botryosphaeria obtuse* and *B. dothidea* have also been reported as pathogens of pistachio in South Africa. The major objectives of the present study were to establish whether either insect pest harbours fungal propagules belonging to these and other potential pathogens of pistachio. Secondary objectives were to determine the seasonal distribution of *S. flava* and if weather patterns have an influence on *N. natalensis* and *S. flava* flight activity. Isolations conducted on agar from insect specimens of both species at various sampling times revealed a total of 12 and 8 genera of mycelial fungi from *N. natalensis* and *S. flava*, respectively. Although neither *B. obtusa* nor *B. dothidea* were isolated from either insect species, species of *Alternaria, Dreschlera* and *Mucor* were most prominent on *N. natalensis* while *Fusarium* spp. were most frequently isolated from the heads and faeces of *S. flava*. Fungal propagules were observed on the exoskeleton of *N. natalensis*, especially on the legs. As the mean temperature decreased towards March and April, the total number of *N. natalensis* individuals tended to increase while numbers of *S. flava* peaked during the warmer summer months of November to February. The results of this study should be very useful in the implementation of an integrated pest management programme for pistachio cultivation in South Africa.

1.0 Introduction

Pistachio nuts are considered one of the prime edible nuts of the world. The pistachio tree (*Pistacia vera* L.) is native to the western parts of Asia and became domesticated in several Mediterranean countries some 2000 years ago. It was introduced in South Africa in 1990 and it is today considered to be one of the country's most important new crops. Currently, approximately 1000 hectares are under intensive cultivation at Green Valley Nuts (GVN) (29° 35'S, 22° 56'E) on the bank of the Orange River near Prieska in the Northern Cape. The project is funded by the Industrial Development Corporation (IDC) of South Africa and is known to be one of the largest and most expensive commitments to a new crop in South Africa. The tree is dioecious and prefers areas that have cool winters and hot long summers.

There are several insect pests and diseases that cause extensive damage on *P. vera* trees. *Nysius natalensis* (false chinch bug, Hemiptera: Lygaeidae: Orsillinae) (Fig. 1) is the most abundant of the four species of *Nysius* recorded from South Africa (Slater 1964). *N. natalensis* has been known to be a pest on wheat, onions, leeks, garlic, alfalfa and sunflowers (Schaefer and Panizzi, 2000) and has also been found to occur on wild host plants. In fact, the abundance of wild host plants, including weeds, influence their pest status (Du Plessis and Byrne, 2000). This is demonstrated by *Nysius vinitor*, a native pest of several plants found throughout Australia and a serious pest on sunflower (*Helianthus annuus*) (Broadley and Rossiter, 1982). According to McDonald and Farrow (1988), *N. vinitor*'s pest status is largely as a result of its capacity to colonise plants rapidly and attack crops *en masse*. Obviously, this may be the case for other *Nysius* species as well. *Nysius ericae* has been shown to transmit the yeast *Nematospora coryli*

to healthy green mustard plants (Burgess, Dueck and Mckenzie, 1983). *N. raphanus* is known to cause damage to pistachio trees in California (Statewide integrated pest management program, 2000). *N. natalensis* has been observed to be dominant in *P. vera* orchards in South Africa and since other closely related species are known to be pests on other crops, it was essential to investigate their relationship to pistachio trees.

Numerous new diseases of *P. vera* have been reported in South Africa for the first time (Swart and Botes, 1995; Blodgett and Swart, 1998). The fungal pathogens associated with some of these diseases have also been associated with insect pests on other crops. The possibility that insects in pistachio orchards at GVN disseminate these fungi therefore needs to be established. Analysing *N. natalensis* activity is important in relation to when and where potential dissemination of fungal propagules will occur. The objectives of the present study with regard to *N. natalensis* were firstly, to isolate and identify fungi associated with *N. natalensis* in specific orchards at GVN, secondly, to determine whether *N. natalensis* harbours fungal propagules by utilising scanning electron microscopy (SEM) and thirdly to determine whether weather patterns have an influence on *N. natalensis* activity on pistachio trees.

Sparrmannia flava (Coleoptera: Scarabaeidae: Melolonthinae) (Fig. 2) has also been observed as an important pest on pistachio at GVN, causing severe feeding damage to shoots. In general, *Sparrmannia* species are widely distributed in the arid and semi-arid regions of southern Africa. Adults are very distinctive, mostly yellow-brown, robust and often furry. *S. flava* is the most widespread of the 25 species, occurring in arid and semi-arid regions of South Africa, Botswana, Namibia, Zimbabwe and Zambia (Scholtz, 1988). Once again, similarly to *N. natalensis*, analysing *S. flava* activity is also important

in relation to when and where potential dissemination of fungal propagules will occur. With regards to *S.flava*, the objectives of the present study were also, firstly, to determine whether pathogenic fungi are associated with the insects, secondly, to determine the seasonal distribution of *S. flava*, and thirdly, to determine whether weather patterns and lunar phases have an influence on *S. flava* flight activity.

2.0 Material and Methods

Collection of insects for fungal isolation. Sampling of *N. natalensis* was conducted on two separate occasions (February and March 2001) in a *P. vera* orchard at GVN, Prieska by means of a light trap. Sampling of *S. flava* was conducted on two separate occasions (November and December 2001) at the same site and same light trap. The orchard consisted of 16 hectares of 9-year-old *P. vera* trees.

An aspirator (pooter) was used to sample *Nysius* specimens and these were subsequently deposited into individual Polytop™ bottles. To prevent possible cross contamination from fungi associated with individual insects, the aspirator was dipped into 70% ethanol for 30 seconds and allowed to air dry before capturing the next insect. Polytop™ bottles containing live *Nysius* specimens were taken to the lab and placed in a freezer at -75°C for 5 minutes in order to kill the insects without harming fungal propagules. In total, 200 bugs were captured and screened for fungi over a period of 2 months.

S. flava were hand collected, deposited into Polytop™ bottles and immediately placed in a freezer for approximately 12 hours at -75°C. In total 150 beetles were collected, 75 for each month.

Isolation of fungi. Specimens of *N. natalensis* were transferred to individual Petri-plates (65 mm diameter) containing potato dextrose agar (PDA) (Difco) amended with streptomycin sulphate (0.33 g/1000 ml water) and incubated at 25°C in a light-dark cycle of 12 hours each. When fungal colonies became visible to the naked eye they were transferred to corn meal agar (CMA) (Difco) for the purpose of identification and quantification.

Specimens of *S. flava* were removed from Polytop™ bottles in the lab and by squeezing the insect with a tweezer, faecal matter was collected for each insect individually. The heads of the beetles were removed by using a small pair of scissors (sterilised in alcohol after every cut). Heads and faecal matter were aseptically transferred to individual Petri-plates (65 mm diameter) containing PDA (Difco) amended with streptomycin sulphate (0.33 g/1000 ml water) and incubated at 25°C in a light-dark cycle of 12 hours each. A total of 150 beetles was treated in this manner.

Scanning electron microscopy. To confirm that *N. natalensis* harbour fungal propagules, scanning electron microscopy (SEM) was conducted. *N. natalensis* specimens were placed in a freezer at -10°C for an hour and then freeze-dried for 2 hours. Specimens were fixed onto SEM stubs by means of a plastic adhesive, sputter coated with gold and observed with a JOEL SEM. All body parts were examined for any signs of fungal propagules.

N. natalensis damage levels. It was necessary to determine whether *N. natalensis* caused any significant damage to pistachio nuts. Net bags were used to enclose a cluster of nuts, ten insects were placed inside, this was repeated ten times on separate trees. A

control treatment consisted of a bag with a cluster of nuts without any *N. natalensis*. After one month the percentage of damaged pistachio nuts per cluster were quantified.

Seasonal distribution of Nysius natalensis. Sweeps were carried out in cover crop rows between pistachio trees to determine the population numbers of *N. natalensis*. Sweep sessions were conducted monthly from December 2001 to April 2001 and for each session two hundred sweeps were made. Different species of wild and planted grasses grow in the cover crops and are trimmed randomly. Collection of *Nysius* was conducted in five different orchards at GVN and at Remhoogte (SE 29 23 ca), a neighbouring farm. Collected material was sorted and analysed quantitavely and qualitatively.

N. natalensis present in the canopies of *P.vera* trees were collected by means of a insecticide fogger (C. R. Haddad, personal communication*). Data collected from April 2001 to April 2002 was used, excluding June and August 2001, when no data was collected. The process of fogging consisted of laying out a white sheet (36 m^2) under a tree canopy and then spraying the tree with the aid of a backpack fogger. Dichlorovos (150 ml/100 litres) was used as knockdown agent. A waiting period of five minutes followed fogging to allow the insecticide to take affect, where after the tree was shaken to allow all insects to fall onto the sheet. Insects were then hand collected and placed in 50 ml plastic bottles containing 70% ethanol. Insects were sorted and quantified in the laboratory.

Sparrmannia flava flight activity. The *S. flava* specimens were sampled every night from October 2000 until March 2002 by using four light traps throughout the GVN orchards. The light traps consisted of 4 vertical white planks with a fluorescent light in

*C. R. Haddad, Zoology and Entomology, University of the Free State, Bloemfontein.

the centre. Situated below the light source is a funnel leading to a large plastic container into which the beetles fall when flying into the white surface illuminated by the light source. Sampled beetles where placed in 100 ml plastic jars and quantified.

3.0 Results and Discussion

Isolation of fungi from Nysius natalensis. Fungi were isolated from all *N. natalensis* specimens at both sampling dates (Table 1). In total, 12 genera of mycelial fungi were isolated, of which *Alternaria* spp., *Dreschlera* sp. and *Mucor* sp. were most prominent in both samples (Table 1). *Alternaria alternata* causes Alternaria late blight on pistachio (Teviotdale, Michailides and MacDonald, 2001) and also occurs at GVN towards the end of the growing season. *N. natalensis* could therefore potentially disseminate *A. alternata* to pistachio at GVN. Three fungi were unique to the first batch of insects, *viz. Chaetomium* sp., *Helminthosporium* sp. and *Phoma* sp.. *Phoma* sp. has also been associated with a stem disease of pistachio at GVN although the species is still undetermined (W.J. Swart, personal communication*). A *Cladosporium* sp. was unique to the second batch whilst 20.4% and 21.8% of the fungi in the first and second sample were unidentified fungi or sterile mycelium, respectively. The remaining fungal species isolated are not known to be pathogenic on pistachio trees.

Nysius ericae is capable of transmitting the pathogenic yeast, *Nematospora coryli* internally to seeds in healthy green mustard plants in Canada (Burgess, Dueck and McKenzie, 1983). The yeast which was originally isolated from hazel nuts, is restricted to warmer parts of the world, and is transmitted by piercing-sucking insects (Batra,

*W. J. Swart, Plant Sciences, University of the Free State, Bloemfontein.

1973). It is important to note that *N. ericae* are reported to over-winter beneath piles of weeds and other debris in or along the margins of cultivated fields (Blatchley, 1926). These locations are also ideal for fungal pathogens to germinate. *Aspergillus* spp. frequently infest, infect and sporulate on pistachio litter, such as fallen pistachio fruit and male inflorescences, throughout summer in pistachio orchards in California (Doster and Michailides, 1994). This increases the amount of *Aspergillus* spp. inoculum in orchards resulting in greater numbers of mouldy and mycotoxin-contaminated pistachio nuts. Michailides and Morgan (1996) found that Hemiptera such as *Leptoglossus clypealis* (Hemiptera: Coreidae), *Thyanta pallidovirens* (Hemiptera: Pentatomidae), and *Liorhyssus hyalinus* (Hemiptera: Rhopalidae), when caged with fruit clusters sprayed with spores of *Botryosphaeria dothidea*, resulted in higher levels of infected fruit than on sprayed or non-sprayed fruit clusters not caged with insects. It is therefore evident that *N. natalensis* are important potential disseminators of fungal pathogens in pistachio orchards in this study

Isolation of fungi from Sparrmannia flava. Fungi were isolated from all specimens of *S. flava*. In total 8 genera of mycelial fungi were isolated from *S. flava*, of which the *Fusarium* spp. were the most prominent in both head and faecal isolates (Table 2). Nalli and Balmas (1997) reported *Fusarium semitectum* as the causal agent of canker and wilt of pistachio trees. However in this study, the species of *Fusarium* were not determined. Isolations from the head and the faeces of *S. flava* yielded similar fungi, except for a *Mucor* sp., which was only isolated from the heads, and a species of *Penicillium*, which was only isolated from the faeces. Isolations from the head represent fungal propagules disseminated externally and which could probably have been

accumulated from infected material, as was the case with *Aspergillus* sporulating on pistachio litter (Doster and Michailides, 1994). Isolations from the faeces represent fungal propagules disseminated internally that could have been acquired from the soil. The adults are nocturnal and during the day seek shelter in underground burrows.

The life cycle of *S. flava* lasts about one year. Adults emerge from their burrows in the ground after the first summer rains. They then mate and lay eggs in the burrows. Larval development then proceeds intermittently, with feeding periods after rain interrupted by quiescent periods during dry spells. Mature larvae over winter, then pupate and emerge as adults within three weeks of the first significant summer rains (Scholtz, 1988). The possibility exists that fungal propagules, whether pathogenic or not, can be deposited while the beetles feed on the plants. Faecal matter also provides a suitable substrate for fungi to grow on and can consequently increase the amount of inoculum within the orchards. Fungi that were common to both *N. natalensis* and *S. flava* (*e.g. A. alternata*) both may serve as an indicator of fungal presence in the pistachio orchards. It is noteworthy that very few *Fusarium* spp. were isolated from *Nysius*, in contrast to *S. flava.* This clearly reflects the soil related ecology of the latter. On the other hand significantly less *Alternaria* spp. were isolted from *S. flava* than from *N. natalensis* probably reflecting plant related ecology for this fungus.

Scanning electron microscopy. Fungal propagules were observed on the exoskeleton of *N. natalensis* (Fig. 3). *In situ*, identification of these fungi is very difficult and most times, impossible. Fungal propagules were observed germinating on the insect exoskeleton or they were acquired while germinating (Fig. 3.1). Similar to *Drosophila* (Chapter 2) most of the fungal propagules were observed on the legs (Fig. 3.2). It is

important to note that *N. ericae* are reported to over-winter beneath piles of weeds and other rubbish in or along the margins of cultivated fields (Blatchley, 1926), where several species of fungi can also thrive. Similarly, *Nysius natalensis* can externally acquire inoculum in a similar manner that is disseminated when the insects migrate. Fruiting bodies (Fig 3.3, Fig 3.4), that are containers or carriers of spores, were also observed on various locations on the exoskekleton of *N. natalensis*. Similarly to *Drosophila* spp. (Chapter 2), setae of *N. natalensis* are suited for adherence of fungal propagules. The importance of setae as attachment sites for fungal propagules probably originated in the Mid-Devonian where spores with complex ornamentation suggested animal dispersal, since the spines and retrorse hooks may have functioned as mechanical attachment to the setae of arthropods (Kevan, Chaloner and Savile, 1975 in Kevan and Baker 1983).

Nysius natalensis injuriousness. In the United States of America false chinch bugs (*Nysius raphanus*) can be a serious problem on newly planted pistachio trees causing young trees to wilt and die, whilst feeding on older trees can cause leaves to drop (Statewide integrated pest management program, 2000). In this study the percentage of nuts damaged *N. natalensis* ranged from 10% to 43% per cluster (Fig. 4). According to Michailides and Morgan (1996) the higher the incidence of fruit with insect punctures the greater the incidence of infected fruit and fruit bearing pycnidia. As a consequence of *N. natalensis* feeding on nuts, definite similarities were observed regarding these symptoms. In California, the incidence of epicarp lesions originating from insect feeding through the base of the fruit is high (Michailides, Rice and Ogawa, 1987). In this study the most prominent symptom of *N. natalensis* feeding was a dark lesion approximately 3-4mm wide, with sap exuding from the wound. Sap present in the centre of a feeding puncture

is characteristic of a puncture resulting from Hemiptera feeding (Michailides and Morgan, 1996). Such wounds are susceptible to pathogen infection. Epicarp lesions were also observed on the base (stem) and the apex of the fruit, areas that are easily accessible to insects with piercing-sucking mouthparts. It is therefore evident that *N. natalensis* may feed on nut bunches, and to a certain degree on sprouting leaflets, since slight feeding damage was also observed on these parts. Fruits that were not exposed to *N. natalensis* in the control had no observable damage.

According to Norris and Kogan (1980, in Michailides, Rice and Ogawa 1987), the thickening of the cell wall and increased toughness of plant tissues due to lignification causes interference with feeding and oviposition of insects. Thus, lignified pericarp tissues act as physical barriers against successful puncturing of pistachio fruits by piercing-sucking insects (Michailides, Rice and Ogawa, 1987). However, as the lignification process accelerates rapidly in the apical portion of the fruit, several Hemiptera preferentially feed at the fruit base, where a small area remains relatively soft due to delayed lignification and is vulnerable to insect punctures early in the season (Michailides, Rice and Ogawa, 1987).

Seasonal distribution of Nysius natalensis. The seasonal distribution of *N. natalensis* was analysed in relation to temperature and rainfall. The mean temperature in Figure 5 was calculated by using the average of the maximum and minimum temperature of three days before and on the sampling day. The total rainfall was the sum of the rainfall on the same four days.

A gradual increase in *N. natalensis* populations was observed from December 2001 to April 2002. Since the trees in Orchard 1 and Remhoogte are the oldest, they

were observed to bear significantly more fruit than the rest of the orchards and yielded the highest number of *N. natalensis* individuals compared to other orchards (Fig. 5). As mean temperature decreased towards March and April, the total number of individuals for all orchards tended to increase. A negative correlation was existed between temperature and the number of individuals (Fig. 5) ($r = -0,473$, $p < 0.05$). Figure 5 also shows that high amounts of rainfall resulted in the capture of fewer individuals. All the individuals in all the orchards therefore had a significant negative correlation ($r = -0.435$, $p < 0.05$) with rainfall. Since the orchards were irrigated, rainfall within the cover crops of orchards is to a certain degree irrelevant. It can be speculated that *N. natalensis* migration from crops to surrounding vegetation may be dependant on rainfall. *N. natalensis* may have the same behaviour as *N. vinitor* which, according to Kehat and Wyndham (1973), utilises summer crops as an extension to a normal range of temporary native habitats, between which they may move during a season. Furthermore, they suggest that this movement is migratory behaviour that is usually cued by the commencement of an unfavourable environmental condition. Rainfall could therefore result in surrounding vegetation becoming more receptive to *N. natalensis* feeding. As a result *N. natalensis* has a larger home range in which to roam and feed and as a result less *N. natalensis* would be present within the orchards.

The mean temperature in Figure 6 was also calculated by using the average of the maximum and minimum temperature per day, three days before and on the sampling day. The rainfall in Figure 6 represents the total rainfall for the same period as the temperature. The seasonal distribution of *N. natalensis* was analysed in relation to temperature and rainfall. *N. natalensis* numbers in pistachio tree canopies are low in

winter but high in spring and early summer (September, October and November) and autumn (February, March and April). No correlation existed between any of the environmental factors and the number of individuals on the trees (Fig. 6).

There was a strong positive correlation ($r = 0.966$) ($p < 0.05$) between the sweeping and fogging data of the Remhoogte site. This may be due to the fact that the pistachio orchard at Remhoogte is 1.5 hectares compared to orchards at GVN that are 16 hectares in size, thus providing *Nysius natalensis* with a small area for movement within the orchard. Remhoogte is also isolated giving *N. natalensis* nowhere to migrate to, except to the surrounding vegetation and probably reflects a preference of *N. natalenisis* to migrate to irrigated areas. The populations of *N. natalensis* are important, since the more insects present, the higher the chances are of fungal dissemination. Differences in insect numbers between Orchard 1 and Remhoogte can therefore possibly be attributed to the physical isolation, smaller orchard size (easier colonized by insects) and the age of the latter locality. At GVN, *N. natalensis* have the choice of either migrating to neighbouring orchards or to surrounding vegetation, or both. These assumptions support the views of Ramesh (1984), who states that minor movement patterns of juveniles and adults of *N. vinitor* within and between weeds and neighbouring crops could explain consequential population shifts.

Pistachio orchards adjacent to open grassveld and native vegetation may encounter problems due to hemipterous insects, which continuously invade the pistachio orchards as surrounding vegetation deteriorates. In addition, uncontrolled weed cover within orchards could operate as a stepping-stone for hemipterous insects (nymphs and adults) from the weeds to the pistachio trees (Michailides, Rice and Ogawa, 1987). *N.*

ericae have a tendency to congregate when feeding and it has been reported that they secrete an odour (Milliken, 1916), which is possibly an aggregation pheromone, or their feeding activity on the plant results in the release of an attractive substance by the plants (Burgess, Dueck and McKenzie, 1983) which influences population numbers.

Sparrmannia flava flight activity in relation to the environment. Numbers of *S. flava* individuals tended to peak during the warmer summer months of November, December, January and February during both the 2000-2001 and 2001-2002 seasons. The numbers of individuals during the first season tended to be higher than in the second season (Fig. 7). This variation can be attributed to differences in survival rates, alternating cropping regimes and weather differences between the years (Allsopp and Logan, 1999). However, this might also be attributed to improved control techniques at GVN. The light traps that seem to attract the most number of individuals, were Trap 1 and Trap 4 (Fig. 8). Trap 4 was situated in the proximity of two riverbanks within an orchard with Augrabies silt soil ecotope and seemed to attract slightly more individuals than Trap 1 (Fig. 7). Trap 1 was situated in an orchard with a Namib soil ecotope and close to the Orange River (Fig. 8). The soil type and to certain extent the distance from the river thus had an affect on the occurrence *S. flava*. The area in which Trap 4 is situated is part of a flood plain. The higher moisture content of the soil and the ecological requirements of deep, sandy soil for *S. flava* to complete their life-cycle, explains the high number of individuals captured in this area. Trap 2 and Trap 3 also attracted *S. flava* individuals, but to a lesser extent. Since these traps were situated at different orchard locations with different soil types and pistachio cultivars, the numbers of *S. flava* captured at these sites differ. Trap 2 is situated in an orchard with a Namib

soil ecotope, but is situated further from the rivers and with shallower soils. Trap 3 is situated far from the Orange River in an orchard with a more Namib/calcareous soil ecotope.

S. flava are known to occur in sandy areas. The Namib soil ecotope bears very sandy soils of which clay content of the topsoil (orthic A horizon) and subsoil (regic sand) is lower than 5 % (Soil Classification Working Group, 1991; Le Roux, Ellis, Merryweather, Schoeman, Snyman, Van Deventer and Verster, 1999). Regic sand is young, sandy, unconsolidated parent material that originates from aeolian deposits. Namib soils have a very high base status and characteristically it is a very fertile soil. Only the Namib/calcareous ecotope contains lime. The soil component of the Augrabies Silt ecotope is the Augrabies soil form. The topsoil (orthic A horizon) and subsoil (neocarbonate B horizon) of Augrabies soils are sandy and the carbonate horizon is the main component of the soil morphology. This soil type therefore has a very high pH (pH > 8.0) and the solubility of plant nutrients such as phosphor, zinc, iron and manganese is therefore very low. Variation in the number of individuals for each of the four traps may also be partially due to the cultivar of the surrounding trees, insecticide use and other farming practices. However, in Australia species composition of chafers in crops appears highly influenced by innate characters of the site, especially soil type (Allsopp and Logan, 1999). At GVN the first adults emerge during late October, but only at Trap 4. The adults in the traps in the other orchards tend to emerge during November. No *S. flava* were captured during the winter months.

At the study site flight patterns of *S. flava* were analysed in relation to temperature, rainfall, wind and moon phases. In both Figures 9 and 10 where this is

shown, the total percentage of *S. flava* per day was the sum of the percentage of individuals for each orchard (seven point moving average, three days before and after a specific date) divided by four. Seven point moving averages of temperature, rainfall and wind speed were calculated to make the trends more apparent. The temperature shown is the moving average of the mean minimum and maximum temperature of seven days, three days before and three days after a specific day. The rainfall is the total rainfall per day converted into the moving average of seven days (three days before and three days after a specific day). Since the orchards are irrigated the *S. flava* residing within the orchards are independent of rainfall. However, those residing outside the orchards are dependant on rainfall.

The avoidance of adversity is an influential factor in the timing of the life cycles of many insects (Wolda, 1988). Chafer flight strategies are thus also critical in this regard (Allsopp and Logan, 1999). In the 2000 - 2001 season (Fig. 9) in the first half of Box A, the first emergence of *S. flava* took place late in October 2000 without any specific trigger, while temperatures were below 20°C. The sudden appearance of *S. flava*, the same time every year can be due to their biological clock, which drives their regular metabolic and behavioural rhythms (Alpatov, Zotov, Tsernyshev and Rietveld, 1999). In the second half of Box A the temperature increased and simultaneously the percentage of individuals increased as the moon is approached full phase. Since *S. flava* are nocturnal, the phase of the moon should play a vital role in their flight activity and the predatory pressure exerted on them. Nocturnal insects to a certain extent escape predatory pressure due to their activities taking place in darkness. If the moon is in full phase, the safety offered by darkness decreases and the possibility of predatory pressure

increases and therefore the flight activity of *S. flava* should be less. However, in Box A (and throughout this survey) it appeared that rainfall and temperature were of greater importance in determining *S flava* activity and therefore these factors probably override the affects of the lunar phase (S. vdM. Louw, personal communication*). In Box B the slight rainfall and low temperatures results in a slight peak in the percentage of individuals. As soon as there was an increase in temperature combined with rainfall the first of two dramatic peaks occured as seen in Box C. Simultaneously, the moon was approaching full phase as the significant peak in percentage of individuals of approximately 60% occured. In Box D a different pattern can be observed. After slightly more rainfall than previously, with temperatures averaging lower than 25°C, and just after the full moon, the percentage of individuals increases and reaches the highest peak at 65%. Once again the rain and temperature override the affects of the lunar phase. In Box E there is a dramatic slump in numbers, albeit that there is an increase in temperature and that the moon is full. In Box F the wind speeds reach 3.5 $m.s^{-1}$ and the percentage of individuals seems to decrease, together with rainfall and an increase in mean temperature. By now most of the adults have ceased flying and in Box G temperatures average below 25°C and rainfall is present, but there is no *S. flava* activity.

During the second season (Fig. 10), the *S. flava* individuals were active over a longer period of time (*i.e.* November 2001 to February 2002). In Box A the first adults emerged immediately after a fair amount of rainfall when temperatures are below 25°C and more or less when the moon is full. Box B starts with good rainfall, increasing

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temperatures and a rapid increase in the percentage of individuals. During this period there is a constant high percentage of individuals and simultaneously there is a slight increase in mean temperature and the moon is approaching full phase. Box C shows some rain and an increase in temperature, reaching means of over 25°C and just following on full moon, again resulting in a flush in the percentage of individuals. Box D has very similar patterns, except that there was more rain and the increase in percentage of individuals coincides with full moon. In Box E temperatures increase and winds speeds are slightly higher. There is now a total decline in *S. flava* activity. Following this, there is a bout of rain, followed by a slight, short flush of individuals. Hereafter, in mid-March, *S. flava* cease flying altogether.

The flight patterns of *S. flava* are evidently influenced by weather. According to Ferreira, Ravenscroft and McKinlay (1998), variation in flight activity of a chafer in New Zealand during spring and summer months were related to temperature and humidity, but these variables only explained 27% of the activity variation. Usually after rain, in this study, there is a slight increase in temperature and then a sharp increase in flight activity. On a few occasions there is also only rainfall, but no increase in temperature that also results in a slight increase in flight activity (Fig. 10, Box C). This largely agrees with Scholtz (1988) who states that adult *S. flava* emerge after the first summer rains, mate and then lay eggs.

Allsopp and Logan (1999) found four general patterns of seasonal activity of different species of adult Scarabaeidae associated with sugarcane in Australia. These are a brief spring activity, a brief summer activity, a prolonged summer activity and a prolonged spring-autumn activity. *S. flava* in South Africa evidently resorts under the

prolonged summer activity period, with their adults fly during the summer months and feeding on leaves, causing extensive damage to pistachio trees. This strategy allows adults to take advantage of moist soil conditions for oviposition as a result of spring-summer rain and to feed on new leaf growth. Light traps, similar to those employed at GVN, have been advocated for the control of chafers that are pests of sugarcane (Jarvis, 1923).

4.0 Conclusion

N. natalensis and *S. flava* are both potential disseminators of fungi, of which some might be potentially pathogenic on *P. vera.* Scanning electron microscopy confirmed the presence of fungal propagules on the exoskeleton of *N. natalensis. N. natalensis* can be considered to be a potential threat to younger trees and to a certain extent may even cause damage to pistachio nuts. Rainfall may influence the number of *N. natalensis* individuals within the orchards indirectly, by extending the feeding range into other surrounding vegetation. It was confirmed that *N. natalensis* populations peak towards the end of summer. *S. flava* flight activity peaks during four months of the year in the summer season. Soil type has an influence on the occurrence of *S. flava* captured at the different traps in the different areas. Temperature, rainfall, windspeed and to a certain extent lunar phases have an influence on the flight activity of *S. flava.*

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Table 1. Fungal taxa isolated from *Nysius natalensis*.

Fungi	Percentage recovery from insects	
	Sample 1	**Sample 2**
Alternaria alternata	14.4	19.7
Alternaria sp. 1	8.6	13.4
Aspergillus sp.	1.3	1.4
Bipolaris sp.	4	1.4
Chaetomium sp.	2	0
Cladosporium sp.	0	0.7
Drechslera sp.	25	22.5
Epicoccum sp.	2.6	4.3
Fusarium sp.	4	4.3
Helminthosporium sp.	1.3	0
Mucor sp.	11.8	7
Penicillium sp.	0.7	2.1
Phoma sp.	2.6	0
Trichoderma sp.	1.3	1.4
Unidentified fungi	20.4	21.8

Table 2. Fungal taxa isolated from *Sparrmannia flava*.

Fungi	Percentage recovery from insects	
	Heads	**Faeces**
Alternaria alternata	5.7	7.5
Aspergillus sp.	1.9	4.7
Chaetomium sp.	2.8	7.4
Cladosporium sp.	1	0.9
Curvularia sp.	4.8	1.9
Fusarium sp. 1	43	28.4
Fusarium sp. 2	16	26.3
Fusarium sp. 3	22	21
Mucor sp.	2.8	0
Penicillium sp.	0	1.9

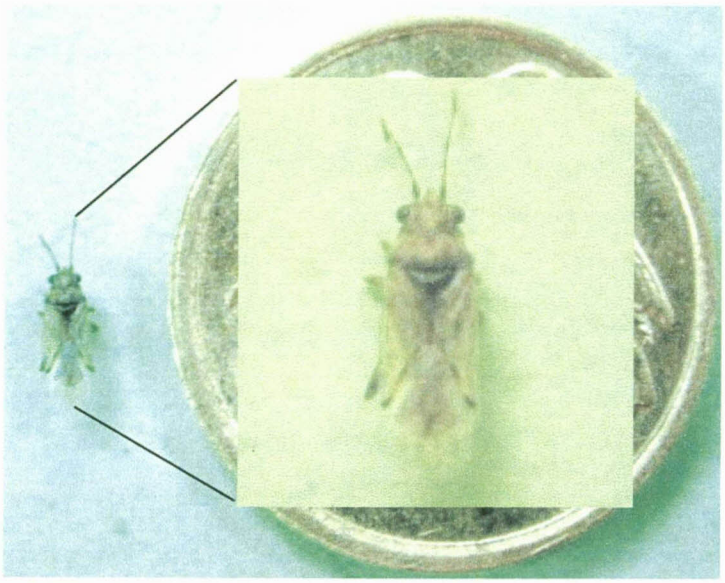

Figure 1. *Nysius natalensis* (Hemiptera: Lygaeidae)

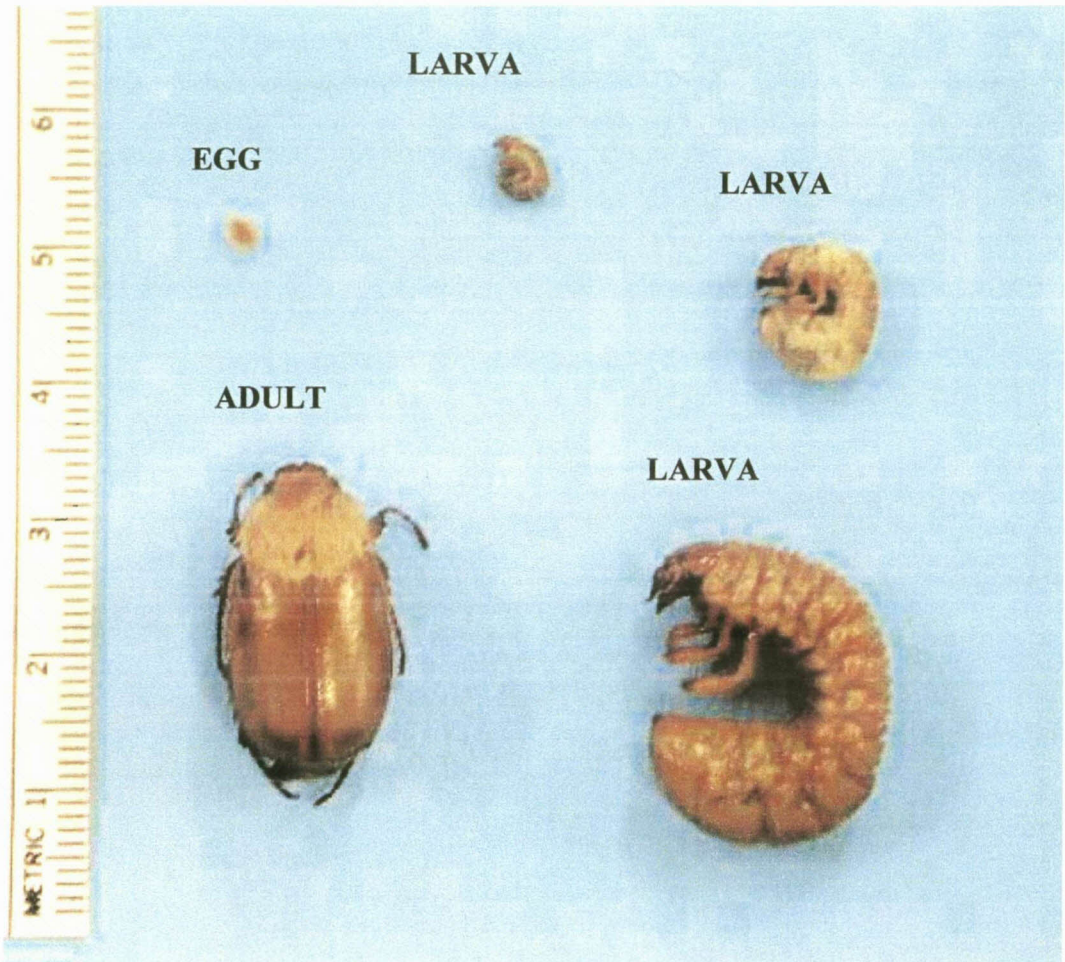

Figure 2. Life cycle of *Sparrmannia flava.*(Coleoptera: Scarabaeidae)

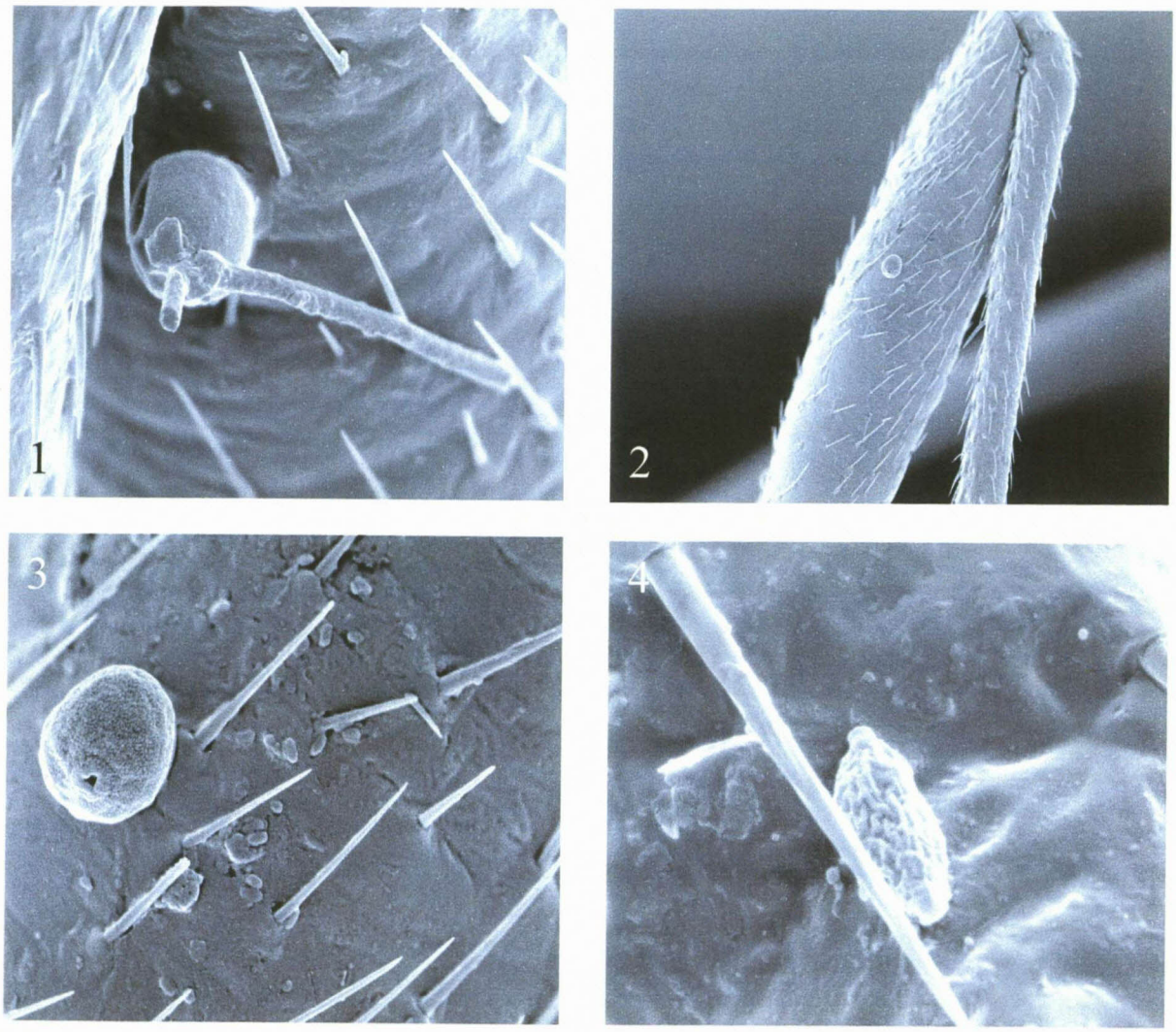

Figure 3 (1-4). Scanning electron microscopy (SEM) of fungal propagules on the exoskeleton of *Nysius natalensis.*

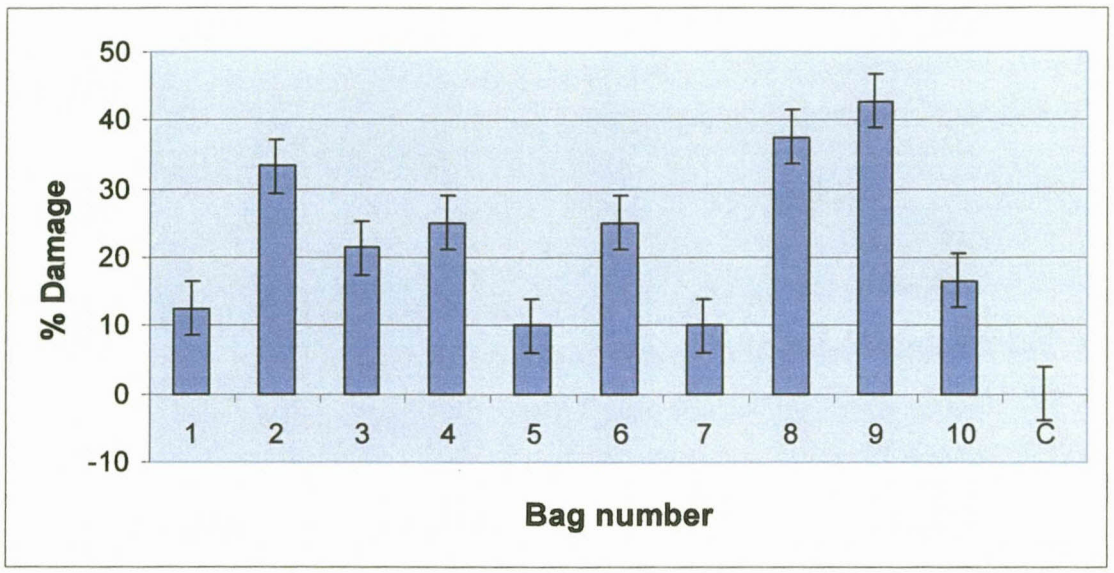

Figure 4. Percentage of pistachio nuts damaged by *Nysius natalensis* feeding. Lines on bars represent standard error.

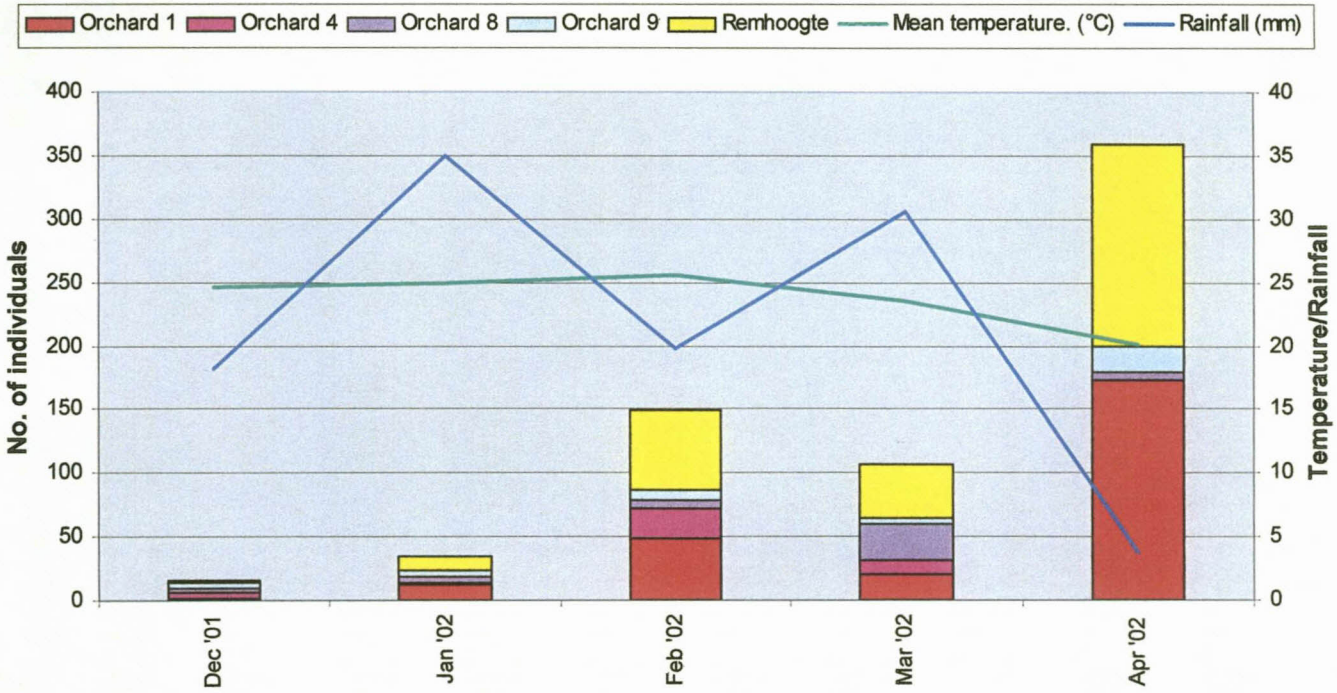

Figure 5. The number of *Nysius natalensis* individuals in cover crops of pistachio orchards, total rainfall and the mean temperature from December 2001 to April 2002. Number of individuals can be read on the primary y-axis and total rainfall and mean temperature can be read on the secondary y-axis

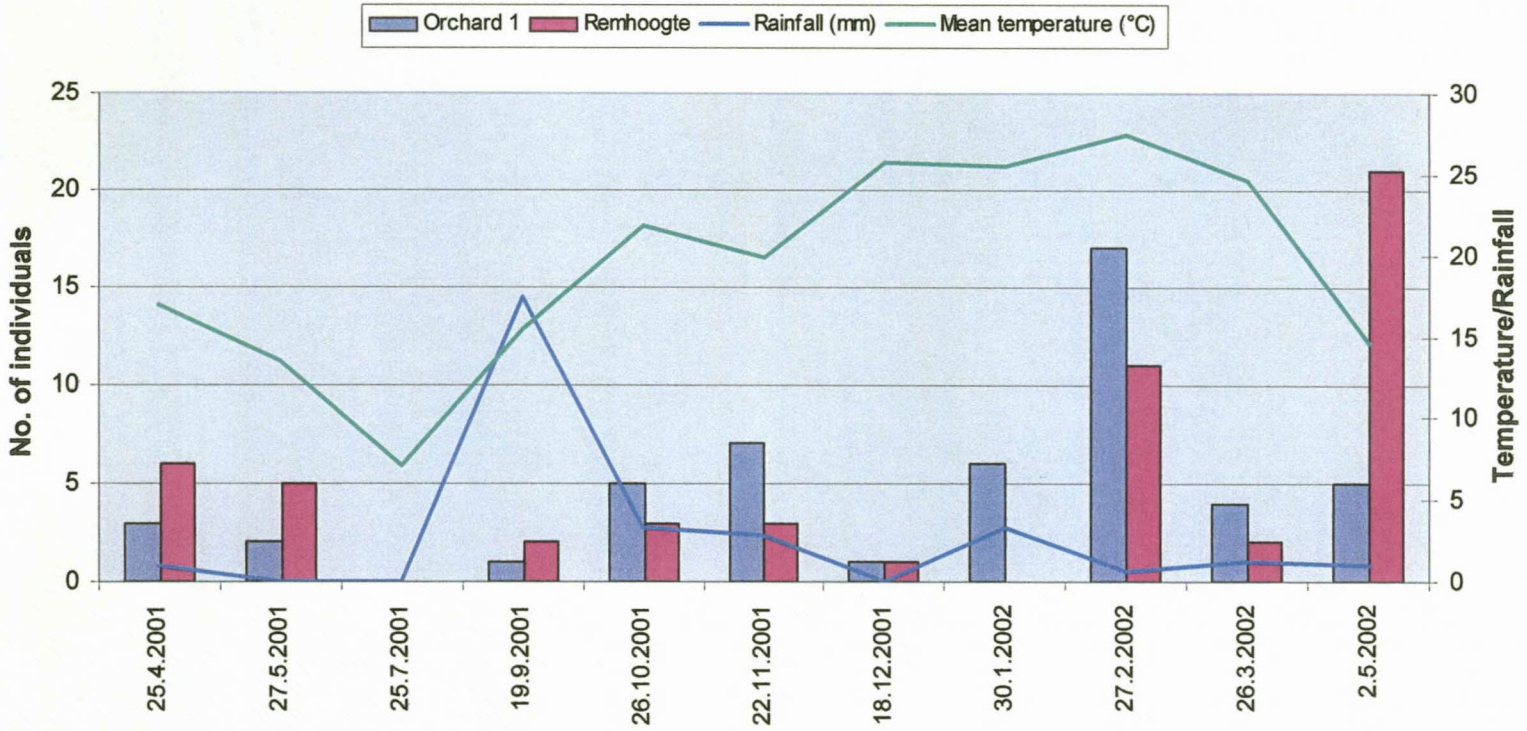

Figure 6. The number of *Nysius natalensis* in the canopies of pistachio trees, rainfall and temperature. Number of individuals is read on the primary y-axis and temperature and rainfall are read on the secondary y-axis.

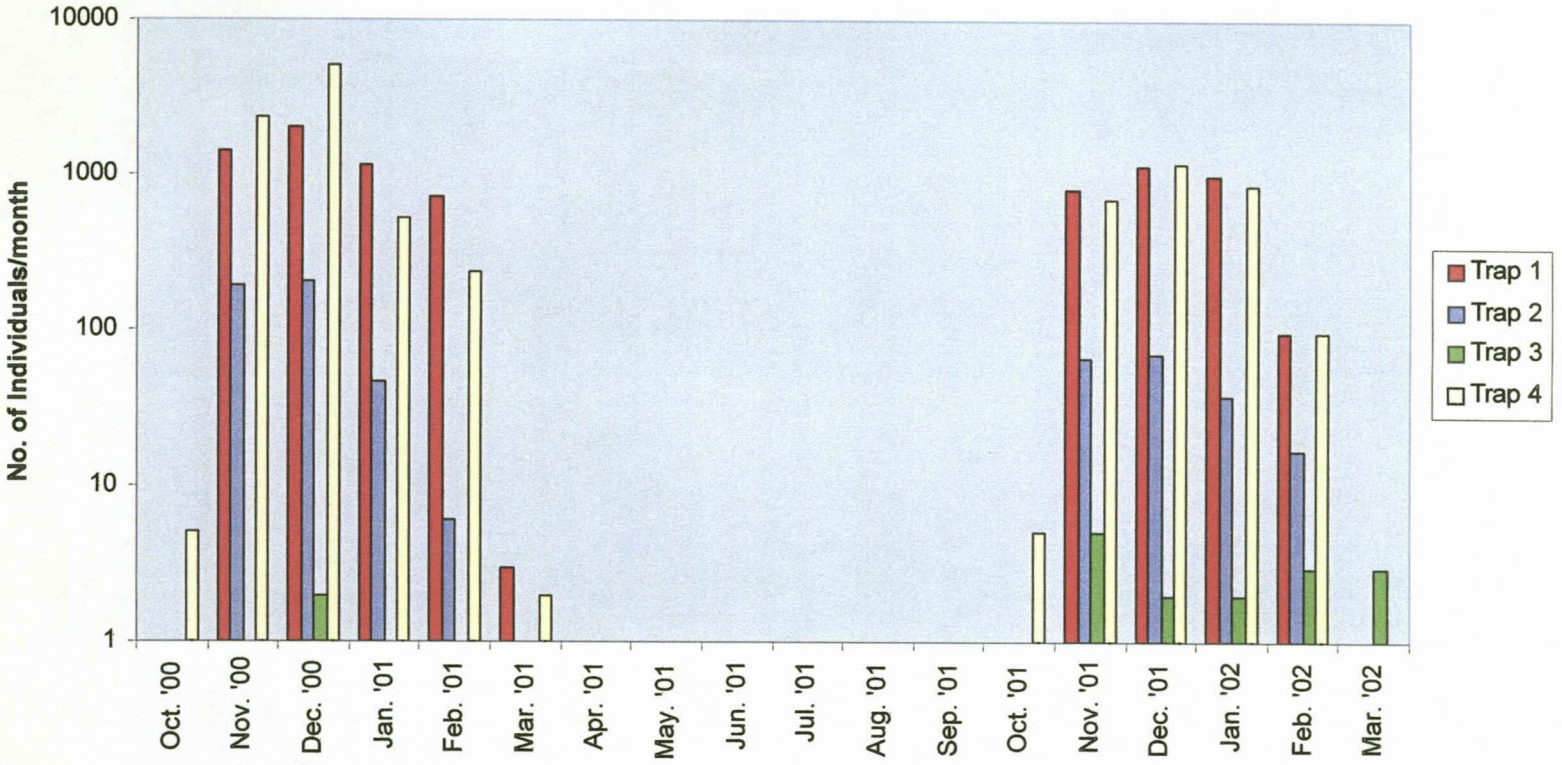

Figure 7. The monthly total number of individuals of *Sparrmannia flava* captured per light trap at Green Valley Nuts.

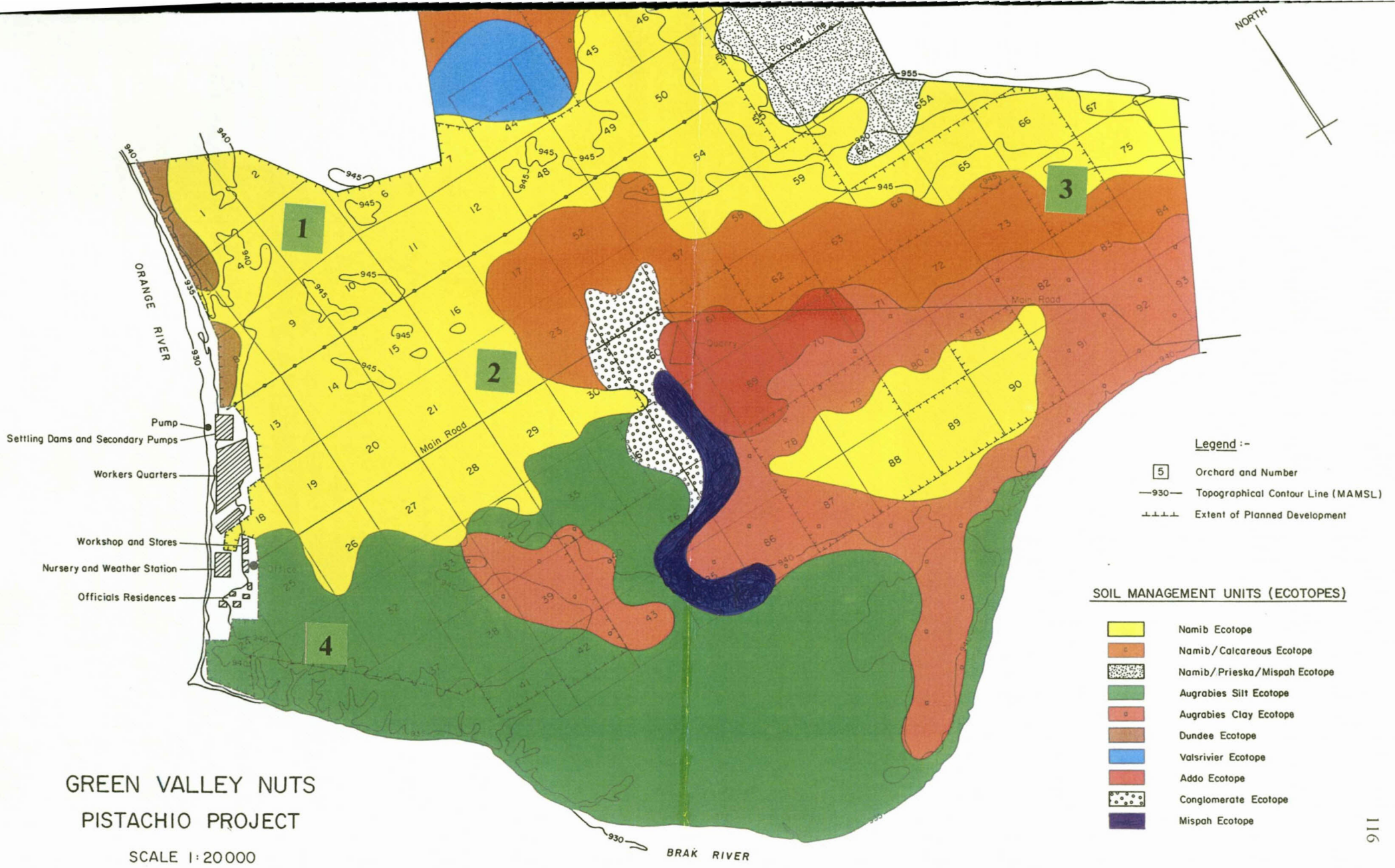

Figure 8. A map of Green Valley Nuts showing the different soil types on the farm and the location of the light traps.

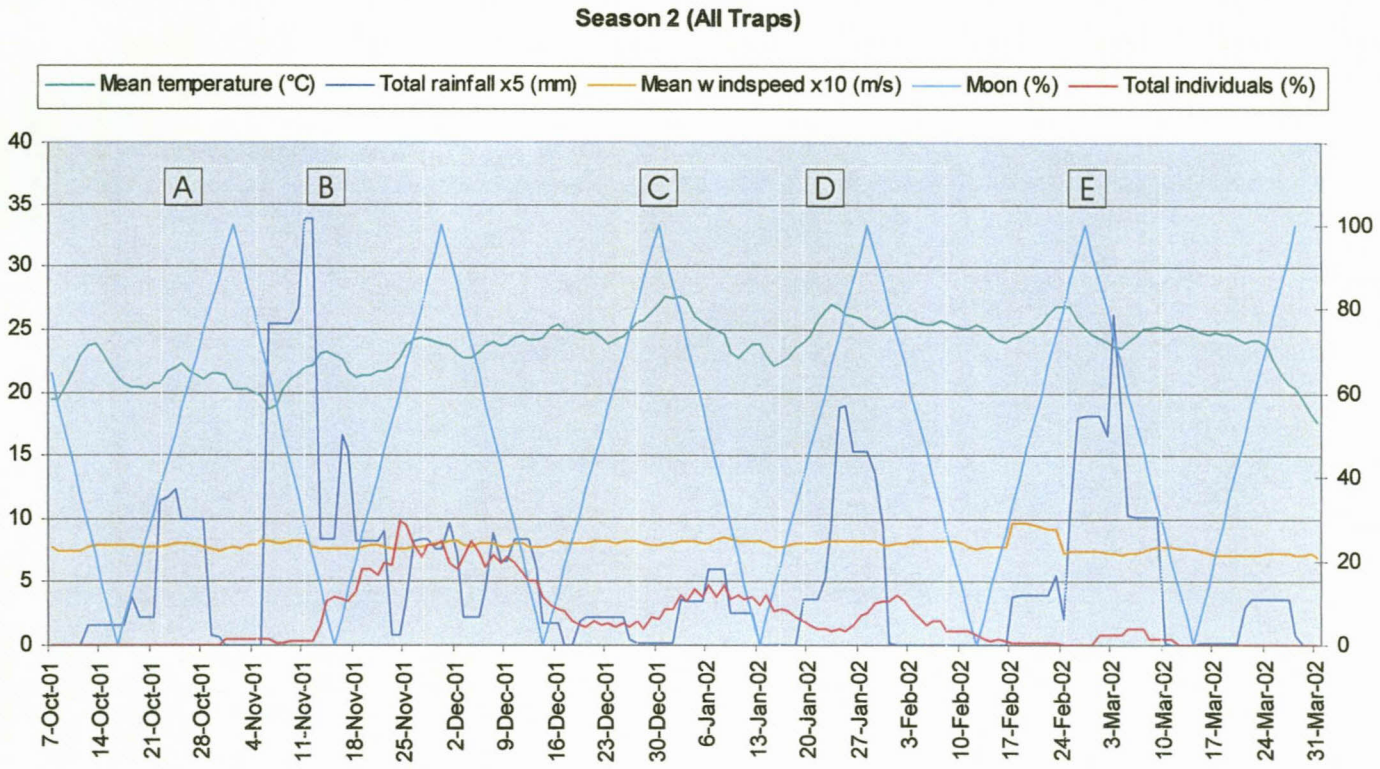

Figure 10. Total percentage of *Sparrmannia flava* recorded at four light traps at Green Valley Nuts in relation to environmental factors and lunar phase. Temperature, rainfall and windspeed are read on the primary y-axis and the percentage of individuals are read on the secondary y-axis. Boxes marked A - E are for explanatory purposes in the text. Rainfall and windspeed are multiplied by a factor of 5 and 10, respectively, to bring these variables into context with the y-axis.

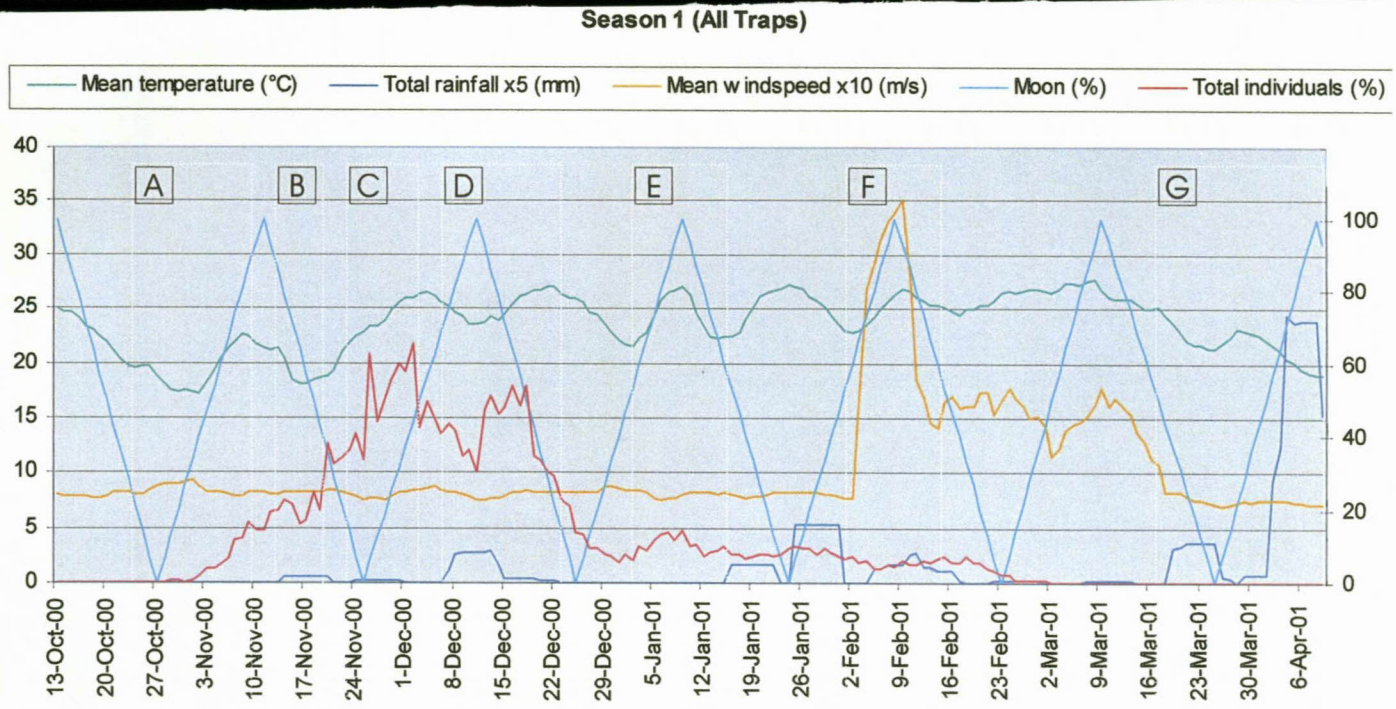

Figure 9. Total percentage of *Sparrmannia flava* recorded at four light traps at Green Valley Nuts in relation to environmental factors and lunar phase. Temperature, rainfall and windspeed are read on the primary y-axis and the percentage of individuals are read on the secondary y-axis. Boxes marked A-G are for explanatory purposes in the text. Rainfall and windspeed are multiplied by a factor of 5 and 10, respectively, to bring these variables into context with the y-axis.

CHAPTER 4

Coreidae as disseminators of fungal phytopathogens to *Cajanus cajan* (Fabaceae)

Abstract

Pigeonpea (*Cajanus cajan*) is an important new crop in South Africa. A number of new diseases have been reported on pigeonpea in South Africa including, a rust (*Uredo cajani*), powdery mildew (*Ovulariopsis ellipsospora*) and *Cercospora* leaf spot (*Mycovellosiella cajani*). *Clavigralla tomentosicollis* (Hemiptera: Coreidae) is one of the most prevalent phytophages interacting with pigeonpea in South Africa. For the successful cultivation of pigeonpea, it is imperative that organisms responsible for yield reduction be studied intensively, especially when considering that insects can harbour and disseminate propagules of fungal pathogens. In the present study, individual specimens of *C. tomentosicollis* were captured, killed by freezing and plated onto corn meal agar to allow for fungal growth. Fungi were consistently isolated from *C. tomentosicollis* and overall, nine genera and fourteen species of fungi were isolated. The insect was also able to transfer viable fungal propagules that cause disease symptoms to healthy pigeonpea plants. Bugs also showed a preference for certain cultivars. Observation of insects using scanning electron microscopy (SEM) confirmed that, fungal propagules were distributed on various parts of the exoskeleton. This study is unique in terms of Coreidae harbouring fungi that are potential pathogens of pigeonpea.

1.0 Introduction

Pigeonpea (Fabaceae: *Cajanus cajan* (L.) Millspaugh) is a nutritious food source for both humans and animals in many developing countries. It is probably native to India having been brought to Africa where different varieties have been developed. It is grown singly or as a hedge plant in home gardens in a number of provinces in South Africa. All the crops produced are consumed locally as a green vegetable by the Asian community and as dry grains for making soup by the African community. The fruit consists of a pod containing a variable number of seeds, depending on the cultivar. Although pigeonpea is not produced widely in South Africa, 120-150 tonnes of pigeonpea seed, processed as oil dhal, is imported into South Africa every month at an annual cost of about a million US dollars (C. Mathews, personal communication*).

Numerous pigeonpea cultivars, that differ in height, habit of growth, colour of flower, time of maturity, shape of pods, and colour, size, and shape of seed, are available (Duke, 1981; Reddy, 1990). Pigeonpea is remarkably drought resistant, tolerating dry areas with less than 650 mm rainfall per annum. It produces seed profusely under dry conditions, as the crop matures in the early stages of development, the incidence of pest damage is low (Duke, 1981). However, many insects feed upon the seeds and other parts of the plant when the plant is more mature causing extensive damage (Reed and Lateef, 1990).

Numerous diseases have been observed on pigeonpea in South Africa. These include the rust (*Uredo cajani*) (Swart, Mathews and Saxena, 2000) and Powdery mildew (*Ovulariopsis ellipsospora*) (Crous, Philips and Baxter, 2000) while *Cercospora* leaf spot (*Mycovellosiella cajani* = *Cercospora cajanii*) has also been observed on plants

*C. Mathews, P/Bag X11318, Nelspruit 1200, Mpumalanga

in Mpumalanga, South Africa (W.J. Swart, personal communication*). *Mycovellosiella* leaf spot is known to cause yield losses as high as 75% to 85%, depending on location and season (Reddy, Raju, Sharma, Nene, and Macdonald, 1993). Combined attacks of *Mycovellosiella* leafspot and powdery mildew have been reported to cause 32% yield losses in Malawi (Subrahmanyam, 1994).

Clavigralla tomentosicollis (Ysterbek, Hemiptera: Coreidae,) (Fig. 1) is by far the most widespread and damaging insect occurring on pigeonpea in Africa (Jackai and Hammond, 1983; Ajayi, Ezueh, Tabo, Asiegbu, Laxman-Singh and Singh., 1995). *C. tomentosicollis* occurs throughout sub-Saharan Africa and belongs to the pod sucking bug complex (PSB complex), which consists of various combinations of Coreidae and Alydidiae species (Schaefer and Panizzi, 2000). The feeding mechanism of *C. tomentosicollis* involves injecting enzymatic saliva and sucking out the digested seed contents, in the process either leaving an unattractive scar on the pod or causing pods to dry out and shrivel prematurely, thus destroying the seed (Schaefer and Panizzi, 2000; Okeola and Machuka, 2001). *C. tomentosicollis* have also been shown to migrate from wild host plants to cultivated fields (Chiang and Singh, 1988). According to Chiang and Jackai (1988) two or more pairs of *C. tomentosicollis* adults per 10 cowpea plants can cause a significant reduction in yield.

It is imperative that any organisms responsible for yield reduction in pigeonpea be studied if the full potential of pigeonpea cultivation in SA is to be realised. This is especially relevant when considering the fact that an insect such as *C. tomentosicollis* can also harbour and disseminate propagules of fungal pathogens. The objectives of this study were therefore to firstly, isolate and identify fungi associated with *C.*

*W. J. Swart, Plant Sciences, University of the Free State, Bloemfontein.

tomentosicollis, secondly to investigate the possible transmission of fungal diseases by *C. tomentosicollis* and finally, to test for cultivar feeding preferences in *C. tomentosicollis.*

2.0 Materials and Methods

Collection of insects. Sampling of *C. tomentosicollis* was conducted on two occasions [March 2001 (Sample 1) and April 2001 (Sample 2)] at an experimental pigeonpea plot on the experimental farm of the University of the Free State, 18 kilometres northwest of Bloemfontein (South Africa). The plot was 23.0 x 11.9 m in size and consisted of four equal sized blocks. Nine cultivars were planted randomly relative to each other within each block. *C. tomentosicollis* were hand collected and expressed into Polytop™ bottles and immediately placed in a freezer for approximately 1 hour. In total 200 bugs were collected, 100 during each sampling period.

Isolation of fungi. The bugs were transferred to individual Petri-plates (65 mm in diameter) containing potato dextrose agar (PDA) (Difco) amended with streptomycin sulphate (0.33 g/1000 ml water) and incubated at 25°C in a light-dark cycle of 12 hours each. When fungal colonies became visible with the naked eye they were transferred to corn meal agar (CMA) (Difco) for the purpose of identification, quantification and further experimentation.

Scanning electron microscopy. To confirm the harbouring of fungal propagules on the *C. tomentosicollis* exoskeleton scanning electron microscopy was conducted. The bugs were placed in a freezer at -10°C for an hour and then freeze-dried for 2 hours. Specimens were fixed onto SEM stubs by mean of a plastic adhesive and sputter coated with gold before being observed. All body parts were examined for signs of fungal propagules.

Dissemination potential experiment. Since several fungi were isolated from *C. tomentosicollis,* and some of the genera are pathogenic to pigeonpea, it was essential to test whether *C. tomentosicollis* is able to disseminate fungi to pigeonpea plants. The experiment consisted of two replications and was carried out in the laboratory. *C. tomentosicollis* were hand collected and placed in plastic containers. Both the pigeonpea plants and the hemipterans were sprayed with a 5 % Sporekill™ solution. *C. tomentosicollis* individuals were subsequently artificially infested with fungal isolates obtained from *C. tomentosicollis*, by placing the insects on agar culture media that containing cultures of the respective isolates for thirty minutes. Five *Fusarium* species, two *Alternaria* species and one *Curvularia* species were used for infestation of the insects.

Individual pigeonpea plants were enclosed in netting and five *C. tomentosicollis* individuals (five insects per plant) infested with the same isolate were placed inside the netting, allowing them access to the plants. Since most of the pods had already reached maturity and were starting to dry out all the pods were removed from the plants. Although *C. tomentosicollis* generally feed on the pods of the plants, they were also observed to feed on the stem and shoots of the plant. After allowing *C. tomentosicollis* to feed for 20 days, isolations were made from all the parts of the plant that showed any symptoms of disease. Fungi isolated in this manner were compared to the original isolate with which the insects had been infested. The control treatment consisted of pigeonpea plants covered in netting, but without the inclusion of any insects.

Cultivar preference experiment. Whilst sampling *C. tomentosicollis* in the field, certain pigeonpea cultivars were observed to host more hemipterans than others. It was

thus important to test whether *C. tomentosicollis* had any feeding preferences for certain cultivars. The experiment was conducted in the glasshouse and repeated twice. Feeding trials were conducted by placing five plants of different cultivars in a single net cage and testing it against a single cultivar of each, thus giving *C. tomentosicollis* a "choice" in cultivars and "no choice" with only one cultivar. Twenty insects were inserted into each cage. Observations were then made upon which pigeonpea cultivar the hemipterans would feed.

3.0 Results and Discussion

Isolation of fungi. Fungi were consistently isolated from *C. tomentosicollis* (Table 1) and overall nine genera and fourteen species of fungi were isolated. *Alternaria alternata*, *Curvularia* sp., *Drechslera* sp., *Fusarium* sp.3, *Fusarium* sp.4 and *Gonatobotrys* sp. were dominant in isolations (more than 10%) from both samples. *Helminthosporium sp.* was only isolated in Sample 1. Unidentified fungi comprised 6% and 4% in Sample 1 and Sample 2, respectively. *Alternaria alternata*, *A. tenuissima*, *Cladosporium oxysporum*, *C. cladosporioides*, *Fusarium pallidoroseum* (*F. semitectum*), *F. avenaceum*, *F. equiseti* and *F. udum* are listed as pathogens of pigeonpea (Statewide integrated pest management project, 2000). Since similar fungal genera were isolated from *C. tomentosicollis* in the present study, the assumption can be made that *C. tomentosicollis* disseminates fungal propagules of potentially pathogenic species. Additionally, Egwuatu and Taylor (1979) state that feeding punctures of *C. tomentosicollis* facilitate secondary infection by microorganisms. According to Jones (1953) and Daugherty (1967, in Jackai, 1984) damaged pigeonpea seeds are usually discoloured at the location of stylet insertion, a phenomenon associated with the infection

of a fungus, possibly related to *Nematospora coryli*, during feeding. Since feeding requires insertion of stylets into the pod, seed damage will result either from the action of histolytic enzymes or the accompanying fungal infection, whether feeding actually takes place or not, assuming that the findings on *N. coryli,* or any other pathogen, are applicable to the insect (Jackai, 1984).

Scanning electron microscopy. Fungal propagules were observed all over the exoskeleton of *C. tomentosicollis* (Fig. 2). Identification of the fungal spores is very difficult and virtually impossible. Some of the observed propagules were germinating on the exoskeleton of the insect (Fig. 2.1), thus proving that they were fungi and not bacteria or other debris. The exoskeleton of *C. tomentosicollis* is very rigid and bears setae in numerous places which are ideal for trapping fungal propagules. Fungal propagules were even observed on the eyes of *C. tomentosicollis* (Fig. 2.2). Most importantly, several fungal propagules were observed on the mouthparts of *C. tomentosicollis* (Fig. 2.3), which increases the possibility of *C. tomentosicollis* facilitating infection of pigeonpea pods by means of feeding wounds. Figure 2.4 illustrates the potential for other microorganisms, such as bacteria, to adhere to the tarsus of the insect. Compared to Drosophilidae and Lygaeidae in the previous chapters, *C. tomentosicollis* seems to harbour more fungal propagules both quantitatively and qualitatively. It is assumed that this is due to the setae present on the exoskeleton, as well as the larger size of *C. tomentosicollis.*

Dissemination potential experiment. Since various species of fungi were isolated from *C. tomentosicollis*, it was important to investigate their potential in transmitting the fungi to pigeonpea. Numerous different symptoms were observed and these were

summarised in terms of occurrence on plant part, with a short description of the symptoms observed (Table 2). All the symptoms differed in terms of the area where they were found and their appearance. *Fusarium* sp. 2, *Fusarium* sp. 3, *Fusarium* sp. 4 and *Alternaria* sp. 1 were all isolated from lesions on pigeonpea plants and correspond with the original fungi that were used to artificially infest *C. tomentosicollis*. With regard to *Fusarium* sp. 2 (Fig. 3.1), symptoms were observed on both the leaves and the stem, but only isolations from stem tissue matched the original isolate. *Fusarium* sp. 3 (Fig. 3.2) was isolated from the discoloured part in the pith of the stem. This is very significant, and suggests that fungal propagules on the mouthparts of *C. tomentosicollis* were transferred whilst individuals were feeding on the stem of the plant. *Fusarium* sp. 4 (Fig. 3.3) was isolated from discoloured leaves of the plant, but it is doubtful whether these symptoms can be attributed to the original isolate with which the insects had been infested. *Alternaria* sp. 1 (Fig 3.4, Fig 3.5) was isolated from lesions on the leaves but it is assumed that accidental transfer (*e.g.* spores harboured on legs that rub off) of fungal propagules took place.

According to Jackai (1984) female individuals of *C. tomentosicollis* generally require greater amounts of energy in order to meet the requirements for egg production, and therefore they feed more than males do. They thus cause more punctures and greater seed damage than male individuals. The assumption can therefore be made that females have a greater potential to cause disease on pigeonpea than males do.

Cultivar preference experiment. C. tomentosicollis was observed feeding on all five cultivars (Fig. 4, Fig. 5) and no real differences were observed between cage one (Fig. 4) and two (Fig. 5). All cultivars used were of the short duration genotype. Minja,

Shanower, Silim and Karuru (2000) showed that spraying a short-duration genotype (ICPL 87091) with ultra low volume (ULV) insecticides resulted in lower pest incidence than was the case with medium and long duration genotypes. In this study cultivars ICPL 85090 and ICPL 87 seemed to attract the most *C. tomentosicollis* in both replications, whilst cultivars ICPL 151 and ICPL 871 were observed to have comparatively less visitation. Cultivar ICPL 87091 had the least visitation in both instances. The reason for the preference for specific cultivars by *C. tometosicollis* in this study is not known. Logically the percentage of insect damaged seeds per pod was affected by the cultivar. In a study by Munthali (1995) the greatest damage (59.4%) was found on PQ14, while the least (38.0%) was on ICP9145.

According to Reed and Lateef (1990) pest preference for cultivars can only be considered when there is a choice of cultivars available. For example, plant lectins are known to have anti-insect properties (Czapla and Lang, 1990). Evidence has shown that a gene encoding antimetabolic properties in *Sphenostylis stenocarpa* (African yam bean) has potential for providing cowpea with resistance against *C. tomentosicollis* (Okeola and Machuka, 2001). According to Chiang and Jackai (1988) the pod wall of pigeonpea may be a primary line of defense either as a physical or chemical entity due to the presence of secretory ducts that contain a tannin-like material. The outer epidermis of the pod wall also has many secretory hairs that contain yellow oil and similar features are found on the leaves. Cultivar differences in terms of these morphological characteristics can therefore be expected to influence insect feeding preference. It is thus evident that characteristics of non-preference should be considered and implemented in future resistance-breeding techniques. The natural enemies of *C. tomentosicollis* include Coleoptera, Hymenoptera,

Diptera and Hemiptera in countries such as Kenya, Malawi, Tanzania and Uganda (Minja, Shanower, Aro, Nderitu and Songa, 1999). Munthali (1995) found that pigeonpea pods from a crop sown in early summer had the lowest proportion of damaged seeds, suggesting that this was the most suitable date for minimising seed damage by pod feeding insects under Malawian conditions. However, this may be dependant on the climatic conditions at the time.

4.0 Conclusion

C. tomentosicollis can be considered a potential disseminator of fungi, of which some might be potentially pathogenic on *Cajanus cajan* Furthermore, strong evidence exists that *C. tomentosicollis* can be considered to be a vector of fungal pathogens to the pigeonpea plant as a whole. Scanning electron microscopy confirmed the presence of fungal propagules on the exoskeleton of *C. tomentosicollis* and the bug can be considered to be a potential threat to pigeonpea yields and the entire health of the plant. It is evident that there is a definite preference for certain cultivars by *C. tometosicollis* and thus characteristics of non-preference should be considered and implemented in future resistance breeding programs.

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Table 1. Fungal taxa isolated from *C. tomentosicollis.*

Fungi	Percentage recovery from insects	
	Sample 1	**Sample 2**
Alternaria sp. *1*	11.2	10.6
Alternaria sp.*2*	6.8	4
Cladosporium sp.	0.8	3.6
Curvularia sp.	12	16
Drechslera sp.	7.5	11.4
Epicoccum sp.	3.8	2.1
Fusarium sp. *1*	4.5	7
Fusarium sp. *2*	6	6.8
Fusarium sp. *3*	9	12
Fusarium sp. *4*	9.8	16
Fusarium sp. *5*	6.8	4
Gonatobotrys sp.	12.8	0.4
Helminthosporium sp.	1.5	0
Mucor sp.	1.5	2.1
Unidentified fungi	6	4

Table 2. Description of symptoms and plant parts from which isolations were made and matched up with original isolate.

	Damage or Symptoms	Isolation Match
Fusarium sp. 1	Leaf discolouration	0
Fusarium sp. 2	Leaf discolouration and lesion on twig	Twig
Fusarium sp. 3	Internal stem discolouration	Stem
Fusarium sp. 4	Shoot and leaf discolouration	Leaves
Fusarium sp. 5	Nothing	0
Alternaria sp. 1	Leaf discolouration	Leaves
Alternaria sp. 2	Twig lesion	0
Curvularia spp.	Nothing	0
Controls (x5)	Nothing	0

Figure 1. *Clavigralla tometosicollis* (Hemiptera;Coreidae)

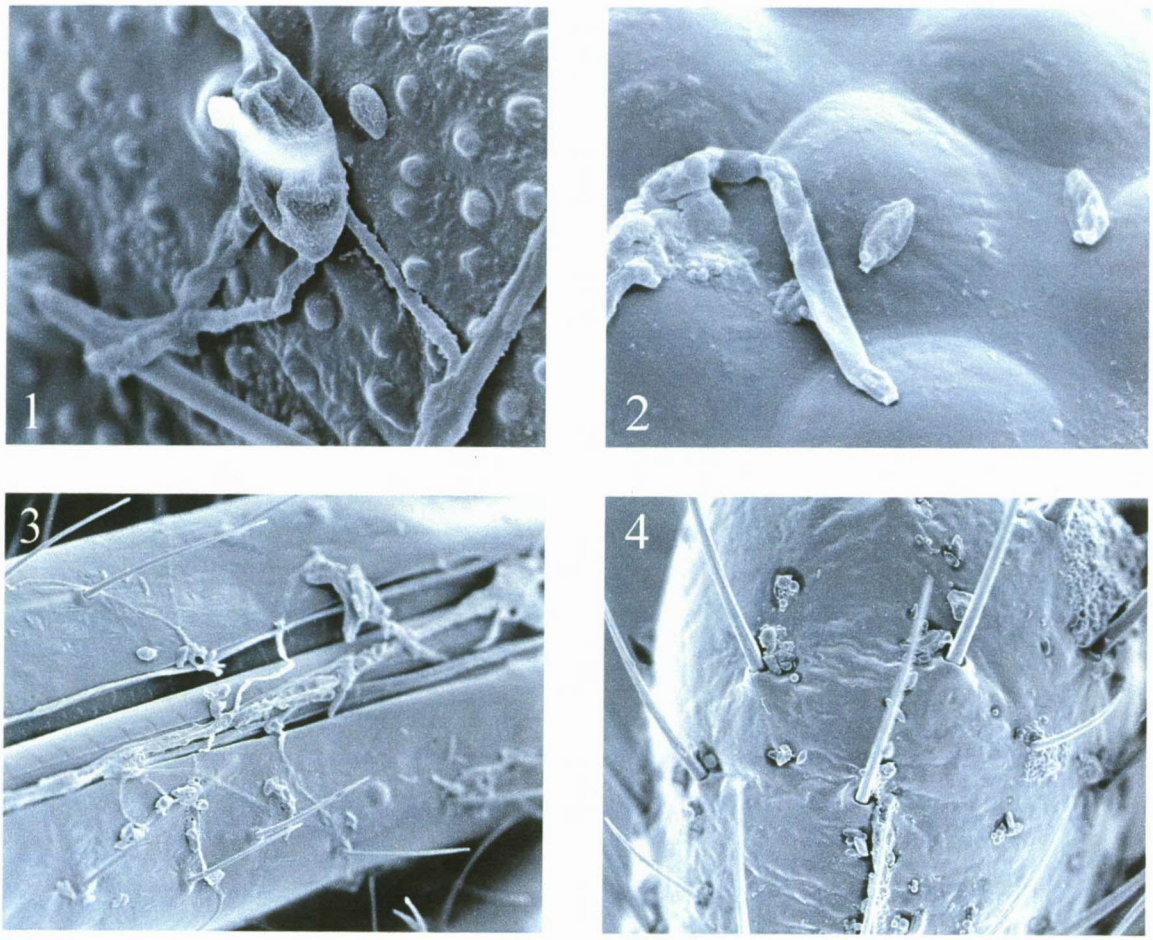

Figure 2 (1-4). Scanning electron microscopy (SEM) of fungal propagules on the exoskeleton of *Clavigralla tomentosicollis.*

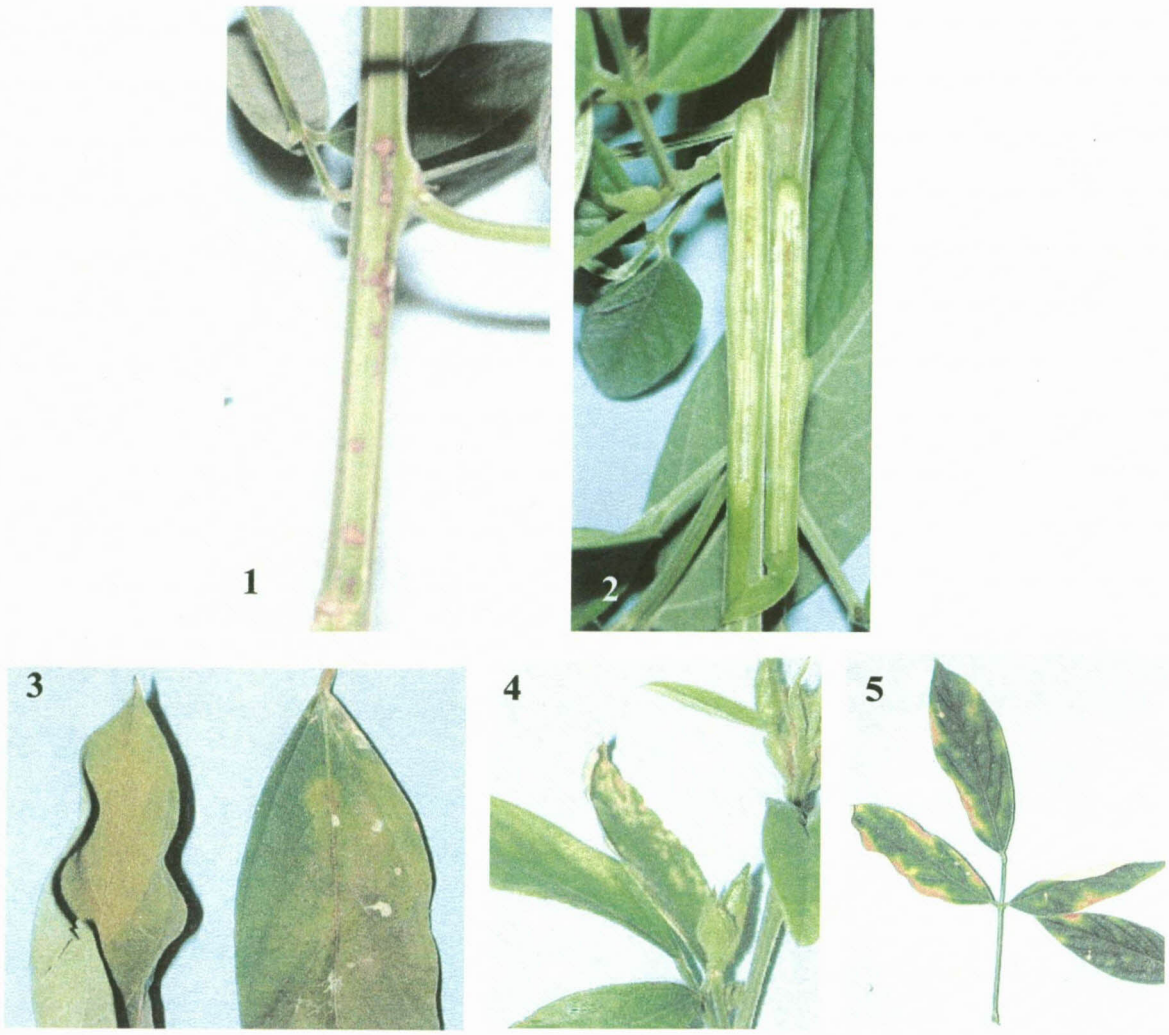

Figure 3 (1-5). Symptoms of possible fungal infection on leaves and stem of pigeonpea.

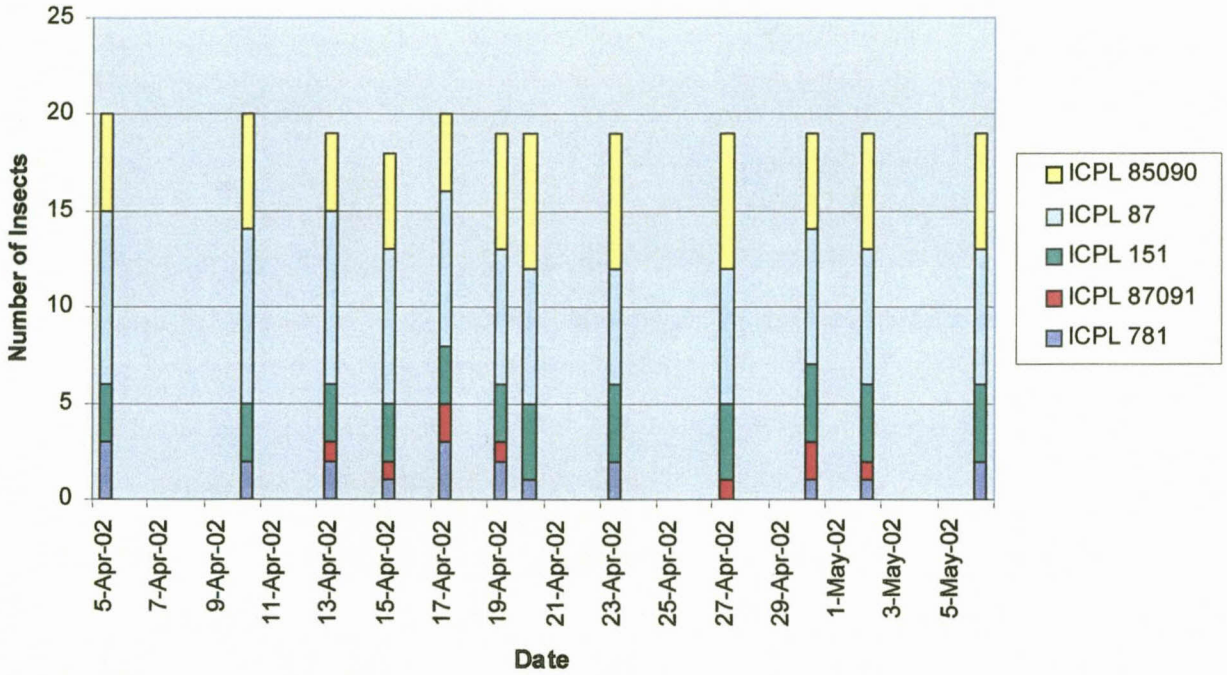

Figure 4. Number of insects in cage 1 feeding on a specific pigeonpea cultivar on specific observation dates.

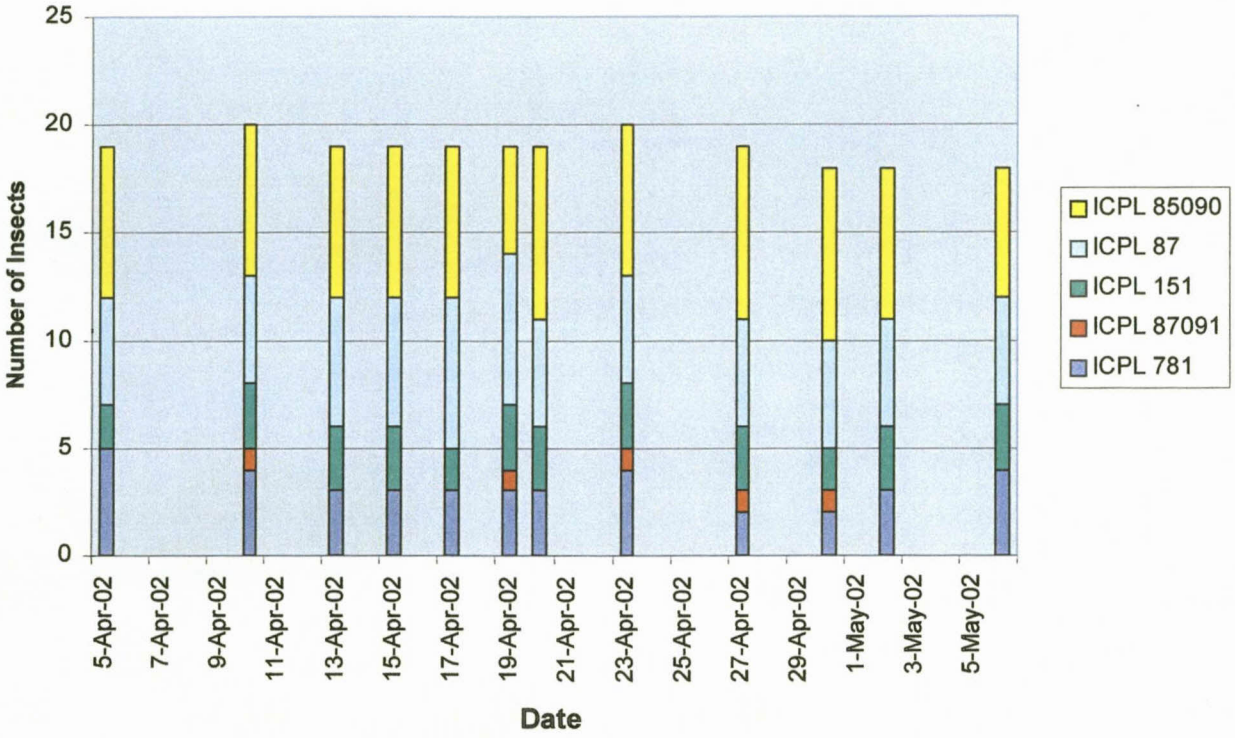

Figure 5. Number of insects in cage 2 feeding on a specific pigeonpea cultivar on specific observation dates.

CHAPTER 5

General conculsions

The present investigation has clearly demonstrated that numerous species of fungi are disseminated by certain insect pests associated with three new crops *i.e.* cactus pear, pistachio and pigeonpea, being planted in South Africa. It was also conclusively proven that many of these fungi had the ability to cause disease symptoms on the crop frequented by its specific host insect. Whether the relationship between the insects and the fungi isolated from them has any bearing on the ecology or life cycle of the insects, or the fungi themselves, is difficult to ascertain from this study. However, the fact that these insect-fungal associations were firmly proven, be they incidental or of a secondary nature, is nevertheless an extremely important consideration in the context of developing an integrated pest management programme for the respective crops.

Given the fact that the insect species studied all have the status of being potential pests on the crops with which they are associated, has significant implications for the protection of the respective crops from phytophagy and disease. Insects that only casually visit plants for shelter, pollination, etc. would obviously have a far lower frequency of association than insects that feed on a crop. The chances of casual visiting insects transferring fungal propagules that are potentially pathogenic to the crop are also negligible. Insects that require crops for food, have a far higher frequency of association and thereby a greater chance of transferring pathogenic propagules to the plant. They also have an additional negative impact on crop health in that they cause feeding wounds that provide easy access to opportunistic pathogens that would normally not be able to infect the plant through unwounded tissue. Of the fungi isolated from insects many, such as *Alternaria* spp., *Fusarium* spp. and *Penicillium* spp., have in fact been shown to be opportunistic pathogens on various crops.

Besides the pest status of an insect, other characteristics pertaining to the biology and morphology of the insect can have both a qualitative and quantitative influence on the fungal component that it harbours and disseminates. These characteristics can therefore be expected to influence the probability of a specific insect harbouring fungi able to cause disease on the plant or which are simply harmless saprophytes. Assuming that a potential fungal pathogen is the most common associate of a specific insect, the above characteristics can also influence disease severity that the fungus is able to induce by virtue of the degree to which the insect is infested with the particular fungus. Interactions between individual characteristics can also be expected to occur, which will in turn also influence disease incidence. The most obvious characteristics of the insects studied in the present investigation that can be expected to play a role in the transmission of phytopathogens and disease incidence are as follows:

Size of the insect. It could probably be assumed that the larger the insect, the larger the surface area of its exoskeleton and therefore quantitatively and qualitatively more fungal propagules will be harboured by the insect. This assumption was tested by considering the observations made when using SEM. Of the four insect taxa that were observed using SEM, *Clavigralla* sp. have from pigeonpea harboured significantly more fungal propagules on its exoskeleton than any other species. This was confirmed by isolations where it also yielded the most fungal isolates per 100 insects and also the most fungal spp. per 100 insects (Table 1). Judging by the relatively large number of fungal propagules that only the heads and faeces of the much larger *Sparmannia flava* (Scarabaeidae) from pistachio harboured, it seems logical to suggest that had the entire body of *S. flava* been plated out, significantly more fungal propagules, as well as a

greater variety would have emerged. It was also interesting to note that despite yielding a similar number of isolates to the other insects, the diversity of fungal species isolated from *S. flava* heads and faeces was notably less than the other three taxa given that these substrates represent very small and specialized parts of the insect. *Fusarium* spp. were the most prominent fungi isolated from *Sparrmannia flava*, a finding that is consistent with its soil dwelling nature.

Table 1. Qualitative and quantitative summary of fungi isolated from four insect taxa.

	Drosophila spp.	*Nysius natalensis*	*S. flava* heads & faeces	*Clavigralla tomentosicollis*
Number of fungal isolates per 100 insects	*D. hydei*= 103 *D. melanogaster* = 113	119	103	137,5
Mean number of fungal spp. per 100 insects	*D. hydei* = 12 *D. melanogaste r* = 8	12	6	14,0

Setae. It would be safe to assume that the denser the setae on an insect's body, the higher the possibility of harbouring fungal propagules. All the insects in this study had setae, but the number, size and location on the body were different. For example, *S. flava* had the most setae and *Drosophila* spp. the least. Setae can also be expected to influence the ability of an insect to harbour fungal propagules, irrespective of its size. For example, a small insect with many setae should be able to harbour more propagules than a large insect with few setae. Thus, *S. flava* can be expected to have the advantage of harbouring more fungal propagules, since it has relatively more setae and a larger size than the other three taxa.

Mouthparts. The morphology of insect mouthparts can also be expected to play a definitive role in the transmission of fungal propagules, as well as in the possibility of

allowing fungi to gain entry to the plant via feeding wounds. For instance, Drosophilidae, which have sponging-sucking mouthparts, has less chance of transmitting or allowing secondary infection fungal propagules to infect the plant, than Coreidae and Lygaeidae, that have piercing-sucking mouthparts, or Scarabaeidae, which have biting-chewing mouthparts. However, it was observed that most fungal propagules were situated on the legs of Lygaeidae and Drosophilidae and very few were observed on the mouthparts and other body parts.

Environmental association. The habitats and niches that insects occupy or visit will have a definite influence on the quantitative and qualitative composition of the mycoflora that they harbour externally. Drosophilidae adults and larvae are associated with fermenting fruit and feed on microorganisms such as fungi and yeasts. Lygaeidae are, amongst others, associated with leaf litter, where fungi are known to thrive, whilst Scarabaeidae are associated with soil and feed on dung (*i.e.* secondary plant material) and humus where fungi are also prevalent. The Coreidae are also associated with wild plant hosts. Thus, Drosophilidae and Lygaeidae have the highest potential to acquire fungi from their environment. The respective habitats of these insects are related to proper crop management techniques. In this regard, sanitation in the cropping system is important to reduce any potential refugium of fungal pathogens that may be disseminated by the insects that frequent them.

Metamorphosis. Drosophilidae and Scarabaeidae are holometabolous insect groups, whilst Lygaeidae and Coreidae are both hemimetabolous insect groups. With hemimetabolous insects moulting would result in fungal propagules being discarded with the exuviae. The exuviae can be assumed to contribute to the dissemination of fungal

propagules and there is even a possibility that the exuviae may come into contact with plants (as has been observed with regard to Coreidae), providing the propagules with a chance to infest the plant. After the insect has moulted, it should be free of fungal propagules, and it can then reacquire propagules from several sources. Hemiptera moulting varies depending on the family and there may be four to five instars prior to maturity. Holometabolous insects moult in the larval stage and the moulting pattern varies, depending on the family and the dissemination of fungal propagules may be applicable here as well.

Fungal association. All the insects isolated from in this study, yielded more or less the same number of species of fungi, but different species relative to each other. Since Drosophilidae adults and larvae are mostly associated with fermenting and rotten fruit, most of the fungal propagules harboured by them would have been acquired here. The only time that cactus pear fruit could be infested would be when the flies oviposit on them or come into contact with an area of the fruit that is already damaged. Both hemipteran species are associated with wild host plants and, in the case of the Lygaeidae, with leaf litter as well. Furthermore, both species have piercing-sucking mouthparts and therefore infestation is evident either through transmission of fungal propagules attached to the mouthparts or through secondary infestation. Scarabaeidae adults reside subterraneously during the day and many soil-born diseases may be acquired here. These scarabs have biting chewing mouthparts and feed on humus and pistachio leaves, thus fungal transmission to the plant would either occur whilst feeding, or through secondary infestation.

Another important issue in insect-fungus-plant interaction is whether to control the insect or the pathogen. If an insect pest is known to disseminate pathogenic fungi to a crop, proper management of the crop would, for instance, involve resistance against the insect or the pathogen, or control of the insect or the pathogen. If the insect is only involved in the dissemination of pathogen propagules, controlling the insect would only reduce the amount of inoculum marginally and it would in any case eventually be transmitted to the host plant by other means, such as wind, water or even another arthropod. Control of the pathogen in this instance would be a better strategy to follow. However, if the insect is a phytophage of the specific crop and it is also a disseminator of fungal propagules able to infect the crop through feeding wounds, then control of the insect would probably be the best option.

Since the insects under investigation were found harbouring potentially pathogenic fungal propagules, certain useful data may be gleaned from this phenomenon that could be useful in an integrated pest management program. For instance, insects could be used as indicators of a specific fungal pathogen present in a plot or orchard. The presence of specific insects can therefore aid growers in predicting whether there are any potential diseases present in the orchard as well as the amount of inoculum. Spore traps, which are usually used to sample fungal presence in cropping systems, have limitations in that they cannot sample fungi that are primarily soil borne. These can be manually placed at different levels or different locations in the crop canopy but they obviously do not cover the complete living space of an insect, whether it be cover crops, surrounding vegetation the crop itself, humus or soil. Since the idea of using insects as indicators of fungal presence in cropping systems is controversial, research should be conducted

whereby fungi from spore traps are compared with these isolated from insects associated with different plant parts.

SUMMARY

This study investigates insect-fungal-plant interactions on three new crops (*viz.* cactus pear, pistachio and pigeonpea) in South Africa. Isolation of *Drosophila. melanogaster* and *D. hydei* flies from cactus pear (*Opuntia ficus-indica*) orchards showed that these two species harbour fungi. Inoculations of specific fungi obtained from the two *Drosophila* species onto fruit and cladodes of cactus pear showed that some of the fungi had the ability to cause rot and may even be pathogenic. Scanning electron microscopy (SEM) confirmed the harbouring of fungi on the bodies by of the two *Drosophila* species and it also showed that most of the fungal propagules were present on the legs of the flies. An insect succession study showed that both Drosophilidae flies and Nitidulidae beetles play a vital role in the degradation of the fruit by contributing to the rate of fruit decomposition and by spreading yeasts and fungi that cause rot. Further to this a dissemination potential experiment with Drosophilidae also showed that these flies are capable of transmitting fungi, which are capable of causing rot on *O. ficus-indica* fruit. Isolation of *Nysius natalensis* bugs collected in *Pistacia vera* (pistachio) orchards showed that this species harbours fungi. Additionally, isolation of the heads and faeces of *Sparrmannia flava* beetles collected in pistachio orchards showed that this species also harbours fungi. More specifically, both these species harbour fungi known to be pathogenic to *P. vera*. SEM of *N. natalensis* confirmed that they harbour fungi on the exoskeleton and that most of the fungal propagules were present on the legs of the insects. It was also shown that *N. natalensis* cause feeding lesions on pistachio nuts, thereby providing access for fungal infection. Analysis of *N. natalensis* confirmed that their numbers are higher in late summer and that cover crops and surrounding vegetation

have an influence on their population dynamics. Analysis of *S. flava* showed that they have a peak season during summer and that their flight activity is influenced by a combination of temperature, rainfall, windspeed, soil type and lunar phases. Isolation of *Clavigralla tomentosicollis* bugs collected in a *Cajanus cajan* (pigeonpea) cultivation showed that this speceis harbours fungi. Some of these fungi are known to be pathogenic to *C. cajan*. Scanning electron microscopy (SEM) confirmed the harbouring of fungal propagules on the exoskeleton of *C. tomentosicollis*. It was also shown that fungal propagules were present all over the body, most importantly the mouthparts. A dissemination potential experiment showed that this species is capable of transmitting fungal proapgules to pigeonpea plants. *C. tomentosicollis* prefers some pigeonpea cultivars above others.

OPSOMMING

Hierdie studie ondersoek die insek-swam-plant interaksie op drie nuwe gewasse (tewete turksvy, pistachio en duifert) in Suid-Afrika. Isolering van *Drosophila melanogaster* en *D. hydei* vlieë vanaf turkvy (*Opuntia ficus-indica*) boorde toon dat hierdie twee spesies swamme huisves. Inokulasies van spesifieke swamme, wat vanaf die twee *Drosophila* spesies verkry is, op die vrugte en blaaie van turksvye toon dat sommige van die swamme die vermoë besit om verrotting te veroorsaak en dat hulle selfs patogenies mag wees. Skandeer elektron mikroskopie (SEM) bevestig dat swamme op die liggame van die twee *Drosophila* spesies gehuisves word en toon ook dat die meeste van die swampropagules op die bene van die vlieë voorkom. 'n Insek suksessie studie toon dat beide Drosophilidae vlieë en Nitidulidae kewers 'n belangrike rol vervul in die degradasie van die vrugte deur by te dra tot die tempo van vrugontbinding en deur die giste en swamme wat verrotting veroorsaak te versprei. Bykomend tot hierdie toon 'n verspreidingspotensiaal eksperiment met Drosophilidae ook dat hierdie vlieë die vermoë besit om die swamme, wat die verrotting van turksvyvrugte veroorsaak, oor te dra. Isolering van *Nysius natalensis* besies wat in *Pistacia vera* (pistachio) boorde versamel is toon dat hierdie spesie swamme huisves. Daarby toon die isolering van die koppe en faeses van *Sparrmannia flava* kewers, wat ook in pistachio boorde versamel is, dat hierdie spesie ook swamme huisves. Beide hierdie spesies huisves spesifieke swamme wat daarvoor bekend is dat hulle patogenies op *P. vera* is. SEM van *Nysius natalensis* bevestig dat hierdie spesie swamme op hul eksoskelet huisves en dat die meeste van die swampropagules op die bene van die insekte voorkom. Daar is ook aangetoon dat *N. natalensis* voedingsletsels op pistachio neute veroorsaak, wat toegang tot swaminfeksies verskaf. 'n

Voorkomsanalise van *N. natalensis* bevestig dat hul getalle gedurende laat somer hoër is en dat dekgewasse en omliggende plantegroei 'n invloed op hul populasiedinamika het. 'n Voorkomsanalise van *S. flava* toon dat hulle 'n piekseisoen gedurende die somer het en dat hul vlugaktiwiteit deur 'n kombinasie van temperatuur, reënval, windspoed, grondtipe en maanfase beïnvloed word. Isolering van *Clavigralla tomentosicollis* besies, wat in 'n *Cajanus cajan* (duifert) aanplanting versamel is, toon dat hierdie spesie swamme huisves. Sommige van hierdie swamme is daarvoor bekend dat hulle patogenies op *C. cajan* is. SEM bevestig die voorkoms van swampropagules op die eksoskelet van *C. tomentosicollis.* Daar is ook getoon dat swampropagules orals op die insekliggaam, en veral belangrik op die monddele, voorkom. 'n Verspreidingspotensiaal eksperiment toon dat hierdie spesie die vermoë besit om swampropagules na duifert oor te dra. *C. tomentosicollis* verkies sekere duifertkultivars bo ander.