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DISTRIBUTION OF *RETROACIZZIA MOPANI* AND ITS NATURAL ENEMIES IN TSHIKUNDAMALEMA AREA, LIMPOPO PROVINCE, SOUTH AFRICA

By

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DECLARATION

I, Rofhiwa Isaac Mmbengeni, hereby declare that the dissertation for the Master of Science in Agriculture degree at the University of Venda, submitted by me, has not been submitted previously for a degree at this or any other University; that it is my own work in design and in execution, and that all reference material contained therein have been duly acknowledged.

Signature (Student):…………………………………… Date:………………………………….

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DEDICATION

I dedicate this research dissertation to God, Lindelani Agnes Mmbengeni, Azwidohwi Makhoshi, Funanani Terresia Mudau, Pretty Takalani Siphuma and Listhani Nemaguvhuni.

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ABSTRACT

Colophospermum mopane (Fabaceae), commonly known as mopane tree, is an indigenous tree species in Southern Africa, often being the dominant species in semi-arid areas. It plays an integral part in the improvement of communities' livelihoods when it is harvested for firewood and mopane worms which are edible insects are collected from the tree. *Colophospermum mopane* is host to many insects, among them *Retroacizzia mopani*, a leaf pest. *Retroacizzia mopani* produces lerps which are protective exudates that shield the insect from predation, but lerps also reduce the photosynthetic area of mopane leaves. This study aimed to determine the distribution of the African mopane psyllid, *R. mopani* and its natural enemies in the Tshikundamalema area, in Limpopo Province. The study assessed the presence of lerps on *C. mopane,* as well as determining the effect of site, tree height, tree density, tree position, branch size and neighbouring trees on *R. mopani* infestations at three sites. Sample of leaves infested with *R. mopani* were collected at six different sites and reared in the laboratory to determine the prevalence of *R. mopani* natural enemies and the levels of parasitism. The site, tree density, tree position, and branch size had an effect on the *R. mopani* infestations. The results showed that as the tree density, tree height and branch size increased, *R. mopani* infestation also increased. Neighbouring trees had no effect on *R. mopani* infestations. Trees found at the edge of the forest were more heavily infested compared to those that were inside the forest. All the three sites had low levels of parasitism by the natural enemy, *Psyllaephagus arytainae* Prinsloo*.* This indicates that *P. arytainae* has little effect on population dynamics of the psyllids.

Key words: *Colophospermum mopane*, insect distribution, natural enemies, parasitism, *Psyllaephagus arytainae*, *Retroacizzia mopani*.

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CHAPTER 1: GENERAL INTRODUCTION

1.1. Background information

Indigenous trees play a fundamental role in improving the livelihoods of many people in rural areas. Many indigenous trees provide shade for both people and livestock. Parts of indigenous trees such as bark, leaves and fruits are used for many purposes. The fruits of indigenous tree+s are eaten raw or used for brewing alcoholic drinks and making products such as hand lotions. In addition, cooking oils are extracted from the fruits of some indigenous trees. Livestock and herbivorous insects also feed on different parts of trees, such as roots, leaves, fruits and flowers. In Tshikundamalema, South Africa, indigenous trees such as *Sclerocarya birrea, Berchemia discolor* and *Colophospermum mopane* are some of the most valued trees for the community because they are used as sources of food and income.

Colophospermum mopane (Benth) J. Léonard, generally known as the mopane tree, belongs to the family Fabaceae/Leguminosae. *Colophospermum mopane* is the only species in the genus *Colophospermum.* It is native in Africa, and has been introduced into India (Orwa *et al*., 2009). In South Africa, the Limpopo and Mpumalanga Provinces, mopane vegetation covers about 23 000 km^2 (Mapaure, 1994). In the Kruger National Park of South Africa, where most of *C. mopane* trees are found, about 50% of the vegetation is *C. mopane* (Fenton, 1983).

Colophospermum mopane plays an integral part in improving the livelihoods of many rural communities. Most people from rural villages rely on the tree for firewood. In some cases, villagers make fences, kraals, structures used for grain storage and houses from the tree (Coates-Palgrave, 1988). The wood is economically valuable and in many markets, it is sold as charcoal for braais (barbecues) (DWAF, 2007). The tree is also used for medicinal purposes (Coates-Palgrave, 1988). Products such as mopane caterpillar and fruits have become commercialized. The caterpillar of the mopane moth, *Imbrasia belina,* is collected from the tree by rural dwellers, dried and sold to subsidise household incomes (Lucas, 2010). There is some knowledge about the utilization of indigenous trees by communal people who rely on these trees for their livelihoods. However, there is no information about biotic factors that affect the productivity of these trees.

Colophospermum mopane is also a host plant for *Retroacizzia mopani* or *Arytiana mopanei,* commonly known as mopane psyllids (Oppong *et al*., 2010). The nymphal stages of *R. mopani* and the adult *R. mopani* feed through sucking the phloem sap from *C. mopane* leaves. *Retroacizzia mopani* psyllid only feeds on *C. mopane.*

Retroacizzia mopani psyllids are very small, usually about 1.5 mm to 4.5 mm and their legs are specifically designed for jumping (Scholtz and Holmes, 2008). Psyllids are monophagous or oligophagous insects because they feed on one or closely related plant species (Scholtz and Holmes, 2008). Both the nymph and adult psyllids feed on the sap in the plant tissue and they feed exclusively on perennial dicotyledonous plants (Scholtz and Holmes, 2008). The symptoms that are produced by psyllids feeding include distortion of the leaves, discolouration and curling of leaves. This leads to a reduction in the photosynthetic area of leaves. The lerps of *R. mopani* cause death of the mesophyll cells on the leaf tissue and where lerps are abundant on a leaf, leaves appear blackish around the edge of the lerps (Oppong *et al.*, 2010).

1.2. Problem statement

Colophospermum mopane is a host plant for *Retroacizzia mopani,* an insect which feeds on the tree. However, *R. mopani* has the potential to severely reduce the leaf area of *C. mopane,* but there is not much work done on the biology and ecology of this species, which is assumed to be the only indigenous lerp in South Africa (Dr. B.P Hurley, pers. comm.).

1.3. Justification

Colophospermum mopane is one of the most widely utilized trees in the Tshikundamalema area. Most people harvest the larvae of *Imbrasia belina* from mopane trees for food and for commercial purposes. The mopane worm is a source of income and food in the Tshikundamalema area, and a psyllid, *Retroacizzia mopani*, has the potential to severely reduce the leaf area of *C. mopane.* The nymphal stage of the psyllid constructs a lerp that seems to protect it from desiccation and predation. The lerps discolour the leaves, and where they occur, the leaves curl up. The presence of psyllids on the trees has the potential to affect the prevalence of the mopane worm and the consequent commercial activities linked to the mopane worm. There is no information on ecological factors such as the effect of site, distance of trees from the open space, closeness of the trees, and tree density on the infestation levels of *R. mopani* on *C. mopane.* In addition, little is known about the prevalence and the parasitism levels of the natural enemies of *R. mopani* on *C. mopane.* This study aimed to assess factors influencing the distribution of the psyllids and their natural enemies at three locations in Tshikundamalema.

1.4. Research questions

- What is the prevalence of *Retroacizzia mopani* and its natural enemies in the Tshikundamalema area?
- What are the factors that affect the infestation levels and distribution of *Retroacizzia mopani* in the Tshikundamalema area?

- Do neighbouring trees influence the *Retroacizzia mopani* infestations levels?

1.5. Aim and objectives

The main aim of this study was to determine the abundance and distribution of *R. mopani* and its natural enemies and the biotic factors affecting these parameters at three sites in Tshikundamalema, Limpopo Province of South Africa.

The objectives were:

- To assess the prevalence of *Retroacizzia mopani* and its natural enemies at three sites in the Tshikundamalema area.
- To assess factors influencing the infestation levels and distribution of *R. mopani* on *C. mopane* in the habitat.
- To determine if neighbouring trees have an effect on *R. mopani* infestation levels.

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CHAPTER 2: LITERATURE REVIEW

2.1. Importance of indigenous trees in ecosystems and the effect of insect pests on trees

Indigenous trees are very important in the ecosystem. This is because trees are a habitat for many insects. In addition, they are a food source for some herbivores. Trees help in improving the organic matter of the soil when they shed their leaves. These leaves act as mulch because the soil gets covered, thus reducing the moisture loss in the soil. Trees also serve as windbreaks for crops, as they protect flowers and fruits on crops from being blown away by heavy winds. They control soil erosion by minimizing the pressure of water on the soil as trees trap the pressure of rains by their enlarged canopies (Reaves, 2001). Trees play an intrinsic role in the purification of air through absorption of carbon dioxide, sulphur dioxide, nitrous oxides and other pollutants (Alliance for Community Trees, 2011). They reduce ozone emissions from vehicles through absorption of carbon dioxide from the atmosphere (Alliance for Community Trees, 2011).

Apart from being important in the ecosystem, there are many essential benefits derived by the communities from the trees. Several parts of indigenous trees, such as bark, leaves and roots are used as medicines. Rural dwellers harvest firewood from indigenous trees. Some indigenous trees are fruit trees and are often the only available fruit source of high nutrients (Mojeremane and Tshwenyane, 2004). Some insects which feed on indigenous trees are edible; for example, mopane worms are harvested by many people in rural communities for food and for commercial purposes.

Insects are diverse in the forest ecosystem and are essential in forest ecosystems. Insects serve as food for wildlife and some insects act as natural enemies of other insects. Insects also interact with trees in several ways: for example, insects such as bees are important for pollinating trees (Laurence, 2010). However, some insects cause tremendous damage to trees through feeding on the various parts of the trees (Hamid, ODell and Katovich, 1997). For example, herbivorous insects pierce and suck many parts of the plants, such as leaves, shoots, roots, buds and flowers, while other insects chew these parts. These interactions interfere with the physiological functioning of the trees. When insects negatively interact with trees, changes in the tree condition occur. The changes that result from the tree-insect interactions can be physical and physiological. For example, a defoliated tree can change its physical structure and its physiology can be affected since there will be loss of leaves, which can lead to a low productivity of flowers or fruits. In addition, insects reduce the growth rate of trees, weaken trees, or kill trees and some are vectors of tree pathogens (Hamid *et al.,* 1997). Moreover, weakened trees are more vulnerable to attack by other diseases. Some pathogens require openings for them to enter the tree and initiate disease and this can occur when insects damage leaves, stems and bark of trees.

2.2. Distribution of *Colophospermum mopane* and factors affecting its occurrence

Colophospermum mopane grows naturally in Democratic Republic of Congo, Angola, Gabon, Botswana, Malawi, Zambia, Mozambique, Namibia, Swaziland, Zimbabwe, South Africa, and Lesotho, and it is an exotic tree species in India (Orwa *et al*., 2009). The native distribution of *C. mopane* is shown in Figure 1.

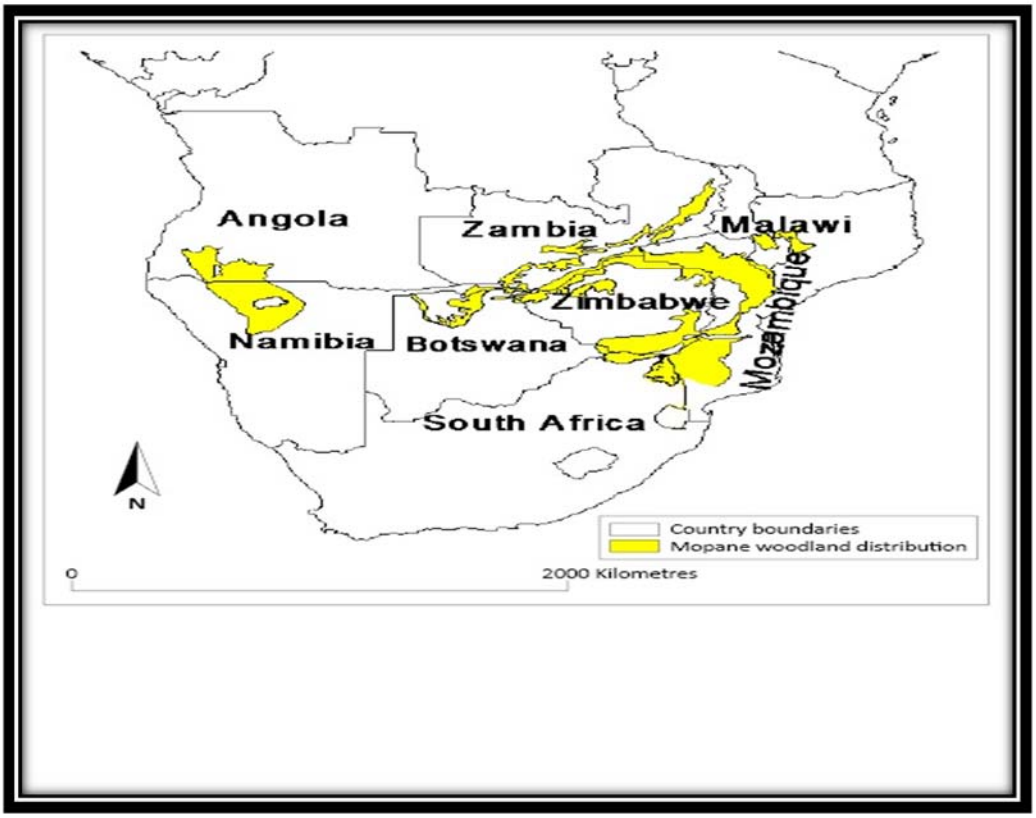

Figure 1. A Map showing the native distribution of *Colophospermum mopane* (Extracted from http://www.abcjournal.org/index.php/ABC/article/view/152/1431 on 20/03/2017)

In Malawi, the area covered by mopane is 9% of the country, 6% in Zambia, 15% in Botswana and 26% in Zimbabwe (Melusi and Mojeremane, 2012). The tree covers an area of almost 555 000 km^2 under woodlands in Southern Africa. In South Africa, it contributes about 50% of the vegetation in the Kruger National Park (Fenton, 1993).

2.3. Description and biology of *Colophospermum mopane*

Colophospermum mopane (Fabaceae) is known as mopanie, butterfly, ironwood, turpentine, balsam, mopane, mopani, mwane, chanye, kapalamamba, lupanye, mpane, sanye, and mophane, depending on the place where it is found (Orwa *et al*., 2009). The tree normally grows 4-18 m high, but sometimes it grows up to 25 m (Orwa *et al.*, 2009) depending on climatic conditions. It usually has one, or sometimes more straight boles, with a diameter of up to 100 cm (Orwa *et al.*, 2009). The bark of the tree is grey to black in colour (Palgrave, 1983). The tree has wide spreading branches (Orwa *et al.*, 2009). The leaves are alternate, compound and drooping, having two leaflets, stipules are ovate, up to 5 mm long (Orwa *et al.*, 2009). Flowers are greenish white to yellow in colour and the flowering occurs from October to March, but in some areas the tree can take many years without flowering (Orwa *et al.,* 2009). The fruit has a flattened pod and contains one seed which almost fills the fruit (Palgrave, 1983). The fruiting occurs from March to June (Orwa *et al*., 2009).

Colophospermum mopane is a species which can tolerate drought and is found mostly in regions characterized by sandy soils (Palgrave, 1983). The geographical distribution of *C. mopane* in Southern Africa seems to be influenced by climatic (Okitsu, 2005) and edaphic factors (O'Connor, 1992). In Southern Africa, the mopane woodland is found in areas where the altitude ranges from 300-1000 m, the mean maximum temperatures in summer of almost 30 ^{o}C and a mean annual rainfall of between 400-700 mm (Palgrave, 1983). It is found over most hot low-lying areas of sub-Saharan Africa, mostly on shallow soils (Orwa *et al.,* 2009). It can also survive under alkaline and poorly-drained soils much better than many tree species (Orwa *et al*., 2009).

2.4. Importance of *Colophospermum mopane* in rural communities

Colophospermum mopane has multiple purposes in rural communities. The leaves provide fodder for many animals, for example, cattle browse on the leaves eagerly and even eat the dry pods off the ground (Palgrave, 1983). In the past, mopane seeds were used by humans as famine food (Palmer and Pitman, 1972). The tree is used for preparing numerous medicines. It can also be used to treat both human and livestock diseases (Palmer and Pitman, 1972; Madzibane and Potgieter, 1999; Mashabane *et al.*, 2001). The fibres from the chewed leaves are used to treat wounds and to stop bleeding wounds (Melusi and Mojeremane, 2012). A decoction of the leaf is used in treating sore eyes and headaches, by dropping of the decoction in the eye and washing the head with the decoction respectively (Roodt, 1998). The bark of the tree is used to treat stomach pains, syphilis, diarrhoea and venereal diseases in South Africa (Roodt, 1998). The VhaVenda in South Africa use the roots to stop gums from bleeding, and twigs are used as chew-sticks for cleaning teeth (Roodt, 1998).

Colophospermum mopane also plays an integral role as food and for commercial activity for many Southern African people, as it hosts the larvae of the moth *Imbrasia belina* which feed on the tree. Rural dwellers harvest the larvae of *I. belina* and use them for food (Kozanayi and Frost, 2002). Some people in South Africa make large profits from selling the mopane worms, which contributes to food security. Many people value the mopane tree because of its wood used for fire (Mashabane *et al.,* 2001). It is also used in the construction of houses, fences, kraals, furniture, hand tools and grain storage structures because it is a hardwood that is resistant to termite attack (Palgrave, 2002). *Colophospermum mopane* wood is also harvested and sold as charcoal (DWAF, 2007).

2.5. Insect pests on *Colophospermum mopane*

There is very little documentation on the diversity of insect pests which affect *Colophospermum mopane*. However, two insect pests have been recorded in literature, namely *Retroacizzia mopani* and *Imbrasia belina. Imbrasia belina* feed almost exclusively on the mopane tree leaves and *R. mopani* feeds by sucking the phloem sap of *C. mopani* (Oppong *et al.,* 2010). *Imbrasia belina* has been recorded as a food source to many rural communities in Southern African countries (Palgrave, 2002). Although it is a pest on *C. mopane,* authors have focused more on its role in the livelihoods of Southern African people than its pest status. According to Oppong *et al.* (2010), the feeding of *R. mopani* on *C. mopani* leaves may reduce the rate of photosynthesis. This implies that *R. mopani* may be a pest if its consumption of leaves affects the growth of a tree. Both *I. belina* and *R. mopani* are potentially important pests on *C. mopane* when there is excessive feeding on leaves, or where they occur in large numbers resulting in excessive defoliation*.*

2.6. Importance of using morphospecies in insect identification

Insects can cause enormous damage to the plants and trees. However, not all insects are pests, because some insects play an essential role in controlling the insect pests in a habitat. It is quite difficult to tell which insect is beneficial and which is a pest. Hence, insect identification is very important. Proper insect identification is important for knowing the exact insect species that is causing either damage to the trees and/or the insect that is beneficial for controlling the insect pest.

Insects are the most diverse and abundant species on earth and have different ways of adapting to environments and surviving (Scholtz and Holmes, 2008). It is estimated that there are over a million species of insects in the world (Scholtz and Holmes, 2008). However, only a few have been identified to species level. The use of parataxomonists or biodiversity technicians has been suggested to reduce this taxonomic impediment. Parataxonomists adopted the use of recognizable taxonomic units also known as morphospecies rather than formally described species (Derraik *et al*., 2010). This is because use of morphospecies in insect identification does not require identification of insects to species level but rather focuses on grouping insects into their morphological characteristics that are easily recognisable

(Barratt *et al*., 2003). Also, the classification of insects to morphospecies plays a critical role in most cases where there is a time and financial burden and in areas where detailed taxonomic information is scarce (Derraik *et al*., 2010).

2.7. Factors influencing insect infestations and abundance of insects in trees

Insect distribution and abundance may be affected by the habitat or vegetation type and how the trees are distributed at a particular site, which can also affect their numbers. In an ecosystem, the landscape structure and quality of a particular habitat may influence the insect species interactions, ecology and behaviour (Tscharntke *et al*., 2002, cited in Steffan-Dewenter *et al.*, 2002). Tree size and density may also affect insect prevalence. However, studies about the effect of tree size and tree density on insect infestations are very limited.

2.7.1 Site, neighbouring trees, surrounding vegetation and tree density

The relationship between trees and insects can be affected by the degree of isolation of habitat as well as the size of that habitat (Summerville and Crist, 2003). According to Debinski *et al*. (2001, cited in Summerville and Crist, 2003), insect species diversity and abundance are influenced by the size of the habitat and the degree of isolation of a particular habitat, implying that insect diversity and abundance increase as the habitat size increases, when the habitat is not isolated.

Insects (particularly specialist insect pests) are more abundant in areas of low plant species diversity (Root, 1973). Herbivorous insect abundances are higher in plant monocultures because specialist insect pests find their host-plants easily and can remain within their habitat (Root, 1973). Root (1973) stated that in monocultures, generalist predators are normally less in abundance than in the area where there is high plant species diversity. This is because in monocultures the effectiveness of general predators which regulate herbivore populations under natural conditions is reduced (Root, 1973).

Artz and Waddington (2006) assessed the effects of neighbouring tree islands on insect pollinator diversity and density, as well as on pollination of a wet prairie species, *Asclepias lanceolata* (Apocynaceae). Their results showed that the number of insect species present and pollinator abundance in the *A. lanceolata* decreased with increasing distance from tree islands. However, the distribution of insects within neighbouring trees may also be affected by the capability of insects flying from a single tree to another. Artz and Waddington (2006) found that there was a higher diversity and abundance of insect pollinators on small scale tree islands than on large scale tree islands.

Neighbouring trees can affect the availability of resources for insect herbivores (Dudt and Shure, 1994). In addition, neighbouring trees can affect insect herbivore communities and damage by modifying that particular microclimate (Corcket *et al*., 2003). There is a possibility that neighbouring trees affect insect herbivores communities through their chemical cues because different tree species have different odours. Interactions between trees, insect herbivores and predators may occur between neighbouring trees or just within plant/tree scale (Gripenberg and Roslin, 2007). Giffard *et al*. (2013) indicated that the interaction between plants, insect herbivores and predators are influenced by the surrounding vegetation. The colonisation of insect herbivores on trees and the foraging behaviour of natural enemies are influenced by neighbouring plants (Giffard *et al*., 2013). Giffard *et al* (2013) investigated the effect of neighbouring plants on insect abundance in uncaged oak seedlings and found that there was a strong indirect effect of neighbouring vegetation on plant-insect-predator interactions. White and Whitham (2000) stated that neighbouring vegetation increases the probabilities of infestations by insect herbivores and when the insect herbivores are high in the neighbouring vegetation, the probability of causing damage to the other neighbouring vegetation increases, too. Neighbouring plants may also decrease infestations on a plant by specialist insects through host-finding disruption (Root, 1973).

The density of plants or trees may affect the insects associated with an individual tree species within a habitat. However, there is very little documentation concerning the effect of tree density on insect infestations. The pine processionary moth (PPM), *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae), is a serious pest of pine trees that defoliates pine leaves in North Africa and Southern Europe (Régolini *et al*., 2014). According to Régolini *et al* (2014), *T. pityocampa* infestation levels depend on stand characteristics. The *T. pityocampa* infestation levels are higher in older stands with low density of trees (Régolini *et al*., 2014). There is a negative association between tree density and levels of insect herbivores. This means that infestation is normally lower at high tree density than at low tree density. According to Geri and Miller (1985, cited in Régolini *et al*., 2014), the chances of an individual tree being attacked by *T. pityocampa* would decrease in denser stands because of dilution process. Insect herbivores may be associated with their host trees through visual cues, such as shape or colour (Régolini *et al*., 2014). Régolini *et al* (2014) also indicated that the chances of *T. pityocampa* infestations depend on perception of insects on the physical or chemical cues of the host tree. However, Davis (1975) showed that as plant density increases, monophagous insect abundance also increases.

2.7.2 Tree size

Insects on trees can be influenced by the ontogeny and growth of their host-tree in many ways (Campos *et al*., 2006). This implies that as the height of a tree increases, changes in microclimate also occur and the insect population densities within that microhabitat increases (Basset *et al.,* 2001). As the height of a tree increases, the more tree branches develop in the canopy and that provides more sites for insects. According to Basset (1992), tall trees produce more fresh leaves than shorter ones; therefore, taller trees may support more herbivorous insects than shorter trees. For Hymnoptera, the numbers of insects such as ants are influenced by tree growth and, consequently, tree growth also influences the availability of food (Davidson *et al*., 2003).

According to Basset (1992) and Basset *et al* (2001), more insect herbivore species are found in the canopy. Campos *et al* (2006) found higher density and higher abundance of insect herbivores on taller trees than on shorter ones. This seems to support the findings that there is a positive relationship between tree size and abundance of insect herbivores. This suggests that the high abundance on taller trees is because taller trees usually have larger canopies which provide insects with a higher quantity of food for insects. In addition, herbivores prefer taller trees because they have more new leaves and these leaves are more nutritious than older leaves (Nichols-Orians, 1991). Insect herbivores on taller trees may easily move from one tree canopy to the next because of the canopy intersection. This is because taller trees normally have bigger canopies than shorter trees.

Anadenanthera macrocarpa (Fabaceae) are mimosa-like timber trees that are native to the Brazil Rain Forest (Campos *et al*., 2006). This perennial tree species can grow up to 40m tall. Their interaction with insects was studied by Campos *et al* (2006) who assessed the effect of tree height on ants and the abundance of insect herbivores that feed on *A. macrocarpa*. The study showed that from all the insects collected from all 30 individual trees of *A. macrocarpa* between 3-40 m tall, the tree height significantly affected the insect abundance. However, tree size does not only affect abundance and species richness of ants and other insect herbivores, it also affects the composition of assemblages of insects that feed on *A. macrocarpa* (Campos *et al.*, 2006). The species richness and abundance of ants and insect herbivores increased with the increase in tree height. According to Campos *et al.* (2006), the size of the crown of the tree also affects the abundance of sap-sucking insects and the abundance of all insect herbivores since bigger crowns usually have more food compared to smaller crowns. Campos *et al* (2006) found that most ants were found on taller trees, while very few of them were found on shorter trees. Likewise, most of insect herbivores species were on tall trees than on shorter trees. The reason tall or larger trees have more insects than shorter trees could be because of their greater productivity and structural complexity, and more available and diverse food resources (Price, 1992).

The effect of tree size on insect infestation levels was also observed on pine trees. *Thaumetopoea pityocampa* attacked both the shorter pine trees and taller trees in the forest. It was found that regardless of where trees are located within the stand, the chances of trees being infested by *T. pityocampa* increased as the tree diameter increased (Régolini *et al*., 2014). There is also a strong correlation between tree size and insects as *T. pityocampa* infestations were higher on taller trees than on shorter ones (Régolini *et al*., 2014).

Stigmacoccus garmilleri Foldi (Hemiptera: Margarodidae) is a scale insect which feeds on oak trees (*Quercus* spp.) in highland forests of Veracruz, Mexico. The distribution of *S. garmilleri* infestations and any distinction in honeydew production at different tree heights of *Quercus* spp. showed that higher densities of the scale insects and infestation levels were higher in taller trees than in shorter ones (Gamper *et al.,* 2011).

2.7.3 Position of tree within a habitat

The distance of trees from the open space (tree positions) can influence insect infestations. However, there is little documentation about how tree position affects the infestation levels of insects. According to Dulaurent *et al*. (2012), the position of trees affected *T. pityocampa* infestation levels. Their study showed that higher infestations of *T. pitycompa* were at the pine stand edges compared to pine trees within the stand. This shows that the pine trees at the edge of the habitat are more vulnerable to attack by *T. pitycompa.* Similar observations were found in another study which showed that trees situated within the edge of the stands are highly infested compared to trees located within the centre of the stand (Régolini *et al*., 2014). Kautz *et al*. (2013) also indicated that other forest insects such as the bark beetle *Ips typographus,* have higher rates of infestations at spruce forest edges.

2.8. General biology and description of psyllids

The Psylloidea are small insects in the order Hemiptera (SubOrder Homoptera), normally 1.5 mm to 4.5 mm long, rather similar to miniature cicadas in appearance and are usually known as ‘jumping plant lice’ (Scholtz and Holmes, 2008). White and Hodkinson (1985) defined them as belonging to six families but recently some studies show that they are classified in eight Families (Aphalaridae, Carsidaridae, Calohyidae, Homotomidae, Liviidae, Phacopteronidae, Pysllidae and Triozidae) (Burckhardt and Ouvrard, 2012).

Psyllids reproduce sexually and are oviparous (Scholtz, 1986; cited in Oppong *et al* 2009). The adult psyllids are active jumpers but can fly for a very short distance on the plants (Scholtz and Holmes, 2008). Female psyllids can lay up to 500 eggs (Oppong *et al.,* 2006) and have incomplete metamorphosis. The development from eggs to adults takes seven stages, which are the eggs, five nymphal instars and the adult. The eggs are laid in masses, either superficially or embedded in clusters or singly (Oppong *et al*., 2009). When laid in clusters, they are usually found in rows, circles or scattered (Dreistadt *et al*., 1999; cited in Oppong *et al.,* 2009). In some psyllid species like *R. mopani*, eggs can be seen as black spots on the leaflets, and their size ranges from 250-360 μm long and 100-125 μm wide (Oppong *et al*., 2009). The eggs of *R. mopani* are characterized by a cone shape with a reticulate-sculptured membrane surrounding them and are black in colour (Oppong *et al*., 2006). *Retroacizzia mopani* females lay eggs on both mature and senescent leaves between July and September, when temperatures are between 11.4 °C and 23.5 °C (Oppong *et al*., 2009).

The nymphs of psyllids construct lerps that are attached to the leaf and it is presumed that the lerps protect the psyllids from natural enemies and desiccation (Oppong *et al.,* 2010). Many psyllids feed exclusively on perennial dicotyledonous plants and are classified as monophagous or oligophagous. The hind legs of psyllids are well-developed for jumping and the forewings are leathery and patterned, and generally transparent (Scholtz and Holmes, 2008). The adults normally do not move when not feeding but are very active jumpers when disturbed (Oppong *et al.,* 2010). The life-span of an adult psyllid takes between five to eight days in the laboratory (Oppong *et al*., 2006).

Few studies have been done on the biology and ecology of psyllids in ecosystems. Acacia psyllids, also called acacia suckers, *Acizzia uncatoides* (Hemiptera: Psyllidae), attack tree species from Fabaceae (acacia species) and are devastating pests of ornamental mimosas and *Albizia* species (Malumphy *et al*., 2007). This species forms lerps and feeds on the leaves and stems (Malumphy *et al.*, 2007). The *Acizzia uncatoides* eggs are almost 0.25mm long with a yellow to orange colour and are laid both individually and in groups on the underside of leaves and on buds (Malumphy *et al.*, 2007). *Acizzia uncatoides* eggs hatch and develop through about five nymphal growth stages before maturing into winged adults (Malumphy *et al.*, 2007). Younger *A. uncatoides* nymphs have a creamy yellow colour with reddish eyes but the older nymphs are yellow to orange or greenish to yellow on the head, and have brown wing pads and abdomens, whilst the adults are brown and greenish to dark brown in colour and 2-3 mm long (Malumphy *et al.*, 2007). In Italy, up to eight overlapping generations were recorded on acacia species in a year (Malumphy *et al.*, 2007). *Acizzia uncatoides* forms dense colonies on the growing tips of acacia species resulting in chlorosis, defoliation and die back (Malumphy *et al.*, 2007). *Acizzia uncatoides* also causes poor growth of the plant. Both adult and nymphs excrete large quantities of honeydew on the plant, and this can affect the growth and development of the acacia plant (Malumphy *et al.*, 2007).

There are many psyllids species that are associated with different tree species other than *Acizzia uncatoides*. These include *Platycorypha nigriviga, Eucalyptolyma maideni, Glycaspis brimblecombei, Cacopsylla tobirae, Trioza eugeniae,* and *Diaphorina citri*. All these psyllids produce sticky lerps (Kabashima *et al.*, 2014) and they are pests to important trees, such as, eucalyptus and citrus trees. Studies have shown that most of these psyllids cause common damages to the trees, such as distortion of terminals or shoots and premature defoliation (Kabashima *et al.*, 2014).

2.8.1. Distribution of the *Retroacizzia mopani* eggs and lerps on *Colophospermum mopane* leaves

Life cycle of many psyllids species have been studied, most importantly, those species that are involved in reducing the productivity of trees, for example *Eucalyptus* species and on agricultural crops grown specifically for commercial purposes. Very little has been published on the biology of *Retroacizzia mopani* (Homoptera: Psyllidae) (synonym: *Arytaina mopanie*), a pest of *Colophospermum mopane* (Oppong *et al*., 2009; Oppong *et al.,* 2010) probably because *R. mopani* is not a serious pest on crops of agricultural importance (Hodkinson, 1974 cited in Oppong *et al*., 2009).

According to Oppong *et al* (2009), *R. mopani* eggs are laid in clusters on leaflets, which might imply that more than one *R. mopani* female uses the same leaflets for oviposition and a lot of eggs are laid on the mopane leaves. After the egg hatching, the first nymphal stage searches for a feeding site on the host plant where it pierces the phloem tissue with its proboscis (Oppong *et al.,* 2010). Immediately when the nymphs start feeding, they begin to construct lerps covering their entire body and this first nymphal stage is normally found on the secondary leaf veins (Oppong *et al.,* 2010). The second nymphal stage is between the secondary veins and this is usually after their lerps have opened. Third, fourth and fifth nymphal stages are found on and along the main leaf veins, then *R. mopani* exits the lerps when it is an adult.

During the lerp construction of *R. mopani,* lerps are placed at various positions depending on the developmental stage of the nymphs (Oppong *et al*., 2010). Lerps are mostly positioned along leaf veins, where *R. mopani* nymphs usually remain when feeding (Oppong *et al*., 2010). Oppong *et al* (2010) found that *R. mopani* lerps are more on adaxial leaf surface than on the abaxial surface; however, they further stated that the difference was not significant. The lerps of *R. mopani* are mostly glued at one end to the surface of leaflet and loose at the opposite end (Oppong *et al*., 2010).

2.8.2. Damage caused by *Retroacizzia mopani* on *Colophospermum mopane*

The nymphs cause damage on the leaves through feeding on the phloem sap (Ernst and Sekhwela, 1987; cited in Oppong *et al.,* 2009), and this leads to black and reddish-brown spots on the surface leaf area (Oppong *et al.*, 2006). The feeding of the psyllid causes twisting, curling of young leaves and the death of new shoots (Grafton-Cardwell *et al*., 2006). Van Wyk (1972, cited in Sekhwela, 1989) reported that lerps can cover a large area on the leaflets surface when the infestation is very high. This can result in a reduction of light penetration in the leaves. Urquhart and Stone (1995, cited in Oppong *et al.,* 2010), also affirm that this damage may play a major role in limiting the growth of the plant since leaf curling and discolouration can limit the rate of photosynthesis. *Retroacizzia mopani* nymphs and lerps cause significant damage on the leaflets. Both nymphs and lerps appear black or reddish brown showing some patches around the feeding sites (Oppong *et al*., 2010). *Retroacizzia mopani* lerps also show black sooty mould on *C. mopane* leaves covering the whole lerp (Oppong *et al*., 2010). This lerp covering can also reduce the rate of photosynthesis.

2.9. Natural enemies of psyllids species belonging to the Psyllidae family

In the ecosystem, populations of insect pests are regulated naturally by natural enemies in their habitats. Some of the enemies include parasitic wasps, parasitoids, parasites and predators. *Cacopsylla pyri* is a pest of pear trees in Europe. Due to its pest status on pears it has been exposed to biological control by the psyllid special predator *Anthocoris nemoralis* (F.) (Hemiptera: Anthocoridae) (Erler, 2004). General predators in pear orchards include ants, spiders and mirids (*Pilophorus gallicus*). Natural enemies such as spiders and mirids (*P. gallicus*) are expected to have the greatest impact on the regulation of the population of *C. pryi* (Sanchez and Ortin-Angulo, 2012). Some insect species responsible for lowering the population of *C. pyri* are the parasitoid *Trechnites insidiosus* (Sanchez and Ortin-Angulo, 2012).

The common natural enemies of many psyllids species are tiny parasitic wasps that belong to the Order Hymenoptera (Kabashima *et al.*, 2014). Many studies on psyllids were conducted on *Eucalyptus* species, perhaps because *Eucalyptus* species are normally grown for commercial purposes. Literature has shown that most of the species of the eucalypt psyllids are normally controlled by *Psyllaephagus* species (Hymenoptera: Encyrtidae); for example, the control of *Eucalyptolyma maideni,* a pest of *Eucalyptus citriodora* and *Eucalyptus maculata* is *Psyllaephagus parvus*; and for *Ctenarytaina eucalypti* is *Psyllaephagus pilosus* (Kabashima *et al.*, 2014). Hence many psyllids seem to be affected by *Psyllaephagus* species.

Very little is known on parasitoids associated with *R. mopani*. The natural enemies of *R. mopani* that are known are predators such as the larvae of the brown lacewing (Neuroptera:

Hemerobiidae), *Hyperolius marmoratus taeniatus*, coccinellid beetle and ants (Oppong *et al.*, 2006).

2.9.1. Predators of psyllids

Predators are zoophagous insects that hunt, catch, kill and eat insects at their various stages of growth, and are often larger and stronger than their prey. Insect predators are carnivorous. During their life time from the immature stage to maturity they consume more than one prey insect (Underwood, 2005). According to Underwood (2005), carnivorous insect species may differ in stages as some are only predatory at the immature stages (such as Neuroptera: Chrysopidae; Diptera: Culicidae), others are predators as adults (Coleoptera: Scarabidae such as *Cremastocheilus sp.*; Hymenoptera: Tiphiidae), and others are predators throughout their entire life cycle. Almost every insect order has a predatory species. However, some insect orders are virtually completely predatory, for example Neuroptera and Odonata (Underwood, 2005). Some predators such as *Olla vincetoxicum-nigrum, Curinus coerinus coeleus, Harmonia axyridis,* and *Cycloneda sanguinea* and the lacewings (*Ceraeochrysa sp.* and *Chrysoperla sp.*) are common predators that prey on psyllid colonies (Qureshi and Stansly, 2012). The acacia psyllid population on acacia species were reported to decrease in June-July due to predation by pirate bug species (*Anthocoris nemoralis*), lady beetle (*Diomus pumilio*) and many other predators, including a brown lace wing, *Hemerobius sp.* (Paine and Dreistadt, 2007).

2.9.2. Parasitoids on psyllids

Over 100 000 insect species have been described in the Hymenoptera (Scholtz and Holmes, 2008), with Encyrtidae reported to contain endoparasitoids of many insects. Encyrtids prefer to feed on homopteran insects and most of the encyrtids are known to regulate the population of scale insects, mealybugs and other related insects from the suborder Homoptera. The blue-gum psyllid population is reduced by a tiny parasitic wasp (*Psyllaephagus pilosus*) and this parasitic wasp only attacks the blue gum psyllid (Paine and Dreistadt, 2007).

2.9.2.1. Tamarixia radiata

Some psyllids are vectors of disease. *Diaphorina citri* Kuwayama (Asian citrus psyllid) (Homoptera: Psyllidae), a pest of citrus, affects the growth of trees and eventually the infested trees may die. *Diaphorina citri* transmits *Liberibacter asiaticus*, a bacterium that causes greening disease in citrus (Garnier *et al*., 2000; Huang *et al*., 1984). In some countries such as Reunion Island and Taiwan, *Tamarixia radiata* was found to be a potential parasitoid that dramatically reduces populations of *D. citri* (Chien and Chu, 1996; cited in Garnier *et al.,* 2000). *Tamarixia radiata* (Hymenoptera: Eulophidae) is a common ectoparasitoid that attacks fifth-instar *Diaphorina citri* (Psyllidae), a serious insect pest of citrus trees (Chien *et al.,* 1991). *Tamarixia radiata* is native to northern India and is considered as a special parasitoid (Zuparko *et al.,* 2011). Adult *T. radiata* are black with widely separated eyes (Onagbola *et al*., 2009). Male *T. radiata* are smaller than the female in length and wing area and the adult female is tiny and normally lays a single egg, occasionally two, underneath of the nymphal stages of *D. citri* (Grafton-Cardwell and Daugherty, 2013). It can deposit as many as 300 eggs (Hoy *et al.,* 2006). When the *T. radiata* eggs hatch, the larvae eventually kill its host, the *D. citri* nymph. According to Hoy *et al.* (2006), the size of instars of the *T. radiata* ranges from first instar to the fourth instar (0.28 mm to 1.14 mm long, and 0.11 mm to 0.59 mm wide). The pupation occurs within the mummified *D. citri* nymph, then an adult *T. radiata* emerges from the thorax or head of the mummified *D. citri* nymph (Hoy *et al.,* 2006).

Female *T. radiata* parasitizes the *D. citri* younger nymphs (Skelley and Hoy, 2004), and may obtain protein for the development of their eggs by feeding on haemolymph from *D. citri* nymphs, which is extracted via ovipositor-induced punctures (Hoy *et al.,* 2006). Each female *T. radiata* is capable of killing over 500 *D. citri* through host parasitism and feeding when females insert their ovipositor into the psyllid to make a hole and then suck up the liquid that oozes out (Hoy and Nguyen, 2001). In Pakistan, the species (*T. radiata*) was reported to have very high parasitism rate, and because of such high levels of parasitism on *Diaphorina citri*, it was then introduced to several countries, among them the United States and Brazil (Pluke *et al*., 2008; León and Setamou, 2010).

2.9.2.2. Psyllaephagus species

Psyllaephagus (Hymenoptera: Encyrtidae) is a diverse genus having more than 200 described species and it may have over 1000 species in total (Noyes and Hanson, 1996). Most of the species from this genus are endemic to Australia. Almost all species in the genus *Psyllaephagus* are parasitoids that attack and kill the nymphs of species within the superfamily Psylloidea and just a few of them are hyperparasitoids that prey on other primary *Psyllaephagus* species (Noyes and Hanson, 1996). *Psyllaephagus* species are often found on species of *Eucalyptus* (Myrtaceae). Because of their effectiveness and being potential parasitoids, *Psyllaephagus* species are used for biological control to attack psyllids on eucalypts trees (Paine *et al*., 2000). *Psyllaephagus bliteus* is a monophagous parasitic wasp that feeds on the Australian red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae), a pest of eucalyptus (Paine and Millar, 2002; Paine *et al*., 2000). Female *P. bliteus* oviposits in the nymphs of the *G. brimblecombei* and eggs of *P. bliteus* hatch within the host psyllids. A larva of *P. bliteus* feeds on the psyllid and makes an exit hole within the lerp when ready to exit. *Psyllaephagus bliteus* has a record of supressing the population of psyllids pests in some countries, including the USA and Mexico (Chauzat *et al*., 2002).

Psyllaephagus pilosus is a parasitoid of eucalyptus psyllid, *Ctenarytaina eucalypti* (Hemiptera: Psyllidae), a pest of *Eucalyptus* species (Noyes and Hanson, 1996). *Psyllaephagus pilosus* is native to Australia and Tasmania (Hodkinson, 1999). Dahlsten (1996) reported that adult females of *P. pilosus* normally lay a single egg in or on the *C. eucalypti* nymph (Hodkinson, 1999). According to Dahlsten (1996), the larva of *P. pilosus* feeds within *C. eucalypti* until the *insect* dies. Dahlsten (1996) further indicated that the pupation period of the *P. pilosus* takes place within the mummified *C. eucalypti* and the adult *P. pilosus* makes an exit hole when ready to exit. *Psyllaephagus pilosus* has been studied and proven to be very effective in supressing and regulating the *C. eucalypti* population on red gum trees and in prevention of mass outbreaks that result in death and die-back of plants (Hodkinson, 1999). Because of its effectiveness in controlling *C. eucalypti*, it has been used successfully for biological control in many countries including, USA, Britain, France and Eire to *C. eucalypti* (Hodkinson, 1999).

2.9.3 Factors affecting parasitism

The population of organisms in an ecosystem is never stable. In the ecosystem, insect populations fluctuate due to many factors, such as availability of food in the habitat and weather conditions. For example, if it rains heavily some insects will die. However, insect population growth may be regulated by the use of insecticides as well as the presence of potential natural enemies. According to Salatic (1963, cited in Schmidt *et al.*, 2003), the population of potential parasitic insects that regulate insect host population depends on factors such as sex ratio, reproductive potential, environmental parameters and host-finding ability. Hence, when the population of parasitic insects is high, it could imply that the environmental conditions and sex ratio of the parasitic insects is favourable for them to increase in numbers. The fluctuations in population of parasitoids or parasitoids densities can be affected by predators, abiotic stress factors and hyperparasitism as well as by predacious insects (Schmidt *et al.*, 2003). Also these factors may prevent the successiveness or effectiveness of parasitoids in the field (Schmidt *et al.*, 2003).

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CHAPTER 3: MATERIALS AND METHODS

3.1. Sites descriptions

3.1.1. Field study sites descriptions

The study sites were located in the Tshikundamalema Area, Mutale Municipality, Vhembe District, Limpopo Province, South Africa (Latitude 22° 29’ 48” S, Longitude 30° 37ˈ 99” E; 22^{o} 38’ 4” S, 30^{o} 51’ 25.87” E), 90 km from the town of Thohoyandou. The altitude of the area is 600 m above sea level. Tshikundamalema has a hot semi-arid climate, with high temperatures most of the year, where the daily temperatures range from 25-40°C in summer whilst winter temperatures range between 20-25°C. The area receives a mean annual rainfall of 350-400 mm. Most of the land is used for agricultural purposes, such as cultivation of maize and sorghum, and for grazing of livestock.

Zwigodini 1 and Zwigodini 2 (22° 29’ 48” S, 30° 37’ 99” E) are mopane woodland. These two sites are dominated by mopane trees and are separated by a tarred road. The sites differed in the number of trees, where there were more mopane trees at Zwigodini 1 than Zwigodini 2. The area surrounding the study sites had other drought tolerant indigenous trees such as *Sclerocarya birrea* (marula tree), *Berchemia discolor* (brown ivory) and *Adonsonia digitata* (baobab tree). Figure 3.1 shows an aerial view of site 1 (Zwigodini 1) and site 2 (Zwigodini 2) in the Tshikundamalema Area. Both sites are situated along the road to The Big Tree, a site of historical and tourist interest.

The third site where the field study was conducted was Domboni (22^{o} 38’ 4” S, 30^{o} 51’ 25.87” E). This site is located along the road to Tshipise Sagole in the Tshikundamalema Area. The road that passes through this site is a gravel road. The site is characterised by sandy soils. The land opposite the study site is used for cultivation of sorghum and livestock grazing. This study area had a lower density of mopane trees compared to Zwigodini 1 and Zwigodini 2. Trees were scattered especially from the middle of the site towards the edge of the site. The site was characterized by an abundance of thorny acacia bushes. Figure 3.2 shows an aerial view of site 3 (Domboni area) in the Tshikundamalema Area.

Figure 3.1. An aerial map showing the sampling sites at Zwigodini 1 and Zwigodini 2 in the Tshikundamalema Area

Figure 3.2. An aerial map showing the sampling site at Domboni Area in the Tshikundamalema Area

3.1.2. Site descriptions for sample collection for laboratory study

In addition to the three sites mentioned in 3.1.1, three other sites were selected. The other three selected sites were Guyuni, Ka-Dzumeri and Ka-Rhangani. Guyuni is a site located in Tshikundmalema Area, near Guyuni Primary School (22^{o} 43’ 36.584” S, 30^{o} 28’ 38.248” E). The vegetation is characterised by mopane woodland with sandy soils. Figure 3.3 shows an aerial view of Guyuni Area in the Tshikundamalema Area.

Figure 3.3. An aerial map showing Guyuni Area in the Tshikundamalema Area

Ka-Dzumeri is situated in Giyani Area, Limpopo Province, South Africa. The geographical coordinates of this site are 23^{o} 36’ 0” S, 30^{o} 44’ 0” E. The vegetation is mopane woodland, with sandy soils. Figure 3.4 shows an aerial view of Ka-Dzumeri Area.

Figure 3.4. An aerial map showing Ka-Dzumeri Area.

Ka-Rhangani is located near Giyani Area, Limpopo Province, South Africa (23° 33’ 35.078” S, 30° 42’ 42.113” E). The site is characterised by sandy soils. The vegetation cover is mopane woodland. Figure 3.5 is an aerial view of Ka-Rhangani Area.

Figure 3.5. An aerial map showing Ka-Rhangani Area

3.2. Sample collection of *Retroacizzia mopani* on *Colophospermum mopane*

3.2.1. Assessment of infestations of *R. mopani* at sites and tree height

Three sites, Zwigodini 1, Zwigodini 2 and Domboni, were used for assessment of the infestations of *Retroacizzia mopani* on *Colophospermum mopane.* At each site, quadrats 80 m^2 were marked, within each quadrat 20 m^2 subplots were divided. The subplots were divided in order to easily assess the trees for infestations of *R. mopani* to avoid duplication of the trees which were already assessed. Since some trees were clumped together, it was decided that bushes within a 30 cm radius would count as one tree. The height of the trees was also measured as either short (less than 1.5 m) or tall (above 1.5 m). The trees were assessed for infestation by recording the presence or absence of lerps.

A sampling data recording sheet (Figure 3.6) was designed and used for recording. Trees which were infested were recorded as "Y", to indicate the presence of lerps, while those which were not infested were recorded as "N", to represent the absence of lerps in both short and tall trees. The figure below indicates how the data recording sheet was designed.

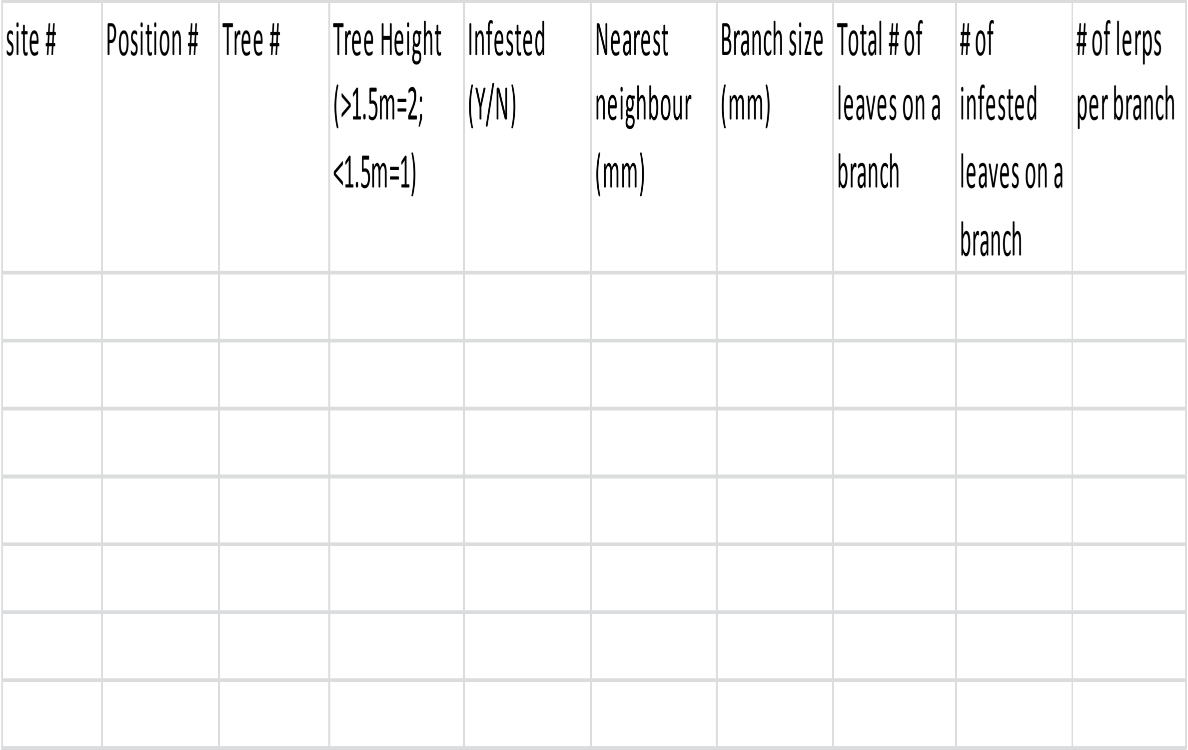
Site No…	Subplot No…	Tree height (<1.5m)	Infested? (Y/N)	Tree height (>1.5m)	Infested? (Y/N)

Figure 3.6. Example of a sampling recording sheet for levels of infestation

3.2.2. Assessment of the infestation levels of *R. mopani on* neighbouring trees and tree position

The study on the influence of neighbouring trees on infestation was conducted at two sites (Zwigodini 1 and Zwigodini 2). Out of the 16 subplots at each site, four subplots were chosen diagonally across the site to represent the subplot for assessing the effect of distance of trees from an open area (tree position). The first tree position was 0-20 m from the open space (a tarred road); the second position was 20-40 m from the open space; the third position was within 40-60 m from the open space, and the fourth position was 60-80 m from the open space. The total number of trees was counted within each selected 20 m X 20 m subplot, recording both the trees below 1.5 m and above 1.5 m.

Information about neighbouring trees was generated by measuring the distance between trees in order to assess whether the nearest neighbouring tree had an effect on the chances of other trees being infested by *Retroacizzia mopani.* Five tall and five short trees were also selected randomly to determine the effect of neighbouring trees on the density of lerps. Each tree was marked for the measurement of a single branch size. The total number of leaves on each marked branch was counted and recorded on the data recording sheet. The total number of leaves with lerps was also counted, as well as the total number of lerps on the infested leaves. Figure 3.7 shows the format of the sheet that was used to record the sample collections.



site #	Position #	Tree #	Tree Height (>1.5m=2; <1.5m=1)	Infested (Y/N)	Nearest neighbour (mm)	Branch size (mm)	Total # of leaves on a branch	# of infested leaves on a branch	# of lerps per branch

Figure 3.7. Sampling recording sheet for neighbouring trees

All the marked branches were collected into separate black refuse bags and taken to the laboratory for observation of emerging insects.

3.2.3 Comparison of parasitism levels of *Retroacizzia mopani* at different sites

Samples of mopane leaves were collected from November 2014 to August 2015 from different places (Zwigodini 1, Zwigodini 2, Domboni, Guyuni, Dzumeri, and Ka-Rhangani) on different dates. Samples were collected at Zwigodini 1, Zwigodini 2 and Domboni at one time point every season of the year and were collected in November 2014, February 2015, May 2015, July 2015 and in August 2015. Samples which were collected at Guyuni, Dzumeri, and Ka-Rhangani were collected only once for the duration of the study for psyllid and parasite emergence. The samples at Guyuni were collected in July and the samples collected at Dzumeri and Ka-Rhangani were collected in August 2015, during winter, when the infestation levels were high.

The branches of mopane trees with infested leaves were collected using a hand cutter and placed in brown paper bags. Each paper bag was labelled with the place of collection and the date of collection. The samples were taken to the laboratory for further studies.

3.3 Rearing of insects in the laboratory

Leaves with lerps were taken to the laboratory. The leaves were placed in plastic containers covered with nylon mesh and secured by rubber bands to prevent emerging natural enemies and psyllids from escaping. Inside each container was a foam wrap with slits which was placed on top of water. *Colophospermum mopane* leaves were placed through the slits to be in contact with water in order for the leaves to be kept moist. The leaves were examined to determine if they were free from other insect materials, other than *R. mopani* lerps, before placing them in containers. Any other insect materials, such as eggs, cocoons and webs were removed from leaves with lerps. *Colophospermum mopane* leaves were pushed through the slit in the foam wrap to be in contact with water, leaving the portion with lerps on top of the foam wrap, to avoid the lerps getting in contact with water. Infested *C. mopane* leaves were counted for the number of lerps in each container to determine the percentage parasitism. Each container was labelled with the date when the sample was collected and sites where the branch/leaves were taken. The containers were monitored each second day for any insects emerging, and all the emerging insect were counted and recorded. Figure 3.8 shows the ventilated containers for rearing insects, covered with nylon mesh.

Figure 3.8. Set-up of ventilated containers for rearing insect covered with nylon mesh

Insects were also reared by putting the infested branches containing lerps in containers. These were labelled with the site, tree position and branch number. The branches in the jars were covered with a nylon mesh and tied with two rubber bands to prevent emerging natural enemies from escaping through the nylon mesh. The total number of lerps on each branch was recorded before rearing. The monitoring was done every second day. All emerging insects were picked from the containers using fine forceps and preserved in 75% ethanol in vials. Figure 3.9 shows the set-up of infested branches covered with nylon mesh.

Figure 3.9. Set-up showing infested branches covered with nylon mesh

3.4. Insects identification

3.4.1 Preliminary insect identification

Preliminary insect identification was done in the postgraduate laboratory in the School of Agriculture. Insects were observed under a dissecting microscope to view the wing venation and orientation of the wing marginal veins, the segment of the antennae, the shape of the eyes, position of the legs on the prothorax, mesothorax, on the metathorax. The identification was done to family level using identification keys (Scholtz and Holmes, 2008).

3.4.2 Confirmation of the insect specimen identification

Confirmation of insect identification was done at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya. The insect specimens were viewed under a dissecting microscope and the identification was confirmed using the identification keys from by Scholtz and Holmes (2008). The identifications were confirmed by Dr Copeland, an insect taxonomist at ICIPE.

3.5 Data analysis

Data analysis was done using Excel programme for investigating the effect of site, tree density, position and tree height on the probability of a particular tree being infested, using the regression model. The Logistic regression model was used in predicting the odds of a tree being infested among the parameters (site, tree height, tree density, and tree position), stating whether these parameters have a positive or negative effect on infestation levels.

$$\text{Log}\,(p / (1-p)) = B\,(\text{Constant}) + B1x1 + B2x2 + B3x3 \ldots\ldots + Bnxn$$

Where $\text{Log}\,(p / (1 - p)$ is the probability of infestation;

p is the probability of occurrence of insects;

$(1 - p)$ is the probability of non-occurrence of insects;

B (constant) is the coefficient constant;

$BnXn$ is the coefficient of parameters measured, which are site, tree density, tree height and tree position.

A Cross Tabulation package from the SPSS program was used to determine the infestation levels at the three sites. It was used to compare whether the infestation levels is significant or not significant amongst these sites. The mean number of leaves per branch and the mean number of lerps per leaflet were calculated as:

$X = Xni/n$,

where X is the mean number of lerps per leaflet and

Xni is the total number of leaves per branch or total number lerps per leaflet.

The relationship between neighbouring trees and infestation levels were analysed using a correlation analysis.

Reference

Scholtz, C.H. and Holmes, E. 2008. Insects of Southern Africa. Butterworth Publishers, South Africa.

CHAPTER 4: PREVALENCE OF *RETROACIZZIA MOPANI* AND ITS NATURAL ENEMIES IN LIMPOPO PROVINCE

Abstract

The prevalence of *Retroacizzia mopani* and its natural enemies were studied in the field in Tshikundamalema at three locations. Leaves infested with lerps were taken to the laboratory to observe emergence of both psyllids and their natural enemies. The study showed that the infestation levels at three sites differed significantly, with Zwigodini 1 being the most heavily infested site (93.6%). The infestation levels were higher on mopane woodland sites (93.6% and 88.6%) than on thorny acacia vegetation (70.5%). *Psyllaephagus arytainae* Prinsloo, a natural enemy of *R. mopani,* emerged from the collected samples. Total parasitism levels from this natural enemy were low, ranging from 0 - 6.2% amongst all the sites, with the highest parasitism level recorded at Domboni. The finding suggests that although there is presence of natural enemies on some sites, the parasitism levels were not significant to colonise the psyllids in large numbers.

Key words: *Colophospermum mopane*, natural enemies, parasitism, *Psyllaephagus arytainae, Retroacizzia mopani*.

4.1. Introduction

Psyllids are small insects (1.5-4.5 mm) that suck the phloem sap from the plant leaves and shoots (Scholtz and Holmes, 2008). They belong to the order Homoptera and family Psyllidae (Scholtz and Holmes, 2008). Adult psyllids are active jumpers but can fly for a very short distance on the plants (Phillips, 1996). They are generally classified as monophagous insects because they feed on one or closely related host plant species. They can be easily noticed when they are present on trees, usually through the lerps which they construct on leaves and/or shoots, depending on the species of psyllid. The lerp is assumed to be for the protection of the *R. mopani* nymph against desiccation and natural enemies. The mopane psyllid, *Retroacizzia mopani,* is a pest of the mopane tree, *Colophospermum mopane,* a tree which is one of the most valuable trees in the Tshikundamalema area, Limpopo Province.

The biology of *Retroacizzia mopane* has been well-studied by Oppong *et al.* (2009 and 2010). According to Oppong *et al.* (2009), *R. mopani* females lay eggs on both mature and senescent leaves between July and September, when temperatures are between 11.4°C and 23.5°C and when the rainfall is as little as 10.8 mm a month. After egg-hatching, the nymphs start searching for feeding sites on *C. mopane* and start feeding (Oppong *et al.* 2010). The nymphs will construct lerps which completely cover their body. There are five nymphal stages of *R. mopani*: the first and second nymphal stages are commonly found on the secondary leaf veins, whilst the third, fourth and fifth stages are typically found on and along the main leaf veins (Oppong *et al.* 2010). When the psyllid reaches adulthood, it exits the lerp.

Retroacizzia mopani adult and nymph feeding causes damage to the leaves through the sucking of phloem sap from the leaves, causing discolouration and curling of the leaves. *Retroacizzia mopani* nymphs can also play a role in limiting the growth of the *C. mopane* because it constructs a lerp, which reduces the rate of photosynthesis on the leaves.

The most common parasitoids species of psyllids are from the genus *Psyllaephagus* (Hymenoptera: Encyrtidae), which is known to have over 200 described species and may have over 1000 species in total (Noyes and Hanson, 1996). *Retroacizzia mopani*, is preyed on by predacious species such as *Prinia subflava* and *Onychognathus morio* (larvae of the brown lacewing, Neuroptera: Hemerobiidae), *Hyperolius marmoratus taeniatus*, coccinellid beetles, and ants (Genus: Crematogaster) (Oppong *et al*., 2006). Compared to plantation tree species, limited information is available on the prevalence of psyllids on indigenous trees as well as their natural enemies in the habitat. The aim of this study was to assess the prevalence of *Retroacizzia mopani* and its natural enemies in the Tshikundamalema area, Limpopo Province of South Africa.

4.2. Research questions

The study aimed to answer the following research questions:

- What is the prevalence of *Retroacizzia mopani* and its natural enemies in the Tshikundamalema area?
- What is the parasitism rate of *R. mopani* in the Tshikundamalema area?

4.3. Materials and Methods

4.3.1. Study sites

Field assessments of psyllid prevalence were carried out at three study sites (Zwigodini 1, Zwigodini 2 and Domboni) in the Tshikundamalema area, Limpopo Province of South Africa (Latitude 22^0 29’ 48” S, Longitude 30^0 37ʹ 99” E; 22^0 38’ 4” S, 30^0 51’ 25.87” E). Samples of mopane branches infested with *Retroacizzia mopani* were collected at six different sites, where four of the sites (Zwigodini 1, Zwigodini 2, Guyuni and Domboni) were in the Tshikundamalema area and the other two sites were near Giyani (Ka-Dzumeri and Ka-Rhangani), Limpopo Province. All the collections of the infested branches were taken to the laboratory for rearing to determine insect emergence.

4.3.2. Assessment of the psyllids and natural enemies on mopane trees

At each sampling site, quadrats measuring 80 m X 80 m were marked. Within each quadrat, 16 subplots measuring 20 m X 20 m were demarcated. Subplots within each quadrat were marked for ease of counting and assessment. Mopane trees were assessed for presence of lerps on both short (less than 1.5 m tall) and tall (more than 1.5 m) trees. The information obtained from these assessments was used to determine the infestation levels at three sites (Zwigodini 1, Zwigodini 2 and Domboni). These sites were also used to collect mopane branches which were infested with *R. mopani*. The branches of mopane trees with infested leaves were collected using a hand cutter and placed in brown paper bags. Each paper bag was labelled to indicate the place and date of collection

4.3.3. Emergence of psyllids and natural enemies

The rearing of insects was done following the methods described in Section 3.2 of this dissertation. All the containers were labelled, with the date of sample collection and sites where the leaves were taken. The monitoring of insect emergence was done on each container every second day. All emerging insects were picked from the containers using fine forceps and preserved in 75% ethanol in vials. The total number of psyllids and natural enemies emerging were recorded to determine the parasitism levels of natural enemies on psyllids amongst sites. The insects were also identified as described in Section 3.4 of this dissertation.

4.3.4. Data analysis

The rate of parasitism was determined by using the formula:

$\frac{\text{NEE}}{\text{PE+NEE}}$ X 100 at each of the sampled site,

where: NEE is the sum of natural enemies emerged; and

PE is the sum of psyllids emerged.

The infestation levels at sites were generated by the Cross Tabulation package from the SPSS program (SPSS 2010, Version 19.0).

4.4. Results

4.4.1. Insect identification

A total of 701 insects emerged from the lerps. These insects were identified using morphological characteristics. The morphological characteristics of the parasitoid wasps were reduced wing venation, antennal segments, the articulation of middle coxa, and the size of the marginal vein. The size of abdomen and colour of the insects were further used to classify them to morphospecies. The confirmation of the insect identification to morphospecies was done at the International Centre of Insect Physiology and Ecology (ICIPE) in Kenya and at the Agricultural Research Council (ARC) in South Africa. The psyllids were identified by counting the number of tarsal segments, antennal segments and the colours of their various parts to see whether the psyllids emerged match the description of *Retroacizzia mopani* described by Pettey (1925).

Figure 4.1 below shows one of the psyllids (Homoptera: Psyllidae) specimen which emerged from the lerps. The insect had long antennae with eight segments, two tarsal segments; forewings were transparent but thicker than hindwings. The head was pale yellow with reddish brown eyes. The segments of thorax, abdomen, membranous wings and legs had a pale yellow colour, and the claws of all legs were black in colour. Ninety-eight percent of all insect specimens emerged were identified and confirmed as *Retroacizzia mopani* (*Arytainae mopanei*).

Figure 4.1. A picture of *Retroacizzia mopani* taken in the lab

Two percent of the insects which emerged from the samples belong to the family Encyrtidae (Hymenoptera) (Figure 4.2). The parasitoid wasps showed a visible pronotum when an insect is observed from above, the size of the mesopleura was large, the development of tibia was strongly developed. The position of the middle coxa was articulated anteriorly to the mesosternal midline, while the mesoscutum was dorsally convex. The wing venations were greatly reduced and the marginal veins and postmarginal veins were short. The stigma veins were relatively equal to the post-marginal vein in size and formed an acute angle at the side of the postmarginal vein. The visual morphological traits indicated that all the 14 parasitoid wasps that emerged from the lerps belong to Encrytidae.

Parasitoid insects were classified into three different insect morphospecies. Six of them had a metallic brown colour with a long abdomen, one of these morphospecies had a metallic brown colour with a very short abdomen which is half the length of the thorax, and the other seven parasitoids had a metallic green colour on the entire body but with a long abdomen. However, all their forewings covered the entire abdomen. Further identification at ARC (Agricultural Research Council) in Pretoria, South Africa confirmed all the morphospecies to be *Psyllaephagus arytainae* Prinsloo (Hymenoptera: Encyrtidae).

Figure 4.2. A picture of *Psyllaephagus arytainae* Prinsloo (Hymenoptera: Encyrtidae)

4.4.2. Parasitism levels of *Retroacizzia mopani* by natural enemies at sites

Fourteen of the 701 insects that emerged were parasitoids (*Psyllaephagus arytainae* Prinsloo, Hymenoptera: Encyrtidae) and 687 were psyllids (*Retroaccizzia mopani,* Homoptera: Psyllidae). The levels of parasitism for each site were also calculated. Figure 4.3 and Table 4.1 show the levels of parasitism at each of the six sites. The site which had the highest levels of parasitism of *R. mopani* was Domboni with 6.2%, followed by Zwigodini 1 which had 2.4% of parasitism levels and Zwigodini 2 with low levels of parasitism of 0.6%. No parasitism was recorded at the other three sites (Ka-Dzumeri, Guyuni and Ka-Rhangani). The parasitism levels differed significantly among the three sites (Fig 4.3), with very low levels of parasitism at three sites, ranging from 0.6% to 6.2%. Parasitism levels at Domboni were significantly more than the other sites, followed by Zwigodini 1 and Zwigodini 2, which also differed significantly from each other.

Table 4.1. Comparison of parasitism levels at six sites in Tshikundamalema and Giyani

Samples collected at	# of psyllids emerged	% of psyllids emerged	% of parasitism
Zwigodini-Madifha 1	242	97,6	2,4
Zwigodini-Madifha 2	181	99,4	0,6
Ka-Rhangani	99	100	0
Ka-Dzumeri	8	100	0
Domboni	106	93,8	6,2
Guyuni	51	100	0
Total count	687	98,4	1,6

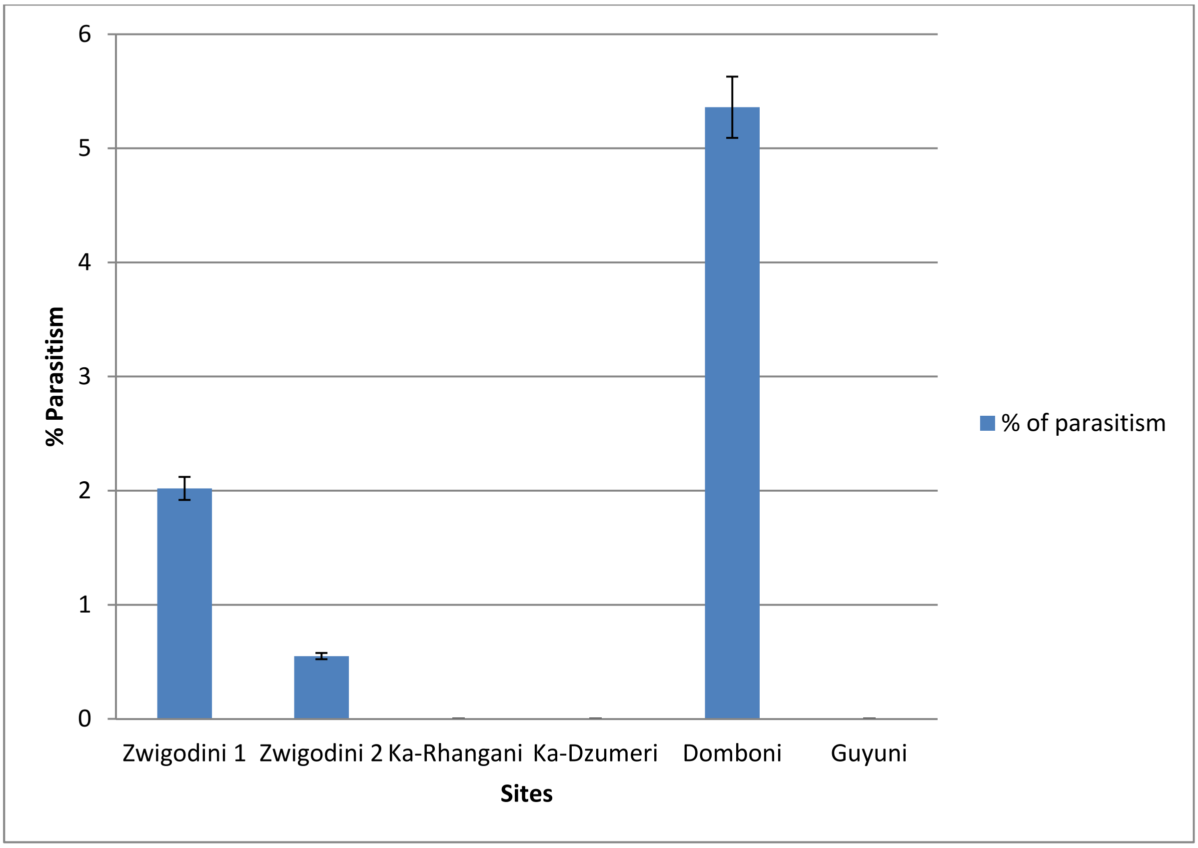


Figure 4.3. Comparison of parasitism levels at six sites in Tshikundamalema and Giyani

4.4.3. Psyllid infestation levels at the three sites

Infestation levels at the three sites (Zwigodini 1, Zwigodini 2 and Domboni) were compared using Cross Tabulation. Table 4.2 shows the data of mean percentage of *Retroacizzia mopani* infestations on *C. mopane* between sites. Zwigodini 1 had 93.6% infested trees and 6.4% were not infested. At Zwigodini 2, 88.6% of the trees were infested whilst 11.4% were not infested. The results for Domboni showed that 70.5% of the trees were infested and 29.5% of trees were not infested.

Table 4.2 Mopani psyllids infestation levels at three sites in Tshikundamalema

Sites		Infestation Level		Total
		Not Infested	Infested	
Zwigodini 1	Number of trees sampled	75	1092	1167
	% of infestations	6.4%	93.6%	100.0%
Zwigodini 2	Number of trees sampled	97	755	852
	% of infestations	11.4%	88.6%	100.0%
Domboni	Number of trees sampled	71	170	241
	% of infestations	29.5%	70.5%	100.0%

Figure 4.4 shows that the percentage of trees with lerps, ranging from 70.5% to 93.6%, with the highest percentage of infestations at Zwigodini 1. However, there were no significant differences in infestation levels between Zwigodini 1 and Zwigodini 2, but infestations were significantly lower at Domboni.

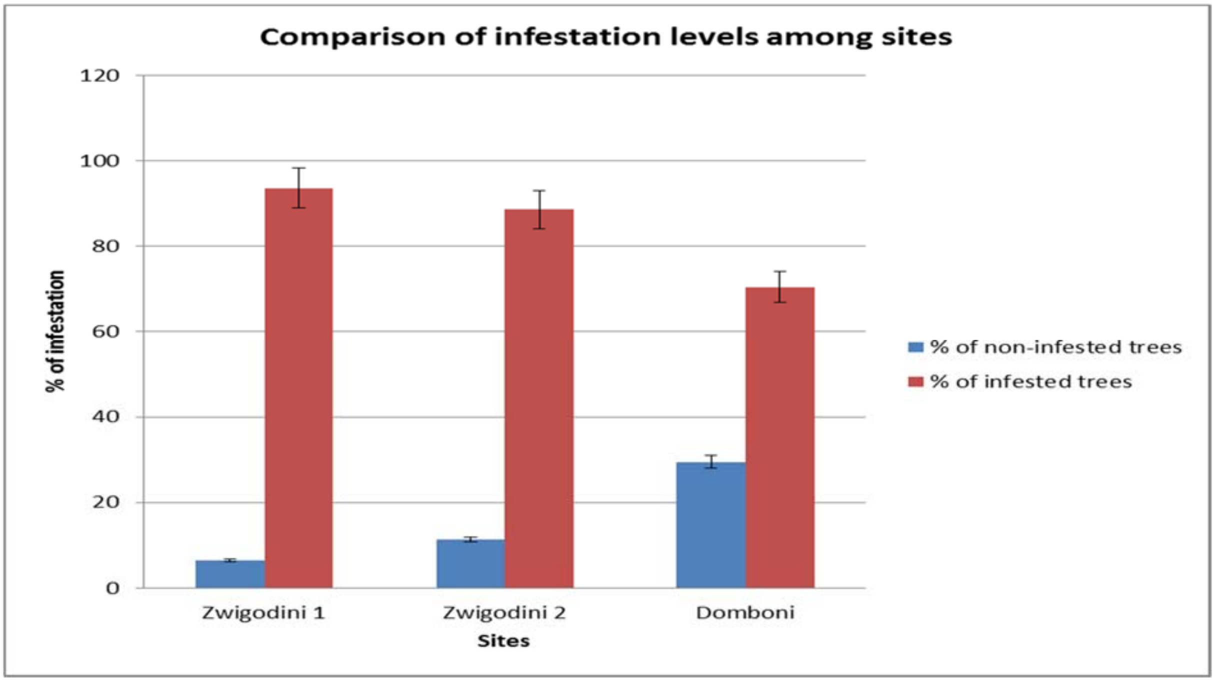


Figure 4.4. Infestation levels of psyllids on mopane trees at three sites in Tshikundamalema

4.5. Discussion

The results from this study showed that the prevalence of psyllids was higher than their natural enemies in Tshikundamalema area. Most of the insects which were in the lerps in the field were *Retroacizzia mopani* and a very few natural enemies emerged. The identity of the psyllid as *Retroacizzia mopani* was confirmed by matching the descriptions of Petty (1925). A parasitoid of *Retroacizzia mopani* in Tshikundamalema was identified as *Psyllaephagus arytainae*. This is supported by Noyes and Hanson (1996), who reported that almost all species within the genus *Psyllaephagus* are parasitoids that attack and kill the nymphs of psyllids species. The genus *Psyllaephagus* has been documented in literature attacking the *Eucalyptus* psyllids species (Paine *et al*., 2000). Most species of *Psyllaephagus* are very common parasitoids of psyllid species (Prinsloo, 1981). Some other psyllids species which are parasitized by *Psyllaephagus* species include *Diaphoria solani, D. tenebrosa* and *Agonoscena* species (Prinsloo, 1981). The results from this study support the findings of Prinsloo (1981), who showed that the parasitoid species associated with *R. mopani* was *Psyllaephagus arytainae*.

The parasitism of the psyllids was generally very low, ranging from 0% at three sites (Guyuni, Ka-Dzumeri and Ka-Rhangani) to 6.2% at the other three sites (Domboni 6.2%, Zwigodini 1 2.4%, and Zwigodini 2 0.6%) (Fig. 4.3). This is unlike other *Psyllaephagus* species which often have a high parasitism levels on the psyllids species. For example, *P. mesohomotomae*, a parasitoid of cacao psyllids, was shown to parasitise up to 87.9% of cacao psyllids (Lebel and Modeste, 2007).

The infestation levels of *Retroacizzia mopani* differed with sites and the differences were significant among the sites. Although the infestation was higher at Zwigodini 1 and Zwigodini 2 compared to Domboni area, the levels of parasitism on psyllids at these sites were lower than Domboni area. The results could suggest that the infestation levels of *R. mopani* at Zwigodini 1 and Zwigodini 2 were higher because of low levels of parasitism at those sites.

4.6. Conclusions

In Tshikundamalema area, *Retroacizzia mopani,* a psyllid pest of *Colophospermum mopani* tree is parasitized by a hymenopteran parasitoid, *Psyllaephagus arytainae.* Further studies are needed to investigate the role of *P. arytainae* in regulating populations of *R. mopani*.

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CHAPTER 5: FACTORS THAT AFFECT THE INFESTATION LEVELS AND DISTRIBUTION OF *RETROACIZZIA MOPANI* IN TSHIKUNDAMALEMA AREA

Abstract

The effect of site, tree density, tree height and tree position on infestation of mopane trees by psyllids was determined at three sites in Tshikundamalema. The number of trees at each site was counted. The infestation levels were measured at two tree heights (short: up to 1.5m; tall: more than 1.5m), as well as at different tree positions within a quadrat. The logistic regression model was used to analyse the data. Site, tree density, tree height and tree position had an effect on *R. mopani* infestations, and infestation levels were influenced by site ($p < 0.0001$). Tree density showed a positive relationship between the sites and the infestation levels: the infestation levels increased as tree density increased (Logistic Regression Model = 0.873 + 1.805*Tree density Zwigodini 1 + 1.179*Tree density Zwigodini 2). There was a positive relationship between the tree height and the infestation levels; the infestation levels increased as the tree height increased (Logistic Regression Model = 1.303 + 1.377* Tall trees). There was a negative relationship between the tree position and infestation levels; the further away trees were from an open space, the lower the infestation level (Logistic Regression Model = 3.807 - 1.640*Position no.4 - 0.892*Position no.3 -0.563*Position no.2). The findings from the study showed that the infestation levels of *R. mopani* are affected by site, tree position, tree height and tree density.

Key words: *Colophospermum mopane*, infestation levels, tree density, tree height, tree position.

5.1. Introduction

The abundance and distribution of insects in the habitat can be regulated by several biotic and abiotic factors and their interactions. Most of the important physical environmental factors that affect the abundance, distribution and ecology of insects are temperature and humidity (Savopoulou-Soultani *et al*., 2012). Among the biotic factors, insect distribution and abundance may be affected by the habitat or vegetation type and how the trees are distributed in such sites. In an ecosystem, the quality of a habitat may influence the insect species interactions, ecology and insect behaviour (Tscharntke *et al*., 2002).

Tree size may affect insect abundance and distribution in many ways. Numerous studies have documented the relationship between tree height and insect abundance. Basset *et al.* (2001) indicated that as the tree height increased, changes in microclimate also occurred. Therefore, the composition and population densities of insects within that microhabitat are affected. According to Basset (1992), tall trees produce more fresh leaves than shorter ones. Taller trees may support many herbivorous insects compared to shorter trees. Davidson *et al*. (2003) also found that tree growth influenced the availability of food. This means that taller trees would have more food available for insects than shorter ones because taller trees have more leaves, more insects would be expected on taller trees than on shorter trees.

The infestation and distribution of insects may be affected by the distance of trees from the open space (tree position) within a habitat. Although there has not been a study on the effect of tree position on psyllids distribution, literature shows that tree position has an effect on insect infestation levels and distribution. These effects were recorded by Dulaurent *et al*. (2012), who found that the pine processionary moth (PPM) *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae), a pest of pine trees, heavily infested pines at the stand edge compared to those pines within the stand. Similar observations were recorded by Régolini *et al* (2014), who found that pine trees situated at the edge of the stands are more infested than pine trees located within the centre of the stand.

Colophospermum mopane is the host for *Retroacizzia mopani,* an insect that feeds on the tree. The psyllid constructs a lerp which is assumed to reduce the photosynthetic area of the tree. There is no information on biotic factors (site, tree density, tree position and tree height) that impact infestation levels of *R. mopani.* The aim of this study was to investigate factors that affect the infestation levels and distribution of *R. mopani* in the Tshikundamalema area of the Limpopo Province.

5.2. Research question

- Is the distribution of *R. mopani* in a habitat affected by site, tree density, tree height and tree position?

5.3. Materials and Methods

The study was carried out at three study sites: Zwigodini 1, Zwigodini 2 and Domboni, as described in Chapter 3 of this dissertation.

5.3.1. Assessment of *Retroacizzia mopani* infestations were done at different sites and tree density, different tree heights and at different tree positions

Zwigodini sites were mopane woodland and Domboni was mainly thorny acacia woodland. The assessments for infestation were carried out following the procedures described in Chapter 4.

Two tree heights were used to assess effect of tree height on infestation levels: tall trees which were above 1.5 m, short trees were less than 1.5 m at all three sites. The presence or absence of lerps on each individual tree was recorded. The data obtained from these assessments was used to determine the effect of the tree height on *R. mopani* infestations.

Retroacizzia mopani infestations were recorded at the two sites (Zwigodini 1 and Zwigodini 2). Each site had 16 subplots and four were chosen diagonally across the site to represent the subplot for assessing the effect of distance of trees from the open area (tree position). The description of these positions was given in detail in Section 3.2.2. Individual trees within each subplot were assessed for the presence and absence of lerps.

5.4. Data analysis

Data was analysed using the Logistic Regression Model to investigate the effect of site, tree density, tree height and tree position on the infestation levels of *R. mopani.* The model was used to predict whether these parameters (site, tree density, tree height and tree position) had a positive or negative effect on infestation levels. The Microsoft Excel spreadsheet programme was used to plot the graphs and in making the summary tables for the data collected during the study.

5.5. Results

5.5.1. Effect of site on *Retroacizzia mopani* infestation levels

The effect of site on *R. mopani* infestation levels was assessed at three sites (Zwigodini 1, Zwigodini 2 and Domboni) with different vegetation structures. Zwigodini 1 and Zwigodini 2, which were mopane woodlands, had higher infestation levels of *R. mopani* than Domboni, which was thorny acacia woodland. Table 5.1 shows the infestation levels according to tree height. The trees with the highest infestation levels for *R. mopani* were at Zwigodini 1 (93% infestations), followed by Zwigodini 2 (88% infestations) and the Domboni area had the lowest infestation levels (70% infestations) of *R. mopani.*

Table 5.1. Infestation levels of *Retroacizzia mopani* at different sites on different tree heights in Tshikundamalema

Site	No. of trees	Tree height (<1.5 m)	Infested? (Y/N)		Tree height (>1.5 m)	Infested? (Y/N)		% of short trees infested	% of tall trees infested	Av. % of all infested per site
			Y	N		Y	N			
Zwigodini 1	1167	342	308	34	825	785	40	90,1	95,2	92,6
Zwigodini 2	852	235	184	50	600	547	53	78,6	91,2	84,5
Domboni	241	82	29	53	158	140	19	35,4	88,1	61,75

Table 5.2 shows a comparison of infestation levels at three sites using the logistic regression model. Site has a significant effect on the infestation levels of *R. mopani* on mopane trees ($p< 0.0001$). The probability of infestations occurring at Zwigodini 2 and Domboni is lower than at Zwigodini 1. Results showed that the probability of occurrence of infestations at Zwigodini 2 is 0.5 (50%) compared to Zwigodini 1 whilst the probability of occurrence of infestations at Domboni is 0.2 (20%) compared to Zwigodini 1. There is a 95 % probability of occurrence of infestations between 11.4-73.3%.

From the exponential coefficient, the equation of Logistic Regression Model = 2.678 – 0.626*Zwigodini2 – 1.805*Domboni, shows that the coefficients of Zwigodini 2 and Domboni are negative which shows that Zwigodini 1 has higher infestation levels compared to these two sites. However, a negative coefficient of Zwigodini 2 is higher than of Domboni, which means Zwigodini 2 is more infested than Domboni area.

Table 5.2. A comparison of infestation levels at three sites using Logistic regression model

Effect/Source	Coeff.	Odd Ratio	Std. Err.	p-value	Lower bound (95%) Conf.	Upper bound (95%) Conf.
Zwigodini 1	0.000	1.000	0.000			
Zwigodini 2	-0.626	0.535	0.161	< 0.0001	0.390	0.733
Domboni	-1.805	0.164	0.185	< 0.0001	0.114	0.236
Constant	2.678					

5.5.2. Effect of tree density on *Retroacizzia mopani* infestation levels

Tree density had an influence on the infestation levels of *R. mopani.* Table 5.1 shows that the highest infestation levels of *R. mopani* were at Zwigodini 1, which had 1167 trees and the lowest infestation levels were at Domboni area, which was the site with the lowest tree density (241 trees). These results showed that as the density of trees increased, so the infestation levels of *R. mopani* increased, as would be expected.

The effect of tree density on *R. mopani* infestations was tested using logistic regression in predicting probability of occurrence of *R. mopani* infestations (Table 5.3). The p-value for each regression effect due to tree density is smaller than 0.05, which shows that tree density had a significant effect on infestation. The probability of infestations at Zwigodini 2 is higher than at Domboni. From the exponential coefficient, the equation of Logistic Regression Model = 0.873 + 1.805*Tree density Zwigodini 1 + 1.179*Tree density Zwigodini 2. This equation shows that the coefficient of tree density of Zwigodini 1 and Zwigodini 2 are positive, which shows that Domboni area with low tree density was less infested than these sites. Also, Zwigodini 1 shows high coefficient compared to Zwigodini 2, this shows Zwigodini 1 was more infested than Zwigodini 2.

Table 5.3. A comparison of effect of tree density on infestation levels at three sites using Logistic regression model

Effect/Source	Coeff.	Odd Ratio	Std. Err.	P-value	Lower bound (95%) Conf.	Upper bound (95%) Conf.
Tree density-Domboni	0.000	1.000	0.000			
Tree density-Zwigodini 2	1.179	3.251	0.178	< 0.0001	2.294	4.606
Tree density-Zwigodini 1	1.805	6.081	0.185	< 0.0001	4.232	8.738
Constant	0.873					

5.5.3. Effects of tree height on *Retroacizzia mopani* infestation levels

Table 5.1 shows that the percentages of infestations on trees of height above 1.5 m were higher than for trees which were less than 1.5 m in height. Figure 5.2 shows the relationship between tree height and *R. mopani* infestations. The results show that tree height (shorter trees and taller trees) had no significant effect on infestation levels at Zwigodini 1 and Zwigodini 2 because the error bars are overlapping at these sites (Fig. 5.2). At Domboni, shorter trees had significantly less infestation than taller trees.

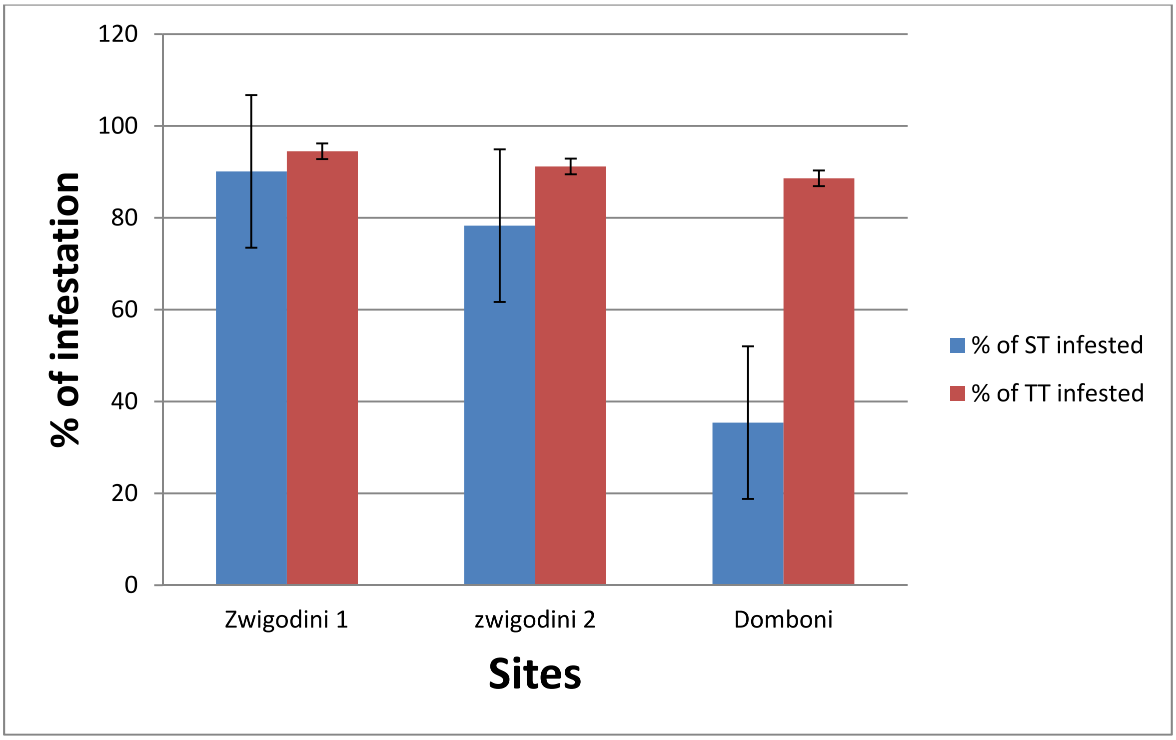

Figure 5.2. Relationship between tree height and *Retroaccizia mopani* infestations

Table 5.4 shows the overall comparison of infestation levels at different tree heights. The Logistic regression model was used in predicting the effect of tree height on infestation levels. Results showed that tree height has a significant effect on infestation levels of *R. mopani* ($p<0.0001$). The overall combination result of all sites in Table 5.4 shows that taller trees were more infested than shorter trees. The coefficient variables show that the equation of Logistic Regression Model = 1.303 + 1.377* Tall trees. The coefficient of tall trees is positive which shows a positive relationship between the tall trees and the infestation levels; that is, the infestation levels increase as the tree height increases.

Table 5.4 Comparison of effect of tree height on infestation levels using Logistic regression model

Effect/Source	Coeff.	Odd Ratio	Std. Err.	P-value	Lower bound (95%) Conf.	Upper bound (95%) Conf.
Short trees	0.000	1.000	0.000			
Tall trees	1.377	3.964	0.139	< 0.0001	3.016	5.211
Constant	1.303					

5.5.4. Effects of tree position on infestation levels

Table 5.5 shows the comparison of *R. mopani* infestations at different tree positions. The results showed that tree position 4 had significantly less infestation than the other three tree positions ($p<0.05$). Also, the probability of occurrence of *R. mopani* infestations, compared to tree position 1, were 57%, 41% and 19% in position 2, 3, and 4 respectively (Table 5.5).

Table 5.5. A comparison of infestation levels at different tree positions using Logistic regression model

Effect/Source	Coeff.	Odd Ratio	Std. Err.	P-value	Lower bound (95%) Conf.	Upper bound (95%) Conf.
Position no.-1	0.000	1.000	0.000			
Position no.-2	-0.563	0.569	0.801	0.482	0.119	2.735
Position no.-3	-0.892	0.410	0.793	0.260	0.087	1.938
Position no.-4	-1.640	0.194	0.765	0.032	0.043	0.869
Constant	3.807					

From the exponential coefficient, the equation of Logistic Regression Model = 3.807 - 1.640*Position no.4 - 0.892*Position no.3 -0.563*Position no.2. The equation shows that the coefficients of tree position 4, 3, 2 are negative and position 4 had the lowest coefficient than other tree positions, which shows that the further the tree position is from the open space, the less the infestation levels of *R. mopani*. Figure 5.3 and Table 5.6 show infestation levels at different positions, showing that there is a decline in infestation levels from position 1 to position 4. Even though tree position has an effect on the infestation levels of *Retroacizzia mopani*, these differences were not significant among positions 1, 2 and 3, but they all had significantly more infestation than position 4 which was furthest away from the open space.

Table 5.6. Number of trees and infestation levels at different positions

	Total no. of trees	% of trees infested
Position 1	92	97,8
Position 2	213	95.8
Position 3	175	94.8
Position 4	146	89.7

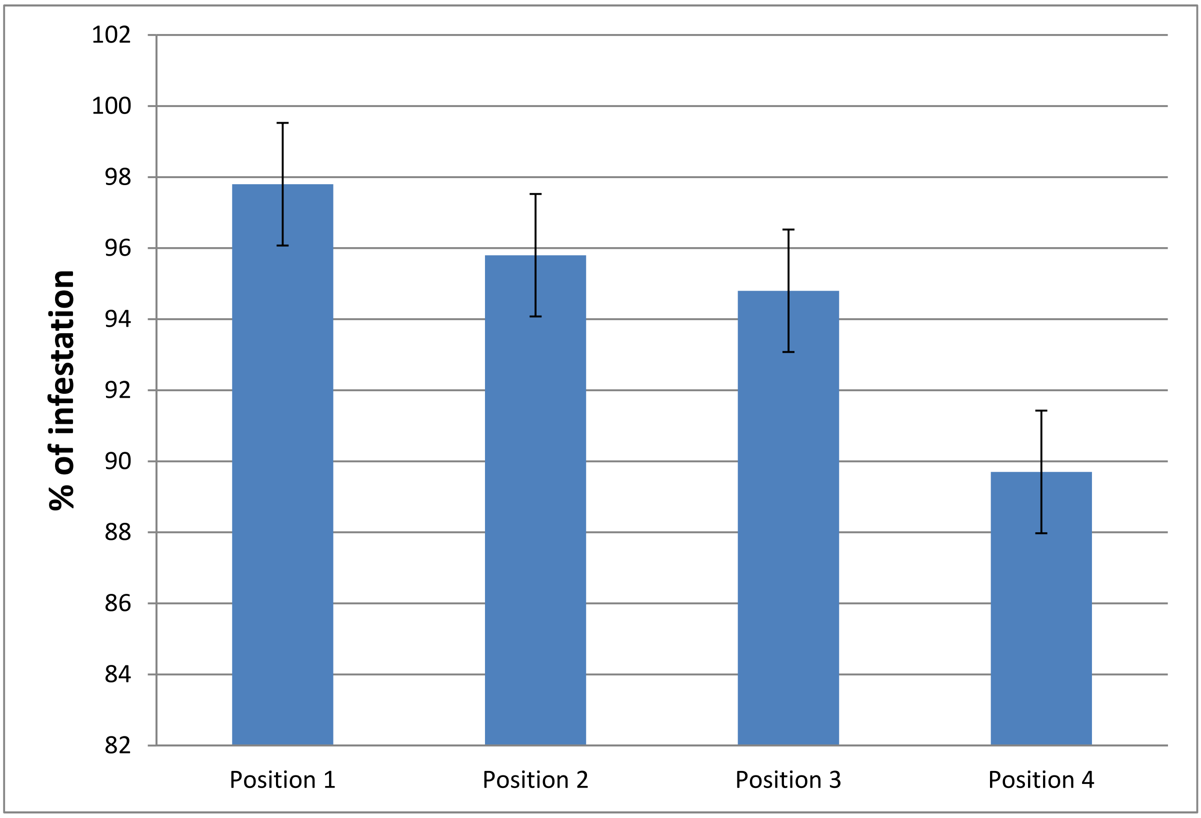

Figure 5.3. A comparison of the effect of position of trees from the open space on infestations

5.6. Discussion

The findings from this study have shown that site has an effect on infestation levels of *R. mopani.* These results suggest that the infestation levels were significantly higher at Zwigodini 1 than Zwigodini 2 and Domboni because the number of trees at Zwigodini 1 was higher than the rest of these sites. Also, Zwigodini 2 was heavily infested than Domboni because Domboni had fewer trees than Zwigodini 2, supporting that number of trees in these sites could have played a role in the infestation levels. The results have also shown number of trees in each position could have also played a role in the infestation levels. This is because the infestation increased as the number of trees was low, which could mean that insects had colonised many trees on positions with low tree densities.

Trees were more heavily infested with *R. mopani* at sites with highest tree density and Zwigodini 1. The results are supported by the logistic regression equation which showed a positive relationship between the tree density and the infestation levels. These results differ from the findings by Régolini *et al* (2014) who stated that *Thaumetopoea pityocampa* infestation levels were higher in older stands with low density of trees. They also stated that the chances of an individual tree being attacked by *T. pityocampa* would decrease in denser stands because of dilution process. However, results from the current study are in agreement with findings by Davis (1975) who showed that as plant density increased, insect abundance also increased.

The results showed that tree height had no significant effect on infestation levels where tree density was higher. The effect of tree height on infestation levels seemed to have an effect where tree density was lower, and taller trees were more heavily infested. These results are supported by Campos *et al* (2006) who found more insect herbivores on taller *Anadenanthera macrocarpa* trees than on shorter ones. This likelihood of infestations on taller trees could be because taller trees are more likely to have more food resources than shorter trees.

The comparison of *R. mopani* infestations at different tree positions showed that tree position had variable effects on infestation levels of *R. mopani*. The results showed that trees closest to the open space were more heavily infested with *R. mopani* than trees furthest away from open spaces. Dulaurent *et al* (2012) found that *Thaumetopoea pityocampa* infestation levels increased at the pine stands edges than for pine trees within the stand. This finding was also supported by Régolini *et al* (2014), who stated that trees situated within the edge of the stands were more highly infested compared to trees located within the heart of stand. These findings suggest that visual cues could have affected the infestation levels.

5.7. Conclusions

Infestation levels of mopane trees by psyllids were directly proportional to the tree density: as the density of trees increased the infestation levels increased. The effect of tree height on infestation levels seemed to depend on tree density. Infestation levels decreased from the edge of the forest to the centre of the forest where infestations were more likely to be found on trees closer to the open space and decreased the further away trees were from the open space.

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CHAPTER 6: THE EFFECT OF NEIGHBOURING TREES AND BRANCH SIZE ON PSYLLID INFESTATIONS IN TSHIKUNDAMALEMA

Abstract

The effect of neighbouring trees and branch size on *Retroacizzia mopani* infestations on *Colophospermum mopane* was studied in the field at Tshikundamalema area, Limpopo. Four subplots were demarcated within each of the two sites (Zwigodini 1 and Zwigodini 2). The distance between neighbouring trees and branch sizes were measured, and infestations of *R. mopani* on the trees recorded. Leaves on measured branches were also counted and lerps present on those branches were counted to determine the percentage infestation per branch. The results were analysed using correlation analysis. The results of the relationship between neighbouring trees and *R. mopani* infestations showed a Pearson Correlation coefficient of -0.756 and -0.792 at Zwigodini 1 and Zwigodini 2 respectively, showing that there is a very strong negative relationship between infestation levels and neighboring trees. This negative relationship implies that as the distance between trees increased, the *R. mopani* infestations decreased. The results also imply that when the trees are densely distributed, the infestations increased. The percentage of infested leaves per branch was high; however, the lerp density was very low on each of the leaves. The mean numbers of infestations showed that as the branch size of a tree increased, *R. mopani* infestations also increased, showing a positive relationship between branch size of mopane trees and infestation levels. This means neighbouring trees and branch size have effect on psyllid infestations.

Key words: *Colophospermum mopane,* infestation levels, neighbouring trees, *Retroacizzia mopani*.

6.1. Introduction

The effect of neighbouring trees and branch size on psyllid infestations can be an interesting study to find how nearness of trees and their branch size influence the psyllids on mopane trees. Artz and Waddington (2006) assessed the effects of neighbouring tree islands on insect pollinator diversity and density, as well as on pollination of a wet prairie species, *Asclepias lanceolata* (Apocynaceae). Their results showed that the number of insect species present and pollinator abundance in *A. lanceolata* decreased with increasing distance from tree islands. They suggested that the decline in abundance of the insect could be attributed to the foraging ecology and behaviour of insects. For both short tree islands and larger tree islands, the number of documented individuals was highest at 25 m away from tree islands and declined with increasing distance, suggesting that overall pollinator activity was highest on neighbouring tree islands (Artz and Waddington, 2006).

There have been various studies on how neighbouring vegetation influences the probabilities of infestations of insects on the main tree species (White and Whitham, 2000). However, the studies do not document the influence of distance between trees of the same species on the infestation levels of insects on those trees. Therefore, the aim of this study was to determine the effect of the nearest neighbouring trees on insect infestations. The objective was to establish if there were any relationships between nearness of trees (neighbouring trees) and infestations, as well as determining if branch size influenced infestation levels on trees.

6.2. Research questions

- Is there any relationship between neighbouring mopane trees and infestation levels of *Retroacizzia mopani*?
- Does branch size of the mopane trees influence *Retroacizzia mopani* infestation levels?

6.3. Materials and Methods

6.3.1. Assessment of *Retroacizzia mopani* infestation levels on neighbouring trees

Four subplots per quadrat were chosen for the study at Zwigodini 1 and Zwigodini 2 in the Tshikundamalema area. The same subplots which were chosen and used for determination of the effect of tree positions on *R. mopani* infestations were also used to determine the relationship between neighbouring trees and *R. mopani* infestations as described in Chapter 3, section 3.2.2 second paragraph of this dissertation.

Graph paper was used to map the trees within each selected subplot. The mapping was done by measuring distances between trees and transferring information of those distances to the graph paper. All trees within the chosen subplots were assessed for infestations. The effect of neighbouring trees and branch size on infestation levels were analysed through correlations analysis.

6.3.3. Assessment of psyllids infestations on branches of mopane trees

Individual trees within subplots were selected at random to study the relationship between branch sizes and density of lerps. Five tall trees and five short trees were selected using random numbers generated from Microsoft Excel. A branch on each tree was marked for counting the number of lerps on the leaves. The number of infested and non-infested leaves was determined to find the average percentage of lerps per branch as well as the average lerp density per leaf. The mean number of infested leaves per branch and the mean number of lerps per leaflet were calculated using the mean formula:

X= Sum of Xni/n,

where X is the mean number of lerps per leaflet or number of infested leaves per branch;
Xni is the total number of leaves per branch or total number of lerps per leaflet and
n is the total number of leaves.

6.4. Results

6.4.1. The relationship of neighbouring trees on infestation levels

Figure 6.1 shows the distribution of trees of *C. mopane* tree at Zwigodini 1 (Site1) and Zwigodini 2 (Site 2). The vertical arrow shows the open space while the horizontal arrow indicates the positions from the open space. The distances between the closeness of trees were measured, and trees were also assessed for the presence and absence of *R. mopane* infestation. Each dot represents a tree, or clustered trees. The distribution patterns of trees varied considerably within the subplots, and ranged from random distribution, through clumped to aggregations.

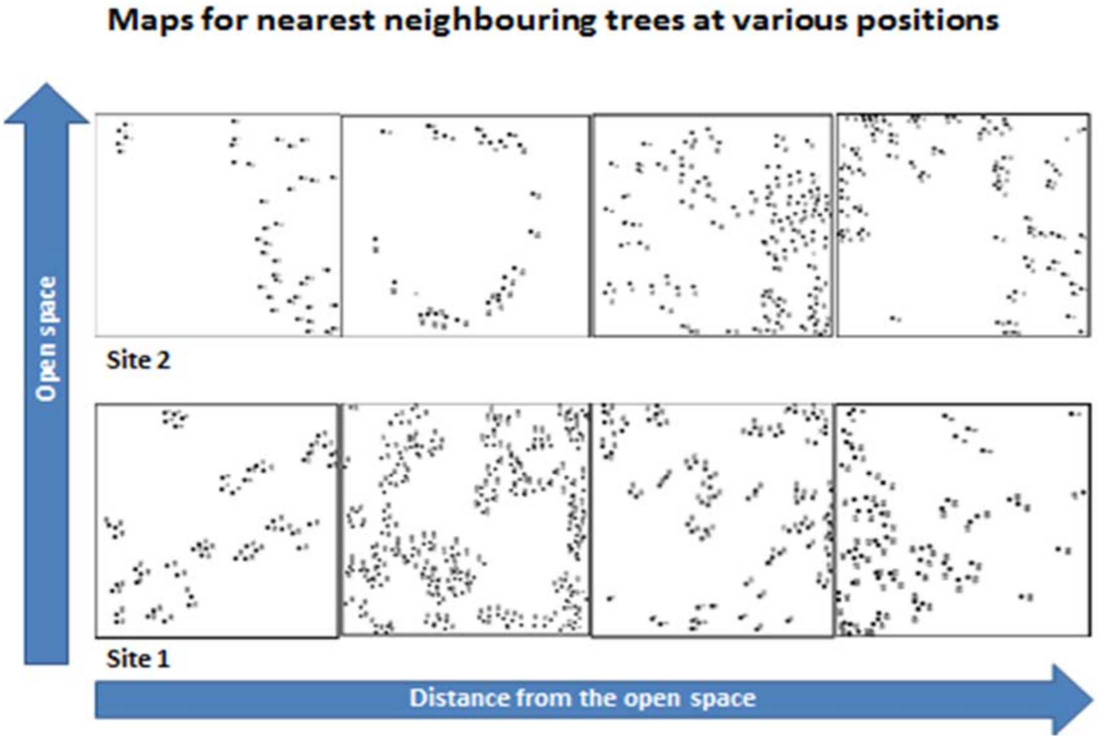

Figure 6.1. A map showing the distribution of *C. mopane* trees in different positions from Zwigodini 1 and Zwigodini 2

Table 6.1 shows the total number of trees in position 1, 2, 3 and 4 at Zwigodini 1 and Zwigodini 2 respectively. The highest number of trees was recorded in Zwigodini 1 in subplot 2, followed by Zwigodini 2 subplot 3. The lowest number of trees recorded was at Zwigodini 2 in subplot 2. Within each subplot, more trees were recorded at Zwigodini 1, except at position 4 where there were more trees at Zwigodini 2. There were more trees recorded at Zwigodini 1 than at Zwigodini 2.

Table 6.1 Total number of mopane trees per position at Zwigodini 1 and Zwigodini 2

Position Number	Number of trees/site			
	Zwigodini 1		Zwigodini 2	
	Total Tree Count	% of infestations	Total Tree Count	% of infestations
1	50	98	42	97
2	187	97.9	26	80.9
3	77	93.5	98	95.9
4	63	100	83	81.9
Total Tree Count	377		249	

Table 6.2 shows the infestation levels of psyllids at specified distances at both sites. An arbitrary distance of 50 cm was used and the number of lerp infestations within each range was counted. The highest number of infestations was recorded within the 51-100 cm range at both sites, and the number of lerp infestations declined as the distance between trees increased. The number of lerps was in the single digits where trees were at distances greater than 300 cm from each other. The number of infestations generally declined the further apart trees were from each other.

Table 6.2 Number of lerp infestations recorded at each site within defined distances from neighbouring trees.

Distance (cm)	**Total infestation in Zwigodini 1**	**Total infestation in Zwigodini 2**
0-50	69	31
51-100	136	71
101-150	60	46
151-200	36	42
201-250	23	16
251-300	12	13
301-350	9	5
351-400	5	5
401-450	3	3
451-500	7	1
500-550	5	2
551-600	2	0
601-650	2	1
Above 650	2	5

The relationship between infestation level and distance between trees was determined using the Pearson Correlation coefficient (Table 6.3 and Table 6.4), which gave a value of -0.756 for Zwigodini 1 and -0.792 for Zwigodini 2. These values indicated a very strong negative relationship between infestation levels and neighbouring trees. As the distance between trees increased, the infestations of *R. mopani* decreased. This seems to indicate that distance between neighbouring trees affected infestation levels.

Table 6.3 Correlation analysis showing the relationship between neighbouring trees and infestation level at Zwigodini 1

		Neighbouring tree	Site 1
Neighbouring tree	Pearson Correlation	1	-0.756**
	Sig. (2-tailed)		.001
	N	15	15
Zwigodini 1	Pearson Correlation	-0.756**	1
	Sig. (2-tailed)	.001	
	N	15	15

**. Correlation is significant at the 0.01 level (2-tailed).

Table 6.4 Correlation analysis showing the relationship between neighbouring trees and infestation level at Zwigodini 2

		Neighboring tree	Site 2
Neighboring tree	Pearson Correlation	1	-0.792**
	Sig. (2-tailed)		.000
	N	15	15
Zwigodini 2	Pearson Correlation	-0.792**	1
	Sig. (2-tailed)	.000	
	N	15	15

**. Correlation is significant at the 0.01 level (2-tailed).

6.4.2. Branch infestation levels.

The infestation levels of *R. mopani* on *C. mopane* branches were assessed by marking a branch and counting the number of leaves per branch and the number of infested leaves and the number of lerps on the leaves on the marked branches. Numbers of all infested leaves were counted and all the number of lerps on them was also counted, recorded and their means were calculated.

6.4.2.1. The relationship between branch size and *Retroacizzia mopani* infestations

The percentage infestation was determined from 80 trees by marking a single branch from each tree and counting the number of infested leaves and the number of non-infested leaves on each branch.

Table 6.5 and Figure 6.2 show the parameters measured at each subplot position. The results show that the average branch sizes of taller trees were longer than those of shorter trees. Average branch sizes of taller trees ranged from 47.7 mm to 72.6 mm and of shorter trees from 38.7 mm to 56.3 mm. The results also showed that trees with longer branches in all the positions had more lerps than those with shorter branches (longer branches had a range of 5 to 10 lerps and shorter branches, 4 to 8 lerps per branch). Also, taller trees were heavily infested than shorter trees in all positions. In position 1, longer branches had more lerps per branch (mean, n = 7) than shorter branches (mean, n = 4). However, the percentage of infested leaves per branch was high on short branches with 12.3% compared to long branches. In position 2, shorter branches (mean, n = 8) were less infested than longer branches (mean, n=10) and the percentage of infested leaves were higher on longer branches with 10.8%. The mean number of lerps was higher on longer branches in position 3 than on shorter branches. However, the percentage of infested leaves was high on shorter branches (11.8%) than on longer branches (9.4%). Average number of lerps per branch and percentage average of infested leaves per branch was the same in position 4 on both longer branches and shorter branches. In general, longer branches of *C. mopane* had more lerps than shorter branches, and shorter branches had the highest infestation in all the positions.

Table 6.5 Summary data of the relationship between the tree height, branch sizes and *R. mopani* infestations

Position	Tree Height (Short=S; Tall=T)	Branch Size Average (mm)	Average # of leaves on a branch	Average # of infested leaves on a branch	% of infested leaves per branch (%)	Average # of lerps per branch
1	T	72,6	71	5,5	7,7	7
	S	54,5	26,3	3,2	12,3	4
2	T	52,85	54,15	5,85	10,8	10
	S	53,1	41,85	4,35	10,4	8
3	T	47,7	50	4,7	9,4	7
	S	38,7	27,2	3,2	11,8	4
4	T	66,9	54	4,3	8	5
	S	56,3	36,1	2,9	8	5

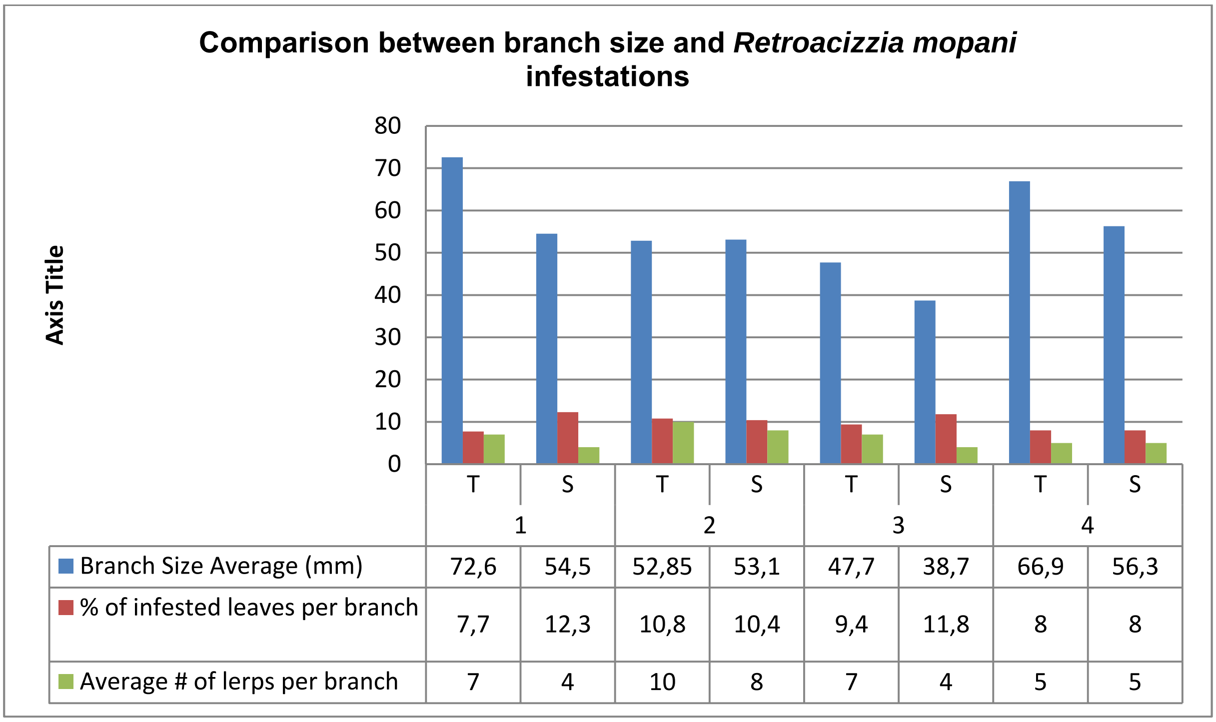

Figure 6.2. The relationship between the branch sizes and *Retroacizzia mopani* infestations

6.5. Discussion

The findings from this study showed that the infestation level of *R. mopani* on *C. mopane* is influenced by neighbouring mopane trees (nearness of the trees to each other). There were different distribution patterns of trees such as clumped distribution, scattered distribution, aggregated and random distribution. Analyses of the results showed that there was a strong negative correlation between infestations and distance between trees (Tables 6.3 and 6.4). This means that infestation levels increased as the distance between neighbouring trees decreased. The closer the trees were to each other, the greater the chance of the trees being infested with the lerp psyllids. When trees are clumped together, they are more likely to be infested with *R. mopani* because there is a greater chance of lerps moving between closer trees than if the trees are further apart. These results are in agreement with Artz and Waddington (2006) who stated that the number of insect species and pollinator abundance in the *Asclepias lanceolata* (Apocynaceae) decreased with increasing distance from tree islands. According to Debinski *et al*. (2001), infestation is lowest when the size of forest is small and trees are isolated. This suggests that infestation on trees that were further apart were low due to such trees being isolated from other trees. In addition, the decrease in infestation levels on trees which are further apart may be affected by other tree species that can interfer with the visual cues of the insects in identifying the host mopane trees.

When determining the relationship between the branch size and infestation levels at various positions, the study found that the infestation levels varied at different tree positions. In all the positions, as expected, taller trees had longer branches compared to shorter trees. It was found that longer branches had the highest number of lerps than shorter branches (Table 6.5 and Fig. 6.2), except in position 4 where both the shorter and longer branches had the same number of lerps. However, the infestation levels were higher in short branches compared to long branches. Longer branches had more lerps in almost all the positions because there were more leaves to colonize than on shorter branches. According to Davidson *et al*. (2003), tree growth affects the availability of food for insects. These suggest that the infestation levels of *Retroacizzia mopani* increased as the branch size decreased, implying that higher infestation levels would be expected on shorter branches because shorter branches have fewer food sources and insects might compete for such limited resources.

6.6. Conclusions

The results of the study indicated that the distance between neighbouring trees were inversely proportional to the *R. mopani* infestations; as the distance between trees increased, *R. mopani* infestations decreased. The branch size affected the infestations of the mopane psyllids and shorter branches were more heavily infested than longer branches. However, it is not clear why the infestation levels were higher on shorter branches than on longer branches.

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CHAPTER 7: GENERAL DISCUSSION AND CONCLUSIONS

The confirmed identification of psyllids as *R. mopani* matched the descriptions given by Petty (1925). All the natural enemies found were encrytid parasitoids, identified by the Agricultural Research Council in South Africa as *Psyllaephagus arytainae*.

Prinsloo (1981) reported that *R. mopani* is parasitized by the parasitoid *P. arytainae*. Amongst many psyllids, the species that were reported as being attacked by *Psyllaephagus* species were *Eucalyptus* psyllid species. For example, parasitism rates by *Psyllaephagus pilosus* in Australia of 50-100% were recorded on the blue gum psyllid in 1994 (Paine *et al.,* 2000). *Psyllaephagus* species have been used successfully in biological control of psyllids. The *Psyllaephagus* species such as *P. mesohomotomae*, a parasitoid of cacao psyllids, has been recorded to parasitize up to 87.9% of cacao psyllids (Lebel and Modeste, 2007). However, in contrast, this study showed low levels of parasitism by *P. arytainae,* ranging from 0 - 6.2% in the habitat, and thus this parasitoid is unlikely to play a major role in regulating the population of *R. mopani* in the study area (Fig. 4.3).

The study showed that infestation levels of *R. mopani* differed among sites and were affected by the density of mopane trees. The highest infestation levels were recorded where the density of mopane trees was higher, and the lowest infestation levels were at low tree density (Table 5.5.2). These findings from the study are in agreement with results by Davis (1975), who reported that as nettle density increased, insect abundance also increased. In contrast, these findings differ with the findings by Régolini *et al* (2014), who stated that *Thaumetopoea pityocampa* infestation levels were higher in older stands with low density of trees and decreased at dense tree stands.

Tree height had no significant effect on infestation levels where tree density was higher. The infestation levels were significant where the tree density was low, but taller trees were significantly more heavily infested than shorter trees (Fig. 5.2). Campos *et al* (2006) also found more insect herbivores on taller *Anadenanthera macrocarpa* trees than on shorter ones.

The position of tree relative to open spaces had an effect on infestation levels of *R. mopani*. The infestation levels of *R. mopani* were higher on trees closest to the open space than on trees furthest away from open spaces. These results were supported by Dulaurent *et al* (2012), who found that *Thaumetopoea pityocampa* infestation levels were higher on the pine trees at the stand edges than on pine trees within the stand. These results were also supported by Régolini *et al* (2014), who stated that trees situated within the edge of the stands were more highly infested compared to trees located within the centre of the stand. These studies suggest that some ecological factors such as visual cues and ability of insects to recognize the mopane trees at various distances could have influenced the likelihoods of infestations. This is supported by Artz and Waddington (2006) who suggested that the decrease in insect abundance could be attributed to their ecology and behaviour. Visual cues could influence the ability of insects to see the mopane trees where there is interference of other trees, possibly explaining why the trees far from other mopane trees were less infested than those mopane trees which were close to each other.

This study showed that the distance between neighbouring trees had an effect on *R. mopani* infestations. There was a very strong negative correlation between neighbouring trees and the infestations. As the distance between *C. mopane* trees increased, the infestation decreased. Therefore, the results suggested that when the mopane trees are closer to each other, the level of infestation of *R. mopani* would be expected to increase. These results are supported by Artz and Waddington (2006) who stated that the number of insect species and pollinator abundance in the *Asclepias lanceolata* (Apocynaceae) were higher at shorter distances than at longer distances. It is possible that *R. mopani* is affected by several ecological factors, visual cues and chemical cues when identifying its host in the habitat.

Infestation levels of *R. mopani* were affected by branch size. The infestation levels were higher on shorter branches than on longer branches. However, longer branches had more infested leaves than the shorter branches. This suggests that lerp numbers were higher on longer branches of the mopane trees because longer branches had more leaves compared to shorter branches. This implies that long branches are more likely to have many lerps than shorter branches of the mopane trees.

Conclusions

The findings from the study showed that *Retroacizzia mopane* is parasitized by *Psyllaephagus arytainae* (Hymenoptera: Encrytidae). The parasitism levels of *R. mopani* by *P. arytainae* were low in the habitat. The infestation levels of mopane trees by the lerp psyllids were influenced by the tree density: as the density of trees increased, infestations also increased. It seems that the density of trees influenced the infestation levels based on tree height. At high mopane tree density, tree height had no effect on infestation levels. However, at low tree density, taller trees were more likely to be infested than shorter trees. Levels of infestation were also influenced by the tree positions relative to open spaces. The closer trees are to the open spaces, the higher the likelihoods of infestations. The relationship between the distance between neighbouring trees and infestation levels showed that as the distance between neighbouring mopane trees increased, the infestations of *R. mopani* decreased.

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