PEST STATUS OF LEUCAENA PSYLLID, *Heteropsylla cubana* Crawford (HOMOPTERA: PSYLLIDAE) AND BIOLOGICAL CONTROL AGENTS IN EASTERN TANZANIA

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EXTENDED ABSTRACT

Heteropsylla cubana has caused damaging effects to *Leucaena leucocephala* in Tanzania since its outbreak in 1992. The major objectives of the study were; to determine population density of *H. cubana*, mummies of *T. leucaena* and *P. yaseeni*, indigenous predators associated with *H. cubana*, infestation density and shoot health of *L. leucocephala* resulting from *H. cubana* attack in Morogoro and Tanga region. The Point Centre Quarter method was employed to select *L. leucocephala* for observation of *H. cubana*, mummies, indigenous predators, infestation and shoot health. R and Excel program software were used in data analysis to obtain descriptive statistics of observed data. The mean number of eggs, small nymphs, medium nymphs, large nymphs and adults per 15cm terminal shoot were 14.24, 11.77, 8.78, 4.79 and 2.81 in Morogoro and 11.40, 8.16, 5.80, 3.72 and 2.42 in Tanga respectively. The population density of eggs differ significantly among crown levels (upper, middle and lower) and not significant among dbh classes (1-5 cm, 6-15 cm and >15 cm) in Morogoro. The situation was different in Tanga where there was no significant difference among crown level and dbh classes. The interaction between dbh classes and crown level was not significantly different in both Morogoro and Tanga for eggs population density. The mean number mummies of *T. Leucaenae* and *P. yaseeni* were 2.33 and 1.68 in Tanga and 2.64 and 2.1 in Morogoro respectively. The dominant indigenous predators found were spiders followed by ladybird beetles, dragonflies and lacewings for adult and regenerants *L. leucocephala*. The infestation density and shoot damage were slightly high in Morogoro compared to Tanga for adults and regenerants *L. leucocephala.* The study has found good shoot health and small injury to *L. leucocephala*. Farmers are advised to plant *L. leucocephala* for various usesas psyllid's population is no longer a problem.

DECLARATION

I, **LYIMO, PAULO JOHN** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and it has neither been, nor concurrently being submitted for higher degree awards in any other institution.

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(Supervisor)

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DEDICATION

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ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
CABI	Centre for Agriculture and Biosciences International
cm	Centimeters
dbh	diameter at breast height
Df	Degree of freedom
EcoSM	Ecosystems Science and Management
FAO	Food and Agriculture Organization of the United Nations
FBD	Forestry and Beekeeping Division
Ha	Hectare
IPM	Integrated Pest Management
KFRI	Kenya Forestry Research Institute
m.a.s.l	metres above sea level
MNRT	Ministry of Natural Resources and Tourism
MRSEP	Morogoro Region Socio Economic Profile
NBS	National Bureau of Statistics
NFTA	Nitrogen Fixing Tree Association
SUA	Sokoine University of Agriculture
TAFORI	Tanzania Forestry Research Institute
Tsh	Tanzania Shillings
URT	United Republic of Tanzania
USA	United States of America

DISSERTATION STRUCTURE

This dissertation consists of four chapters. Chapter one describes background information on Leucaena psyllid*, Heteropsylla cubana* Crawford globally, Africa and in Tanzania, problem statement and justification, objectives. Chapter two (paper one) describes population density of *H. cubana*, infestation density and shoot health of *Leucaena leucocephala* (Lam.) de Witresulting from *H. cubana* attach in Morogoro and Tanga region. Chapter three (paper two) presents abundance of *H. cubana* mummies of *Tamarixia Leucaenae* Boucek and *Psyllaephagus yaseeni* Noyes, percentage of parasitization of *H. cubana* and abundance of indigenous predators in Morogoro and Tanga regions. Chapter four consists of key areas for further studies and specific recommendations forfarmers practice agroforestry, foresters, forest entomologists, NGOs and government in general.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 An Overview of Exotic Forest Insect Pests in Tanzania

Most introduced organisms, including fungi, insects and mammals, cause of major. In some cases their impacts have been worldwide (Speight and Wainhouse, 1989). The spread of exotic forest pests to foreign countries is mostly accidental. Many of the insects now established in different parts of the world originated from Europe (Campbell and Schlarbaum, 2014). Some of the early introductions into the new world, in the late nineteenth and early twentieth centuries due to importation of European trees by the large immigrant population to USA and Canada. Some of these insects became serious pests of the native forests (Madoffe and Petro 2011). In Tanzania, most exotic tree species are attacked by insects, however the intensity of attacks varies.

1.1.1 *Leptocybe invasa* Fisher and La Salle (Hymenoptera: Eulophidae): *Eucalyptus chalcids*

An outbreak of the gall-forming invasive wasp, *Leptocybe invasa* Fisher and La Salle (Hymenoptera: Eulophidae), commonly called blue gum chalcid (Plate 1) damage eucalyptus plantations throughout the world (Mendel *et al*., 2004). It is native to Australia and was first reported in the Middle East in 2000 and has spread to most Mediterranean countries and many of the *Eucalyptus* areas in northern and Eastern Africa (Mutitu, 2003; Mendel *et al*., 2004; Thu, 2004). The wasp occurs in several countries including Algeria, Iran, Israel, Italy, Jordan, Kenya, Morocco, Spain, Syria, Turkey, Uganda and Tanzania (Mutitu, 2003 and Petro *et al.,* 2014). In Tanzania, *L. invasa* infestation was first recorded on young *Eucalyptus camaldulensis* Dehnh*, E. tereticornis* Smith and *E. grandis* Hill ex

Maidenin Tabora and Shinyanga in early 2005. Infestation was also reported in *E. grandis* clonal trial grown in Kibaha, Pwani Region and Mombo and Korogwe in Tanga Region (Petro, 2009).

The wasp lays eggs in the petiole and midrib of leaves and stems of young shoots that leads to gall formation, which further damages growing shoot tips and leaves of eucalypts, resulting in quicker abscission of leaves and drying up of shoots. Severely affected eucalypts show gnarled appearance, stunted growth, lodging, die back and sometimes tree death (Mendel *et al*., 2004; Nyeko, 2005; Protasov *et al*., 2008; Kumari *et al*., 2010). The infestation is more severe on seedlings in nurseries and young (1–3 year old) plantations than on older trees (Nyeko, 2005). Suitable hosts of *L. invasa* include several *Eucalyptus* species and their hybrid clones (Mendel *et al*., 2004; FAO, 2009; Thu *et al*., 2009; Mutitu*et al*., 2010).

Plate 1: Gall wasp, *Leptocybe invasa*. Source: (Rigi *et al*., 2014)

Leptocybe invasa infestation significantly impacted growth and biomass production in *E. grandis* and *E. saligna* in Tanzania (Petro *et al.,* 2014). Control measures included application of agrochemicals and fertilizers to induce leaf formation. Suggested pest management options in Kenya included quarantine, cultural control methods and Kenya Forestry Research Institute (KEFRI) plans to initiate a biological control program as a permanent solution (Mutitu and Mukirae, 2004).

1.1.2 *Pineus boerneri* Annand (Homoptera: Adelgidae): Pine woolly aphid

The Pine Woolly Aphid, *Pineus boerneri* Annand (Plate 2) feeds on the shoots of *Pinus* species, at times causing tip dieback. The aphid occurs in Africa, Australia, Europe, New Zealand and North and South America. This pest is native to Europe, where it damages various species of pines. *Pineus boerneri* attacks 50 pine species in Africa, of which 41 species were introduced in Eastern and Southern Africa and nearly 30 species were recorded as furnishing food for the pine woolly aphid (Madoffe, 1989). Towards the end of 1984 nearly all pines plantations in Tanzania were infested, at different degrees of attack (Madoffe and Day, 1995). In East Africa, planted pines are *Pinus patula, P. elliottii* and *P. kesiya* of which *P. kesiya* and *P. patula* appear more susceptible to attack than other pines grown (Odera, 1991). Biology, ecology and economic importance of this pest were studied in Kenya, Zimbabwe and South Africa (Barnes *et al*., 1976; Zwolinski, 1989).

The commonest control method of *P. boerneri*is by practicing proper silviculture e.g. sites amelioration and use of resistant pines. Biological control has also been used successfully for example in Tanzania, native predators such as the *Coccinellids* sp., *Chaelemens* sp., *Chilocorus* sp. and *Rodolia* sp. Reduced aphid population in some pine plantations in the Sao Hill, West Kilimanjaro and Meru Forest projects (Kisaka, 1990). Various exotic predators which were evaluated for control of *Pineus boerneri* includes*Leucopis nigraluua, L. manii, L. tapiae, Ballia eucharis, Scymnus* speciesand *Tetraphleps raoi*

Ghauri (Hemiptera: Anthocoridae). Most of these predators fed on the aphids and suppressed the pest.

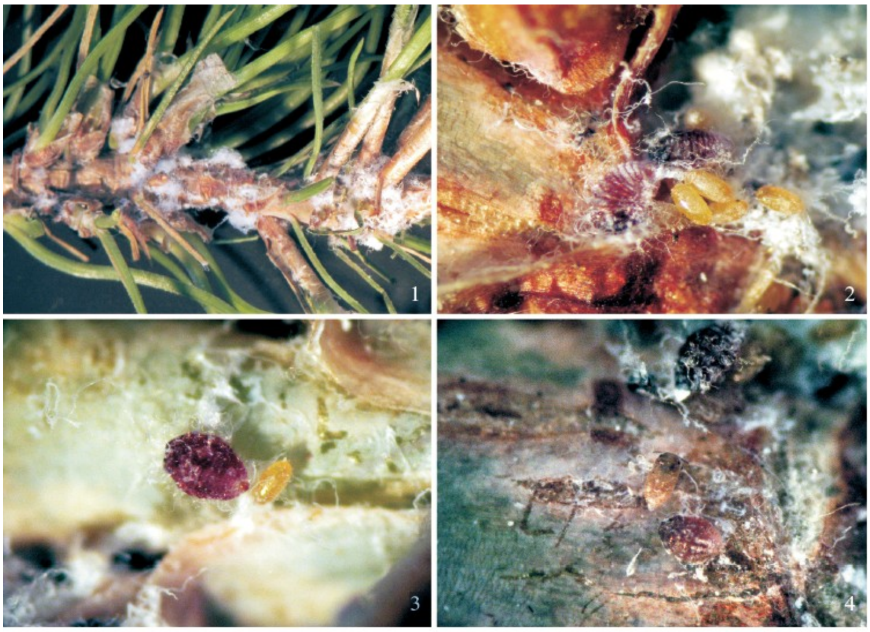

Plate 2: 1–4. One year old *P. taeda* seedling bearing colonies of *P. boerneri*. 1, white woolly wax secreted by adults and nymphs; 2. Apterous adult female and eggs covered with wax; 3, Apterous adult female and egg; 4. Nymphs. Source: (Lazzari and Cardoso, 2011)

However, Kisaka (1990) reported that *Tetraphleps raoi* predator was not very effective as it did not reduce population sufficiently to prevent tree mortality in contrast to Madoffe (2006). Similarly some chemicals were reported to suppress the pest though they are expensive and not environmentally friendly (Lazzari and Cardoso, 2011).

1.1.3 *Cinara cupressivora* Watson and Voegtlin (Lachnidae: Homoptera): Cypress aphid

Cypress aphid*, Cinara cuppressivora* Watson and Voegtlin (Plate 3) is a significant pest of cypress species. It is believed to have originated on *Cupressus sempervirens* from

Eastern Greece (Alleck *et al*., 2005). It was first recorded in Africa, from northern Malawi in 1986. Since then it spread rapidly throughout East and southern Africa causing significant damage. It first appeared in Kenya in 1990 and the most recent record is from Ethiopia in 2004. Damage to hosts includes browning and defoliation, which in some cases causes dieback and tree death (FAO, 2007). In Tanzania, symptoms of damage on cypress trees by *C. cupressivora* were observed earlier in Musoma in 1986 (Murphy *et al*., 1990). The *C. cuppressivora* is considered to be one of the most damaging introduced insects, where it has caused extensive damages to planted cypress forests. A secondary problem caused by aphid feeding is the copious quantities of honey dew which encourages the growth of sooty mould. Watson *et al*. (1999) reported that *C. cuppressivora* has seriously damaged commercial and ornamental plantings and native stands of *Cupressus, Juniperus, Widdringtonia* and other *Cupressaceae* in Africa, Italy, Jordan, Yemen, Mauritius and Colombia. *Cinara cuppressivora* caused a loss of commercial plantations in East and South Africa and seriously affected supply of domestic wood in the region (Ciesla, 1991).

Over 75 000 ha of *C. lusitanica* in Kenya, 15 000 in Tanzania and 4600 in Uganda were infested by *C. cuppressivora* to variable damage levels ranging from slight to severe (Mwangi, 2002). Therefore, it was estimated that *C. cuppressivora*caused an annual loss of growth increment worth 13.5 million USD and killed 41 million USD worth of trees in Africa (Murphy, 1996).

Threat from this pest forced the Tanzanian Government to stop planting *C. lusitanica* while most of the mature plantations were clearfelled in 1970’s and 1980’s (Madoffe, 2006). The pest can be controlled by silviculture methods e.g. thinning, proper site selection and selection of resistant trees. Chemical control is only feasible on small areas such as hedges (Madoffe and Day, 1995).

Plate 3: Cypress aphid, Cinara cuppressivora. Source: (FAO, 2007)

Classical biological control using a parasitoid, *Pauesea* species showed some positive results in Kenya and Uganda (Allard *et al.,* 1994). The parasitoid was released in early 1990's and records showed that in the late 1990s it spread and established in northern Tanzania (Kilimanjaro and Arusha) where *C. lusitanica* is widely planted. In spite of lack of a systematic survey to evaluate the status of the pest, research showed severity of the attack is diminishing and the Government has relaxed its ban on replanting of Cypress, while many individuals have continued planting the species (Madoffe, 2006).

1.1.4 *Eulachnus rileyi* Williams (Homoptera: Lachnidae): Pine Needle Aphid

Pine Needle Aphid, *Eulachnus rileyi* Williams (Plate 4) attacks several species of *Pinus*. Typically, this insect causes only minor damage where it has been introduced, however, it has the potential to cause serious damage. Heavy infestations cause needles to turn yellow and drop prematurely, resulting in growth reduction (FAO, 2007). This needle infesting aphid of European and North America origin was for the first time discovered in Zambia, Zimbabwe and South Africa in the late 1970’s but the species subsequently spread to Tanzania, Kenya and Malawi where pines are grown (Katerere, 1984). Like the *P. boerneri*, *P. patula* and *P. elliottii* seem to be particularly more susceptible. The infested needles turn yellow and could be lost prematurely and the aphids produce copious quantities of honeydew, which induce a cover of sooty moulds on heavily infested trees (Madoffe, 2006). In Tanzania, the pest is found in most pine growing plantations and Sao Hill forest plantation has the most serious attacks (Madoffe, 1989).

Plate 4: Pine Needle Aphid, *Eulachnus rileyi*. Source: (Goszczyński and Budzińska 2010)

However, there is no available information about the quantitative effect of the pine needle aphid on its pine host in Tanzania, Kenya, South Africa and the actual damage to pines is slight than that caused by the pine woolly aphid. Massawe (1991) described *T. raoi* as the most important predator of *Pineus* species. *Leucopis tapeae* could also have some prospects for management of this pest. Proper site selection, proper silvicultural practices and use of resistant pine species could also reduce ravages from this pest.

1.1.5 *Phoracantha semipunctata* Fabricius and *Phoracantha recurva* Newman (Coleoptera: Cerambycidae): Eucalyptus bark beetles

These two longicorn beetles are native to Australia and were accidentally introduced in many parts of the world including South Africa and East Africa where *Eucalyptus* are widely grown (Bubala *et al.,* 1989). It attacks both growing trees and green logs (Annecke and Moran, 1998). Attacks can cause considerable damage to physiologically stressed trees sometimes killing them. *Phoracantha semipunctata* (Plate 5 (1)) is a serious pest in Zimbabwe, Malawi and Zambia and to a lesser extent in Southern part of Tanzania (Annecke and Moran, 1998).

In 1989 the beetles were widely distributed in Zambia. Out of the 54 plantations inspected during 1980 -1983, they were present in 32 plantations corresponding to 94% of the area of 25 000 hectares under eucalypts in Zambia (Bubala *et al*., 1989). All *Eucalyptus* species grown in Zambia are susceptible to the attack by *P. semipunctata* whereby in South Africa, host plants of *Phoracantha* beetles are *E. grandis, E. saligna, E. diversicolor, E. paniculata and E. maculata.* Control of Phoracantha beetles includes stripping the bark and destroying the infested branches of felled trees (Annecke and Moran, 1998). *Phoracantha semipunctata* is mostly closely related to *P. recurva* which has been recorded in the Northern Jarrah Forest (Wang, 1995). The two species are distinguished by the elytra; *P. semipunctata* has dark reddish brown or blackish brown elytra with the following yellowish brown markings: 1 narrow zigzag fascia at sub-base, 1 wide, more or less straight fascia just before middle and 1 oval or irregular spot on disc before apex (Wang, 1995) (Plate 4 (1)). In comparison, the elytra of *P. recurva* are pale yellow to yellowish brown with a narrow incomplete zigzag band which is reduced to a small spot before the middle of each elytra (Plate 4 (2)). *Phoracantha semipunctata* does not have long hairs and sensilla filiformia on each antennal segment (Faucheux, 2011), as well as spines on the front dorsal side of hind femur, and the presence of barbs on the backs of lower leg segments (Wang 1995).

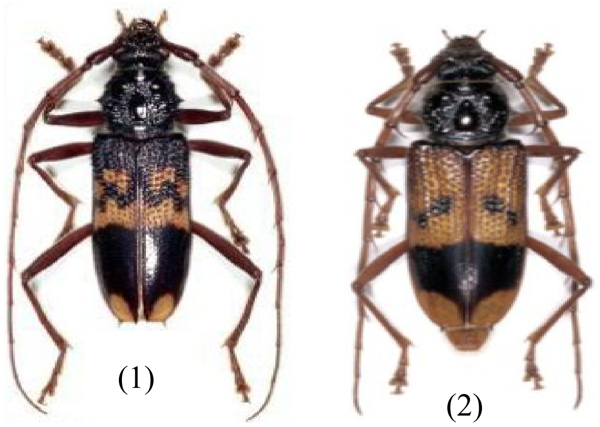

Plate 5: Eucalyptus bark beetles; (1) *Phoracantha semipunctata* (Hoskovec 2010), and (2) *Phoracantha recurva* (Paine *et al.,* 2009)

In contrast, *P. recurva* (Plate 5 (2)) have very dense and long golden hairs arising from the underside of each antennal segment and the hind femora with strong dense spines on the front-dorsal side.

1.2 Study Insect: Leucaena psyllid, *Heteropsylla cubana* Crawford

1.2.1 Biology and ecology of *H. cubana*

Showler and Melcher (1995) provided a synopsis of *H. cubana* biology and life cycle (Plate 6) which is somewhat variable depending upon the region and habitat. Immediately after hatching, first-instar nymphs begin to feed gregariously near the oviposition site. As the nymphs grow through five instars to adulthood, they colonize and feed on other terminal portions of stems, branches and petioles of young leaves. Eggs, nymphs and adults can be found together on shoot terminals. Each female can produce 300-500 eggs, with an average of 241 eggs. Eggs are mostly laid on the upper surface of unfolded leaflets, attached by a posterior pedicel. The incubation period for eggs seems to differ from area to area, but it is generally 2-5 days. *Heteropsylla cubana* populations are normally fluctuating quite widely over time and different levels of pest abundance occur in different parts in the same tree, according to the differences in the growth stages of *Leucaena* species. Apparently, Leucaena trees are vulnerable to high infestation of *H. cubana* in the stage of producing new shoots (San Valentin, 1988). In India, the new shoot has been usually observed by heavy infestations of up to 3000 nymphs and adults of *H. cubana*per 15 cm of terminal shoot (Nair, 2007).

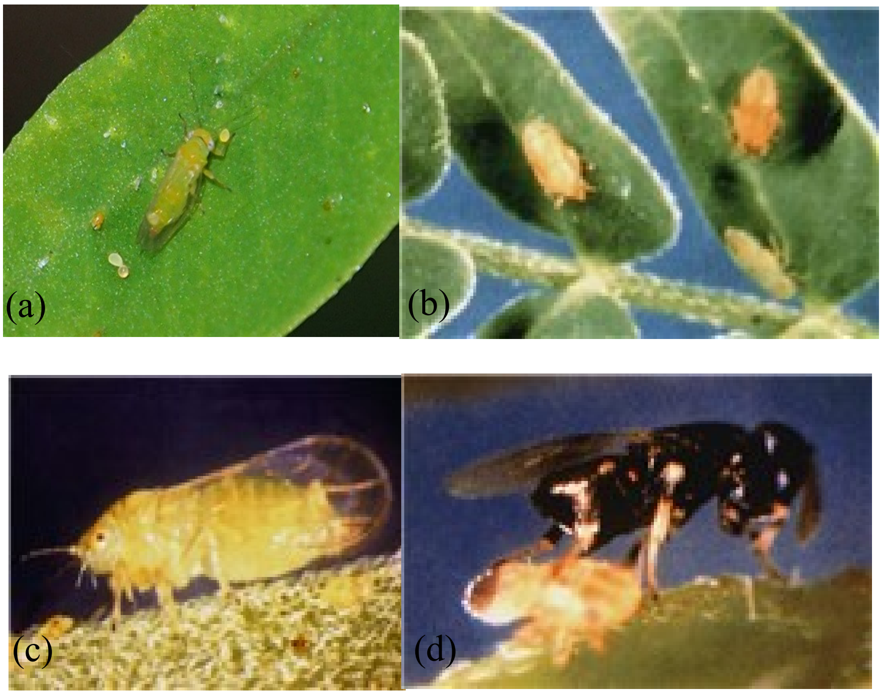

Plate 6: Leucaena psyllid, *H. cubana*; (a): Eggs and Nymph, (b): Nymphs on Leucaena, (c): Adult and (d): Adult *Psyllaephagus yaseeni* Noyes, parasite of *H. cubana* (Source: CABI, 2013)

1.2.2 Global spread and damage of *H. cubana*

The first spread of *H. cubana* from its natural habitat was Hawaii in 1984 and later spread to Asia in 1985 and in 1992 were noticed in the African continent, in Tanzania, Kenya, Uganda and Burundi and by 1994 in Sudan and Zambia (Geiger *et al*., 1995; Ogol and Spence, 1997; Madoffe and Petro, 2011; Ahmed *et al.,* 2014). Most of *Leucaena* plantings in Tanzania were severely damaged by *H. cubana* in late 1980s (Kisaka, 1994) and continued in 2000 (Madoffe *et al.,* 2000). Farmers in some parts of Tanzania including Morogoro and Tabora abandoned planting Leucaena due to severe attacks. Alternative species such as *Leucaena diversiflora* Schlecht*, L. pallid* Britton, *L. collinsi* Britton*, Gliricidia sepium* Jacq and *Calliandra calothyrsus* Meisn were sought but were not well accepted by farmers due to their limitation as a fodder (Sorensson and Brewbaker 1987; Edward *et al.,* 2006).

1.2.3 Status of *H. cubana* in Tanzania

Heteropsylla cubana has been damaging *L. leucocephala* in Tanzania since its outbreak in 1992. Cultural, genetic and chemical controls were tried in some localised areas, however without success (Madoffe, *et al.,* 2000). *Leucaena leucocephala* is important in maintaining soil fertility through nutrient cycling, provision of fuel wood and building materials, fodder and in environment conservation in Tanzania (Lulandala and Hall, 1987). Leucaena is found in most part of Tanzania including Morogoro, Tanga, Tabora and Shinyanga (Madoffe and Petro, 2011; Msangi *et al*., 2002). The occurrence of the devastating *H. cubana* has discouraged the spread of Leucaena-based fodder production technology since it's outbreak on the coast of Tanzania in July-August 1992 (Madoffe and Petro, 2011; Msangi *et al*., 2002). Two hymenopterous parasitoids, *Tamarixia Leucaena* Boucek and *Psyllaephagus yaseeni* Noyes were introduced from Trinidad to

Tanzania in 1995 and 1996 to control *H. cubana* (Madoffe and Petro, 2011). Preliminary survey in late 1990's showed that the parasitoids were well established and were spreading from the epicentre while there were some remarkable declines of the *H. cubana* populations (Madoffe, *et al.,* 2000; Madoffe and Petro, 2011).

1.2.4 Impacts of *H. cubana*

Heteropsylla cubana limited the use leucaena as a potential forage crop in Malaysia and to a lesser extent in Australia. In Indonesia and the Philippines, Leucaena was key to the development of more intensive, stable and sustainable farming systems for smallholder (Napompeth, 1994; Ahmed *et al.,* 2014). *Heteropsylla cubana* has also had a significant impact on exports of leaf meal from Thailand and the Philippines. In Indonesia, considerable economic loss was recorded on cocoa, coffee and vanilla as a result of defoliation of Leucaena that was planted to provide shade for these crops (Napompeth, 1994).

Production losses due to *H. cubana* damage costed Central Queensland beef industry an excess of $2 million per year in Australia (Mullen *et al.,* 1998). In Morogoro region, the average household economy loss due to *H. cubana* attack were estimated to be 54 125 Tanzania shillings per year for loss of pole and timber (Johansson, 1994).

1.2.5 Control of *Heteropsylla cubana*

1.2.5.1 Biological control

Biological control involves the use of natural enemies of a pest or disease to help keep its numbers in check. Biological control efforts against *H. cubana* were successfully by using specific natural enemies such as the predators, *Curinus coeruleus* and *Olla v-*

nigrum and the parasitoids, *P. yaseeni* and *T. leucaenae* in Asia-Pacific Region and Africa (Madoffe *et al.,* 2000; Shivankar *et al.,* 2010; Madoffe and Petro, 2011). In additional, several arthropod natural enemies were associated with the *H. cubana*, the most dominant being spiders, lacewings, dragonflies, ladybird beetles and ants in Tanzania (Madoffe *et al.,* 2000).

1.2.5.2 Chemical control

Several insecticides, such as carbaryl, carbosulfan, cyhalothrin and bifenthin, have showed equivocal results, although in some cases insecticides suppressed *H. cubana* adults but spared the nymphs (CABI, 2013). Other studies indicated that some pesticides were effective. However, the possibility of residual pesticides on leucaena fodder residues on and financial costs discouraged pesticide use (Krishnamurthy *et al.,* 1989; NFTA, 1988; CABI, 2013). In India, Insecticides such as endosulfan, phosalone, quinalphos and monocrotophos (all at 0.05%), controlled *H. cubana* for at least two weeks and adult pest infestations were reduced but nymph populations were unaffected (Krishnamurthy *et al.,* 1989; CABI, 2013). Thus, the insecticides controlled *H. cubana* for a short period (Krishnamurthy *et al.,* 1989). Chemical control is uneconomical and eliminates predators and parasites (NFTA, 1988).

1.2.5.3 Cultural methods or ecological management

Cultural methods create conditions inhospitable for the development of damaging numbers of pests. These include matching tree species with suitable growing sites, intermediate harvests to maintain tree vigor and timely harvesting of plantations at maturity (FAO, 2001). Cultural control methods are diverse and rely on a good understanding of the pest species ecology in relation to the plant production system (Alao *et al.,* 2011). Some cultural methods, such as grazing and pruning, can be used to manage *H. cubana* and should be investigated further (Soon *et al.,* 1989). Pruning and grazing of new Leucaena shoots preclude the use of the tree for shade estate crops. However, in one Malaysian grazing new shoots by livestock reduced *H. Cubana* populations temporarily. There are no reports of other cultural control tactics that are effective against *H. cubana* (CABI, 2013).

1.2.5.4 Host Plant Resistance

Breeding aims to produce superior new genotypes by combining the desirable attributes of 2 or more parents (Shelton, 2008). There is considerable scope for hybridisation among Leucaena species and several naturally occurring hybrids have been reported in the native range in Central America (Hughes, 1998). The University of Hawaii initiated a hybrid-breeding program with *Leucaena* in the early 1980s. Since then hundreds of inter- and intra-specific crosses were developed (Shelton, 2008). Early research with artificial hybrids concentrated on crosses among the tetraploid accessions, *L. leucocephala*, *L. pallida* and *L. diversifolia*, as these were highly cross-compatible (Sorensson and Brewbaker 1987). Artificial interspecific F1 hybrids between *L. pallida* and *L. leucocephala* (KX2 hybrids) have exhibited good *H. cubana* resistance, exceptionally high biomass yield (the result of heterosis or hybrid vigour) and broad environmental adaptation (Mullen *et al*., 2003).

1.2.5.5 Integrated Pest Management (IPM)

Integrated pest management systems combines decision-making and pest management tools directed against a pest or pest complexes in various stages of development. IPM is an approach for reducing the impact of insects or other pests in any ecosystem. Integrated pest management was employed to control successfully *H. cubana* in East Africa through use of biological methods, resistant species like *L. deversiflora, L. pallida, L. collinsi*, cultural methods and alternative species such as *Gliricidia* and *Calliandra* species (Madoffe, 2006). Similar programmes were used successful in Asia and IPM was suggested as an essential approach for managing the *H. cubana* in Africa (Napompeth, 1994).

1.3 Justification

Damage caused by *H. cubana* was the most important the limitation in 1990s to the productivity of *L. leucocephala* in Eastern Tanzania. *Heteropsylla cubana*can be controlled easily by a wide range of systemic, broad-spectrum insecticides (Barrientos *et al.,* 1991; Rao, 1995). However, the use of insecticides is uneconomical to small scale farmers and poses health and environmental risks and may limit the build-up of the *H. cubana* natural enemies (Ahmed *et al.,* 2014; Heydon and Affonso, 1989).

Biological control agents may suppress *H. cubana* populationsin economically and environmentally manner (Napompeth, 1994). The parasitoids, *T. leucaenae* Boucek (Eulophidae) and *P. yaseeni* Noyes (Encyrtidae) were introduced in Tanzania to manipulate population of *H. cubana*. The two parasitoids were introduced in Tabora, Western Tanzania in February 1996 and *T. leucaenae* was introduced in Morogoro and Tanga, Eastern Tanzaniain July and August 1995. However, the spread and establishment of *P. yaseeni* in Morogoro and Tanga, Eastern Tanzania are not well known. Despite the economic importance of Leucaena, the effectiveness of biological control agents for *H. cubana* and the fundamental physiological ecology of the tree and *H. cubana* remain poorly understood (Geiger and Andrew, 2000). In additional, little has been done to identify other potential natural enemies of the *H. cubana*in Tanzania (Kisaka, 1994; Madoffe *et al.,* 2000).

A study by Madoffe *et al.* (2000) reported a decline of *H. cubana* population which was associated with *T. leucaenae* and *P. yaseeni*. Reconnaissance surveys conducted in April, 2015 in Morogoro and Tanga showed a decline in attacks of *L. leucocephala* by *H. cubana*. Furthermore, *T. leucaenae* and *P. yaseeni* were recorded in almost all *L. leucocephala* growing areas. However, the general visual observations were not correlated population density of both *H. cubana* and mummies of *T. Leucaenae* and *P.* yaseeni. Infestation density and shoot health of *L. leucocephala* resulting from *H. cubana* attack are also not known.

The findings from this study provide valuable information about whether the release of *T. Leucaenae* and *P. yaseeni* has manipulated significantly population of *H. cubana*. The information would form a basis for advising the farmers on whether to continue planting *L. leucocephala* or look for alternative fodder and multipurpose trees.

1.4 Objectives

1.4.1 Overall Objective

To assess population density, infestation density and biological control of *Heteropsylla cubana* in *Leucaena leucocephala* growings in Eastern Tanzania

1.4.2 Specific Objectives

i. To establish population density of *Heteropsylla cubana* in Morogoro and Tanga regions.

ii. To determine abundance of mummies of *Tamarixia leucaenae* and *Psyllaephagus yaseeni* establishment in Morogoro and Tanga regions.

iii. To determine abundance of indigenous predators associated with *Heteropsylla cubana* in Morogoro and Tanga regions.

iv. To assess infestation density and shoot health of *Leucaena leucocephala* resulting from *Heteropsylla cubana* attack in Morogoro and Tanga regions.

1.5 Hypotheses

H_{o1}: There is low population density of *Heteropsylla cubana* in Morogoro and Tanga regions.

H_{o2}: There is a low mummy of *Tamarixia leucaenae* and *Psyllaephagus yaseeni* establishment in Morogoro and Tanga regions.

H_{O3}: There is no indigenous predators associated with *Heteropsylla cubana* in Morogoro and Tanga regions.

H_{O4}: There is no infestation density and shoot damage of *Leucaena leucocephala* resulting from *Heteropsylla cubana* attack in Morogoro and Tanga regions.

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CHAPTER TWO

MANUSCRIPT 1

2.0 Infestation and Population Density of Leucaena psyllid, Heteropsylla cubana (Homoptera: Psyllidae) on Leucaena leucocephala in Eastern Tanzania

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Abstract

The invasion of *Heteropsylla cubana* has restricted the utilization of the important multi-purpose tree *Leucaena leucocephala* in Tanzania. The objectives of the study were to determine population density of *H. cubana*, to assess the infestation density and shoot health of *Leucaena leucocephala* resulting from psyllid attach in Morogoro and Tanga region. The Point Centre Quarter method was employed to select *Leucaena leucocephala* for observation. R and Excel program software were used in data analysis. The results of the study showed that mean number of eggs, small nymphs, medium nymphs, large nymphs and adults per 15cm terminal shoot were 14.24, 11.77, 8.78, 4.79 and 2.81 in Morogoro and 11.40, 8.16, 5.80, 3.72 and 2.42 in Tanga respectively. The population density of eggs differed significant among crown level and not significant among dbh class in Morogoro, differently in Tanga were not significantly among crown level and among dbh classes. The interaction between dbh classes and crown level was not significant in both Morogoro and Tanga for eggs population density. There was no significance difference of nymph and adult population density among crown level and among dbh classes in Morogoro and Tanga. The infestation density and shoot damage were slightly high in Morogoro compared to Tanga for both adults and regenerants *L. leucocephala.* The study has found good shoot health and small injury to *L. leucocephala* as a result of low population density of *H. cubana*. Farmers are advised to plant *L. leucocephala*for various use without any fear of *H. cubana* as its population is no longer a problem.

Key words: *Heteropsylla cubana*, *Leucaena leucocephala,* Infestation density and Shoot health

Introduction

Leucaena leucocephala (Lam.) de Witis a multipurpose tree widely grown in the tropics (Ahmed *et al.,* 2014; Nair, 2007). *Leucaena leucocephala*is highly used throughout much of Asia and Africa as a multipurpose legume tree which provides a source of fodder, fuelwood, shade for estate crops, reforestation, timber, erosion control and nitrogen fixation (Napompeth, 1994). However, invasion of Leucaena psyllid, *Heteropsylla cubana* Crawford limited all these benefits and it has reduce production of *L.leucocephala* by 50-70% in humid regions and 20-50% in sub-humid environments (Mullen and Shelton, 2003). *Heteropsylla cubana* is a tiny yellow-green insect in the family Psyllidae of the order Homoptera. *Heteropsylla cubana* feeds on young growing shoots of several plant species related to the genera *Mimosa*, *Piptadenia* and *Leucaena*. It is native to Central and South America. The first spread from its natural habitatwas recorded Hawaii in 1984 and later Asia in 1985 and East Africa in 1992 (Ahmed *et al.,* 2014; Nair, 2007; Madoffe and Petro, 2011). *Heteropsylla cubana* damages plants when both the nymphs and adults suck from the developing shoots and young foliage. Heavy infestations leads to defoliation of the plant and stop growth, although older leaves are not directly damaged by psyllid. However, *H. cubana* produces sticky fluid exudates that promote growth of sooty mould on leaves and limits photosynthesis (Shelton, 2008).

During 1986, the economic loss due to *H. cubana* attacks was estimated at more than 316 million USD in Indonesia. Malaysia imported over 48 000 tons of leaf meal at an estimated cost of over 20 million USD for pig and poultry feeds due to attack of Leucaena fodders (CABI, 2013). Also, *H. cubana* infestation in northern and southern Queens land reduced Leucaena production by at least 55%. In India, over 200 000 seedlings were destroyed by *H. cubana* in 1988 (CABI, 2013; Napompeth, 1994). In Tropical America, the socio-ecological and economic impacts of *H. cubana* are negligible largely because *L. leucocephala* is not cultivated with the same intensity as in Asia and because of complexes of natural enemies which co-evolved with *H. cubana* (CABI, 2013). *Heteropsylla cubana* arrived in East Africa in August 1992 and restricted the utilization of the important multi-purpose tree *L. leucocephala* (Madoffe and Petro, 2011). *Leucaena leucocephala* is found in many parts of Tanzania and is usually planted along farm boundaries and in homesteads for fodder, soil fertility improvement and fuelwood (Msangi *et al*., 2002). Most Leucaena stands were affected after the invasion of *H. cubana* (Madoffe *et al.,* 2000).

In planning efforts to control *H. cubana* through biological approachin Kenya and Tanzania, the Asia-Pacific experience was considered the best option (Ciesla and Nshubemuki, 1995; Madoffe and Petro, 2011). It involved biological control using two hymenopterous parasitoids *Tamarixia leucaenae* Boucek and *Psyllaephagus yaseeni* Noyes introduced from Trinidad and Tobago. They were released in Tanzania and Kenya and both species are well established in Tanzania, have spread over large areas and they appear to be effective against their hosts (Madoffe *et al*., 2000; Madoffe and Petro, 2011).

There is some reduction in *H. cubana* population and Leucaena shoot damage in some areas, which could be attributed to the parasitoids (Madoffe *et al*., 2000). However, little is known aboutcurrent population density of *H. cubana*, infestation density and shoots health of *L. leucocephala* since the release of *T. leucaenae,* and *P. yaseeni*. Additionally, the hypothesis that the decline of *H. cubana* population and damage in Tanzania were due to the introduced hymenopterous parasitoids is yet to be proved (Madoffe *et al*., 2000; Madoffe and Petro, 2011). Population density of *H. cubana*, infestation density and shoot health of *L.leucocephala* were investigated. The study was based on two hypotheses that; (1) There is a decline in population density due to release of *T. leucaenae* and *P. yaseeni* and (2) There is a decline in infestation density and improved shoot health of *L. leucocephala* due to release of *T. leucaenae* and *P. yaseeni* in Eastern Tanzania.

Materials and Methods

Description of study areas

Studies were conducted in selected sites of Morogoro and Tanga regions, as described in Table 1 below.

Table 1: Description of study areas

Region	Location name	Latitude	Longitude	Altitude (m)
Morogoro	SUA farm	6°.822097	37°.661160	500.7
	Melela A	6°54'53.6"	37°26'0.61"	488.19
	Melele B	6°55'84.6"	37°19'.61"	486.9
Tanga	Mlingano	5^0.6667	38^0.91667	87.12
	Tanga dairy farm	$5^0$15'S	$39^0$15	65.34
	Ziwani	5°.3354	38º.5494	236.43

Heteropsylla cubana parasitoids were released in these sites in the 1995. Spread and establishment of the psyllid was assessed in 1999 by Madoffe *et al.* (2000). Morogoro region lies between latitude 5^{o} 58" and 10^{o} 0"to the South of the Equator and longitude 35^{o} 25" and 35^{o} 30" East of Greenwich. The region has an area of 72 939 km^{2}and population of 2 218 492 (MRSEP, 2006; NBS Census, 2012). Morogoro is pre-dominantly a Miombo woodland and mountainous vegetation area. The region receives an annual average rainfall of 600-1200 mm and average annual temperature varies between 18^{o}C on the mountains to 30^{o}C in river valleys (MRSEP, 2006). Morogoro region has mostly sandy clay loams in the topsoils and clays in subsoils.

Tanga Region is situated between 4° and 6° below the Equator and 37° to 39° 10' east of the Greenwich Meridian. Tanga occupies a total area of 26 677 km^{2} with a population of 2 045 205 (NBS Census, 2012). The region is characterized by bushland, palm gardens, village cultivations and estates (mainly sisal), natural forest and shrub thickets and open savannah grassland with scattered trees and scrub thickets. Tanga region is characterized by mean annual rainfall of 1200 mm and mean monthly temperatures range between 19^{o}C and 33^{o}C. Tanga soils are sandy in the coastal belt, clay to loamy in the hinterland and leached mineral laterite in the highlands (Swai *et al*., 2005).

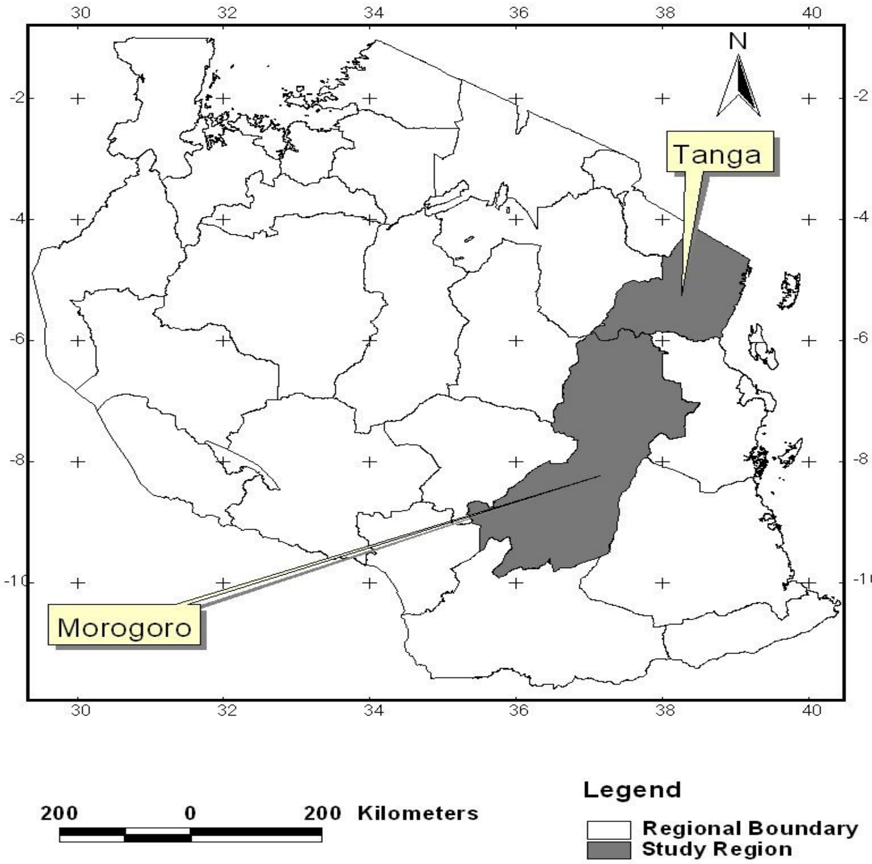

Figure 1: Map of the study regions

Methodology

Sampling Design

The Point Centre Quarter method (PCQ) was employed to assess population of *H. cubana*, incidence and damage (Marisa, 2015). Five sampling points were established in each site; one at center and other four at the corners of site (Figure 2). Four quadrants were established in each sampling point. *Leucaena leucocephala* tree and regenerants near the sampling point at each quadrant were sampled and recorded. *Leucaena leucocephala* were first grouped into two clusters of regenerants (diameter at breast height (dbh)< 1 cm) and adult species (dbh≥ 1 cm). Adult Leucaena trees were then categorized into three dbh classes i.e., (1-5 cm), (6-15 cm) and (>15 cm). Tree in each class were then divided into three crown levels (upper, middle and lower level). Two Leucaena trees for each dbh classes and two regenerants were sampled in each quadrant.

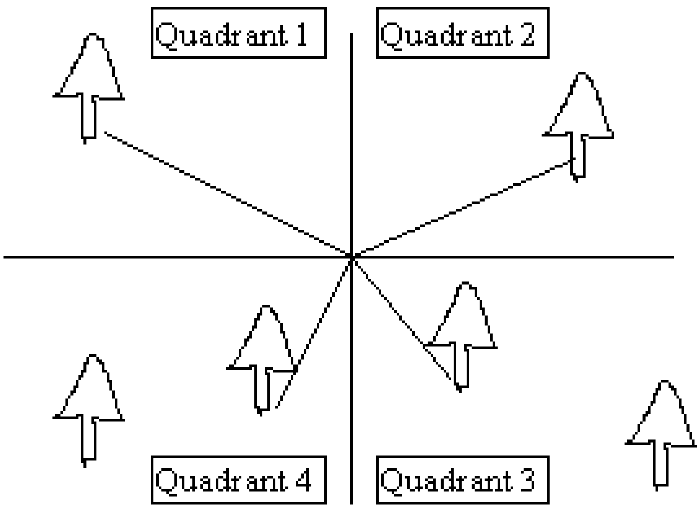

Figure 2: Layout of sampling point on the study area (Marisa, 2015).

Determining population numbers of *H. cubana*

Data were collected from 05:30-10:00 am in the morning, when adult *H. cubana* were less active. One 15cm growing shoot was randomly selected and sampled from each crown level for adult individuals (*L. leucocephala* with dbh≥ 1 cm) and two 15cm growing shoot of two regenerants (*L. leucocephala* with dbh< 1 cm) in each quadrant. A total of 2 160 and 240 adult individuals and regenerants *L. leucocephala* were sampled. The shoots were carefully cut and put into a polythene bag (destructive sampling) and put in a refrigerator overnight to immobilise the nymphs and adult psyllid. The collected shoots were washed carefully with a help of brush and ethanol (70%) to a petri-dish to remove the insects (eggs, nymphs and adults) and indigenous predators. A dissecting microscope was used to observe when sorting and counting eggs, nymphs and adult insects. Nymphs were then scored as small (yellow in colour, first and second instars), medium (blackish in colour, third and fourth instars), or large (greenish in colour, fifth instars).

DeterminingInfestation density and shoot health due to *H. cubana*

Infestation density, nymph population and shoot health of *L. leucocephala* were recorded using empirical score as modified from Bray and Woodroffe, 1988 (Table 2).

Table 2: Scores for infestation, shoot health and nymph population counts

Tree infestation ratings	Nymph population score	Shoot health score
1 - No infestation	0- None	1-No damage
2 - Light infestation	1 - 1-5 nymphs	2- Slight damage
(Loss of < 25% of young Leaves)	2 - 6-30 nymphs	3 - Heavy damage
3 - Moderate infestation	3 - 31-100 nymphs	4 - Dead
(Loss of 26 to 50% of young Leaves)	4 - >100 nymphs	
4 - Heavy infestation	5 - >100 nymphs extends to stem	
(Loss of >75% of young Leaves)		
5 - Severe infestation		
(Blackening stem with total leaves loss)		

Source: (Modified from Bray and Woodroffe, 1988)

Data Analysis

R version 3.2.3 and Microsoft excel computer software programs were used in data analysis. Descriptive statistics were used to determine mean population density of *H. cubana*. Two way Analysis of Variance (ANOVA) at 5% level of significance was used to statistically test the equality of means population density differences in *H. cubana* and infestation density between dbh classes and crown parts.

Results

Population density of *H. cubana* in Morogoro and Tanga Regions

Figures 3 and 4 show mean numbers of *H. cubana* individuals in Morogoro and Tanga regions respectively. Mean numbers of eggs, small nymphs, medium nymphs, large nymphs and adults per 15cm terminal shoot were 14.24, 11.77, 8.79, 4.79 and 2.81 in Morogoro and 11.41, 8.16, 5.59, 3.73 and 2.43 in Tanga respectively. Numbers of eggs and small nymphs were higher than the other *H. cubana* stages (Table 2 and 3; Figure 3 and 4). Mean numbers of larger nymphs and adults were lowest in all examined dbh classes. The mean numbers of *H. cubana* in Morogoro and Tanga decreased from one stage to another for adults *L. leucocephala* (Figure 6). The same population trend was observed for regenerants *L. leucocephala* (Figure 5). Results showed significant difference in number of eggs among crown levels (*F*=5.768, df=2, P=0.003) but not among dbh classes (*F*=2.872, df=2, P=0.057) in Morogoro region. In Tanga region, number of eggs were not significant different among crown level (*F*=0.061, df=2, P=0.941) and dbh class (*F*=0.816, df=2, P=0.443). The interaction between DBH and crown level was not significant different in both Morogoro (*F*=0.484, df= 4, P=0.748) and Tanga (*F*=0.612, df= 4, P=0.654) regions.

There was no significant difference in number of small nymphs among crown levels (*F*=0.367, df=2, P=0.693) and among dbh classes (*F*=2.317, df=2, P=0.100) in Morogoro (Table 6). Likewise, there was no significant difference inpopulation density of small nymphs among crown level (*F*=0.005, df=2, P=0.995) and among dbh class (*F*=0.813, df=2, P=0.444) in Tanga region. The interaction between dbh and crown level was not significantly different in both Morogoro (*F*=0.805, df=4, P=0.522) and Tanga (*F*=0.763, df=4, P=0.550) for small nymph population density. The results showed no significance difference of numbers of adult *H. cubana* among crown levels (*F*=0.235, df=2, P=0.156). However, dbh classes had a significant effect (*F*=3.379, df=2, P=0.035) in Morogoro (Table 7). In addition, there was no significance difference in number of adult *H. cubana* among crown levels (*F*=0.235, df=2, P=0.790) and among dbh classes (*F*=0.813, df=2, P=0.727) in Tanga (Table 8). The interaction between dbh and crown level was not significantly different in both Morogoro (*F*=0.257, df=4, P=0.905) and Tanga (*F*=0.514, df=4, P=0.725) regions.

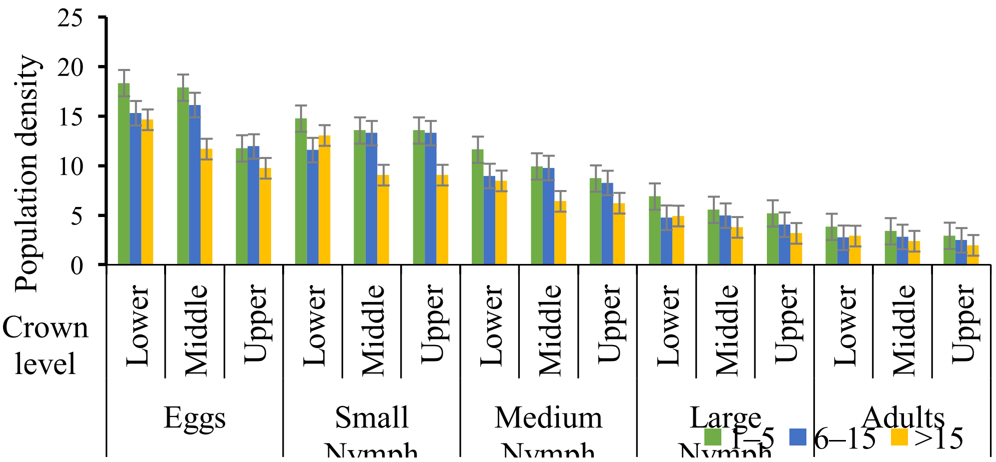

Figure 3: Mean number of eggs, nymphs and adults of *H. cubana* for adults *L.leucocephala* in Morogoro region

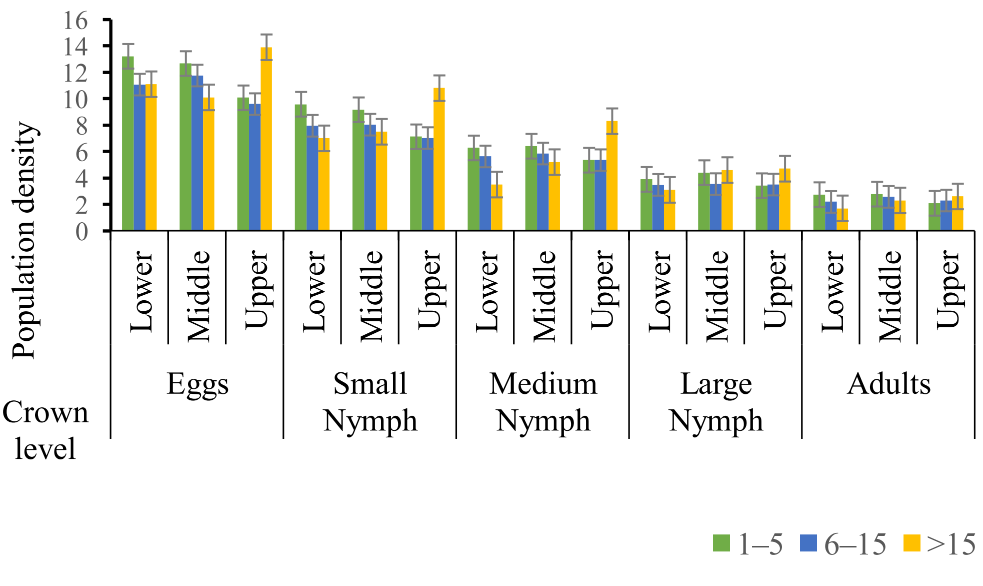

Figure 4: Mean number of eggs, nymphs and adults of *H. cubana* for adults *L. leucocephala* in Tanga region

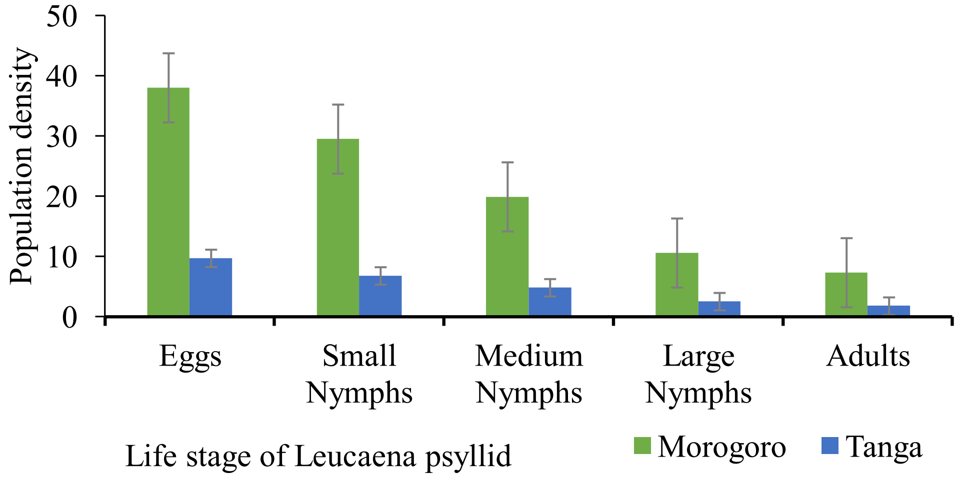

Figure 5: Mean number of eggs, nymphs and adults of *H. cubana* for regenerants *L. leucocephala* in Morogoro and Tanga regions

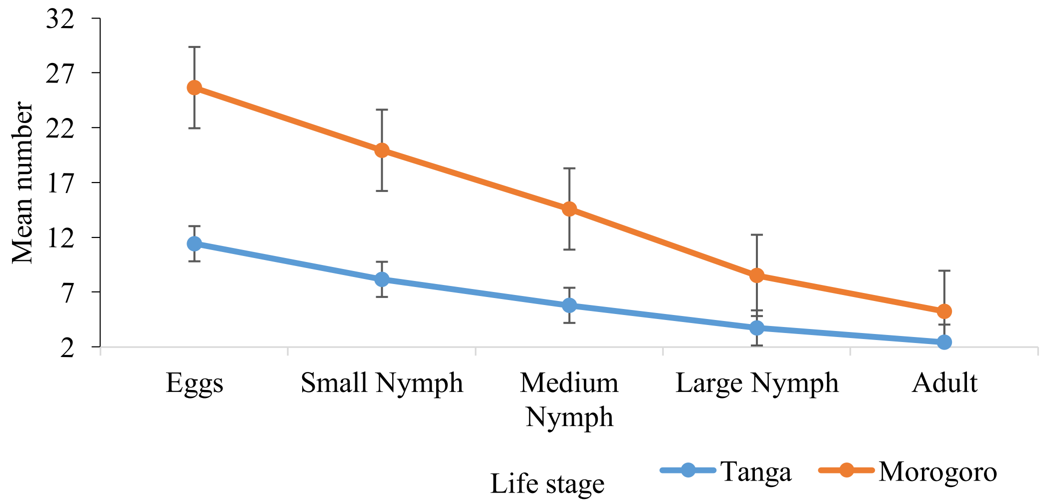

Figure 6: Mean trend of *H. cubana* life stages adults *L. leucocephala* in Morogoro and Tanga regions

The nymph population counts through subjective ratings (Table 1) showed most growing shoots of adult *L. leucocephala* had 6–30 nymphs followed by adults with 1–5 nymphs

(Figure 7 and 8). Regenerants *L. leucocephala* had 1–5 nymphs in Morogoro and 6–30 nymphsin Tanga region (Figure 9).

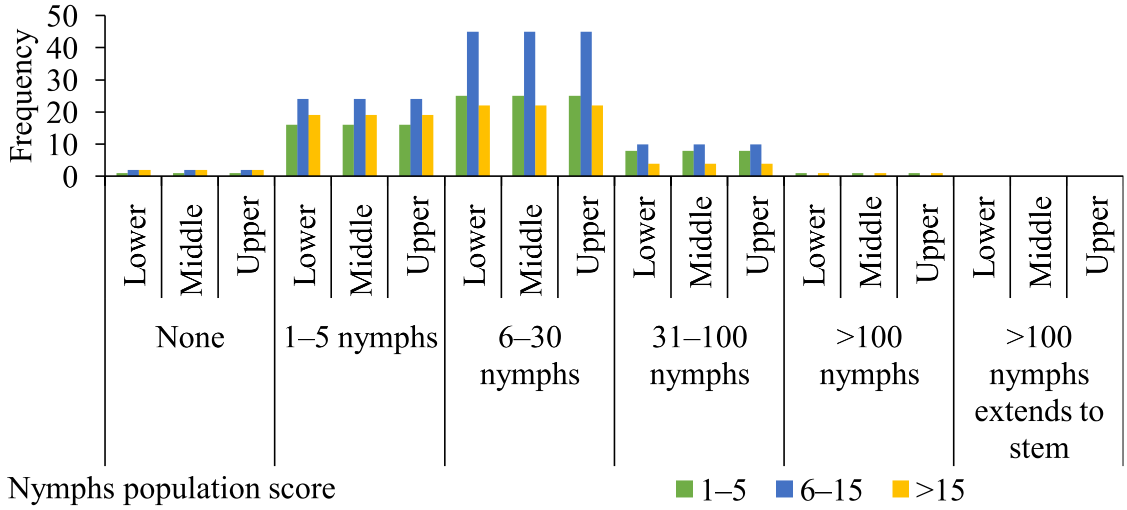

Figure 7: Nymph population counts through subjective ratings for adults *L. leucocephala* in Morogoro

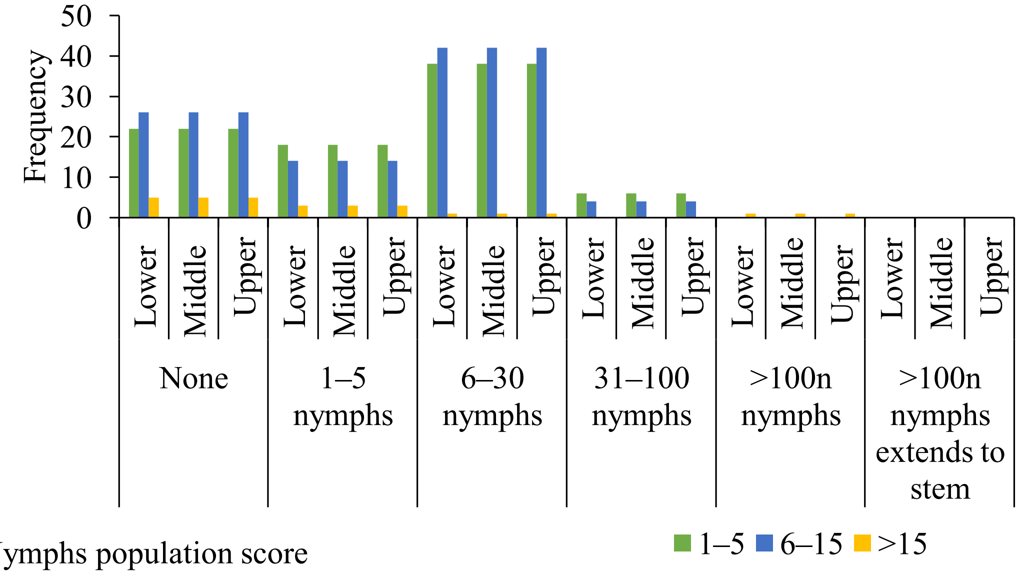

Figure 8: Nymph population counts through subjective ratings for adults *L. leucocephala* in Tanga

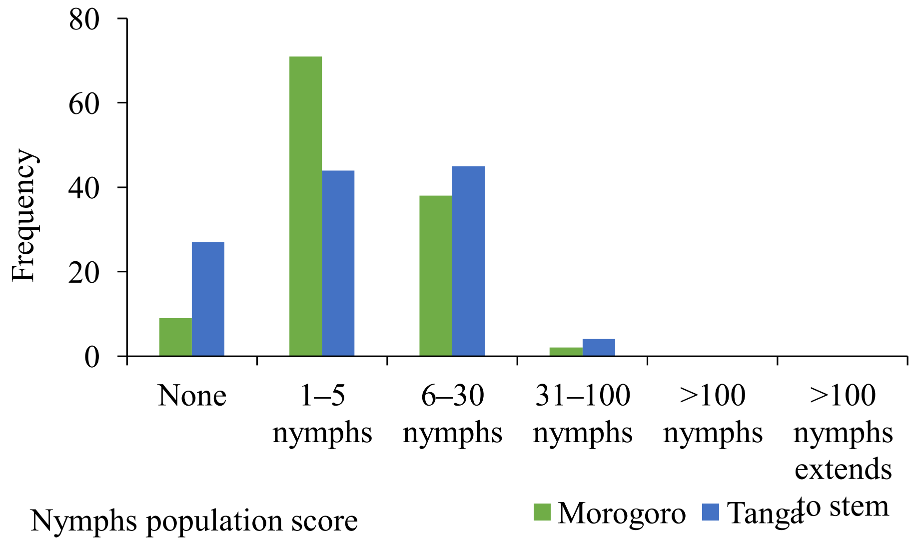

Figure 9: Nymph population counts through subjective ratings for regenerants *L. leucocephala* in Morogoro and Tanga regions

Incidence of *H. cubana*on *L. leucocephala* in Morogoro and Tanga Regions

The results revealed that there was generally slightly higher incidence of infestationin Morogoro compared to Tanga for both adults and regenerants *L. Leucocephala* (Figure 10 and 11). A high proportionof adults *L. leucocephala* were lightly infested in all three dbh classes in Morogoro and Tanga (Figure 10). A high proportion of regenerants of *L. leucocephala* were lightly and moderately infested in Morogoro andin Tanga (Figure 10). The results showed that there wasno significant relationship between dbh classes and infestation density in both Morogoro and Tanga. Chi- square results for association between dbh classes and infestation density were not significant different for adults and regenerants *L. leucocephala* in Morogoro and Tanga region (Table 3).

Table 3: Chi- square results for association between dbh classes and infestation density in Morogoro and Tanga regions

Region	dbh classes	χ^2 value	Df	P- value
Morogoro	1–5	20.0	16	0.220
	6–15	15.0	12	0.241
	>15	20.0	16	0.220
	Regenerants	20.0	16	0.220
Tanga	1–5	15.0	12	0.241
	6–15	10.0	8	0.265
	>15	15.0	12	0.241
	Regenerants	15.0	12	0.241

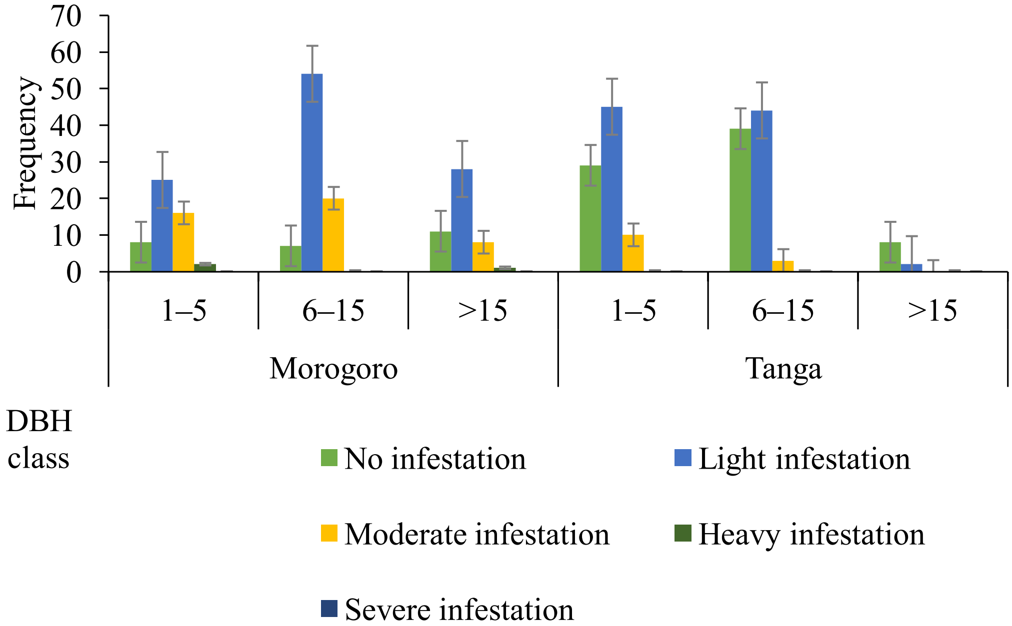

Figure 10: Infestation frequency of *H. cubana* on *L. leucocephala* in dbh classes in Morogoro and Tanga regions

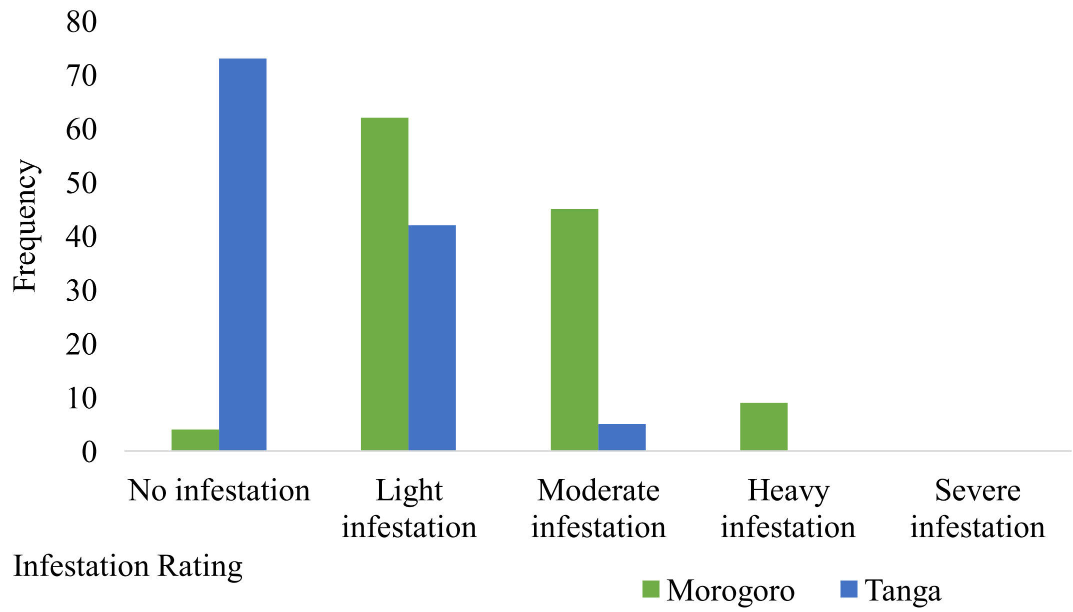

Figure 11: Infestation frequency of *H. cubana* on regenerants *L. leucocephala* in Morogoro and Tanga

Shoot Damage of *L. leucocephala* in Morogoro and Tanga Regions

The results showed thata high proportion of shoots of adults *L. leucocephala* were slightly damaged in all three dbh classes in Morogoro and Tanga (Figures 12 and 13). The Chi-square test results shows a significant difference between dbh classes and shoot health of adults *L. leucocephala* for all three dbh classes in Morogoro and Tanga regions (Table 4). The shoot health for regenerants *L. leucocephala* was not significant different in Morogoro and Tanga regions (Table 4).

Table 4: Chi- square test results shows a significant difference between dbh classes of shoot health in Morogoro and Tanga regions

Region	dbh classes	χ^2value	Df	P- value
Morogoro	1–5	24	6	0.001
	6–15	36.0	9	0.0001
	>15	36.0	9	0.0001
	Regenerants	12.0	9	0.213
Tanga	1–5	36.0	9	0.0001
	6–15	36.0	9	0.0001
	>15	36.0	9	0.0001
	Regenerants	12.0	9	0.213

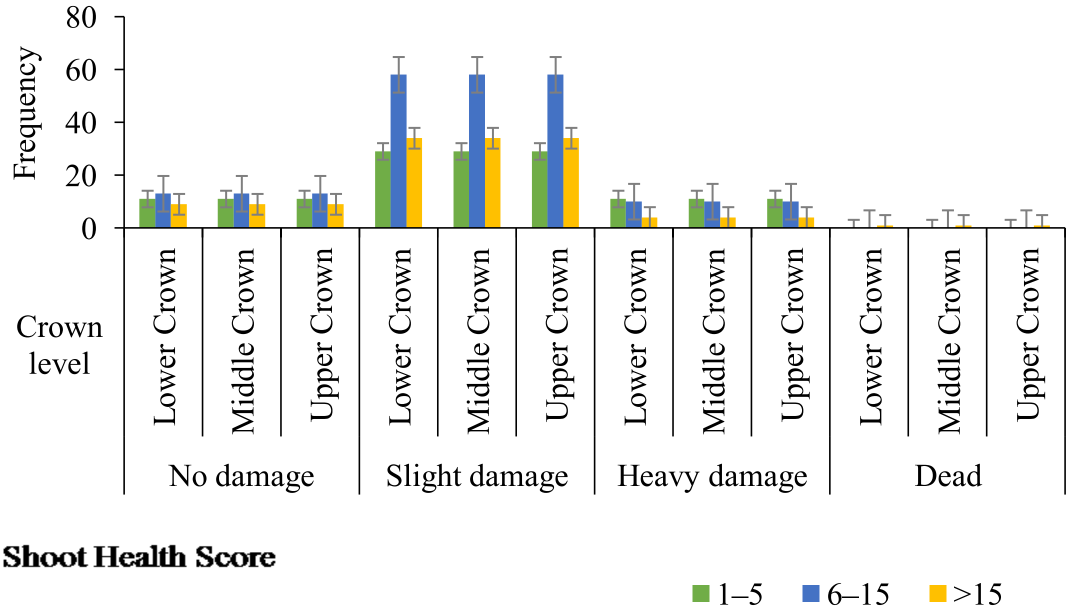

Figure 12: Shoot health frequency in different dbh classes and crown parts for adult *L. leucocephala* in Morogoro

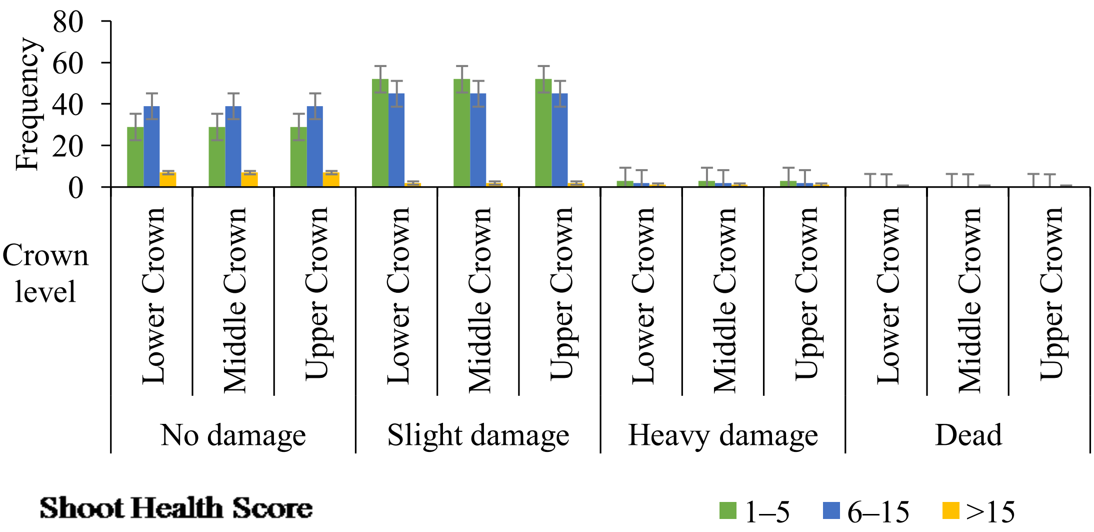

Figure 13: Shoot health frequency in different dbh classes and Crown parts for adult *L. leucocephala* in Tanga

Discussion

During this study a low population density of *H. cubana* was found compared to what was reported in other studies. In India, the new shoots were heavily infested with up to 3000 nymphs and adults per 15 cm of terminal shoot (Nair, 2007), which is very high compared to this study found. In Australia, it was reported field collected stem tips from all *L. leucocephala* individuals had *H. cubana* eggs of average 234±58 eggs/shoot (Shelton, 2008).

Over 90% of samples also had approximately 75-80 younger (instars 1-2) and older (instars 3-5) nymphs/shoot. However, the present study (in contrast to above reported studies) were carried out in sites where *T. leucaenae* and *P. yaseeni were* introduced in 1995/1996 (Madoffe *et al*., 2000; Madoffe and Petro, 2011). Ahmed *et al.,* 2014; Geiger and Andrew, 2000 and Shivankar *et al.* (2010) reported that *T.leucaenae* and *P.yaseeni* are one of successful biological control agentsagainst *H. cubana* in native and exotic locations.

The results of this study showed that *H. cubana* prefers cooler climates. During the study Morogoro had cooler climate than Tanga. Austin *et al*. (1996) and Castillo *et al*. (1997) reported high psyllid numbers throughout the year at Southeast Queensland and upland regions in Hawaii due to cooler climates. The current studyfound high number of egg and small nymphs compared with medium nymphs, large nymphs and adults. Similarly, Madoffe *et al.* (2000) found that small nymph populations were consistently higher than the other two instars and larger nymphs had the lowest populations in the same studied localities. This study found a decline in number at each life stage of *H. cubana* from eggs to adults which resemble same trend reported by Madoffe *et al.* (2000). The lower mean number of adult psyllid could be also due to escaping during cutting growing shoot to polythene bags for laboratory observation. In a study, one year done by Bruzas (1983) in South Africa, experienced higher number of eggs than nymph or adult *Pineus boerneri.*

The mean total numbers of *H. cubana* were not significantly different among dbh classes. This is because *H. cubana* prefer new growing shoots and new shoots were available at each dbh classes. In contrast, Madoffe and Petro (2011) reported a significant difference in numbers of *H. cubana* between middle and old age classes but not between middle and old age class. The difference is due to difference mode of attack by *P. boerneri* and *H. cubana* is highly seasonal in its occurrence (Ahmed *et al.,* 2014) and if food is available, cool temperatures could increase the psyllid populations (Bray, 1994; Madoffe and Massawe, 1994; Napompeth, 1994). In other studies, dry season led to tree stress and made trees susceptible to even moderate psyllid population (Larsson, 1989). Other studies showed that psyllid population was affected by temperature, moisture, humidity and exposure to wind (Ahmed *et al.,* 2014; Geiger and Andrew, 2000; McAuliffe, 2008) and the ups and downs of the *H. cubana* populations were related to an optimum cooler temperature range and the availability of tender shoots in Hawaii (Ahmed *et al.,* 2014).

Generally, the mean number of *H. cubana* per 15cm terminal shoot in Morogoro and Tanga for lower, middle and upper crown part were statistically insignificant which is similar to that reported by Madoffe and Petro (2011) that the infestation by *P. boerneri* between crown parts was not significant for *Pinus patula* and *Pinus elliottii*. Despite this, there was a slightly high population density in the lower crowns, which contrasted Petro and Madoffe (2011) who reported that middle crown part had higher total mean number of *P. boerneri*, followed by lower crown part and upper crown parts.

There was slightly high infestation density in Morogoro compared to Tanga for both adults and regenerants *L. leucocephala* which could be a result of high population density of *H. cubana* in Morogoro compared to Tanga. *Heteropsylla cubana* infestations are determined largely by the presence of nymphs and adults on the growing shoots of *L. leucocephala* (CABI, 2013). The slight infestation density in Morogoro and Tanga was a result of a role played by *T. leucaenae*, *P. yaseeni* and indigenous predators. Similarly, Madoffe *et al.* (2000) and Madoffe and Petro (2011) reported the declining *H. cubana* population recorded was probably due to hymenopterous parasitoids, *T. leucaenae* and *P.yaseeni* attack. In the Asia-Pacific Region, the release of *T.leucaenae* and *P.yaseeni* were reduced populations of *H. cubana* to their present low levels (Ahmed *et al.,* 2014; Nair, 2007). The insignificance difference between dbh classes and infestation density in both Morogoro and Tanga was due to availability of growing shoots for *H. cubana* feeding in each dbh class. The heavy level of shoots damage occurred in Morogoro compared to Tanga, after that the population fluctuation in *L. leucocephala*. Generally, shoot damage decreased in Morogoro and Tanga since release of parasitoids. Female *H. cubana* mostly like to lay eggs on very young shoots where they are lodged between the folds of the developing leaflets (CABI, 2013; Shelton, 2008). The regenerants *L. leucocephala* have high number *of* suitable growing shoot compared to adults *L. leucocephala*. That's why shoot health for regenerants *L. leucocephala* were similar in Morogoro and Tanga regions. These results are in agreement with Geiger and Andrew (2000) and Chazeau *et al*. (1989) who reported that, *H. cubana* populations increased only in the presence of young *L.leucocephala* leaves and new growing shoots.

Conclusion

The study investigated low *H. cubana* population at all life stages including eggs, small nymphs, medium nymphs, large nymphs and adults compared to previous studies.This could be due to role played in combination of *T. leucaenae* and *P. yaseeni*, indigenous predators and environmental factors.The study has found good shoot health and slightly infestation to *L.leucocephala* as a result of low population density of *H. cubana* in Morogoro and Tanga regions.

Recommendations

Based on the results from this study and experiences from other studies, it is recommended that; farmers should plant *L. leucocephala* for various uses without any fear about *H. cubana* as its population is no longer a problem. Further studies should be conducted on the status of *H. cubana* to other localities where the hymenopterous parasitoids were not released. In addition, studies to assess the seasonal population density of *H. cubana* and investigation on the effect of abiotic factors such as rainfall, temperature, wind velocity and others on *H. cubana* are suggested.

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CHAPTER THREE

MANUSCRIPT 2

3.0 Biological control and Parasitism of Leucaena psyllid, Heteropsylla cubana Crawford (Homoptera: Psyllidae) in Eastern Tanzania

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Abstract

Biological control offer potential solutions against *Heteropsylla cubana*is economically feasible and environmentally desirable. The objectives of the study were to quantify abundance of mummies of *Tamarixia leucaenae* and *Psyllaephagus yaseeni* and percentage of parasitization of *H. cubana* and to identify indigenous predators of the *H. cubana* in Morogoro and Tanga region. The Point Centre Quarter method was employed to select *Leucaena leucocephala* for observation. R and Excel program software were used in data analysis. The mean number of *H. cubana* mummies of *T. Leucaenae* and *P. yaseeni* were 2.33 and 1.68 in Tanga and 2.64 and 2.1 in Morogoro per 15cm terminal shoot respectively. This study found rate of parasitism of small nymph and medium nymph were 0.15% and 0.14% in Morogoro and 0.16% and 0.11% in Tanga of *P. yaseeni* and *T. leucaenae* respectively. The dominant indigenous predators were *Neoscona theisi*, *Araneus inustus*, *Coccinella transversalis*, *Chilocorus circumdatus*, *Coelophora inequalis*, *Menochilus sexmaculatus*, *Synonycha grandis*, *Harmonia sp* and *Chrysoperla* *sp.* This study revealed that *T. Leucaenae* and *P. yaseeni* have been established successfully in Eastern Tanzania. The observed mean mummies was low compared to previous studies. Farmers are advised to plant *L. leucocephala* for various use without any fear about *H. cubana* as the introduced hymenopterous parasitoids has controlled *H. cubana* population. We recommend further investigations to quantify number of *H. cubana* preyed on by individual indigenous predators in Tanzania to realize whether the indigenous predator is potential or not in controlling *H. cubana*.

Key words: Parasitism, *Leucaena leucocephala, Tamarixia leucaenae, Psyllaephagus yaseeni*, *Heteropsylla cubana*and Mummies

Introduction

Leucaena leucocephala (Lam.) de Wit is one amongst many multipurpose trees that has been promoted for fodder production in Tanzania (Lulandala and Hall, 1987). The tree is found in many parts of Tanzania and usually planted along farm boundaries and in homesteads for fodder, soil fertility improvement and fuelwood (Msangi *et al*., 2002). The outbreak of the devastating Leucaena psyllid, *Heteropsylla cubana* Crawford discouraged the spread of leucaena based fodder production technology and many other uses in the country. Most Leucaena stands were lowered after invasion of *H. cubana* (Madoffe *et al.,* 2000). Effort to control *H. cubana* via biological control in Kenya and Tanzania, the Asia-Pacific experience was considered the best option (Ciesla and Nshubemuki, 1995; Madoffe and Petro, 2011). Hymenopterous parasitoids *Tamarixia leucaena* Boucek (Hymenoptera: Eulophidae) and *Psyllaephagus yaseeni* Noyes (Hymenoptera: Encyrtidae) introduced from Trinidad and Tobago and released in Tanzania and Kenya are well established in Tanzania, have spread over large areas and they appear to being effective against their hosts (Madoffe *et al*., 2000; Madoffe and Petro, 2011). *T. leucaenae* is a solitary ectoparasitoid that lay eggs behind the hind coxae of third or fourth instar *H. cubana* nymphs (Patil *et al*., 1993). Oviposition by *T. leucaenae* appears to inhibit further nymphal development. *P. yaseeni* is a solitary endoparasitoid which attacks *H. cubana* instars first and second, which continue to develop until instar fifth when they mummify (Patil *et al.,* 1993).

Reduced *H. cubana* population and Leucaena shoot damage in some areas, could be attributed to the parasitoids and indigenous predators (Madoffe *et al*., 2000). However, little is known on abundance of *H. cubana* mummies of *T.Leucaenae* and *P. yaseeni* and percentage of parasitization of *H. cubana* in Africa, including Tanzania (Geiger and Gutierrrez, 2000). In addition, little has been done to identify the indigenous predators of the *H. cubana* in Tanzania and what already reported was not at species level (Kisaka, 1994; Madoffe *et al*., 2000). Therefore, this study aimed at identifying and determining the abundance of indigenous predators associated with *H. cubana*, abundance of *H. Cubana* mummies of *T. Leucaenae* and *P. yaseeni* and percentage of parasitization of *H. cubana*in Morogoro and Tanga.

The study was based on two hypotheses that: (1) There are several indigenous predators associated with *H. cubana*; (2) There will be high abundance of *H. cubana* mummiesof *T. Leucaenae* and *P. Yaseeni* (3) Thereis a high rate of parasitism of *H. cubana*. This results from present study will be useful to agroforesters, plant protectionists, conservationists, livestock keepers, and farmers whom have abandon planting *L. leucocephala* in the country to establish and use of Leucaena for multipurpose uses such as a source of fodder, fuelwood, shade for estate crops, reforestation, timber, erosion control and nitrogen fixation. Also communities will fully understand indigenous predators associated with *H. cubana* and look forward to conserve them for present and future generation.

Materials and Methods

Description of study area

Studies were conducted in selected sites of Morogoro and Tanga region, namely; Morogoro: SUA farm, Melela A and B; Tanga: Mlingano, Tanga dairy farm and Ziwani (Table 1).These sites were used in 1990s for the release of two *H. cubana* parasitoids and later assessed for their spread and establishment (Madoffe *et al.,* 2000). Morogoro region lies between latitude 5^o 58" and 10^o 0"to the South of the Equator and longitude 35^o 25" and 35^o 30" the East Greenwich with an area of 72, 939 square kilometers of the total Tanzania mainland and population of 2218492 (MRSEP, 2006; NBS Census, 2012). Tanga Region is situated at the extreme northeast corner of Tanzania between 4° and 6° degrees below the Equator and 37° - 39° 10' degrees east of the Greenwich Meridian. The region occupies an area of 26677 km^2 with a population of 2045205 (NBS Census, 2012).

Sampling Design

The Point Centre Quarter method (PCQ) was employed to assess population of *H. cubana* and parasitoids, damage rate and shoot health (Marisa, 2015). Five sampling points were established in each site, where one at center and other four at the corners of site (Figure 2). In each sampling point, four quadrants were established. The adults and regenerants *L. leucocephala* near sampling point at each quadrant were sampled and recorded. *L. leucocephala* were grouped into two cluster of regenerants (dbh< 1 cm) and adult species (dbh≥ 1 cm). Adult Leucaena trees were categorized into three dbh classes i.e., (1-5 cm), (6-15 cm) and (>15 cm) and then into three crown levels (upper, middle and lower level). In each quadrant, two adults *L. leucocephala* for each dbh classes and two regenerants were sampled.

Determining abundance of mummies of *T. Leucaenae* and *P. yaseeni*

Data were collected during early morning (05:30-9:00am). One 15cm growing shoot was randomly selected and sampled from each crown level for adult species and two 15cm growing shoot of two regenerants in each quadrant. The shoots were carefully cut into a polythene bag (destructive sampling) and put in a refrigerator to ensure its flesh for observation. The collected shoots were washed carefully with the help of a brush and ethanol (70%) into a petri-dish to remove mummies of *P. yaseeni* and *T. leucaenae*. In the Laboratory mummies of *P. yaseeni* and *T. leucaenae* were counted under a dissecting microscope.

Determining abundance of indigenous parasitoids

The collected growing shoots were washed carefully with the help of a brush and ethanol (70%) into a petri-dish to remove indigenous predators associated with *H. cubana*. The laboratory indigenous predators were counted under magnifying hand lens for identification of indigenous predators to species level.

Data Analysis

R version 3.2.3 and Microsoft excel computer software programs were used in data analysis. Descriptive statistics were used to determine mean abundance of *H. cubana* mummies of *P. yaseeni* and *T. leucaenae* and indigenous predators associated with *H. cubana*. Analysis of Variance (ANOVA) at 5% level of significance was used to determine whether the means abundance in *H. cubana* mummies of *P. yaseeni* and *T.*

leucaenae between dbh classes and growing sites are different. The percentages of parasitization were calculated from mummy and 5th-instar counts using the method of Luck *et al.* (1988):

Percentage parasitism = (Number of mummies /shoot)

(Correction factor) (Number of fifth instar/shoot

With

Corretation factor = 3.8

Results and discussion

Results

Abundance of Mummies of *Tamarixia leucaenae* and *Psyllaephagus yaseeni* establishment in Morogoro and Tanga regions

The mean numbers of *H. cubana* mummies of *P. yaseeni* and *T. leucaenae* per 15cm terminal shoot were 2.33 and 1.68 in Tanga and 2.64 and 2.1 in Morogoro respectively (Table 4). Results showed that the abundance of mummies of *Psyllaephagus yaseeni* in Morogoro was not statistically different among crown levels (F=1.21, df=2, P=0.298) and among dbh classes (F=1.29, df=2, P=0.27) in Morogoro. In Tanga mean numbers of *H. cubana* mummies of *P. yaseeni* were not significantly among crown levels (F=0.448, df=2, P=0.639) and among dbh classes (F=1.41, df=2, P=0.246). The interaction between dbh and crown level was not significantly different in both Morogoro (F=1.21, df= 4, P=0.302) and Tanga (F=0.827, df= 4, P=0.51). The abundance of *H. cubana* mummies of *T. leucaenae* were not statistically different among crown levels (F=1.28, df=2, P=0.278) and among dbh classes (F=0.98, df=2, P=0.37) in Morogoro. In Tanga *H. cubana* mummies of *T. leucaenae* were not significantly among crown levels (F=0.19, df=2, P=0.82) and among dbh classes (*F*=1.227, df=2, P=0.294). The interaction between dbh and crown level was not significantly different in both Morogoro (*F*=0.928, df= 4, P=0.447) and Tanga (*F*=1.101, df= 4, P=0.355). There was high mean numbers of mummies in regenerants *L. leucocephala* than in adult *L. leucocephala* for both *P. yaseeni* and *T. leucaenae* in Morogoro and Tanga (Figure 15, 16 and 17).

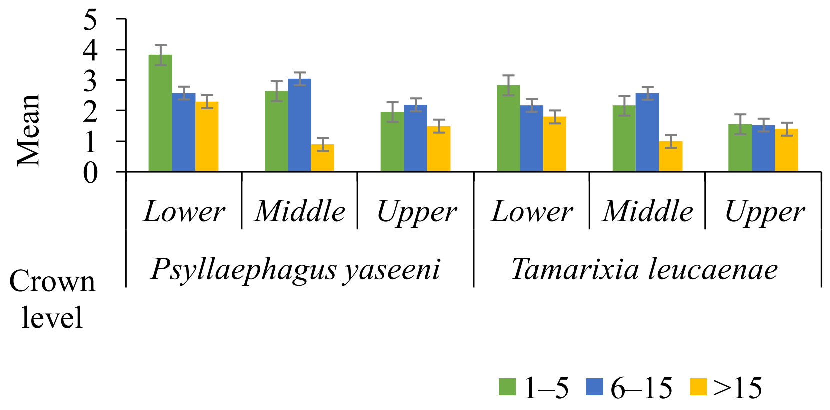

Figure 14: Mean numbers of *H. cubana* mummies of *P. yaseeni* and *T. leucaenae* for adult *L. leucocephala* in Morogoro regions

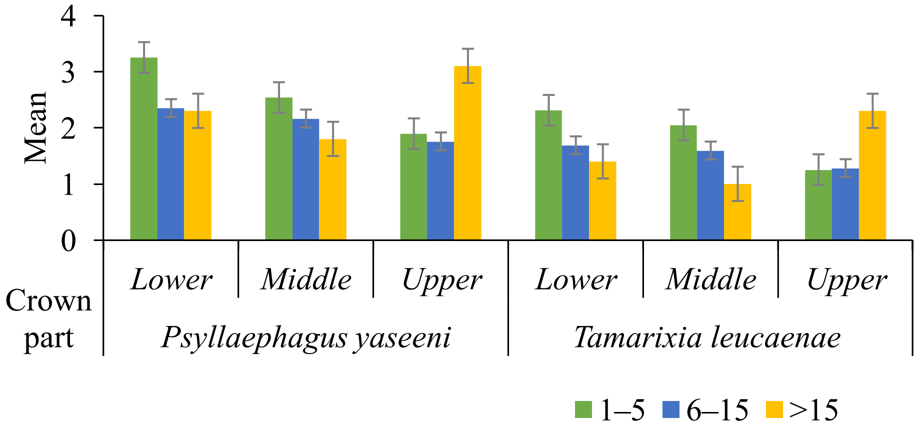

Figure 15: Mean number of *H. cubana* mummies of *P. yaseeni* and *T. leucaenae* for adult *L. leucocephala* in Tanga regions

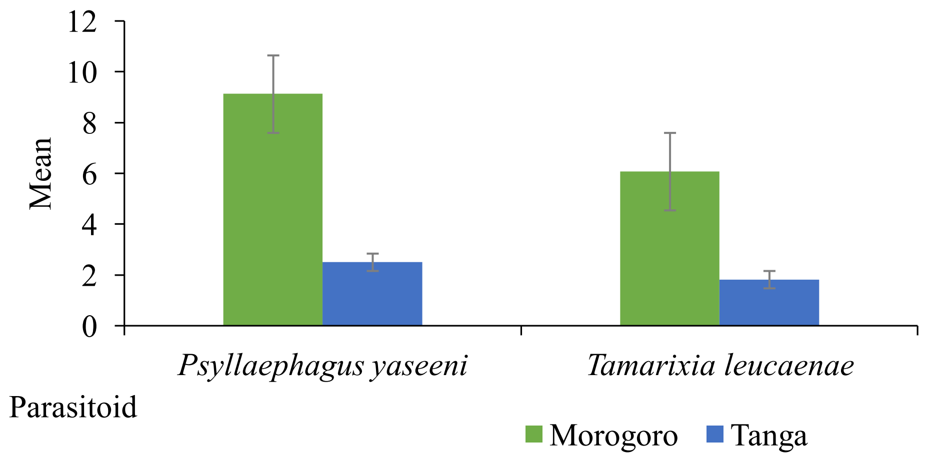

Figure 16: Mean number of *H. cubana* mummies of *Psyllaephagus yaseeni* and *Tamarixia leucaenae* for regenerants *Leucaena leucocephala* in Morogoro and Tanga regions

Abundance of indigenous predators associated with *H. cubana*in Morogoro and Tanga regions

The dominant indigenous predators found were spider (*Neoscona theisi* and *Araneus inustus*) (71.11%) of which about 60.44% observed in Tanga and 10.67% in Morogoro (Table 6), followed by ladybird beetles (*Coccinella transversalis*, *Chilocorus circumdatus*, *Coelophora inequalis*, *Menochilus sexmaculatus*, *Synonycha grandis* and *Harmonia* species) (22.67%) of which 14% in Morogoro and 8.67% in Tanga, un identified dragonfly (5.11) of which 3.67 in Morogoro and 1.33% in Tanga and lacewing (*Chrysoperla sp.*) (1.11%) which was only observed in Morogoro for adult *L. leucocephala* (Figure 10 and 11). The almost the same was observed for regenerants as the predator was spider 37.02% and 22.98%, followed by ladybird beetles 15.74% and 15.74%, dragonfly 2.12% and 5.11% in Tanga and Morogoro respectively while Lacewing 1.28% was observed in Morogoro only for regenerants *L. leucocephala*.

Parasitism Percentage of *H. cubana*in Morogoro and Tanga regions

The result found rate of parasitism of small nymph and medium nymph for adults *L. leucocephala* were 0.15% and 0.14% in Morogoro and 0.16% and 0.11% in Tanga for *P. yaseeni* and *T. leucaenae* respectively (Table 7). The rate of parasitism of small nymph and medium nymph for regenerants *L. leucocephala* were 0.47% and 0.41% in Morogoro and 0.47% and 0.31% in Tanga of *P. yaseeni* and *T. leucaenae* respectively.

Results showed that rate of parasitism of small nymph by *P. yaseeni* was not statistically significant different among dbh classes in Morogoro (F= 0.478, df=2, P=0.621) and in Tanga (F= 2.95, df=2, P=0.055). The rate of parasitism of medium nymphs was significantly different among dbh classes in Tanga (F=3.07, df=2, P=0.049) but not in Morogoro (F=0.495, df=2, P=0.610). The rate of parasitism of small nymph and medium nymph for regenerants were high compared to adult *L. leucocephala* (Table 7).

Table 5: Percentage of parasitization of *H. cubana* for adult *L. leucocephala* in Morogoro and Tanga regions

Region	dbh classes	% Parasitism	
		Psyllaephagus yaseeni	*Tamarixia leucaenae*
Morogoro	1–5	0.16	0.14
	6–15	0.16	0.13
	>15	0.13	0.16
	Mean total	0.15	0.14
Tanga	1–5	0.18	0.12
	6–15	0.14	0.09
	>15	0.12	0.08
	Mean total	0.16	0.11

Discussion

The study found low mean mummies compared to that reported by Madoffe *et al.* (2000), which was 10 and 11 mummies per growing shoot in Tanga and Morogogro respectively.

The decline in mummies were due to the decline of *H. cubana* population as a result of role played by *P. yaseeni* and *T. leucaenae*, indigenous predators and environmental factors. The trend of mummies for both *P.yaseeni* and *T. leucaenae* in the three dbh classes and localities were not different from that of *H. cubana* population for both regenerants and adults *L.leucocephala*. The low abundance of both *H. cubana* and mummies in Tanga were due to unfavourable climatic condition which does not support *H. cubana* production. This concur with other studies which shows that *H. cubana* population is affected by temperature, moisture, humidity and exposure to wind (Ahmed *et al.,* 2014; Geiger and Andrew, 2000; McAuliffe, 2008) and the ups and downs of the psyllid populations which in turn affect mummies production are related to an optimum cooler temperature range and the availability of tender shoots in Hawaii (Ahmed *et al.,* 2014).

This study found a low rate of parasitism compared to 0.23% using mummy count reported by Geiger and Andrew (2000). The low rate of parasitism was due to few host available for parasitism. At both localities, parasitism rates showed increased with nymph density which was different from that of Geiger and Andrew (2000), that parasitism increased with no density. The low parasitism rates 0.18%-0.08% (Table 3) of the *H. cubana* population in this study were similar to those observed in Hawaii (Uchida et al., 1992) and appear insufficient to suppress psyllid populations. After exposing *T.leucaenae* to psyllid nymphs in quarantine, only one parasitized nymph was obtained and the entire culture eventually perished. As a result, there was no further attempt to utilize this species in Thailand (Napompeth, 1994). *Tamarixia radiata* Waters on was introduced from Reunion to Taiwan between 1984 and 1988 for control of *Diaphorina citri* Kuwayama and has caused a substantial reduction in populations of the psyllid, with up to 100%

parasitism recorded (Chien *et al*., 1989). The current study revealed that the found indigenous predators associated with *H. cubana* are similar with previous studies reported. Indeed, the study did not prove that these indigenous natural enemies were feeding on the psyllid, consequently contributing to the declining psyllid population. Madoffe *et al.* (2000) found several arthropod natural enemies living in association with the *H. cubana* being spiders, ladybird beetles, ants, dragonflies and lacewings. In contrast to this study, ants were not found in association with *H. cubana*. The found indigenous predators were reported as important predators in South East Asia Pacific Region and Central America and (Ahmed *et al.,* 2014, Nakahara *et al*., 1987; McClay, 1990; Napompeth, 1994) although there is still no quantitative evidence on the role of indigenous predators against *H. cubana* (Shivankar *et al*., 2010).

The study by Geiger and Andrew (2000) were reported predominant coccinellid predators at all study sites were *Menochilus sexmaculatus* (F.), followed by *Oenopia sauzeti* Mulsant and *O.kirbyi* Mulsant (highland site only) and *Micraspisdiscolor* (F.). *Coccinella transversalis* F. and *Micraspis lineata* (Thunberg) were occasionally observed at the valley site. Numerous spider and dragonfly species, 4 species of ants, vespid wasps, syrphids, reduviids, mirids, 1 *Geocoris* species and lacewings were also observed preying on *H. cubana,* but they were not abundant. This concur with current study found in Morogoro and Tanga.The study revealed a positive relationship and statistically significant between population density of *H. cubana* and mummies of *T.leucaenae* and *P.yaseeni* establishment in Tanga and Morogoro, which means that the high population density of *H. cubana*was related to the mummification.

Conclusions

This study has shown that *T. leucaenae* and *P. yaseeni* has been established successfully in Eastern Tanzania, despite the fact that *P. yaseeni* were neither released in Tanga nor Morogoro. Mummies population found lower significantly compared to what was reported soon after the release of the *T. leucaenae* and *P. yaseeni* in 1995/1996. The findings from the present study revealed that dominant indigenous predators of *H. cubana* in Tanzania are *Neoscona theisi*, *Araneus inustus*, *Coccinella transversalis*, *Chilocorus circumdatus*, *Coelophora inequalis*, *Menochilus sexmaculatus*, *Synonycha grandis*, *Harmonia* species, *Chrysoperla* species and several unidentified dragonfly and mantispid.

Recommendations

Farmers are advised to plant *L. leucocephala* for various use without any fear about *H. cubana* as *T. leucaenae* and *P. yaseeni* has significantly manipulate psyllid's population. Due to time constraint this study has not manage to assess the temporal nature of both mummies of *T. leucaenae* and *P. yaseeni*, further investigation is recommended. Also, other investigation should look on the effect of abiotic factors such as rainfall, temperature, wind velocity and others onmummies of *T. leucaenae* and *P. yaseeni*. The amount of *H. cubana* preyed by found indigenous predators and predator dynamics is not well known. Further investigation should look on the interaction of indigenous predators and *H. cubana*.

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CHAPTER FOUR

4.0 CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

Based on the findings from this study the following conclusions are made;

i. The mean number of *H. cubana* population density was very low compared to other studies due to role played in combination of *Tamarixia leucaenae* and *Psyllaephagus yaseeni*, indigenous predators and environmental factors.

ii. The study has found good shoot health and slightly infestation to *Leucaena leucocephala* as a result of low population density of *H. cubana*.

iii. This study has shown that *T. Leucaenae* and *P. yaseeni* has been established successfully in Eastern Tanzania, despite the fact that *P. yaseeni* were neither released in Tanga nor Morogoro.

iv. The study has found low psyllid mummies compared to what was reported soon after the release of the *T. Leucaenae* and *P. yaseeni* in 1995/1996.

4.2 Recommendations

Based on the results from this study and experiences from other studies, it is recommended that;

i. Farmers should now again plant *L.leucocephala* for various use without any fear about *H. cubana* as its population is no longer a problem.

ii. Farmers and others who practice agroforestry should conserve the found indigenous predators in order to continue keeping in check *H. cubana* population in checkfor present and future generation.

iii. Further study should be conducted on what extent found indigenous predators consume *H. cubana* as well as the status of *H. cubana* to other localities where *T. Leucaenae* and *P. yaseeni* were not released.

iv. Since, due to time constraint this study has not manage to assess the temporal nature of both *H. cubana*, mummies of *T. Leucaenae* and *P. yaseeni*, further investigation should look on this.

v. Also, other investigation should look on the effect of abiotic factors such as rainfall, temperature, wind velocity and others on both *H. cubana* and *T. Leucaenae, P. yaseeni* as well as indigenous predators. Additionally, studies on indigenous predators dynamics is highly recommended.