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The role of agroforestry tree species in biocontrol of the cabbage pest *Plutella* *xylostella* – A field study in Kenya

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– A field study in Kenya

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Abstract

Pest control in agriculture is a necessity and the use of chemicals is the method by choice for most farmers. But the use of chemicals comes with a price; the pests develop resistance and the chemicals pollute the environment. It is therefore of urgent importance to shift pest control from chemicals to other methods. Biological control is an alternative which can prove to be as effective as chemicals but without the negative effects. In Kenya, the agriculture sector is the backbone in the economy, contributing to the livelihood of near 80% of the population. One of the most common vegetables grown in the country is cabbage, but due to various pests, the production is threatened. The most severe pests affecting cabbage is the diamondback moth, *Plutella xylostella*, DBM for short. If left uncontrolled, the moth larvae can lead to massive yield losses, and in Kenya entire yields have been destroyed. This study aimed to investigate if the use of different tree species used in an agroforestry system affected the presence of two natural enemies to DBM, parasitoids and insectivorous birds.

Cabbage plants, *Brassica oleracea* var. *capitata*, were grown specifically for the study and distributed to different plots fulfilling specific study criteria regarding presence of one of three tree species. Observations of DBM-larvae presence and collection of pupae were made at regular intervals during a period of two weeks. Due to bad weather conditions during the study: drought during the initial phase leading to plants dying, followed by rainy nights hampering the expected DBM population growth, the DBM abundance was much lower than expected. Due to these circumstances leading to reduced number of replicates when entire plots had to be abandoned and DBM densities were low, statistical tests of data could not be performed. Nevertheless, caged plants seemed to have an effect, indicating that birds did contribute to the reduction and the loss was slightly higher on *Macadamia* plots.

Keywords: DBM, Cabbage, Agroforestry

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1 Aim and hypothesis

1.1 Aim

This study consisted of two parts where the first part was a field study and the other the interviewing of local farmers. The aim of the field study was to investigate if the tree species in an agroforestry system indirectly affects the abundance of the diamondback moth (*Plutella xylostella* (L.) (Lepidoptera: Plutellidae)), by focusing on effects on two types of natural enemies: insectivorous birds and parasitoids. The local farmers hosting the experiment were interviewed to get an overview of the farm practice in the region, how common the tree species are and if biocontrol is something that the local farmers have experienced. The study was limited to farms with the tree species *Eucalyptus* spp., *Grevillea robusta* and *Macadamia integrifolia*.

1.2 Hypothesis

Various tree species growing adjacent to a cabbage field (plot) affects the occurrence of two types of natural enemies of the diamondback moth (DBM) differently due to their various morphology and growing strategy. The hypothesis was that *Macadamia*, with its large canopy and sweet nectar, would attract more natural enemies of *Plutella xylostella* than the other tree species.

2 Introduction

2.1 Kenya

Kenya, named after the country's highest mountain, Mount Kenya, is an equatorial country located on the Eastern side of Sub-Saharan Africa, along the coast of the Indian Ocean (Fig. 1). Both the country's geography and climate vary greatly - from low plains to highlands and a climate ranging from savannah in the South to tropical rainforests along the coast and in the western part of the country. Most of the land is very dry, 82% of the country is semi-arid or arid (Kenya Land Alliance (KLA), 2015).

The rains fall mainly during the two rainy seasons which are between March/April to May/June and October to November/December and precipitation is most plentiful in the highlands with an average of 1200 mm/year (Kenya Land Alliance, 2015). The highlands are due to this ideal for farming and some of the best agricultural regions in Africa are found here (CIA, 2019).



*Figure 1. Map of Africa where Kenya is highlighted.
(Picture: CIA)*

2.1.1 Agriculture in Kenya

The agricultural sector is a crucial part of Kenya's economy and around 75% of the 53 million residents works at least part time in the sector (CIA, 2019). The sector

contributes, both directly and indirectly, to approximately 50% of the Gross Domestic Product, provides the livelihood of over 80% of the population and contributes to 65% of the country's export earnings (The Food and Agriculture Organization (FAO), 2019). The semi-arid areas support mixed crops and livestock breeding, while ranching, wildlife conservation and pastoralism are common in the arid areas since crop failures are common there (KLA, 2015). The arable lands, mainly in the highlands, support a large production of cash crops such as coffee and tea, and food crops such as maize, cassava, tomatoes, kale and cabbage (CIA, 2019).

However, the sector does not only contribute with food and income, it is also the reason to one of Kenya's current environmental issues, which is the degrading quality of fresh water due to agrochemical residues (CIA, 2019).

Cabbage

One of the most grown vegetables in Kenya is cabbage (*Brassica oleracea* var. *capitata*) (Ogol and Makatiani, 2007). It is grown all over the country, but primarily in the Central and Rift Valley provinces at altitudes 800-2000 meters above sea level (Macharia et al., 2005). The main produce comes from small scale farmers, which grow it for own consumption and as a cash crop, not from industrial scale farms. Most of the crops are sold locally at markets in the vicinity of where it is produced. Cabbage belongs to the family Brassicaceae that includes, varieties such as broccoli (*B. oleracea* var. *italica*), cauliflower (*B. oleracea* var. *botrytis*), and kale (*B. oleracea* var. *acephala*) (Seif and Nyambo, 2013). The global production of cabbage is close to 71.5 million tonnes (MT). Asia is the main producer with 54.1 MT (75.7% of the world's total production), Europe second with 11.4 MT (16 %). The African continent comes in at 3.35 MT (4.7%) where Kenya alone produces 690 kT (approx.1%), which represents 20.6% of the African production of that crop (FAO-STAT; 2017).

The yield of cabbage, as well as other vegetables from the Brassicaceae family, is often severely reduced by the damage inflicted by different pests.

2.1.2 Embu county

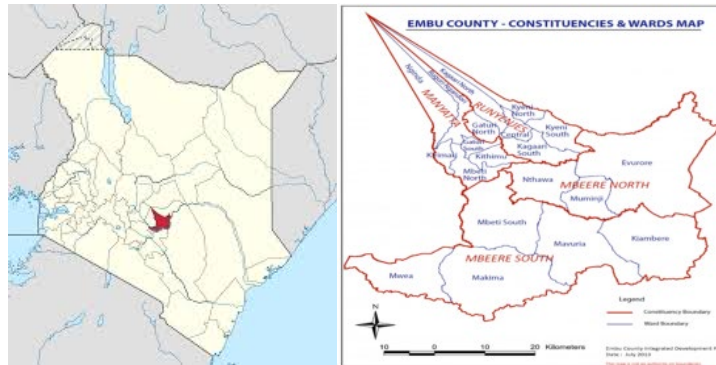


Figure 2. Left: Location of Embu County in Kenya (Picture: Wikipedia, 2019). Right: Embu County (Picture: embu.go.ke, 2019).

Embu county is in the northeast part of Kenya, approximately 120 kilometres from the capital city of Nairobi. It is located on the foothill of Mount Kenya. In the lower regions, the agro-ecological conditions are not optimal due to a semiarid climate, while the higher regions, closer to Mount Kenya, have a greater potential as a result of higher annual precipitation and lower temperatures (Climate Risk Profile for Embu County, 2017). Embu county is dominated by small-scale farmers, and the main food crops grown here are maize, beans, sweet potatoes, kale and cabbage while cash crops and industrial crops include coffee, *Macadamia* nuts, cotton and tea (Embu.go.ke).

Embu county has a bimodal rain pattern with two distinct rain seasons - one between March and June and the other between October to December. The county has great differences in altitudes, and the annual precipitation varies depending on height above sea level, with an average of 1120 mm and a mean temperature of 20.2 °C (Embu.go.ke; Climate-Data.org)

2.2 Agroforestry

According to the UN, the world population will reach 9.1 billion by the year 2050 and in order to produce food in enough quantities, the intensification of agriculture is a necessity. However, the area of arable land is decreasing as a result of improper use and management such as overgrazing, the removal of crop-residues, excessive tillage and forest conversion, as well as the overuse of chemicals for pest control and soil fertilization. This has led to severe land degradation. Today, 50% of all land world-wide suitable for vegetation, has been transformed into agricultural land (Zomer et al., 2016) which is affecting the conditions for agriculture negatively in numerous ways. For instance, the conversion from forest into agricultural land leads

to a more simplified landscape and hence a decrease in biodiversity with the result of a lower number of natural enemies of crop pests in the fields (Dainese et al., 2019). Often, natural enemies of pests are very effective in keeping the population below economically damaging levels and destruction of the natural relationship between pest and control agent can lead to pest outbreaks (Thacker 2002). A more sustainable land use is central and one way to achieve this is by the implementation of agroforestry.

Agroforestry is a cropping system in which woody perennials are planted together with, or in proximity to food crops or pastures but can also be achieved when farming is introduced to existing forests. The trees provide the farmer with both direct and indirect benefits. Some of the direct ones are providing fodder for livestock, wood and fuel, as well as products to sell such as fruits and nuts and often an increased yield. In arid and semi-arid areas, such as sub-Saharan and East Africa, agroforestry practices may increase the yields by 100% (Branca et al., 2013). Indirect benefits are, among others, enhanced soil fertility, soil- and water conservation and windbreak where the lower wind speed reduces both transpiration and soil erosion. Further, the habitat complexity in these systems is higher, leading to a greater biodiversity (Guenat et al., 2019). Many studies have shown that complexity and presence of trees in agricultural habitats can improve regulation of different pests by increasing the abundance of natural enemies, both vertebrates, for example birds and bats, and invertebrates such as insects, spiders, and mites (Hawkins et al., 1997; Guenat et al., 2019; Linden et al., 2019). As an example, Guenat et al. (2019) found that the abundance of both aphids and caterpillars were lower in fields where shade trees were present. In a study by Karp et al. (2013) the number of birds active in pest control were higher in systems with a higher number of trees. The number of parasitoids and pest reduction have been found to increase in complex surroundings such as forests, forest edges and road verges Bianchi et al. (2008). For some parasitoid species, forests are even considered necessary habitats.

Agroforestry is of global interest, promoted by organisations like FAO and The European Agroforestry Federation (EURAF). It is widely used on the African continent including in Kenya where trees are intercropped in the fields at most small-scale farms. One drawback with agroforestry is that it is not easily compatible with industrial production and therefore more often practiced by small-scale farmers.

2.3 Trees in Agroforestry

Depending on the purpose of the tree species planted, different characteristics are desirable. If the trees are going to be used on a farm with livestock, the trees have to be free of toxic substances and preferably have a high protein content to work as fodder while trees that will be used as windbreaks should have a deep rooting system, keeping them anchored to the ground. Some factors that applies for most common agroforestry trees are that they should be easily established, easy to propagate, be fast growing and preferably have a deep root system to prevent them from competing with the crop for water and nutrients. They should also provide the farmer with something; may it be food or products to sell such as timber or charcoal. Three tree species, commonly planted in the area where this study was conducted, are further presented below.

2.3.1 *Eucalyptus* spp.

Eucalyptus (family Myrtaceae) is native to Australia and is the most widely cultivated tree in the world. The *Eucalyptus* genus includes more than 900 species and numerous hybrids.

It was introduced to Kenya 1902, during the railroad expansion in the country, as a fast growing source for wood material (Oballa and Konuche, 2010).

It can grow in all agro-ecological zones from dry lowlands to wet highlands, and on a variety of soils, even infertile sands and heavy clays. Today, *Eucalyptus* trees are grown and sold mainly as building materials, but also as fuelwood, windbreaks and transmission poles. The fact that it is both fast growing and has a high commercial value makes it a popular tree planted at small farms throughout Kenya (Kluthe and Chen, 2017). However, since the tree has an extensive root system it may compete for nutrients and water with crops, especially during scarce condition, and water sources nearby *Eucalyptus* plantations have been observed to dry out. Further, studies have shown that compared to native forests *Eucalyptus* plantations have a lower species richness (Calviño-Cancela, 2013; Goded et al. 2019). Considering these facts, *Eucalyptus* is not ideal to grow in agroforestry systems, but for the sake of its high cash potential it is still frequently planted (Oballa and Konuche, 2010).

2.3.2 *Grevillea robusta*

Grevillea robusta (family Proteaceae) is an evergreen tree native to Australia. It was introduced to Kenya in the late 19th century as windbreak and shading in coffee and tea plantations. It is a common tree in agroforestry systems, and it is grown in 19 of the country's 42 districts and it is the dominating tree species on the eastern and

southern slopes of Mt. Kenya. In Embu county, 37% of the trees on 254 randomly selected farms in a study were *Grevillea* (Muchiri, 2004).

The reasons for its popularity are many; it is easily established and propagated, it grows rapidly, and it provides economically viable products such as firewood, charcoal and timber. Moreover, it has a high tolerance to pests and pathogens and a deep rooting system, leading to it being a tree well suited for agroforestry systems since it can withdraw water and nutrients from horizons beneath the crop-rooting zone. Due to its cluster roots, it is also well adapted to grow in soil with little available nutrients (Muchiri, 2004; ANPSA, 2013). The final height is between 12-25 m and the fernlike leaves can grow up to 30 centimetres long. When fallen to the ground, these hinder evaporation, leading to a high soil moisture. They also provide the soil with a rich mulch, leading to a high amount of organic matter. During drought the branches are also used as fodder for livestock.

When mature the tree starts to produce yellow flowers, which flowers throughout the year. These are a popular food source for bees, and other pollinating insects, and therefore good at farm with apiculture, and they are also attractive to birds (ANPSA, 2009).

2.3.3 *Macadamia integrifolia*

Macadamia integrifolia is a large, evergreen tree belonging to the family Proteaceae. As the previously mentioned tree species, it is indigenous to Australia and was introduced to Kenya in 1946.

It can grow up to 18 metres tall and has a wide foliage of up to 15 metres. It has a cluster of small roots, which is increasing the surface area of the root system for maximum absorption, leading to the ability to grow on very dry soils.

The trees are grown for their nuts, which are sold both locally and exported to other countries. Kenya is the fourth largest producer of *Macadamia* nuts (Gitonga et al., 2011) and 10% of all *Macadamia* nuts are produced in the country. Areas with a large production are Embu, Meru, Kiambu, Kirinyaga and Nyeri (Ondabu et al., 2007).

The pollen of its flowers is attractive to bees, hence constituting a good tree to grow at farms with apiculture. The nut shells can be used as fuel, and the tree is excellent for shading due to its dense foliage. It can be used in inter-row with other cash-crops, making it a popular tree in agroforestry systems.

2.4 Diamondback Moth

In agriculture, pests and diseases have always been a concern, regardless of crop or climate. Every year, they are the cause of pre- and postharvest losses of up to 50%, globally (Thacker 2002). One species, causing huge losses in its host crops, is *Plutella xylostella*, also known as the diamondback moth (DBM). Its host range is species belonging to the family *Brassicaceae*. A somewhat dated report by Macharia et al. (2005) states that the yearly yield loss to DBM in Kenya is between 31-35%. Losses of entire yields has also been reported (Muthamia et al., 2011) and in South-east Asia, losses of 90% during DBM outbreaks has occurred (Sarfraz et al., 2005). Annually, DBM is estimated to cost the global economy 4-5 billion dollars (Furlong et al., 2013). Despite an increased use of pesticides, which is the main procedure of tackling the concern in conventional farming systems, the losses to pests are still great. In some highland areas of Malaysia, farmers apply pesticides up to twice a week during the growing period of three months. The pesticides used are many times non-selective and often a mixture of different types. However, this way of controlling the pest is not limited to Malaysia but used in most tropical countries (Furlong et al., 2013). The positive effect of pesticides, which often is a higher yield, needs to be related to its negative effects. Except for it being an economic expense for the farmer it may pose direct health risks often as a result of improper protective equipment. Further, the use has a negative impact on the environment with contamination of both soils and water where the pollution of groundwater is a global problem. The use also disrupts the biological control by the killing of natural enemies as well as killing off other non-harmful or beneficial insects (Kahuthia-Gathu et al. 2008; Wasim Aktar et al., 2009; Mithöfer and Waibel, 2011). Another major issue associated with an extensive use of pesticides is that it promotes the selection for pesticide resistance. DBM has a very high ability to develop resistance and it was the first crop pest reported to develop resistance to DDT and microbial *Bacillus thuringiensis* insecticides, (Talekar and Shelton, 1993). Further, it has shown resistance to all major groups of insecticides (Trocza et al., 2012; Furlong et al., 2013).

2.4.1 Biology of the Diamondback Moth

The female DBM uses volatiles to locate a suitable host plant (Couty et al., 2006) where oval shaped and white-yellowish eggs are placed mainly in concavities, like the base of the leaves, or along the veins primarily on the leaf underside. The larvae that hatch cause severe damage due to their great appetite. The larvae have four instars, and the first instar lives as a leaf-miner while second, third and fourth instar feeds on the whole plant tissue. When the last larval stage is ready to pupate, it creates a thin, white cocoon attached to the underside of the leaf.

The DBM larva is oligophagous, and in addition to cabbage, it feeds on weeds and cultivated plants containing mustard glucosides (Talekar and Shelton, 1993).

Although being weak active flyers the pest is considered to be the most universally spread Lepidopteran. This is since they can migrate and invade crops as far as at other continents by passive wind dispersal. Eggs, larvae and pupa can also be spread to new areas through plant materials (Talekar and Shelton, 1993).

In tropical countries, the brassica production is continuous throughout the year, and in these regions DBM can have up to 20 generations (Talekar and Shelton, 1993).



Figure 3. Left: DBM larvae feeding on host plant. Note the characteristic windowlike holes (Picture: agric.wa.gov.au). Right: A heavily infected cabbage (Picture: Infonet-biovision.org).

2.4.2 Biological Control of the Diamondback Moth

In biological control, living organisms are utilised for suppressing the impact, or quantity, of a certain pest, making the pest population less abundant and hence reducing the damage level it would otherwise cause. Natural enemies can either deliberately be introduced to a new area, where the pest is present, or the environment, where specific enemies live, can be modified to improve their survival conditions (Thacker 2002). A great number of different natural enemies of DBM have been reported, including vertebrates and invertebrates (e.g. spiders, insect predators and parasitoids). In this study the focus will be on parasitoids and insectivorous birds.

Parasitoids

Parasitoids can be either external feeders, called ectoparasites or internal feeders, endoparasites. It is primarily the larval stage that is feeding on the host, but in some species also the adults are feeding on the host. However, in general adult parasitoids feed on nectar and other sugar-rich resources, such as honeydew. The most important groups of parasitoids of DBM belong to Hymenoptera and two studied species are *Diadegma semiclausum* and *Cotesia plutellae* (Momanyi et al., 2006). According to a study by Hawkins et al. (1997) parasitoids are among the most effective

killers compared to both predators and pathogens, especially during mid/late larval and pupal stage. For example, under greenhouse conditions, *Diadegma*, may have a parasitism rate of up to 95% (Miranda Ortiz, 2011). After the introduction of the parasitoids *Cotesia plutellae* and *Diadromus collaris* in St Helena, a remote island outside the western coast of Africa, the reduction was so great that the pest status of DBM became insignificant (Momanyi et al., 2006). Parasitoids are highly sensitivity to insecticides (Furlong et al., 2004; Bommarco et al., 2011; Jonsson et al., 2012) and the occurrence of parasitoids correlates with the farming practice, which Furlong et al. (2004) shows in a study where they compared the prevalence of parasitoids in different farming systems.



Figure 4. To the left; a pupa of diamondback moth, *Plutella xylostella*, parasitised by *Diadegma semiclausum* (Picture: pestnet.org). To the right is an unparasitized pupa (Picture: ipm-info.org). Note the clear difference in colour and the slight difference in shape.

Insectivorous birds

In Kenya there are many birds that prey on different types of insects. According to a study made by Ndang'ang'a et al., (2013) in the Nyandarua County, central Kenya, 45% of the 195 bird species in the agricultural area were predominantly insectivorous. A study in the tropical farmland of Kenya have estimated that insectivorous birds consumed a minimum of 1000 kg pest invertebrates per square kilometre a year (Ferber et al., 2013). Several studies where birds have been excluded from the crop have shown a significantly higher abundance of both pests and reduction in leaf area comparing to control plants, suggesting that birds are important as biological control agents of crop pests (Ndang'ang'a et al., 2013; Linden et al., 2019). In a study by Ndang'ang'a et al. (2013) the exclusion of birds in kale plantations led to both a high increase in the number of pests, in this case aphids and thrips, with damage to the leaf up to three times as high as on control plants. A study by Karp et al. (2013) showed that birds were responsible for the control of coffee-berry borer on plantations in Costa Rica, leading to a significant economic benefit for the farmer.

Method

2.5 Choosing Tree Species

To be able to get an overview of what tree species were common on the farms and in Embu county field visits were done. Based on these visits, together with consultation by supervisors and university staff, three tree species for the study were selected: *Grevillea*, *Eucalyptus* and *Macadamia*. A total of 11 farms suitable for the study was chosen.

2.5.1 The plots

The plot location at each farm was selected in fields where the neighbouring trees were dominated by one of the chosen tree species: *Grevillea*, *Eucalyptus* or *Macadamia*. For the species to be considered dominant, its proportional tree cover had to be a minimum of 50%. Since the tree species have different growing strategies regarding canopy size and trunk height, a minimum of five *Grevillea* or *Eucalyptus* trees, respectively, had to border the plot. For *Macadamia*, with much larger canopy, two were considered adequate. On farms with more than one plot established a minimum distance of 50 metres between plots was used and the same tree line could only border one plot.

2.6 Study Design

The research was conducted between April and June 2019. Five of the plots were in the Mutunduri area, in the northern part of Embu county and ten in Njikuri area, near Embu town.

In late April uniformly sized seedlings of cabbage (variety Queen F1), from a nearby nursery, were planted into sixteen plots of 3.7 x 3.7 m (12 x 12 feet).

The plan was to have five replicates of each tree species, but it turned out to be difficult. Although being a common agroforestry tree, *Eucalyptus* is usually not planted in proximity to growing crops due to its tendency to compete for water and nutrients. Therefore, only four suitable plots next to a line of *Eucalyptus* trees were found, and three out of these four plots could be used in the study. This was due to that the seedlings on one of the plots were eaten by Noctuid moth larvae *Agrotis spp*, leaving only 13 seedlings. Since both *Agrotis spp* and *Plutella xylostella* belong to the genera Lepidopteran, treatment of the plot with insecticides was not possible since it would negatively affect *P. xylostella* as well.

Next to *Grevillea* trees, five plots were established as planned and all could be included in the study. At one of the farms, the tree species composition surrounding the plot changed (plot 4). The farmer cut down most of the trees between establishment of the plants and the first observation. This severely changed the habitual conditions for the possible bio-controlling factors surrounding the plot. Despite this, the plot was still used in the study as there was enough trees left fulfilling the method criteria.

Six *Macadamia* plots were established. Out of these six, one plot was excluded as a result of extensive damage done by chickens eating the seedlings, another plot withered due to unknown reasons, and yet another had to be excluded when the farmer planted competitive crops in the same plot, hampering the seedlings growth and changing the original study conditions. The result was that three of the original six plots were used in the study.

The plots were placed 0.9 m (3 feet) from the nearest trunk on the *Grevillea* and *Eucalyptus* fields. Since *Macadamia* trees are lower and often has a larger canopy area which shades the ground below and therefore makes it unsuitable for planting, the plots were placed 0.9 m (3 feet) from the outer foliage instead of the trunk. Each plot contained 56 plants at a row and plant spacing of 60 cm and 45 cm respectively. At planting approximately 5 grams of granulated fertilizer (NP 23:23) was applied to each seedling, and mixed with manure from the farmers own farm animals. Depending on supply, two types of manure was used – goat or cow. During week one, dead or damaged plants were replaced by new seedlings of the same variety to keep the test population intact.

The seedlings were allowed to grow for an additional five days, followed by adding 20 grams of granulated top dressing (27% N) per seedling.

Once the seedlings were established a total of six cabbage plants were selected for data collection and sampling. To get the conditions as equal as possible, the plants chosen for data collection were located in the same positions on all plots; one middle-plant, one corner-plant and one row-plant. Three of these were covered with a conically structured mesh cage (mesh size 2.5 x 2.5 cm). These cages were used to

shield plants from predatory birds, but still let the DBM as well as parasitoids and other natural enemies of the DBM, enter (Fig. 5).

Each cage-enclosed plant had an equivalent plant exposed to birds and other natural enemies.

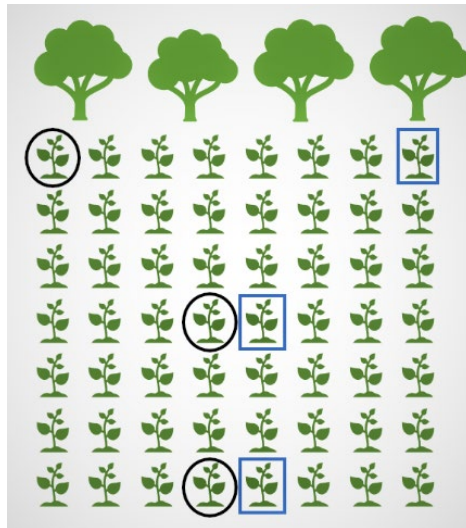


Figure 5. General plot layout with the 56 seedlings planted in 7 rows with 8 plants in each row. The seedlings marked with a circle were un-caged while the seedlings marked with a square were caged.

2.6.1 Data collection

One week after the cages were put into place the first sampling was executed. To evaluate the DBM population and damage on cabbage, the plants were checked for the presence of immature DBM and the number of DBM larvae counted and recorded.

The initial plan was to register the number of larva once a week for six weeks but due to drought the plants were planted three weeks later than planned and the time available for plant establishment was reduced to three weeks. The monitoring of larvae was instead repeated twice a week for two weeks, a total of four times. To be able to assess the level of parasitism by parasitoids, DBM pupae found in the plots were collected. Pupae were collected not only from the six study plants, but also from plants with visible feeding damage. The larval feeding behaviour, leaving characteristic irregular-, window-like holes on the leaves directed which ones to check for pupae. Pupae were collected during the whole period at all the plots in connection to the observations of larvae.

When a pupa was found it was placed in a marked plastic container and brought indoors and kept until either moth or parasitoids emerged and their incidences were recorded.

During the final inspection all plants in the plots were physically monitored for prevalence of DBM and the number of surviving plants was recorded.



Figure 6. Left: DBM pupae attached to the underside of a leaf of cabbage. Right: One late and one early instar DBM on the underside of a cabbage leaf.

2.6.2 Bird observations

As insectivore birds are natural enemies to the DBM, bird observations were a part of the study.

Twice a week, after the counting of larva and collection of pupae, bird observations were conducted where signs of bird activities between visits, such as faeces on or near the cabbage plants, were noted.

Once during the study, a more thorough investigation of the surrounding bird life was conducted. Around 9 am, before entering the field, the surrounding trees were scanned from a hidden location in relation to each plot for a period of approximately 20 minutes. A bird encyclopaedia and a pair of binoculars were the tools used to obtain data.

Attempts were made to identify the species. Bird activities, like birds flying down from the surrounding canopies to the plot to feed on larvae, were also noted.

2.6.3 Farm inventory and other observations

To see if the surrounding environment might influence the occurrence of the different biocontrol agents, as well as the occurrence of DBM, a thorough inventory of each plots' surroundings was made. The inventory was performed in the area right next to the plot and as far as could be seen from standing at the plot. The percentage

of the tree species bordering the plot was estimated through an ocular observation. Since shade is an important factor when it comes to pest abundance it was noted if the plot had sun/shade when the sun was in zenith.

2.7 Interview with farmers

A questionnaire was constructed to gather empirical data from farmers (see questions in Appendix). The farmers were asked about their farm practice, their experience regarding biocontrol on the farms, what types of pests they had problems with and what types of trees that were growing at the farms. The interviews were held at the farm in connection to data-collection and a local extension officer assisted during the interviews and translated when necessary.

3 Results

3.1 Occurrence of Biocontrol Agents

3.1.1 Parasitoids

In this study only one of the 62 collected pupae were found to be parasitized. Four pupae did not develop into full-grown moths (Table 1). Figure 4 illustrates the darker colour and different shape of the parasitized pupae compared to the non-parasitized pupae, which is usually clearly identifiable. The risk that the remaining unhatched pupae were parasitized are therefore very slim. Classification by ocular examination of the pupa and the adult parasitoid that emerged suggests it to belong to the *Diadegma* genus, but the species is unclear.

Table 1. Total number of pupa hatching into either DBM or parasitoids.

	Number of pupae	DBM	Parasitoids	Unhatched
<i>Eucalyptus spp.</i>	13	11	1	1
<i>Grevillea robusta</i>	26	24	0	2
<i>Macadamia integri- folia</i>	23	22	0	1
Total amount:	62	57	1	4

3.1.2 Insectivorous birds

A decreasing trend in larval presence was observed on uncaged plants in contrast to increasing or static prevalence on caged plants. Since many of the plants used for data collection had no larvae at all, the percentage of affected plants with or without cage was used for a better overview (Fig. 7a-c).

The *Eucalyptus* plots showed the least difference between caged plants and uncaged controls (Fig. 7a). However, there were a lower number of larvae on uncaged plants indicating that birds, or other larger predators unable to feed on larvae when the cage was present, did contribute to the reduction of DBM in these fields.

In plots planted next to trees of *Grevillea* there were greater difference between control plants and caged ones with a trendline clearly and steadily decreasing over time on plants accessible to birds and other vertebrate predators (Fig. 7b). On caged plants, where birds could not access their prey, the incidence of larvae seemed to increase instead.

On plots next to *Macadamia* trees, the percentage of infested plants were low during the beginning of the study while nearly 80% were infested at the end and the increasing trendline was steep (Fig. 7c). For uncaged plants, the trendline was slightly decreasing and natural enemies of DBM kept the population nearly constant. The great difference between the trendlines for caged and uncaged plants indicating a relatively high predation pressure by presumably birds.

The location of the cages within the plot did not seem to influence the predation of larvae in any of the tree species since there were not any clear differences between the paired plant treatments within the individual plots.

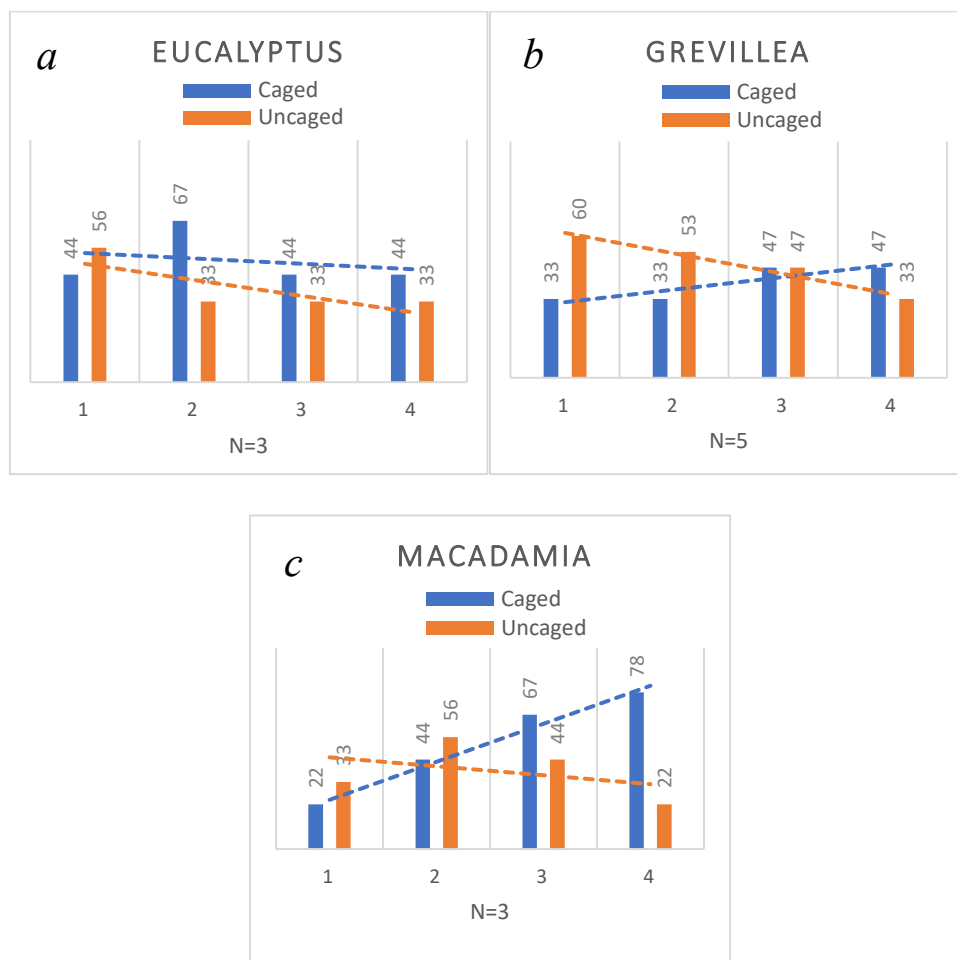


Figure 7a-c. Percentage of caged and un-caged plants with diamondback larvae present at the four occasions of data collection. The x-axis represents the different occasions of data collection.

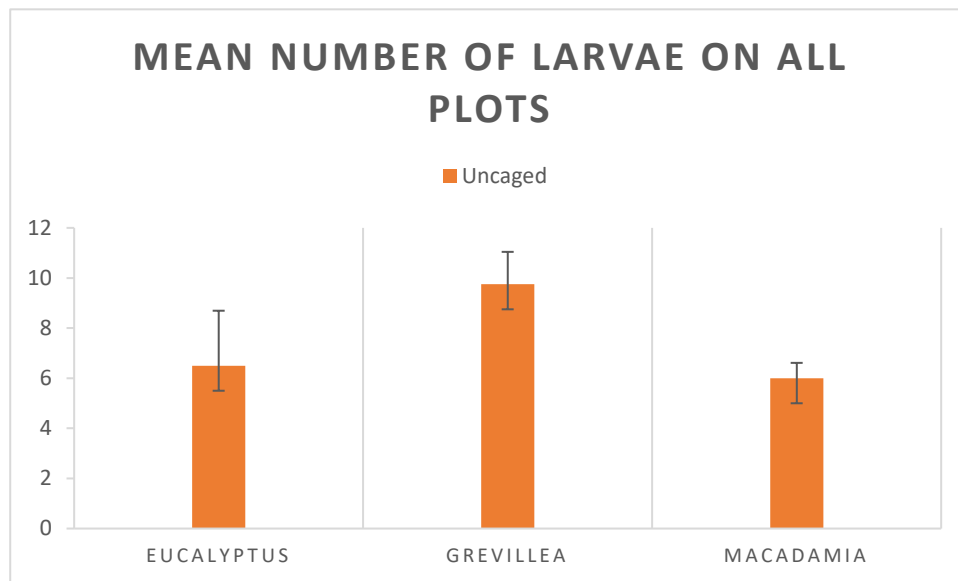


Figure 8. Mean number of larvae over time on all plots with standard error.

3.1.3 Bird observations

When inspecting the plots, a check for birds and their nests was done, including one more thorough observation at each plot. Few or no birds were observed in the plots during the counting of larvae. Birds could be heard at some plots, regardless of tree species but activity with birds flying between surrounding trees and bushes were mainly where *Macadamia* and *Grevillea* trees were grown.

Nor were there signs of nests in the nearby trees or in other tree species surrounding the plots. Despite the use of binoculars and an identification key, no specific species could be identified.

3.2 Farm inventory

As shown in the Tables 2-4 below, the most common crop was maize, which was grown by all the farmers. Flowering shrubs was surrounding many of the fields.

At *Macadamia* farms (Table 4), there was not as much shrubs and neighbouring trees. Neither were there much flowering weeds next to the plots.

The survival rate of plants differed between the tree species. At *Macadamia* plots, there was a tendency of higher plant survival with less variation (Fig. 9)

At plots adjacent to *Eucalyptus*, the survival rate of plants differed greatly between plots (Fig. 11). The general conditions, such as tree composition, shading and irrigation, differed considerably between farms (Table 2-4).

Table 2. Description for each plot regarding crops, the composition of trees and bushes surrounding the plot and an estimate of the dominant tree species percentage of the total number of trees for the farms with mainly *Eucalyptus* trees.

<i>Eucalyptus</i>	Crops	Surroundings	Comments
Plot 1	Maize, banana, avocado, napier grass, coffee.	Flowering shrubs was surrounding the field and the ground covered by flowering weeds	~75% of the trees by the plot were <i>Eucalyptus</i> of varying ages. Some <i>Grevillea</i> and an avocado tree. The plot was in full sun.
Plot 2	Maize, coffee, banana, beans and napier grass.	Flowering shrubs was surrounding the field and the ground covered by flowering weeds	~75% <i>Eucalyptus</i> . Other trees were <i>Grevillea</i> and indigenous trees. The plot was in full sun.
Plot 3	Maize, squash, beans and cabbage.	Flowering shrubs was surrounding the field	~90% <i>Eucalyptus</i> . Other trees were <i>Grevillea</i> and an avocado tree. The plot was shaded.

Table 3. Description for each plot regarding crops, the composition of trees and bushes surrounding the plot and an estimate of the dominant tree species percentage of the total number of trees for the farms with mainly *Grevillea* trees

<i>Grevillea</i>	Crops	Surroundings	Comments
Plot 1	Maize, banana, sweet potatoes, sugar cane, beans.	Flowering shrubs was surrounding the field and the ground covered by flowering weeds	~75% were <i>Grevillea</i> , many passion fruit-trees. The plot was fully shaded.
Plot 2	Maize, coffee, banana, beans and napier grass.	Flowering shrubs was surrounding the field and the ground covered by flowering weeds	~90% were <i>Grevillea</i> and the rest indigenous trees. The plot was in full sun.
Plot 3	Maize, squash, beans and potatoes.	Flowering shrubs was surrounding the field	~90% were <i>Grevillea</i> and the rest indigenous trees. The plot was partly shaded.

<i>Grevillea</i>	Crops	Surroundings	Comments
Plot 4	Maize, cabbage, potatoes.	Flowering shrubs was surrounding the field and the ground covered by flowering weeds	~90% were <i>Grevillea</i> and the rest indigenous trees. The plot was in full sun.
Plot 5	Maize, beans, coffee, banana trees.	Flowering weeds and lot of grass covering the surroundings near the plot.	100% <i>Grevillea</i> trees. The plot was shaded.

Table 4. The conditions for each plot regarding crops. At farms with *Macadamia* no other crops species were grown near the plots due to their large foliage.

<i>Macadamia</i>	Crops	Surroundings	Comments
Plot 1	Maize, sweet potatoes, napier grass, banana	Very few neighbouring trees and bushes. Some flowering weeds nearby the plot.	100% <i>Macadamia</i> trees. The plot was in full sun.
Plot 2	Coffee, maize, sweet potatoes, banana	Very few neighbouring trees and bushes	100% <i>Macadamia</i> trees. The plot was in full sun.
Plot 3	Maize, beans, kale.	The plot had a lot of flowering shrubs along one of the sides of the plot.	100% <i>Macadamia</i> trees. The plot was in full sun.

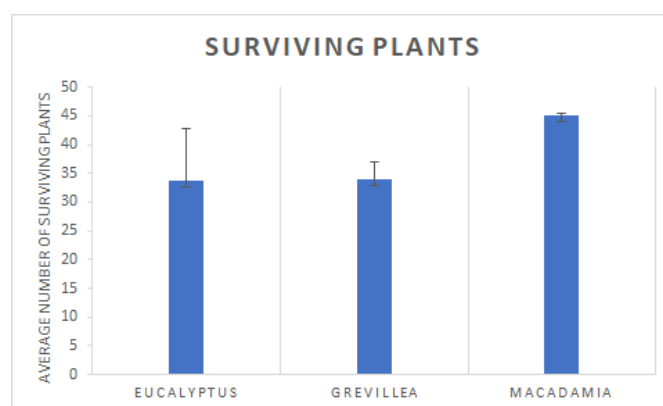


Figure 9. The average number of surviving plants for each tree species with standard error.

3.3 Other observations

Aphids were abundant on most of the plots and in many of them aphids were the most destructive pest regarding both plant damage and number of affected plants. Other species of larvae were observed feeding on the cabbage in two of the *Macadamia* dominated plots. Grasshoppers' feeding on plants were problematic in most plots. Not only were other pests observed, but also natural enemies of these. Spiders, as well as ground dwelling insects were a common sight in all fields observed. Further, lady bugs and their caterpillars as well as heteropteran were observed both on the ground and on the cabbage plants.

3.4 Interviews with farmers

Seven farmers were interviewed. The size of the farms ranged between 2-5 ha and common crops were maize, beans, sweet potatoes and napier grass. All the farmers mentioned aphids as the most difficult crop pest, followed by whiteflies, which five of the farmers stated having problems with. Other pests mentioned were fall armyworm, DBM, stalk borers, cutworms, nematodes, ants and stink bugs. The way of controlling these pests was by a frequent use of pesticides.

One farmer did mention alternative ways to control the pests, such as the use of ashes, planting spring onions and American Marigold, but did not use this instead of pesticides, but together with them.

Five of the farmers were unaware of any specific biological control agents while two mentioned birds. One farmer mentioned once seeing a bird feeding on pests in the field but could not remember which type it was.

The most preferred tree at the farms was *Grevillea* due to its versatile nature – both as a source of income and as fodder for livestock. Fruit trees such as Avocado and *Macadamia* came second and was planted because of their high economical potential. *Eucalyptus* was also mentioned by many farmers as a “good” tree, but was only the most preferred by one farmer, which planted it as “savings” for the future. Other farmers also mentioned *Eucalyptus* as a good source of income but chose not to plant it due to its poor compatibility with the crop in agro-systems.

4 Discussion

This study could not support that parasitoids contribute to the regulation of DBM in the field but supported that insectivorous birds decimated the population for all three tree species, with *Macadamia* and *Grevillea* preferred over *Eucalyptus*. The visual bird observation supports this fact since there was more bird activity by *Macadamia* and *Grevillea* plots.

At plots next to *Macadamia* trees, there was a considerable difference in percentage of affected plant between the two groups and there was also a big difference in the total number of larvae between the two groups, which indicates a high predation pressure on the uncaged plants, presumably by birds. *Macadamias* morphology with a large canopy and flowers full of nectar make it a popular tree for both insects and birds. The abundance of birds between the species was noted during the observations, and it was especially palpable when comparing *Macadamia* to *Eucalyptus*. *Macadamia* plots were surrounded by the voices of different bird species while the silence was apparent at plots where *Eucalyptus* dominated the tree composition. Therefore, the fact that *Eucalyptus* plots showed the least difference between caged plants and the uncaged controls (Fig. 7a), was no surprise. As earlier studies have shown, both the species richness and number of birds in *Eucalyptus* plantations are lower comparing to both natural forests but also other plantations of trees (Calviño-Cancela, 2013; Goded et al., 2019) and may explain the lack of bird observations around these plots. As described, there are great differences in morphology and growing strategies between the two species. *Eucalyptus* are grown close together and grows tall, outcompeting other crops, leading to not much understory vegetation under the trees. Neither do they produce flowers that are attractive to birds and insects. Although birds did not seem to play a major role in the decimation of larvae population in *Eucalyptus* plots, something did lead to a decrease of the population over time. As shown in table 2, a lot of the surrounding ground to these plots were

covered by both flowering weeds and shrubs, which may work as shelter and alternative food source for other natural enemies of the pest, such as ground dwelling insects or rodents. However, birds should not be fully excluded as control agents of DBM in these plots. The mean total number of larvae on caged plants was higher than on uncaged plants, indicating a presence of predators excluded by the cage, for example birds but also rodents or lizards.

The trendlines representing the plots next to *Grevillea* trees, with a clear decrease of larvae exposed to avian predators and an increase where they were protected indicates a predation by birds or other larger predators on these plots. *Grevillea*, with its flowers, is a species popular by both birds and insects. During the regularly observations, birds were observed to a higher extent around and in those trees compared to *Eucalyptus* trees. One thing that did not affect the outcome of the result was placing of the cages within the plot. Not in any of the tree species were there any clear differences between the paired plant treatments within the individual plots.

Earlier studies have shown that parasitoids are important natural control agents of DBM, something not observed in this study. The number of parasitized pupae was below 2% and hence parasitoids was not a contributing factor to the decimation of DBM larvae. Although the result was a bit surprising it was not totally unexpected due to several factors. For instance, the overall low number of DBM larvae may lead to a low concentration of herbivore-induced volatiles emitted when the plant is attacked which parasitoids use to locate their host. Another factor that might explain the low number is that all the farms in the study used chemical pesticides as their primary control measure of crop pests. At conventional farms, the abundance of parasitoids were substantially lower than at farms using other methods for pest control which were shown in a study by Bommarco et al. (2011). Another possible explanation is that the population of parasitoids had not had time to establish a viable population during the short amount of time that data collection took place.

Although there are indications that *Macadamia* is the most preferred tree by birds, the data is not statistically tested due to limited data which was a result of numerous unfortunate circumstances. One of the most important was the low number of replicates. This was the result of several factors – drought, varying caretaking between farms, plants competition with weeds, insects other than DBM eating the seedlings, leading to the loss of several plots. There was also a wide variation in the number of surviving plants with an average number at plots next to *Grevillea* and *Eucalyptus* around 60% and at *Macadamia* around 80%. Further, the number of larvae was very low and many times there were no larvae at all at the plants, leading to few replicates in each plot as well. Despite efforts to making the plots as similar as possible, the plots differed substantially. This led to difference in for example shading and this

effect could be seen clearly in the *Eucalyptus* plots. While two plots, number 2 and 3, were in full sun during the hottest hours, the third plot was shaded. On the shaded plot, the seedling grew better, had a higher survival rate and showed less damage by DBM as well as by other pests. This difference led to a very high contrast between surviving plants at plots next to *Eucalyptus* trees (Fig. 9).

Since there was a loss of larvae that was not due to parasitoids or birds, pitfall traps or other types of traps to monitor the abundance of ground dwelling arthropods (Carabids, spiders) would be interesting. Since parasitoids and insectivorous birds were the natural enemies in focus of this study, no collection of other species of invertebrates on the plots were made.

There were many plants chosen for data collection that did not have any larvae, making it difficult to follow the predation. An improvement of the method would be to place a fixed number of larvae at each plant and keep the amount by either add or remove larvae between the occasions of counting. Lastly, since many of the factors in such study requires a lot of time, and many unexpected hurdles may occur, a longer period would be preferable.

Although this study could not statistically support the hypothesis that different trees in an Agroforestry system affect natural enemies of DBM differently it did show that insectivorous birds seem to contribute to the decimation of larvae in the field and that Macadamia trees seemed to be the most preferred tree species. However, birds are often unwelcome guests in the field since some bird species feed on grains and/or vegetables and thus regarded as pests. But, even when considering the losses caused by granivorous and omnivorous birds, the positive effects made by the insectivorous is greater (Linden et al., 2019). Increasing their number in agroecosystems would be beneficial from a biological control perspective. By informing farmers about the positive effects' birds may have on pest reduction this might hopefully lead to a more forgiving attitude towards the presence of birds in the fields, which in turn might lead to a reduced use of pesticides.

Appendix

Questionnaire

1. Name:
2. Age:
3. What types of crops are grown at the farm?
4. Do you experience any problems with crop pests? If yes, what kind of pests?
5. What methods are used to control the pests?
6. Are you aware of any biological control agents? If so, which ones?
7. What types of trees are growing at the farm?
8. Which is the most preferred tree at the farm? Why?

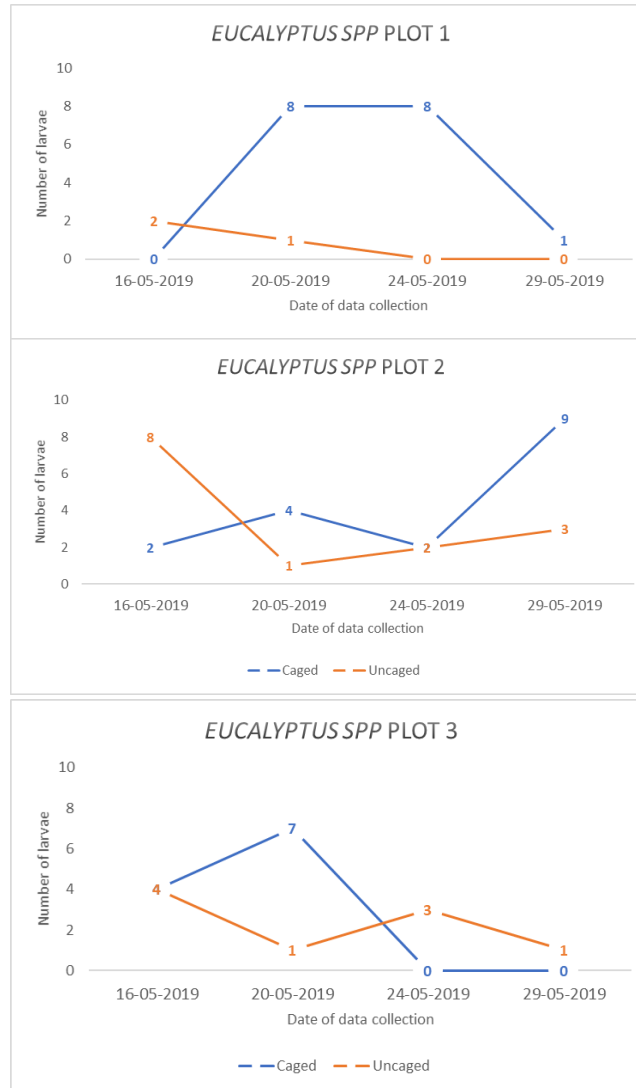
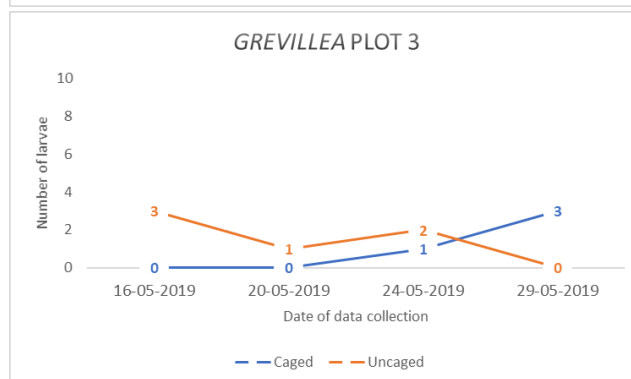
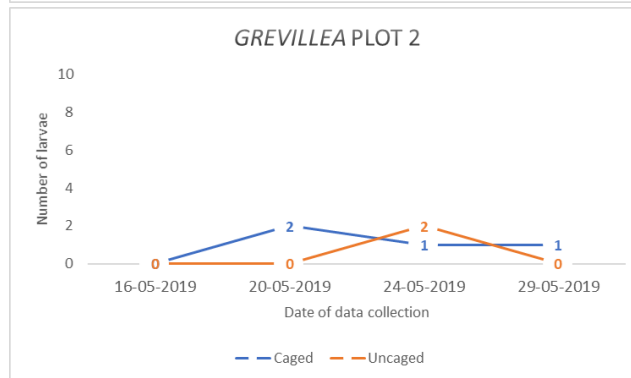
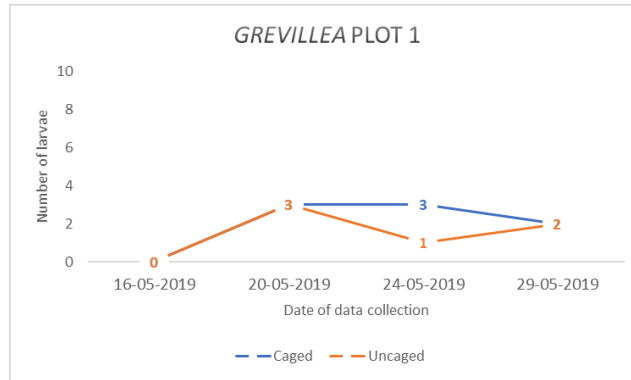


Figure 10. Total number of larvae on study plants; three plants with and three without cage.



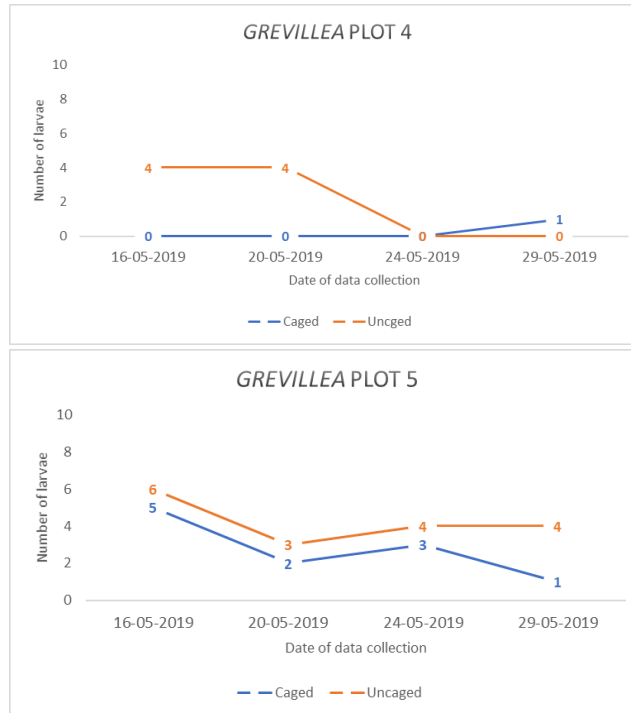


Figure 11. Total number of larvae on all six study plants; three with and three without cage

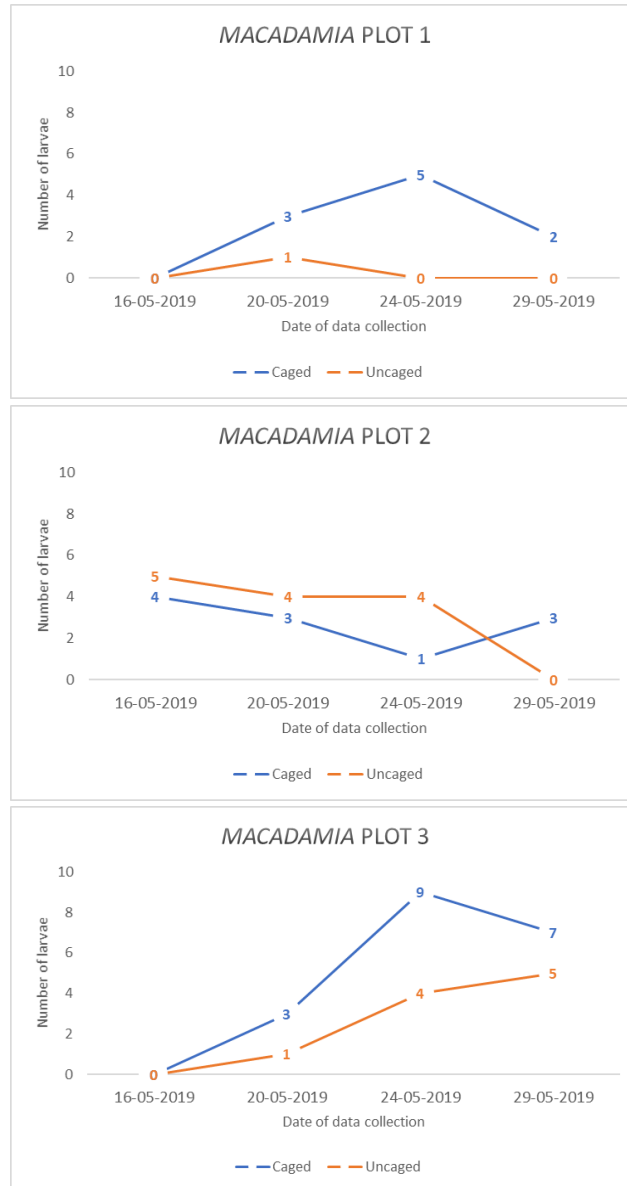


Figure 12. Total number of larvae on the study plants at each inspection; three plants with and three without cage.

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