Understanding spatial structuring and the role of domestication in the development of sustainable harvest techniques of Mopane worms (*Gonimbrasia belina*).

By

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DECLARATION

I, **Zwannda Nethavhani**, declare that this research proposal "Understanding spatial structuring and the role of domestication in the development of sustainable harvest techniques of Mopane worms (*Gonimbrasia belina*)." is my original work and has not been submitted for any degree at any other university or institution. The thesis does not contain another persons' writing unless specifically acknowledged and referenced accordingly.

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ABSTRACT

Mopane worm (*Gonimbrasia belina* Westwood) is an indigenous edible insect that periodically has population eruptions in Mopane tree (*Colosphermum mopane*) dominated vegetation. This insect is a valuable source of food and has become an economic commodity for commercial harvesters. Despite its importance, little is known of the population dynamics of mopane worm. Considerable attention has been paid to the nutritional and social dimensions of mopane worm consumption. However, anecdotal evidence suggests there is a decline in the spatial extent of their distribution due to unsustainable utilization, land transformation, and commercialization. This is paralleled with a decrease of harvest yield, thereby affecting local communities, commercial harvesting operations, and market product availability. Partial domestication of this species may be a way of ensuring sustainable and reliable utilization of this edible insect because it improves natural survival rates. Here I explore mopane worm ecology by mainly focusing on understanding spatial structuring and the role of domestication in the development of sustainable harvest techniques of Mopane worms (*G. belina*). Spatial structuring of mopane worms was investigated at both a fine (10m x 10m) and medium-scale (1 km^2) at sites with and without commercial harvesting. Mopane worm populations were experimentally treated through transplant experiments and manipulating access of predators to $1^{st} - 3^{rd}$ instar larvae. The role of tree characteristics (height, canopy volume and number of stems) on both spatial and experimental treatments were explored using generalized linear mixed models. Moran's Eigenvector Maps (MEM's) were used to represent spatial structures at various scales and the role of soil. Relative to control, seeding of worms was successful in establishing new populations in unoccupied areas while transplant and application of sleeve nets had no significant effect on larval survival. Populations at sites where no harvesting takes place were spatially structured, while this was not true where commercial harvesting takes place. Canopy volume also accounted for egg packets distribution although the relationship was ambiguous. The density of larvae increased with an increase in sodium concentration in soil. Seeding of the population provides a viable option for the sustainable utilization of mopane worms and educating people on how to domesticate mopane worms. This study also highlights that populations of mopane worms are not only clumped in time but also in space and at various spatial scales.

Keywords: Meta - population, mini-livestock, mopane worms, life cycle, entomophagy.

ACKNOWLEDGEMENTS

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CHAPTER 1: INTRODUCTION

1.1. Human Populations: Size and Growth

The world's population is predicted to grow from 6.3 billion in 2002 to 9 billion in 2050 (Charles *et al.* 2010). More than half of the annual global population increase in 2002 occurred in India, China, Pakistan, Bangladesh, Nigeria, and the United States (Cohen *et al.* 2003). Approximately, 5.1 billion lived in developing countries with an annual growth rate of 1.46%, eight times that of developed countries.

Africa is the second largest and second most crowded continent on earth, with an estimated population of 1.2 billion people in 2016. Population growth in most African countries is around ~2% annually (Pelser & Kherehloa, 2000), peaking in Uganda and Nigeria and the continent's population is predicted to reach 2.4 billion by 2050 and more than 4 billion in 2100 (Gerland *et al.* 2014).

Human population growth is the principal cause of major environmental impacts across the earth and is linked to increases of pollution deposited in the land, water, atmosphere, and simultaneously increase demand on the outputs of available agro-ecosystems (FAO, 2015). An increase in the human population decreases available arable land and resources (Pelser & Kherehloa, 2000). With the decrease in natural resources and land for other feeding activities practiced by people, feeding the current population and the 9 billion people expected on planet earth in 2050 (Charles *et al.* 2010) will take extraordinary steps and innovative advances in technology. These steps include the use of Non-timber forest products (NTFPs) as an alternative source of food. Non-timber forest products are biological products acquired from the wild that are used for human well-being (Shackleton & Shackleton, 2004 and MEA 2005). For example, intensive consideration of edible insects around the world as an alternative food for feed is one step towards food security. More studies on Non-timber forest products (NTFPs) are required, including their sustainable production and harvesting to ensure future availability. Approximately 85% of rural households make use NFTPs which include wild spinach, fuelwood, wooden utensils, grass hand-brushes, edible fruits, wood for construction, bushmeat, wild honey, reeds for weaving, and twig hand-brushes (Shackleton and Shackleton, 2004). These products contribute 5 - 90% of total household income (Shackleton *et al.* 2008; Delang, 2006; Te Velde *et al.* 2006; Kamanga *et al.* 2009; Pokorny *et al.*

2012) and play a vital role in improving food security, employment and the nutritional status in rural areas (Shackleton & Shackleton, 2004).

Edible insects are primarily a category of non-timber forest products, harvested for both commercial and subsistence use particularly in rural areas (Shackleton & Shackleton, 2004; Rumpold & Schlüter, 2013; Halloran *et al.* 2014). They are an important potential source of food and feed and considered as a means of supporting the ever-growing population (FAO, 2013; Van Huis *et al.* 2013; Halloran *et al.* 2014; Tao & Li, 2018). These insects represent an important seasonal source of protein and a normal part of the diet of a large proportion of the human population (Illgner and Nel, 2000; van Huis *et al.* 2013; Rumpold & Schlüter, 2013). Even though the consumption of insects is of less importance to the western world, they are significantly important to other cultures and for human health (Van Huis *et al.* 2013; Anankware *et al.* 2014).

Limpopo for example, is one of the poorest provinces in South Africa with high rates of food insecurity (Shisana *et al*. 2013) but is also home to some of the most economical and nutritionally important edible insects in Southern Africa (Shackleton and Shackleton 2004; Teffo *et al.* 2007; Dovie *et al.* 2002, Dzerefos *et al.* 2013). In the Blouberg of the Limpopo province, there are at least 30 insects considered edible and consumed by about 91% of households around the area (Egan *et al.* 2009).

1.2. Entomophagy
Entomophagy is the practice of consuming edible insects as food and has now become a global phenomenon (Gahukar, 2011). This practice is heavily influenced by culture and religion (FAO, 2013) and can be a solution to reducing malnutrition. Edible insects are considered a naturally renewable resource providing nutritional, economic and ecological benefits to communities (Wang *et al.* 2006; Chen *et al.* 2008; Zhou *et al.* 2009; Gahukar, 2011; Ji *et al.* 2012). In addition, insect protein is healthier than animal proteins and more efficient at capturing energy from primary production (i.e. plants) (FAO, 2013). The Food and Agriculture Organization (FAO) initiated policies and recommendations of using insects as an alternative source of protein for feeding people as they provide proteins and other nutrients like fats, calories, vitamins, and minerals (Yen, 2009; FAO, 2010a; FAO, 2010b). Adding insects to the human diet can alleviate diseases caused by diet deficiencies in most vulnerable groups in society (Kwiri *et al.* 2015).

In 2009, there were over 1500 known edible species globally (Yen, 2009), this increased to over 1900 species in 2012 (van Huis, 2013). The most commonly consumed insects at global level are termites, grasshoppers, stink bugs, bees, mopane worms, jewel beetles, ants, wasps, beetles, flies, white-grubs and crickets (Ramos-Elorduy, 2006; Toms & Nonaka, 2005; Egan, 2013). Insect-eating seems to be culturally universal even though it varies with location, types of insect populations and ethnic group (Johnson, 2007).

For South and North America, 679 edible insect species have been recorded, compared to 524 in Africa, 349 in Asia, 152 in Australasia, and 41 from Europe in 2005 (Ramos-Elorduy, 2005). Even though the American continent recorded the highest number of edible insect species, Africa had the highest number of countries (36) valuing entomophagy, followed by Asia (29), America (23), Australasia (14) and Europe (11). Considerable numbers of insect species are consumed in the Central Africa Republic (~96 species with locust, grasshoppers, and caterpillars as most consumed species) particularly in the Congo Basin (Roulon-Doko, 1998 in FAO 2013). In Asia, Thailand was one of the countries with many edible insects, with 81 edible insects reported available as snacks and canned products (particularly cooked crickets, cooked silkworm pupae, and cooked bamboo worms). Studies from Thailand showed that insects are consumed throughout the year and varied between months (Yhoung-Aree & Viwatpanich, 2005).

Caterpillars are consumed globally because they are among the world's most diverse groups of edible insects. Mopane worms are consumed in Africa, witchetty grubs and bamboo caterpillar in Australia while the bamboo caterpillar is popular in Thailand as well (Meyer-Rochow, 2005). Termites are mostly consumed on the African continent (Paoletti *et al.* 2003). Stinkbugs are consumed throughout Mexico (Ramos Elorduy & Pino, 2002), southern Africa and Southeast Asia even though the species differ (DeFoliart, 2002; Nonaka, 2007; Dzerefos *et al.* 2013). Different species of grasshoppers are largely consumed in the African continent and Asia (Nonaka, 2009; Kinyuru *et al.* 2010).

Besides being harvested for private consumption, monetary value is also derived through the bartering sale of harvests (Gondo *et al.* 2010). The selling and consumption of edible insects are advocated as a source of food because of their abundance and low ecological footprint during production compared to livestock (Anankware *et al.* 2015). Edible insect domestication is not necessarily a land-based activity; hence it does not require land clearing for production expansion.

They emit fewer greenhouse gases and fewer ammonia emissions than most livestock (Anankware *et al.* 2015). Given the reduced cost and ecological footprint in producing edible insects, domestication of edible insects has become an important issue and will require more studies (Feng *et al.* 2017).

1.3. Domestication

Domestication is a sustained multi-generational relationship in which one group of organisms assumes a significant degree of influence over the reproduction and care of another group to secure a more predictable supply of resources from that group. The process of insect domestication is not only limited to large-scale production units but can also be used on a small-scale in the backyard (Halloran *et al.* 2014). For example, Black Soldier Flies (*Hermetia illucens*) naturally occur in high abundance around manure piles of large poultry, pigs, cattle and on organic wastes like coffee bean pulp, vegetables, distillers' waste and fish offal. They are essential for reducing manure mass, moisture content and offensive odors off manure and many other organic wastes. Black soldier flies also provide feed for cattle, pig, poultry and fish (Newton *et al.* 2005). Furthermore, their high crude fats can be converted to biodiesel where growing 1000 larvae of this species on 1 kg of cattle manure, pig manure and chicken manure produce 36 g, 58 g and 91 g of biodiesel respectively (Li *et al.* 2011). Therefore, there is a necessity of developing more insect farming techniques to ensure the availability of insects that both subsistence and commercial harvesters can depend on for sustainable global food supply and other important uses.

Insect domestication was mostly reported on the Asian continent (FAO, 2013). Thailand was reported as one of the countries with successful insect farming. Crickets, palm weevils and mealworms were among the most farmed species in Thailand (Hanboonsong *et al.* 2013). About 43 tons of palm weevils were produced by 120 farmers in Thailand in 2011 alone using a traditional method of collecting beetles, allowing them to oviposit on palm trees and then harvesting the final larvae (Hanboonsong *et al.* 2013). Besides being harvested for subsistence, palm weevil frass collected and sold as organic fertilizers. Approximately 7 500 tons of crickets were also produced in Thailand through farming processes engaging about 20 000 farmers annually (Hanboonsong *et al.* 2013).

The most commonly known domesticated insect products are honey and silk (van Huis *et al.* 2013). Silkworm was farmed for the production of silk and sold to earn a living while their pupae are a

delicacy in China, Japan, Thailand, and Vietnam (van Huis *et al.* 2013). Bees produce honey as well as pollination needed by farmers from both small and large farm scales (Potts *et al.* 2016). Bees produce about 1.2 million tons of commercial honey per year (FAO, 2009b) while more than 90 000 tons of silk were produced from silkworms (Yong-woo, 1999). Over 90% of the 250 000 flowering plant species depend on pollinators and through domesticated bees alone, 15% of these species are pollinated (van Huis *et al.* 2013). The motivation behind farming of edible insects is their importance which benefits both subsistence and commercial farmers. Greater control on predicting the availability of targeted species, increasing availability and sustainable production which can meet increased demand with less exploitation are some of the benefits associated with domestication.

In Africa, mopane worm is harvested exclusively from wild populations. Natural populations however already experience mortality due to predation and parasitization by natural enemies, and this either results in low harvestable yields (Gardiner, 2003; Ghazoul, 2006 in Hope *et al.* 2006) or overharvesting leads to population crashes (i.e. Hartland-Rowe, 1992). Domestication of the mopane worm may be a way of ensuring sustainable and reliable utilization of this edible insect species.

1.4. Mopane worms

1.4.1. Mopane worm (*G. belina*) harvesting and uses
Mopane worms are harvested when they reach their final instar stage. Populations are bivoltine in nature and two harvests are possible per year depending on the amount of rainfall (Illgner & Nel, 2000). During this process, worms are collected from and under mopane trees (Stack *et al.* 2003). Gloves are used as protection against the worm's sharp spines during harvesting and degutting (Taylor, 2003). The use of gloves is a universal practice and is confirmed to be the same in Zimbabwe, Botswana and South Africa (Gashe and Mpuchane, 1996). Following the process of harvesting, worms are degutted, boiled with saltwater and dried in the sun to avoid spoiling (Tom & Thangwana, 2005; Ghazoul, 2006 in Hope *et al.* 2009).

The fifth instar worm has a long history of being an important traditional delicacy in many parts of southern African countries (Stack *et al*. 2003). Before commercialization became common, mopane worms were harvested only for subsistence and nutritional purposes. Studies have shown that 67% of the interview respondents ranked mopane worms as a very important source,

(Sekonya, 2016). Studies done on the nutritional value of mopane worms revealed that these worms contain more protein, calcium, iron, zinc and less in fat compared to traditional livestock (Hobane; 1995; Madibela *et al.* 2009; van Huis *et al.* 2013; Rumpold & Schlüter, 2013; Kwiri, 2015). The worm is a recommended supplement for high cereal diet for infants (Ohiokpehai *et al.* 1996) and food for anaemia patients (Akpalu *et al.* 2009). They are consumed as a snack or relish, roasted, fried or cooked in a stew with other vegetables (Ballantine, 2000). These worms are also used as animal fodder. They have amino acids that are important for supporting the immune system of parasitized animal (gut parasites) and help also with foetal growth and production of milk for mammals (Madibela *et al.* 2009 & Hoskin *et al.* 2002).

Other than harvesting for human and animal feed (e.g. pets, pigs, poultry, and fish), mopane worms are an important source of income (Makhado *et al.* 2014). Approximately 9.5 billion mopane worms are harvested for trading annually in southern Africa (Greyling & Potgieter, 2004; Halloran, 2014). Data that was collected from southern Zimbabwe shows that many households harvest worms and approximately 79-95% of these households sell or exchange some of the worms they collected for goods they need (Gondo *et al.* 2010). Several tons of mopane worms were annually exported to Zambia and Zimbabwe from Botswana and South Africa (Thomas, 2013 and Ghazoul, 2006 in Hope *et al.* 2009). Thomas (2013) reported that a 50 kg maize meal sack of worms cost R716.95 in Namibia in 2013. In South Africa, the price of an 80 kg maize meal sack of worms ranged between R752.26 and R1002.98 in 2005 (Makhado *et al.* 2014). The cross-border trade between Botswana, South Africa, and Zimbabwe is estimated at between R422 million and R638 million per year (Makhado *et al.* 2014). At Thohoyandou markets in Limpopo province, these worms were sold for between R 5.00 and R 10.00 per 100 g, R 20.00 per 200 g and R 1200.00 - R 1500.00 for an 80 kg bag of worms and Makhado *et al.* (2009) reported an annual selling turnover of R20 000.00.

1.4.2. Biology of the mopane worm

Gonimbrasia belina belongs to a group of Emperor moths from the Saturniidae family (Pinhey, 1972; Oberprieler, 1995). The wingspan of the female moth ranges from 105mm to 125mm and 100-125mm for males (Oberprieler, 1995). The base colors of these moths are chestnut-brown and orange-brown to pale yellow and greyish, others range from greyish-green through chocolate brown. White and black bands isolate the eyespots on both fore- and hind wings. The orange eyespots on the hind wings are much larger than the eyespots on the forewings. Populations are

bivoltine with adult moths emerging late in February and late in November (Oberprieler, 1995). Moths only live for a few days during which they do not feed (they do not have functional mouthparts). During this time males search for female by following pheromones released by females. After mating, female moths lay their eggs in a cluster of about 30 to 355 eggs on the upper and lower surface of the host plant (mopane tree) leaves (Ditlhoho, 1996).

Eggs hatch within ten days and larvae pass through five larval stages of growth. Each instar stage lasts for about 5-7 days. Larvae feed predominantly on mopane tree leaves and grow up to 80 mm in length and about 10 mm in diameter (Oberprieler, 1995). They are black in color with round scales in indistinct, alternating reddish, yellowish and whitish bands and they are armed with short reddish or black spines (which first appear from the third instar) covered with fine white hairs (Pinhey 1972). During their first three instar stages, they cluster together in groups of 15-200(Courtney 1984) (Fig. 1) when feeding and disperse during the fourth and fifth instar stages. Aggregation behavior in mopane larvae aids early instars in thermoregulation and water balance (Klok & Chown 1999). The final (fifth) instar worms find a place next to a host tree, burrow 15cm deep into the soil to pupate and pupae remain underground until the next rainy season then emerge as a moth to begin the cycle again (Ghazoul, 2006 in Hope *et al.* 2009).

Figure 1: Second and third instar stages in aggregation (Source: Zwannda Nethavhani).
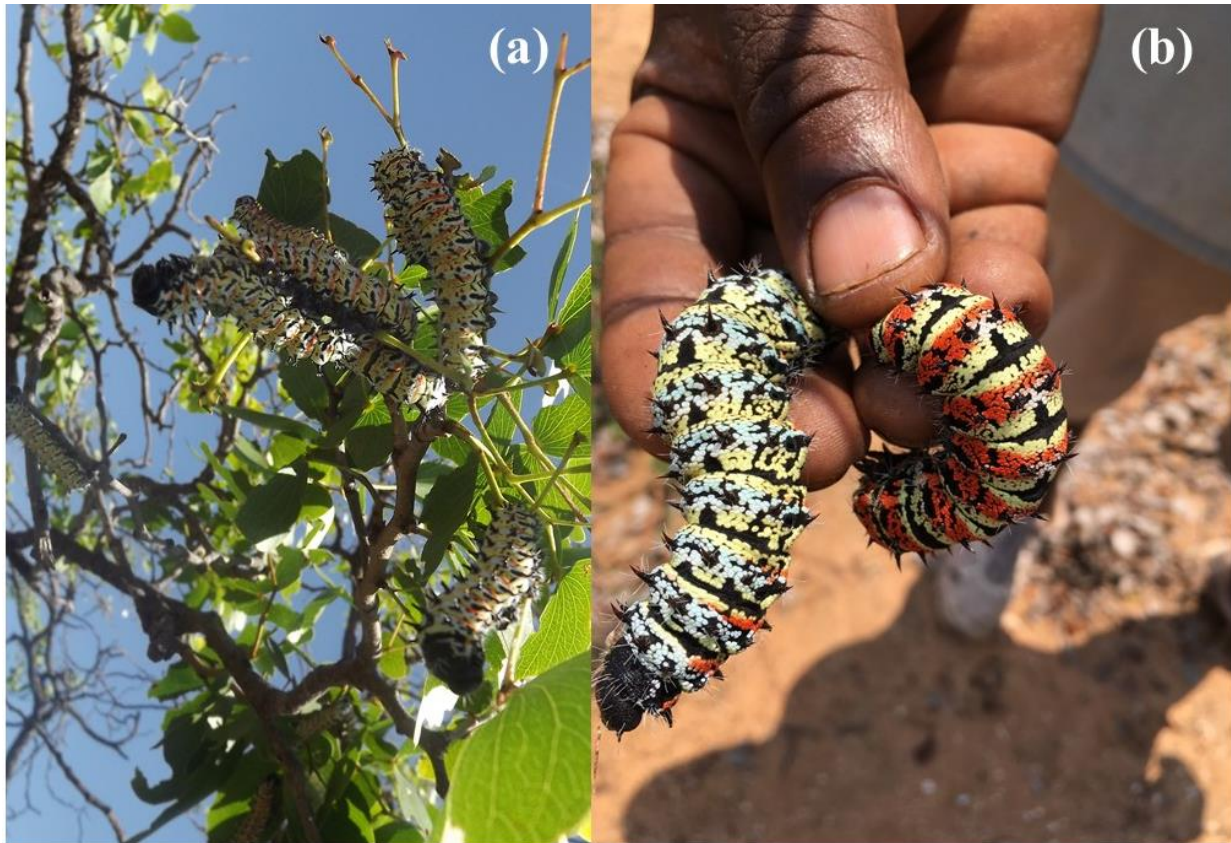
Figure 2: (a) fourth instar stage worms and (b) fifth instar stage prior to pupation (Sources: Zwannda Nethavhani & Prof. S.H Foord).

1.4.3. Population dynamics of mopane worms

During an insects' life cycle, factors that affect insect demographics vary with their life histories (Price 1997; Veldtman *et al.* 2007b). Identifying these factors and their effects on the species strengthens our understanding of how their populations are structured in time and space (Veldtman *et al.* 2007a). Spatial structuring deals with how populations of species are distributed in space and time at a certain scale. It sheds light on the population dynamics of a species and how both the species and its habitat can be conserved (Collinge, 2010). Given that there is no data on mopane worm population dynamics, even descriptive studies should provide valuable information and knowledge.

Mopane worm distribution follows that of their host plant (Appendix 1), and its life cycle consists of seven different life stages that may be subjected to different mortality factors. Studies reported both top-down (predation, harvesting pressure, parasites) and bottom-up (habitat, host plants, food

availability, climatic conditions) factors contributing to interannual variability of mopane populations (Gardiner, 2003, Makhado *et al*., 2009, Makhado *et al*., 2012). These factors influence their survival at each stage differently and may result in different behaviors and preferences throughout the life cycle which may lead to different distribution patterns of this species (Price, 1997 and Veldtman *et al.* 2007b). If the effects of each factor on the outbreak of mopane worms are identified, it may be possible to predict where and when outbreaks will occur.

Climatic factors were documented to play an important role in maintaining the population dynamics of Lepidoptera species (Hartland-Rowe, 1992). Temperature and rainfall are the two main climatic factors driving the population outbreak of mopane worms (Sekonya, 2016; Dube & Phiri, 2013 and Hartland-Rowe, 1992). Temperature regulates the time for the emergence of moths' for the next generation and rainfall provides adequate food (host plant leaves) which larvae will feed upon. Although rainfall has been known as an important factor driving the outbreaks of the mopane worms, no quantitative assessment of this relationship exists.

Mopane trees as host plants of mopane worms are also significant plant species within their range and among the most widely utilized plant species in South Africa providing various products (Makhado *et al.* 2014). These products include construction materials, firewood, livestock feed, medicinal use (Musvoto *et al.* 2007; Thomas, 2013; Makhado *et al.* 2016). Mopane trees are also important in the dry-season for browsing herbivores (Hooimeijer *et al.* 2005). Mopane worms inhabit and predominantly feed on the leaves of these trees, hence the availability of these trees in high densities is required to provide sufficient food for high densities of worms. The densities of worms decrease as vegetation composition of mopane veld changes through bush clearing for firewood (Hobane, 1995; Roberts, 1998).

Even though there seems to be a co-evolutionary relationship between mopane trees and mopane worms, where trees provide food for worms and worms aid in nutrient cycling (Appendix 2). Mopane worms also exert heavy defoliation on their host plants and thereby also affect other herbivores dependent on *C. mopane* (Fig. 3) (Ditlhogo, 1996; Adelabu *et al.* 2012). In 4000ha mopane veld near Messina in Limpopo Province for example, worms consumed leaf matter 13 times more than elephants in six weeks and the same was documented in the Kruger National Park density over a 12 month period (Styles, 1996). In cases where host plants are defoliated before larvae complete their growth and development, some starve to death, this only happens to larvae

which do not find alternate hosts (*Carissa* spp., *Diospyros* spp., *Ficus* spp., *Sclerocarya* caffra, *Terminalia* spp. and *Trema* spp.) to feed on to complete their development (Ditlhogo, 1996).
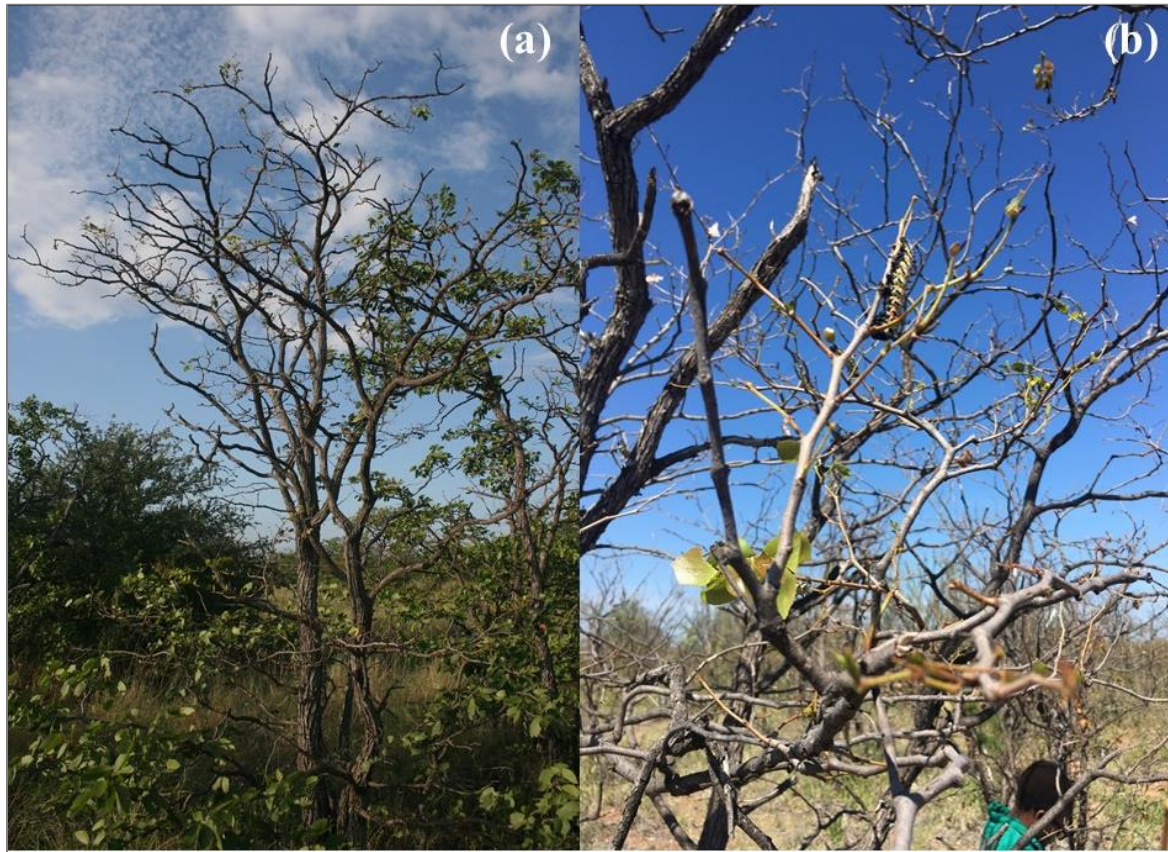
Figure 3: Mopane trees defoliated by worms (Source: Prof S.H Foord).

Habitat structure and host tree characteristics are important in the life cycle of many lepidopteran species (Ellingson & Andersen, 2002; Hrabar *et al.* 2009; Veldtman *et al*. 2007a). These host tree characteristics include; tree size, tree density, canopy size and tree connectivity (Williams *et al.* 2001; Hrabar *et al.* 2009). Population densities are reported to be higher in connected patches compared to isolated ones, patches with a high number of trees with large canopy size for sufficient food and trees tall in height for minimizing predation attack mostly by herbivores (Hrabar *et al.* 2009; Khaliq *et al.* 2015). Mopane female moth oviposition preference is likely in habitats with tall large trees with large canopy size (Hrabar *et al.* 2009).

Because of the income that can be generated through trading of worms, thousands of people are now engaged in harvesting (Stack *et al*. 2003; Thomas, 2013). Although this has not been quantified before, an increase in the number of harvesters is likely to lead to overharvesting. As

with any ecosystem service of a primary natural product, it becomes an ecological problem if people harvest more than they should (Rebe, 1999; Ashipala *et al.* 1996). Because the resources are shared there is also a competition for access, resulting in a tragedy of the commons, the common resource is not protected because harvesters maximize personal gain. This can be made worse by harvesting early instar worms which threaten future populations (e.g. Yen, 2015). The harvesting pressure continues from year to year in the absence of regulations for sustainable harvesting, with competition also rising between local people and outsiders (Ashipala *et al.* 1996; Lucas, 2011). A study done in Mexico showed that out of 30 edible insect species, a total of 14 species are threatened by the current level of harvesting because of the commercialization of their consumption (Ramos-Elorduy, 2006). During the mass harvesting process, host plants (mopane trees) experience branches breakage to reach mopane larvae (Stack *et al*. 2003; Gullan *et al.* 2005) as people attempt to speed up collection.

Except for human destruction, mammalian herbivores are responsible for changing habitat structure through grazing in fenced properties. In the mopane veld, elephants were documented as causing mopane tree destruction (De Klerk, 2009). They break branches, strip bark and roots which reduce the number of mopane trees, changes vegetation composition and delay tree growth rate in some cases (De Klerk, 2009). Given that worms predominantly feed on mopane tree leaves, the change in the composition of mopane veld will affect their survival as well as abundance.

Earlier work on domestication at farm-scale found that parasites, viral and bacterial diseases were major factors affecting mortality of mopane larvae (Gardiner, 2003). In order to circumvent irregular and unpredictable outbreaks (Hope *et al.* 2009), domestication was attempted at both the farm and household scale in Zimbabwe (Gardiner, 2003; Ghazoul, 2006 in Hope *et al.* 2009). Populations were maintained in shadehouses and protective shade cloth sleeves were used to cover branches of the host plant protecting larvae from heat, drought, and predation which contribute to larvae mortality in the wild (Gardiner, 2003). This was a large-scale (a farm) experiment which only ran for three years, implying that the long-term feasibility of the techniques used is still unknown.

This three-year study found that pupae can live for a maximum of two years (Gardiner, 2003). During the first year of the experiment, fifth instar larvae prior were collected and put in different soil substrate to test for the influence of the substrate on the survival of pupae. Sandy substrate had

a positive effect on the survival of pupae, however, a viral attack on the pupae was discovered. Subsequently, the use of boxes and tins in the previous studies were practiced to reduce mortality of pupae. These facilities helped to prevent the spread of viral diseases and parasitoid attack as pupae are isolated in small groups and stored within laboratories (Gardiner, 2003; Ghazoul, 2006 in Hope *et al.* 2009). For egg production, adult mating within boxes and egg house was effective compared to shadehouse and hand mating. Eggs were then stored in containers, some pinned and stapled to leaves within bags to protect them from parasites. Larvae were moved to trees covered with bags (sleeve nets), egg house and trees within shadehouses one day after hatching. Larvae within bags survived until second and third larval instar provided if conditions are not hot. The high mortality of larval stages (II-IV) was recorded in egg house while the survival rate of larvae was better in the shadehouse, while the combination of sleeve nets and the shadehouse was most successful (Gardiner, 2003). Larvae that were later added to captive populations introduced viral diseases, resulting in the collapse of the captive population. This together with the build-up of parasites and disease during the rainy season was the main threat to domesticated populations. The survival of larvae within shadehouses and those protected with sleeve nets were significantly higher than those in wild.

Small/household scale versions of the farm-scale study were done at three sites (Ndiweni, Kapeni and Dombodema) (Ghazoul, 2006 in Hope *et al.* 2009). Parasitic wasps (*Mesochomys pulchriceps* and *Pediobius sp)* were found to parasitize eggs of *G. belina* moths and identified during the collection of egg packets (Ghazoul, 2006 in Hope *et al.* 2009). Eggs were stored in tins, tied to trees and protected with 20% shade cloth, net sleeves and removed after the third larval stage as done by Gardiner (2003). It was difficult to assess the difference in larval survival between facilities as larvae move to neighboring trees for foraging however, larvae within bags were reported to develop better compared to control populations in the wild.

Soil type can also be an important determinant of pupal distributions of worms. This factor influences the number of larvae that pupate successfully (Gardiner, 2003). A study done on the emperor moth species, *Panolis flammea*, highlighted the importance of pupation substrate on pupal survival (Leather, 1984). An experiment was done to test soil substrate suitability on pupation of worms where different soils were placed in different boxes to assess the effect of substrate on pupal mortality. The fifth larval instars were placed on these boxes and preferred sandy soil for

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pupation (Gardiner, 2003). Gardiner (2003) explained that the high survival of pupa in sandy soils may be related to the low water retention capacity of the substrate, reducing drowning of the pupa. Sandy soils also do not produce a microclimate suitable for bacterial, fungal and viral growth which may detrimentally affect pupa survival (Gardiner, 2003). Dry (areas which are not waterlogged) and soft (not hard) conditions of sandy soil are more suitable for fifth instars to dig into when they molt into the pupa stage and allows for easier emergence (Gardiner, 2003).

Ghazoul (2006) recommended small-scale farming since large-scale were more vulnerable to viral and bacterial diseases. Small-scale (household) farming had a positive outcome and people from the communities were educated on how to farm worms. Both studies (Gardiner, 2003; Ghazoul, 2006 in Hope *et al.* 2009) highlighted the possibilities of unfavorable weather conditions playing a role during the domestication of mopane worms, although no climatic conditions records were made. The small-scale production(domestication) increase both subsistence consumption and commercial farming. It is therefore better to have many small-scale than a single large-scale in an area or a farm (Ghazoul, 2006 in Hope *et al.* 2009).

Given that attempts at increasing the survival and harvestable population of the mopane worm has had limited success, it is clear that the distribution patterns and spatial structure of populations should be better understood. This is particularly evident from the tendency of their natural enemies to heavily impact dense populations (positive density dependence) originating from sporadic population outbreaks (Gardiner, 2003; Ghazoul, 2006 in Hope *et al.* 2009). In contrast to their cultural and economic importance, very little quantitative data exists that explores their ecology and how populations are structured in space. Given that pests and diseases of mopane worms are strongly influenced by larval densities, any attempts to increase population numbers should be measured relative to natural population structure and density. This study will therefore aim to;

- o Develop an understanding of mopane larvae population spatial structuring at fine and medium scales contrasting areas where harvesting is not allowed with those that allow for commercial harvesting.
- o Develop techniques to increase availability and reduce variability in mopane worm supply for harvesting in both space and time under natural uncontrolled conditions by exploring;
  - ▪ The effectiveness of sleeve nets to reduce larval instar I -III mortality in the field.

- The effectiveness of transplanting (introducing eggs to unoccupied trees in areas occupied by *C. mopane*) and seeding (introducing eggs to areas unoccupied by any populations) of mopane worms.

CHAPTER 2: METHODOLOGY

2.1 Study area

Study sites were located in the Limpopo valley, south of the border between South Africa and Zimbabwe (Fig. 4a) within Musina local municipality (Fig. 4b). The climate of the area is characterized by summer rainfall with very dry winters and mean annual precipitation ranging between 300 to 400 mm (Mucina & Rutherford, 2006). The region is dominated by *Colophospermum mopane*, *Combretum apiculatum*, *Terminalia sericea* particularly in areas with deep sandy soils and *Grewia flava* which mainly grows in clumps of bush and in rocky areas (Mucina & Rutherford, 2006).

The study was conducted across three properties, Venetia Nature Reserve (Fig. 4c), Mapesu Private Game Reserve (Fig. 4e) and Joubert farm (Fig. 4f). Manipulative field experiments and the medium-scale distribution study of mopane worms was done in the Venetia Limpopo Nature Reserve (Fig. 4c). This reserve lies south of the Mapungubwe National Park and is owned by the De Beers group. It covers approximately 33 000 ha and includes megafauna such as elephant and rhinoceros (O'Connor, 2014).

Studies of fine-scale spatial structuring (Fig. 4d) of mopane egg packets and worms were done across three properties. Two of them, Venetia and Mapesu private game reserve were near each other (approximately 7Km) (Fig. 4b). No harvesting of larvae is allowed in either Venetia and Mapesu. The vegetation, topology, and climate of Mapesu (approximately 7 200 ha) are similar to that of the Venetia Reserve, the third property (Joubert farm, Fig. 4f) host commercial and subsistence harvesters whenever there is an outbreak. It is situated 125km east of the Venetia and Mapesu reserves and is also dominated by *C. mopane*.
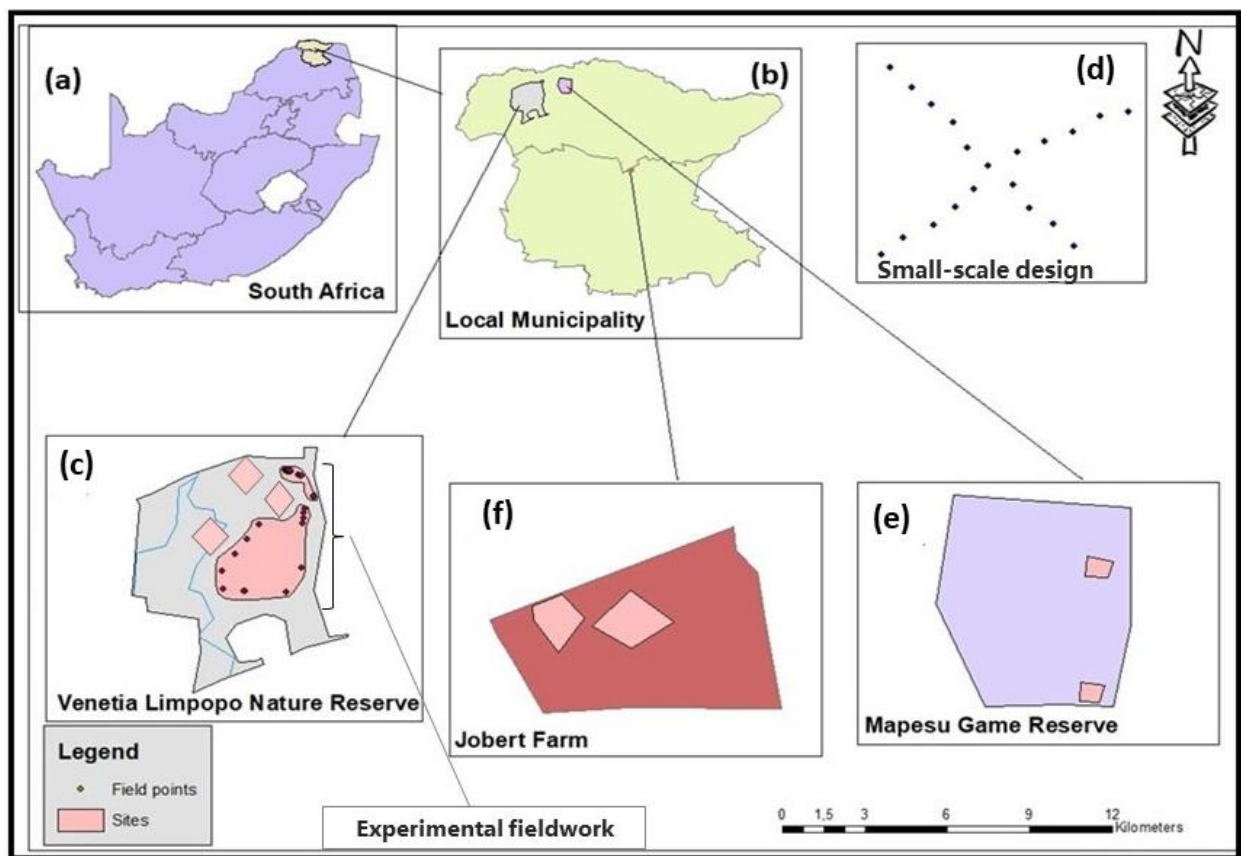
Figure 4: A map of South Africa (a) showing the local municipality (b), study sites -property (Venetia Limpopo Nature Reserve (c), Mapesu Game Reserve (e), Joubert farm (f), sampling design (d) and experimental field points.

2.2 Sampling designs

2.2.1 Manipulative field experiment
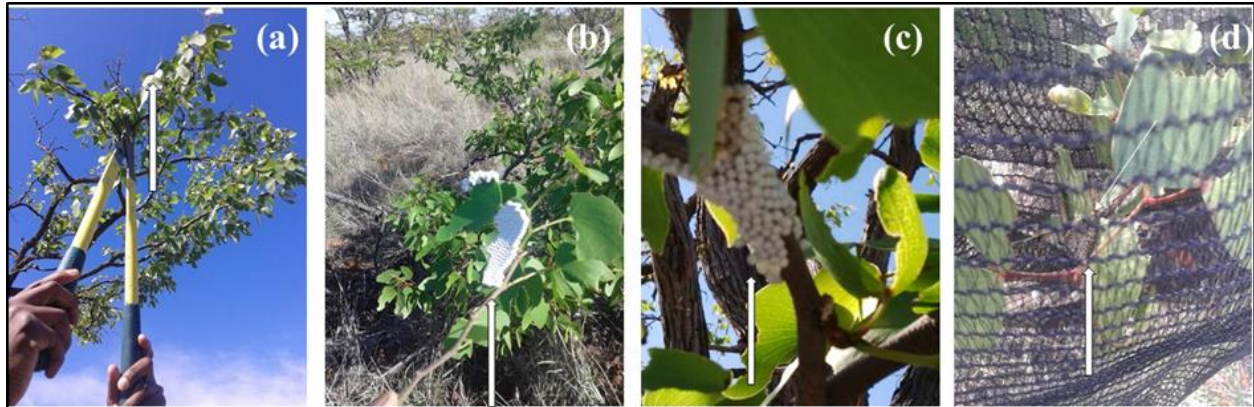
Figure 5: Cutting of mopane tree branches with eggs and 1st larval stage worms from occupied sites to transplant on to unoccupied trees within occupied site and seed some on unoccupied trees within unoccupied sites (a), mopane egg packets for transplant and seeding (b), control (c) and application of sleeve nets to transplants (transplant & sleeve nets) – eggs/1st instars transferred to unoccupied trees within occupied sites and application of sleeve nets to branches with eggs/1st instars found on occupied trees within occupied sites – sleeve nets (d) (source: Zwannda Nethavhani).

Experimental field trials were conducted at Venetia Limpopo Nature Reserve in November/December of 2017 (23 November - 18 December 2017). I focused on the eggs and early instars (I-III instar stages) before they disperse as they are easily monitored because of their aggregation behavior. In each site, mopane trees were scanned for 10 minutes and the presence or absence of mopane larvae or eggs noted. Based on this initial survey, a total of 21 sites were selected. Nine of these sites (which were > 300 meters apart) had mopane worms present and 12 sites (which were > 2 km apart) where they were absent (Appendix 3). For this trial, 116 trees were used at the nine sites where mopane worms were present, and 37 trees stratified across the 12 sites where it was absent (Appendix 4). Mopane worms were then exposed to either of these four tree treatments:

(a) Controls: Nine sites within the population outbreak were identified, in each site, three trees were chosen based on the availability of egg packets or larvae (27 trees in total). In each tree,

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a branch with egg packets/1st instar larvae was chosen and tagged with a ribbon to represent the control. All egg packets and larvae on a chosen branch were counted.

(b) Transplant experiments (introducing eggs to unoccupied trees in sites occupied by mopane woms worms): Within the nine sites mentioned above, 27 trees (three per site) which had no eggs or larvae, received egg packets tied to branches (one branch per tree).

(c) Sleeve trials: For five of the same nine sites mentioned above with mopane worms present, 15 trees (three per site) were chosen and one branch with egg packets or 1st instars were covered with sleeve nets (1m × 1m) made of 40% shade cloth, tied at the base of the branch to prevent predator access. At the other four sites, 10 trees without larvae (two trees in two sites and three trees in two sites) were chosen, egg packets or 1st instar larvae were tied to one branch of each tree and covered with a sleeve net tied at the base of the branch (Appendix 3 & 4).

(d) Seeding (introducing eggs to areas unoccupied by any mopane worm populations) experiments: Egg packets were seeded to 37 trees stratified across 12 sites (which were > 2 km apart) where there were no mopane larvae observed. Egg packets or 1st instar larvae were tied to a branch of each tree.

For every tree used in the tree treatment experiment, height, number of stems, canopy height and width (canopy volume) were recorded, all larvae in this experiment were monitored and counted weekly until they dispersed (IV instar). All nets were removed from *C. mopane* branches at the end of the third instar larval stage.

2.2.2 Fine-scale abundance and distribution

These surveys were done during the April 2018 population outbreaks. A total of seven sites were surveyed across three properties (Fig. 4c; e and f), three in Venetia (site A, B, and C), two in Mapesu (site D and E) and two on the Joubert farm (site G and P). Sites consisted of four 500m transects perpendicular to each other. Plots (10×10m) were set out at 100m intervals along each transect (Fig. 6). All trees within each plot were surveyed. The number of egg packets and their cardinal position on the tree was recorded for each tree as well as height, canopy height and width (canopy volume). The regular sampling design employed here improves the ability of the Moran's eigenvector used to model the spectral decomposition of spatial structures within the plots (Brind'Amour *et al*. 2018).
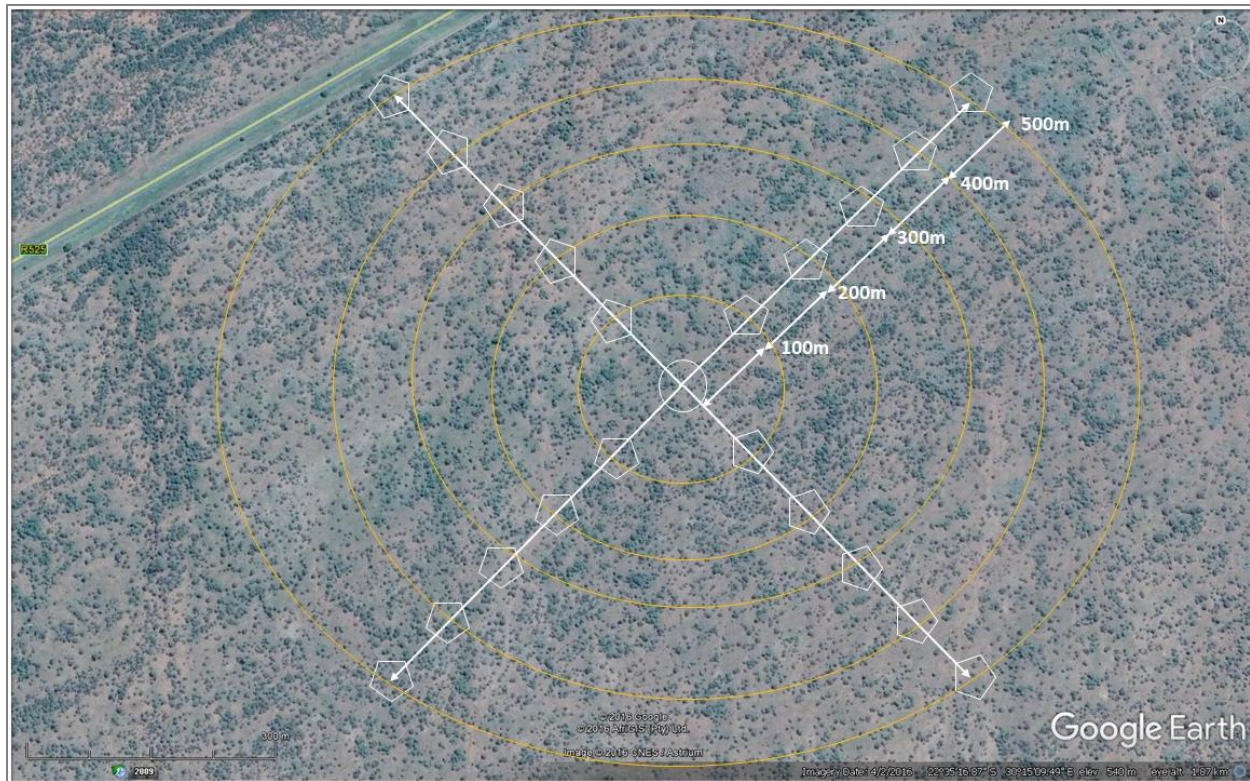
Figure 6: Fine-scale abundance and distribution sampling design performed in three properties (Venetia Limpopo Nature Reserve, Mapesu Private Reserve and Joubert Farm).

2.2.3 Medium-scale sampling

As mentioned earlier, outbreaks of worm populations in Venetia are concentrated in certain areas. Both the November and April outbreaks were largely concentrated to the north-eastern corner of the reserve. The remaining ¾ of the reserve was largely unaffected by the outbreak although these areas were almost completely dominated by *C. mopane*. Outbreaks seem to be localized at large scales and although unpredictable in time, they seem to be predictable in space with major population outbreaks recurring in specific areas (pers comm, from both the reserve managers). This survey was done at the end of the April 2018 population outbreaks. Leaf damage (defoliation) on *C. mopane* trees were noted at 123 plots (10m × 10m) which were > 2 km apart and stratified across the Venetia reserve (Fig. 7.1a & b), to quantify the availability of mopane worms in the reserve. Each plot was scanned for ten minutes, and larval damage per tree was recorded: high (completely defoliated tree), medium (worms fed upon large parts of a tree but not completely defoliated), low (only a small part of a tree was fed upon, mostly at the tips) and no damage (tree was not fed upon). Then, defoliation per tree was given scores; high = 3 (high defoliation is equivalent to high larval abundance), medium = 2 (a lower number of mopane worms fed on these trees – medium larval abundance), low = 1 (low defoliation – a very low number of mopane worms fed on these trees – low larval abundance), nothing = 0 (no leaf damage indicates no mopane worm fed on these trees) and these scores were used as an index of larval abundance per plot. We used the average abundance per plot for analysis. Out of 123 sampled plots, 33 soil samples were taken from randomly selected plots using a soil corer (Fig. 8). Soil samples consisted of four subsamples (~15 cm deep each) (Figure 8). These soil samples were mixed and sent for analysis to the Agricultural Research Council – Institute for Soil, Climate and Water (ARC – SCW) laboratory in Pretoria for concentrations of K, Ca, Mg, P and Na, pH and texture (percentage sand, silt, and clay).
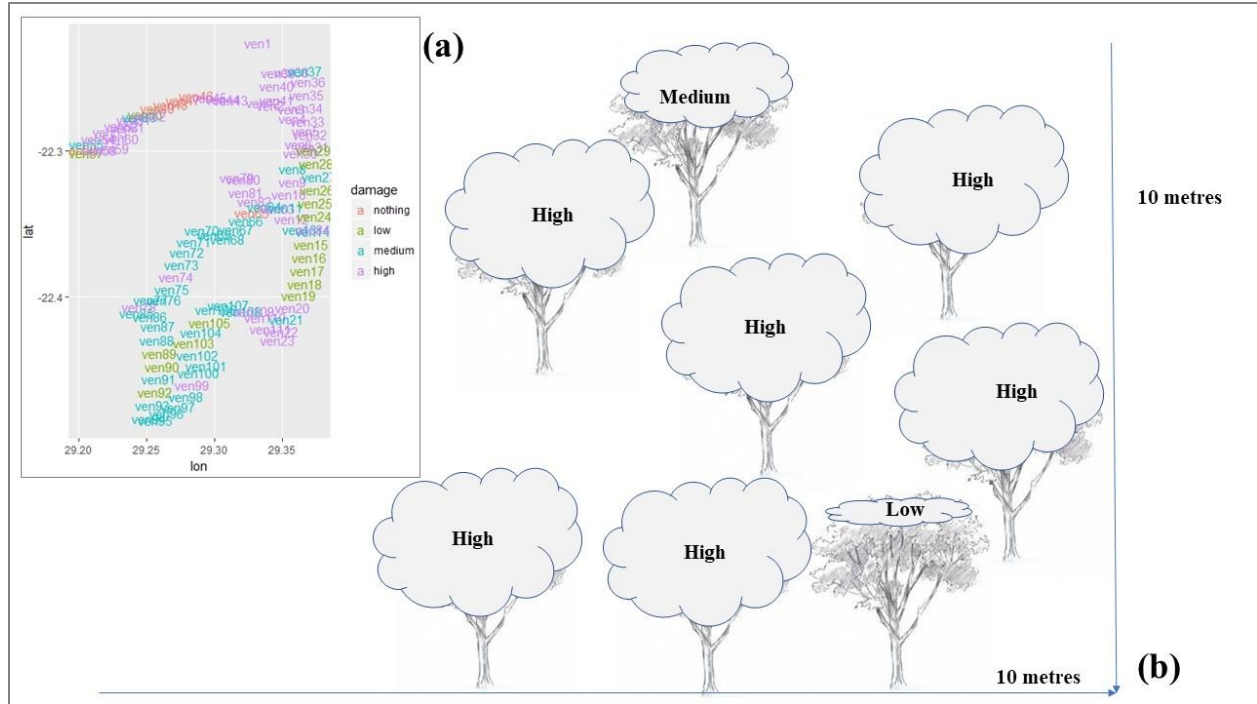
Figure 7.1: Medium-scale sampling design, representing tree defoliation (high-completely defoliated tree, medium-worms fed upon large parts of a tree but not defoliated, low-only a small part of a tree was fed upon, mostly at the tips and no damage-tree was not fed upon) (b), with scores (3, 2, 1 and 0 respectively) representing larval abundance per site from 123 sites which were > 2 km apart (a), 'ven' code represents a site and color represents overall defoliation per site (high-purple, medium-blue, low-blue and nothing-red).
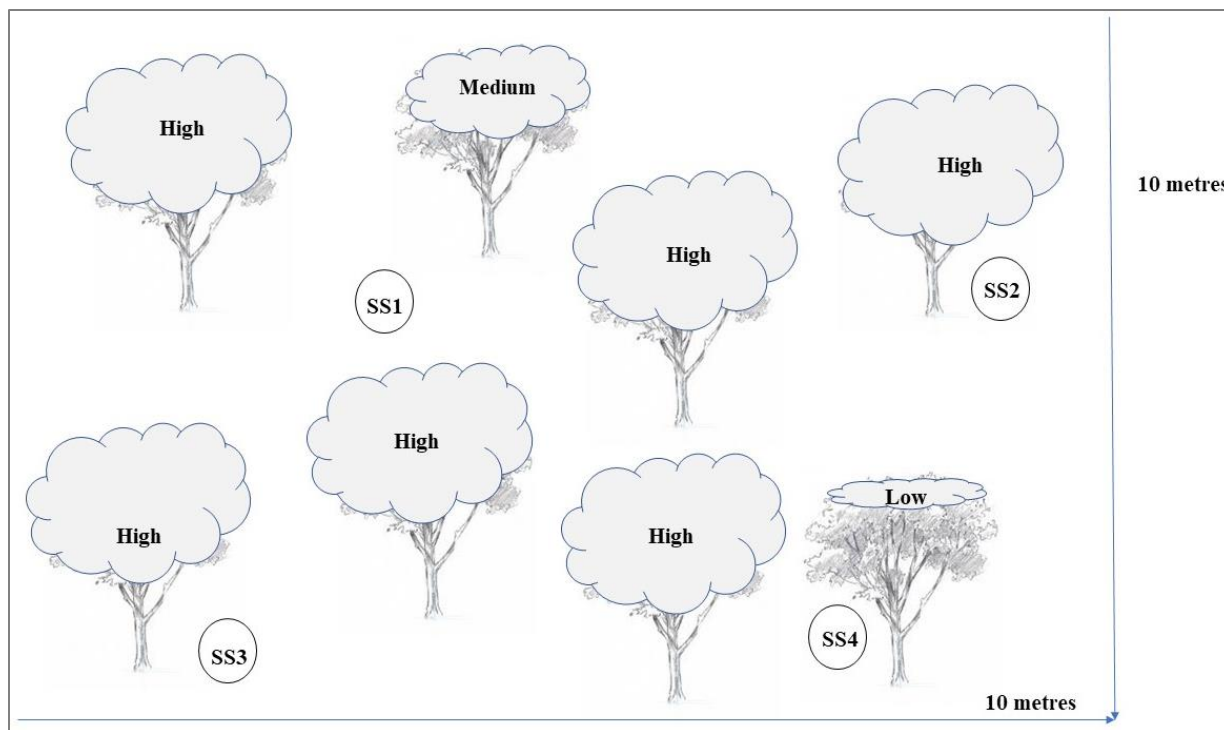
Figure 7.2: Sampling design, representing tree defoliation (high - completely defoliated tree, medium - worms fed upon large parts of a tree but not defoliated, low - only a small part of a tree was fed upon, mostly at the tips and no damage-tree was not fed upon), with scores (3, 2, 1 and 0 respectively) representing larval abundance per site from 33 sites which were randomly selected. SS1 – SS4 represent four subsoil samples taken per site.

Figure 8: Soil sampling in 33 plots, soil sample collection(a) and mixing soil subsamples in a bag (b) (Source: Zwannda Nethavhani).

2.3 Statistical analysis

All analyses were done using R (R core team, 2018).

2.3.1 Field trials

The response of larval abundance to treatments (seeding, transplant, sleeves, and transplants & seeding) was modelled using generalized linear mixed models (GLMM) with the function 'glmer' in the package lme4 (Douglas *et al.* 2015) using a log-link function with Poisson error distributions, site (21 groups) was included as a random factor. All model residuals were inspected for assumptions including heteroscedasticity, normality, independence, and overdispersion.

Analysis for each period was done separately. The relationship between larval abundance and tree characteristics were modelled using generalized linear models (GLM) with the function 'lmer' in the package lme4 (Douglas *et al.* 2015) with a quasi-poisson distribution for overdispersion.

2.3.2 Fine-scale spatial distribution of egg packets

The seven sites were analyzed individually. The response of egg packet abundance to tree height, canopy volume, tree density, and space was modelled using generalized linear models (GLM) with a log-link function and a quasi-poisson distribution as the data were over-dispersed. Spatial relations were modelled using Moran's Eigenvectors Maps (MEMs – Appendix 5) as predictor variables in the package adespatial (Dray *et al*. 2018). MEMs are orthogonal synthetic variables that provide a decomposition of the spatial relationships among sampling sites based on a spatial weighting matrix (Dray *et al.* 2012). They decompose spatial relationships to components (eigenvectors) representing variation at specific spatial scales. Each axis of the MEM defines a synthetic periodic pattern of spatial variation, automatically arranged from large to fine-scale patterns related to the eigenvalue of the MEM axis (Dray *et al.* 2006). The outcome of a set of n sampling sites will be n-1 MEM axes, enough to complete the spectral decomposition of space. Positive eigenvalues represent positive spatial autocorrelation. This spectral decomposition can then be used to represent spatial relationships between study sites in statistical analysis.

The first step in deriving MEMs is to create the spatial neighborhood or connectivity matrix for the sampling design based on the geographical coordinates of sites. Gabriel graphs were used to define the neighborhood of plots in each of the sites using the function 'gabrielneigh' in the package spdep (Bivand *et al.* 2013). A spatially weighted matrix was then created based on distances between plots using the function 'nb2listw' in the package spdep.

Moran's eigenvector maps (MEMs) were computed using the function 'mem' in the package adespatial (Dray *et al*. 2018). The significance of the Moran's I for each of the eigenvectors was calculated and only the significantly positive eigenvectors retained, i.e. eigenvectors that represent significant positive spatial autocorrelation. These MEMs were then included as spatial predictors in the model of egg packet abundance. The function 'glmutli' in the package glmulti (Vincent, 2013) was used to identify the best combination of MEMs. The interactions of MEMs were not included in the model. The MEMs of the best model were then combined with tree metrics and analyzed using GLM to identify the combination of environmental (tree height, density, canopy

volume and number of stems) and spatial (MEMs) that best-explained egg packet abundance. The best and next best models were compared using analysis of deviance. If models did not differ significantly the simplest model was retained as the best model.

2.3.3 Medium-scale distribution and abundance of larvae

The relation between larval presence/absence and soil properties in 33 sites were modelled using generalized linear models (GLM) with the function 'lme' in the package lme4 (Douglas *et al.* 2015) using logit-link function and binomial error distribution. Collinearity between soil parameters was explored using Pearson's correlation coefficients between each of the variables (Appendix 6) with a correlation coefficient larger than 0.5 were considered co-linear, and the only the variable considered to biologically more relevant was retained. For this analysis; P, Na, pH, and Sand were retained.

The spatial relations in 33 sites with soil correspondence data and 123 sites without soil data were modelled using MEM. The significance of the Moran's I for each of the eigenvectors was calculated and only the positive significant eigenvectors retained and used as spatial predictors. The function 'glmutli' in the package glmulti was used to identify the best combination of MEMs that explaining the spatial distribution in the presence of larvae. These MEMs were then combined with soil metrics and analyzed with glmulti to identify the combination of soil properties and spatial (MEM's) that best-explained mopane larvae presence. The best and next best models were compared using analysis of deviance. If models did not differ significantly the simplest model was retained as the best model.

CHAPTER 3: RESULTS

3.1 Experimental field trials

At the start of the experiment, all categories had significantly fewer larvae than the control sites (Table 1, Fig. 9). Seeding sites had lower larval abundance compared to other treatments because most of the seeded populations were eggs (Table 1). The abundance of larvae covered with sleeves decreased relative to the control over the period of the study, while the abundance of larvae in treatments that were both transplanted and covered sleeves marginally increased relative to that of control at sites (Table 1). Transplants on their own seem to have been the least successful while seeding performed the best with the abundance of larvae approximating that of the control sites after three weeks (Table 1). Of all tree characteristics included in the analysis, tree height was the only variable that was significantly related to larval abundance, with larval abundance increasing related to tree height (Table 2).

Table 1: Modelled (GLMM) response of larvae to four different treatments with site as a random factor over a period of three weeks. The reference category was control

Treatments	23/11/2017	05/12/2017	18/12/2017
Intercept	5.432 (***)	4.200 (***)	3.955 (***)
Seeding	-4.025 (***)	-0.286 (*)	-0.068
Sleeve	-0.089 (*)	-0.281 (***)	-0.287 (***)
Sleeve & transplant	-0.247 (***)	-0.214 (***)	-0.134 (**)
Transplant	-0.496 (***)	-0.477 (***)	-0.503 (***)
	Model: larvae abundance ~ treatments + (1|site)		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
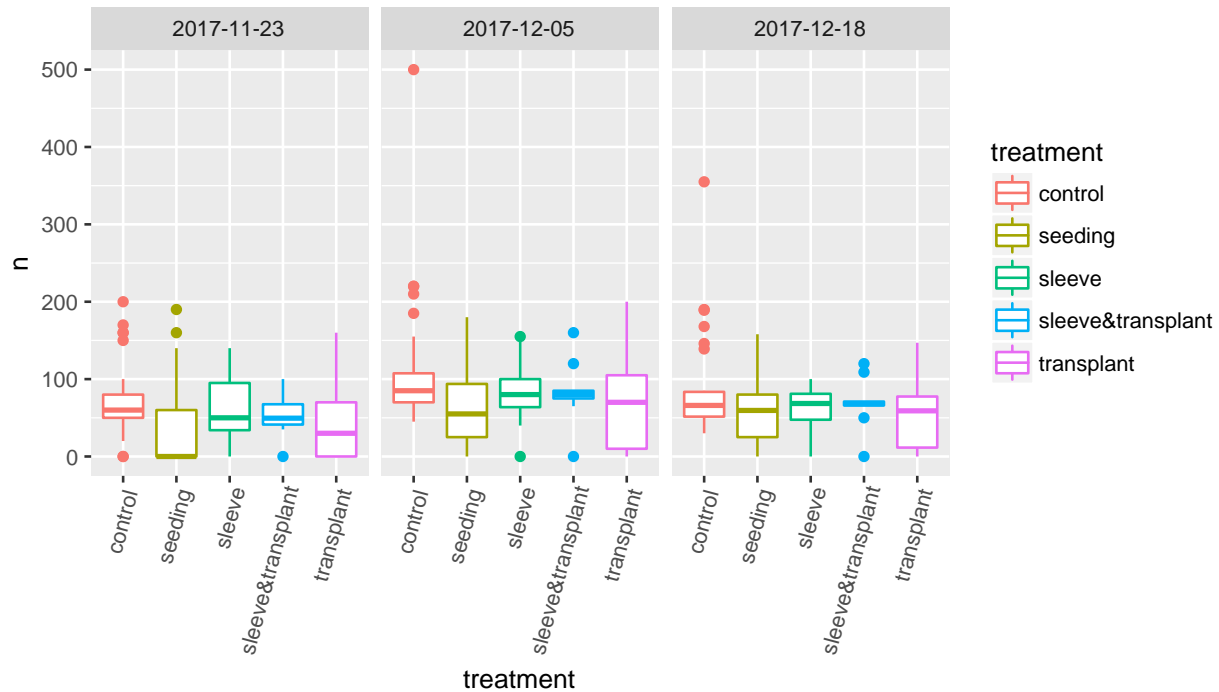
Figure 9 Abundance of mopane larvae (n) in control, transplanted, seeded, sleeved, sleeve and transplanted sites, monitored (counts of mopane larvae) for four weeks with three counts during the November/December outbreak of 2017.

Table 2: Modelled (GLM) response of larval abundance to different tree characteristics over a period of three weeks. Reference variable was an intercept.

Response variables	Estimates
Intercept	3.669±0.198
Tree height	0.104±0.049 *
Stems	-0.037±0.022
Canopy volume	0.001±0.001

'*' $P < 0.05$

3.2 Fine-scale egg packet abundance and distribution

Egg packets at five of the seven sites (three in Venetia and two at Mapesu – where no commercial harvesting was permitted) were spatially structured with significant positive spatial autocorrelation at various scales (Table 3). MEMs included in the models were the larger eigenvectors which

suggest large and medium scale spatial patterns within the plots. The best model for site A included two MEMs and a significant negative relationship with canopy volume. Egg packet abundance was positively related to two MEMs as well as canopy volume (Table 3). Space alone explained the distribution and abundance of egg packets in site B and included only MEM2 and MEM3. Similarly, site D included two large scale MEMs (MEM1 and MEM3) but also MEM5, that point to finer scale structuring of egg packets in this site. While site E had two finer scale MEMs (MEM6 and MEM7) included in its best model. Egg packets at the Joubert farm had no spatial structuring and none of the environmental variables explained the observed distributions (Table 3).

Table 3: Generalized Linear Model with quasi-Poisson error distribution showing the effect of space and host tree characteristics on egg packets abundance at fine scales.

Site	Model to explain egg pack abundance	AIC (best)	AIC (second best)
A	~ MEM1** + MEM3 + canopy volume** Egg packet abundance ~ $e^{0.009(\pm0.005)MEM1 +}$ 0.602($\pm$0.196)MEM3 - 0.268($\pm$ 0.207)canopy volume	126.29	127.25
B	~ MEM2 + MEM3* Egg packet abundance ~ $e^{0.279(\pm0.311)MEM2}$ - 0.721($\pm$0.311)MEM3	73.27	73.27
C	~ MEM1 + MEM6+ canopy volume Egg packet abundance ~ $e^{-0.152(\pm0.209)MEM1 +}$ 0.056($\pm$0.0.236)MEM4 + 0.006($\pm$ 0.002)canopy volume	120.94	121.86
D	~MEM1* + MEM3* + MEM5 Egg packet abundance ~ $e^{0.542(\pm0.302)MEM1 +}$ 0.663($\pm$0.302)MEM3 - 0.008($\pm$ 0.302)MEM5	68.63	68.66
E	~ MEM6 + MEM7 Egg packet abundance ~ $e^{0.224(\pm0.219)MEM6+}$ 0.387($\pm$0.219)MEM7	82.96	83.92
G	-	-	-

P	-		-		-

Significant codes: < 0.001 '***'; 0.01 '**'; 0.05 '*'; 0.1 '.' 0.1;' ' >0.1

3.3 Medium-scale patterns in the larval distribution

The significant terms in the Generalized Linear Model (GLM) fitted to the larvae per site were related to three soil variables (soil elements, pH and texture). The larval presence had a positive and significant relationship with space and sodium concentration (Table 4a) for the best model. There was a negative relationship between larval presence, sand and potassium while space was positively correlated with larval abundance in the second-best model (Table 4a).

Table 4a: Two best fitting GLMs with Poisson distribution showing the effect of soil elements, pH and soil texture on larvae abundance.

Response variable	Model to explain larvae abundance	AIC
larval abundance	~ Na* + MEM1* + MEM3* + MEM11** larvae abundance $\sim e^{\,0.288(\pm 0.154)Na\,+\,0.314(\pm 0.211)MEM1\,+\,0.247(\pm 0.189)MEM3}$	68.78
larval abundance	~MEM1 + MEM11* + Sand + P larvae abundance $\sim e^{\,0.199\,(\pm 0.100)\,MEM1\,+\,0.217\,(\pm 0.087)\,MEM11\,-\,0.015\,(\pm 0.098)\,Sand\,-\,0.037\,(\pm 0.098)\,P}$	75.89

Significant codes: < 0.001 '***'; 0.01 '**'; 0.05 '*'; 0.1 '.' 0.1;' ' >0.1

Modelled response for the observed presence of larvae/larvae abundance across Venetia Limpopo Nature Reserve (123 sites) point to considerable spatial structuring. (Table 4b).

Table 4b: Best fitting GLM with Poisson distribution showing the effect of space on larvae abundance in all 123 sites.

Response variable	Model to explain larvae abundance
larval abundance	~ MEM6*** + MEM11*** + MEM12* + MEM17*** + MEM19* + MEM21*** + MEM31***
	larvae abundance ~ $e^{0.362(\pm0.054)MEM6 - 0.202(\pm0.054)MEM11 + 0.113(\pm0.054)MEM12 + 0.256(\pm0.054)MEM17 + 0.117(\pm0.054)MEM19 - 0.226(\pm0.054)MEM21 - 0.227(\pm0.054)MEM31}$

Significant codes: < 0.001 '***'; 0.01 '**'; 0.05 '*'; 0.1 '.' 0.1;' ' >0.1

CHAPTER 4: DISCUSSION

This study took the first step in understanding the spatial structuring and domestication of mopane worms. Spatial structuring of mopane worm populations at the finest scale of this study, (< 1 ha) was evident at all the sites where they were not commercially harvested (VLNR & Mapesu) while no spatial structuring was evident for the site where commercial harvesting takes place on an annual basis (Joubert farm). Although ambiguous and only included into models for two of the plots – the only other variable that accounted for egg packets distribution was canopy volume. There was significant spatial structuring of worms at medium scale. The density of worms increased with an increase in sodium. Unexpectedly, worm densities decreased with increase in sand and higher levels of potassium. Seeding of worms was successful in establishing new populations in 100 % of unoccupied areas. The transplant of worms to increase the biomass in occupied areas was relatively unsuccessful. Densities of worms on transplant technique kept on decreasing relative to control and other techniques throughout the experiment. Application of sleeve nets to natural populations did not have any significant effect on reducing mortality of worms for the first three instar stages. Interestingly, transplants of worms protected with sleeve nets (sleeve net & transplant) in occupied areas improved relative to transplant only (transplant), during the period of the study and worm density was positively related to tree height.

4.1 Spatial structuring at fine and medium scales

This is the first study to explore the spatial structuring of mopane worms at various scales, evidence for positive spatial autocorrelation of mopane worm abundance was observed at both fine and medium scales. The spatial structuring observed at fine and medium scale disappears during commercial harvesting.

Environmental variables played a limited role in explaining the distribution of egg packets and mopane worm larvae (Table 3) and were limited to five instances where tree density had a positive impact on egg packet density and one instance of tree canopy volume (Table 3). This suggests that trees are selected for oviposition because of their proximity to other host trees. It is possible that egg-burdened female moths do not have great flight distances which result in moths depositing eggs on trees which are in close proximity to where they have emerged, as explained by the strong spatial structuring of egg packets (e.g. *Gonometa* species female moth, Veldtman *et al.* 2007b). As much as 20 egg packets were observed on a single tree during the study. Although processes behind the oviposition behavior pattern of female mopane moths are currently unclear, Hrabar *et al.* (1999)

found that female moths prefer trees with large canopy volume for oviposition. The direction of the wind in the current study might be one of the processes behind mopane female moth oviposition behavior, and larvae distribution (Greyling & Potgieter, 2004 cited in Thomas, 2013). This implies that when female moths emerge from pupae, they mate and fly to the direction of the wind (in a swarm activity) where they deposit eggs on available host trees irrespective of a tree being occupied before. The potential for intra specific competition between larvae when eggs hatch does not seem to affect female choice of oviposition trees. In contrast, galling sawfly females avoid oviposition sites which are already used, this result in the regular dispersion of eggs deposited and less of competition on larvae when they hatch (Price *et al.* 1995).

However, a male moth might have the ability to fly far (from one subpopulation to another) to mate with other female moths which influences gene flow between these populations. Similar to males of a related species, they fly some distance for mating with females (Veldtman *et al.* 2007, and has also been documented for *Gonometa podocarpi*, see Okelo 1972).

Although this study found limited evidence for the role of canopy volume in affecting egg packet abundance, Hrabar *et al.* (2009) showed that canopy volume was the primary determinant of oviposition sites although they did not include a spatial component in their study. Ballabeni *et al.* (2001) & Khaliq *et al.* (2015) also argued that oviposition is bound to occur on and near trees that provide larvae with enough food for survival and growth. Furthermore, oviposition behavior (related to canopy volume) is the function of larval distribution for sufficient food source and avoiding natural enemies (Hrabar *et al.* 2009). Thus, the distribution of larvae will be determined by the distribution of deposited eggs which is governed by large tree canopies.

The spatial structuring and environmental control of mopane worm abundance were absent from sites where commercial harvesting takes place. Although it was not quantified, overharvesting of worms results in the lack of spatial structuring. Mostly, a small number of remaining isolated adults' larvae after harvesting survives to pupate with the collapse in spatial structuring. Arguably it is the clustering of larvae that attract harvesters in the first place, as it increases the ease of collection during harvesting. The consequence of large-scale commercial harvesting on the ability of populations to persist over time is something that requires further study.

The commercial harvesting of mopane worms in an area implies the availability even in a smaller number of the resource even though factors governing their availability and density remain

unknown. Previous work has recorded high sodium concentrations in the body and gut content of mopane worm larvae (Pillay, 2016). This may translate to a high concentration of sodium in feces, hence soil with high sodium concentration (increased through feces of mopane worm) might approximate the distribution of this species. This indicates the significance of Lepidoptera larvae that produce large quantities of frass as ecosystem engineers (i.e. Hartland-Rowe, 1992; Styles, 1996). In the current study, deposited feces would have considerable influence on the environment because most trees in these sites were aggregated (high density) with high canopy volumes. Stands with *C.mopane* trees are expected to have natural populations of mopane worms since the distribution of mopane worms is known to follow that of the host tree as their life-cycle fully depend on these trees (Ditlhogo, 1996; Styles, 1996). Results from the current study showed that not all mopane veld have natural populations but when they appear, they are mostly aggregated. Contrary to the current study, none of the soil elements were significantly related to the distribution of both mopane trees and *G. belina* (Mufandaedza *et al.* 2018) while clay soil texture was found to govern the distribution of mopane trees.

The second-best model also showed that worms were spatially structured. Sandy soil and phosphorus explained the distribution of worms even though the relationship was negative. This relation contradicts other studies where sandy soil is considered the best texture for larvae to pupate and emerge (Gardiner, 2003; Ghazoul, 2006 in Hope *et al.* 2009). It is possible that these factors are more important for the occurrence and distribution of *C. mopane* trees than the mopane worm, hence the contrasting findings reported here.

4.2 Manipulation techniques (treatments)

Having few larvae in transplant treatment could have resulted from the numerical response of predators and increased prevalence of disease. Natural enemies (predators and parasites) aggregate in high number in prey hotspot because they have particularly critical senses for prey location (Walde & Murdoch, 1988; Hassell, 2000; Veldtman & McGeoch, 2004). The numerical response of predators and parasites profoundly affects, and possibly even regulate the numbers of prey (Price, 1997; Padmalatha *et al.* 2003; Johns *et al.* 2016).

An increase in prey density increases predators' survival and reproduction resulting in an increase of the predator population, which in turn eats more prey, e.g. as larch sawfly (*Pristiphora erichsonii*) was preyed upon by birds (Buckner & Turnock's 1965) and as the density increased, the numerical response increased through reproduction and immigration. Fifty percent of the

34

silkworm (*G. rufobrunnea)* eggs were attacked by parasites while up to 70% of young larvae were preyed upon by insect predators in the natural system (Hartland-Rowe, 1992). More than 90% of *G. rufobrunnea* pupae was predated in an outbreak with a large number of pupae per tree (Veldtman *et al.* 2007b).

In contrast to other mopane worm studies (Styles, 1995; Ditlhogo *et al.* 1995; Styles & Skinner, 1996), the presence of parasites and viral disease were noted as main factors reducing populations throughout mopane worm life cycles (Gardiner, 2003). In the present study, no vertebrate predators mentioned in previous studies were observed feeding on worm populations, only large amounts of unidentified parasitoid wasps bred out of collected eggs (Appendix 7). Larvae were only monitored up to the third instar, however, and the natural sources of mortality mentioned above may have been missed later in the life cycle of monitored populations. Parasitoids (from orders Diptera and Hymenoptera) and diseases have also been reported as main factors responsible for the mortality of wild silkworm larval stages (Veldtman *et al*. 2004; Mbahin *et al*. 2010).

Several studies found the significant improvement in survival of larvae protected by sleeve nets (Gardiner, 2003; Riana, 2004; Mbahin *et al.* 2010). The use of sleeve nets was found to be effective in preventing access of natural enemies to natural (wild) populations of silkworm (*Aphane panda*) (Mbahin *et al.* 2010), as the number of larvae recorded was higher in sleeve nets compared to larvae in the wild systems. Sleeve nets were effective also in reducing the mortality rate in early instar stages of mopane worms, most particularly sleeve nets applied within built shadehouses, this combination prevented easy access of major invertebrate and vertebrate predators (Gardiner, 2003).

Application of sleeve nets to tree branches only in the occupied area failed to reduce the mortality of young instar stage larvae. Insect such as ants and stinkbugs were observed in occupied sites and there may have been an opportunity to access the applied sleeve nets which may be responsible for preying on mopane worms adding to a mortality rate of young instars. This indicates that the material of the net was not appropriate for this experiment (the mesh size was too big to exclude small natural enemies). However, other possibilities are that eggs were attacked before application of sleeve nets which might be the reason for larval density reduction in this technique throughout the experiment. The attack before application of sleeve nets is evidence of natural enemy response in an occupied area (Walde & Murdoch, 1988; Hassell, 2000). Parasitic egg wasps may track high

egg densities in mopane veld. Thus, with the availability of parasites on eggs, sleeve nets could not be effective on population which was already parasitized. This may thus require that eggs are sourced from adult moths that are mated in captivity so as to exclude egg parasitoids. Such a strategy would require further scientific study.

Due to the aggregated distribution of *G. belina* moth eggs, large tracts of mopane veld did not have mopane worms at all. Areas with less or no prey have fewer or no natural enemies and referred to as "enemy-free space" (Veldtman *et al.* 2007b). Insects could therefore deposit their eggs on non-host trees to gain refuge from natural enemies making them less predictable to find (Novotny *et al.* 1999). *Gonometa rubofrunnea* was abundant on non-host trees (Veldtman *et al.* 2007b) and there were reduced parasitoid attacks on larvae of *Liriomyza helianthin* that utilized novel plants (Gratton& Welter, 1999). These taxa contrast with that of Mopane worms however, whose foodplant occurs in large tracts of almost continuous mopane trees with localized mopane worm outbreaks. The absence of natural enemies in unoccupied tracts of mopane veld could explain why seeding in these areas was relatively more successful than any of the other treatments. Interestingly, populations were established in 100% of the sites where they were introduced through seeding. Hartland-Rowe (1992) observed similar trends in *Gonometa rufobrunnea,* a large moth that feeds on mopane trees and undergoes outbreaks (Veldtman *et al.* 2007b) although their seeding was successful in establishing new populations in only 50% of the sites. Seeding of mopane worms was successful sites from which they were absent (Ghazoul, 2006 in Hope *et al.* 2009). It has been shown that natural enemy responses can in part be predicted by insect herbivore life history (Veldtman *et al.* 2007b).

Sandy soil texture dominated areas with natural populations which might be evidence that it is an important factor in the distribution of mopane worms at an area, hence the hardness of the soil explains the sustainability of newly established populations during pupation. This appeared to be true, particularly in areas with natural and reared populations of worms (Gardiner, 2003). Sandy soil was found to be suitable soil texture for burrowing of worms prior to pupation and emergence of pupae (Gardiner, 2003). All populations were only monitored up to the third instar and cannot be used to infer long-term establishment. Further studies should focus on monitoring the complete cycle that includes pupal survival.

There is no literature on spatial structuring of mopane worms and little on their domestication which call for further quantitative studies on mopane worms. Mopane worm populations are randomly distributed in space. This pattern is due to various factors resulting in spatially patchy structure, suggesting certain interactions within and between the species and the factors. In the current study, mopane worm dynamics are largely driven by both top-down processes (more specifically natural and human predation) and bottom-up processes (tree density and soil characteristics playing a very limited role). The spatial structure of mopane worms might differ with different land use categories due to both top-down and bottom-up factors available in a land use category. Therefore, additional study sites with different land use categories (privately owned-cattle farm, conservation areas, and communal land) may be important in identifying all possible factors driving the population dynamics of mopane worms at various scales. This will include quantifying these factors (e.g. natural, herbivores, human predation, the density & connectivity of host trees, soil texture and environmental conditions) and their effects on mopane worm population dynamics in different land use categories. Such information would also be beneficial to aid the domestication process, explain the environmental importance of mopane worms and give directions on how to conserve the worms and their natural habitats. With comprehensive information on lifecycle, dynamics, movement and behavior from further studies, spatial pattern models to estimate and predict events in locations not sampled before may be developed.

Information on the impact of regulations on sustainable harvesting of mopane larvae and utilization mopane trees is also lacking. A recent questionnaire study showed a significant relationship between land category and presence of regulations governing the harvesting process in Zimbabwe (Mufandaedza *et al*. 2013). There was a significant difference in the quantity of worm harvested between the reserve and communal area, farm and communal area, resettlement area and the communal area which might be brought by presence and absence of regulations on these properties. Hence harvesting must be monitored to ensure that harvesters do not deplete the resource and its host. The presence of regulations will further help in the conservation of habitats (mopane veld) and mopane worms.

Future studies must also investigate the relatedness (genetic interchange) between the localized populations. Studies on Nuclear DNA could give an insight into the movement of mopane male moths. Currently, no genetic data studies exist. This will require the development of nuclear

markers. Although such studies are labor intensive and expensive, requiring genotyping of large numbers of samples to be able to draw any conclusions, this work of this insect species is required (pers comm, from Dr. Barbara van Asch).

References

Adelabu, S., Mutanga, O., & Cho, M. A. (2012). "A review of remote sensing of insect defoliation and its implications for the detection and mapping of *Imbrasia belina* defoliation of mopane woodland," *African J. Plant Science and Biotechnology*, 6, 1–13.

Akpalu, W., Muchapondwa, E., & Zikhali, P. (2009). Can the restrictive harvest period policy conserve mopane worms in southern Africa? A bioeconomic modelling approach. *Environment and Development Economics*, 14(05), 587-600.

Anankware, P. J., Fening, K. O., Osekre, E., & Obeng-Ofori, D. (2015). Insects as food and feed: a review. *Int J Agric Res Rev*, *3*(1), 143-151.

Ballabeni, P., Conconi, D., Gateff, S., & Rahier, M. (2001). Spatial proximity between two host plant species influences oviposition and larval distribution in a leaf beetle. *Oikos*, 92(2), 225-234.

Ballantine, J. (2000). Man Eating Bugs: The Art and Science of Eating Insects/Material World: A Global Family Portrait. *Teaching Sociology*, *28*(3), 261.

Bivand, R. S., Hauke, J., & Kossowski, T. (2013). Computing the Jacobian in Gaussian spatial autoregressive models: An illustrated comparison of available methods. *Geographical Analysis*, 45(2), 150-179.

Buckner, C. H., & Turnock, W. J. (1965). Avian predation on the larch sawfly, *Pristiphora erichsonii* (Htg), (Hymenoptera: Tenthredinidae). *Ecology*, 46(3),223-236.

Charles, H., Godfray, J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M. and Toulmin, C. 2010. Food Security: The Challenge of Feeding 9 Billion People. Science (327) 812.

Chen, X.M., Feng,Y., & Zhang, H. (2008*)* Review of the nutritive Value of Edible Insects: In Forest Insects as Food: Humans Bite Back (eds. P.B. Durst, D.V. Johnson, R.N. Leslie & K. Shono), *Chiang Mai, Thailand*, 85–92.

Cohen, J. E. (2003). Human population: in the next half century. *Science*, 30 (5648), 1172-1175.

Collinge, S. (2010). Spatial Ecology and Conservation. *Nature,* 1(69):24

DeFoliart, G. R. (1995). Edible insects as minilivestock. *Biodiversity & Conservation*, 4(3), 306-321.

DeFoliart, G.R. (2002). The human use of insects as food resource: a bibliographic account in progress. Wisconsin, USA, Department of Entomology, University of Wisconsin-Madison.

De Klerk, M. (2009). Individual based modelling: a study of the correlation between Mopane caterpillar outbreaks and the movement of African elephants.

Delang, C. O. (2006). Economic valuation of non-marketed wild edible plants in Thailand. *Environmental Conservation*, 32(4), 285-287.

Ditlhogo, M. (1996). The ecology of Imbrasia belina (Westwood) in north-eastern Botswana. In *Proceedings of the first multidisciplinary symposium on phane*,18, 46-68.

Dovie, D. B., Shackleton, C. M., & Witkowski, T. F. (2002). Direct-use values of woodland resources consumed and traded in a South African village. *The International Journal of Sustainable Development & World Ecology*, 9(3), 269-283.

Dray, S., Legendre, P., & Peres-Neto, P. R. (2006). Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbor matrices (PCNM). *Ecological Modelling,* 196,483-493.

Dray, S., Pélissier, R., Couteron, P., Fortin, M. J., Legendre, P., Peres-Neto, P. R., & Dufour, A. B. (2012). Community ecology in the age of multivariate multiscale spatial analysis. *Ecological Monographs*, *82*(3), 257-275.

Dray, S., David, B., Guillaume, B., Daniel, B., Sylvie, Clappe, G. G., Thibaut, J., Guillaume, L., Pierre, L., Naima, M., & Helene H Wagner (2018). adespatial: Multivariate Multiscale Spatial Analysis. R package version 0.3-0. https://CRAN.R-project.org/package=adespatial

Douglas, B., Martin, M., Ben, B., & Steve W. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1-48. doi:10.18637/jss.v067.i01.

Dzerefos, C. M., Witkowski, E. T. F., & Toms, R. (2013). Comparative ethnoentomology of edible stinkbugs in southern Africa and sustainable management considerations. *Journal of Ethnobiology and Ethnomedicine*, *9*(1), 20.

Egan, B. A., Olivier, P. A. S., & Addo-Bediako, A. (2009). Towards integrating indigenous knowledge on culturally significant insects into local schools. *Calabash: Indigenous Studies Journal*, *3*, 65-79.

Egan, B. A. (2013). Culturally and economically significant insects in the Blouberg Region, Limpopo Province, South Africa (Doctoral dissertation, University of Limpopo).

Ehler, L. E. (2002). An evaluation of some natural enemies of *Nezara viridula* in northern California. *BioControl,* 47, 309-325.

FAO (Food and Agricultural Organization) (2003). Non-wood news: Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO. (2009b). Biodiversity and nutrition, a common path. Rome.

FAO. (2010a). Forest insects as food: humans bite back Proceedings of a workshop on Asia-Pacific resources and their potential for development 19-21 February 2008, Chiang Mai, Thailand.

FAO. (2010b). Biodiversity and sustainable diets: united against hunger. Report presented at World Food Day/World Feed Week, 2-5 November 2010, Rome.

Food and Agriculture Organization (FAO), 2013. The contribution of insects to food security, livelihoods and the environment. FAO, Rome, Italy.

FAO. (2015). http://www.fao.org/forestry/edibleinsects/en/

Gahukar, R.T. (2011). Entomophagy and human food security, *International Journal of Tropical Insect Science*. 3(30), 129-144.

Gardiner, A.J. (2003) Internal Final Report: Mopane Woodlands and the Mopane Worm: Enhancing Rural Livelihoods and Resource Sustainability, DFID Project No. R7822. The Domestication of Mopane Worms (*Imbrasia belina*). Veld Products Research & Development, Gaborone, Botswana.

Gashe, B. A., & Mpuchane, S. F. (1996). Phane: Proceedings of the First Multidisciplinary Symposium on Phane. *Gaborane, Botswana: University of Botswana*.

Gerland, P., Raftery, A. E., Ševčíková, H., Li, N., Gu, D., Spoorenberg, T., Alkema, L., Fosdick, B. K., Chunn, J., Lalic, N., Bay, G., Buettner, T., Heilig, G. K., & Wilmoth. J. (2014). World population stabilization unlikely this century. *Science,* 346, 234-237.

Ghazoul, J. (2006). Mopane woodlands and the mopane worm: enhancing rural livelihoods and resource sustainability. *Forest Research Programme Report*, 7822.

Gondo, T., Frost, P., Kozanayi, W., Stack, J., & Mushongahand, M. (2010). Linking knowledge and practice: assessing options for sustainable use of mopane worms (Imbasia belina) in southern Zimbabwe. *Journal of Sustainable Development in Africa,* 12(1), 281-305.

Gratton, C., & Welter, S. C. (1999). Does "enemy-free space" exist? Experimental host shifts of an herbivorous fly. *Ecology*, 80(3), 773-785.

Greyling, M., & Potgieter, M. (2004). Mopane worms as a key woodland resource: the use, trade and conservation of *Imbrasia belina*. *Indigenous forests and woodlands in South Africa: policy, people and practice*, 575-589.

Gullan, P.J., Cranston, P.S., & McInnes, K.H. (2005). *The Insects*, Wiley-Blackwell.

Halloran, A., Muenke, C., Vantomme, P., & van Huis, A. (2014). Insects in the human food chain: global status and opportunities. *Food Chain,* 4(2), 103-118.

Hanboonsong, Y., Jamjanya, T., & Durst, P. B. (2013). Six-legged livestock: edible insect farming, collection and marketing in Thailand. *RAP publication*, *3*.

Hartland-Rowe, R. (1992). The biology of the wild silkmoth *Gonometa rufobrunnea* Aurivillius (Lasiocampidae) in north-eastern Botswana, with some comments on its potential as a source of wild silk. *Botswana Notes and Records*, *24*, 123-133.

Hassel, M. P. (2000). Host-parasitoid population dynamics. *Animal Ecol*ogy 69, 543-566.

Hobane, P. A. (1995). The gathering, processing, consumption and trade of edible caterpillars in Bulilimamangwe District. *CASS Occasional Paper*, 67.

Hooimeijer, J.P., Jansen, F.A., De Boer, W.F., Wessels, D.C.J., Van Der Waal, C., De Jong, C.B., Otto, N.D., & Knoop, L. (2005). The diet of kudus in a mopane dominated area, South Africa. *Koedoe* 48: 93–102.

Hope, R. A., Frost, P. G., Gardiner, A., & Ghazoul, J. (2009). Experimental analysis of adoption of domestic mopane worm farming technology in Zimbabwe. *Development Southern Africa*, 26(1), 29-46.

Hoskin, S. O., Lobley, G. E., Coop, R. L., & Jackson, F. (2002). The effect of cysteine ad glutamine supplementation on sheep infected with *Trichostrongylus colubriformis*. In *Proceeding-New Zealand Society of Animal Production*, 62, 72-76.

Hrabar, H., Hattas, D., & Du Toit, J. T. (2009). Intraspecific host preferences of mopane moths (Imbrasia belina) in mopane (*Colophospermum mopane*) woodland. *African Zoology*, 44(2), 131-140.

Illgner, P., & Nel, E. (2000). The Geography of Edible Insects in Sub-Saharan Africa: a study of the Mopane Caterpillar. *The Geographical Journal*, 166(4), 336-351.

Ji, Y.J., Kong, X.F., & Yin, Y.L. (2012). Insect nutritional values and its application in livestock and poultry production. *Natural Product Research and Development*, 24, 220–223.

Johns, R. C., Flaherty, L., Carleton, D., Edwards, S., Morrison, A., & Owens, E. (2016). Population studies of tree-defoliating insect in Canada: a century in review. *The Canadian Entomologist*, 148(S1), S58-S81.

Johnson, D.V. (2007). The contribution of edible forest insects to human nutrition and to forest management: current status and future potential, with emphasis on the Asia-Pacific Region. Workshop Background Report. 74 pp.

Jones, V. P. (1995). Reassessment of the role of predators and *Trissolcus basalis* in biological control of southern green stink bug (Hemiptera: Pentatomidae) in Hawaii. *Biol. Control*, 5, 566-572

Kamanga, P., Vedeld, P., & Sjaastad, E. (2009). Forest incomes and rural livelihoods in Chiradzulu District, Malawi. *Ecological Economics*, 68(3), 613-624.

Khaliq, A., Amad, M. H., Ullah, R., & Anas, M. (2015) Behavioral ecology of oviposition in insect-A dumpy review. Sky Journal of agricultural research, 4(1), 001-007.

Klok, C., & Chown S. (1999). "Assessing the benefits of aggregation: thermal biology and water relations of anomalous Emperor Moth caterpillars." *Functional ecology*, 13(3), 417-427.

Kinyuru, J.N., Kenji, G.M. & Muhoho, S.N. (2010). Nutritional potential of longhorn grasshopper (*Ruspolia differens*) consumed in Siaya District, Kenya. *Journal of Agriculture, Science and Technology*, 12(1), 1–24.

Koppel, A. L., Herbert, D. A., Kuhar, T. P., & Kamminga. K. (2009). Survey of stink bug (Hemiptera: Pentatomidae) egg parasitoids in wheat, soybean, and vegetable crops in southeastern Virginia. *Environmental Entomology,* 38, 375-379.

Kwiri, C. W., Muredzi, P., Tongonya, J., Gwala, W., Mujuru, F., & Sha, R. (2015). Mopane worm (Gonimbrasia belina) utilisation, a potential source of protein in fortified blended foods in Zimbabwe: A review. *Glob. J. Sci. Front. Res*, 14.

Li, Q., Zheng, L., Cai, H., Garza, E., Yu, Z., & Zhou, S. (2011). From organic waste to biodiesel: black soldier fly, *Hermetia illucens*, makes it feasible. *Fuel*, 90 (4), 1545–1548.

Lucas, T. L. (2011). *The evolution and impacts of mopane harvesting: perceptions of harvesters in central Botswana* (Doctoral dissertation).

Madibela, O. R., Mokwena, K. K., Nsoso, S. J., & Thema, T. F. (2009). Chemical composition of Mopane worm sampled at three sites in Botswana and subjected to different processing. *Tropical animal health and production*, 41(6), 935-942.

Makhado, R. A., Von Maltitz, G. P., Potgieter, M. J., & Wessels, D. C. (2009). Contribution of woodland products to rural livelihoods in the northeast of Limpopo Province, South Africa. *South African Geographical Journal*, 91(1), 46-53.

Makhado, R., Potgieter, J. M., Wessels, D., Saidi, A., & Masehela, K. (2012). Use of mopane woodland resources and associated woodland management challenges in rural areas of South Africa. *Ethnobotany Research and Application*, 369-380.

Makhado, R., Potgieter, M., Timberlake, J., & Gumbo, D. (2014). A review of the significance of mopane products to rural people's livelihoods in southern Africa. *Transactions of the Royal Society of South Africa*, 69(2), 117-122.

Makhado, R., Potgieter, J. M., & Luus-Powell, W. (2016). Nutritional value of *Colophospermum mopane* as source of browse and its chemical defences against browsers: a review. *Journal of Animal and Plant Science*, 26(3).

Mbahin, N., Raina, S. K., Kioko, E. N., & Mueke, J. M. (2010). Use of sleeve nets to improve survival of the Boisduval silkworm, Anaphe panda, in the Kakamega Forest of western Kenya. *Journal of insect science*, *10*(1), 6.

Meyer-Rochow, V. B. (2005). Traditional food insects and spiders in several ethnic groups of Northeast India, Papua New Guinea, Australia, and New Zealand. *Ecological implications of minilivestock: Rodents, frogs, snails, and insects for sustainable development*, 385-409.

Mucina, L., & Rutherford, M. C. (2006). The vegetation of South Africa, Lesoto and Swaziland. Strelitzia 19. South African National Biodiversity Institute, Pretoria. *Memoirs of the Botanical Survey of South Africa*.

Mufandaedza, E., Moyo, D. Z., & Makoni, P. (2013). Management of Non-Timber Forest Products Harvesting: Rules and Regulations Governing (*Imbrasia belina*) Access in South-Eastern Lowveld of Zimbabwe. *Academia Journal of Agricultural Research,* 1(10), 193-203.

Mufandaedza, E., Moyo, D. Z., & Makoni, P. (2018). Effect of Soil Type and Foliar Factors on the Distribution of *Imbrasia belina* in the South-Eastern Lowveld of Zimbabwe. *Scientifica,* 2018.

Musvoto, C., Mapaure, I., Gondo, T., Ndeinoma, A., & Mujawo, T. (2007). Reality and references in community mopane (Colophospermum mopane) woodland management in Zimbabwe and Namibia. *International Journal of Social Sciences*, 1(3), 173-177.

Newton, L., Sheppard, C., Watson, D.W., & Burtle, G. (2005). Using the black soldierfly, *Hermetia illucens*, as a value-added tool for the management of swine manure. *Animal and Poultry Waste Management Center, North Carolina State University, Raleigh, NC*, 17.

Nonaka, K. (2007). Hebo yellow jackets: from the fields to the dinner table: a delightful culinary experience.

Nonaka, K. (2009). Feasting on insects. (Special issue: trends on the edible insects in Korea and Abroad.). *Entomological Research,* 39(5): 304–312.

Novotny, V., Basset, Y., Auga, J., Boen, W., Dal, C., & Drozd, P. (1999). Predation risk for herbivorous insects on tropical vegetation: a search for enemy-free space and time. *Australian Journal of Ecology*, 24, 477–483.

Ohiokpehai, O., Bulawayo, B. T., Mpotokwane, S., Sekwati, B., & Bertinuson, A. (1996). Expanding the use of mopane, a nutritionally rich local food. *In Proc. Multidisciplinary Symposium. Mopane. University of Botswana and Kalahari Conservation Society*, 111-130.

Oberprieler, R. G. (1995) The Emperor Moths of Namibia. *Sigma Press, Pretoria.*

O'Connor, T. G., & Page, B. R. (2014). Simplification of the composition, diversity and structure of woody vegetation in a semi-arid African savanna reserve following the re-introduction of elephants. *Biological conservation*, *180*, 122-133.

Padmalatha, C., Singh, A. J. A. R., & Jeyapauld, C. (2003), Predatory potential of syrphid predators on banana aphid, *Pentalonia nigronervosa* Coq. *Journal of Applied Zoology*. 14, 140-143.

Paoletti, M.G., Buscardo, E., Vanderjagt, D.J., Pastuszyn, A., Pizzoferrato, L., Huang, Y.S., Chuang, L.T., Glew, R.H., Millson, M. & Cerda, H. (2003). Nutrient content of termites (*Syntermes* soldiers) consumed by Makiritare Amerindians of the Alto Orinoco of Venezuela. *Ecology of Food and Nutrition*, 42(2): 177–191.

Pelser, A. J., & Kherehloa, T. (2000). Some causes and strategies pertaining to land degradation in southern Africa. South African Journal of Agricultural Extension, 29(1), 24-46.

Pillay, S. (2016). Mopane worms (*Imbrasia belina*) as indicators of elemental concentrations in a trophic system (Doctoral dissertation)

Pinhey, E. C. (1972). Emperor Moths of south- and south-central Africa.

Potts, S. G., Ngo, H. T., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V., Garibaldi, L. A., & Vanbergen, A. (2016). The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production.

Ramos-Elorduy, J. (2006). Threatened edible insects in Hidalgo, Mexico and some measures to preserve them. *Journal of Ethnobiology and Ethnomedicine*, *2*(1), 51.

Pokorny, B., Johnson, J., Medina, G., & Hoch, L. (2012). Market-based conservation of the Amazonian forests: revisiting win–win expectations. *Geoforum*, *43*(3), 387-401.

Price, P. W. (1997). *Insect ecology*. John Wiley & Sons.

Rebe, M. (1999) *The Sustainable Use of Mopane Worms as a Harvestable Protein Source for Human Consumption: Local Perceptions*, MSc thesis, University of Pretoria, South Africa.

Roberts, C. (1998). Long-term costs of the mopane worm harvest. *Oryx*, 32: 6-8.

R Core Team (2018). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*. URL https://www.R-project.org/.

Rumpold, B. A., & Schlüter, O. K. (2013). Nutritional composition and safety aspects of edible insects. *Molecular nutrition & food research*, *57*(5), 802-823.

Sekonya, J. G. (2016). *Mopane worm use, livelihoods and environmental change in Limpopo Province, South Africa* (Masters dissertation, University of Cape Town).

Shackleton, C., & Shackleton, S. (2004). The importance of non-timber forest products in rural livelihood security and as safety nets: a review of evidence from South Africa. *South African Journal of Science*, 100(11-12), 658-664.

Shackleton, S., Campbell, B., Lotz-Sisitka, H., & Shackleton, C. (2008). Links between the local trade in natural products, livelihoods and poverty alleviation in a semi-arid region of South Africa. *World Development*, 36(3), 505-526.

Shisana, O., Labadarios, D., Rehle, T., Simbayi, L., Zuma, K., Dhansay, A., Fabe,r M., & the SANHANES-1 Team. (2013). *South African National Health and Nutrition Examination Survey* (SANHANES-1),145.

Stack, J., Dorward, A., Gondo, T., Frost, P., Taylor, F., & Kurebgaseka, N. (2003). Mopane worm utilisation and rural livelihoods in Southern Africa. In *international conference on rural livelihoods, forests and biodiversity, Bonn, Germany,* 19-23.

Styles, C. V. (1995). Notes on the bird species observed feeding on mopane worms. *Birding in Southern Africa,* 47, 53-54.

Styles, C. V., & Skinner, J. D. (1996). Possible factors contributing to the exclusion of saturniid caterpillars (mopane worms) from a protected area in Botswana. *African Journal of Ecology*, 34(3), 276-283.

Styles, C.V. (1996) 'The biological ecology of *Imbrasia belina* (Saturnidae) with reference to its behaviour, physiology, distribution, population dynamics, impact within mopane veld and utilization within South Africa', in B.A. Gashe and S.F. Mpuchane (eds), *Proceedings of the First Multidisciplinary Symposium on Phane. Gaborone, Botswana: NORAD.*

Tao, J., & Li, Y. O. (2018). Edible insects as a means to address global malnutrition and food insecurity issues. *Food Quality and Safety*, *2*(1), 17-26.

Taylor, F. (2003). Mopane worm utilisation and rural livelihoods in Botswana. Preliminary field survey report.

Te Velde, D. W., Rushton, J., Schreckenberg, K., Marshall, E., Edouard, F., Newton, A., & Arancibia, E. (2006). Entrepreneurship in value chains of non-timber forest products. *Forest Policy and Economics*, 8(7), 725-741.

Teffo, L. S., Toms, R. B., & Eloff, J. N. (2007). Preliminary data on the nutritional composition of the edible stinkbug, *Encosternum delegorguei Spinola*, consumed in Limpopo province, South Africa. *South African Journal of Science*, 103(11-12), 434-436.

Thomas, B. (2013). Sustainable harvesting and trading of mopane worms (*Imbrasia belina*) in Northern Namibia: an experience from the Uukwaluudhi area. *International Journal of Environmental Studies*, 70(4), 494-502.

Toms, R. B., & Nonaka, K. (2005). Harvesting of insects in South Africa and Japan—Indigenous knowledge in the classroom. *Online: www. scienceinafrica. co. za.*

Van Huis, A. (2013) Potential of insects as food and feed in assuring food security. *Annual Review of Entomology*, 58, 563–583.

Van Huis, A., Itterbeeck, J.V., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013) Edible Insects: Future Prospects for Food and Feed Security. *Food and Agriculture Organization of the United Nations*, Rome.

Veldtman, R. & McGeoch, M. A. (2004). Spatially explicit analyses unveil density dependence. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(1556), 2439–2444.

Veldtman, R., McGeoch, M.A., & Scholtz, C.H. (2004). Parasitoids of southern African wild silk moths (Lepidoptera). *African Entomology*, 12, 117–122.

Veldtman, R., McGeoch, M. A., & Scholtz, C. H. (2007a). Fine-scale abundance and distribution of wild silk moth pupae. *Bulletin of Entomological Research*, 97(1), 15-27.

Veldtman, R., McGeoch, M. A., & Scholtz, C. H. (2007b). Can life history and defence traits predict the population dynamics and natural enemy responses of insect herbivores? *Ecological Entomology*, 32, 662-673.

Vincent, C. (2013). glmulti: Model selection and multimodel inference made easy. R package version 1.0.7. https://CRAN.R-project.org/package=glmulti.

Walde, S. J., & Murdoch, W. W. (1988). Spatial density dependence in parasitoids. *Annual Review of entomology*, *33*(1), 441-466.

Wang, D., Zhang, Q., & Zhai, S.W. (2006) Advances in the application of insect protein, chitosan and fatty acids to animal nutrition and feed. *Journal of Northwest Forestry University*, 21, 135–138.

Williams, I. S., Jones, T. H., & Hartley, S. E. (2001). The role of resources and natural enemies in determining the distribution of an insect herbivore population. *Ecological Entomology,* 26, 204-211.

Yen, A. L. (2009). Entomophagy and insect conservation: some thoughts for digestion. *Journal of Insect Conservation*, 13(6), 667-670.

Yen, A. L. (2015). Insects as food and feed in the Asia Pacific region: current perspectives and future directions. *Journal of Insects as Food and Feed,* 1(1), 33-55.

Yhoung-Aree, J., Puwastien, P., & Attig, G.A. (1997). Edible insects in Thailand: an unconventional protein source? *Ecology of Food and Nutrition*, 36, 133–149.

Yong-woo, L. (1999). Silk reeling and testing manual. *FAO Agricultural Services Bulletin*, 136.

Zhou, Q., Li, Z.R., Liu, J., Lin, Q., Wang, C.K., & Jiang, X. (2009). Effect of *Periplaneta americana* meal on immunity and antioxidation of broliers. *Journal of Fujian Agriculture and Forestry University (Natural Science Edition)*, 38, 175–180.

Appendices

Appendix 1 Distribution of *Colophosphermum mopane* (blue icons) and sites visited with mopane worm outbreaks in 2016 and 2017 (yellow icons).
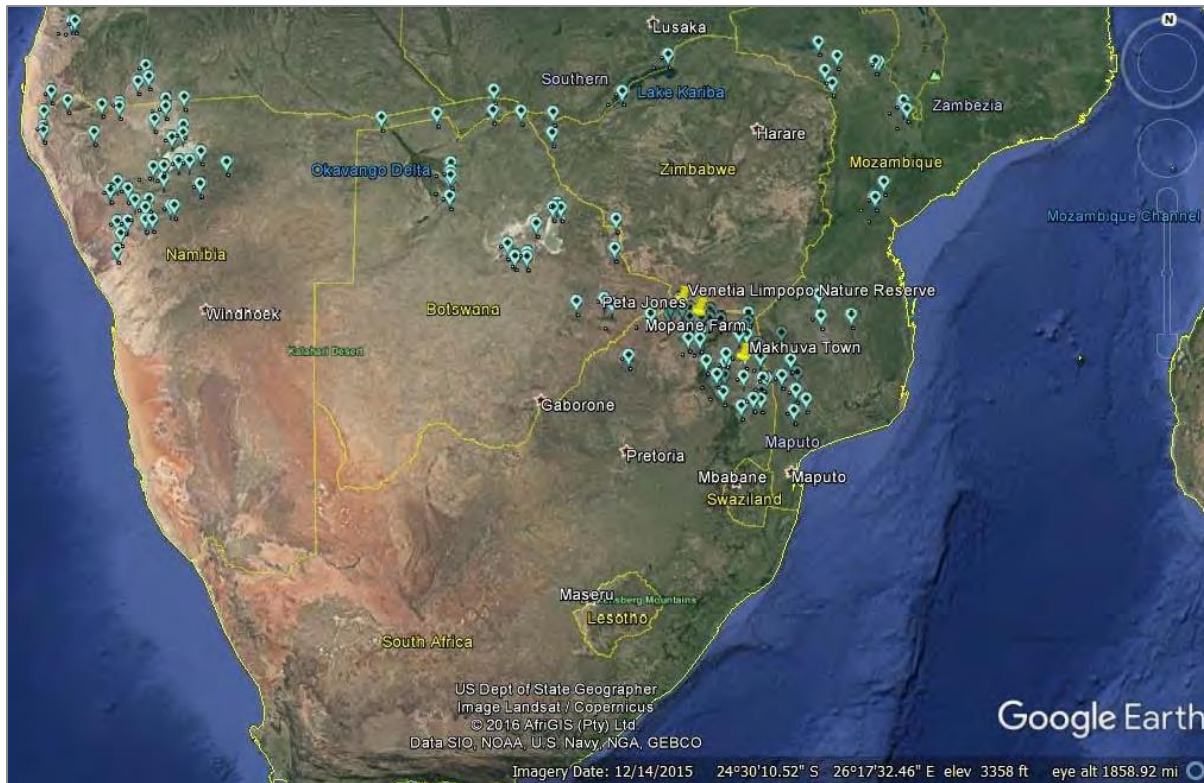
**Appendix 2** Mopane worm feces deposited on the ground

Appendix 3 Field trial design with control as a reference category, transplant indicating moving eggs and 1st larval stage worms to unoccupied tree within occupied sites, sleeve representing application of nets to branches with eggs and 1st larval stage worms, sleeve & transplant representing application of nets to transplanted eggs and 1st larval stage worms and seeding treatment, referring to moving eggs and 1st larval stage worms to unoccupied sites.

| Mopane present	Number of sites	Trees/site	Total trees
Control	9	3 (in 9 sites)	27
Transplant	9	3 (in 9 sites)	27
Sleeve	5	3 (in 5 sites)	15
Sleeve & transplant	4	2 (in 2 sites) + 3 (in 2 sites)	10
Mopane absent	**Number of sites**	**Trees/site**	**Total trees**
Seeding	12	3 (in 11 sites) + 4 (in 1 site)	37

Appendix 4 Sampling design in sites with mopane worms population (S – sleeve net, C -control, T – transplant, ST – sleeve net & transplant). Each letter represents a tree within nine sites (which were > 300 meters apart) with worm population

Appendix 5 Moran Eigenvector Maps (MEMs for fine-scales study – for all seven sites), the increase in size of squares correspond to increasing positive value (black) and increase in negative values (white) of the eigenvector (each square represents a plot). Plots with same color indicate similarity in the abundance of mopane worms, and the size of squares indicate the level of similarity, thus small squares represent low similarity while large ones represent high similarity.
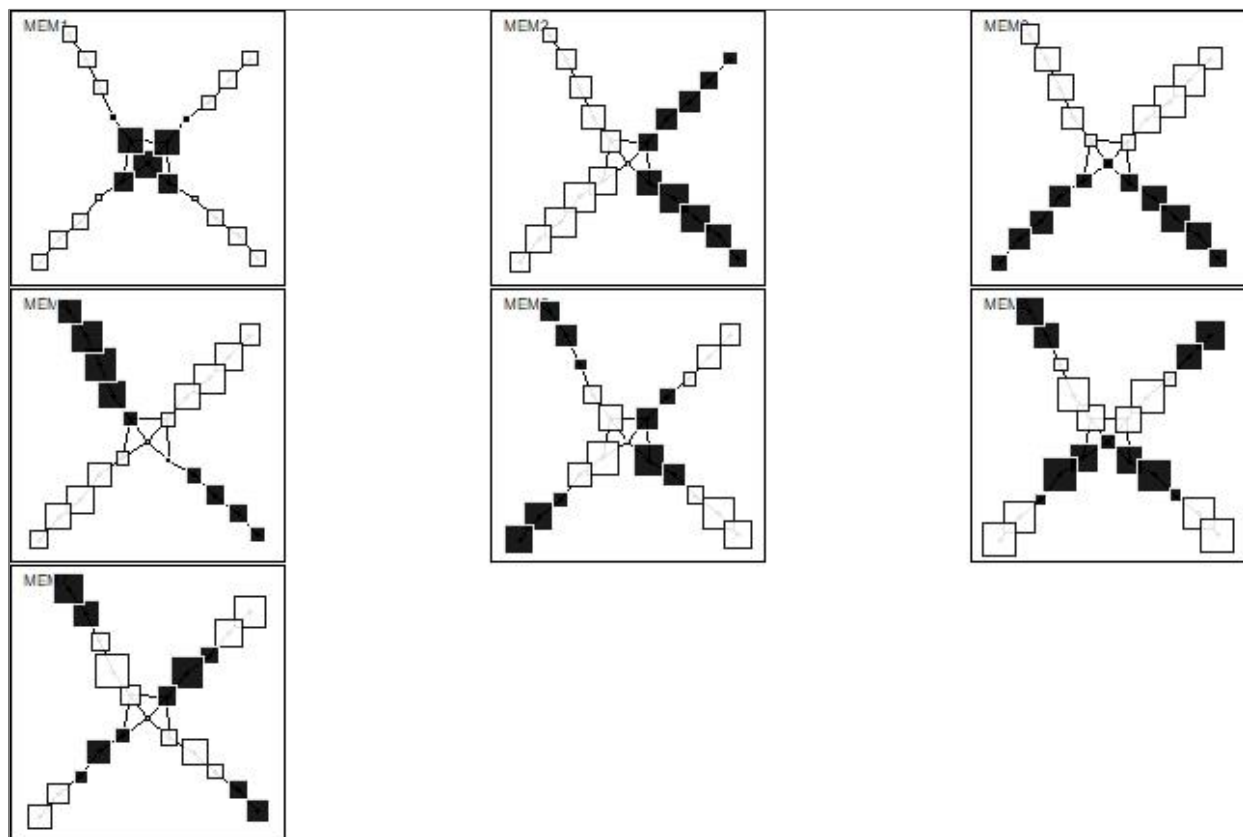
Appendix 6 Correlation coefficients between variables which were soil elements (K, Ca, Mg, P and Na), pH and soil texture percentage (sand, silt and clay)
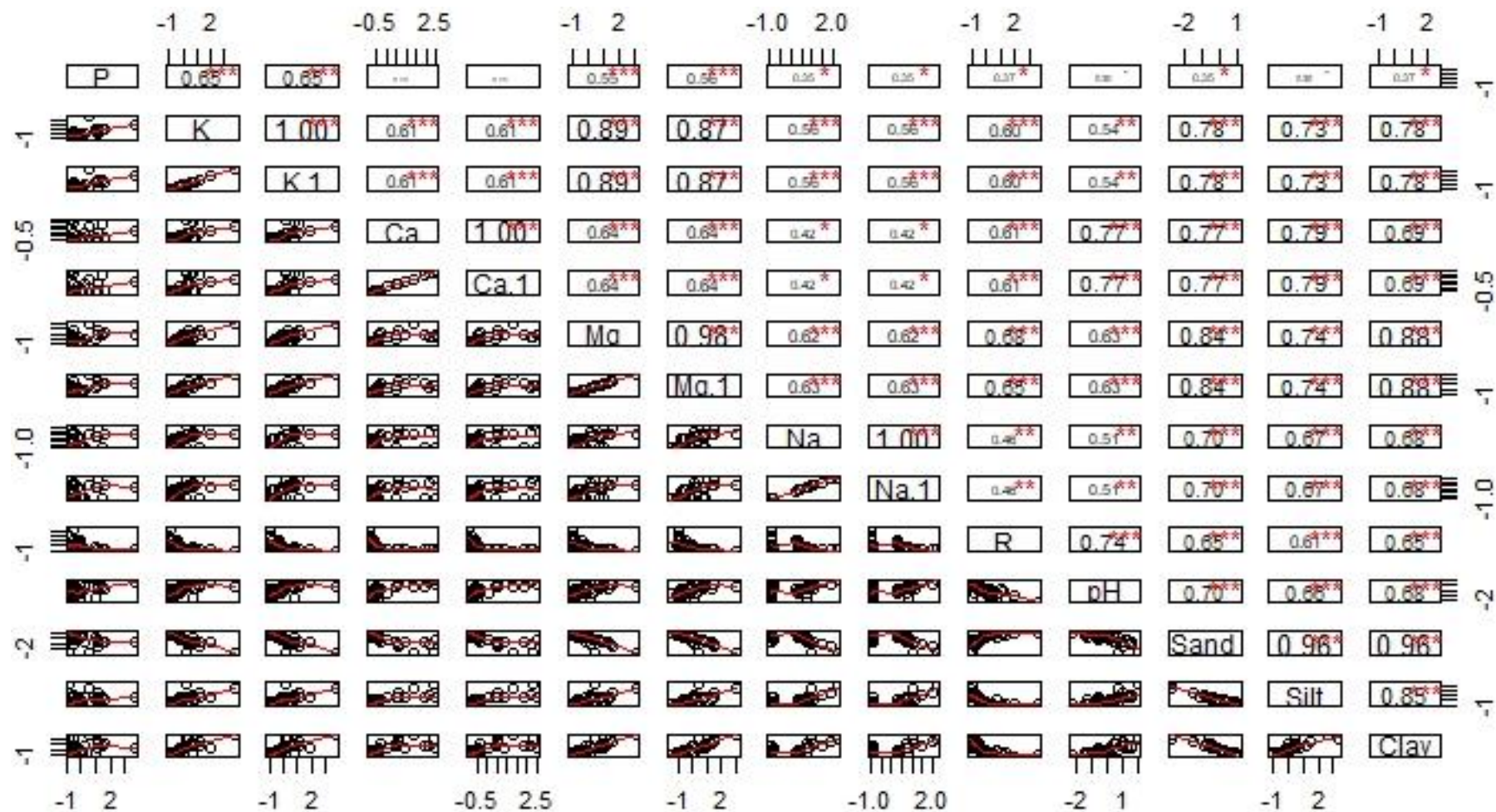
Appendix 7 Parasitic wasps that emerged from egg packets collected during the surveys.

Appendix 8 Fine-scale plots description

Site	Plots	Description
Venetia Limpopo Nature Reserve	A, B and C	No harvesting of larvae takes place in this reserve.
Mapesu Private Game Reserve	D and E	No harvesting of larvae takes place in this reserve.
Joubert Farm	G and P	Commercial and subsistence harvesting of larvae take place in this farm.

Appendix 9 Moran Eigenvector Maps (MEMs for medium-scale study – for 123 sites), the increase in size of squares correspond to increasing positive value (black) and increase in negative values (white) of the eigenvector (each square represents a plot). Plots with same color indicate similarity in the abundance of mopane worms, and the size of squares indicate the level of similarity, thus small squares represent low similarity while large ones represent high similarity.
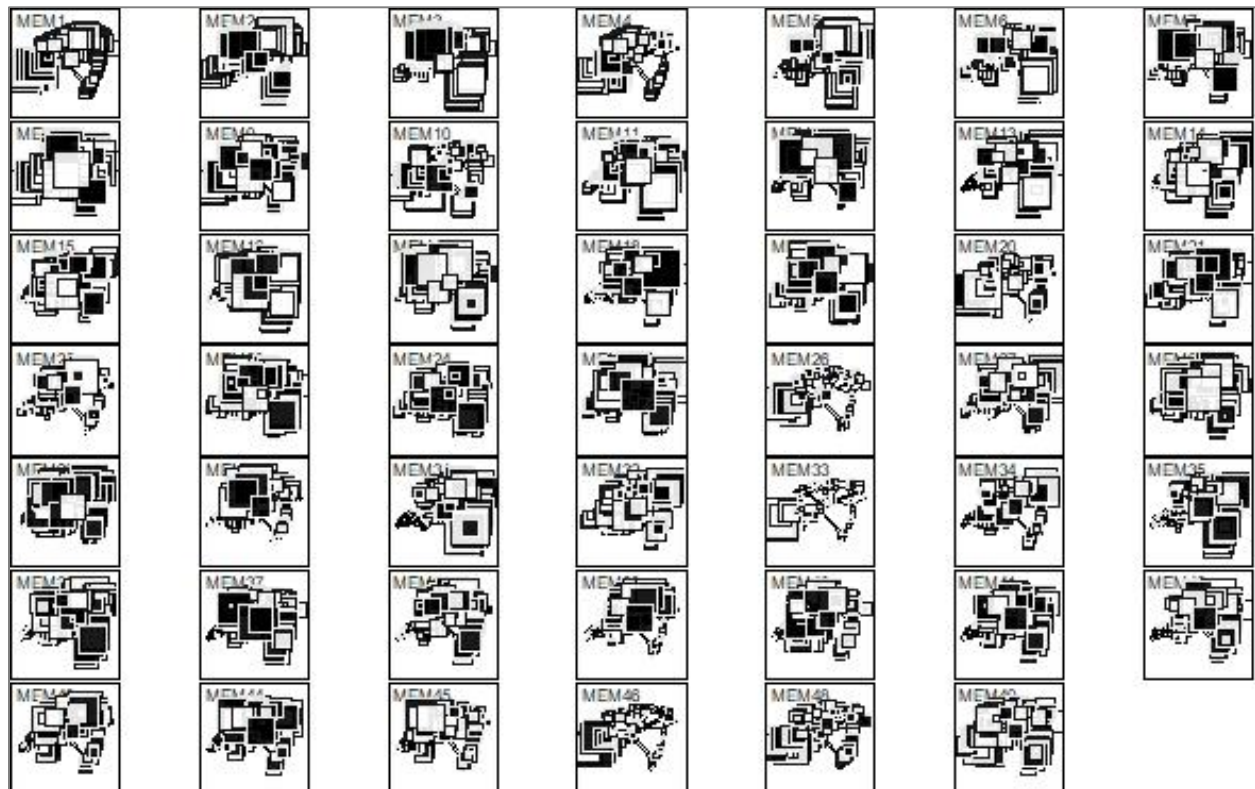
Appendix 10 Application of sleeve nets to branches with eggs and 1st larval stage worms

Appendix 11 Eggs within sleeve nets (a) and stickbug on egg packet (b) transplanted in occupied areas

Appendix 12 Moran Eigenvector Maps (MEMs for medium-scale study – for only 33 sites with soil analysis), the increase in size of squares correspond to increasing positive value (black) and increase in negative values (white) of the eigenvector (each square represents a plot). Plots with same color indicate similarity in the abundance of mopane worms, and the size of squares indicate the level of similarity, thus small squares represent low similarity while large ones represent high similarity.