CHAPTER 4

INSECT ASSOCIATES OF CERATOCYSTIS ALBIFUNDUS AND PATTERNS OF ASSOCIATION IN A NATIVE SAVANNA ECOSYSTEM IN SOUTH AFRICA

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ABSTRACT

Species of *Ceratocystis sensu lato* (*s.l.*) include important plant pathogens such as *C. albifundus*, that causes a serious wilt disease of non-native, plantation-grown *Acacia mearnsii* trees in Africa. The aim of this study was to identify the insects associated with *C. albifundus* in South Africa and thus to consider the means by which the pathogen spreads. Insects were collected weekly for 77 weeks in a native ecosystem using modified pitfall traps. Trapped insects were identified and fungi were isolated using carrot baiting and by plating them onto malt extract agar. Fungi were identified using morphological characteristics and DNA sequence comparisons. Three different nitidulid beetles (Coleoptera: Nitidulidae), *Brachypeplus depressus*, *Carpophilus bisignatus* and *Ca. hemipterus* were collected, of which the most common were the *Carpophilus* spp. Two *Ceratocystis* spp., namely *C. albifundus* and *C. oblonga* were isolated from all three insect species. Insect numbers and fungal isolates decreased significantly in the colder months of the year. Of the two *Ceratocystis* spp., *C. oblonga* was most abundant occurring on 0.5% of the *Carpophilus* spp. *C. albifundus* was isolated from 1.1% of the *Brachypeplus* individuals and from 0.01% of the *Carpophilus* individuals. This study presents the first record of insects associated with *C. albifundus* and *C. oblonga* and it provides an indication of environmental influences on fungal and insect populations, which could contribute to future disease management.

4.1. INTRODUCTION

Species of *Ceratocystis sensu lato* (*s.l.*) includes many plant pathogens, the majority of which infect trees (Kile 1993). This group of fungi has been known for more than a century with the type species, *Ceratocystis fimbriata* Ellis & Halstead, described in 1890 as the causal agent of black rot of sweet potato (*Ipomoea batatas* L.) (Halsted 1890). *Ceratocystis fimbriata* is widely recognised as representing a complex of cryptic species, some of which have been described recently. Examples of important pathogens in the *C. fimbriata s.l.* species complex are *C. platani* (J.M. Walter) Engelbrecht & T.C. Harrington (Engelbrecht & Harrington 2005), *C. cacaofunesta* Engelbrecht & T.C. Harrington (Engelbrecht & Harrington 2005) and *C. albifundus* Morris, De Beer & M. J. Wingfield (Wingfield *et al*. 1996). Other well-known pathogens accommodated in *Ceratocystis s.l.*, not related to *C. fimbriata s.l.,* include the oak wilt pathogen *C. fagacearum* Bretz (Bretz 1952) and *C. paradoxa* (Dade) Moreau, which causes disease of numerous crops (Kile 1993). There are also numerous species that are best known as agents of sap stain in lumber, or that have not been shown to be pathogenic (Kile 1993).

Ceratocystis spp. are well adapted to being vectored by insects. In this regard, there are two discrete groups in the genus. Those that produce fruity aromas, have casual vectors such as nitidulid beetles (Coleoptera: Nitidulidae) and flies (Diptera) (Moller & DeVay 1968a, Kirisits 2004). A second suite of species, such as *C. polonica* (Siemazko) Moreau and *C. laricicola* Redfern & Minter live in a mutualism with conifer-infesting bark beetles (Coleoptera: Scolytidae) and do not produce fruity

aromas (Harrington & Wingfield 1998). Other than the production of fruity aromas, *Ceratocystis* spp. are well adapted for dispersal by insects, having long ascomatal necks that give rise to sticky masses of spores that stick easily to insect bodies. It is thought that these long necks not only reduce the competition of surrounding fungi by bearing their spore drops above the competing fungi, but that they could also influence the type of insects that vector them (Malloch & Blackwell 1993). An interesting related adaptation is that some species compensate for short perithecial necks by the production of their spore masses in thread-like tendrils (Wingfield 1993).

Aside from the bark beetle associated species, the species of *Ceratocystis* that has been considered most closely in terms of its insect vectors is the oak wilt pathogen *C. fagacearum*. Numerous studies have shown that nitidulid beetles are the primary vectors of this fungus (Gibbs 1980, Gibbs & French 1980, Juzwik & French 1983). Transmission of *C. fagacearum* by nitidulid beetles is significant in overland spread of the fungus and the establishment of new infection centers (Cease & Juzwik 2001). The beetles are attracted to sporulating mats on recently killed oak trees, and after feeding on these mats, they are covered in fungal propagules which they subsequently spread to other trees (Juzwik & French 1983).

Various insects that are associated with *Ceratocystis* spp. either feed on sap or on the fungi themselves. After the insects have been attracted to the fungi by the fruity odors that they produce (Lanza & Palmer 1977), the spores are ingested or they adhere to their bodies. These insects are attracted to the sweet sap associated with fresh wounds on plants and the fungi are thus transferred to a new substrate as in the case of nitidulid beetles transmitting *C. fimbriata* (Moller & DeVay 1968a). There is also

some evidence that the insects play a role in the over wintering of the fungi, that do not persist on wounds for very long (Moller & DeVay 1968a). Insects are further believed to play a significant role in the spermatization of the fungi with which they are associated, as has been shown in the case of *C. fagacearum* (Thompson *et al.* 1955).

Ceratocystis albifundus resides in the *C. fimbriata s.l.* species complex and it causes a serious wilt disease of non-native *A. mearnsii* de Wild in eastern and southern Africa (Morris *et al.* 1993, Roux *et al.* 2005). The fungus was first discovered in South Africa in the 1980's and was initially treated as *C. fimbriata* (Morris *et al.* 1993) until DNA sequence comparisons became available and it was recognized as a discrete taxon (Wingfield *et al.* 1996). Population biology studies, its occurrence on several native African tree families, as well as reports only from Africa suggest that *C. albifundus* is most likely an African fungus (Roux *et al.* 2001, Barnes *et al.* 2005, Roux *et al.* 2007).

Very little is known regarding the biology of *C. albifundus*, despite the fact that it is an important pathogen. Because of its fruity aroma, it has been assumed that it is vectored by insects similar to those associated with other *Ceratocystis* spp. that produce attractive aromas. The aim of this study was to identify possible insect associates of *C. albifundus* in South Africa. Furthermore, we considered the seasonal occurrence of these vectors in a typical native savanna ecosystem.

4.2. MATERIALS AND METHODS

4.2.1. Study areas

Two study areas were selected for this investigation. One was located on the Leeuwfontein Collaborative Nature Reserve (Leeuwfontein) approximately 60km north-east of Pretoria, Gauteng Province, South Africa. This study area included three sites (S 25° 23' 38.3", E 028° 37' 19.5"; S 25° 22' 39.1", E 028° 37' 23" & S 25° 22' 37.2", E 028° 37' 38.2") and is situated in native savannah vegetation. The area was selected because *C. albifundus* has previously been isolated from several native tree species in this reserve (Roux *et al*. 2007) and due to its close proximity to the laboratory.

The second study area, selected for comparison of insect species, was situated ~40 km south-west of Piet Retief, Mpumalanga Province, South Africa (S 26° 58' 68.5", E 030° 54' 28.3"). This study area, consisting of a single site, is within a plantation of non-native *A. mearnsii* trees, between two compartments. The one compartment was six-years-old and the second compartment had recently been clear felled. The two aforementioned study areas are referred to as the "native" and "non-native" study areas.

4.2.2. Traps and bait

Before commencement of the main trials, several different trap and bait types were tested in pilot trials. The different baits tested included fermenting dough, bananas, pineapples and a mixture of all three baits. The traps that were tested included funnel traps, panel traps and a modified pitfall trap (Southwood & Henderson 2000). Based on results of the pilot trials, freshly cut pineapple (1cm^2 blocks) was selected as the

ideal bait and modified pitfall traps were used for the main experiments. These traps (Figure 1) consisted of a removable cup-shaped bottom section (115mm diameter) in which the bait was placed beneath a sieve to prevent the insects from coming into direct contact with the bait. The top section of the trap consisted of a tube (with 3mm diameter holes) fitting into the cup, which allowed insects to enter the tube. The tube was sealed with a lid (Figure 1) to prevent rainwater from entering the trap or the insects from escaping. The traps were fastened to trees at a height of approximately 1.5m with adjustable straps.

4.2.3. Collection of insects from the "Native" study area

Trapping and collection of insects on Leeuwfontein commenced in mid winter, 2005 (30 June), and proceeded over a period of 77 weeks. The bait was replaced and insects were collected weekly throughout the study period. Insects were removed from the traps with an aspirator and transported to the laboratory in individual glass vials in a cool box at ~5°C. Insect specimens were grouped based on morphological characteristics, counted, and representative samples were mounted or preserved in 70% alcohol for identification.

4.2.4. Collecting of insects from "Non-native" study area

Trapping of insects in the plantation of non-native *A. mearnsii* trees near Piet Retief was undertaken to consider the possible insect associations of *C. albifundus* in commercial plantations of non-native trees, specifically to compare to insects obtained in the native ecosystem. Sampling was performed for only a week during summer 2006 (23-26 April). For this portion of the study, the same trap design, bait and trapping protocol as that used for the native study area, was used. The bait was

replaced and insects collected daily for four days. Insect numbers were not calculated for this portion of the study, as the aim here was not to monitor insect numbers or environmental conditions but merely to compare insect species to those in the native habitat.

Insects were also collected from under bark flaps on the stumps of recently felled *A. mearnsii* trees. This made it possible to compare the incidence of fungus/insect associations for insects collected from traps and insects collected from the stumps. This was also done to confirm that the fungal isolation techniques used were effective. Sampling for this part of the study was done during January (summer) 2008.

4.2.5. Presence of fungal propagules on insect bodies

Insect exoskeletons were inspected for the presence of fungal propagules using scanning electron microscopy (SEM). A total of 25 specimens of each of the morphological groups of insects collected from the "native" area were examined. Specimens were dried in self-indicating silica gel for five days. Once the critical drying point (CDP) was reached, specimens were mounted on stubs using double-sided carbon tape. Half of the specimens were mounted on their dorsal sides and the remaining specimens were mounted on their ventral sides. Specimens were coated with gold using a Polaron E5200C sputter coater (Watford, England) and examined using a JSM-840 scanning electron microscope (JEOL, Tokyo, Japan).

4.2.6. Isolation of fungi

Three methods were used to isolate *Ceratocystis* spp. from the collected insects. Equal numbers of insects were used for each of the three isolation methods. For one

third of the insects, the exoskeletons were surface disinfested by submerging the insects in 96% ethanol for 1 min, in undiluted bleach for 1 min and in 70% ethanol for 1 min and thereafter rinsing them in sterile water. Insects were then macerated onto 2% Malt Extract Agar (MEA) (Biolab, Merck, Midrand, South Africa) amended with 100mg/liter (100ppm) Streptomycin sulphate (Sigma, Steinheim, Germany) to inhibit bacterial growth and incubated at 25°C for two to seven days. Plates were then examined using a dissection microscope.

Another third of the insects were killed with forceps and placed directly onto the surface of 2% MEA amended with Streptomycin sulphate with either the dorsal or ventral sides facing the agar surface. Samples were then incubated for two to four days at 25°C after which plates were examined using a dissection microscope. Pure cultures were obtained by making hyphal tip transfers.

With a third of the insects, five to 10 individuals were placed between two carrot discs, approximately 10 mm thick (Moller & DeVay 1968b). The carrot discs were prepared by soaking in distilled water amended with 300mg/liter (300ppm) Streptomycin sulphate for 10min. The carrot discs, containing the insects, were incubated at 25°C for four to seven days. Once ascomata had formed on the carrot surfaces, single spore drops were transferred to 2% MEA amended with 100mg/litre (100ppm) Streptomycin sulphate in order to obtain pure cultures. Only one isolate of the various fungal species were isolated from each individual insect. All isolates obtained in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

4.2.7. Identification of isolates

Ceratocystis isolates obtained from the insects were grouped based on culture morphology after seven days of growth at room temperature on 2% MEA. Isolates were identified based on structural morphology following published descriptions for *Ceratocystis* spp. Fungal structures were mounted on glass slides in lactophenol and examined under a Zeiss Axioskope (Carl Zeiss Ltd., Germany) microscope and images were captured using a HRc Axiocam digital camera and Axiovision 3.1 software (Carl Zeiss Ltd., Germany).

Identifications of the *Ceratocystis* isolates were confirmed by comparing DNA sequences for two isolates of each morphospecies, with those previously published. Three gene regions were used for the DNA sequence comparisons. These were the Internal Transcribed Spacer regions (ITS1, ITS2), including the 5.8S rDNA region, amplified using primers ITS1 and ITS4 (White *et al*. 1990), part of the Beta-tubulin (*β*-tubulin) gene region using primers Bt1a and Bt1b (Glass & Donaldson 1995) and the Transcription Elongation Factor 1α (EF-1α) region using the primers EF1F and EF2R (Jacobs *et al*. 2004). Polymerase chain reaction (PCR), sequencing, purifying, data processing and data analyses protocols were the same as those used by Van Wyk *et al*. (2006).

4.2.8. Statistical analyses of data

Data collected in this study included the number of insects collected per week on Leeuwfontein, the number of fungal isolates obtained from these insects, as well as various climate variables. After insects had been collected and divided into groups based on morphological characteristics, the total number of insects residing in each

morphological group was enumerated. Once fungal isolates had been obtained from the insects and identified, the total number of each species was noted.

Climate data were obtained from the Range and Forage Department, Roodeplaat research station of the Agricultural Research Council (ARC) (S 25° 60' 41", E 028° 35' 42"), approximately 30km from Leeuwfontein. The weather data collected included rainfall (mm/day), daily maximum temperature (°C), daily minimum temperature (°C), wind speed (M/s), maximum daily relative humidity (%) and minimum daily relative humidity (%).

For the purpose of statistical analyses, the data were divided into two sets. One dataset was used to determine which variables influenced the number of fungal isolates obtained during the study period. Data for the number of fungal isolates were transformed into binary values where values below zero were converted to zero and those greater than zero to one. Data were subjected to the stepwise logistic procedure determining the predictive variables using SAS (SAS Version 8.2, 2001).

The second data set was used to determine which climatic variables had an influence on the number of insects collected during the study period. Data were subjected to a stepwise regression on the predictive variables using SAS (SAS Version 8.2, 2001). The significance level for entry of variables into the model was set at 0.05 and the significance level for variables remaining in the model was set at 0.1.

The fungus / insect association was calculated by deviding the total number of fungal isolates (per individual fungul species) by the total number of insects (per individual insect species) collected. This value was converted to a percentage value by multiplying the association level calculated by 100.

4.3. RESULTS

4.3.1. Collection of insects from the "Native" study area

Insects were found in the traps at Leeuwfontein throughout the study period, with 38,262 insects collected over the 77 week study period. A clear seasonal trend was observed in the number of insects collected, with the lowest numbers of insects (4,752) collected in the winter and spring (June 2005 – October 2005 and 22 June 2006 – 26 October 2006) seasons respectively (Figure 2).

At the start of the 2005/2006 summer (mid October), the number of insects increased gradually and these fluctuated over the summer months, gradually decreasing from early May 2006 to stabilize at relatively low numbers in winter 2006 (June – September). A similar trend was observed for the 2006 summer, with the number of insects increasing from the end of October. A total of 33, 510 insects were collected during the summer and autumn months during the study period. In contrast to the summer of 2005/2006, the number of insects collected in the summer of 2006 increased more rapidly at the beginning (October) of the 2006 summer season (Figure 2).

Three main groups of insects were identified based on morphological characteristics. These represented two *Carpophilus* spp. (28,252 insects) and one *Brachypeplus* sp.

(2,041 insects). The two *Carpophilus* spp. were identified as *Ca. bisignatus* Boheman, and *Ca. hemipterus* L. (Figure 3). The *Brachypeplus* sp. was identified as *B. depressus* Erichson (Figure 3). Other insects collected in the traps included Coleoptera, Diptera & Hymenoptera (7969). The numbers of insects collected for each of the two *Carpophilus* spp. differed significantly with *Ca. bisignatus* being more abundant (21,188) than *Ca. hemipterus* (7,064).

4.3.2. Collection of insects from "Non-native" study area

The same three species of nitidulid beetles (*Ca. bisignatus*, *Ca. hemipterus*, and *B. depressus*), collected in the native study area, were found in the *A. mearnsii* plantations. Other insects collected in the traps included Coleoptera and Diptera. Mainly *Ca. hemipterus* (6) and *B. depressus* (23) were collected in 2008 from under bark flaps from the stumps of recently felled trees.

4.3.3. Presence of fungal propagules on insect bodies

Scanning electron microscopy revealed hat shaped ascospores on the dorsal sides of seven insects (Figure 4). These spores had similar morphology to those produced by *Ceratocystis* spp. isolated from the insects, varying from 4µm–6µm in size. Ascospores were observed on two of the 25 *B. depressus* beetles scanned. Of the 50 *Carpophilus* beetles examined, ascospores were observed on only two *Ca. hemipterus* and three *Ca. bisignatus* individuals. The spores occurred singly on the insects and were not abundant. No spores were observed on the ventral sides of the beetles.

4.3.4. Identification of isolates

The *Ceratocystis* spp. obtained in this study represented two species. Some isolates were easily identified as *C. albifundus* based on morphology because this species is the only *Ceratocystis* sp. known to produce ascomata with light-coloured bases. This is in contrast to the second species obtained that had white, fast growing colonies that turned brown with time, with dark coloured ascomatal bases and granular hyphae. It was not possible to accurately distinguish these isolates from closely related species such as *C. savannae* Kamgan-Nkuekam & Jol. Roux (Kamgan *et al*. 2008), necessitating DNA sequencing to confirm their identity.

Analyses of DNA sequences showed that isolates with light coloured ascomatal bases grouped within the *C. albifundus* clade (Bootstrap support of 100%). Isolates that could not be identified based on morphology alone grouped in a clade (Bootstrap support, 98%) with the recently described species, *C. oblonga* R. N. Heath & Jol. Roux (Heath *et al*. 2009), distinct from all other species in the *C. moniliformis s.l.* Hedgcock group.

4.3.5. Association of fungi with insects

Of the three methods used to isolate *Ceratocystis* spp. from the insects, only two yielded positive results. When the insects were surface disinfested, they yielded only bacteria and, therefore, these insect numbers were excluded from the levels of association calculations. *C. oblonga* was isolated from the insects that were placed directly on agar as well as those placed between the carrots, while *C. albifundus* was obtained only using the carrot baiting technique. For the calculation of association levels for *C. albifundus*, only the number of insects placed between carrots was included.

In total, 22 of the 680 (3.2%) *B. depressus* individuals used in the carrot baiting yielded isolates of *C. albifundus*. Two isolates were obtained from 2,354 *Ca. hemipterus* (0.08%) and one isolate was obtained from 7,062 (0.02%) *Ca. bisignatus* individuals. In contrast, 137 of the 18,832 (0.73%) *Carpophilus* spp. yielded *C. oblonga* and only three (0.44%) of the *B. depressus* individuals yielded this fungus. Of the *C. oblonga* isolates obtained from *Carpophilus* spp., 29 isolates were from *Ca. hemipterus* and 108 isolates were from *Ca. bisignatus* (Table 1).

Both the insect numbers and success of fungal isolation showed considerable seasonal fluctuation (Figure 2). *Ceratocystis* spp. were obtained from the insects only during the summer months, with the first isolates obtained in the 22nd wk (24-30 November 2005) of sampling. Successful isolation of *Ceratocystis* spp. ceased in week 48 (1-8 June 2006) of sampling. *Ceratocystis* spp. began to reappear in isolations at the beginning of summer in week 75 (30 November 2006) of the sampling period (Figure 2). A total of 165 *Ceratocystis* isolates were obtained from 30,293 insects during the entire study period. However, as one third of the insects (10098) had been surface sterilized and no fungal isolates were expected from them the effective number of insects from which *Ceratocystis* isolates could be expected was 20,195.

Isolation of *Ceratocystis* spp. from the insects collected from traps in the non-native study area gave similar results to those obtained from the native study site. In this area, *C. oblonga* (11 isolates) was isolated only from *Carpophilus* spp. and all but one of the *C. albifundus* isolates (7) originated from the *Brachypeplus* sp. with one isolate obtained from *Ca. bisignatus*.

The six *Ca. hemipterus* and 23 *B. depressus* collected from under bark flaps of stumps from recently felled *A. mearnsii* trees in 2008 yielded a total of 38 *Ceratocystis* isolates. Six of the *Ca. hemipterus* individuals yielded six *C. oblonga* isolates and three of the same insects gave rise to three *C. albifundus* isolates. Twenty-one *C. oblonga* isolates and eight *C. albifundus* isolates were collected from the 23 *B. depressus* insects collected from under the bark flaps. No *Ca. bisignatus* were obtained from the stumps (Figure 3).

4.3.6. Statistical analyses of data

Analyses of one climate data set, to determine which variables influenced the number of fungal isolates obtained, indicated that there were linear relationships present. Analyses indicated that the number of *C. oblonga* isolates obtained was influenced by minimum daily relative humidity (Chi-Square=10.81, P=0.001, Degrees of freedom=1). The association of predicted probabilities and observed responses for this interaction produced a concordance percentage of 78.4%. Analyses further indicated that the number of *C. albifundus* isolates obtained was influenced by an interaction between wind speed and the number of *B. depressus* collected (Chi-Square=9.31, P=0.0023, Degrees of freedom=1). The association of predicted probabilities and observed responses for this interaction produced a concordance percentage of 95.2%.

Analyses of the second data set, to determine which climatic variables influenced the number of insects collected, showed the presence of linear relationships. With regard to the number of *B. depressus* collected, a number of variables had a linear relation

(F-value=13.64, R-square=0.5426). These included daily maximum temperature, maximum daily relative humidity, daily minimum temperature, minimum daily relative humidity, the interaction between daily maximum temperature and maximum daily relative humidity and the interaction between daily minimum temperature and minimum daily relative humidity. However, only daily maximum temperature (t-value=2.81, P=0.0064, Df=1) and maximum daily relative humidity had significant probability values (t-value=2.73, P=0.0080, Df=1).

With regard to the number of *Carpophilus* spp. collected, only two of the tested variables had a linear relationship (F-value=13.43, R-square=0.4896). These were daily maximum temperature and maximum daily relative humidity. The linear relation between the climatic variable, daily maximum temperature, and the number of insects collected was significant (t-value=4.55, P>0.0001, Df=1). The linear relationship with maximum daily relative humidity identified was, however, not supported by the probability value (t-value=-0.29, P=0.7762, Df=1).

4.4. DISCUSSION

Prior to this study, nothing was known regarding the mode of spread of the wilt pathogen *C. albifundus*. We have thus confirmed that, similar to other *Ceratocystis* spp., *C. albifundus* has associations with nitidulid beetles. Virtually nothing is known of the biology of nitidulid beetles in South Africa and this study was, therefore, also the first to consider the influence of climate on the population numbers of these insects in the country and to identify some of the fungi associated with them. Results

also provide valuable information that might be used to limit spread of the disease of *A. mearnsii* caused by *C. albifundus* in South Africa.

Three insect species were identified as associates of *C. albifundus* and *C. oblonga* collected in this study. These were *B. depressus, Ca. hemipterus* and *Ca. bisignatus*. Very little is known regarding these insects in Africa and none has been reported to be associated with fungi in South Africa. *Brachypeplus depressus* has, however, been reported to be the most common *Brachypeplus* sp. in Africa (Kirejtshuk & Barcley 2007).

Carpophilus spp. have previously been reported as vectors of *Ceratocystis* spp. (Moller & DeVay 1968a, Juzwik & French 1983). *Carpophilus hemipterus* in particular, is a known agricultural pest and has been reported as an associate of *C. fimbriata* (Moller & DeVay 1968a). No evidence could, however, be found of prior associations between fungi and *Ca. bisignatus* and *B. depressus*.

It was interesting that only two species of *Ceratocystis* were isolated from insects in this study. Various other *Ceratocystis* spp. are known to occur on wounds of trees in the areas studied. These include *C. savannae* in the native study area (Kamgan *et al*. 2008), *C. obpyriformis* R.N. Heath & Jol. Roux and *C. polyconidia* R.N. Heath & Jol. Roux in the non-native study area (Heath *et al*. 2009). All of these fungi occur on wounds and are presumably vectored by insects similar to those encountered in this study. The results may suggest that there could be some host specialisation in terms of the species of *Ceratocystis* vectored, or that some level of competition exists between these fungi. Alternatively, these *Ceratocystis* spp. may occur at much lower

levels than *C. albifundus* and *C. oblonga*. Our data could also have been skewed by the low number of *Ceratocystis* spp. recovered from the insects collected in traps.

Ceratocystis oblonga was more commonly isolated from nitidulid beetles than was *C. albifundus*. *Ceratocystis oblonga* is a species recently described from cut stumps of non-native *A. mearnsii* in South Africa (Heath *et al.* 2009). The relatively large numbers of isolates of *C. oblonga* compared to those of *C. albifundus* could be due to differences in the odours produced by the two fungi, with *C. oblonga* being more attractive to the insects than *C. albifundus*. Alternatively, competition between the fungi on the substrate or isolation technique could have influenced these results. *Ceratocystis oblonga* grows fast (83mm in 7d) (Heath *et al.* 2009) compared to the relatively slow growing *C. albifundus* (20mm in 8d) (Wingfield *et al.* 1996) on 2% MEA. This could account for the fact that *C. albifundus* was more frequently isolated from insects subjected to carrot baiting than from insects placed on 2% MEA plates. This study represents the first report of *C. oblonga* from an other organism than *A. mearnsii* and it expands the geographic range of this fungus. It was predominantly isolated from the two *Carpophilus* spp. The study performed in the *A. mearnsii* plantations showed that *C. oblonga* has a percentage association with *Carpophilus* spp. of 100%. A large number of isolates of *C. oblonga* (21) also originated from *B. depressus* (23) collected from *A. mearnsii.*

Differences in isolation success were observed between insects collected in traps compared with those collected from stumps of recently felled *A. mearnsii* trees. Isolations from insects collected from bark flaps resulted in an association of 100% for *C. oblonga* with *Ca. hemipterus* and 91% with *B. depressus*. *Ceratocystis*

albifundus had a 50% association with *Ca. hemipterus* and 35% association with *B. depressus*. These levels of association were noticeably higher than those obtained for *C. albifundus* with *B. depressus* (3.2%) and *Carpophilus* spp. (0.03%) collected in traps in the native study sites. Similar results have been reported in previous studies with *C. fagacearum* where low associations (0.7%) were found between *C. fagacearum* and *Ca. truncatus* Muttay collected from traps in contrast to high association levels (79.8%) obtained from insects collected from fungal mats (Norris 1956, Gibbs 1980, Juzwik & French 1983). The lower recovery rates of *Ceratocystis* spp. from insects collected in traps could be due to the fact that the insects and spores are exposed to harsh environmental conditions during flight possibly reducing the viability and number of spores. In contrast, insects collected from fungal mats would not have been exposed to the same severe environmental conditions. The fact that we isolated the *Ceratocystis* spp. from free flying insects collected in traps, and from insects obtained from fungal mats, clearly shows that these insects play a role in the spread of these fungi.

Climatic factors had a significant influence on the population fluctuation of the nitidulid beetles. Not surprisingly, these insects were much more abundant in the spring and summer months (87%) than in the colder winter months (12%). These observations are similar to those for studies on the transmission of *C. fagacearum* by free-flying and fungus-mat-inhabiting nitidulids in Minnesota (Yount *et al*. 1955, Juzwik & French 1983). In those studies, it was shown that nitidulid beetle numbers are strongly influenced by temperature, with the insects over wintering under the bark of trees, in debris or in rotting fruit on the soil surface during the winter (James *et al*. 1995, Hossain & Williams 2003). Although we did not consider where these insects

might occur during the winter, they most likely occupy similar niches in South Africa to those of the better-studied Northern Hemisphere species.

Climatic factors had an influence of the number of fungal isolates obtained. For *C. oblonga*, minimum relative humidity was the only climatic variable with a significant influence on isolation. Interestingly, the number of *C. albifundus* isolates obtained was influenced by wind speed. This result was unusual as the only instance where winds have been reported to play a role is in the dissemination of fungal spores in the frass of insects (Iton 1960) and it might be incidental and an aberration.

Ceratocystis albifundus infects wounds on trees and results of this study suggest strongly that nitidulid beetles are the primary vectors that move the pathogen from one tree to another. *Acacia mearnsii* trees require corrective pruning to improve growth form after establishment and to correct damage caused by animal grazing (Dunlop & Goodricke 2000). We assume that these wounds are visited by nitidulid beetles that then transmit the pathogen. Given that the insects appear not to be active in the winter months, pruning should be undertaken only during this period to restrict the spread and infection by the fungus.

In this study we showed that *C. albifundus* and *C. oblonga* are involved in a relationship with three nitidulid beetles that most likely act as their vectors. It seems that these relationships are not specific as both fungal species were isolated from more than one insect species. This is not surprising as both the fungi produce fruity odours and are expected to have a loose association with the insect vectors (Himelick & Curl 1958). Vectorship of these insect however needs to be confirmed by further

studying the frequency of contaminated beetles visiting commonly known infection courts, the number of contaminated beetles emerging from diseases material and experimental caging studies. There are at least 11 *Ceratocystis* spp. known from Africa, however, no research regarding the possible insect associations of these species or their role in the biology and possible control strategies of the fungal pathogens have been performed. Further research is required into the possible insect associations of these species on the continent as this could provide valuable information to facilitate management and control strategies.

REFERENCES

Barnes, I., Nakabonge, G., Roux, J., Wingfield, B. D. & Wingfield, M. J. (2005). Comparison of populations of the wilt pathogen *Ceratocystis albifundus* in South Africa and Uganda. *Plant Path*ology **54**: 189– 195.

Bretz, T. W. (1952). The ascigerous stage of the oak wilt fungus. *Phytopathology* **42**: 435-437.

Cease, K. R. & Juzwik, J. (2001). Predominant Nitidulid species (Coleoptera: Nitidulidae) associated with spring oak wilt mats in Minnesota. *Canadian Journal of Forest Research* **31**: 635-643.

Dunlop, R. W. & Goodricke, T. G. (2000). Black Wattle (*Acacia mearnsii*). In D. L. Owen (ed.), South African Forestry Handbook 2000. The South African Institute for Forestry. Menlopark, South Africa.

Engelbrecht C. J. B. & Harrington, T. C. (2005). Intersterility, morphology and taxonomy of *Ceratocystis fimbriata* on sweet potato, cacao and sycamore. *Mycologia* **97:** 57-69.

Gibbs, J. N. (1980). Role of *Ceratocystis piceae* in preventing infection by *Ceratocystis fagacearum* in Minnesota. *Transactions of the British Mycological Society* **74**: 171-174.

Gibbs, J. N. & French, D. W. (1980). The transmission of oak wilt. USDA Forest Service Research Paper, NC-186. North Central Forest Experiment Station, U.S. Department of Agriculture, St. Paul, Minnesota.

Glass, N. L. & Donaldson, G. C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. *Applied Environmental Microbiology* **61**: 1323-1330.

Halsted, B. D. (1890). Some fungous disease of the sweet potato. *Agricultural College Experiment Station Bulletin* **76**: 1-32.

Harrington, T. C. & Wingfield, M. J. (1998). The *Ceratocystis* species on conifers. *Canadian Journal of Botany* **76**: 1446-1457.

Heath, R. N., Meke, G., Mbaga, A., Wingfield, B. D., Wingfield, M. J. & Roux, J. (2009). *Ceratocystis* species on *Acacia mearnsii* and *Eucalyptus* spp. in eastern and southern Africa including six new species. *Fungal Diversity* **34**:(*in press*).

Himelick, E. B. & Curl, E. A. (1958). Transmission of *Ceratocystis fagacearum* by insects and mites. *Plant Disease Reporter* **42**: 538-545.

Hossain, M. S. & Williams, D. G. (2003). Phenology of Carpophilus beetle populations (Coleoptera: Nitidulidae, *Carpophilus* spp.) in fruit dump in Northan Victoria. *Australian Journal for Experimental Agriculture* **43**: 1275-1279.

Iton, E. F. (1960). Studies on a wilt disease of cacao at River Estate. II. Some aspects of wind transmission. In Annual Report on Cacao Research 1959-1960 : pp. 47-58. St. Augustine, Trinidad: Imperial College of Tropical Agriculture, University of the West Indies.

Jacobs, K., Bergdahl, D. R., Wingfield, M. J., Halik, S., Seifert, K. A., Bright, D. E. & Wingfield, B. D. (2004). *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. *Mycological Research* **108**: 411-418**.**

James, D. G., Vogele, B. & Faulder, R. J. (1995). Seasonal abundance of *Carpophilus* spp. (Coleoptera: Nitudulidae) in fallen citrus fruit in the Murrumbidgee Irrigation Area of Southern New South Wales. *Plant Protection Quarterly* **10**: 103-106.

Juzwik, J. & French, D. W. (1983). *Ceratocystis fagacearum* and *C. piceae* on the surface of free-flying and fungus-mat-inhabiting nitidulids. *Phytopathology* **73**: 1164-1168.

Kamgan Nkuekam, G., Jacobs, K., De Beer, Z. W., Wingfield, M. J. & Roux, J. (2008). *Ceratocystis* and *Ophiostoma* species, including three new taxa, associated with wounds on native South African trees. *Fungal Diversity* **29**: 37-59.

Kile, G. A. (1993). Plant diseases caused by species of *Ceratocystis sensu stricto* and *Chalara*. In M. J. Wingfield, K. A. Seifert and J. F. Webber (eds), *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology, and Pathogenicity. 173-183. APS Press, St. Paul, Minnesota.

Kirejtshuk, A. G. & Barclay, M. V. L. (2007). On *Brachypeplus* (*Brachypeplus*) *parallelus* and *B.* (*B.*) *euparallelus* sp. n. from Africa with notes on synonymy in the subgenus *Brachypeplus* (Coleoptera: Nitidulidae: Cillaeinae). Studies and reports of District Museum Prague-East. *Taxonomical Series* **3**: 115-128.

Kirisits, T. (2004). Taxonomy and systematics of bark and ambrosia beetles. In: *Bark and woodboring insects in living trees in Europe, a synthesis.* (Lieutier, F., Day, K. R., Batisttisti, A., Grégoire, J-C, & Evans, H. F. eds.). Kluwer Academic Publishers. The Netherlands.

Lanza, E. & Palmer, J. K. (1977). Biosynthesis of monoterpenes by *Ceratocystis moniliformis*. *Phytochem*istry **16**: 1555-1560.

Malloch D. & Blackwell, M. (1993). In: Wingfield, M. J., K. A. Seifert and J. F. Webber (eds.) *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology, and Pathogenicity. pp 21-25. APS Press, St. Paul, Minnesota.

Moller, W. J. & DeVay, J. E. (1968a). Insect transmission of *Ceratocystis fimbriata* in deciduous fruit orchards. *Phytopathology* **58**: 1499-1507.

Moller W. J. & DeVay, J. E. (1968b). Carrot as a species-selective isolation medium for *Ceratocystis fimbriata*. *Phytopathology* **58**: 123-126.

Morris, M. J., Wingfield, M. J. & De Beer, C. (1993). Gummosis and wilt of *Acacia mearnsii* in South Africa caused by *Ceratocystis fimbriata*. *Plant Pathology* **42**: 814-817.

Norris, D. M. (1956). Association of insects with the oak tree and *Endoconidiophora fagacearum* Bretz. PhD thesis. Iowa State College, Ames. pp. 284.

Rexrode, C. O. & Brown, D. (1983). Oak wilt. Forest Insect and Disease leaflet. 29**.** US Department of Agriculture Forest Service.

Roux, J., Harrington, T. A., Steimel, J. P. & Wingfield, M. J. (2001). Genetic variation in the wattle wilt pathogen *Ceratocystis albofundus*. *Mycoscience* **42**: 327-332.

Roux, J., Heath, R. N., Labuschagne, L., Kamgan Nkuekam, G. & Wingfield, M. J. (2007). Occurrence of the wattle wilt pathogen, *Ceratocystis albifundus* on native South African trees. *Forest Pathology* **37**: 292-302.

Roux, J., Meke, G., Kanyi, B., Mwangi, L., Mbaga, A., Hunter, G. C., Nakabonge, G., Heath, R. N. & Wingfield, M. J. (2005). Diseases of plantation forestry trees species in Eastern and Southern Africa. *South African Journal of Science* **101**: 409-413.

SAS institute (2001). SAS Statistical Software, SAS/STAT User's Guide, Version 8.2, SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA.

Southwood, T. R. E. & Henderson, P. A. (2000). Ecological Methods – 3rd Edition. 213-246pp. Blackwell Science Ltd.

Thompson, N. E., Hadley, B. L. Jr. & Jeffrey, A. R. (1955). Transmission of *Endoconidiophora fagacearum* by spore-infested nitidulids caged on wounded healthy oaks in Pennsylvania. *Plant Disease Reporter* **39**: 58-60.

Van Wyk, M., Van der Merwe, N. A., Roux, J., Wingfield, B. D. & Kamgan Nkuekam, G. & Wingfield, M. J. (2006). Population genetic analyses suggest that the *Eucalyptus* fungal pathogen *Ceratocystis fimbriata* has been introduced into South Africa. *South African Journal of Science* **102**:259-263.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis M. A., D. H. Gelfand, J. J. Sninsky and T. J. White (eds), PCR Protocols: A sequencing guide to methods and applications. pp. 315-322. Academic Press, San Diego

Wingfield, M. J. (1993). Problems in delineating the genus *Ceratocystiopsis*. In Wingfield, M. J., K. A. Seifert and J. F. Webber (eds.) *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology, and Pathogenicity. pp 21-25. APS Press, St. Paul, Minnesota.

Wingfield, M. J., De Beer, C., Visser, C. & Wingfield, B. D. (1996). A new *Ceratocystis* species defined using morphological and ribosomal DNA sequence comparisons. *Systematic and Applied Microbiology* **19**: 191-202.

Yount, W. L., Jeffery, A. R. & Thompson, H. E. (1955). Spores of *Endoconidiophora fagacearum* on the external surface of the body of nitidulids. *Plant Disease Reporter* **39**: 54-57.

Table 1. Association percentages for *Ceratocystis* spp. isolated from three nitidulid species collected from baited pitfall traps in native study area and from under bark flaps on non-native *A. mearnsii* stumps in South Africa.

	Ceratocystis albifundus				*Ceratocystis oblonga*			
	Traps	Nr. of insects assayed	Bark flaps	Nr. of insects assayed	Traps	Nr. of insects assayed	Bark flaps	Nr. of insects assayed
B. depressus	3.2%	680	35%	23	0.44%	680	91%	23
Ca. hemipterus	0.08%	2 354	50%	6	1.2%	2 354	100%	6
Ca. bisignatus	0.02%	7 067	-		1.5%	7 067	-	

Figure 1. Illustration of a section through the trap used in this study.

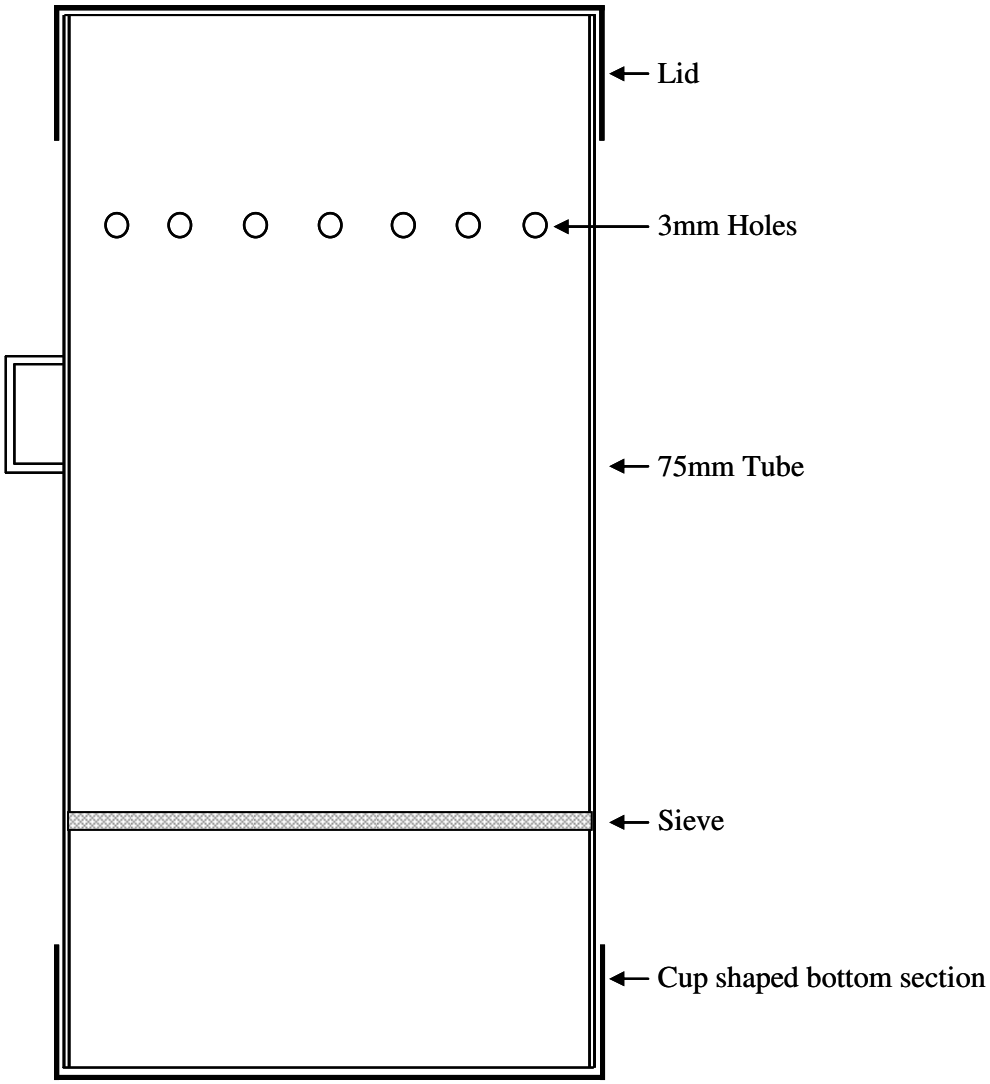

Lid
3mm Holes
75mm Tube
Sieve
Cup shaped bottom section

Figure 2. Graph illustrating the fluctuation of the total numbers of individual insects species and number of *Ceratocystis* isolates collected for the duration of the study.

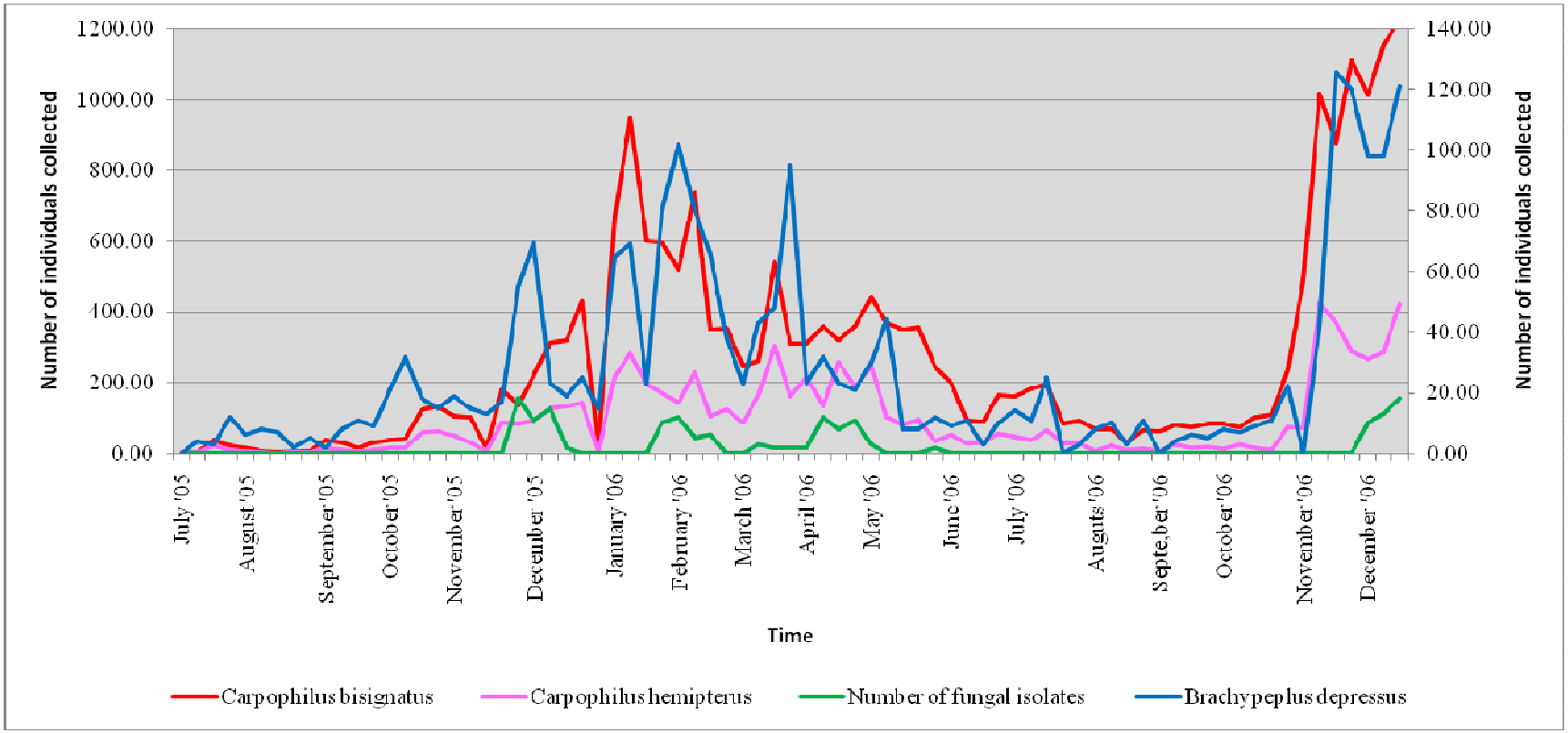

Number of individuals collected
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Number of individuals collected
July '05
August '05
September '05
October '05
November '05
December '05
January '06
February '06
March '06
April '06
May '06
June '06
July '06
Augusts '06
Septe,ber '06
October '06
November '06
December '06
Time
Carpophilus bisignatus
Carpophilus hemipterus
Number of fungal isolates
Brachypeplus depressus

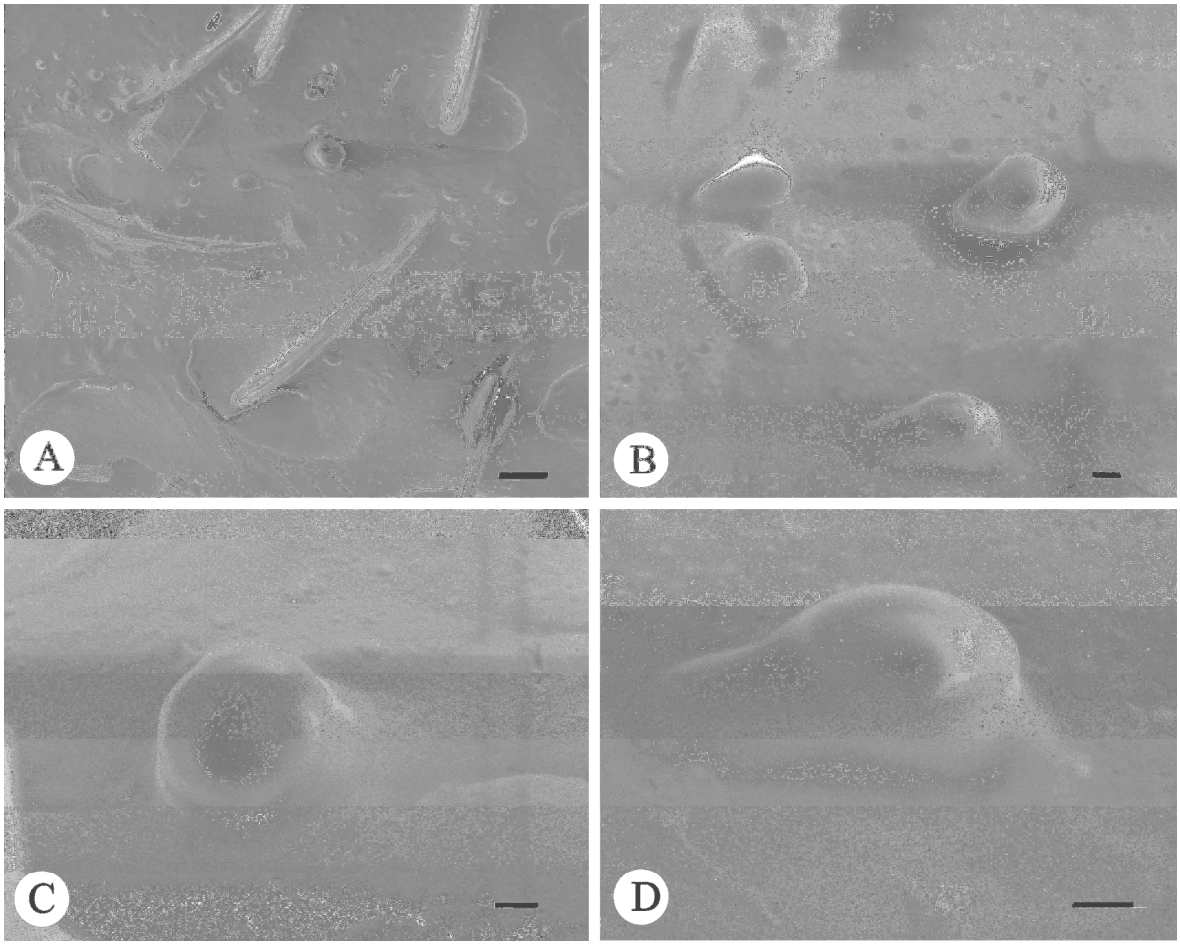
Figure 3. Insects collected from the non-native study site (A) *B. depressus*, (B) *C. hemipterus*, (C) *C. bisignatus*. Scale bar = 1000µm.

A

B

C

Figure 4. Scanning Electron Microscope images illustrating (A) fungal propagules on the insect body, (B) hat-shaped ascospores characteristic of *Ceratocystis* spp. observed on *B. depressus*, (C) hat-shaped ascospores characteristic of *Ceratocystis* spp. observed on *Ca. bisignatus*, (D) hat-shaped ascospores characteristic of *Ceratocystis* spp. observed on *Ca. hemipterus.*


A
B
C
D

Chapter 5

FACTORS INFLUENCING INFECTION OF ACACIA MEARNSII BY THE WILT PATHOGEN CERATOCYSTIS ALBIFUNDUS IN SOUTH AFRICA

ABSTRACT

It is well known that species of *Ceratocystis*, which cause canker and wilt diseases on trees require wounds for infection. In this regard, a number of physical and environmental factors influence the success of wound colonization by these fungi. The aim of this study was to consider the influence of wound age, stem diameter and colonization of wounds by *Ophiostoma quercus* on the success of infection by *Ceratocystis albifundus,* an important wilt pathogen of *Acacia mearnsii* in southern and eastern Africa, under field conditions. This was done by performing controlled inoculations on *A. mearnsii* trees, of different diameters, with *C. albifundus* at different time intervals after wounding and after pre-inoculation of wounds with *O. quercus* at the time of wounding. The success of infection by *C. albifundus* decreased significantly from eight hours after wounding and it was higher on stems of greater diameter. Pre-infection of wounds by *O. quercus* reduced the infection success of *C. albifundus*. The overall results showed that managing wounds created during forestry operations and treating wounds with naturally occurring, non-virulent fungi, such as *O. quercus,* could reduce the prevalence of infections by *C. albifundus*.

5.1. INTRODUCTION

Ceratocystis albifundus De Beer, M.J. Wingf. & Morris (Ascomycetes, Microascales) is the causal agent of Ceratocystis wilt and die-back of non-native plantation-grown *Acacia mearnsii* De Wild. trees in southern and eastern Africa and is considered the most important pathogen of *A. mearnsii* trees in Africa (Morris *et al*. 1993, Wingfield *et al*. 1996 Roux *et al*. 2005). The disease was first reported on *A. mearnsii* in South Africa (Morris *et al.* 1993), but it has subsequently also been recorded from Uganda (Roux & Wingfield 2001), Kenya and Tanzania (Roux *et al.* 2005). Symptoms of infection include gum exudation, wood-discoloration, stem cankers, rapid wilting and tree death (Morris *et al.* 1993, Roux *et al.* 1999). The fungus has been reported from native tree species in Africa, but has not been reported to cause disease on its native hosts (Roux *et al*., 2007).

Ceratocystis spp. require wounds for infection (Norris 1953, Bretz 1952, DeVay *et al*. 1963, Kile 1993). Such wounds can arise from wind (Roux *et al*. 2007), hail damage (Roux *et al*., 1995), growth cracks (Teviotdale & Harper 1991), insect and animal feeding (Walter 1946, Bretz 1952, DeVay *et al*. 1963), harvesting (Teviotdale & Harper 1991, Marin *et al*. 2003) and silvicultural practices such as grafting and pruning (Walter 1946, Teviotdale & Harper 1991, Dunlop & Goodricke 2000). Infection is dependant on a number of physical and environmental factors. For example, species in the *C. fimbriata sensu lato* (*s.l.*) species complex are able to infect their hosts when viable fungal propagules are deposited onto bark wounds (DeVay *et al.* 1968). Other *Ceratocystis* spp., such as *C. fagacearum* (Bretz) Hunt, can only infect if viable fungal propagules are deposited onto freshly exposed wood of the host (Kuntz & Drake 1957). Temporal factors also affect the success of infection by *Ceratocystis* spp. For example, Kuntz & Drake (1957) showed that *C. fagacearum* could not cause infection when wounds were o$lder than 24 hours. Climatic factors such as temperature and relative humidity have also been shown to influence germination of spores and infection by *Ceratocystis* spp. (Cole & Fergus 1956). Gibbs (1980), showed that colonization of wounds by the saprophytic fungus,

Ophiostoma piceae (Munch) H. Sydow & Sydow, prior to infection by *C. fagacearum*, prevented colonization by the pathogen.

Ceratocystis spp. have evolved several strategies to reach wounds and subsequently infect trees. The most common of these is through associations with insects including bark beetles (Coleoptera: Scolytidae) (Webber & Gibbs 1989, Kirisits 2004), nitidulid beetles (Coleoptera: Nitidulidae) (Himelick & Curl 1958, Moller & DeVay 1968, Harrington 1987) and flies (Moller & DeVay 1968, Hinds 1972). These fungi are morphologically adapted to insect dispersal having their ascospores in sticky drops at the apices of long-necked sporocarps (Dowding 1984). Many species of *Ceratocystis*, particularly those not associated with bark beetles (Webber & Gibbs 1989, Kirisits 2004), also produce fruity aromas that attract insects to fungal sporulating structures that subsequently visit fresh wounds on trees. In this regard, *C. albifundus* has recently been reported to be associated with nitidulid beetles (*Carpophilus* spp. and *Brachypeplus* spp.) that visit wounds on native and non-native trees in South Africa (Heath *et al*. 2009).

Infection of *A. mearnsii* by *C. albifundus* has been associated with hail wounds, as well as with pruning wounds on these trees (Roux *et al.* 1995). *Acacia mearnsii* trees typically require corrective pruning to improve growth form after establishment and to correct damage caused by animal grazing (Dunlop & Goodricke 2000). Wounds arising from these operations provide ideal infection sites for *C. albifundus*.

Although selection and breeding for resistance against the disease caused by *C. albifundus* has been promoted, the fungus remains one of the most important pathogens of *A. mearnsii* in South Africa. Therefore, an understanding of the factors involved in the successful infection of wounds by *C. albifundus* could contribute to improved disease management options in plantations. In this study we aimed, 1) to consider the influence of wound age on the success of infection by *C. albifundus* under field conditions, 2) investigated the possible influence of the colonization of wounds by the commonly occurring wound-infecting *Ophiostoma quercus* (Georgév.) Nannf. (De Beer *et al*. 2003) on the success of infection by *C. albifundus* under field

conditions. The third aim of this study was to consider whether stem diameter affects the infection success of *C. albifundus*.

5.2. MATERIALS AND METHODS

5.2.1. Preparation of inoculum

An isolate (CMW4095) of *C. albifundus* collected in 1998 from diseased *A. mearnsii* in South Africa, was selected for artificial inoculations. Only one isolate was used for the study as low variance in virulence between isolates of *C. albifundus* was proven by De Beeer (1994). An *O. quercus* isolate (CMW24164), collected from a four-week-old wound on an *A. mearnsii* stump in 2006, also in South Africa, was selected for tests to consider the effect of pre-infection by this fungus on the infection success of *C. albifundus*. Both isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Pure cultures of the test isolates were grown on 2% (w/v) malt extract agar (MEA) (Biolab, Midrand, South Africa) supplemented with Streptomycin Sulphate (0.001 g/vol, SIGMA, Steinheim, Germany) and incubated at 25ºC under natural day/night conditions for seven days prior to inoculation. Spore suspensions were made by washing spores from the surface of seven-day old cultures, with sterile water. Concentrations of spores were adjusted to 1 x 10^6 spores per ml with sterile water using a Neubauer 1/100 mm haemocytometer (BEOCO, Germany). Suspensions were prepared no longer than ~60 minutes before inoculation and were maintained at ~5ºC prior to application. Viability of the spores in the suspensions was confirmed by plating 1ml of spore suspension onto 2% (w/v) MEA at the time of inoculation and confirming growth after five days.

5.2.2. Wounding and inoculation of trees

The study was conducted in a two-year-old stand of *A. mearnsii* situated 35 km east of Pretoria, South Africa (S 25° 23' 38.3", E 028° 37' 19.5"). The inoculation study was performed using a completely randomised design with regard to treatments as well as the diameter of tree stems. Trees ranging from 10 mm to 32 mm in diameter were

wounded by cutting the stems approximately 50 cm above the soil surface with pruning shears, thereby simulating stem reduction practices applied in silvicultural regimes for *A. mearnsii* cultivation in South Africa (Figure 1a). This method produced a stub with a flush cut exposing the xylem cells of the stems. Inoculations commenced in March 2007 (late summer) and the study was completed in May 2007 (autumn), thus also consistent with the time at which corrective pruning often occurs.

A total of 400 trees were pruned for the experiment. Twenty different treatments were applied, with each treatment applied to 20 trees. The 20 treatments included a water control (negative control), a positive control with *C. albifundus*, a positive control with *O. quercus* and a combination of *C. albifundus* and *O. quercus* inoculated at wounding. Eight treatments included those where *C. albifundus* was inoculated onto wounds two, six, eight, 24, 48 (2 days), 124 (5 days), 196 (8 days) and 346 hours (14 days) after wounding. The remaining eight treatments were fresh wounds which were inoculated with *O. quercus* at the time of wounding, followed by inoculation with *C. albifundus* at two, six, eight, 24, 48 (2 days), 124 (5 days), 196 (8 days) and 346 hours (14 days) after wounding. Inoculations were made by dispensing 2 ml spore suspensions directly onto the wounds using disposable syringes. The entire experiment was repeated once consecutively.

5.2.3. Assessment of infection

The experiments were terminated six weeks after each treatment commenced as this time period has been shown to be sufficient for disease development on trees of similar size under field conditions in South Africa (Roux *et al*. 1999). Inoculated stubs were collected by cutting the stems at the soil surface and transporting the samples in separate plastic bags to the laboratory. In the laboratory, the diameter of each stem was measured and the bark removed. Every inoculated wound was assessed visually for the presence or absence of vascular discoloration. Re-isolations were performed to correlate with visual assessment of *C. albifundus* infection.

5.2.4. Statistical analyses

Statistical analyses were performed using SAS (SAS Version 8.2, 2001). The infection data were converted to binary format with the presence of infection represented by a “1” and the absence of infection represented by “0”. Because this

response variable was binary, a logistic model could be applied to reproduce the logit as a linear model of the predictor variables. The predictor variables were Treatment (a categorical variable with 20 levels), Replicate (a categorical variable with two levels) and Tree Diameter (a continuous variable). The logistic analysis models the variable lin{P/(1-P)}, the logit, as a linear function of the predictor variables, where P is the probability of a specific combination of predictor values to yield an infection.

The significance of each predictor to predict the logit was calculated. The concordance percentage was calculated to serve as a measure of the "goodness of fit" of the applied model. Confidence intervals for the true model parameters were calculated using the estimated parameters and their standard errors. Furthermore, to provide for specific chosen combinations of predictor values (Treatment, Replicates and Tree Diameter), not present in the data, such combinations were added to the dataset while the response variable was absent. This was done to exclude the "spiked" observations when the model was fitted, and to include them when estimated P - values were calculated for the specific added combinations. Finally, 95 % confidence intervals were calculated for the P-values for the "spiked" observations.

5.3. RESULTS

5.3.1. Assessment of infection

The only exterior symptoms observed on inoculated stems, six weeks after inoculation were death of the tips of the stems (Figure 1b) and yellowing of the bark bark and staining of the wood. *Ceratocystis* infection was distinguished from infection by *O. quercus* based on the fact that *C. albifundus* causes light to dark brown streaking (Figure 1d) whereas *O. quercus* resulted in stain, when present, having a distinct blue colour (Figure 1e). The majority (95%) of the 40 negative water controls (Figure 1c) showed no symptoms of *C. albifundus* or *O. quercus* infection. Those control treatments that did show infection (5%) were considered to be due to natural infection since *C. albifundus* naturally occur in the Gauteng Province (Roux *et al.*, 2007). Re-isolation of *C. albifundus* from infected stems followed a similar trend to infection assessed visually (Table 1). The Chi-square probability value showed significant treatment differences within the total dataset ($P < 0.0001$). The predictor variable, replicate, had a significant effect on the analyses ($P = 0.0008$). This could be due to

the trees used in the repeat trial entering the dormancy period due to the change in season (Robinson *et al*., 2004). However, no interaction existed between the predictor variables treatment and replicates (P = 0.4745), therefore, treatment means could be calculated across replicates.

5.3.2.1. Time after wounding

The time after wounding had a significant influence on the infection success of *C. albifundus*. The greatest number of stems with symptoms of *C. albifundus* infection was observed in the 6hr treatment where *C. albifundus* was inoculated alone, with 31 of the 40 trees (77.5%) showing infection. Infection of stems inoculated with *C. albifundus* at the time of wounding, two, six and eight hours after wounding was significantly higher than that of all other treatments ($P \leq 0.0344$). The levels of infection of stems that were inoculated with *C. albifundus* at 124, 196 and 346 hours after wounding were not significantly different from the control treatments for this experiment, nor did they differ significantly from each other (Figure 2).

5.3.2.2. Pre-inoculation with O. quercus

Pre-inoculation of stems with *O. quercus* had a significant influence on the infection success of *C. albifundus*. Stems inoculated with *O. quercus* and *C. albifundus* concurrently at the time of wounding exhibited the highest levels of infection and differed significantly from all other treatments. All other treatments inoculated with *O. quercus* at the time of wounding, and *C. albifundus* at different time intervals thereafter, were significantly different from the control treatment (Figure 3). Pre-inoculation with *O. quercus* at time of wounding and with *C. albifundus* at 196 hours was not significantly different from the control treatments. All other treatments differed significantly from the control treatments.

5.3.2.3. Influence of stem diameter on infection

The diameter of the stems, and thus surface area of the wound exposed to infection, had a significant influence on the infection success of *C. albifundus* ($P = 0.0002$). The analysis of maximum likelihood estimates produced an estimate value of 0.0617 for tree diameter as a predictor variable with one degree of freedom (Figure 4). This indicated that with every 10 mm increase in stem diameter, the probability of infection by *C. albifundus* increased by 6.2%.

5.4. DISCUSSION

Results of this study showed clearly that infection of wounds on *A. mearnsii* by *C. albifundus* is strongly influenced by the condition of the wounds. Wounds became less susceptible to infection over time and after 24 hours they had a very low level of susceptibility. Likewise, pre-inoculation of wounds with *O. quercus* significantly decreased the ability of *C. albifundus* to infect wounds. Potential of infection also increased as the size of the stems increased.

Loss of susceptibility to infection by *C. albifundus* with increasing wound age was not surprising. Similar results have been found in many studies of pathogens, including *C. fimbriata, Leucostoma cincta* and *L. persoonii*, which infect woody plants (Bostock & Middleton 1987, Biggs 1989, Teviotdale & Harper 1991). Specifically in the case of *Ceratocystis*, the results of this study are similar to those on *C. fagacearum* where wounds on oak trees are typically found not to be susceptible to infection after 24 hours (Morris *et al*., 1955, Kuntz & Drake 1957, Gibbs 1980).

The present study did not investigate the possible reasons why wound susceptibility decreases with time. Previous studies attributed loss of susceptibility of wounds to *C. fimbriata* on Almond trees to the fact that the thin film of moisture required on the wound for the germination of spores disappears with time. Other studies have attributed the increase in resistance of wounds to infection with time to the accumulation of an impervious lingo-suberized layer (Biggs 1984, 1985 a, b, 1989, Oven *et al*., 1999, Stobbe *et al*., 2002, Robinson *et al*., 2004). Numerous authors have stated that this layer is responsible for preventing moisture los from the wound as well as preventing fungal infection (Biggs 1992, Woodward 1992). The formation of a necrophylactic periderm has also been reported as a wound response that could prevent fungal infection (Biggs 1985a). It is, however, unlikely that the formation of the necrophylactic periderm is the primary defence against fungal infection as this layer only forms after the impervious lingo-suberized layer has formed (Woodward 1992, Oven & Terelli 1994, Oven *et al*., 1999).

Pre-inoculation of wounds with *O. quercus* had a significant effect on infection with *C. albifundus*. Thus, colonisation of fresh wounds by *O. quercus* approximately two hours prior to infection by *C. albifundus* could reduce the chance of infection by the pathogen significantly. In a similar study, Gibbs (1980) showed that where *O. piceae* was inoculated onto fresh wounds approximately 24 hours prior to *C. fagacearum*, the chance of infection by the pathogen was greatly reduced. *Ophiostoma quercus* was selected for the current study because it is commonly found on wounds on *A. mearnsii* trees (unpublished data) and has not been reported to decrease the value or structural integrity of the timber. Damage caused by sapatain fungi such as *O. quercus* have been reported to be cosmetic (Seifert 1993). Another consideration was that *Ophiostoma* spp. have a biology similar to that of *Ceratocystis* spp. and are also carried to wounds by insects (Brasier 1990, Kirisits 2004). Gibbs (1980), suggested that colonisation of wounds by *O. piceae* may be common in nature, reducing the natural occurrence of oak wilt. The same situation seems to be possible in South Africa in the case of *C. albifundus* infection and the colonisation of wounds with *O. quercus*.

The diameter of the stems inoculated in this study had a significant effect on the infection success of *C. albifundus.* As the stem diameter increased, and thus the size of the exposed wound (horizontal cut) surface area, the probability of infection increased. This could be due to a more rapid drying of the smaller stems compared to the larger stems. The fact that stem diameter has an influence on the infection success of *C. albifundus*, makes the results of this study applicable to the prescribed pruning protocols for *A. mearnsii* trees in South Africa. Current silvicultural prescriptions for the management of *A. mearnsii* suggest that pruning should be undertaken on stems two to four meters tall (Dunlop & Goodricke 2000). If the age at which pruning conducted is reduced, the diameter of the stems would be smaller and infection be less likely.

Successful management of plantation forest diseases relies on a combination of selection and breeding and sound silvicultural practices. The results obtained in this study could assist forestry companies in southern and eastern Africa in formulating silvicultural regimes that could reduce the impact of *C. albifundus* infection and mortality after pruning. The results obtained in this study could also be applied to

better management of numerous tree growing industries. Furthermore, this study also raises the possibility of using *O. quercus* to reduce the infection of recently cut stems by *C. albifundus*. Although this study supports findings obtained in previous studies using other *Ceratocystis* spp., this is the first study of its kind on the African continent and the first to provide possible management prescriptions for the fully regulated forestry management system followed in numerous countries world-wide.

REFERENCES

Biggs, A. R. (1984). Intercellular suberin: Occurrence and detection in tree bark. *International Association of Wood Anatomy Bulletin* **5**: 243-248.

Biggs, A. R. (1985a). Detection of impervious tissue in tree bark with selective histochemistry and fluorescence microscopy. *Stain Technology* **60**: 299-304.

Biggs, A. R. (1985b). Suberised boundary zones and the chronology of wound response in tree bark. *Phytopathology* **75**: 1191-1195.

Biggs, A. R. (1989). Temporal Changes in the infection court after wounding of peach bark and their association with cultivars in infection by *Leucostoma persoonii*. Phytopathology **79**: 627-630.

Biggs, A. R. (1992). Anatomical and physiological responses of bark tissues to mechanical injury. In: Defense mechanisms of woody plants against fungi. Ed. by Blanchette, R. A, Biggs, A, R. Springer Verlag,Berlin.

Bostock, R. M. & Middleton, G. E. (1987). Relation of wound periderm formation to resistance to *Ceratocystis fimbriata* in almond bark. *Phytopathology* **77**: 1174-1180.

Brasier, C. M. (1990). China and the origins of Dutch Elm disease: and appraisal. Plant *pathology* **39**: 5-16.

Bretz, T. W. (1952). The ascigerous stage of the oak wilt fungus. *Phytopathology*. **42**: 435-437.

Cole, H. J. & Fergus, C. L. (1956). Factors associated with germination of Oak wilt fungus spores in wounds. *Phytopathology* **46**: 159-163.

De Beer, C. (1994). Ceratocystis fimbriata with special reference to its occurrence as a pathogen of Acacia mearnsii in South Africa. MSc Thesis. University of the Orange Free State, Bloemfontein, South Africa. Pp 73-89.

De Beer, Z. W., Wingfield, B. D. & Wingfield, M. J. (2003). The *Ophiostoma piceae* complex in the southern hemisphere: a phylogenetic study. *Mycological Research* **107**: 469-476.

DeVay, J. E., English, W. H., Lukezic, F. L., Moller, W. I. & Trujillo, E. E., (1968). Ceratocystis canker of deciduous fruit trees. *Phytopathology* **58**: 949-956.

DeVay, J. E., Lukezic, F. L., English, W. H. & Trujillo, E. E. (1963). *Ceratocystis* canker of stone fruit trees. *Phytopathology* **53**: 873.

Dowding, P. (1984). The evolution of insect-fungus relationships in the primary invasion of forest timber. In: Invertebrate-microbial Interactions (eds. Anderson, J.M., Rayner, A.D.M. and Walton, D.W.H.) Cambridge University Press, New York. In: *Ceratocystis and Ophiostoma;* taxonomy, Ecology and Pathogenicity. Ed. by Wingfield, M.J.; Seifert, K.A.; Webber, J.F. APS Press, St. Paul, Minesota.

Dunlop, R. W. & Goodricke, T. G. (2000). Black Wattle (*Acacia mearnsii*). In: South African Forestry Handbook 2000. The South African Institute for Forestry. (ed. Owen D.L). Menlopark, South Africa.

Gibbs, J. N. (1980). Role of *Ceratocystis piceae* in preventing infection by *Ceratocystis fagacearum* in Minnesota. *Transactions of the British Mycological Society* **74**: 171-174.

Harrington, T. C. (1987). New combinations in *Ophiostoma* and *Ceratocystis* species with *Leptographium* anamorphs. *Mycotaxon* **28**: 39-43.

Heath, R. N., Van Wyk, M., Wingfield, B. D., Wingfield, M. J. & Roux, J. (2009). Insects associated with *Ceratocystis albifundus* in South Africa. *Environmental Entomology* **37**: (in press).

Himelick, E. B. & Curl, E. A. (1958). Transmission of *Ceratocystis fagacearum* by insects and mites. *Plant Disease Reporter* **42**: 538-545.

Hinds, T. E. (1972). Insect transmission of *Ceratocystis* species associated with Aspen cankers. *Phytopathology* **62**: 221-225.

Kile, G. A. (1993). Plant diseases caused by species of *Ceratocystis sensu stricto* and *Chalara*. In: *Ceratocystis and Ophiostoma;* taxonomy, ecology and pathogenicity. Ed. by Wingfield, M.J.; Seifert, K.A.; Webber, J.F. APS Press, St. Paul, Minesota.

Kirisits, T. (2004). Taxonomy and systematics of bark and ambrosia beetles. In: Bark and woodboring insects in living trees in Europe, a synthesis. Ed. by Lieutier, F.; Day, K. R.; Batisttisti, A.; Grégoire, J-C; Evans, H. F. Kluwer Academic Publishers. The Netherlands.

Kuntz, J. E. & Drake, C. R. (1957). Tree wounds and long distance spread of Oak wilt. *Phytopathology* **47**: 22.

Marin, M., Castro, B., Gaitan, A., Preisig, O., Wingfield, B. D. & Wingfield, M. J. (2003). Relationship of *Ceratocystis fimbriata* isolates from Colombian

coffee-growing regions based on molecular data and pathogenicity. *Phytopathology* **151**: 395-405.

Moller, W. J. & DeVay, J. E. (1968). Insect transmission of *Ceratocystis fimbriata* in deciduous fruit orchards. *Phytopathology* **58**: 1499-1507.

Morris, C. L., Thompson, H. E., Hadley, B. L. & Davis, J. M. (1955). Use of radioactive tracer for investigation of the activity pattern of suspected insect vectors of the oak wilt fungus. *Plant Disease Reporter* **39**: 61-63.

Morris, M. J., Wingfield, M. J. & De Beer, C. (1993). Gummosis and wilt of *Acacia mearnsii* in South Africa caused by *Ceratocystis fimbriata*. *Plant Pathology* **42**: 814-817.

Norris, D. N. (1953). Insect transmission of oak wilt in Iowa. *Plant Disease Reporter* **37**: 417-418.

Oven, P. & Torelli, N. (1994). Wound response of the bark in healthy and declining Silver firs (*Abies alba*). *International Association of Wood Anatomy Journal* **15**: 407-415.

Oven, P., Torelli, N., Shortle, W, C. & Zupančič, M. (1999). The formation of a ligni-suberised layer and necrophylactic periderm in beech bark (*Fagus sylvatica* L.). *Flora* **194**: 137-144.

Robinson, R. M., Jensen, G. D. & Morrison, D. J. (2004). Necrophylactic periderm formation in the roots of western larch and Douglas-fir trees infected with *Armillaria ostoyae*. I The response to abiotic wounding in non-infected roots. *Forest Pathology* **34**: 105-118.

Roux, J., Dunlop, R. & Wingfield, M. J. (1999). Susceptibility of elite *Acacia mearnsii* families to the Ceratocystis wilt in South Africa. *Journal for Forest Research* **4**: 187-190.

Roux, J., Heath, R. N., Labuschagne, L., Kamgan Nkuekam, G. & Wingfield, M. J. (2007). Occurrence of the wattle wilt pathogen, *Ceratocystis albifundus* on native South African trees. *Forest Pathology* **37**: 292-302.

Roux, J., Kemp G. H. J. & Wingfield, M. J. (1995). Diseases of Black Wattle in South Africa - A Review. *Suid-Afrikanse Bosboutydskrif* (changed to *South African Journal of Forestry*) **174**: 35-40.

Roux, J., Meke, G., Kanyi, B., Mwangi, L., Mbaga, A., Hunter, G. C., Nakabonge, G., Heath, R. N. & Wingfield, M. J. (2005). Diseases of plantation forestry tree

species in eastern and southern Africa. *South African Journal of Science* **101**: 409-413.

Roux, J. & Wingfield, M. J. (1997). Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa. *Forest Ecology and Management* **99**: 327-336.

Roux, J. & Wingfield, M. J. (2001). First report of Ceratocystis wilt of *Acacia mearnsii* in Uganda. *Plant Disease* **85**: 1029.

SAS Statistical Software (2001). SAS/STAT User's Guide, Version 8.2, SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA.

Seifert, K. A. (1993). Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis*. In: *Ceratocystis and Ophiostoma;* taxonomy, ecology and pathogenicity. Ed. by Wingfield, M. J.; Seifert, K. A.; Webber, J. F. APS Press, St. Paul, Minesota.

Stobbe, H., Schmitt, U., Eckstein, D. & Dujesiefken, D. (2002). Development stages and fine structure of surface callus formed after debarking of living trees (*Tillia* sp.). *Annals of Botany* **89**: 773-782.

Teviotdale, B. L. & Harper, D. H. (1991). Infection of pruning and small bark wounds in almond by *Ceratocystis fimbriata*. *Plant Disease* **75**: 1026-1030.

Walter, J. M. (1946). Canker stain of plane trees. USDA Circular, No. 742.

Webber, J. F. & Gibs J. N. (1989). Insect dissemination of fungal pathogens of trees. In: Insect fungus interactions. 14th Symposium of the Royal Entomological Society of London in collaboration with the British Mycological Society. Ed: by Wilding N.; Collins N.M.; Hammond P.M.; Webber J.F. London Academic Press.

Woodward, S. (1992). Responses of gymnosperm bark tissue to fungal infection. In Defence mechanisms of woody plants against fungi. Ed. by Blanchette, R. A., Biggs, A. R., Springer, Berlin, pp. 62-75.

Wingfield, M. J., De Beer, C., Visser, C. & Wingfield, B. D. (1996). A new *Ceratocystis* species defined using morphological and ribosomal DNA sequence comparisons. *Systematic and Applied Microbiology* **19**: 191-202.

Table 1. Comparison of *C. albifundus* isolated from vascular streaking to visual infection assessed

Treatment	**Number of visual symptoms observed**	**Number of *C. albifundus* isolates obtained**
Water control	2	0
Ceratocystis albifundus, 0 hrs	30	10
Ophiostoma quercus, 0 hrs	15	3
C. albifundus & *O. quercus*, 0 hrs	28	5
C. albifundus, 2 hrs	28	9
O. quercus 0 hrs & *C. albifundus* 2 hrs	15	0
C. albifundus, 6 hrs	31	9
O. quercus 0 hrs & *C. albifundus* 6 hrs	16	1
C. albifundus, 8 hrs	29	5
O. quercus 0 hrs & *C. albifundus* 8 hrs	11	0
C. albifundus, 24 hrs	18	2
O. quercus 0 hrs & *C. albifundus* 24 hrs	9	0
C. albifundus, 48 hrs	9	0
O. quercus 0 hrs & *C. albifundus* 48 hrs	8	0
C. albifundus, 124 hrs	5	0
O. quercus 0 hrs & *C. albifundus* 124 hrs	10	0
C. albifundus, 196 hrs	6	0
O. quercus 0 hrs & *C. albifundus* 196 hrs	5	0
C. albifundus, 346 hrs	3	0
O. quercus 0 hrs & *C. albifundus* 346 hrs	7	0

Figure 1. Wound morphology and symptoms of infection on *A. mearnsii.* (a) typical wound made for the inoculation, (b) external symptoms including dying of the tips of the stems, (c) water control showing no signs of infection, (d) vascular streaking caused by *C. albifundus* (indicated by arrow), (e) vascular streaking caused by *O. quercus* (indicated by arrow) on positive treatment with *O. quercus*..

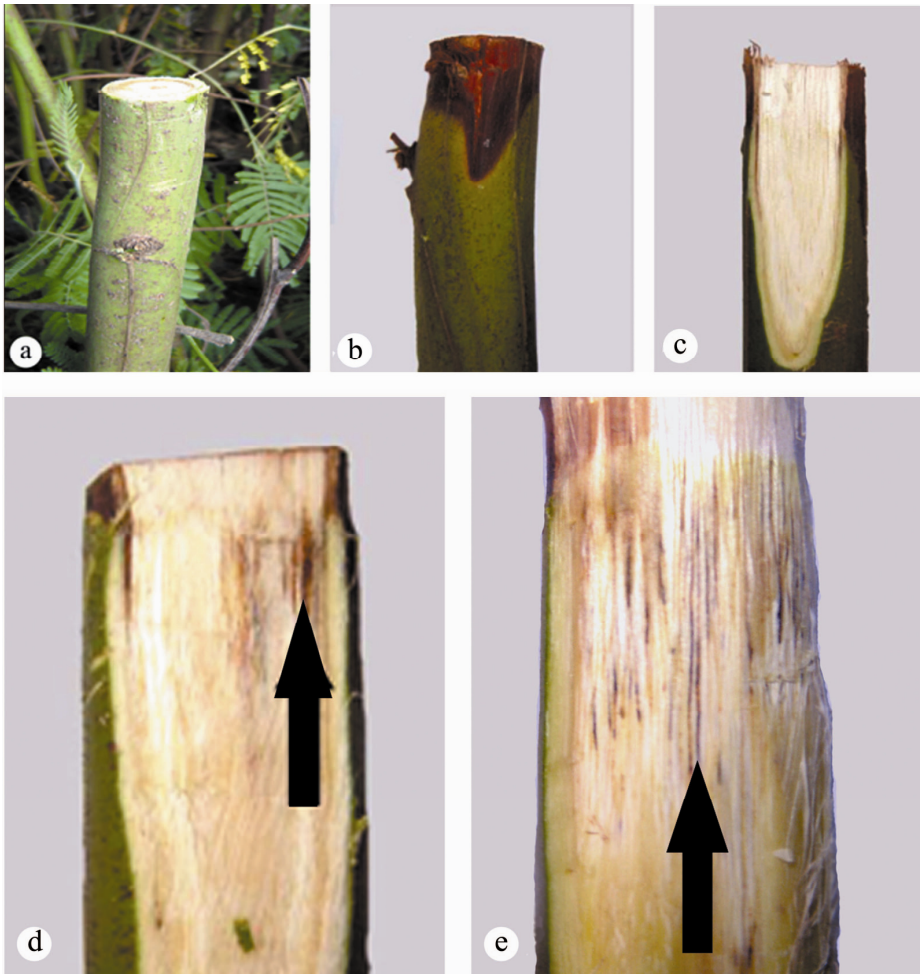

a
b
c
d
e

Figure 2. Number of horizontal stem cuts (wounds) infected with *C. albifundus* assessed visually for each treatment. Different letters above bars indicate treatment results significantly different from each other at a 5 % significance level. Figure shows combined data for both experiments (2x20 trees).

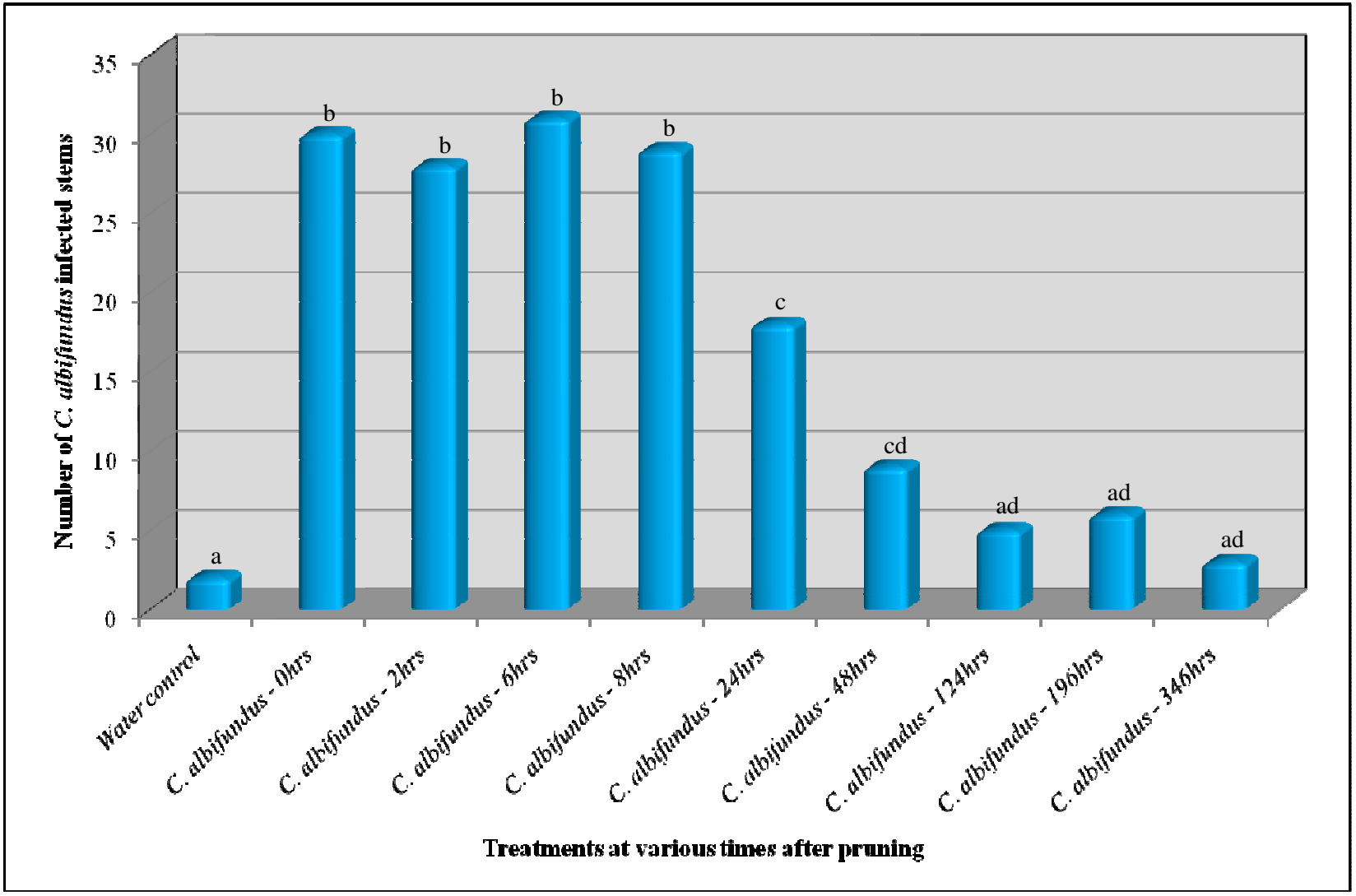

Number of C. albifundus infected stems
35
30
25
20
15
10
5
0
a
b
b
b
b
c
cd
ad
ad
ad
Water control
C. albifundus - 0hrs
C. albifundus - 2hrs
C. albifundus - 6hrs
C. albifundus - 8hrs
C. albifundus - 24hrs
C. albifundus - 48hrs
C. albifundus - 124hrs
C. albifundus - 196hrs
C. albifundus - 346hrs
Treatments at various times after pruning

Figure 3. Number of horizontal stem cuts (wounds) infected with *C. albifundus* assessed visually for each treatment. Different letters above bars indicate treatment results significantly different from each other at a 5 % significance level. Figure shows combined data for both experiments (2x20 trees).

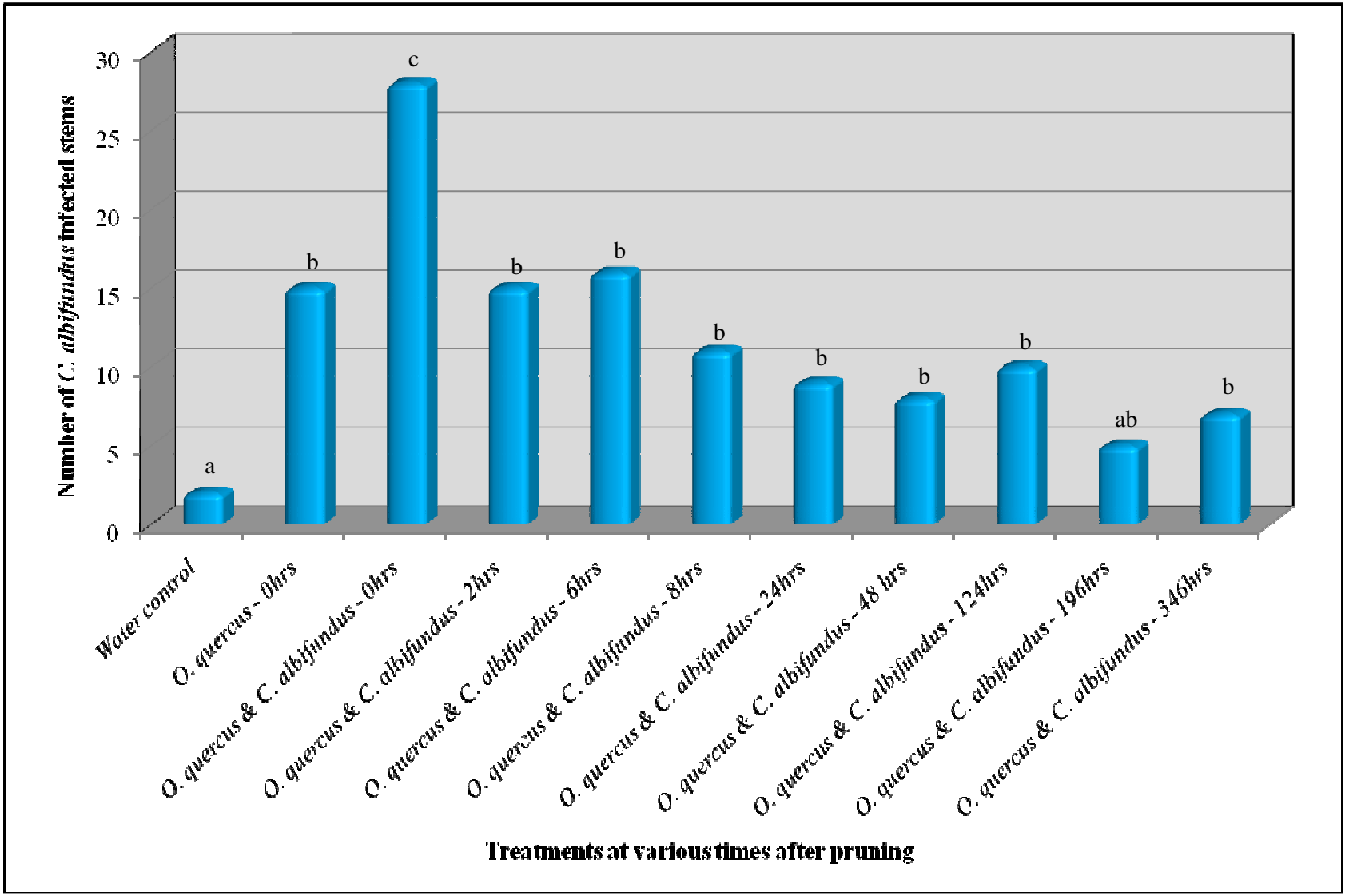
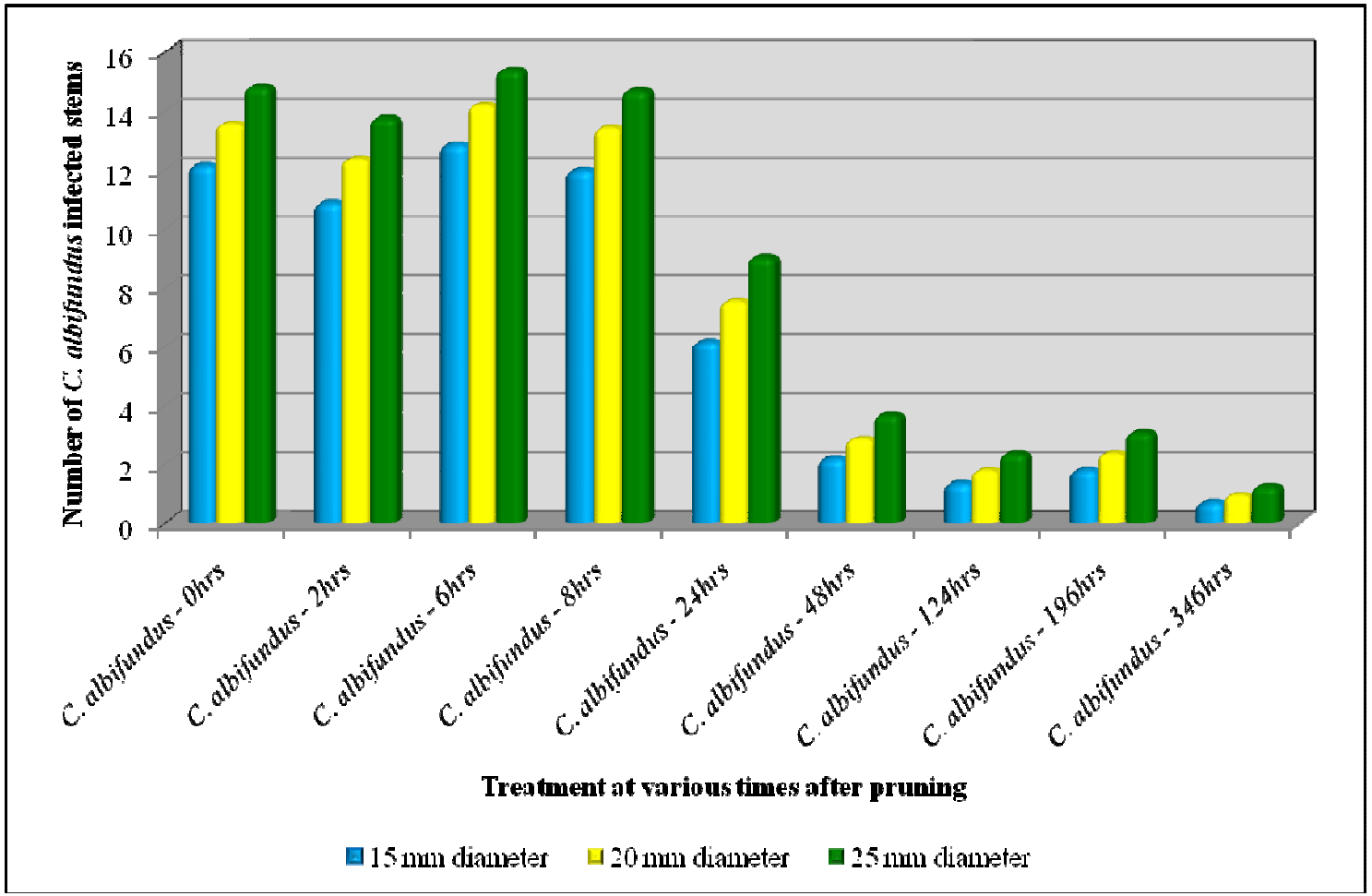

Number of C. albifundus infected stems
30
25
20
15
10
5
0
a
b
c
b
b
b
b
b
b
b
ab
b
Water control
O. quercus - 0hrs
O. quercus & C. albifundus - 0hrs
O. quercus & C. albifundus - 2hrs
O. quercus & C. albifundus - 6hrs
O. quercus & C. albifundus - 8hrs
O. quercus & C. albifundus - 24hrs
O. quercus & C. albifundus - 48 hrs
O. quercus & C. albifundus - 124hrs
O. quercus & C. albifundus - 196hrs
O. quercus & C. albifundus - 346hrs
Treatments at various times after pruning

Figure 4. The effect of diameter on infection success of *C. albifundus* on horizontal stem cuts (wounds) obtained from analysis for specific chosen combinations of the predictor values (Treatment, Replicates and Tree Diameter) at a 5 % significance level obtaining a percentage concordance of 80.5% and Wald Chi-square value of 13.67 and *P* – 0.0002. Figure shows combined data for both experiments (2x20 trees).


Number of C. albifundus infected stems
16
14
12
10
8
6
4
2
0
C. albifundus - 0hrs
C. albifundus - 2hrs
C. albifundus - 6hrs
C. albifundus - 8hrs
C. albifundus - 24hrs
C. albifundus - 48hrs
C. albifundus - 124hrs
C. albifundus - 196hrs
C. albifundus - 346hrs
Treatment at various times after pruning
15 mm diameter
20 mm diameter
25 mm diameter

SUMMARY

The genus *Ceratocystis* includes many important tree pathogens. Some of the well known tree diseases caused by *Ceratocystis* spp. include oak wilt caused by *C. fagacearum* in the USA, Ceratocystis wilt of wattle caused by *C. albifundus* in Africa and canker stain disease of plane trees in Europe caused by *C. platani*. Apart from *C. albifundus,* and the disease it causes on Australian *Acacia* spp. in southern and eastern Africa, relatively little is known regarding the *Ceratocystis* spp. on the African continent. Recent studies have lead to the discovery of several previously undescribed species, as well as a number of new distribution and host reports for *Ceratocystis* spp., especially in South Africa. Many areas of research pertaining to *Ceratocystis* spp., however, still deserve attention in Africa. Studies conducted for this dissertation investigated several aspects pertaining to the *Ceratocystis* spp. occurring on commercially important plantation grown tree species, including the taxonomy, biology and insect associations of the species infecting wounds on *A. mearnsii* and *Eucalyptus* spp.

The first chapter of this dissertation provided a review of the literature pertaining to the biology, ecology and taxonomic history of *Ceratocystis* spp. A strong focus of this chapter was the association of *Ceratocystis* spp. with insects. In this section, examples of known insects associations were mentioned while the levels of association and interaction or interdependence of these associations were discussed. This review highlights the need for research in this field in Africa. To date, only six *Ceratocystis* spp. have been reported from tree species in Africa and no literature is available pertaining to the possible insects associations of these species.

Human population growth is placing increasing pressure on native forests in Africa. To address this problem, extensive plantation forestry programmes, based on non-native tree species, have been established. Pests and diseases, however, are threatening the long term sustainability of these plantations. The threat of host jumps, and the ease with which pathogens move across the globe exasperates this problem. Concern regarding diseases caused by *Ceratocystis* spp. has initiated surveys for this important group of

tree pathogens. Chapter two in this thesis described five previously unknown *Ceratocystis* spp. and one *Thielaviopsis* sp. from four African countries. These species were *C. oblonga*, *C. polyconidia*, *C. obpyriformis*, *C. tanganyicensis*, *C. zombamontana* and *T. ceramica*. As all of these species were able to cause lesions following artificial inoculations on the tree species they were isolated from, they could be pathogenic and pose a threat to these trees in the areas where they are grown. This, however, needs to be tested under field conditions for an extended period of time to determine whether these species are pathogenic or only staining agents. The high number of species described from this single study highlights the need for further research in forestry in Africa.

One of the most serious pathogens threatening non-native *A. mearnsii* trees grown in commercial plantations in Africa is the wilt pathogen, *C. albifundus*. Ceratocystis wilt of wattle commonly affects trees after hail damage, but the fungus is also able to infect through other wounds such as pruning wounds. It has been hypothesised that the fungus is native to the African continent. The discovery of *C. albifundus* on native hosts in Africa enabled us to perform a population study on this fungus using microsatellite markers and for the first time incorporate populations from both native and non-native hosts on the African continent. The results of this chapter support the hypothesis that the fungus has performed a host jump from native hosts to non-native hosts and not the reciprocal. Although results from this study provided some insight into the possible origin of the fungus, it could not provide conclusive proof. Based on the data, it appears that *C. albifundus* originated from a range of native hosts north of South Africa with which it co-evolved. It then established itself on the non-native trees via multiple introductions in a southerly direction.

Ceratocystis spp. are well-known for their insect associations. However, no information regarding this aspect of the biology of *Ceratocystis* spp. was known for Africa. Research conducted for this dissertation identified three insect species in association with *C. albifundus* and the newly described fungus, *C. oblonga*. These were *Brachypeplus depressus*, *Carpophilus hemipterus* and *Ca. bisignatus*. Results show a possible closer association between *C. albifundus* and *B. depressus* and between *C.*

oblonga and *Ca. hemipterus* and *Ca. bisignatus*. However, these fungi form part of a group that are known to have non-specific associations with insects. Both *C. albifundus* and *C. oblonga* could thus be associated with the insects identified in this study. We also provided evidence that climatic factors have an influence on the population size and abundance of the insects associated with the *Ceratocystis* spp. This study not only provided ecological and biological information pertaining to the fungus and the insects, but also provides information that could be applied in the formulation of control strategies to reduce the impact of *C. albifundus* and other *Ceratocystis* spp. in South Africa.

To date, limited control strategies are available for the management of Ceratocystis wilt of *A. mearnsii*. Currently, the only management strategy that is followed is the use of disease tolerant plant families and recommendations to avoid tree wounding as far as possible. Many of the recommendations are based on information from Northern Hemisphere countries and not necessarily the most effective or accurate for Africa. In Chapter five, we investigated the effect wound age and size has on the infection success of *C. albifundus*. We also investigated the influence pre-inoculation of wounds with *Ophiostoma quercus* has on the infection success of *C. albifundus*. Results obtained indicated that both these factors have a significant influence on infection success. The results of this study indicated that infection by *C. albifundus* decreases significantly eight hours after wounding. The study results also indicated that infection of *C. albifundus* increases significantly as the size of the wound increases and that pre-inoculation of wounds with *O. quercus* decreased the infection success of *C. albifundus*.

Research conducted for this dissertation has highlighted the importance of studies focusing on fungal diseases occurring in the commercial plantations of southern and eastern Africa. With a total of six previously undescribed fungal species being reported in this thesis, it makes one question as to what number of fungal species still awaits to be discovered. It also raises the question of how many species on the native flora are able to infect and threaten economically important non-native crops. It has also reiterated the importance of understanding the biology of these fungi and the role this information could play in the formulation of disease control strategies. For the future,

knowledge pertaining to these fungi and their biology in an aim to manage diseases could be achieved by expansive and continued surveys of the commercial plantations. This can only be achieved effectively by the establishment of collaborations between research and forestry institutions and between countries.