Early warning system for the black maize beetle (*Heteronychus arator* Fabricius) in a major maize producing region of South Africa

by

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Submitted in fulfilment of the requirements for the degree of Magister Scientiae in Entomology

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2015

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Declaration

I hereby declare that this dissertation submitted by me for the degree Magister Scientiae at the University of the Free State is my own independent work and that I have not previously submitted the same work at another University / Faculty. I furthermore concede copyright of the dissertation to the University of the Free State.

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Nicolene de Klerk

15 May 2015

Acknowledgements

During the course of this study people from various departments and institutions assisted which led to the completion of this study. Appreciation is expressed to all.

Financial aid was provided by the ARC (Agricultural Research Council) and the Maize Trust. Assistance by ARC staff included Mabel du Toit and Mischack Moroladi. Special thanks to Dr. T.W. Drinkwater. Weather data were obtained from SA (South African) weather services as well as from ARC-ISCW (Agricultural Research Council – Institute for Soil, Climate and Water). Statistical support was provided by Nicolene Thiebaut, Frikkie Calitz and Wiltrud Durand. Thanks to all 99 maize producers that assisted with capturing the insect populations over the years.

To my husband, Adriaan and my children, Angelene, Lee-Ann, Niané, Lichané and Adriaan thank you all for supporting me in the many struggles that faced us throughout the journey. You are amazing.

Special thanks to both Prof. J.B.J van Rensburg and Prof. S. vd M. Louw for the guidance and open mindedness regarding this study as well as professional advice that was always available.

Abstract

Black maize beetle (*Heteronychus arator* Fabricius) (Coleoptera: Scarabaeidae) is economically the most important coleopteran pest that attacks the subterranean part of maize seedlings in South Africa. The sporadic nature of black maize beetle outbreaks led to the need for developing an early warning system. This required improved knowledge on the ecology of this pest. Black maize beetles are nocturnal and are attracted to artificial light. Ninety nine modified Robinson light traps were placed in quarter degree grids (30km x 30km) throughout the eastern part of the maize production area. Weekly captures for three consecutive months from February of every year were preserved in 70% alcohol and counted. The flight pattern of *H. arator* was in this way monitored for 11 consecutive years. Some captured beetles were placed in breeding containers to harvest eggs for ecological studies. L1-larvae with visible blackened hind guts were placed singly into 7cm diameter pots prefilled with varying organic matter concentrations (10% intervals) for both Avalon and Hutton soil forms. Other L1-larvae were also subjected to varying moisture concentrations (10% intervals) in both soil forms (Hutton and Avalon) premixed with 50% organic matter. Data were recorded and analysed. Spatial variation indicated that black maize beetles in most years are limited to a small area (540 000ha) located on the borders of the Free State, Mpumalanga and Gauteng provinces. The long term temporal variation indicated that epidemic outbreaks recur in the same area with 32 year intervals. The next expected outbreak will be during 2041. Short term temporal variation indicated that populations tend to recur at five year intervals but with varying intensities. Through the use of monthly weather variables as well as black maize beetle captures of the previous year (February to end April) a prediction model was developed. This prediction model was able to explain 62.93% of the number of black maize beetles expected to fly during February to end of April. However, the weather station must be within 10km from the sample area. The prediction model developed for black maize beetles indicated that average solar radiation contributed to 26.99% of the total prediction model while minimum temperature contributed another 15.96%. Both variables are related to heat and contributed in total to 42.95% of the 62.93% that is predicted by the model. Larval development in two soil forms (Hutton and Avalon) differed significantly, where the favourable moisture content for Hutton soil ranged from 40% to 80%, whereas in

Avalon soil it was limited to a range of 60% to 70%. This indicated that soil collected in the area known for black maize beetle outbreaks had a larger moisture range suitable for larval development than other areas known for Avalon soil forms. Highly significant black maize beetle larval mass differences were also recorded with variation in organic matter content. The higher the organic matter content the greater the mass gain of the black maize beetle larvae, pupae and pre-adults as well as the time it took to the pre-adult stage. In Hutton soil, all larvae reached maturity at 80% to 90% organic matter content. However, with Avalon soil with an organic matter content of 90% only 40% of the larvae managed to develop into pre-adults. At 100% organic matter content all larvae managed to become the pre-adult stage. This indicated that black maize beetle larvae feeding exclusively on organic matter are able to reach pre-adults and that no-till practice especially in the known distribution area of black maize beetles may lead to a significant increase in black maize beetle numbers. Results indicated that with Hutton soil an 80% organic matter content is needed for larvae to reach maturity unless black maize beetle larvae are able to feed on living plant material such as plant roots. It took approximately five days longer for beetles to emerge from a Hutton soil form with an 80% organic matter content compared to the same soil with 100% organic matter content. This indicated that the lower the organic matter content in soil, the longer it will take for black maize beetles to become pre-adults unless larvae are able to feed on living plant material. By determining organic matter content in the soil and measuring soil moisture levels a more effective prediction model for black maize beetles can be developed.

Uittreksel

Swartmieliekewer (*Heteronychus arator* Fabricius) (Coleoptera: Scarabaeidae) is ekonomies die belangrikste kewerplaag wat die ondergrondse dele van mieliesaailinge in Suid Afrika aanval. Die sporadiese aard van swartmieliekewer uitbraak het die ontwikkeling van ‘n tydige waarskuwing stelsel genoodsaak. Om dit te kon doen moes ‘n deeglike ekologiese studie van die plaag gedoen word. Swartmieliekewers vlieg gedurende die nag en word daarom deur kunsmatige lig aangelok. Nege-en-negentig gemodifiseerde Robinson ligvalle was in kwart graad blokke (30km x 30km) geplaas dwarsdeur die oostelike deel van die mielieproduksie area. Weeklikse vangste vir drie aaneenlopende maande vanaf Februarie van elke jaar was gepreserveer in 70% alkohol en getel. Die vlugpatroon van *H. arator* was op hierdie wyse vir 11 agtereenvolgende jare gemonitor. Data was aangeteken en geanaliseer. Sommige gevangde kewers was in broeihouers geplaas om eiers te oes vir die ekologiese studie. L1-larwes met sigbaar verdonkerde agterlywe was enkel in 7cm deursnee potte wat vooraf gevul was met variëerende organiese inhoud konsentrasies (10% intervalle) vir beide Avalon en Hutton grondvorms geplaas. Ander L1-larwes was ook blootgestel aan variëerende vog konsentrasies (10% intervalle) in beide grondvorms (Hutton en Avalon) wat voorafgemeng was met 50% organiese materiaal. Ruimtelike variasie het aangetoon dat swartmieliekewers in meeste jare beperk was tot ‘n klein area (540 000ha) wat voorkom op die grense van die Vrystaat, Mpumalanga en Gauteng provinsies. Die langtermyn temporale variasie van swartmieliekewer populasies het getoon dat epidemiese uitbrake in dieselfde area uitbreek met 32 jaar intervalle. Die volgende verwagte uitbraak sal gedurende 2041 wees. Korttermyn temporale variasie het aangetoon dat populasietoenames met vyf jaar intervalle voorkom, maar met verskillende intensiteite. Deur die gebruik van maandelikse weersveranderlikes asook swartmieliekewer vangstes van die vorige jaar (Februarie tot einde April) is ‘n voorspellingsmodel ontwikkel. Die voorspellingsmodel kon tot 62.93% van die aantal swartmieliekewers wat verwag word om te vlieg gedurende Februarie tot einde April van die volgende jaar voorspel. Die weerstasie moet wel binne 10km van die monster area wees. Die voorspellingsmodel wat ontwikkel is vir swartmieliekewers het getoon dat gemiddelde sonlig uitstraling tot 26.99% van die totale voorspellingsmodel bygedra het, terwyl minimum temperatuur ‘n verdere 15.96%

bygedra het. Dus, beide veranderlikes is verwant aan hitte en dit het in totaal tot 42.95% van die 62.93% bygedra. Larwale ontwikkeling in twee grondvorms (Hutton and Avalon) het wesenlik verskil waar die gunstige voginhoud vir Hutton grond gestrek het van 40% tot 80% terwyl in Avalon grond dit beperk was van 60% tot 70%. Dit het aangetoon dat grond wat van die area wat bekend is vir swartmieliekewer uitbrake, 'n groter vogreeks geskik vir larwale ontwikkeling het in vergelyke met ander areas bekend vir Avalon grond vorms. Hoogs betekenisvolle swartmieliekewer larwale massa verskille was aangeteken met verskillende organiese inhoud. Hoe hoër die organiese inhoud hoe groter die massa toename van die larwes, papies en volwassenes asook die tyd wat dit geneem het tot volwassenheid. In Hutton grond het alle larwes volwassenheid bereik by 80% tot 90% organiese inhoud. In Avalon grond met 'n organiese inhoud van 90% het egter slegs 40% van die larwes volwassenheid bereik. By 100% organiese inhoud het alle larwes volwassenheid bereik. Dit het aangetoon dat swartmieliekewer larwes wat uitsluitlik net op organiese materiaal voed, volwassenheid bereik en dat geen-bewerkingspraktyke veral in die area bekend vir swartmieliekewer voorkoms tot 'n wesenlike toename in getalle sal bydra. Resultate het aangetoon dat Hutton grond met 80% organiese inhoud nodig is vir larwale ontwikkeling om volwassenheid te bereik, behalwe as larwes op lewende plantmateriaal soos plant wortels, kan voed. Dit het ongeveer vyf dae langer geneem vir kewers om te ontpop in 'n Hutton grondvorm met 'n 80% organiese inhoud in vergelyking met dieselfde grond met 'n 100% organiese inhoud. Dit het aangetoon dat hoe laer die organiese inhoud in die grond hoe langer sal dit neem vir swartmieliekewers om volwassenheid te bereik behalwe as larwes lewende plantmateriaal kan vreet. Deur die organiese inhoud van grond asook die vogvlakke in grond vas te stel kan 'n meer effektiewe voorspellingsmodel vir swartmieliekewers ontwikkel word.

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CHAPTER 1:

The black maize beetle, *Heteronychus arator*, in perspective

Insect pests that attack cultivated crops, especially maize (*Zea mays*) (Cyperales: Poaceae) in South Africa belong to various orders (Robertson, 2012). A pest is adestructive insect or other animal that attacks crops, food and/or livestock of humans (Oxford University Press, 2014). Pest species is grouped into primary and secondary pests. A primary pest is known to attack the host directly, while a secondary pest only attacks the host as a result of the primary attack. Black maize beetle attacks the heart directly and is therefore a primary pest of maize.

A pest species is further grouped according to which feeding guild it belongs to (Robertson, 2012). Thus, pest species with different modes of feeding such as sucking or biting can attack the same plant part. These are grouped together as one feeding guild.

Insect species belonging to the Hemipteran order such as *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae), commonly known as the maize aphid, is mainly responsible for damage to maize leaves (Robertson, 2012). Most insect species that belong to Lepidoptera are in the maize stem guild, such as *Busseola fusca* (Füller) (Lepidoptera: Noctuidae) commonly known as the maize stalk borer, as well as the maize seedling guild such as *Agrotis* spp. (Hampson) (Lepidoptera: Noctuidae), commonly known as cutworms (Robertson, 2012).

Most Coleopteran insect pests such as *Buphonella nigroviolacea metallica* (Jacoby) (Coleoptera: Chrysomelidae) (maize rootworm), *Gonocephalum* spp. (Coleoptera: Tenebrionidae) and wire worms (Coleoptera: Elateridae), attack mainly the root and subterranean part of maize (Robertson, 2012).

Heteronychus arator Fabricius (Coleoptera: Scarabaeidae), commonly known as the black maize beetle, is the most important Coleopteran pest that attack the subterranean part of maize, especially in South Africa (Taylor, 1951). This pest is indigenous to South Africa, but also occurs in Australia, New Zealand, Zimbabwe and Zambia (Drinkwater, 1979). The following literature review includes information obtained from all countries involved.

According to Ormerod (1889) an epidemic outbreak of *H. arator* occurred in the eastern parts of South Africa during 1881 through to 1885. During the early summer of 1946 another wave of *H. arator* attack occurred in newly planted maize fields in the Frankfort district of South Africa (Taylor, 1951). After a period of approximately 32 years (from 1946 to 1977) the species again reached epidemic proportions, again in the Frankfort district of South Africa (Drinkwater, 1982). This attack surged for five successive planting seasons until 1982 (Drinkwater, 1987).

Black maize beetles are confined to a small area situated at the border of the Free State, Mpumalanga and Gauteng provinces of South Africa (Chapter 2 and 3). This area was described in literature as the Frankfort district of South Africa. An aerial map indicates that this area occurs near the Vaal dam (Figure 1.1). This dam has extensive branching over a large area which might explain their preference to this specific area. Extensive branching might contribute to high soil humidity levels found in this region when compared to other regions.

Figure 1.1. Hotspot area of black maize beetles in the Vaal dam area (AfriGIS, 2014).

It is difficult to predict when outbreaks of *H. arator* will occur due to its high injuriousness at low densities, the unpredictability of when it will reach epidemic numbers, the clustered distribution in maize fields, damage that can go undetected due to the concealed and cryptic behaviour of this pest and producers underestimating the importance of subterranean damage to maize (Du Toit, 1998).

Adults are known to cause damage to maize but it is unknown if their larvae can cause damage (Du Toit, 1998). Most damage is caused by the overwintering adults that feed on maize seedlings, planted in spring, but newly emerged adults can also cause damage to maize seedlings (Figure 1.2b) during late-summer months (Du Toit, 1998). Late-evenings these adults emerge from the soil (Figure 1.2a) and attack nearby maize seedlings by burrowing next to it and feeding on the subterranean parts (Figure 1.2c) of the stem (Drinkwater, 1979).

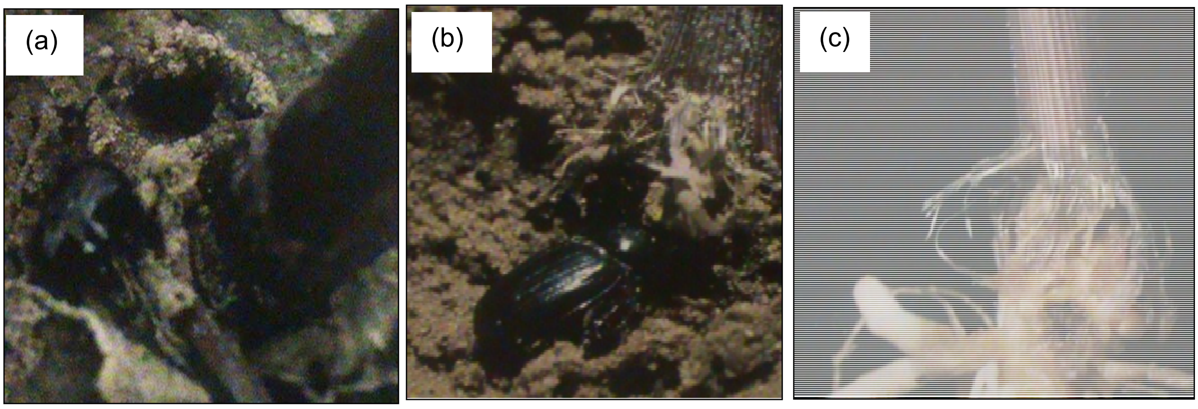

Figure 1.2(a). Exit hole of *Heteronychus arator* near maize seedling, (b) damage by *Heteronychus arator* to maize seedling and (c) damage to the subterranean part of a maize seedling (Du Toit, 1998).

Two distinct types of damage symptoms (Figure 1.3a and b) to the plant can be distinguished (Du Toit, 1998).The first symptom occurs within three weeks after planting where the growth point wilts, resulting in a “dead heart” (Figure 1.3a and b). If severely attacked this plant usually dies or the seedling forms excessive useless tillers at the base of the plant (Du Toit, 1998).

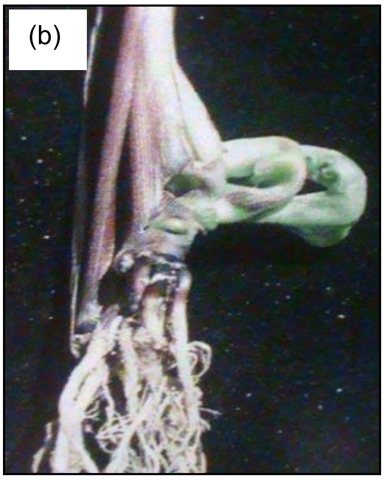

Figure 1.3(a). Above-surface symptom (dead heart) caused by *Heteronychus arator* damage to maize seedling and (b) sub-surface symptom (dead heart) caused by black maize beetle to maize (Du Toit, 1998).

The second symptom is longitudinal yellow stripes on the leaves (Figure 1.4) of the maize plant whereby the plant is only partially damaged. This usually appears between three to four weeks after planting. Older plants normally survive the attack but remain weakened and are prone to lodging (Du Toit, 1998). If the plant is not severely attacked it may continue to grow, but will be stunted and not able to bear any ears (Taylor, 1951). During harvesting, beetles have been found devouring fallen maize ears (Taylor, 1951). Damage always occurs in patches and is mostly found in the elevated portions of the field under attack (Taylor, 1951).

Figure 1.4. Longitudinal yellow stripes on maize leaf caused by *Heteronychus arator* (Du Toit, 1998).

The black maize beetle has an annual life cycle and therefore only one generation occurs annually (Du Toit, 1998). Mating only occurs underground where it can last up to 12 hours (Harington, 1953). Fourteen days after mating, females lay their eggs, singly, in the soil, usually around late-September until mid-April, in South Africa (Taylor, 1951; Drinkwater, 1979). Overwintering adults also lay their eggs during the same period (Drinkwater, 1979).

Newly laid eggs, initially oval in form (Figure 1.5), are white or creamy in colouration and as they mature they become increasingly rounder and larger (Du Toit, 1998). Eggs vary between 1.5-2mm in length and are laid between 10-150mm below the soil surface near the vicinity of maize roots (Du Toit, 1998). Females lay an average of 20 eggs, but they can lay up to 80 eggs per individual per life time (Du Toit, 1998).

Figure 1.5. Eggs of *Heteronychus arator* (Du Toit, 1998).

Three larval stages (L1-L3) (Figure 1.6) are commonly known as white grubs (DuToit, 1998). The larval stages of *H. arator* feed predominantly on decomposing organic matter in the soil (Du Toit, 1998). However, the amount of organic matter in soil needed for larval development is unknown. Larvae are typical scarab larvae and curled like a horseshoe (Figure 1.6) when relaxed (Taylor, 1951). L1 larvae hatch after approximately 19 days depending on the temperature and moisture content of the soil (Du Toit, 1998). The moisture level in soil needed for larval development is unknown. L1 larvae are approximately 5mm long, and have distinct pale brown head capsules and it take approximately 21 days to develop into L2 larvae (Du Toit, 1998). L3 larvae are about 25mm in length and 6mm wide and possess a light brown head capsule (Du Toit, 1998). L3 larvae feed for approximately 52 days and after fully fed and prepare to pupate. The L3 larvae can burrow as deep as 1.5m into the soil where they construct an oval-shaped pupal chamber in the soil (Du Toit, 1998).

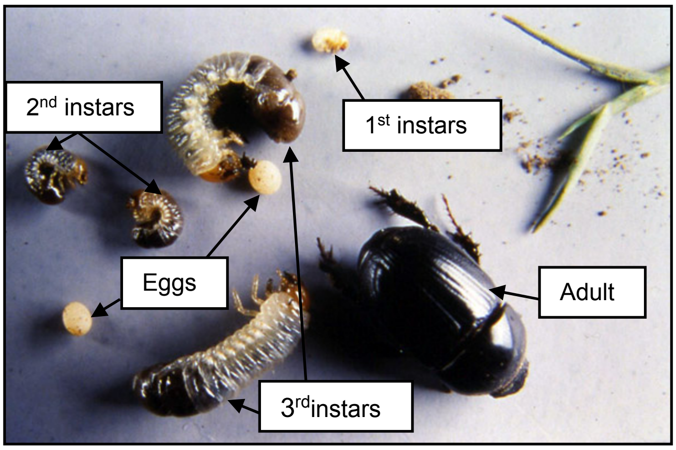

Figure 1.6. Life stages of *Heteronychus arator* (Anon, 2012).

After excreting the content from the hind gut the L3 develop into a pre-pupa, after approximately a week, thereafter it develops into a pupa (Figure 1.7) which is pale yellow in colour and about 15mm in length (Du Toit, 1998). As the pupa develops, it turns from yellow, to brown and then reddish brown (Du Toit, 1998).

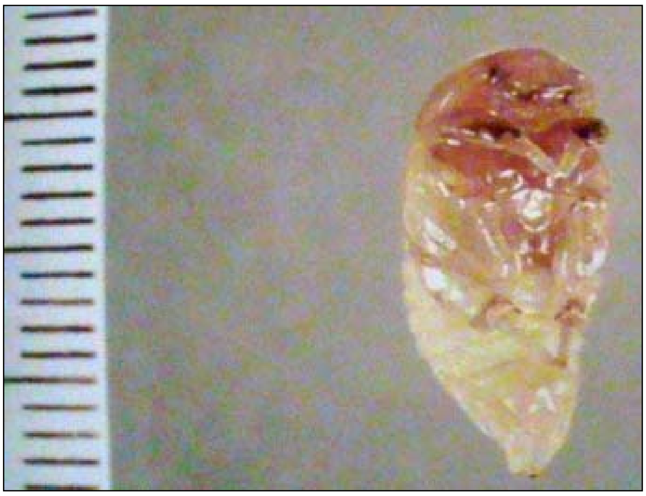

Figure 1.7. Pupa of *Heteronychus arator* (Du Toit, 1998).

Adults (Figure 1.8) are initially reddish brown and emerge after a period of 6 to 30 days (Du Toit, 1998). Soil moisture is a key factor for the emergence of beetles (Du Toit, 1998). If the soil is moist the adults move directly to the soil surface, but if the soil is dry then the adults stay in the pupal chamber until soil conditions improve and become more favourable (Du Toit, 1998). Beetles acquire their distinct shiny black colour within three days after emergence.

Figure 1.8. Adult female (left) and adult male (right) of *Heteronychus arator* (Du Toit, 1998).

Heteronychus arator overwinters as larvae and/or adults in the soil (Drinkwater, 1979). Swarming of beetles occurs at dusk, one or two days after rain, usually during spring and late-summer to autumn (Du Toit, 1998). The distance that the beetles can fly as well as their preference for a particular habitat is unknown (Du Toit, 1998). Adults are nocturnal and are attracted to artificial light just after emerging from the soil, but are usually found in the soil near the soil surface (Du Toit, 1998). Adults spend most of the time underground where they feed on the subterranean parts of maize (Todd, 1964). Adults are not adversely affected by heavy rain and can even tolerate long periods of submersion in water (Todd, 1964).

Larvae are able to move through the soil and are found throughout the field, some are found near moist root systems of maize and sweet grass (*Panicum laevifolium*) but it has not been established if they feed on these plants (Taylor, 1951). Natural food seems to be the humus (organic matter) in the soil (Taylor, 1951). Larval development is dependent on moisture, as they develop faster during the rainy summer months of South Africa (Taylor, 1951). Larvae are more concentrated around maize root systems during the drier months of January and February (Taylor, 1951). White grubs (larvae) show a common clustered distribution and infestations tend to recur annually in the same field (Litsinger *et al.*, 1983). The following factors contribute to the patchy distribution of white grub species: (1) larval tolerance to the narrow range of soil texture and soil moisture, (2) selection of adults' oviposition site by differences in plant cover, (3) prevalence of favourable larval plant hosts and (4) proximity to adult "flight" trees (Litsinger *et al.,* 1983). White grub larvae are confined to light-textured soil types which offer a moisture gradient in the soil horizon, heavier soil types can lead to high mortalities of larvae especially during heavy rain (Litsinger *et al.,* 1983).

Two main flight peaks of beetles (Figure 1.9) are found during a year (Taylor, 1951; Du Toit, 1998). The 1st peak occurs early in the summer, October to December and this peak is most damaging to maize seedlings (Taylor, 1951). This peak consists of overwintering larvae which are the offspring of the later-emerging beetles of the first generation, as well as adults from the second generation (Taylor, 1951). This flight comprises approximately 5% of the total yearly flight (Du Toit, 1998). The 2nd wave occurs during February to April and is the offspring of the early-emerging beetles of the first generation and will continue to occur until May depending on temperature (Taylor, 1951). According to Du Toit (1998) this peak comprises approximately 95% of the total yearly flight.

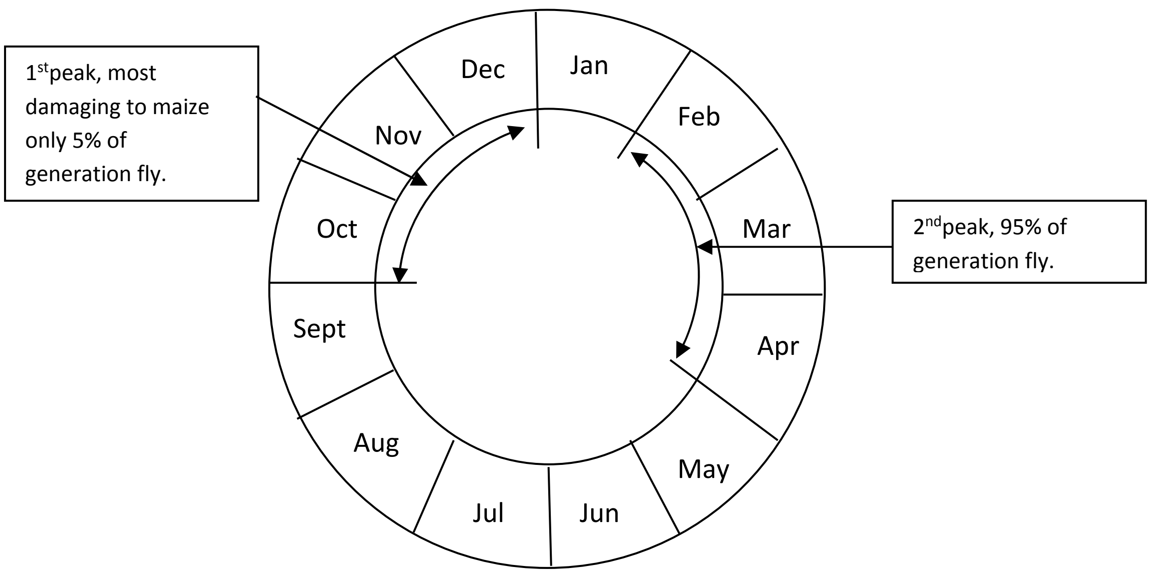

Figure 1.9. Main flight peaks of black maize beetle populations in South Africa (Taylor, 1951; Du Toit, 1998).

1.1 Morphology of *Heteronychus arator*

Distinct morphological differences exist between most Coleopteran adults and their larvae (Figure 1.5). Based on only their morphological characteristics, it is difficult to identify larvae of a species (Dittrich *et al.*, 2006). Molecular diagnostic techniques serve as a complementary technique to match adults and larvae of the same species (Dittrich *et al.,* 2006). Raster patterns (setal patterns found on the last abdominal segment) can also be used to identify the larval stage of the family Scarabaeidae to its adult form (Dittrich *et al.,* 2006).

Heteronychus arator adults are stout-bodied, 12-15mm long and shiny black in colouration. Females are slightly larger than males with slender tarsi (Figure 1.10) at the tip of the frontal legs (Drinkwater, 1979; Du Toit, 1998).

Figure 1.10. Female frontal tarsus (above) and male frontal tarsus (bottom) of *Heteronychus arator* (Anon, 2010).

Frontal legs are adapted (Figure 1.11) for digging (Du Toit, 1998). Their shiny black outer elytra cover their well-developed membranous wings below, which are used during flight (Taylor, 1951). They are strong fliers, but are usually restricted to the soil and only emerge during sunrise to move to the next maize plant to feed until sunset (Taylor, 1951).

Figure 1.11. Adapted frontal tarsus of *Heteronychus arator.*

1.2 *Heteronychus arator* host plants

During 1977, black maize beetles drastically reduced the stand of maize, obliging the producers to replant certain areas of the Frankfort district (Drinkwater, 1979). Reports on attack by these beetles also involved grain sorghum, oats, Italian rye grass, beans, buckwheat, nut grass and even sunflower (Drinkwater, 1979). Additionally, it is found to be a serious pest of potato tubers and pineapple (Venter and Louw, 1978).

Black maize beetle natural hosts such as *Paspalum dilatatum* Poir., a grass species, is a preferred ovipositional host in New Zealand and this grass species also occurs in South Africa (Du Toit, 1997). According to Du Toit (1997), the family Scarabaeidae is the most abundant insect pest in both natural grass fields and maize areas in South Africa. However, scarabaied larval stages are more concentrated in the natural field areas whereas adults are more concentrated in the maize areas which indicate that adults migrate after emergence towards the more acceptable host species, such as maize (Du Toit, 1997). Thus, natural vegetation surrounding maize may act as reservoirs for black maize beetles (Du Toit, 1997).

1.3 Management of *Heteronychus arator*

Current agricultural practices and environmental conditions intensified the seasonality of the family Scarabaeidae (Pardo-Larcarno *et al.*, 2005) but in the long term *Heteronychus arator* is considered to be sporadic (Taylor, 1951). Species-specific control measures can be developed if species identification of larvae exist (Dittrich-Schröder *et al*., 2009). Knowledge of species composition of scarabaied larvae in an area will make management and biological control methods a greater possibility (Dittrich-Schröder *et al.*, 2009). Control is not easy since the adults are active fliers flying away from the point of emergence, mating and ovipositing at the base of the host plant (Carnegie, 1988).

Black maize beetle populations can be controlled naturally by abnormal wet or dry weather conditions (Du Toit, 1998). Eggs are prone to desiccation, therefore under dry conditions, many eggs can die and L1 larvae that venture too close to the soil surface during dry conditions also die of heat stress (Du Toit, 1998). Water logging during wet conditions can also reduce the number of eggs and larval populations significantly (Du Toit, 1998).

There are three different types of control namely cultural-, biological- and insecticidal, which will be discussed.

1.3.1 Cultural control

The best way to control black maize beetle populations is by the use of cultural control methods through tillage during the more vulnerable pupal stage, *i.e.* September to October months of South Africa (Taylor, 1951). Larvae are very susceptible to injury and diseases found affecting larvae could be due to cultivation (Walker, 1968). Mechanical damage and predation by birds can lead to high mortalities in white grub populations when the tillage operations are correctly timed and carried out frequently (Litsinger *et al.*, 1983).

Crop rotation with leguminous crops such as cowpeas and sunflower can also help to control black maize beetles. Where crop rotation practices were done there was no damage reported compared to maize mono-cropping (Taylor, 1951).

1.3.2 Biological control

Biological control through the use of various natural enemies such as scoliid wasps (*Campsomeris pilosella),* tachinid parasitoids (*Microphthalma capensis)*, carabid predators (*Scarites madagascariensis),* fungi (*Beauveria* and *Metarrhizium), Rickettsias* and bacteria such as blue disease (*Coxiella popilliae)*, milky disease (*Bacillus popilliae)* and parasitic nematodes of the genus *Agamermis* sp., *Neoaplectana* sp., *Scarabnema* sp., *Diplogaster* sp. and *Sphaerularia* sp. are viable against *Heteronychus* species. However, beneficial populations are usually too small to cause effective control (Harington, 1953; Walker, 1968; Milner, 1997).

Natural enemies such as predators, pathogens and parasites do not suppress *H. arator* numbers below economic injury levels (EIL's) and guinea fowl that feed on the beetles have little impact on reducing their numbers (Du Toit, 1998). According to Milner (1997), a mycoinsecticide such as the bacterium *Bacillus thuringiensis* variety *japonensis* (a soil-born pathogen), commonly known as Buibui, is pathogenic to scarab larvae and is being developed in the USA and Japan.

Pathogens can be a very effective control strategy against soil insect pests, but a number of key elements must be considered, such as the cheaply *in vitro* mass production of a highly virulent pathogen strain, the availability of equipment for application, an application strategy which ensures sufficient contact between target pest and pathogen, as well as the compatibility of the pathogen strain with prevailing environmental conditions (Milner, 1997). According to Milner (1997), genetically engineered pathogen strains ensure that a wider range of host pests can be targeted and therefore improves field efficacy in the short-term, but if pathogen reproduction on the host is impaired, long-term control will not be achieved.

1.3.3 Chemical control

During 1947, organochlorine insecticides namely DDT and Benzene hexachloride (BHC) were tested and only BHC provided control of black maize beetle populations in maize (Taylor, 1951). Soil moisture and insecticides are closely linked with the type of soil (Walker, 1968). Insecticide treatment should be applied when adults and young larvae are present (Walker, 1968). A long residual insecticide is needed against larvae, while direct application needs to be implemented against the adults (Walker, 1968). *H. arator* adults became resistant to organochlorine insecticides such as chlordane, DDT, dieldrin and lindane during 1974 in Sydney, Australia (Goodyer, 1979).

In South Africa, before 1977, the only seed treatments available against black maize beetle in maize were dieldrin 42,5% wettable powder and gamma-BHC granules (refined BHC) (Drinkwater, 1982). During 1977 phorate 10%, which has a systemic and contact action, was registered as a preventative control measure against black maize beetle in maize and proved more effective than the other registered products on the S.A. market (Drinkwater, 1979; Drinkwater, 1982). Phorate, as well as gamma-BHC (in high dosages), can be phototoxic thus must not come in contact with maize seed (Drinkwater, 1979). However, gamma-BHC granules and chlorpyrifos granules proved to be the best corrective measure, but chlorpyrifos granules are degraded by ultraviolet-rays of the sun thus its use is not advised (Drinkwater, 1982). Various bait formulations such as endosulphan, registered for the control of cutworms in maize, did not reduce the black maize beetle numbers significantly (Drinkwater, 1982).

During 1967, dieldrin, an organocholrine insecticide, was banned due to the adverse toxic effects that it had on the environment and on humans (Blank *et al.,* 1982). In New Zealand, during 1979, isazophos and fensulfothion granules reduced the black maize beetle populations in maize significantly (Blank *et al.* 1982). However, soil types and moisture levels of the soil influenced the effectiveness of these chemicals against black maize beetles (Blank *et al.*, 1982). Granule chemicals also provided better control than liquid chemicals for the control of these beetles in maize (Blank *et al.*, 1982).

Drinkwater (1987) found that carbosulphan EC (emulsfiable concentrate) was the only liquid chemical that reduced the black maize beetle population significantly. Gamma-BHC placed between the soil surface and the seed, as well as with the seed, proved to be more efficient than placing these granules on the soil surface, thus correct placement of granules is vital for the effective control of black maize beetles in maize (Drinkwater, 1987).

Persistent organochlorine insecticides were an effective control method for *H. arator* in maize, but due to insect resistance and prohibition of such chemicals in agriculture prompted the development of other control strategies (Matthiessen and Ridsdill-Smith, 1991). During 1991, imidacloprid, a neonicotenoid seed dressing insecticide commonly known as Gaucho® became commercially available in South Africa for the first time for control of *H. arator* adults in maize (Drinkwater, 2002).

During 1997, approximately 700 000 ha of maize seed was annually chemically-treated, to control black maize beetles in South Africa (Du Toit, 1998). Also during 1997, in South Africa various seed dressings and granular insecticides, two emulsifiable concentrates and only one bait formulation were registered for chemical control of black maize beetle in maize (Du Toit, 1998). The efficacy of chemicals is dependent on factors such as placement and planting depth (Du Toit, 1998). Preventative insect treatments are advisable within the traditional distribution area and curative treatments must be applied beyond this area (Du Toit, 1998).

Neonicotinoid insecticides such as imidacloprid and carbamates, such as benfuracarb, carbosulphan, furathiocarb and thiodicarb seed dressing insecticides are registered for the control *H. arator* adults in the South African maize industry (Drinkwater, 2002). The systemically translocated anti-freedant and/or repellent activity of imidacloprid are more effective for the control of *H. arator* adults in maize compared to the systemically translocated insecticidal activity of the carbamates (Drinkwater, 2002).

Soil fumigation with methyl bromide successfully controlled white grub populations with no effect on the physical properties of the soil, but this method is not operationally cost effective (Zwolinski *et al.,* 1998).

Currently various chemical formulations exist in various classes (Table 1) to control black maize beetles in maize in South Africa, such as carbamates, oximecarbamates, organophosphorus, organophosphorus/pyrethroid, organochlorine and chloro-nicotinyl (Nel *et al.,* 2007).

Table 1.1. Chemical formulations currently registered in South Africa for the control of black maize beetles in maize (Nel *et al.,* 2007).

Chemical class	**Pesticide (common name)**	**Nature (action)**	**Type of formulation**
Carbamate	Benfuracarb Carbofuran Carbosulphan	Systemic Systemic Systemic	CS / LS GR DS/EC
Oximecarbamate	Thiodicarb	Contact	FS
Organophosphorus	Chlorpyrifos Isazofos Phorate Quinalphos Terbufos	Contact Systemic Systemic Penetrant and/or translaminar Systemic	CS/EC/GR/WG GR GR GR GR
Organophosphorus/ Pyrethroid	Chlorpyrifos/ cypermethrin	Contact Contact	EC EC
Organochlorine	Gamma-BHC	Contact	DS
Chloro-nicotinyl (neonicotenoid)	Imidacloprid Thiamethoxam	Systemic and/or repellent Systemic	FS/WS FS/WS

Key to table 1.1 (Nel *et al.,* 2007).

- CS – Capsule suspension – stable suspension of capsules in a fluid (normally intended for dilution in water).
- LS – Solution for seed treatment – a solution for application to the seed either direct or after dilution.
- GR – Granule – a free-flowing solid product of a defined granule size range, ready for use.
- DS – Powder for dry seed treatment – a powder for application in dry state direct to the seed.
- EC – Emulsifiable concentrate – a liquid, homogeneous formulation to be applied as an emulsion after dilution in water.
- FS – Flowable concentrate for seed treatment – a stable suspension for application to the seed either direct or after dilution.
- WG – Water dispersible granule – a formulation consisting of granules to be applied after disintegration and dispersion in water.
- WS – Water dispersible powder – for slurry treatment. A powder to be dispersed at high concentration in water before application as a slurry to the seed.

Carbamate insecticide is a synaptic poison that binds to an enzyme found in the synapse of most organisms. This will result in the continuous stimulation of nerves thus resulting in tremors and uncoordinated movement of the organism (Vallas and Koehler, 2012).

Oximecarbamate, such as thiodicarb, has both oral and contact activities against major lepidopteran, coleopteran, dipteran and hemipteran pests of various crops such as maize (Roberts, 1999). It is a neurotoxic compound that inhibits chlorinesterase enzymes (Roberts, 1999).

Organophosphorus insecticide is a synaptic poison that binds to an enzyme found in the synapse of most organisms. This will result in the continuous stimulation of nerves thus resulting in tremors and uncoordinated movement of the organism (Vallas and Koehler, 2012).

Organophosphorus/pyrethroid insecticides affect the nervous system of most organisms (Vallas and Koehler, 2012). Pyrethroids are anoxic poisons that poison the nerve fibres by binding to proteins found in the nerve fibres (Vallas and Koehler, 2012). These pyrethroids stimulate the nerve continuously which results in tremors and uncoordinated movement of organisms (Vallas and Koehler, 2012).

Organochlorine insecticides cause hyperexocitation in the nervous system of various organisms, which lead to the failure of the respiratory system, thus resulting in death (Coats, 1990).

Chloro-nicotinyl insecticide evolved from nicotine binds to post-synaptic nicotinergic acetylcholine receptors of most organisms thus interfering with normal nerve impulse transmission (Reid *et al.,* 2002). Chloro-nicotinyl insecticides are synaptic nervous system poisons that over-stimulate the nervous system, mostly of insects, resulting in tremors and uncoordinated movement (Vallas and Koehler, 2012).

1.4 Key factors for survival of *Heteronychus arator*

1.4.1 Soil structure

The black maize beetle favours a grey sandy soil, which is rich in humus. This pest has never been found in black, peaty soils (Taylor, 1951). Five different topsoil horizons exist in South Africa (Soil Classification Group, 2009). These include organic, humic, vertic, melanic and orthic (Soil Classification Group, 2009).

Organic O horizon top soil is confined to very small areas in South Africa; it is especially found in estuarine swamps, inland swamps and mountain slopes (Soil Classification Group, 2009). This topsoil is normally black or dark brown in colouration.

Humic A horizon is freely drained topsoil which have accumulated relatively large amounts of organic matter in cool or cold moist climates (Soil Classification Group, 2009). This is dark topsoil that has undergone moderate to strong weathering (Soil Classification Group, 2009).

Vertic A horizon topsoil has both a high clay content and a predominance of smectitic clay minerals (can swell and shrink in response to moisture changes) (Soil Classification Group, 2009). This topsoil is black to very dark in colour (Soil Classification Group, 2009).

Melanic A horizon topsoil is dark in colour and has a lower clay percentage than vertic A horizon topsoil. High clay percentage micaceous, vermiculitic or even kaolinitic clay minerals are present (Soil Classification Group, 2009).

The majority of topsoil found in South Africa belongs to the Orthic A horizon (Soil Classification Group, 2009). Orthic topsoil varies widely in organic carbon content, colour, texture, structure, base status and mineral composition (Soil Classification Group, 2009). For example, the orthic topsoil of Hutton and Avalon differ in their physical and chemical properties, as well in moisture regimes (Soil Classification Group, 2009). Hutton has a reddish colour that is weakly structured and is free from water logging. Avalon is yellow or brown in colour and is more easily formed in sands. The average moisture status is higher where the soil is shallow thus water logging can occur (Soil Classification Group, 2009).

1.4.2 Temperature

At soil temperatures lower than 15°C larvae of the family Scarabaeidae cease to feed (Glogoza *et al.,* 1998). Temperatures above 15°C are most favourable for the development and survival of *H. arator*, with an optimal temperature range between 20-25°C (Matthiessen and Ridsdill-Smith, 1991). If spring-summer temperatures are above average the mortalities in the early instar larvae are less, which results in outbreaks (Matthiessen and Ridsdill-Smith, 1991). Older larvae and adult mortalities still need to be investigated (Matthiessen and Ridsdill-Smith, 1991).

1.5 Distribution of *Heteronychus arator*

Black maize beetle (*H. arator*), previously described as *H. sanctae-helenae* Blanchard, occur throughout South Africa, but is more concentrated in the eastern Highveld, north-eastern and eastern Free state, northern and central Kwa-Zulu

Natal, as well as in the Eastern and Western Cape provinces of South Africa, where they are a serious pest of maize (Harington, 1964; Drinkwater, 1979; Du Toit, 1998).

Scholtz and Holm (2008) classified the black maize beetle (*H. arator*) as follows:

Order:	Coleoptera
Suborder:	Polyphaga
Superfamily:	Scarabaeoidea
Family:	Scarabaeidae
Subfamily:	Dynastinae
Genus:	*Heteronychus* spp.
Species:	*Heteronychus arator* Fabricius

Subfamily Dynastinae, commonly known as the rhinoceros beetles, comprises about 20 genera and 60 species in South Africa (Scholtz and Holm, 2008). Most adult males possess a large horn on the head and thorax, therefore the common name rhinoceros beetle (Scholtz and Holm, 2008). As a rule adults of the subfamily Dynastinae do not feed on living plant material, except for adults from the genus *Heteronychus* (Scholtz and Holm, 2008). Some species from the genus *Heteronychus* are regarded as serious pests of cultivated crops in South Africa such as *H. arator* (Figure 1.12) which feed on maize, potato, wheat, grain sorghum and pineapple (Harington, 1964; Drinkwater, 1979; Du Toit, 1998). *Heteronychus licas* Klug (Figure 1.12), another species within the same subfamily, attacks living plant tissues such as sugar cane (Scholtz and Holm, 2008).

Figure 1.12. *Heteronychus licas* adult (left) and *Heteronychus arator* adult (right) (Drinkwater, 1979).

The first record of *H. arator* in South Africa was before 1860 and the species was introduced to Australia during the 1920's (Harington, 1964) and New Zealand during 1937 (Todd, 1964). Eleven species of *Heteronychus* are known in South Africa, but *H. arator* is regarded as the most economically important coleopteran pest of maize (Drinkwater, 1979).

Six species of *Heteronychus* are known to attack maize in Zimbabwe and Zambia, but *H. licas,* that look similar to *H. arator* but slightly larger, is the most severe (Drinkwater, 1979). It is unknown if *H. licas* attacks maize in South Africa (Drinkwater, 1979).

1.6 Hypothesis

With the growing population and increased demand for food, pressures of maximum production has led to alternative production practices, including the use of pivot irrigation in order to reduce risks associated with droughts. The increase in input costs could also led to the implementation of no-till practises. No-till practises are known to alter the soil composition and preserve available soil moisture which is favoured by all developmental stages of black maize beetle. Ploughing during the pupal stage of the black maize beetle is the most effective cultural control method available and since this is discouraged in favour of no-till practices this control method is no longer an option. Current agricultural practices and environmental conditions may intensify the seasonality of this species.

Currently only preventative insecticidal control measures exist in South Africa and therefore predicting when this pest will occur will help to establish when to employ preventative control measures. Producers currently employ preventative control measures even if black maize beetle numbers are low, resulting in input costs that could have been avoided if producers can be informed of the current status of the black maize beetle population within their area.

Chemical control of black maize beetles could amount to millions of Rand annually. Approximately 700 000 ha (at a cost of approximately R110 ha^{-1}) are treated annually with insecticides to control black maize beetles (between R110 and R116 per 25 kg bag of seed). Producers are continuously being advised accordingly and could therefore save up to R110/ha annually. In years when it is unnecessary to use insecticides (as advised in the past ten consecutive years) the maize producers can save R70 000 000 per year on input costs alone.

Knowledge of the population status will lower the risk of unnecessary use of insecticides when insect numbers are very low and ensure effective control measures in years when it reaches epidemic status. If the mode of action of newly developed insecticides and factors influencing their efficacy under different field conditions are known, optimal efficacy would be ensured by eliminating the unnecessary use of ineffective insecticides and incorrect application methods. The incidence and variation in population levels are poorly understood. This results in the unnecessary use of insecticides at the abovementioned cost in years when beetle numbers are very low, or a lack in control measures in years when it reaches epidemic status.

Global warming may contribute to a shift in beetle abundance and distribution and therefore establishing a prediction model for black maize beetle may help to predict if a shift in beetle distribution and abundance is expected to occur.

This study attempts to develop an early-warning system for black maize beetles in order to lower the risk of unnecessary use of insecticides when insect numbers are very low and ensure effective control measures in years of high population levels.

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CHAPTER 2:

Spatial and temporal variation of *Heteronychus arator*

2.1 Introduction

Spatial variation is defined as the extent or degree to which something varies (differs) in space, whereas temporal variation defines the extent or degree to which something varies over time (White, 2013). This study aims to provide information on geographical and seasonal variation of black maize beetle occurrence, abundance as well as distribution within seasons.

2.1.1 Distribution of *Heteronychus arator*

Black maize beetle (*H. arato*r Fabricius) was discovered in South Africa (Cape province) and described as *Scarabaeus arator* by J.C. Fabricius in 1775 (Bell *et al.*, 2011). This species is currently distributed throughout southern, central and eastern Africa (CAB International, 2000). Black maize beetles were introduced to Australia during the 1920's (Harington, 1964), New Zealand during 1937 (Todd, 1964) and to Brazil, South America during the late 1960's (CAB International, 2000).

Locally black maize beetles (*H. arator*) occur throughout South Africa, but is more concentrated in the eastern Highveld, north-eastern and eastern Free State, northern and central Kwa-Zulu Natal as well as in the Eastern and Western Cape provinces, where the insect is a serious pest of maize, potato, wheat, pineapple and grain sorghum (Harington, 1964; Drinkwater, 1979; Du Toit, 1998).

2.1.2 Lifecycle and flight behaviour

Black maize beetles have only one generation annually and favour a grey sandy soil with high organic matter content (Taylor, 1951). At soil temperatures lower than 15°C larvae of the family Scarabaeidae cease to feed (Glogoza *et al.,* 1998). Temperatures above 15°C are most favourable for the development and survival of *H. arator*, with an optimal temperature range between 20-25°C (Matthiessen and Ridsdill-Smith, 1991). If spring-summer temperatures are above average, the mortality rate of the early instar larvae is lower which results in outbreaks (Matthiessen and Ridsdill-Smith, 1991). Older larvae and adult mortalities still need to be investigated (Matthiessen and Ridsdill-Smith, 1991). According to Bell *et al.*

(2011) the distribution of black maize beetle populations are confined to areas with a mean annual soil surface temperature of 12.8°C.

Adults of black maize beetles are nocturnal and sporadic in occurrence (Taylor, 1951; Du Toit, 1998). The first adults become active during autumn months, only after significant rainfall, onset of warmer soil temperatures (>17°C) and when calm wind conditions are present (Du Toit, 1998; Bell *et al.*, 2011). Flights during spring time tend to be smaller in numbers and are restricted to ≤four evenings per season (Bell *et al.*, 2011). During black maize beetle epidemic years these flights only increase in number and not in frequency (Bell *et al.*, 2011).

Two main flight peaks of black maize beetles are found during each year in South Africa (Taylor, 1951). The 1st peak occurs early in the summer, October to December. This peak is most damaging to maize seedlings (Taylor, 1951). This flight comprises approximately 5% of the total annual flight (Du Toit, 1998). The 2nd peak occurs during February to April and is the offspring of the early-emerging beetles of the first generation and will continue to occur until May depending on soil temperatures (Taylor, 1951). According to Du Toit (1998) this peak accounts for approximately 95% of the total annual flight.

2.1.3 Key factors favored by *Heteronychus arator*

Current agricultural practices and environmental conditions intensify the seasonality of members of the family Scarabaeidae (Pardo-Larcarno *et al.,* 2005). The implementation of no-till practices results in more available soil moisture, especially under drought conditions, when compared to conventional tillage practices in maize (Harrison *et al.,* 1980). All stages of Scarabaeidae are dependent on moisture (Taylor, 1951; Harrington, 1953; Walker, 1968), temperature (Taylor, 1951; Du Toit, 1998; Glogoza *et al.,* 1998) and humus content in the soil (Taylor, 1951; Walker, 1968). Therefore irrigated fields with no-till practices may be more prone to recurring attacks by members of the family Scarabaeidae such as *H. arator*.

2.1.4 Hosts

Natural hosts favoured by *H. arator* include paspalum (*Paspalum dilatatum*) and ryegrass (*Lolium* spp.) (Bell *et al.,* 2011). Both these grass species are a preferred ovipositional site for black maize beetle (Bell *et al.,* 2011). Organic matter in soil serves as feeding stimulant for young black maize beetle larvae and 3rd instar larvae feed on these grass root systems (Bell *et al.*, 2011).

According to Du Toit (1997), the family Scarabaeidae is the most abundant insect pest in both natural grass fields and maize areas in South Africa. However, scarabaied larval stages are more concentrated in natural field areas whereas adults are more concentrated in maize areas which indicate that adults migrate after emergence towards the more acceptable host species, such as maize (Du Toit, 1997). Thus, natural vegetation surrounding maize may act as reservoirs for black maize beetles (Du Toit, 1997). Black maize beetles' dispersal ability during autumn and spring play a substantial role in infesting maize fields, especially during outbreak years (Bell *et al.*, 2011).

2.1.5 Management strategies for *Heteronychus arator*

Control is not easy because the adults are active fliers (Carnegie, 1988). By flying from point of emergence, mating and ovipositioning takes place at the base of the host plant (Carnegie, 1988).

Black maize beetle populations are controlled naturally by abnormal wet or dry weather conditions (Du Toit, 1998). Eggs and L1 larvae are prone to desiccation when too close to the soil surface during hot, dry and windy conditions (Du Toit, 1998). Water logging during wet conditions can also reduce the number of eggs and larval populations significantly (Du Toit, 1998).

Three different types of management strategies exist for black maize beetle namely cultural, biological and insecticidal control.

2.1.5.1 Cultural control

The best way to control black maize beetle populations is the use of cultural control methods, through ploughing during the more vulnerable pupal stage, September to October (Taylor, 1951). Larvae are very susceptible to mechanical damage and apparent diseases found within larvae can be due to cultivation practices (Walker, 1968).

Crop rotation with leguminous crops such as cowpeas and sunflower can also help to manage black maize beetle populations. Where crop rotation practices were done no apparent damage occurred when compared to maize mono-cropping practices (Taylor, 1951). Mechanical damage and predation by birds can lead to high mortalities in white grub populations when the tillage operations are correctly timed and carried out frequently (Litsinger *et al.*, 1983).

2.1.5.2 Biological control

Biological control through the use of various natural enemies such as scoliid wasps (*Campsomeris pilosella),* tachinid parasites (*Microphthalma capensis)*, carabid predators (*Scarites madagascariensis),* fungi (*Beauveria* and *Metarrhizium), Rickettsias* and bacteria like blue disease (*Coxiella popilliae)*, milky disease (*Bacillus popilliae)* and parasitic nematodes of the genus *Agamermis* sp., *Neoaplectana* sp., *Scarabnema* sp., *Diplogaster* sp. and *Sphaerularia* sp. are viable against *Heteronychus* spp. However, populations are usually too small to be effective (Harington, 1953; Walker, 1968; Milner, 1997). Natural enemies such as predators, pathogens and parasites do not suppress *H. arator* numbers below economic injury levels (EIL's) and guinea fowl that feed on the beetles have little impact on reducing their numbers (Du Toit, 1998).

According to Milner (1997), myco-insecticides such as the bacterium *Bacillus thuringiensis* variety *japonensis* (a soil-born pathogen), commonly known as Buibui, are pathogenic to scarab larvae and are being developed in USA and Japan. USDA approved C*ry* 3A toxin (MIR 604) Agrisure®Duracade™ 5122 and 5222 for commercial release during the 2014 planting season against corn rootworm in the European Union (Syngenta, 2013). This toxin is effective against coleopteran pest species but is still not known to be effective against species in the Scarabaeidae family such as black maize beetle.

Pathogens can be a very effective control strategy against soil insect pest populations, but a number of key elements must be considered such as the cheaply *in vitro* mass production of a highly virulent pathogen strain, the availability of suitable equipment for application, an application strategy which ensures sufficient contact with the target pest and pathogen as well as the compatibility of the pathogen strain with prevailing environmental conditions (Milner, 1997).

According to Milner (1997), genetically engineered pathogen strains ensures that a wider range of host pests can be targeted, therefore improving field efficacy in the short-term but if reproduction failure on the host is impaired, long-term control will not be achieved.

2.1.5.3 Chemical control

Currently various chemical formulations exist in various classes (Table 1) to control black maize beetles on maize in South Africa such as carbamates, oximecarbamates, organophosphorus, organophosphorus/pyrethroid, organochlorine and chloro-nicotinyl (Nel *et al.,* 2007).

Table 2.1. Chemical formulations currently registered in South Africa for the control of black maize beetles in maize (Nel *et al.,* 2007).

Chemical class	**Pesticide (common name)**	**Nature (action)**	**Type of formulation**
Carbamate	Benfuracarb Carbofuran Carbosulphan	Systemic Systemic Systemic	CS / LS GR DS/EC
Oximecarbamate	Thiodicarb	Contact	FS
Organophosphorus	Chlorpyrifos Isazofos Phorate Quinalphos Terbufos	Contact Systemic Systemic Penetrant and/or translaminar Systemic	CS/EC/GR/WG GR GR GR GR
Organophosphorus/ Pyrethroid	Chlorpyrifos/ cypermethrin	Contact Contact	EC EC
Organochlorine	Gamma-BHC	Contact	DS
Chloro-nicotinyl (neonicotenoid)	Imidacloprid Thiamethoxam	Systemic and/or repellent Systemic	FS/WS FS/WS

Key to table 2.1 (Nel *et al.,* 2007).

- CS – Capsule suspension – stable suspension of capsules in a fluid (normally intended for dilution in water).
- LS – Solution for seed treatment – a solution for application to the seed either direct or after dilution.
- GR – Granule – a free-flowing solid product of a defined granule size range, ready for use.
- DS – Powder for dry seed treatment – a powder for application in dry state direct to the seed.
- EC – Emulsifiable concentrate – a liquid, homogeneous formulation to be applied as an emulsion after dilution in water.
- FS – Flowable concentrate for seed treatment – a stable suspension for application to the seed either direct or after dilution.
- WG – Water dispersible granule – a formulation consisting of granules to be applied after disintegration and dispersion in water.
- WS – Water dispersible powder – for slurry treatment. A powder to be dispersed at high concentration in water before application as a slurry to the seed.

All these compounds are to be applied preventatively. This unavoidably leads to economically unjustified applications during periods of low beetle occurrence as well as to economically important losses in the absence of chemical control during outbreaks.

This study attempts to provide a more detailed image of the occurrence and distribution of *H. arator* populations as well as perspective on seasonal and geographical variation within a major maize producing region of South Africa.

2.2 Material and methods

2.2.1 Sites

The eastern part of the maize triangle of South Africa (Figure 2.1a) was divided into quarter degree longitudinal by quarter degree latitudinal blocks (approximately 30km x 30km) (Figure 2.1b). Within this block a maize farm (purple circle) was selected as close as possible to the midpoint to ensure even distribution of light traps. A total of 99 modified Robinson light traps were installed within the eastern part of the maize triangle of South Africa.

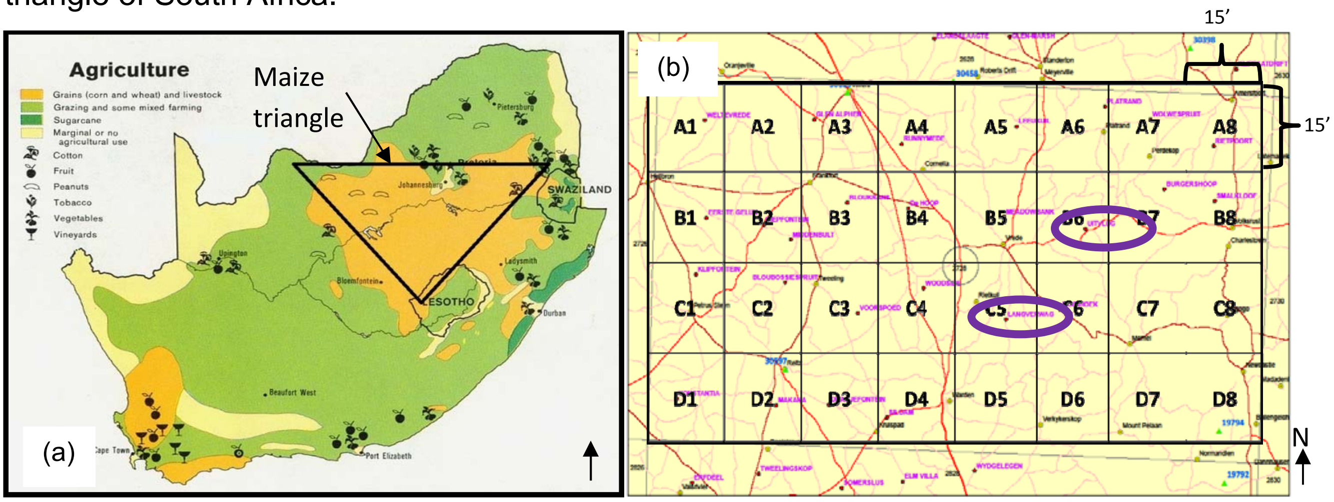


Figure 2.1(a). Maize triangle of South Africa and (b) samples within the map of Frankfort district of South Africa.

2.2.2 Trap design

A modified Robinson light trap (Common, 1959) consisted of a stand, funnel and container (Figure 2.2a). The stand was approximately 150mm high and comprised three triangularly placed steel round tubing of 20 mm in diameter. This was connected by a triangular structure, 300 mm from the base, on which the 20 litre container resided. The top of the stand was connected by a 6mm steel round bar that had been bent into a circle with a diameter of about 400mm in which the funnel resided.

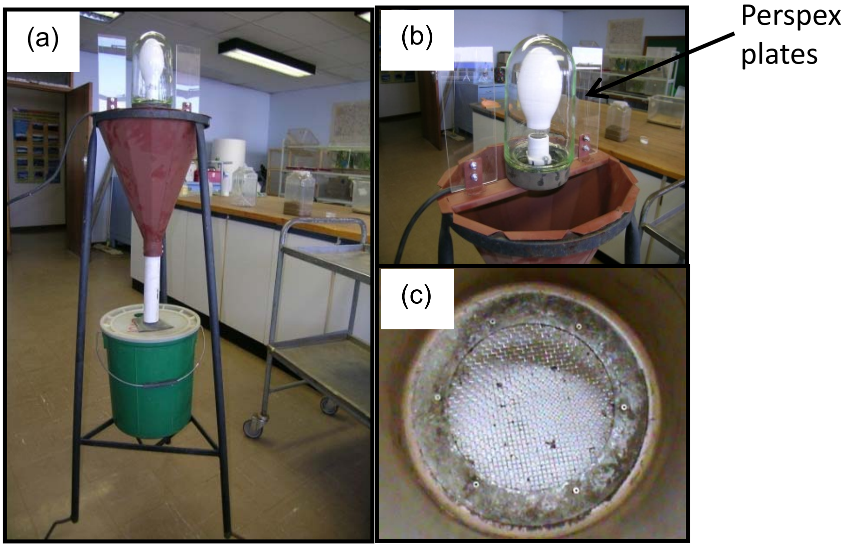


Figure 2.2(a). Modified Robinson light trap used to catch black maize beetles, (b) close-up of the top part of light trap indicating the Perspex plates at opposite ends of the light bulb and (c) the modified inside of the 20 litre container.

The funnel comprised a 1mm steel plate that was bent in the form of a funnel with top diameter of approximately 420mm and a bottom diameter of approximately 50mm. The entire steel structure was coated with anti-corrosion paint. A 50mm PVC pipe of approximately 500mm in length was connected at the base of the funnel which connected the funnel to the 20 litre container. A 2mm flat bar with a length of approximately 420mm was connected across the midpoint of the funnel on which a 220V light bulb with a 160W output was connected into an electrical source. Clear Perspex plates (100mm x 200mm) were fitted on either side of the light bulb (Figure 2.2b) which aided in the capture of nocturnal insects. Additionally, a day-night switch was connected within this connection that contributed to the saving of electricity.

The bottom of the 20 litre container was cut out in a diameter of 150mm. This was replaced with circular stainless steel mesh that was cut into a radius of 160mm. The stainless steel mesh with a wire thickness of approximately 1mm with 4mm x 4mm holes was placed into the previously cut 20 litre container which aided in drainage during the rainy season (Figure 2.2c). The lid of the 20 litre container had a hole of 50mm in the centre which allowed for the PVC pipe to fit through.

2.2.3 Data collection

Total black maize beetle captures per locality from 2002 to 2012 are provided in Table 2.3. Map and block numbers were allocated to each producer within the monitored area (e.g. Figure 2.1b and Table 2.2). Data were grouped into 5 representative areas (Table 2.2). These groups are indicated in 5 different color codes:

Green – Northern Mpumalanga province,

Dark blue - Eastern Free State province,

Light blue – Southern Free State province,

Purple – Western Free State province and

Red – Border of Mpumalanga, Free State and Gauteng provinces (Figure 3, Table 2.2 and 2.3).

Black – Rest.

Table 2.2 Block number allocation of light traps.

Block number	5	6	7	8	1	2	3	4	5	6	7	8
D							2528	2528	2528	2528	2528	2528
A						2628	2628	2628	2628	2628	2628	2628
B					2628	2628	2628	2628	2628	2628	2628	2628
C					2628	2628	2628	2628	2628	2628	2628	2628
D					2628	2628	2628	2628	2628	2628	2628	2628
A				2726	2728	2728	2728	2728	2728	2728	2728	2728
B				2726	2728	2728	2728	2728	2728	2728	2728	2728
C				2726	2728	2728	2728	2728	2728	2728	2728	2728
D			2726	2726	2728	2728	2728	2728				
A		2826	2826	2826	2828	2828	2828	2828	2828			
B		2826	2826	2826	2828	2828	2828	2828				
C		2826	2826	2826	2828	2828	2828	2828				
D	2826	2826	2826	2826								

Data accumulation varied between localities over the 11 year capture period (Table 2.3). This was due to farmers either losing their farms to land relocation processes or selling of farms. The most challenging problem encountered was the hike in electricity prices, during the capture period. This led to a lot of producers rejecting this project. Load shedding also contributed to loss in data over the capture period. These data were not included. Another problem encountered, was producers who were forced or chose to stop maize production in favor of animal production.

Table 2.3. Total seasonal black maize beetle captures per locality from 2002 to 2012.

	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
2528/D3					122	22	377	516	114	32	57
2528/D5	963		864				120		58	18	39
2528/D5.1	565		538	174							
2528/D6	148										
2528/D7	1383	67	1003	851	179	196	415		297	927	0
2528/D8	119	7	125	428	1	257	275		820	401	
2628/A2		422	274	936	60	461	11				
2628/A2.1	139	190	192	223		110					
2628/A3	1083	1984		1244	313		205				
2628/A4	651	1778				199			1809	1219	
2628/A5	4321	80	9542	826	335						
2628/A6	3783	214	1075	1054	33	360	34	748	251	303	
2628/A7	180	498									
2628/A8	146		1156	322	94	397	112	2896	121	29	
2628/B1		1357	250	2132	47						
2628/B2	64		264	670	177	26	702	792	616	176	
2628/B3	2235	217	1315			397	370	557	16		
2628/B4	101	372	658	683	3	35	46	387	96		
2628/B5	1328	371	232	562							
2628/B6	546		976	958	107	91					
2628/B7	194	59	565	1034	285		3			12	
2628/B8			251	205	684	516	255	620	118	34	114
2628/C1	188	38	783	1481	98	17	25	90	2	1	
2628/C2		118	784	770	468						
2628/C2.1				4380	1055	256	5398	10268		41	2487
2628/C3				241							
2628/C4	394	269		532							
2628/C5	347										
2628/C6	286	489		1322							
2628/C7	387	8	433	272		190	367	9728	2121	296	759
2628/C8	872	1632	242	987		698	832	3413	660	209	676
2628/D1	120	94	166		203	44	285	3413	833		
2628/D2	753	2939	10613	2551	490	541	8028	16626	3693	255	2679
2628/D2.1		649	4127	3554	1276	249	1792	7452	3324	96	376
2628/D2.2	1066	736	7724	2794	928	389	289	8951		21	54
2628/D3	1281	276	7579	1590	652	312	7964	15636	2052	361	
2628/D4	947	761	669	697							
2628/D5	879		904	518	56	267					
2628/D6	58	267	154	276		275					
2628/D7	327	654	584	1129	275	160					
2628/D8	274		973	914	105	299					
2728/A1	399	932		833	106						
2728/A2	272	363		665	294	115	1526	7970	681	112	983
2728/A2.1	348	619	4228	2752	414	705	2259	15529		221	2055
2728/A3	1004	729	5702	1949	196	290	3992	16475		71	289
2728/A3.1	408	321	1696	1205	23	18	1353	10770	585	64	
2728/A4	682	125	243	1526	149	242	1114	5787	608	97	82
2728/A5	446	326	363	1203	124	240	271	328	1659	107	188
2728/A6	1612	4856	1548	865	174	485			2997	47	1301
2728/A7	526	503	151	2183	246	580	1802	15318	1162	114	828
2728/A8			137	828	80	74					
2728/B1	167	124	112	306		170	100	927	651	335	449
2728/B2	560	154	147	535	109	241	164	4342		1167	

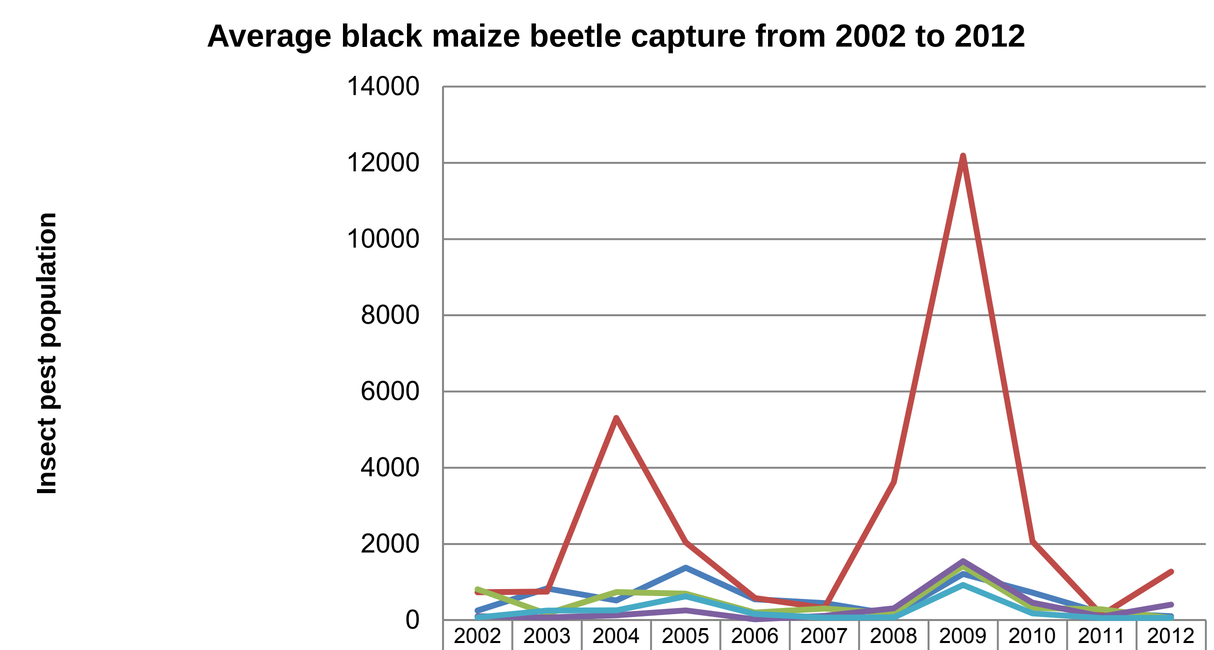
Table 2.3 (continued). Total seasonal black maize beetle captures per locality from 2002 to 2012.

Map/block	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
2728/B3	129		205	1233	579	510	813	4342	1102	136	270
2728/B4			253	798			331				
2728/B5	197				152	522	562	2522		160	
2728/B5.1							26				
2728/B6		547	529	350							
2728/B7						393	55	14156			
2728/B7.1			14	12	3	261	18	170	5	13	46
2728/B8			372	1855	65	344	99	6084	1960	258	64
2728/C1	281	1204		1145		192	795	2200	994		
2728/C2	28	108		708	19		47	111	42	39	
2728/C3	142	1113	913	1423					2045	858	28
2728/C4	266	582	327	1431	216	150	334	1980			
2728/C5	224		212				43			131	
2728/C6			167		55		214	1650	1396	209	
2728/D1	150	92	117	739	113	303	48	240		116	150
2728/D2	715	461		793			74				
2728/D3	802		443	2461	1608	691	145	282	91	26	4
2728/D4	61	936	643	1831	773	547	148	215	218	40	71
2726/A8			184	704	25	297	677	2724	1133	13	55
2726/B8		89	82	323	7	50	167	1944	96	68	1149
2726/C8			191	107	57	63	386	1248	890	209	30
2726/D7	103	47	82	56	10	33		285	151		
2726/D8			91	93			13		25		397
2826/A6	489	39	183	311	94				196	54	0
2826/A7	38	241	795	1261	142	100	79	2218	215	86	96
2826/A8	91	475	319	732	33	32	79	1027	973	134	167
2826/B6		572	140	292	77	89	12	1027	46	2	24
2826/B7	26	367	114	269	134	75	56	266	50	211	119
2826/B8	73			403	185	16	7	50	53	10	41
2826/C6	71		260	995	68	76	181	937	7	4	0
2826/C7	90	60	258	178	606	89	131		46	11	18
2826/C8	23	35	78		41	24	5		20	2	9
2826/D5	137	180	86	435	104	36	197	264	213	274	95
2826/D6	15	8	53	642	120				18	35	84
2826/D7	18	29	71	705	226	106	425	272	110	34	15
2826/D8	164	26	113	881	167	73	126	936	203	58	77
2828/A1	343	598	708	873	1	79		103	510	29	154
2828/A2	667	4990	6218	3498	2357	578	28	805	738	447	811
2828/A3	22	695	594	1032	441	605	25	1743	372	42	302
2828/A4			865	950	427	383	88	1095	502	29	
2828/A5			505	515	298	295	218	733	442		
2828/B1	58	1405	1011	558	126	331	15	900	96	20	
2828/B2	126	237	288	324	236	489			48		
2828/B3				1476	1143	612	285	1173	796	105	89
2828/B4				817							
2828/C1	116		110	631	218				9		

Weekly collected insect captures, by the producers, were emptied into another 2 litre container were they were preserved in a 70% alcohol solution that was filled to the level of the capture. The 2 litre container with the preserved insects was then clearly marked with the date of capture and locality. The capture period ranged from the first week of February to the end of April of each year. After the three month collection period, these captures were collected and the new stock which comprised of the 2 litre containers and 70% alcohol solution was delivered to each site. This was for the following years' capture. After all the sites had been collected the numbers of black maize beetles for each week at each site were recorded. The statistical procedure (Arc-GIs version 10.1) is provided with the results as a necessary step.

2.3 Results and statistics

The average black maize beetle capture (Figure 2.3), ranged from 25 up to 12186 in total over the 11 year capture period for five representative areas. Four of these areas had less than 1700 black maize beetles over the capture period. On the border of Mpumalanga, Gauteng and Free State provinces two long term peaks were identified. The 1st occurred during 2004 when 5307 beetles were captured, and the 2nd during 2009 when the average black maize beetles capture was 12186.



	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Eastern Free State province	253	832	519	1378	545	445	152	1213	724	191	101
Border of Mpumalanga, Free State and Gauteng provinces	733	750	5307	2041	580	319	3622	12186	2067	138	1275
Northern Mpumalanga province	812	169	736	693	198	303	182	1421	321	284	57
Western Free State province	103	68	126	257	25	111	311	1550	459	97	408
Southern Free State province	72	254	260	626	161	64	75	923	179	58	61

Figure 2.3. Average black maize beetle captures at five representative areas from 2002 to 2012.

2.3.1 Spatial Auto-Correlation

Given a set of features and an associated attribute, the Spatial Auto-Correlation evaluates whether the pattern expressed is clustered, dispersed, or random. When the z-score or P-value indicates statistical significance, a positive Moran's I index value indicates tendency toward clustering while a negative Moran's I index value indicates tendency toward dispersion. The Global Moran's I tool calculates a z-score and P-value to indicate whether or not the null hypothesis can be rejected. In this case, the null hypothesis states that feature values are randomly distributed across the study area.

Results obtained (Figures 2.4, 2.5 and 2.6) throughout the 11 year capture period (2002-2012) indicated that black maize beetle populations were distributed in highly significant (P<0.01; z-score>2.58) clusters (positive Moran's Index) throughout the monitored area except for 2003 and 2011. No significant clustering of black maize beetle populations were found during 2003 and 2011 (P>0.05; z-score between -1.65 and 1.65), which indicates that they were randomly (negative Moran's Index) distributed across the study area. In all therefore, black maize beetle populations were not dispersedly distributed across the monitored area over the 11 year period.

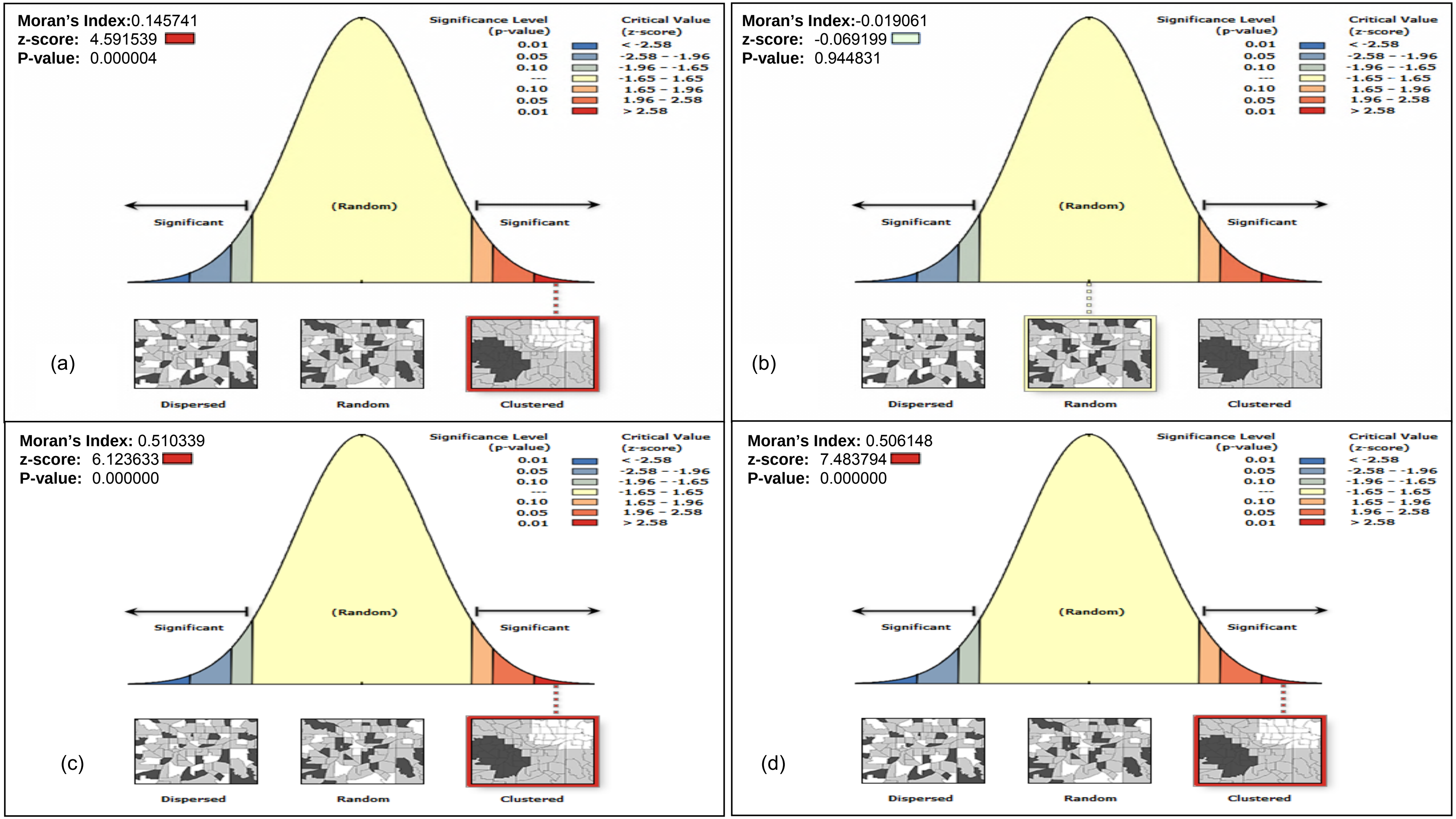


Figure 2.4. Black maize beetle spatial auto correlation during (a) 2002 (74 locations), (b) 2003 (65 locations), (c) 2004 (78 locations) and (d) 2005 (85 locations).

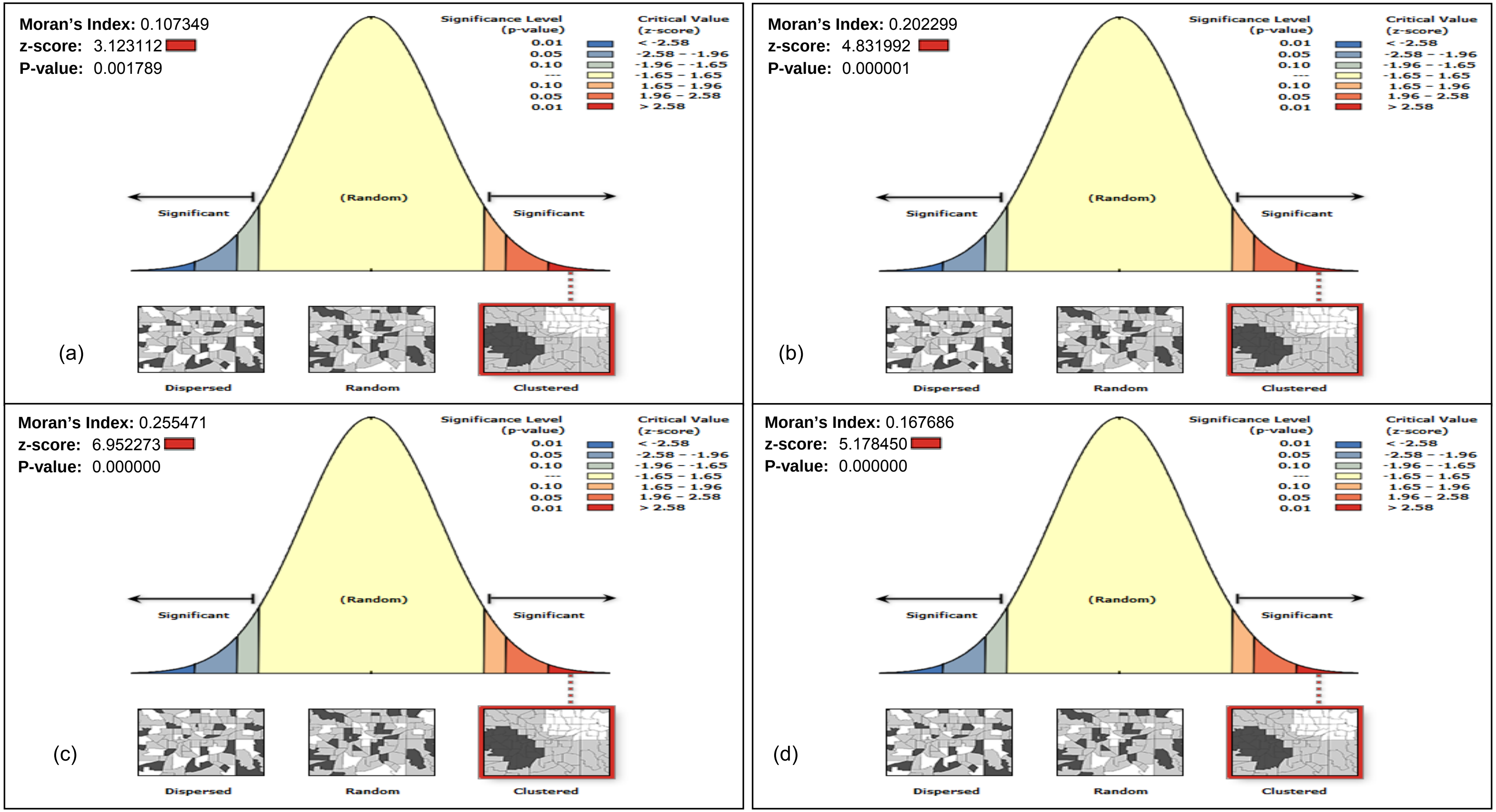


Figure 2.5. Black maize beetle spatial auto correlation during (a) 2006 (72 locations), (b) 2007 (70 locations), (c) 2008 (68 locations) and (d) 2009 (57 locations).

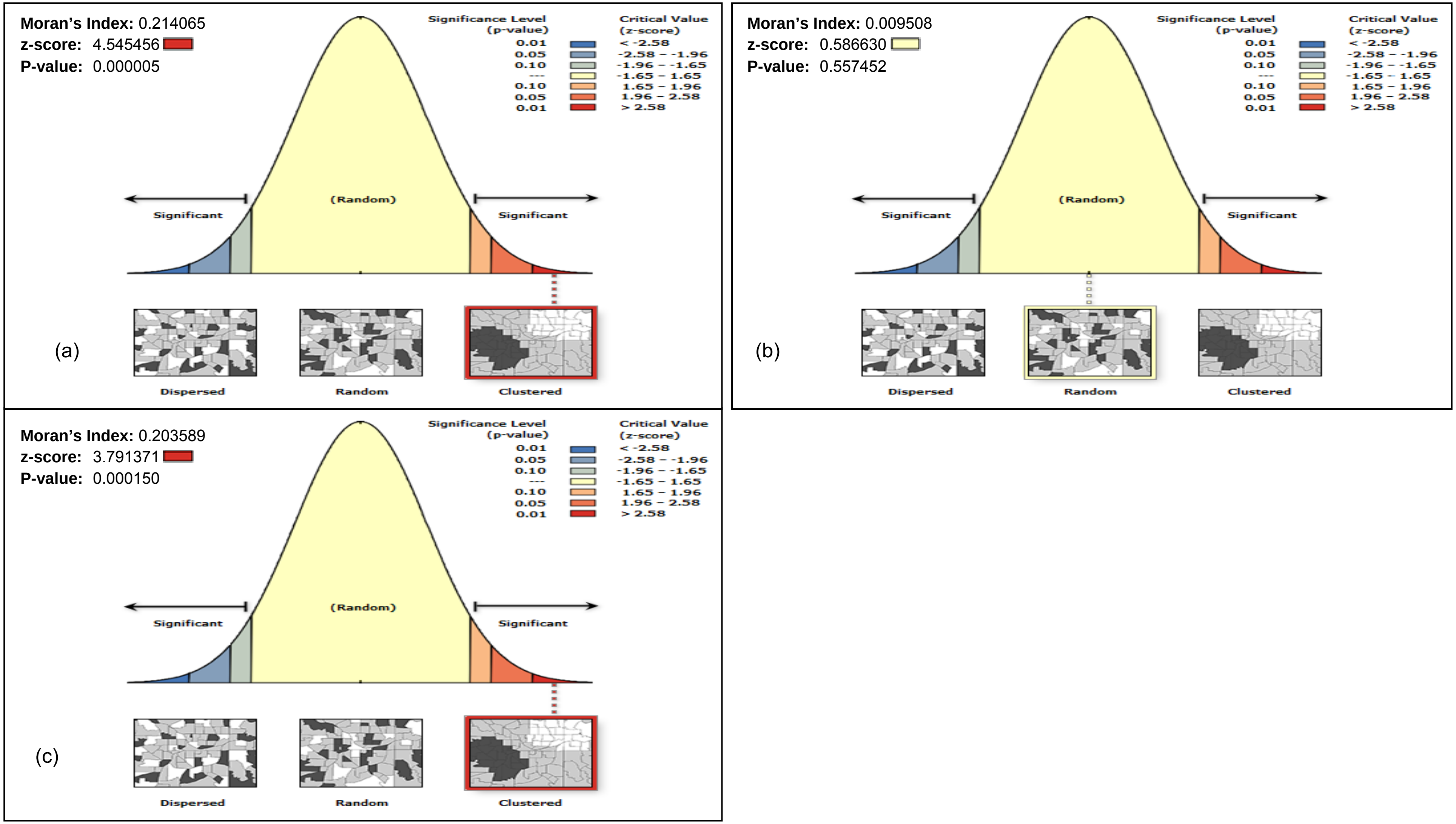


Figure 2.6. Black maize beetle spatial auto correlation during (a) 2010 (61 locations), (b) 2011 (61 locations), and (c) 2012 (46 locations).

2.3.2 Cluster and outlier analysis

The z-scores and P-values are measures of statistical significance which tell whether or not to reject the null hypothesis, feature by feature. In effect, they indicate whether the apparent similarity (a spatial clustering of either high or low values) or dissimilarity (a spatial outlier) is more pronounced than one would expect in a random distribution.

A highly positive z-score for a feature indicates that the surrounding features have similar values (either high values or low values). The COType field in the Output Feature Class will be HH for a statistically significant (P=0.05 level) cluster of high values and LL for a statistically significant (P=0.05 level) cluster of low values.

A low negative z-score (for example, <-1.96) for a feature indicates a statistically significant (P=0.05 level) spatial outlier. The COType field in the Output Feature Class will indicate if the feature has a high value and is surrounded by features with low values (HL) or if the feature has a low value and is surrounded by features with high values (LH).

Cluster and outlier analysis (Figures 2.7 and 2.8) of black maize beetle populations across the study area indicated that similar values with either high (HH: black circles) and/or low (LL: blue circles) values were observed over the 11 year period (2002-2012) except for 2003 and 2011.

Only during 2003, one spatial black maize beetle outlier was identified (HL: yellow circle) south of Mpumalanga with a low negative z-score of -0.069199. This location yielded a higher than expected value of 4856 (Table 2.2, (green circle) and Figure 2.7 (orange circle)) than one would expect from the surrounding locations where low values ranging from 267 to 674 were observed.

Spatial outliers with low black maize beetle numbers occurred at one location during 2005, two locations in 2008, and four locations in 2009 and 2010 as indicated by purple arrows (Figure 2.7 and 2.8).

Only during 2002 similar black maize beetle clustering of significance was found in the north of the monitored area (Figure 2.7, (orange circle)). During six seasons

(2004, 2005, 2008, 2009, 2010 and 2012) similar significant ($P<0.05$) high black maize beetle clustering was found on the border of Mpumalanga, Free State and Gauteng provinces (Figure 2.7 and 2.8). Similar significant low values were found only in the Free State province for three seasons (2007, 2009 and 2010) (Figure 2.7 and 2.8 (blue circles)).Only during 2005, 2006 and 2007 significantly high spatial clustering was found in the eastern part of the Free State province (Figure 2.7 (black circles)). During 2007 and 2010 significant spatial clustering of high black maize beetle numbers were found in the eastern part of the Mpumalanga province (Figure 2.7 and 2.8 (black circles)).

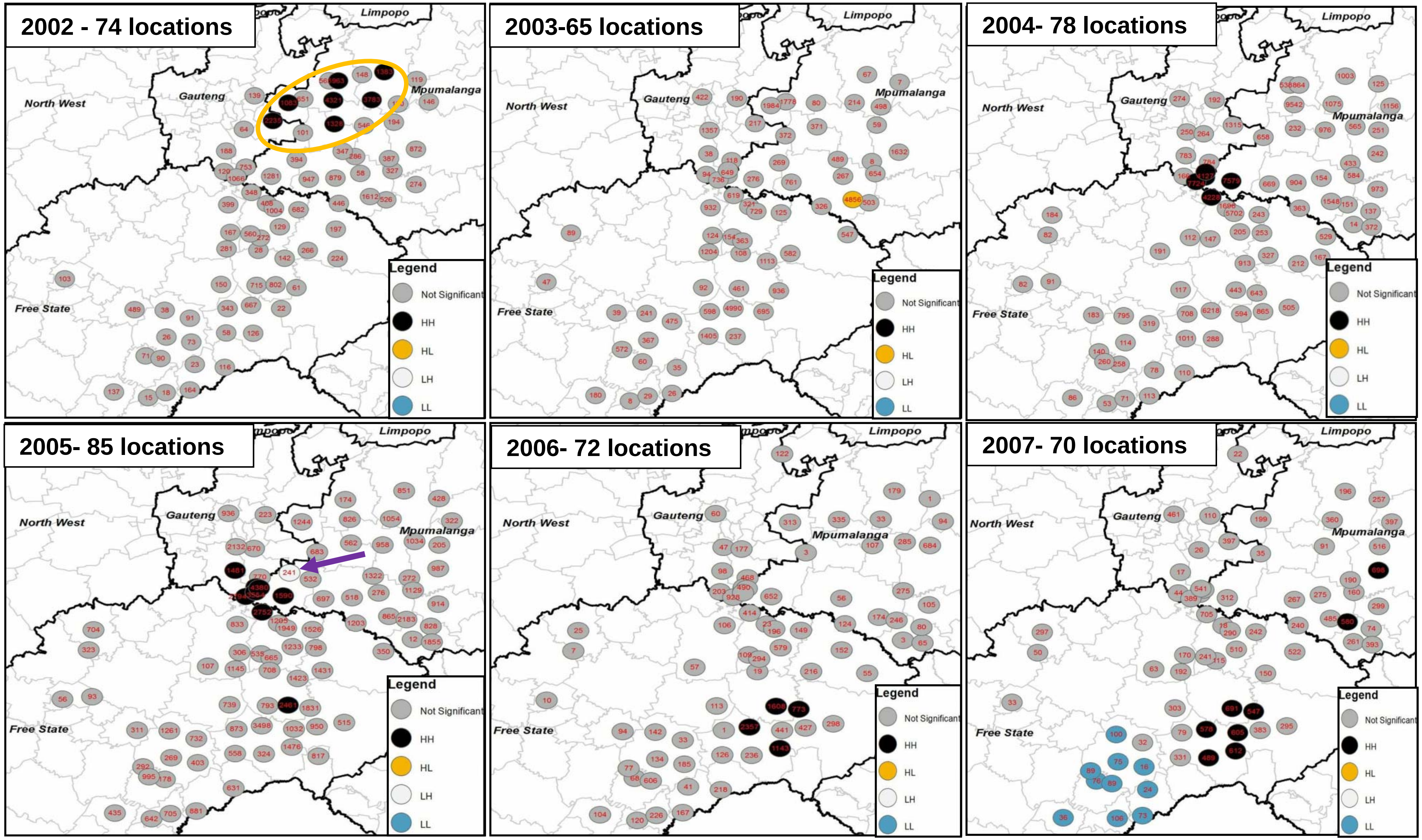


Figure 2.7. Black maize beetle cluster and outlier analysis.

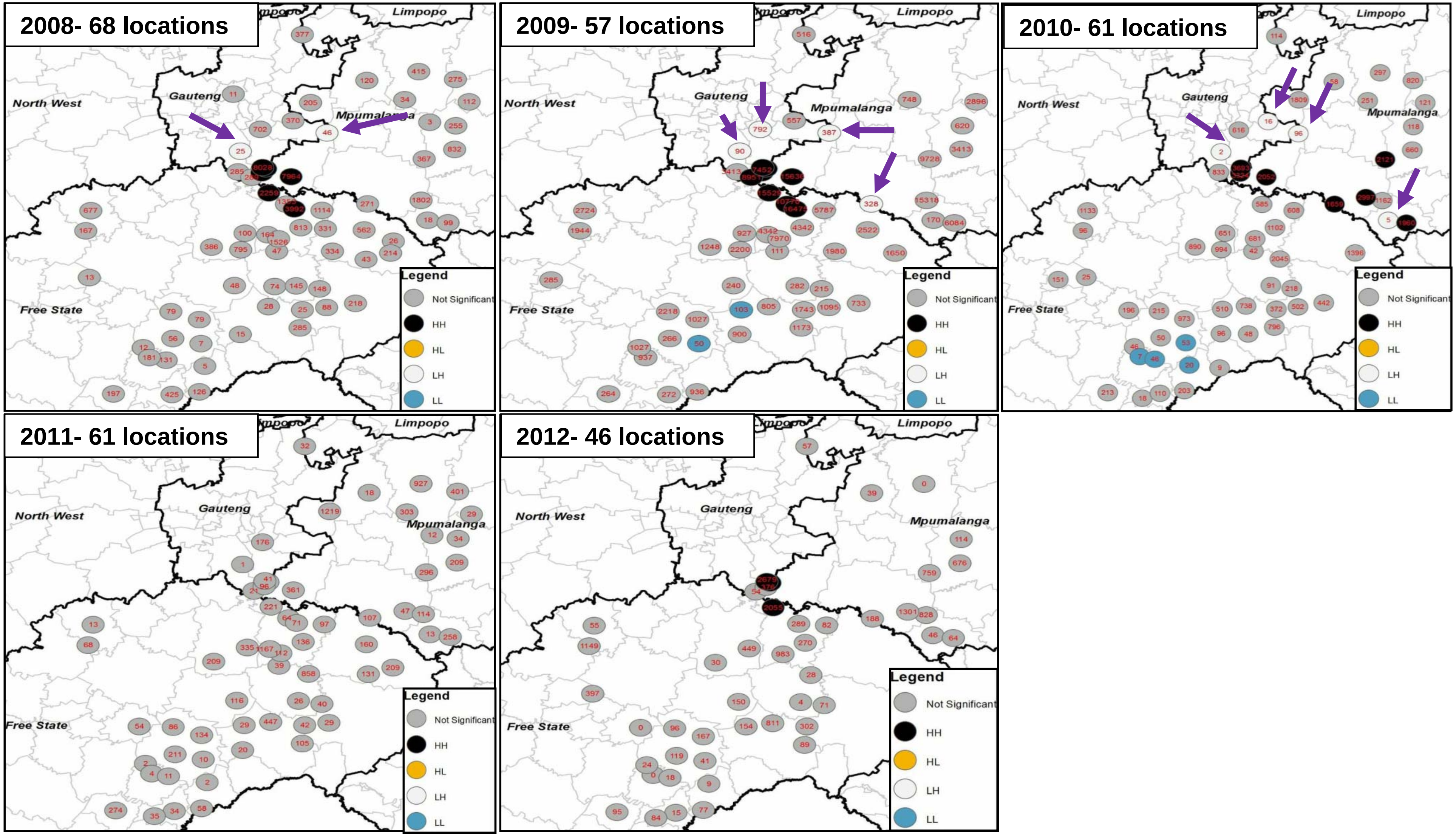


Figure 2.8. Black maize beetle cluster and outlier analysis.

2.3.3 Hot spot analysis

Null hypothesis states that black maize beetle populations are randomly distributed across the study area. Hot spot analysis was used to identify locations where statistical significant groupings of high and low black maize beetle numbers occur within the monitored area of South Africa.

This tool (Hot spot analysis) identifies statistically significant spatial clusters of high values (hot spots) and low values (cold spots). It creates a new Output Feature Class with a z-score and P-value for each feature in the Input Feature Class. It also returns the z-score and P-value field names as derived output values for potential use in custom models and scripts.

The z-scores and P-values are measures of statistical significance which tell whether or not to reject the null hypothesis, feature by feature. In effect, they indicate whether the observed spatial clustering of high or low values is more pronounced than one would expect in a random distribution of those same values.

A high z-score and small P-value for a feature indicates a spatial clustering of high values. A low negative z-score and small P-value indicates a spatial clustering of low values. The higher (or lower) the z-score, the more intense the clustering. A z-score near zero indicates no apparent spatial clustering. The z-score is based on the randomization null hypothesis computation.

Hotspot analyses (Figures 2.9 and 2.10) of black maize beetle numbers were found across the monitored area with varying intensities ranging from hotspots (highly significant black maize beetle clustering of high numbers – red circles) to cold spots (highly significant clustering of black maize beetle clustering of low numbers – blue circles). Not only did these hot- and cold spots vary within the monitored area, but also over the 11 year period (2002-2012). No significant clustering was found during 2003 and 2011 as illustrated in Figures 2.7 and 2.8.

Black maize beetle hotspots occurred mostly on the border of Free State, Mpumalanga and Gauteng provinces over the 11 year period, except for 2007. Cold spots only occurred in Free State province during 2007, 2009 and 2010 (Figure 2.9

and 2.10 (blue circles)). During 2002 and 2004 black maize beetle hotspots also occurred in the north-eastern part of Mpumalanga province (Figure 2.9). In central Mpumalanga less intense black maize beetle clustering (orange circles) was found during 2007. Hotspots only occurred during 2005, 2006 and 2007 in the southern part of the Free State province (Figure 2.9).

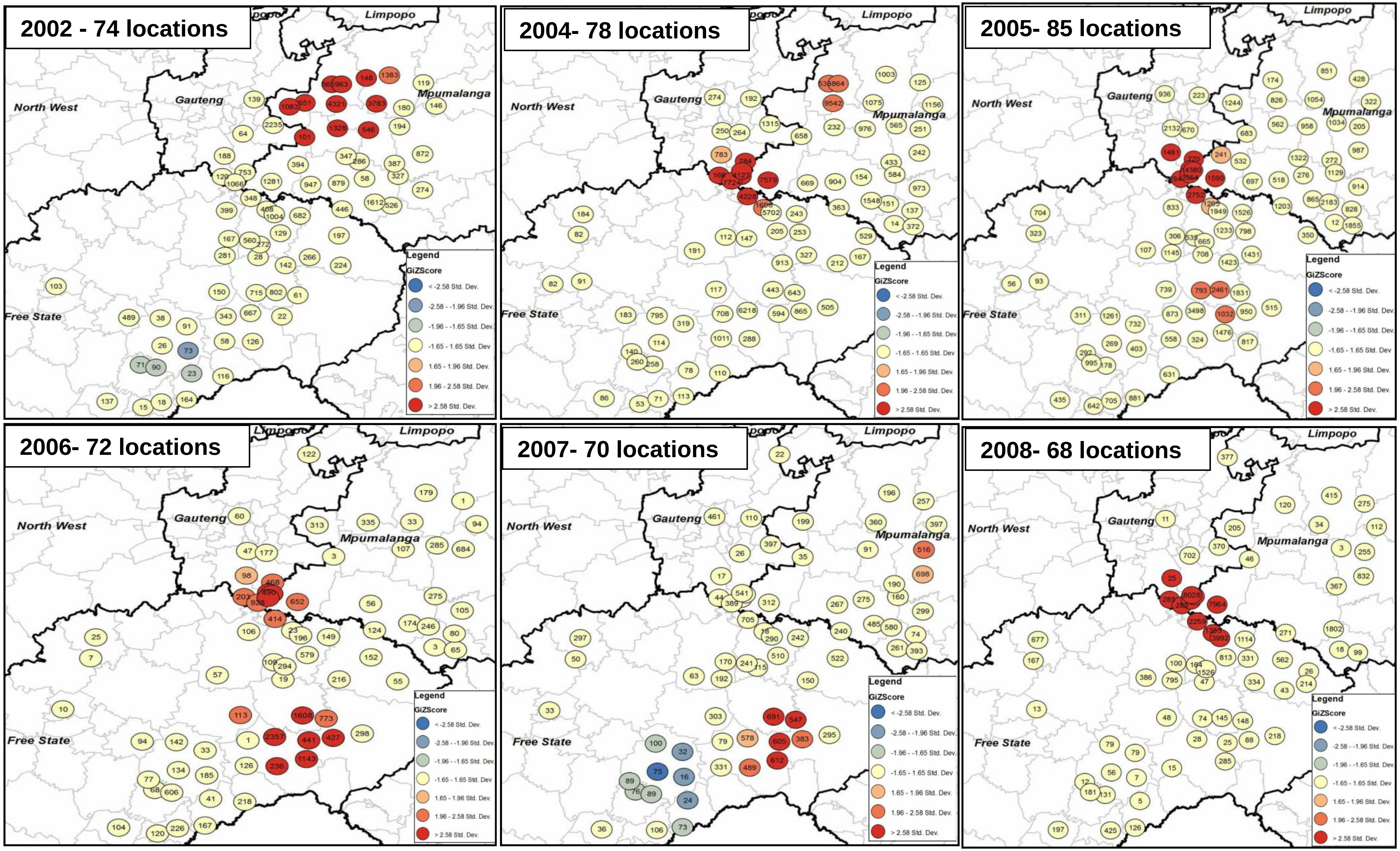


Figure 2.9. Black maize beetle hotspot analyses.

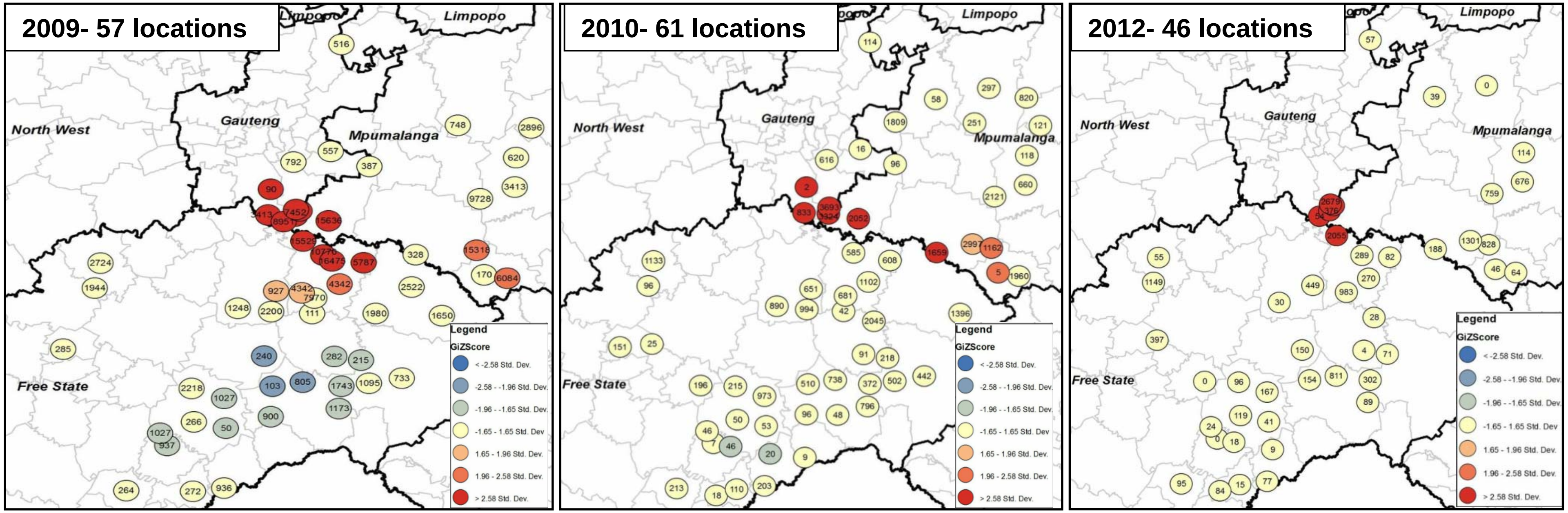


Figure 2.10. Black maize beetle hotspot analyses.

2.4 Discussion

More than 11 years' black maize beetle data indicated highly significant spatial clustering across the study area, not only between locations but also over years. Hotspots and cold spots tended to recur in the same area with minimal variation except for the hotspot found on the border of the three provinces which differed in intensity (Figure 2.9 and 2.10).

This indicates that preventative measures for black maize beetle throughout the monitored area could be drastically reduced, even for localities found on the border of the Free State, Gauteng and Mpumalanga provinces. However, care should be taken when outbreaks do occur (Figure 2.9 and 2.10). Hotspots occur within a small spatial range (approximately 90km x 60km). After a five year period from 2004 to 2009, the average seasonal black maize beetle population doubled in size (5307 to 12186) but only on the border of Gauteng, Free State and Mpumalanga provinces (Figure 2.3).

When above average black maize beetle flights occur during the onset of warmer temperatures (spring months) and maize mono-cropping practices are practiced black maize beetles may be more prone to increase in pest status (Taylor, 1951). Implementing crop rotation practices by means of non-host crops, such as cowpea and sunflower, black maize beetle populations may be reduced significantly. Another effective management tactic through cultivation practices is ploughing during September and October when the most vulnerable pupal stage of the black maize beetle occurs. However, timing and frequent tillage operations are essential for optimal results.

Biological control through the use of GMO (genetically modified organisms) maize, commonly known as Bt (*Bacillus thuringiensis*) maize, is another more effective and efficient alternative than cultural control, especially in the areas where maize mono-cropping practices are practiced. This method is species specific and can therefore be planted especially when beetle numbers rise. Using transgenic maize (when available in the future) is only necessary within the identified hotspot (border of Mpumalanga, Free State and Gauteng province) and only during certain years (five year intervals). This ultimately will lead to significant saving of unnecessary input costs when this trait is released for commercial use and is specifically effective against the adult stage. However, transgenic control is effective only during the early developmental stages of the insect, it is doubtful if GMO crops will control *H. arator* where the adults are the plant feeders.

Current chemical control options for the control of black maize beetle populations in South African maize (Table 2.1) are all preventative methods. This allows for unnecessary use of chemicals during planting, especially when insect numbers are low. Results obtained in this study indicated that preventative chemical control measures can only be used in areas where Mpumalanga, Free State and Gauteng provinces border. This will ultimately lead to a substantial saving in input costs. The area only needs to apply preventative chemicals against black maize beetle populations at five year intervals.

Larval stages of black maize beetle populations can be controlled naturally by abnormal wet or dry weather conditions (Du Toit, 1998). Water logging during wet conditions can also reduce the number of eggs and L1 populations significantly (Du Toit, 1998). By controlling the identified hotspot area by means of flooding, through the use of pivot systems, during autumn months when oviposition is taking place, black maize beetle populations can be reduced significantly. This procedure is, however, very costly but it can be restricted to 5 year intervals. Further study is needed on this matter.

Natural hosts favoured by *H. arator* include paspalum (*Paspalum dilatatum*) and ryegrass (*Lolium* spp.) (Todd, 1964) and both these species act as reservoirs for ovipositional adults. Black maize beetles' dispersal ability during autumn and spring plays a substantial role in infestation of maize fields, especially during epidemic years (Bell *et al.*, 2011). Natural hosts surrounding maize fields within the hotspot area may explain the sudden decreases/increases in beetle abundance as found in this study.

Organic matter in soil serves as feeding stimulant for young black maize beetle larvae (Bell *et al.*, 2011). Current agricultural practices pursue no-till practices. No-till practices alter soil composition and structure resulting in higher organic content and soil moisture (Harrison *et al.,* 1990; Clapperton & Ryan, 2001). This alteration in soil composition and structure may be more favourable for the development of black maize beetle populations, which in turn may lead to more concentrated and intensified outbreaks within an area. It may also lead to the expansion of currently known black maize beetle hotspots. Further investigation is recommended to monitor potential future black maize beetle outbreaks.

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CHAPTER 3:

Predicting black maize beetle population levels

3.1 Introduction

*Heteronychus arato*r Fabricius (Coleoptera: Scarabaeidae) commonly known as the black maize beetle is the most important coleopteran pest that attack the subterranean part of maize (*Zea mays*) (Cyperales: Poaceae) in South Africa (Taylor, 1951). The sporadic nature of the black maize beetle requires the development of a prediction model. This can be done by understanding their distribution patterns, prior occurrences, annual flight pattern as well as the key factors needed for their survival.

3.1.1 Distribution and occurrences of black maize beetle populations in South Africa

Black maize beetle (*H. arator)* occur throughout South Africa, but is more concentrated in the eastern Highveld, north-eastern and eastern Free State, northern and central Kwa-Zulu Natal, as well as in the Eastern and Western Cape provinces of South Africa, where it is a serious pest of maize (Harington, 1964; Drinkwater, 1979; Du Toit, 1998).

According to Ormerod (1889) an epidemic outbreak of *H. arator* occurred in wheat crops, during 1881 through to 1885, in the eastern parts of South Africa. During the early summer of 1946 another wave of outbreaks occurred in newly planted maize fields in the Frankfort district which is situated on the border of Free State, Mpumalanga and Gauteng provinces of South Africa (Taylor, 1951).

After a period of approximately 32 years (from 1946 to 1977) this species reached another epidemic status, again in the Frankfort district, attacking maize (Drinkwater, 1982). This attack surged for five successive planting seasons until 1982 (Drinkwater, 1987). Another attack was expected to occur from 2009 to 2014, based on this 32 year cycle theory. In this study spatial and temporal analysis of black maize beetle populations over 11 years (Chapter 2) indicated populations to recur in the same area.

3.1.2 Annual flight pattern of black maize beetles

Adults of black maize beetles are nocturnal and sporadic in nature (Taylor, 1951; Du Toit, 1998). The first adults become active during autumn months, only after significant rainfall, onset of warmer soil temperatures (>17°C) and when calm wind conditions are present (Du Toit, 1998; Bell *et al.*, 2011). Flights during spring tend to be smaller in numbers and are restricted to less than four evenings per season (Bell *et al.*, 2011). During black maize beetle epidemic years these flights only increase in numbers and not in frequency (Bell *et al.*, 2011).

Two main flight peaks of beetles (Figure 3.1) are found during a year (Taylor, 1951). The 1st peak occurs early in the summer, October to December. This peak is most damaging to maize seedlings (Taylor, 1951). This peak is derived from the overwintering larvae which are the offspring of the later-emerging beetles of the first generation and adults of the second generation (Taylor, 1951). This flight comprises approximately 5% of the annual flight (Du Toit, 1998). The 2nd wave occurs during February to April and is the offspring of the early-emerging beetles of the first generation and will continue to occur until May depending on temperature (Taylor, 1951). According to Du Toit (1998) this peak comprises approximately 95% of the total annual flight.

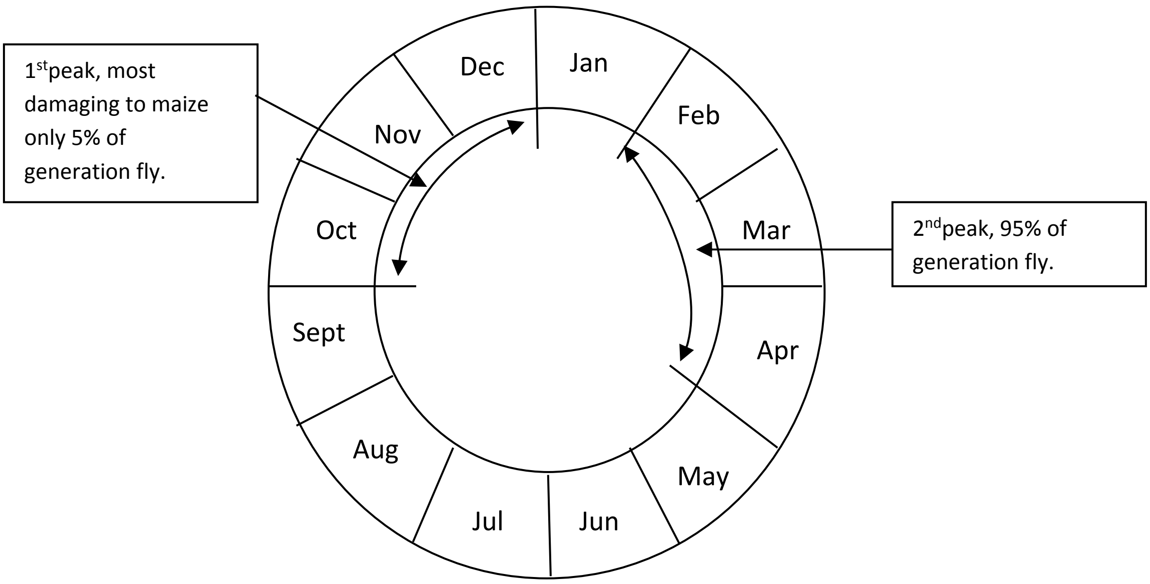


Figure 3.1. Main flight peaks of the black maize beetle population (Taylor, 1951; Du Toit, 1998).

3.1.3 Key factors for the survival of black maize beetle populations

The black maize beetle favours a grey sandy soil, which is rich in humus. This pest was never found in black, peaty soils (Taylor, 1951). At soil temperatures lower than 15°C larvae of the family Scarabaeidae cease to feed (Glogoza *et al.,* 1998). Matthiessen and Ridsdill-Smith (1991) found that the development and survival of *H. arator* is most favourable at temperatures above 15°C, with an optimal temperature range between 20-25°C in Australia. If spring-summer temperatures were above average the mortalities in the early instar larvae were less which resulted in outbreaks (Matthiessen and Ridsdill-Smith, 1991). Older larval and adult mortalities still need to be investigated (Matthiessen and Ridsdill-Smith, 1991). However, these studies have not been done in South Africa.

In Northern Cauca, Colombia current agricultural practices and environmental conditions intensify the seasonality of Scarabaeidae (Pardo-Larcarno *et al.,* 2005). The implementation of no-till practices results in more available soil moisture, especially under drought conditions, when compared to conventional tillage practices in maize (Harrison *et al.,* 1980). All stages of Scarabaeidae are dependent on moisture (Taylor, 1951; Harington, 1953; Walker, 1968), temperature (Taylor, 1951; Du Toit, 1998; Glogoza *et al.,* 1998) and humus content of the soil (Taylor, 1951; Walker, 1968) and irrigated fields with no-till practices may possibly be more prone to intensified attacks by members of the family Scarabaeidae, such as *H. arator*.

In South Africa black maize beetle populations have been recurring in the same area since 1885, with varying intensities over time resulting in epidemic outbreaks during certain years. By calculating the time elapsed during documented epidemic outbreaks of the black maize beetle populations, the long term major trend appears to be an approximate 32 year cycle. The last documented epidemic outbreak occurred during 1977 in the same area (Frankfort district) as previously documented findings. The Frankfort district of South Africa is located on the border of the Free State, Mpumalanga and Gauteng provinces. By adding 32 years from the previous documented finding during 1977 (Drinkwater, 1982) an epidemic outbreak was expected to recur during 2009 in the same area.

Due to the sporadic nature of *H. arator* outbreaks and the fact that only preventative chemical control measures currently exist in South Africa, a need arose for predicting black maize beetle population levels. Since all stages of black maize beetle populations are dependent on moisture, temperature and organic material in the soil and the fact that black maize beetle populations recur in the same area over time (Chapter 2), the development of a prediction model for this pest species seemed to be possible. Since spatial and temporal variation of black maize beetle populations recur in the same area over time (Chapter 2), various weather variables such as evapotranspiration, maximum and minimum temperature, maximum and minimum humidity, solar radiation, rainfall and wind speed were used to explain this variation.

All weather variables described above impact either directly or indirectly on soil moisture and soil temperature which are expected to be key factors for the survival of all stages on black maize beetles. Because of this a prediction model using weather data was developed.

Currently, no black maize beetle prediction model exists anywhere in the world. Such a method would enable farmers to reduce input costs substantially by either controlling maize seed chemically when black maize beetle populations are expected to reach epidemic outbreaks or to plant a non-host crop, such as a legume, during this time. Similarly, if low numbers of beetles can be predicted, the use of predictive chemical control measures can be eliminated, with a consequent saving in input costs.

3.2 Material and methods

3.2.1 Sites

The eastern part of the maize triangle of South Africa (Figure 3.2a) was divided into quarter degree by quarter degree blocks (approximately 30km x 30km) (Figure 3.2b). Within this block a maize farm (purple circle) was selected as close as possible to the midpoint to ensure even distribution throughout the monitored area. A total of 99 modified Robinson light traps were installed within the selected area. Active weather stations (blue circles) were identified within the monitored area (Figure 3.2b) and monthly weather data were collected from these stations. Map and block numbers were allocated to each producer within the monitored area (Figure 3.2b).

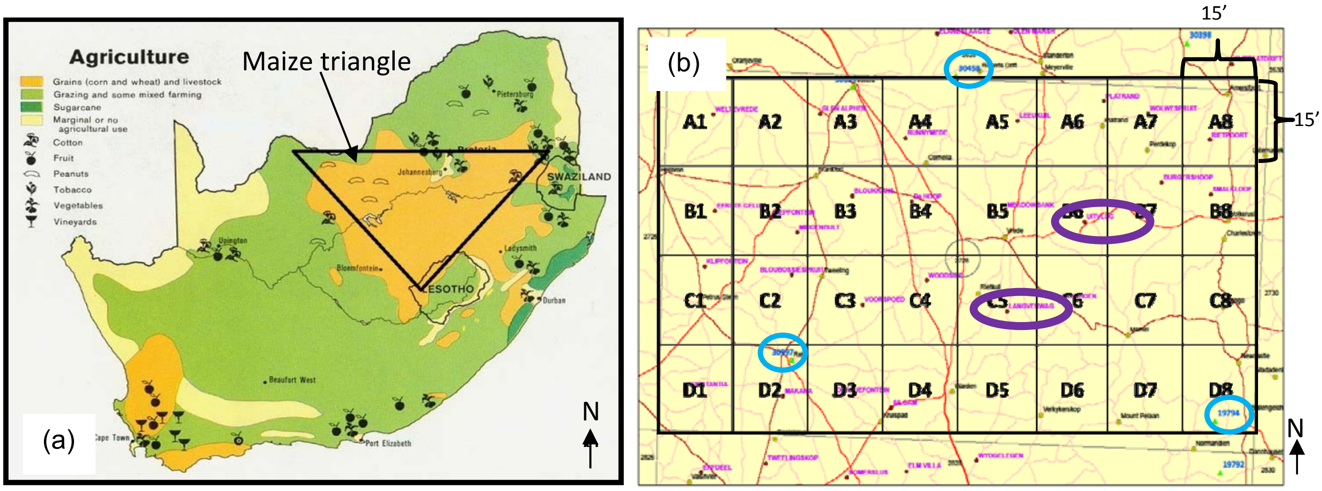


Figure 3.2(a). Maize triangle of South Africa and (b) sampling sites within the map of Frankfort district of South Africa.

3.2.2 Data collection and analysis

Black maize beetles are nocturnal and were captured by means of a modified Robinson light trap (Common, 1959) on a weekly basis from the first week of February to the end of April (13 weeks) for 11 consecutive years (2002-2012). Weekly collected insect captures, by the producers, were emptied into another 2 litre container where they were preserved in a 70% alcohol solution that was filled to the level of the capture. The 2 litre containers with the preserved insects were clearly marked with the date of capture and locality. After the three month collection period, new stock was delivered to each site. This was intended for the following years' capture. After all the sites had been collected the numbers of black maize beetles for each week at each site were recorded.

Weather data were obtained from SA (South African) Weather Services as well as from ARC-ISCW (Agricultural Research Council – Institute for Soil, Climate and Water) (AgroClimatology Staff, 2012). Average monthly data (January 2000 to December 2012) of various weather variables including minimum humidity (MinHum), maximum humidity (MaxHum), minimum temperature (MinTemp), maximum temperature (MaxTemp), rainfall (Rain), solar radiation (Rad), evapotranspiration (Evap) and wind speed (Wind) were obtained.

3.2.3 Buffer zones

Active weather stations within the monitored area were limited and therefore buffer zones (Figure 3.3) surrounding the active weather stations were divided into 10km, 20km, 30km, 40km and 50km areas respectively. Seasonal light trap captures within a 10km and 50 km radius to a weather station, were subjected to forward regression analysis on the independent variables. For prediction of black maize beetle numbers, the multiple linear regression method of forward regression analysis was used. The total black maize beetle capture of the previous season (TotCatchPrev) from 1 February to end April was included as a correctional independent variable since population changes could be expected to be, at least to some extent, determined by the size of the preceding seasonal numbers.

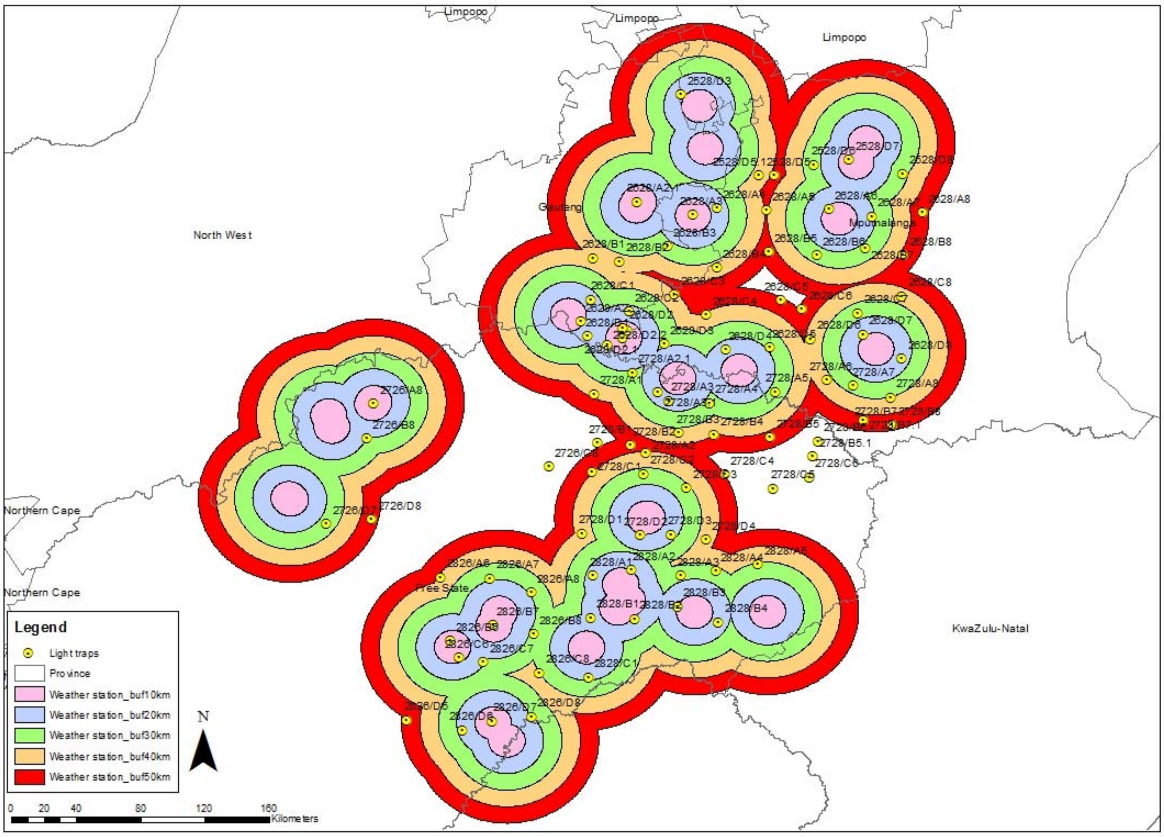


Figure 3.3. Buffer zones surrounding active weather stations in the eastern maize producing part of South Africa.

3.2.4 Subjective grouping of light traps

Map and block numbers were allocated to each producer within the monitored area (e.g. Figure 3.2b and Table 3.1). Data were grouped into five representative areas (Table 3.1). These groups are indicated in five different color codes:

Green – Northern Mpumalanga province,

Dark blue - Eastern Free State province,

Light blue – Southern Free State province,

Purple – Western Free State province and

Red – Border of Mpumalanga, Free State and Gauteng provinces (Figure 3.4, and Table 3.1).

Black – Rest.

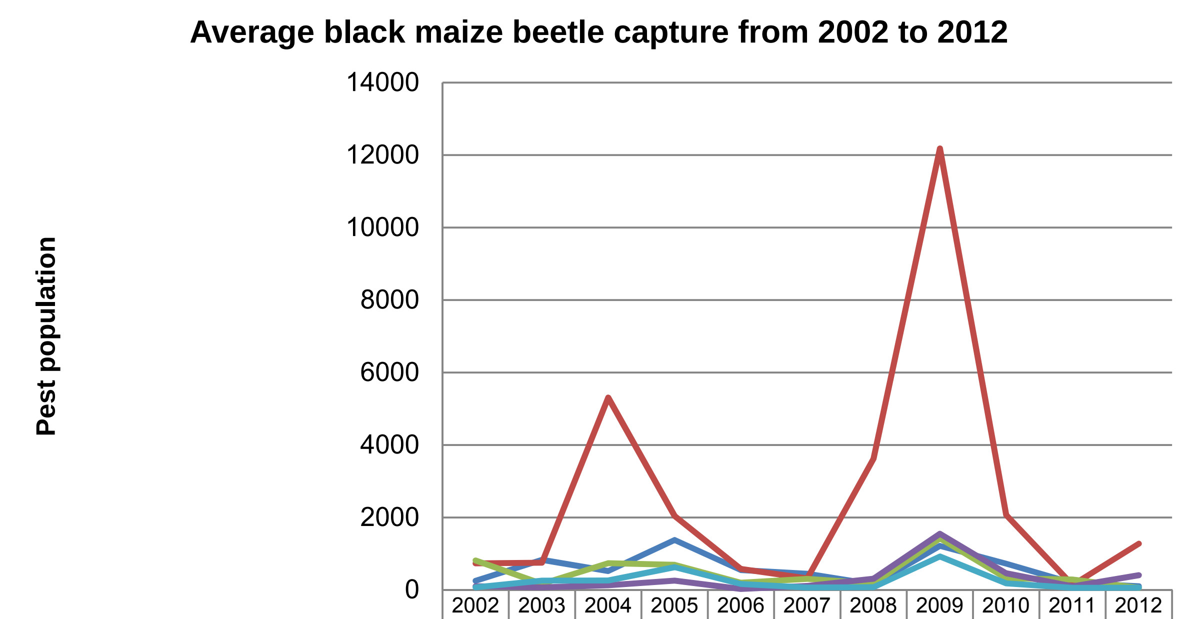
Table 3.1. Block number allocation of light traps.

Block number	5	6	7	8	1	2	3	4	5	6	7	8
D							2528	2528	2528	2528	2528	2528
A						2628	2628	2628	2628	2628	2628	2628
B					2628	2628	2628	2628	2628	2628	2628	2628
C					2628	2628	2628	2628	2628	2628	2628	2628
D					2628	2628	2628	2628	2628	2628	2628	2628
A				2726	2728	2728	2728	2728	2728	2728	2728	2728
B				2726	2728	2728	2728	2728	2728	2728	2728	2728
C				2726	2728	2728	2728	2728	2728	2728	2728	2728
D			2726	2726	2728	2728	2728	2728				
A		2826	2826	2826	2828	2828	2828	2828	2828			
B		2826	2826	2826	2828	2828	2828	2828				
C		2826	2826	2826	2828	2828	2828	2828				
D	2826	2826	2826	2826								

3.3 Results

3.3.1 32 year cycle theory

Black maize beetles captured during the 11 year capture period were divided into five distinct groups (Figure 3.4). Only on the border of the Free State, Mpumalanga and Gauteng provinces did the black maize beetle seasonal captures exceed 2000 and this was only during 2004 - 2005 and 2008 – 2010 (Figure 3.4 (green circles)). During 2009, the number of black maize beetles exceeded the 10000 mark which indicated that an epidemic outbreak had occurred. However, this was confined to a small area located on the border of Mpumalanga, Free State and Gauteng provinces. This area is described in literature as the Frankfort district of South Africa.



	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Eastern Free State province	253	832	519	1378	545	445	152	1213	724	191	101
Border of Mpumalanga, Free State and Gauteng provinces	733	750	5307	2041	580	319	3622	12186	2067	138	1275
Northern Mpumalanga province	812	169	736	693	198	303	182	1421	321	284	57
Western Free State province	103	68	126	257	25	111	311	1550	459	97	408
Southern Free State province	72	254	260	626	161	64	75	923	179	58	61

Figure 3.4. Average black maize beetle captures at five representative areas from 2002 to 2012.

3.3.2 Prediction model

Data were acceptably normal distributed, although the kurtosis was somewhat high. A 10% significance level was used for variables included in the prediction model.

Two regression models were proposed. The first prediction model included light traps that surrounded an active weather station within a 10km buffer zone. The second included light traps within a 50km buffer zone (radius) around an active weather station.

The following model was applied:

$$y = a + \sum_{i=1}^{p} b_i x_i$$

Where:

y = predicted black maize beetle numbers

a = intercept

x_i= weather variables and previous year's beetle capture

b_i = coefficient of the weather variables

p = number of x variables

Regression analysis indicated that a highly significant prediction model (P<.0001) was identified with a R^2 of 62.93% (Table 3.2) at a level of 10%.

Table 3.2. Forward regression analysis for prediction of black maize beetles within a 10km buffer zone.

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	15	908101921	60540128	16.18	<.0001
Error	143	534944225	3740869		
Corrected Total	158	1443046146			
Root MSE	1934.13	R-Square	0.6293		
Dependent Mean	1783.61	Adj R-Sq	0.5904		

Average solar radiation in July played the highest role (20.02%) in predicting beetle numbers for the following year (1 February until end of April) (Table 3.3). Average minimum temperature contributed another 12.23% to the prediction model. Total black maize beetles caught during the previous year in predicting black maize beetle flight the following year contributed 3.19% to the prediction model. Fifteen different variables were identified to contribute to the prediction of black maize beetles for the following year. These variables were able to predict black maize beetles to an accuracy of 62.93%. Numbers found in the variable entered column indicate which month was used for the prediction model e.g. Rad7 = Average solar radiation of July.

The sum of average solar radiation within the prediction model accounted for 26.99% of the 62.93% of the prediction model. The second highest contributing factor was the sum of average minimum temperature which accounted for 15.96% while the lowest contributing factor was average evapotranspiration for September (0.89%).

Table 3.3. Summary of forward regression model for predicting seasonal black maize beetle numbers within a 10km buffer zone.

Step	Variable Entered	Number Variables Included	Partial R-Square	Model R-Square	F Value	Pr> F
1	TotCatchPrev	1	0.0319	0.3928	8.1	0.005
2	Rad7	2	0.2002	0.2002	39.3	<.0001
3	MinTemp4	3	0.1223	0.3225	28.15	<.0001
4	Rad9	4	0.0384	0.3609	9.32	0.0027
5	Rain6	5	0.0309	0.4237	8.19	0.0048
6	MinHum9	6	0.0284	0.4521	7.89	0.0056
7	MinHum7	7	0.0259	0.478	7.48	0.007
8	MinTemp12	8	0.0239	0.5019	7.19	0.0082
9	MaxTemp8	9	0.0226	0.5244	7.08	0.0087
10	Rain2	10	0.0331	0.5575	11.06	0.0011
11	Rad11	11	0.0192	0.5767	6.65	0.0109
12	Wind1	12	0.0182	0.5949	6.57	0.0114
13	Rad3	13	0.0121	0.607	4.47	0.0361
14	MinTemp1	14	0.0134	0.6204	5.07	0.0258
15	Evap9	15	0.0089	0.6293	3.44	0.0658

Parameter estimates (Table 3.4), also known as coefficients, of the various variables used to predict black maize beetle populations were entered into the prediction model below.

$$Y= a+bx_1+cx_2+dx_3+ex_4+fx_5+gx_6+hx_7+ix_8+jx_9+kx_{10}+lx_{11}+mx_{12}+nx_{13}+ox_{14}+px_{15}$$

where:

Beetles predicted (Y) = -2.14 + Evap9(-2120.62) + MaxTemp8(809.24) +MinHum7(111.80) + MinHum9(-227.57) + MinTemp1(537.60) + MinTemp4(279.85) +MinTemp12(-1216.81) + Rad3(228.10) + Rad7(-5.85) + Rad9(111.93) +Rad11(-485.61) + Rain2(15.98) + Rain6(-60.20) + Wind1(1910.53) + TotCatchPrev(0.37).

Table 3.4. Coefficients of various variables used in predicting black maize beetle populations within a 10km buffer zone.

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr> |t|
Intercept	1	-2.14	5674.12	0.00	0.9997
Evap9	1	-2120.62	1143.68	-1.85	0.0658
MaxTemp8	1	890.24	154.83	5.75	<.0001
MinHum7	1	111.80	63.11	1.77	0.0786
MinHum9	1	-227.57	74.43	-3.06	0.0027
MinTemp1	1	537.60	240.41	2.24	0.0269
MinTemp4	1	279.85	128.43	2.18	0.0310
MinTemp12	1	-1216.81	237.55	-5.12	<.0001
Rad3	1	228.10	91.80	2.48	0.0141
Rad7	1	-5.85	211.67	-0.03	0.9780
Rad9	1	111.93	208.51	0.54	0.5922
Rad11	1	-485.61	104.21	-4.66	<.0001
Rain2	1	15.98	6.91	2.31	0.0222
Rain6	1	-60.20	15.54	-3.87	0.0002
Wind1	1	1910.53	520.28	3.67	0.0003
TotCatchPrev	1	0.37	0.07	5.65	<.0001

Lower and upper confidence limits (95%) for each coefficient in the prediction model were calculated (Table 3.5) which can be used to establish the range that the prediction model for black maize beetles can be effective. By entering all lower confidence limits at a level of 95%, together with the average variables across all years (2002 - 2012), will result in the lower limit predicted for beetle numbers. Similarly, by entering the upper limit values in the prediction model will provide the upper limit for prediction.

Table 3.5. Upper and lower confidence limits at 95% of the various coefficients used in the prediction model of black maize beetles.

Variable	**DF**	**Standardized Estimate**	**95% Confidence limits**	
			Lower	**Upper**
Intercept	1	0.00	-11218.00	11214.00
Evap9	1	-0.27	-4381.32	140.08
MaxTemp8	1	0.48	584.19	1196.30
MinHum7	1	0.12	-12.94	236.55
MinHum9	1	-0.30	-374.70	-80.45
MinTemp1	1	0.20	62.38	1012.82
MinTemp4	1	0.18	25.98	533.72
MinTemp12	1	-0.40	-1686.37	-747.25
Rad3	1	0.21	46.64	409.57
Rad7	1	0.00	-424.26	412.55
Rad9	1	0.08	-300.23	524.09
Rad11	1	-0.39	-691.60	-279.61
Rain2	1	0.21	2.32	29.64
Rain6	1	-0.27	-90.93	-29.48
Wind1	1	0.28	882.09	2938.97
TotCatchPrev	1	0.34	0.24	0.51

Using a 50km buffer zone prediction of black maize beetles, indicated that a less accurate prediction model was obtained providing prediction efficacy of 43.76% (Table 3.6) compared to the 62.93% (Table 3.3) for the model above. A total of 26 variables played a role towards the prediction of black maize beetles within a 50km buffer zone. Total beetle captures of the previous year played the highest role (11.13%) in this prediction model.

Table 3.6. Summary of forward regression model for predicting seasonal black maize beetle numbers within a 50km buffer zone.

Step	Variable Entered	Number Variables Included	Partial R-Square	Model R-Square	C(p)	F Value	Pr> F
1	TotCatchPrev	1	0.1113	0.1113	894.62	172.64	<.0001
2	Rain12	2	0.0674	0.1787	724.24	113.16	<.0001
3	Rad11	3	0.0433	0.222	615.69	76.55	<.0001
4	MinTemp4	4	0.0408	0.2627	513.43	76.13	<.0001
5	Rad5	5	0.0364	0.2992	422.33	71.46	<.0001
6	Rad12	6	0.018	0.3171	378.42	36.14	<.0001
7	MaxTemp8	7	0.0103	0.3274	354.21	20.93	<.0001
8	MinTemp12	8	0.0129	0.3403	323.19	26.87	<.0001
9	Rain2	9	0.0229	0.3632	266.76	49.21	<.0001
10	Rain11	10	0.0068	0.37	251.4	14.77	0.0001
11	Rain6	11	0.0085	0.3784	231.73	18.67	<.0001
12	Rad3	12	0.0072	0.3856	215.33	16.03	<.0001
13	Rain7	13	0.0085	0.3941	195.7	19.09	<.0001
14	Wind2	14	0.0065	0.4006	181.2	14.71	0.0001
15	Rain4	15	0.0062	0.4067	167.44	14.19	0.0002
16	Rain8	16	0.0032	0.4099	161.26	7.39	0.0066
17	Wind7	17	0.0037	0.4136	153.77	8.63	0.0034
18	Wind8	18	0.0031	0.4168	147.74	7.34	0.0068
19	MaxTemp4	19	0.0024	0.4192	143.59	5.63	0.0178
20	MinHum8	20	0.0021	0.4213	140.19	4.96	0.0261
21	MinHum11	21	0.003	0.4243	134.46	7.14	0.0076
22	MinTemp1	22	0.0036	0.4279	127.25	8.55	0.0035
23	MaxTemp3	23	0.0031	0.431	121.44	7.28	0.0071
24	MaxHum5	24	0.0025	0.4334	117.16	5.88	0.0155
25	Evap1	25	0.0026	0.436	112.63	6.14	0.0133
26	Wind9	26	0.0016	0.4376	110.55	3.84	0.0502

3.4 Discussion

According to theory black maize beetles are expected to reach epidemic outbreak levels in 32 year cycles. According to this theory another outbreak was expected to occur during 2009. Results indicated that an outbreak occurred during 2009 but was confined to a very small area which was previously described in literature as the Frankfort district of South Africa. This area amounts to 540 000ha (60km x 90km). The next expected black maize beetle outbreak is expected to occur during 2041.

A prediction model for black maize beetle populations was identified using a forward regression model at a 10% significance level. Average monthly weather variables, as well as total black maize beetles captured (1 February to end of April) in the previous year, predicted 62.93% of the black maize beetle flight for the following year from 1 February to end of April when light traps were situated within 10km of an active weather station.

The number of black maize beetles which is known to damage maize during October to December (main cropping season for maize in South Africa) can be calculated because it is described in literature as 5% of the population. Threshold values for beetle damage to a maize crop are still to be investigated. Through the use of light traps and scouting techniques within black maize beetle damaged maize fields, within the same area, a threshold value can be determined for both light trap captures and maize damage caused by black maize beetles. This can then be indicative whether a comparison can be made to the number of black maize beetles captured in light traps and the consequent damage to maize. This still needs further investigation.

The total number of black maize beetles predicted within a 50km buffer zone was less effective than when light traps were within the vicinity 10km of an active weather station. This indicated that the nearer a light trap to an active weather station the more accurate the prediction model would be.

Key factors for the survival of black maize beetles include humus content in the soil, minimum soil temperature and soil moisture (Chapter 4). The prediction model developed for black maize beetles indicated that average solar radiation contributed to 26.99% of the total prediction model while minimum temperature contributed to another 15.96%. Solar radiation is energy emitted from the sun, usually in the form of heat. Thus both variables are related to heat and contributed in total to 42.95% of the 62.93% that is predicted by the model. Therefore minimum temperature has a large impact on the survival of black maize beetle populations, confirming one of the key factors as described in the literature.

All weather variables used were measured above the soil surface. Above surface weather variables either directly or indirectly impact on subsurface variables, such as soil moisture and soil temperature. These subsurface variables are key factors to the survival of black maize beetle populations and as such subsurface variables may prove to be a better source for predicting black maize beetle populations. This still needs to be investigated.

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CHAPTER 4:

Ecological factors affecting *Heteronychus arator*

4.1 Introduction

Larval survival presumably impacts directly on the black maize beetle population growth. Since all stages of the black maize beetle is assumed to be dependent on soil moisture, temperature and humus (organic matter) content of the soil; studies on the larval development may shed light on survival of black maize beetle populations.

Three larval stages (L1-L3), commonly known as white grubs, occur within the Scarabaeidae family and *H. arator* larvae feed predominantly on decomposing organic matter in the soil (Du Toit, 1998). Soil moisture is a key factor for emerging beetles (Du Toit, 1998). If soil is moist enough, beetles eclose and then move directly to the soil surface, but if the soil is dry the adult stays in the pupal chamber until the soil conditions improve (Du Toit, 1998).

H. arator overwinters in the soil as larvae and/or adults during winter months (Drinkwater, 1979). Swarming of beetles occurs at dusk, one or two days after the occurrence of rain, usually during spring and late-summer to autumn (Du Toit, 1998). Beetles are strong flyers, but the distance of flight as well as preference for particular habitats are unknown (Du Toit, 1998). Adults are nocturnal and are attracted to artificial light just after emerging from the soil, but are predominantly found in the soil near the soil surface (Du Toit, 1998).

Adults spend most of the time underground where they feed on the subterranean parts of maize plants (Todd, 1964). Adults are not adversely affected by heavy rain and can even tolerate long periods of submersion in water (Todd, 1964). However, rainfall does affect the duration of the life cycle of the pest (Harington, 1953).

Larvae are able to move through the soil and are found throughout the field, some are found near the moist root systems of maize and sweet grass (*Panicum laevifolium)* but it has not been established if they feed on the latter plants (Taylor, 1951). Natural food seems to be humus (organic matter) within the soil (Taylor, 1951). However, the percentage organic matter needed for larval development is also not clear.

Larvae are more concentrated around maize root systems during the drier months of January and February (Taylor, 1951) where development seems to be dependent on moisture, since in South Africa they develop faster during the rainy summer months (Taylor, 1951).

White grubs in the Philippines show a common clustered distribution and infestations tend to recur annually in the same field (Litsinger *et al.*, 1983). The following factors seems to contribute to the patchy distribution of white grubs: (1) larval tolerance to the narrow range of soil texture and soil moisture conditions, (2) selection of oviposition sites as affected by differences in plant cover, (3) prevalence of favourable larval plant hosts and (4) proximity to adult "flight" trees (Litsinger *et al.,* 1983). Litsinger *et al.* (1983) also state that white grub larvae are confined to light-textured soil types which offer a moisture gradient in the soil horizon. Heavier soil types can lead to high larval mortality during heavy rains (Litsinger *et al.*, 1983).

In South Africa black maize beetle larvae favour a grey sandy soil, which is rich in humus (organic matter) (Taylor, 1951). This pest has never been found in black, peaty soils (Taylor, 1951). Five different topsoil horizons exist in South Africa (Soil Classification Group, 2009). However, the majority of topsoil found in South Africa belongs to the Orthic A horizon. Orthic topsoil varies widely in organic carbon content, colour, texture, structure, base status and mineral composition (Soil Classification Group, 2009). For example, the orthic topsoil of Hutton and Avalon differ in their physical and chemical properties, as well as in moisture regimes. Hutton has a reddish colour that is weakly structured and is free from water logging. Avalon is yellow or brown in colour and is more easily formed in sandy soils. The average moisture status is higher especially where the soil is shallow, thus water logging can occur (Soil Classification Group, 2009).

Current agricultural practices and environmental conditions intensify the seasonality of members of the Scarabaeidae in agro-ecological systems of Northern Cauca, Colombia (Pardo-Larcarno *et al.,* 2005). In the USA it was reported that the implementation of no-till practices results in more available soil moisture, especially under drought conditions, when compared to conventional tillage practices in maize (Harrison *et al.,* 1980). All stages of Scarabaeidae are dependent on moisture (Taylor, 1951; Harington, 1953; Walker, 1968) temperature (Taylor, 1951; Du Toit, 1998; Glogoza *et al.,* 1998) and humus content in the soil (Taylor, 1951; Walker, 1968) and therefore irrigated fields with no-till practices may be more prone to intensified attacks by members of the Scarabaeidae, such as *H. arator*.

The prediction model (Chapter 3) indicated that air humidity and ambient air temperature played a high role towards predicting black maize beetle populations. Spatial and temporal variation (Chapter 2) indicated that black maize beetle populations tend to recur in the same area, but with varying intensities over time. This area is situated on the border of the Free State, Mpumalanga and Gauteng provinces of South Africa. Further study of soil humidity levels in soil (Hutton) collected from that area, as well as sandy soil (Avalon) from another area, may explain the survival of the larval stages of black maize beetles in that area. Organic matter levels seem to be a key factor for larval development and survival but are unknown. Information about organic matter content in different soil forms might explain the reason for the recurrence of black maize beetles in the same area over time. Additionally, this information, as well as information about soil moisture levels needed for larval development, might substantiate or enhance the future prediction of black maize beetle populations. This information can also reduce future monitoring of black maize beetle populations by defining the most suitable soil form with its moisture regime. This information might limit survey areas for future studies on black maize beetle populations.

4.2 Material and methods

4.2.1 Trial design

Three laboratory tests were conducted. A split-split plot as treatment design was done on the dataset, with soil form as main plots and organic matter concentrations or soil moisture levels as sub-plots. The variable (days) were sub-samples of the measurements. There were six replicates for each experimental unit and the trial was monitored at room temperature (±25˚C). Data in all three trials were normally distributed with acceptable homogeneous treatment variances. The treatment means were separated using Fishers' unprotected t-test least significant difference (LSD) and data were tested at the 5% level of significance (Snedecor and Cochran, 1980).

1st trial – Moisture trial

The 1st trial was to determine which soil moisture levels in two major soil types favoured larval development. A Hutton soil form was collected in the known epidemic outbreak area of the Frankfort district and an Avalon soil form was collected in the Vredefort district of South Africa. According to literature black maize beetles were never found in black soils with a high clay percentage. Therefore this soil form was not included in these trials. Both soil forms were sifted and mixed with equal volumes of pre-sifted organic material. Through the use of field capacity, the percentage soil water was calculated for both Avalon and Hutton soil forms.

By determining the dry weight of a soil form and adding water until the maximum water retention capacity of the particular soil form is reached. It is then weighed again and the difference in the maximum weight and minimum weight is then calculated and divided into the intervals that were chosen to be tested (10% intervals).

Water was added on a daily basis in order to maintain the preselected soil moisture levels ranging from 0% to 100% (10% intervals). First instar black maize beetle larvae with a visible darkened hind gut were placed singly in 7cm diameter plant pots filled with organic mixture. Larvae with visible darkened hind guts were selected because they will not easily rupture during handling and sifting procedures. Each of the 11 treatments was replicated six times. Water was added every day at 9h00 to maintain soil moisture levels. Larval mass was determined prior to entry into the different treatments. Thereafter larval mass was recorded twice a week until death of larvae.

2nd trial – Organic matter

The purpose of the 2nd trial was to determine what percentage organic matter mixture was optimal for larval development in each soil form. Equal volumes of pre-sifted organic matter were and added to Avalon as well as Hutton soil. The different organic matter percentages were determined by adding for instance 80% soil and 20% organic matter to a pot volume (100%). Weighted volumes were used to calculate the organic matter content for both soil forms. A 50% soil moisture level was maintained throughout the trial by adding water on a daily basis. First instar larvae with visible darkened hind guts were weighed and placed singly into 7cm diameter planting pots with a depth of 8cm. Larvae were weighed twice a week until death or maturity was reached. Additionally, pupae and beetle weights as well as time to pupation and time to maturity were recorded for both soil forms.

3rd Trial – Pupa and beetle trial

This is an extension of the organic matter trial. It was found that above 80% organic matter content larvae reached maturity. The mass of beetles and pupae found above the 80% organic matter level in both soil forms was recorded. The average time (days) to maturity was also recorded and tested for statistical differences.

4.2.2 Data collection

Black maize beetles are nocturnal and were captured through the use of a modified Robinson light trap (Common, 1959) on a weekly basis from the first week of February to the end of April (13 weeks) at one locality for one season.

Weekly collected insect captures were sorted and approximately 200 black maize beetles were placed into another 10 litre container prefilled with lightly pre-sifted moistened sandy soil. Five whole carrots were submersed into the lightly moistened pre-sifted sandy soil which served as food source. These containers were transported back to the ARC-GCI (Agricultural Research Council – Grain Crops Institute) and kept at room temperature (±25˚C).

Black maize beetle breeding containers were sifted with an ordinary kitchen sieve, twice a week. Carrots were replaced and dead beetles were discarded. Soil was lightly moistened if required. Black maize beetle eggs were harvested, mixed with lightly moistened red Hutton soil (pre-mixed with 50% sifted organic matter) and placed in clear glass poly-top containers, sealed with a plastic lid, at room temperature. Harvested egg containers were clearly marked with quantity and date of harvest.

4.3 Results and discussion

4.3.1 Soil moisture levels

Highly significant differences (Table 4.1) in larval mass were recorded for both soil forms and moisture levels. The interaction between soil forms and moisture levels over time (days) was also highly significant.

Table 4.1. Average larval mass (in g) with different soil forms and at various soil moisture levels over time.

Soil moisture	**Soil form**		
	Hutton	**Avalon**	**Average**
0%	0.0001	0.0001	0.0001
10%	0.0001	0.0001	0.0001
20%	0.0004	0.0001	0.0003
30%	0.0032	0.0002	0.0017
40%	0.3642	0.0006	0.1824
50%	0.1170	0.0023	0.0597
60%	0.1706	0.2210	0.1958
70%	0.1016	0.1285	0.1151
80%	0.1535	0.0031	0.0783
90%	0.0119	0.0005	0.0062
100%	0.0004	0.0001	0.0002
Day 0	0.0042	0.0049	0.0045
Day 7	0.0078	0.0052	0.0065
Day 14	0.0119	0.0058	0.0089
Day 17	0.0128	0.0059	0.0094
Day 21	0.0161	0.0065	0.0113
Day 26	0.0228	0.0065	0.0146
Day 29	0.0411	0.0086	0.0249
Day 32	0.0567	0.0075	0.0321
Day 35	0.0733	0.0109	0.0421
Day 41	0.0926	0.0207	0.0567
Day 44	0.1060	0.0217	0.0639
Day 48	0.1401	0.0291	0.0846
Day 51	0.1599	0.0370	0.0984
Day 54	0.1707	0.0460	0.1083
Day 58	0.1762	0.0532	0.1147

Table 4.1 (continued). Average larval mass (in g) with different soil forms and at various soil moisture levels over time.

Day 61	0.1812	0.0595	0.1204
Day 65	0.1817	0.0642	0.1229
Day 68	0.1851	0.0655	0.1253
Day 73	0.1759	0.0673	0.1216
Day 76	0.1826	0.0662	0.1244
Day 79	0.1572	0.0695	0.1133
Day 83	0.1402	0.0671	0.1037
Day 86	0.1136	0.0649	0.0893
Day 90	0.1020	0.0634	0.0827
Day 93	0.0854	0.0633	0.0744
Day 97	0.0478	0.0676	0.0577
Day 100	0.0467	0.0547	0.0507
Day 104	0.0186	0.0205	0.0195
Day 107	0.0181	0.0071	0.0126
Day 111	0.0157	0.0000	0.0079
Day 114	0.0169	0.0000	0.0084
Day 117	0.0082	0.0000	0.0041
Day 121	0.0000	0.0000	0.0000
Average	0.0839	0.0324	
Source	**F**	**P**	
Soil	1130.4	<.0001	
Rep (Soil)	24.7	<.0001	
Cons	889.62	<.0001	
Soil*Cons	551.64	<.0001	
Rep (Soil*Cons)	15.74	<.0001	
Days	108.94	<.0001	
Soil*Days	31.34	<.0001	
Cons*Days	19.95	<.0001	
Soil*Cons*Days	13.83	<.0001	

Highly significant larval mass differences were found between the two soil forms (Figure 4.1). Average larval mass recorded in Hutton soil (0.0839g) was significantly higher than in Avalon (0.0324g) with a LSD (Least Significant Difference) of 0.017g.

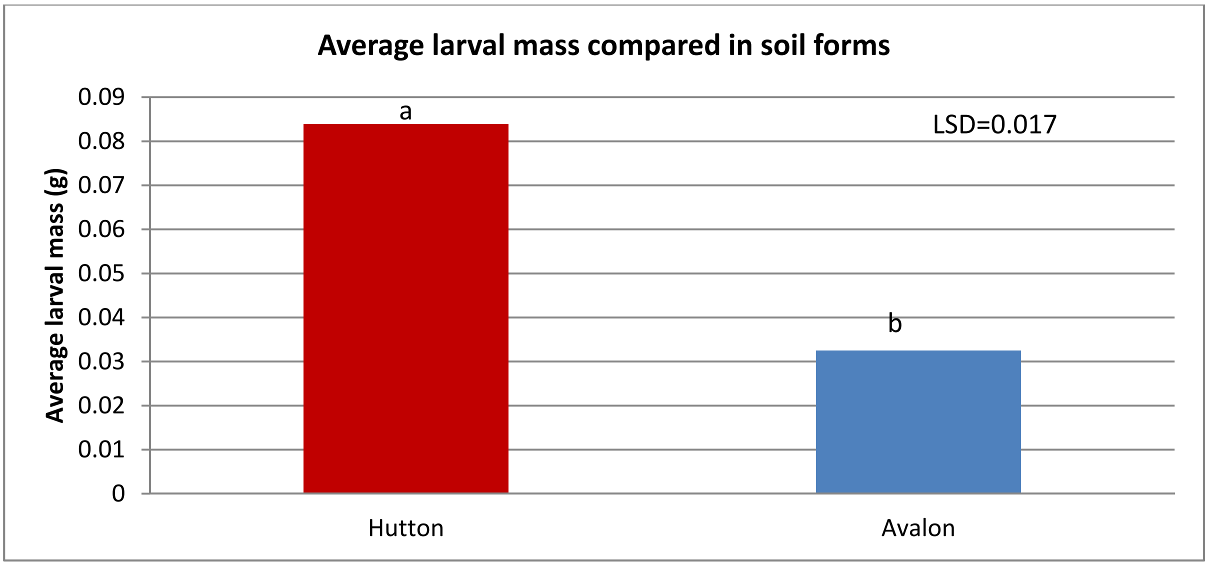

Figure 4.1. Average larval mass recorded in Hutton and Avalon soils.

Average larval mass differed significantly at various moisture levels. Larval mass at 40% moisture level reached on average 0.1824g, compared to 0.1958g at 60% soil moisture level (Figure 4.2).

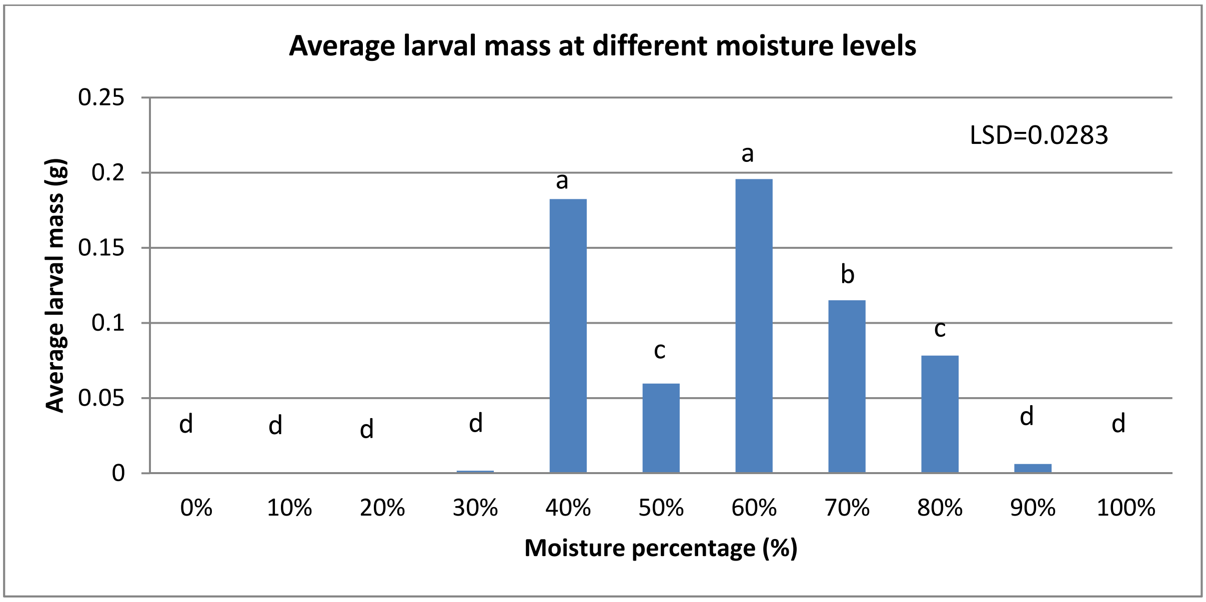

Figure 4.2. Average black maize beetle larval mass at different moisture levels.

Average larval mass differed significantly not only between soil forms, but also between moisture levels where the average larval mass (0.3642g) in a Hutton soil at 40% was the highest (Figure 4.3). Larval mass at 60% soil moisture (0.2210g) in Avalon soil was significantly lower than in the Hutton soil at a 40% moisture level, but was significantly higher than with any other soil moisture combination in Avalon.

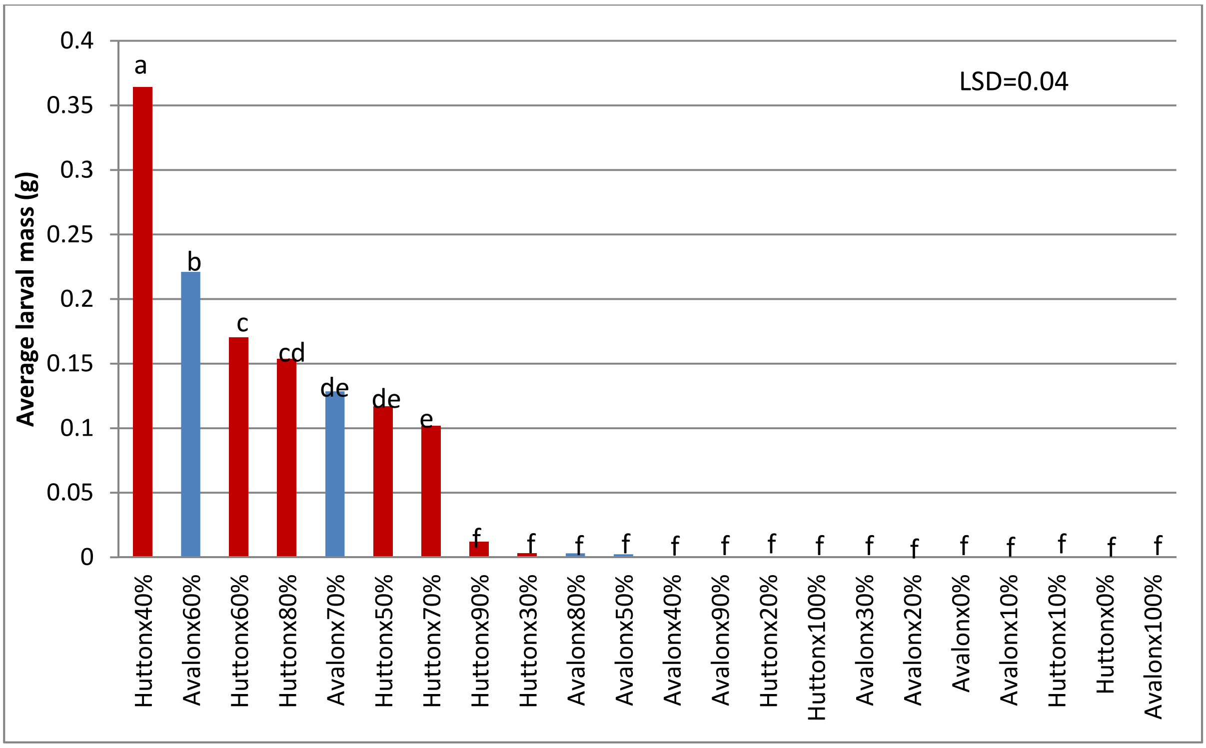

Figure 4.3. Moisture tolerance levels of larvae in two different soil forms.

Average larval mass gain over time indicated that larval growth peaked at day 68 with an average larval mass of 0.1253g (Figure 4.4). After day 68 the larval mass decreased steadily until day 100. There was a highly significant decrease in larval mass found between day 100 and 104 (Figure 4.4 (purple arrows)).

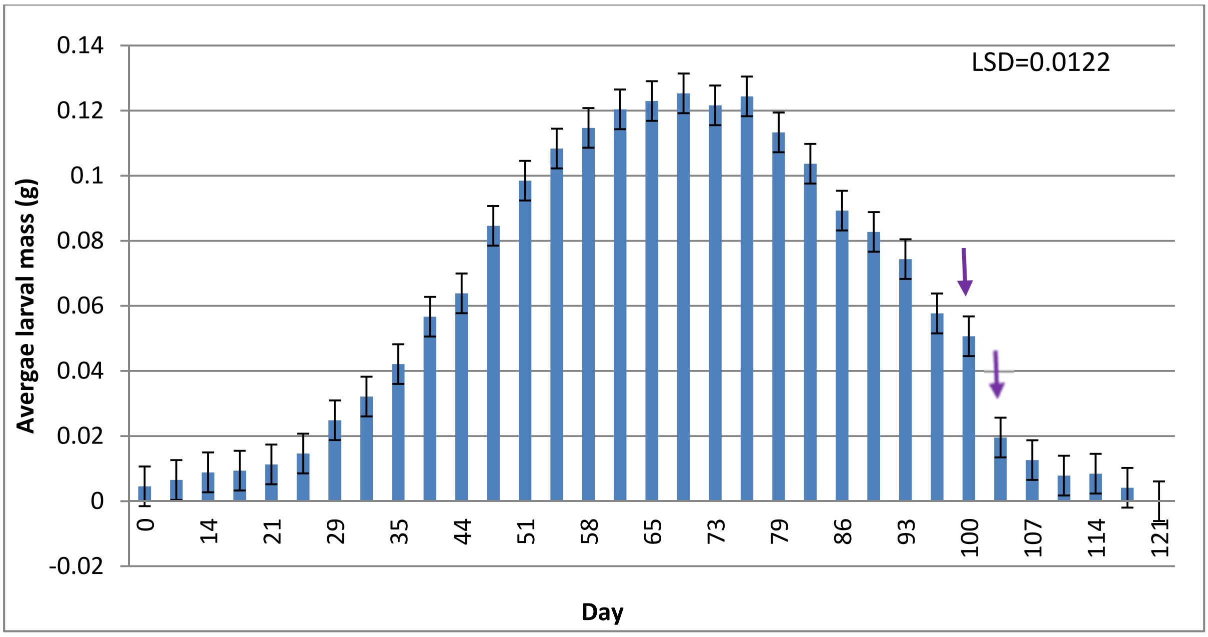

Figure 4.4. Average larval mass gain over time.

Highly significant larval mass differences over time were found between Hutton and Avalon soil forms (Figure 4.5). Average larval mass gain in Hutton soil was significantly higher from day 29 up to day 97 (Figure 4.5 (purple arrows)).

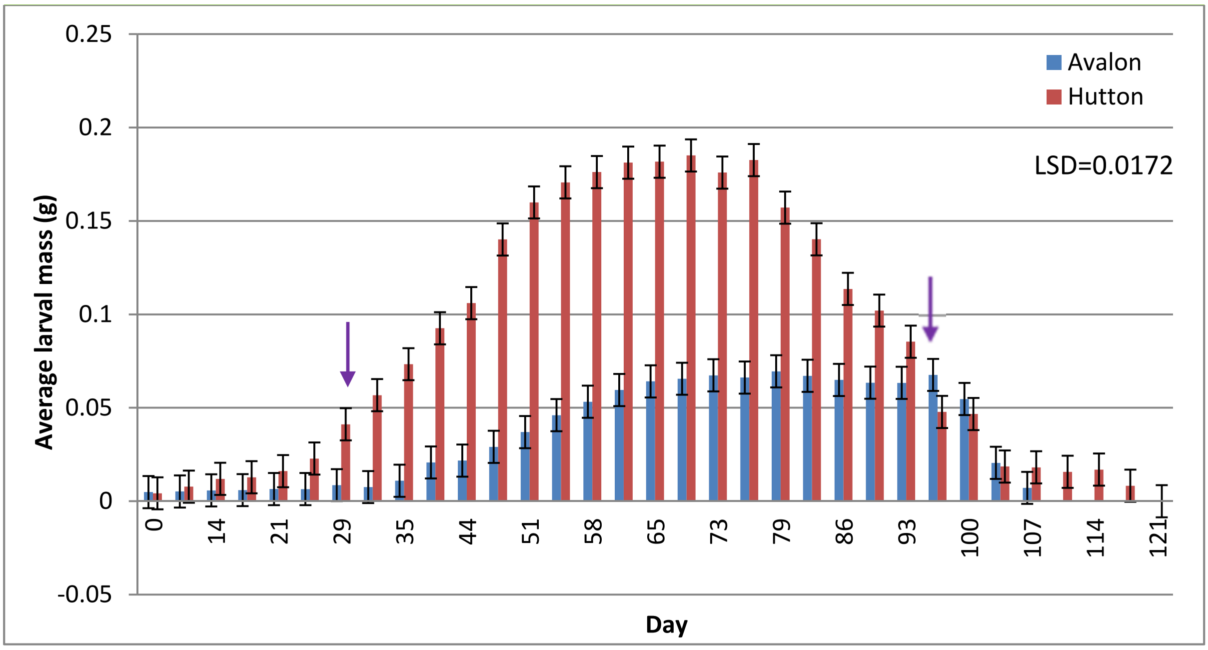

Figure 4.5. Average larval mass gain over time in different soil forms.

Highly significant larval mass differences over time were recorded at various moisture levels (Figure 4.6). The highest average larval mass over time (days) were recorded with a 60% soil moisture level on day 47 up to day 86 (Figure 4.6 (blue circles)). Larvae at 100% soil moisture survived only 35 days. Larvae at 90% moisture level managed to survive up to 51 days. Similarly larvae at 0% to 30% soil moisture levels all died within 35 days.

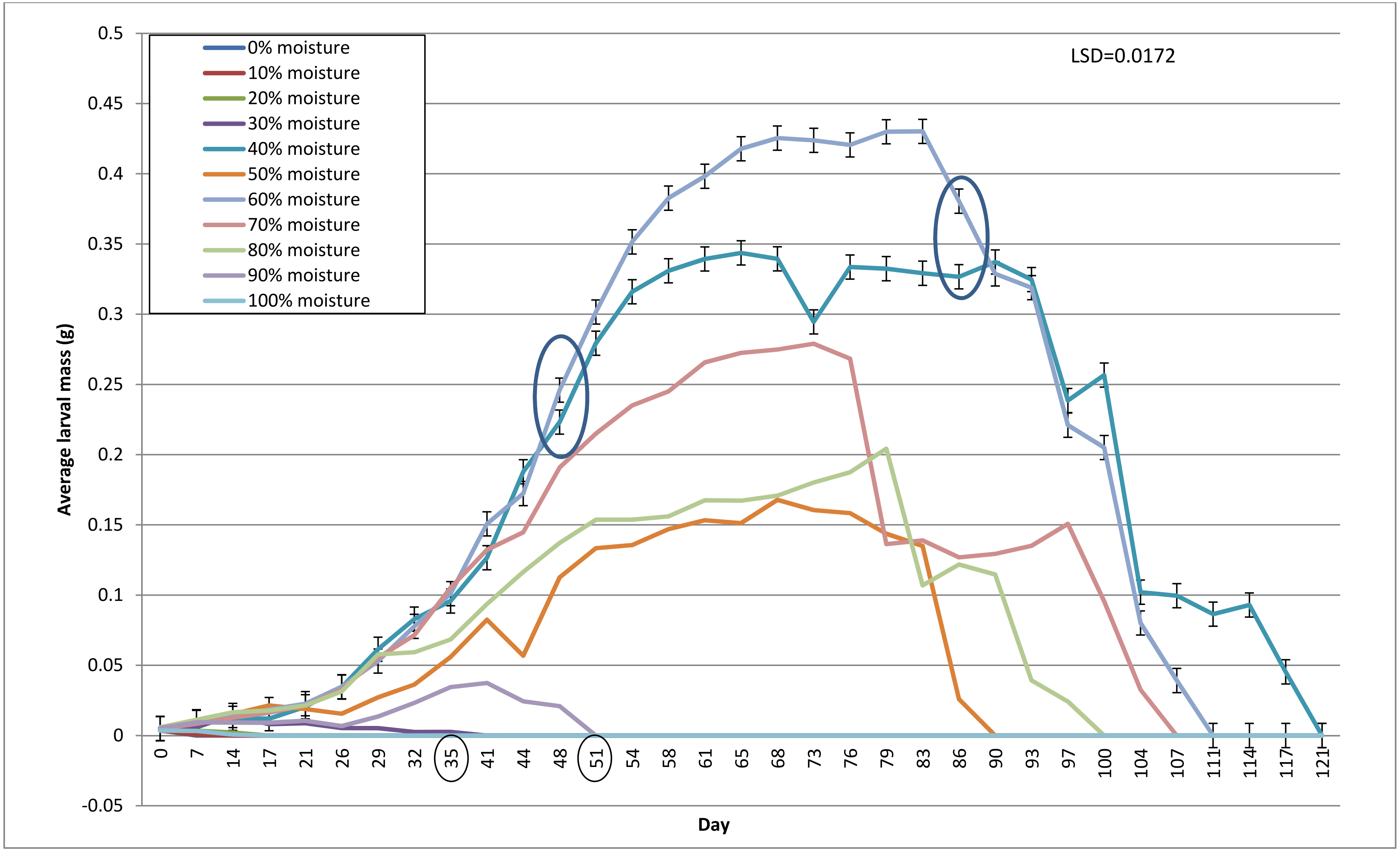

Figure 4.6. Average larval mass at various soil moisture levels over time.

Highly significant differences in larval mass gain over time (days) were found regarding interaction of soil moisture levels with soil forms (Figure 4.7). Larval mass in a Hutton soil with 40% moisture content was significantly higher than with all other combinations. In Hutton soil the larval mass gain was optimal between 40% and 80% soil moisture levels, but with the highest larval mass gain at 40%. Larval survival in Avalon soil ranged between 60% and 70% moisture levels with an optimum at 60%. However, this was significantly lower than in Hutton soil with a 40% soil moisture level.

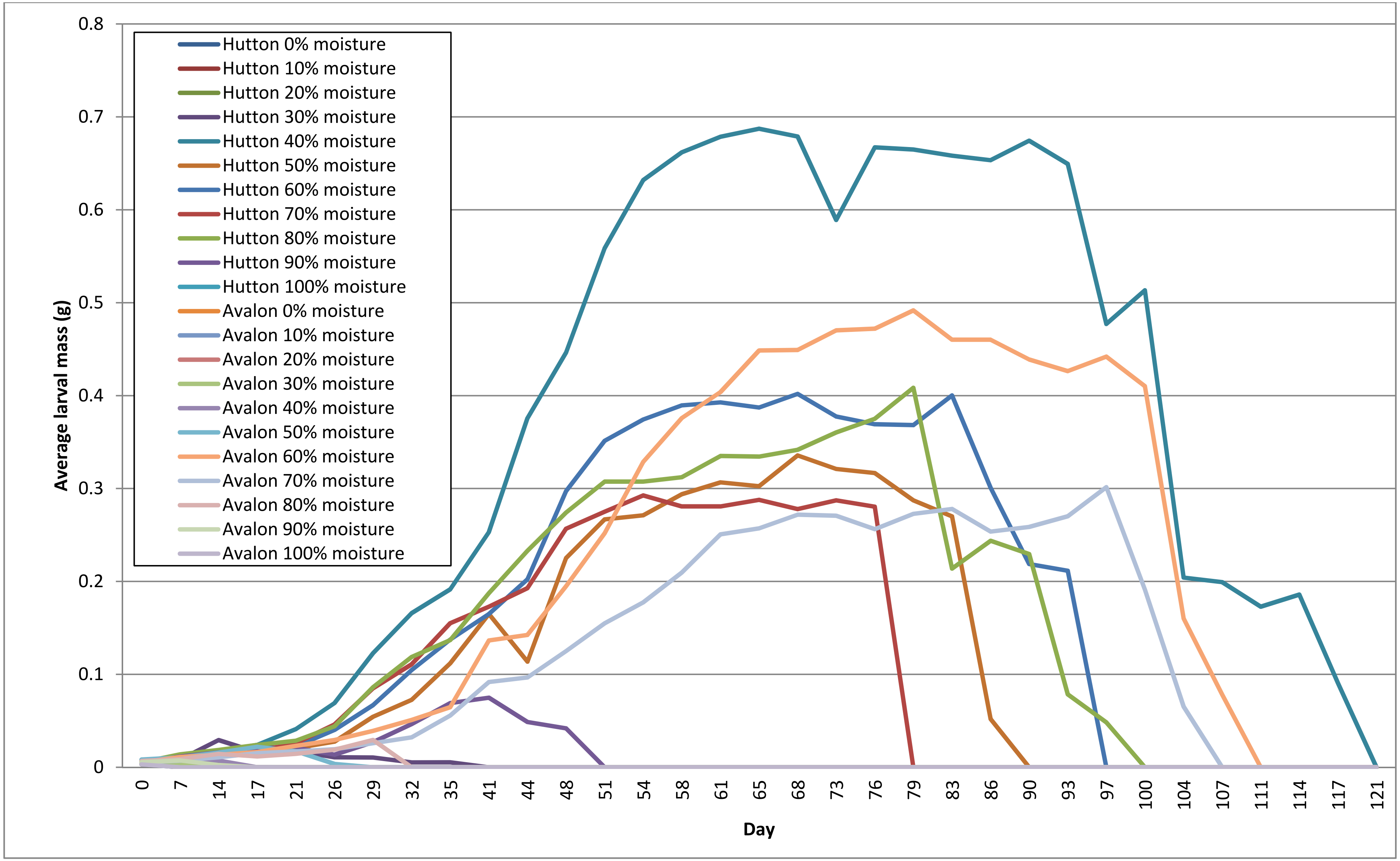

Figure 4.7. Average larval mass at different moisture levels in two soil forms over time.

Discussion

A Hutton soil type was favoured by black maize beetle larvae. From day 29 larvae in Hutton soil was able to feed significantly more than larvae in Avalon soil until day 93. Larvae in a 0% to 30% soil moisture level died within 35 days, as well as at 100% soil moisture. In a Hutton soil larvae were able to survive from 40% to 80% soil moisture levels with an optimal at 40%, while larvae in an Avalon soil survived at moisture levels ranging from 60% to 70% with an optimal at 60%. A Hutton soil form had a larger moisture range suitable for larval development than other soil forms. Larvae also survived better in a Hutton soil form than in Avalon soil forms at different moisture levels. This explains the known occurrence of black maize beetles in the Frankfort area. This information (soil form and soil moisture content) can also be used to calculate the distribution area of black maize beetle populations within a country, thus limiting survey areas and costs for black maize beetle management.

Avalon soil is prone to water logging. This might explain the observed lowered survival rate of black maize beetle larvae outside the Frankfort area. Hutton soil is free from water logging and is therefore better suited for black maize beetle occurrence. According to literature both larval and adults overwinter during the winter months. Black maize beetles in the survey area are found in a summer rain fall area where minimal rainfall occurs during the winter months. This indicates that overwintering larvae (especially L1-L2) will most likely not survive the harsh winter months. However, the 3rd larval stage is able to easily move to a depth of 1.5m below the soil surface (Du Toit, 1998). This larval stage is therefore most likely able to survive during the harsh winter months.

4.3.2 Organic matter levels

Highly significant larval mass differences (Table 4.2) were recorded between organic matter levels as well as for the interaction between organic matter levels over time (days). No significant differences were recorded between soil forms.

Table 4.2. Average larval mass (in g) in different soil forms and at various organic matter content levels over time.

	Soil form		
Organic matter content	**Hutton**	**Avalon**	**Average**
0%	0.0005	0.0002	0.0004
10%	0.0010	0.0005	0.0008
20%	0.0018	0.0006	0.0012
30%	0.0023	0.0035	0.0029
40%	0.0149	0.0041	0.0095
50%	0.0543	0.2423	0.1483
60%	0.2756	0.2227	0.2492
70%	0.3929	0.2846	0.3388
80%	0.3329	0.3225	0.3277
90%	0.3474	0.3417	0.3445
100%	0.3454	0.3454	0.3454
Day 0	0.0073	0.0065	0.0069
Day 7	0.0141	0.0121	0.0131
Day 14	0.0187	0.0140	0.0163
Day 17	0.0230	0.0273	0.0251
Day 21	0.0361	0.0340	0.0351
Day 26	0.0554	0.0457	0.0506
Day 29	0.0814	0.0677	0.0745
Day 32	0.1060	0.0985	0.1022
Day 35	0.1533	0.1325	0.1429
Day 41	0.1878	0.1881	0.1879
Day 44	0.2616	0.2458	0.2537
Day 48	0.2895	0.2927	0.2911
Day 51	0.3062	0.3110	0.3086
Day 54	0.3160	0.3234	0.3197
Day 58	0.3095	0.3223	0.3159
Day 61	0.2742	0.2820	0.2781
Day 65	0.2420	0.2426	0.2423
Day 68	0.2071	0.2176	0.2124
Day 73	0.1857	0.1947	0.1902
Day 76	0.1646	0.1773	0.1710
Day 79	0.1566	0.1655	0.1611
Day 83	0.1361	0.1283	0.1322
Average	0.1606	0.1605	

Table 4.2 (continued). Average larval mass (in g) in different soil forms and at various organic matter content levels over time.

Source	F Value	Pr> F
Soil	0	0.9681
Rep (Soil)	6.13	<.0001
Cons	1209.12	<.0001
Soil*Cons	58.42	<.0001
Rep (Soil*Cons)	6.5	<.0001
Days	272.93	<.0001
Soil*Days	0.52	0.9637
Cons*Days	33.87	<.0001
Soil*Cons*Days	2.44	<.0001

Average larval mass differed significantly between organic matter levels. Organic matter contents in soil from 70% to 100% were statistically the best suited for larval mass gain (Figure 4.8).

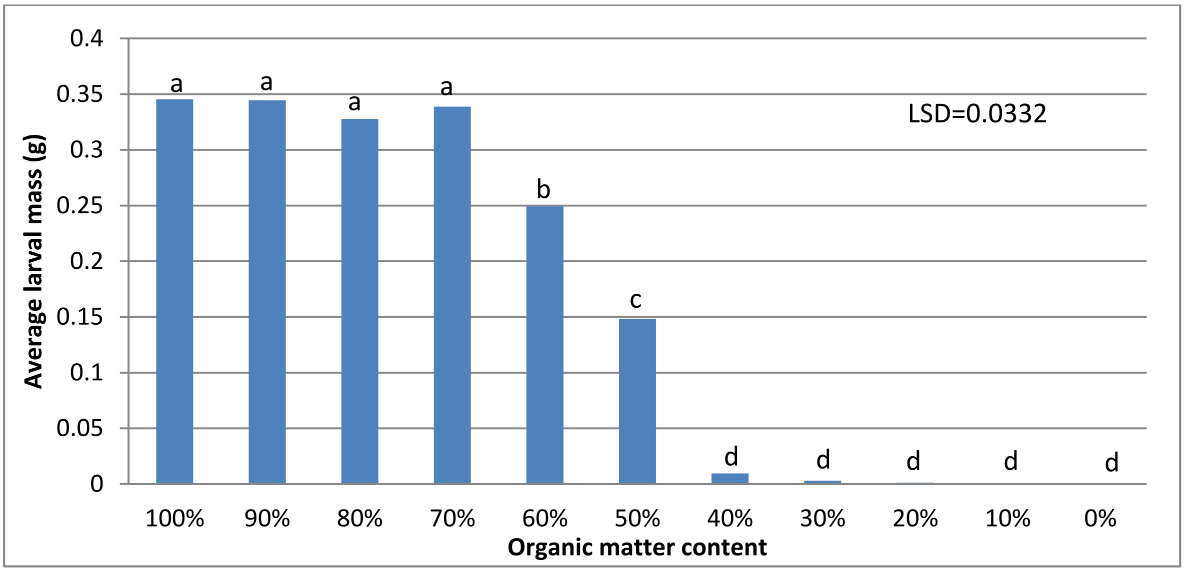

Figure 4.8. Average larval mass in different organic matter contents.

Highly significant larval mass differences were found with both soil forms with different organic matter levels (Figure 4.9). The highest larval mass was recorded in a 70% Hutton organic matter mixture with an average larval mass of 0.3929g. This did not differ significantly from the 90% Hutton organic matter mixture. The best suited organic matter mixture for Avalon soil was between 80% and 100%. Organic matter concentrations less than 50% in the Hutton soil form were not suitable for larval development while less than 40% organic matter in an Avalon soil was unsuitable for larval development.

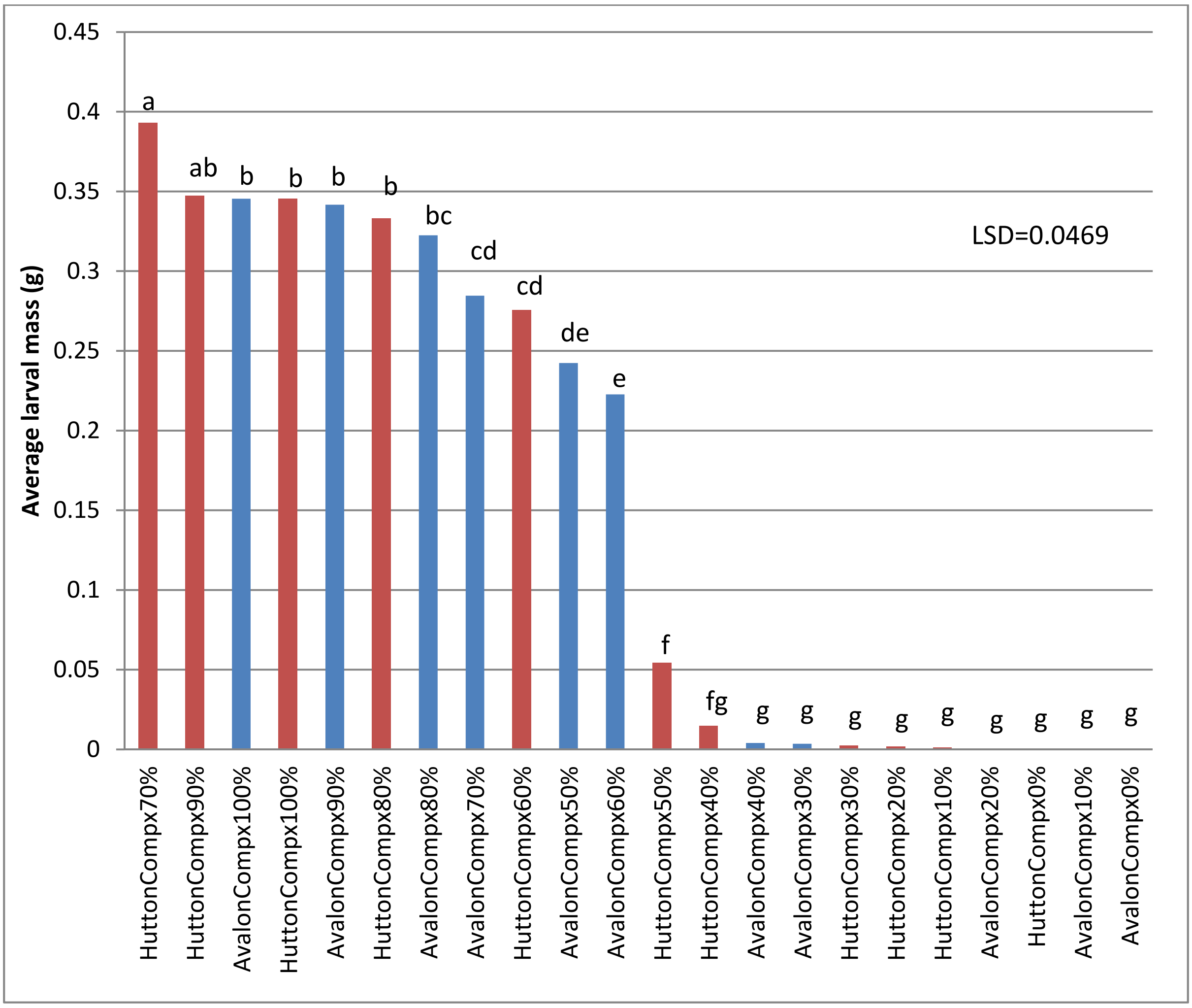

Figure 4.9. Average larval mass in different soil forms with various organic matter levels.

Average larval mass gain over time increased steadily from day zero to day 56 (0.3187g), where-after it decreased steadily (Figure 4.10).

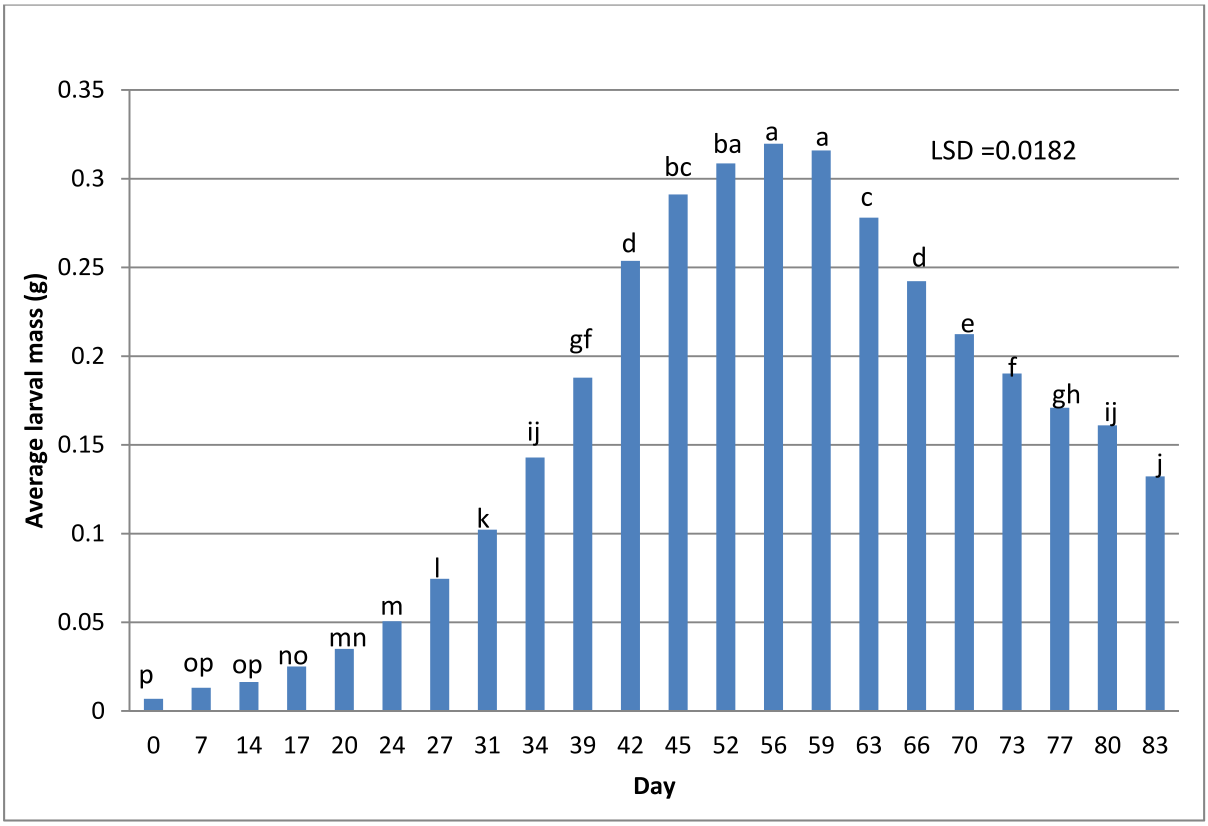

Figure 4.10. Average larval mass over time, irrespective of soil type and organic matter content.

No significant differences in larval mass over time between Avalon and Hutton soils were found (Figure 4.11). The tendency of larval mass gain was similar for both soil forms with a peak recorded at day 56.

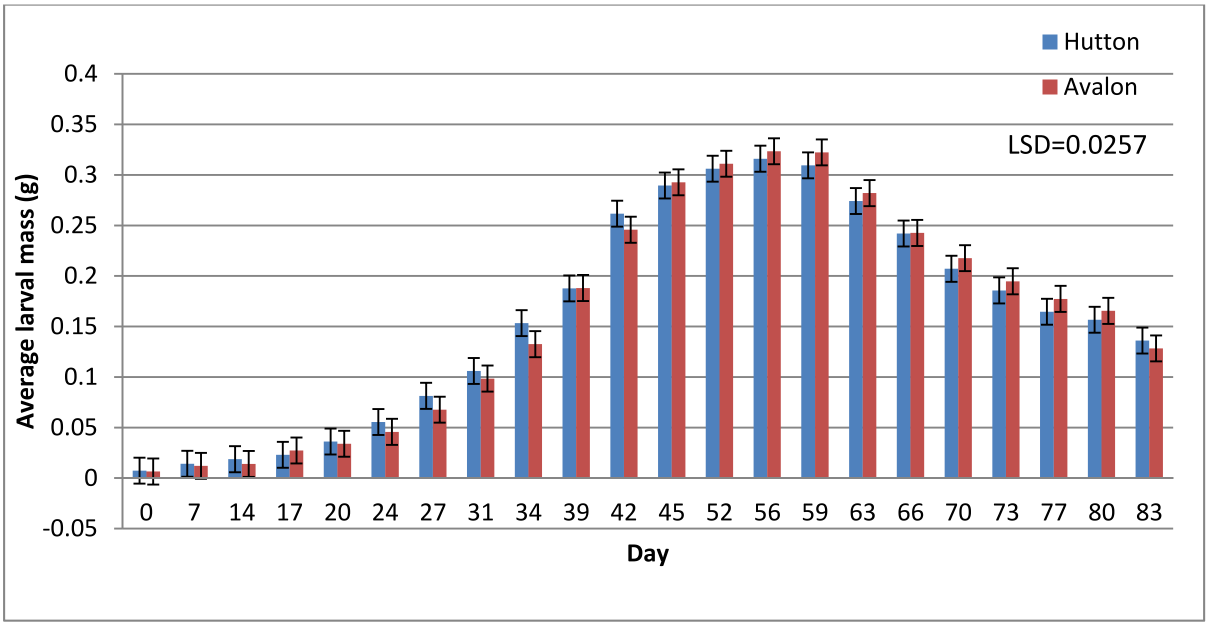

Figure 4.11. Average larval mass gain over time in Avalon and Hutton soil.

Average larval mass at different organic matter levels indicated highly significant differences (Figure 4.12). Over time, larval mass gain was the highest with a 90% organic matter level. However, after the peak of 0.7677g average larval mass during day 56 a steady decline in average larval mass occurred. In a 70% organic matter mixture the larval mass gain was slower and only managed to reach a peak of 0.6196g at day 63. Larval mass loss here was very slow in comparison with the average mass at a 90% organic matter concentration.

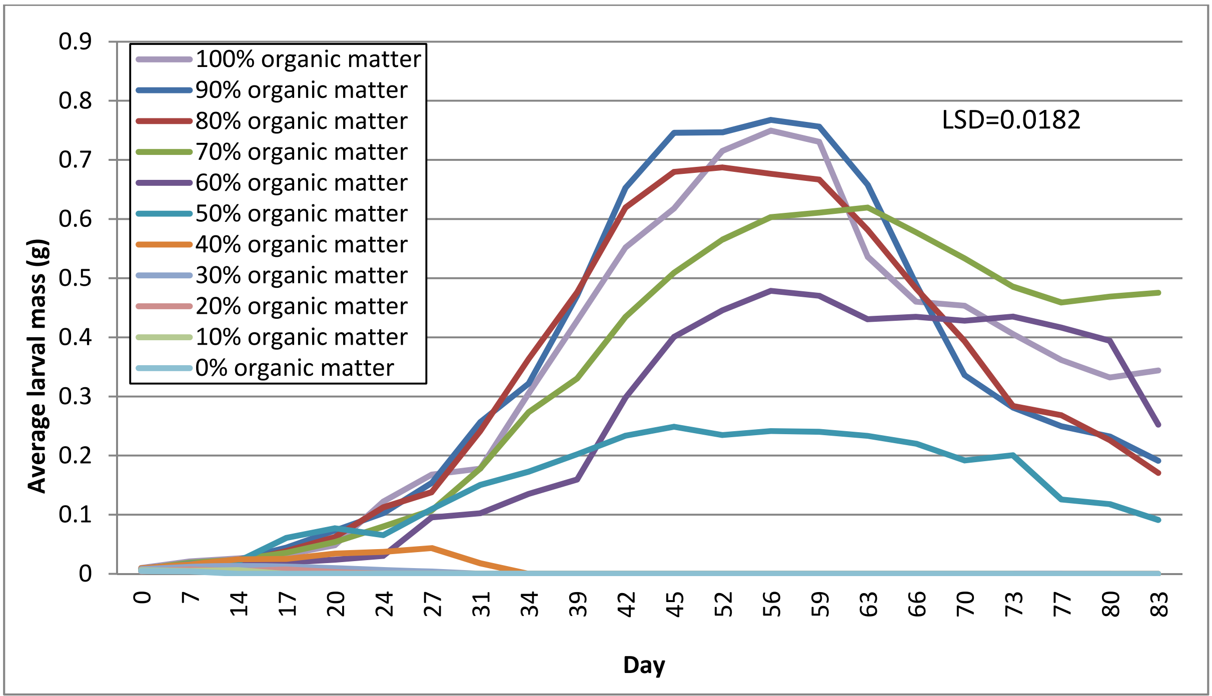

Figure 4.12. Average larval mass with various organic matter contents over time.

Discussion

Significant black maize beetle mass differences were found with different organic matter levels. The higher the organic matter content in the soil the higher the mass gain of the larvae. Larva feeding exclusively on organic matter in the soil needed at least 70% levels to develop into maturity. Black maize beetles are active fliers and can therefore fly from soils which are rich in organic matter to nearby maize fields. This indicated that larval infestation may occur in grass fields, rich in organic material near maize fields. This must still be investigated.

4.3.3 Pupae and beetle formation at different organic matter levels in two soil forms

Pupal mass differed highly significantly (Table 4.3) for organic matter content, but not between soil forms and the average pupal mass was lower in Avalon soil than in Hutton soil. Additionally, decreasing pupal mass was recorded with decreasing organic matter levels for both soil forms.

Table 4.3. Pupal mass (in g) at three organic matter levels found in each of Hutton and Avalon soil forms.

Organic matter content	**Soil form**		
	Hutton	**Avalon**	**Average**
100%	0.4388	0.4388	0.4388
90%	0.3439	0.2758	0.3129
80%	0.3064	0.2196	0.2775
Average	0.3630	0.3336	
Source	**F**	**P**	
Soil type	1.24	0.2817	
Organic matter content	14.99	0.0002	
Interaction (soil x organic matter)	1.06	0.3703	

Highly significant pupal mass differences were recorded between different organic matter levels (Figure 4.13). The highest pupal mass (0.4388g) was recorded with 100% organic matter content.

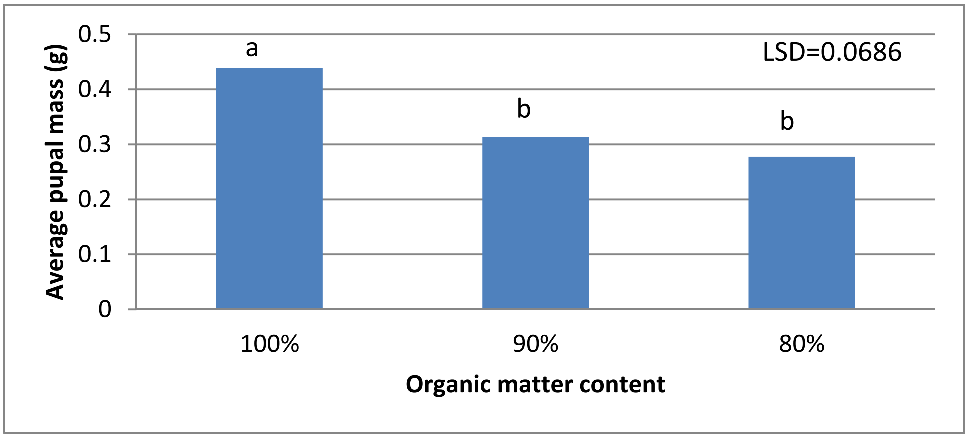

Figure 4.13. Average pupal mass at different organic matter levels, across soil types.

Highly significant pupal mass differences were recorded between different organic matter levels in the two soil forms (Figure 4.14). At a 100% organic matter level in both Avalon and Hutton soils the average pupal mass was the highest (0.4388g).

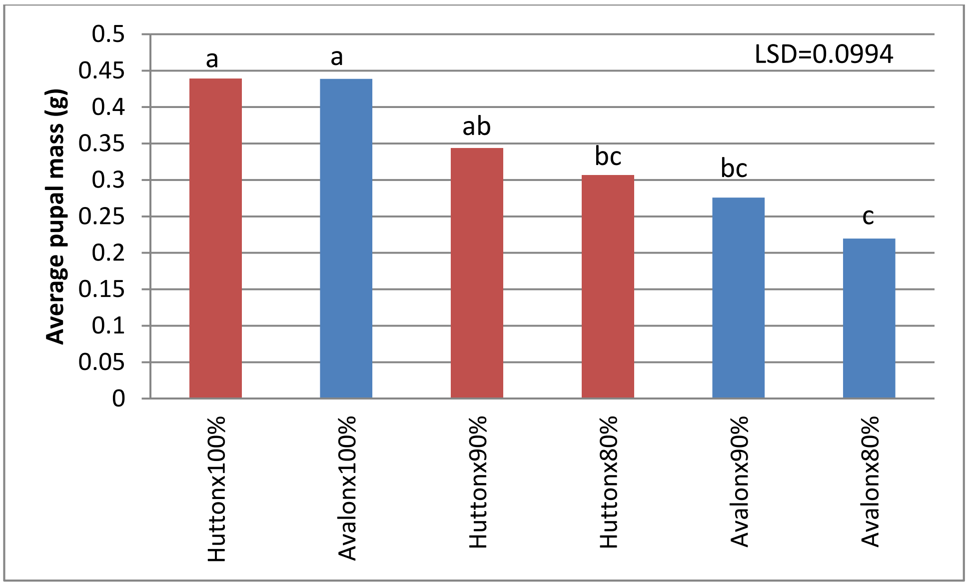

Figure 4.14. Average pupal mass at different organic matter levels within two different soil forms.

No significant differences in time (days) to pupation were recorded (Table 4.4) between soil forms, neither was the interaction with organic matter content significant. Significant differences were recorded only for organic matter content. However, time (days) to pupation increased with decreasing organic matter levels in soil.

Table 4.4. Time (days) to pupation at three organic matter levels in each of Hutton and Avalon soil forms.

Organic matter content	**Soil form**		
	Hutton	**Avalon**	**Average**
100%	62.83	62.83	62.83
90%	64.50	66.80	65.55
80%	68.67	68.33	68.56
Average	65.33	65.43	
Source	**F**	**P**	
Soil type	0.01	0.9391	
Organic matter content	6.91	0.0069	
Interaction (soil x organic matter)	0.71	0.5043	

Highly significant differences in days to pupation were recorded (Figure 4.17). It took on average longer for larvae to form pupae at 80% organic matter content than at 100% organic matter level.

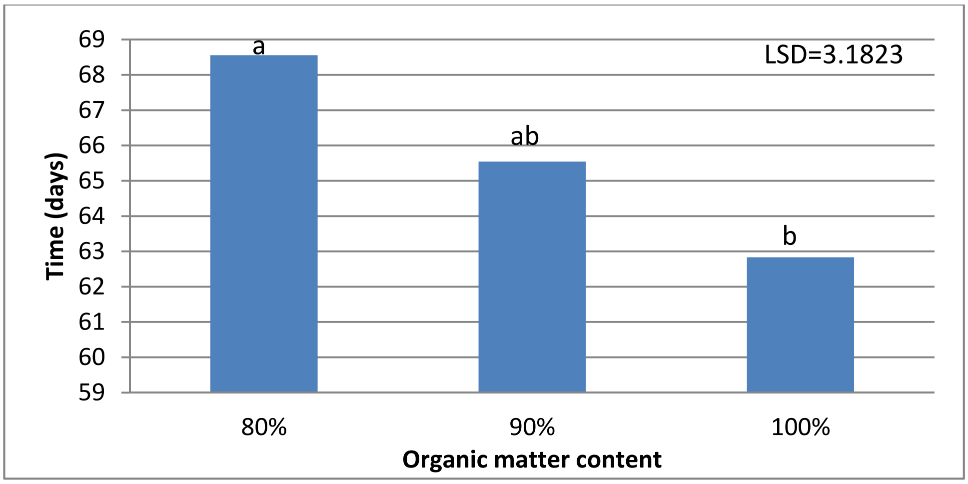

Figure 4.17. Average time (days) to pupation in different organic matter content.

Highly significant differences in days to pupation (Figure 4.18) were recorded between the different soil forms and with different organic matter content. It took on average longer to pupation at lower organic matter concentrations. At 80% organic matter content it took approximately 69 days for larvae to pupate compared to 63 days at 100% organic matter content.

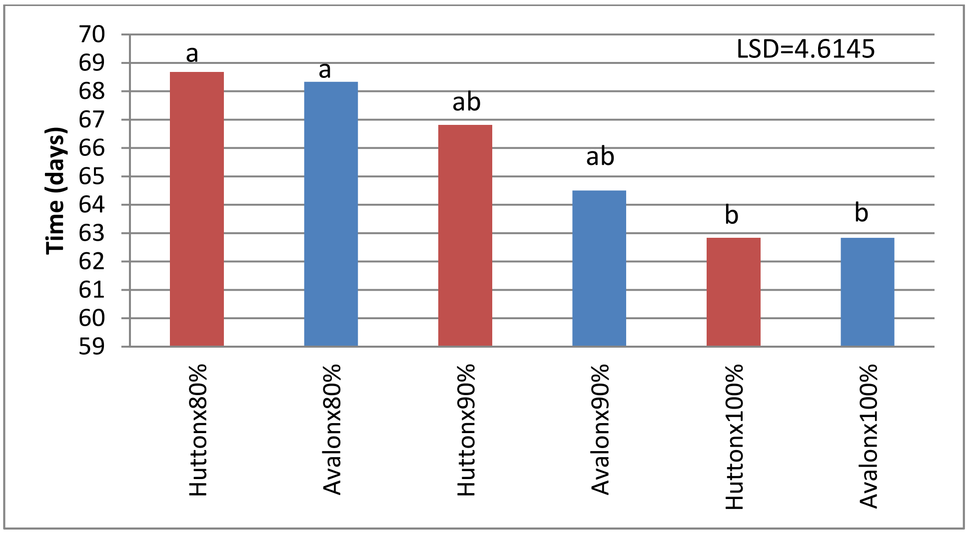

Figure 4.18. Average days to pupation in different soil forms with different organic matter levels.

Beetle mass differed highly significantly (Table 4.5) at various organic matter levels, but not between soil forms. The interaction between soil forms and organic matter was not significant, indicating that soil form responded similarly to organic matter content.

Table 4.5. Average beetle mass (in g) at various organic matter contents in Hutton and Avalon soil forms.

Organic matter content	**Soil form**		
	Hutton	**Avalon**	**Average**
100%	0.2958	0.2958	0.2958
90%	0.2075	0.1417	0.1812
80%	0.1419	-	0.1419
Average	0.2151	0.2342	
Source	**F**	**P**	
Soil type	0.72	0.4115	
Organic matter content	19.66	0.0001	
Interaction (soil x organic matter)	3.33	0.0909	

Average beetle mass (Figure 4.13) at 100% organic matter content was 0.2958g, 0.1812g at 90% organic matter mixture and 0.1419g at 80% organic matter content.

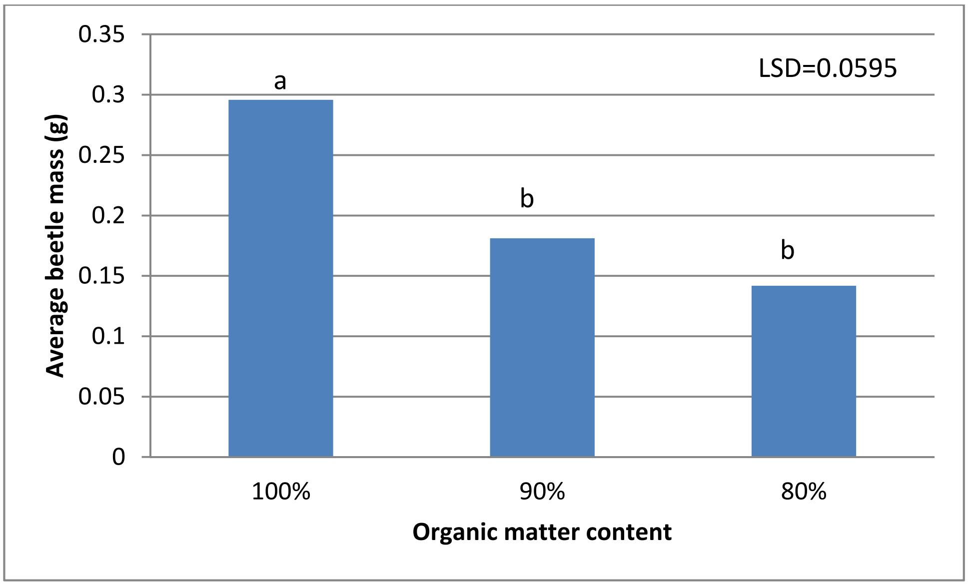

Figure 4.13. Average beetle mass with different organic matter levels.

Beetle mass differed significantly between organic matter levels, but not between soil forms (Figure 4.14). However, average beetle mass was higher at 90% organic matter level in Hutton soil than at the same organic matter level in an Avalon soil form.

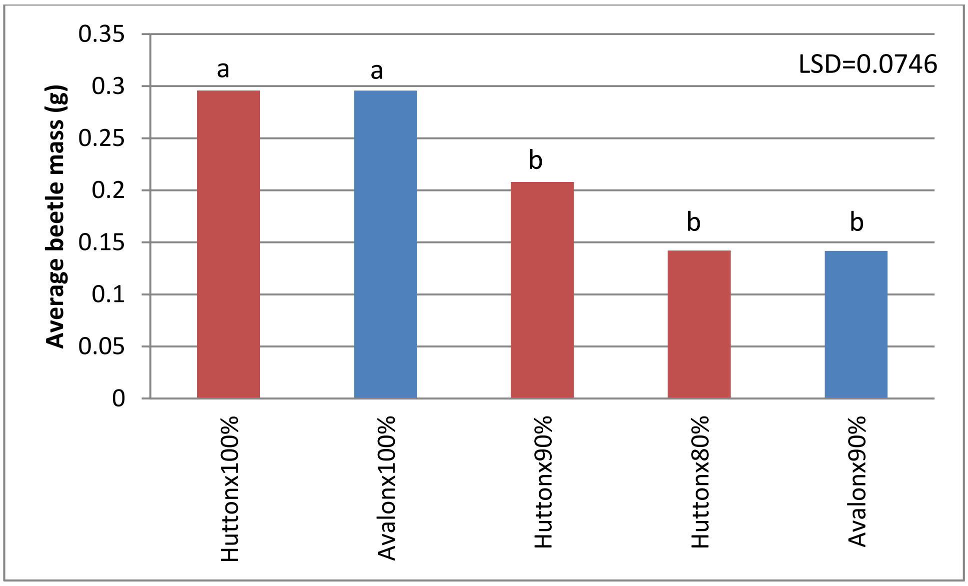

Figure 4.14. Average beetle mass in different organic matter levels in two different soil forms.

No significant differences in the time it took to the pre-adult stage were found between soil forms (Table 4.6), neither was the interaction with organic matter content significant. Significant differences were recorded only for organic matter content.

Table 4.6. Time (days) to beetle maturity at three organic matter levels in each of Hutton and Avalon soil forms.

Organic matter content	**Soil form**		
	Hutton	**Avalon**	**Average**
100%	76.67	76.67	76.67
90%	78.50	79.25	78.80
80%	82.00	-	82.00
Average	79.06	77.70	
Source	**F**	**P**	
Soil type	1.82	0.2009	
Organic matter content	7.24	0.0077	
Interaction (soil x organic matter)	0.00	0.9720	

At an organic matter concentration of 80% it took 82 days for pre-adults to emerge from pupae but at 100% organic matter level it only took on average 76.67 days (Figure 4.15).

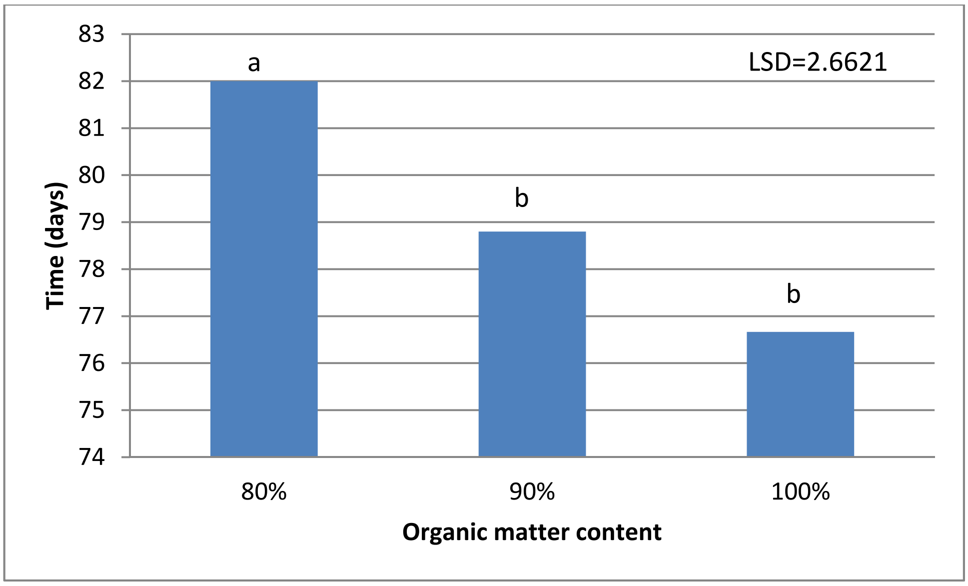

Figure 4.15. Average time (days) to pre-adults in different organic matter levels.

Highly significant difference in time (days) to pre-adults were found with different organic matter content in the two soil forms. It took on average significantly longer to reach maturity at lower organic matter content (Figure 4.16)

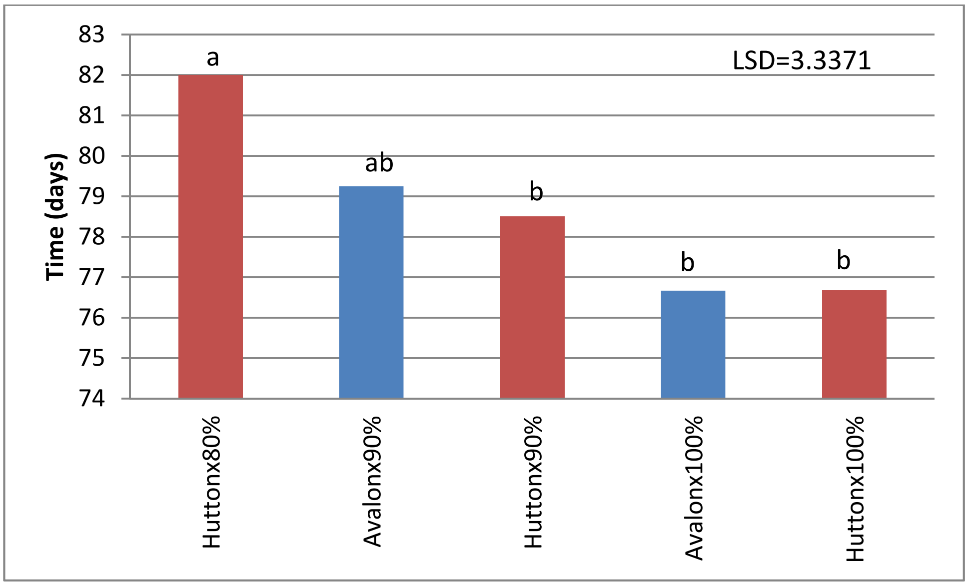

Figure 4.16. Average time (days) to pre-adults with different organic matter levels in two soil forms.

Discussion

The higher the organic matter found in soil, the higher the pupal and pre-adult mass. In Hutton soil, all larvae reached maturity at 80% to 90% organic matter content. However, in the Avalon soil with organic matter content of 90% only four larvae in the trial managed to develop into pre-adults. Pre-adults mass was significantly lower in Avalon soil than in Hutton soil at the same organic matter content. At 100% organic matter content all larvae managed to reach pre-adults. This indicated that black maize beetle larvae feeding exclusively on organic matter are able to reach adulthood and that no-till practice, especially in the known distribution area of black maize beetles may lead to a significant increase in black maize beetle numbers.

To conclude:
No-till practices at first leads to soil compaction, but this compaction are lifted within a few years after continuous years of no-till. The effect of soil compaction on the development of the black maize beetle population still needs to be investigated.

Larvae were able to survive in Avalon soils but with a narrow moisture range (60% to 70%). Therefore, no-till practices (which are known to increase moisture levels and enhance organic matter levels in soil) in Avalon soils may not contribute to an increased black maize beetle population. No pre-adults emerged from the 80% organic matter level soil and at a 90% organic matter content struggled to survive in the Avalon soil. Therefore, larvae are more likely to cause damage to maize in a Hutton soil than in an Avalon soil type.

Black maize beetle larvae favoured a Hutton soil form. A Hutton soil is free from water logging and is thus suitable for larval development. Results indicated that a moisture range in a Hutton soil from 40% to 80% was suitable for larval development. This study showed that a Hutton soil was most favourable to larvae and therefore ≥80% organic matter content and a soil moisture level of 40% are the most suitable for larval development. All larvae reached pre-adults in ≥80% organic matter content in Hutton soil. Pupa and pre-adult mass decreased with decreasing organic material in soils. Pre-adults were also reached much earlier with 100% organic matter content than in soil with lower organic matter concentrations. Results indicated that in Hutton soils an 80% organic matter content is needed for larvae to reach pre-adults, unless black maize beetle larvae are able to feed on living plant material such as plant roots. This still needs to be investigated.

This information substantiates the occurrence of black maize beetles in the area that was monitored (Chapter 2). Therefore, any future survey area for black maize beetles can be limited to Hutton soil.

Additionally, by determining organic matter content in the soil and by measuring soil moisture levels a more precise prediction model for black maize beetles can therefore be developed.

4.5 References

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CHAPTER 5:

Conclusions and recommendations

Black maize beetle (*H. arator*) is considered to be the most economically important coleopteran pest that attacks the subterranean part of maize seedlings in South Africa. Under conditions of localised outbreaks (not coinciding with epidemic years) plant populations are consequently reduced to such an extent that producers are often compelled to replant with another non-host crop such as a legume or sunflower.

The sporadic nature of the black maize beetle as well as the fact that only preventative chemical control measures exist in South Africa lead to unnecessary use of insecticides. Chemical control of this pest could amount to millions of Rand annually. Approximately 700 000 ha (at a cost of approximately R110 ha^{-1}) are treated annually with insecticides to control the black maize beetle. Producers could therefore save up to R110 ha^{-1} annually if they are continuously advised of the black maize beetle status for a forth coming season. In years when it is unnecessary to use insecticides for the control of black maize beetles (as was advised in the past four years) maize producers can save altogether R70 000 000 per year on input costs alone.

Producers currently employ preventative control measures even if black maize beetle numbers are low, resulting in input costs that could have been avoided if producers could be informed of the current status of black maize beetle populations within their area. Currently only preventative insecticidal control measures exist in South Africa and therefore predicting when this pest will occur will help to establish when to employ preventative control measures. Knowledge of the pest status will lower the risk of unnecessary use of insecticides when insect numbers are very low and ensure effective control measures in years when it reaches economically important status. If the mode of action of newly developed insecticides and factors influencing their efficacy under different field conditions are known, optimal efficacy would be ensured by eliminating the unnecessary use of ineffective insecticides and incorrect application methods.

Another effective control method against the black maize beetle is by ploughing during the most vulnerable pupal stage. With the growing human population and increased demand for food, pressures of maximum production have led to minimization of risks in the agricultural sector, resulting in the implementation of pivot irrigation and other means of irrigation, in order to reduce risks associated with droughts. However, no-till practices are being implemented in South Africa because of numerous advantages such as higher soil moisture levels, lowered input costs, as well as increased soil fertility. No-till practises are known to alter the soil composition and increase available soil moisture which is favoured by all developmental stages of black maize beetle. Ploughing during the pupal stage of the black maize beetle is the most effective cultural control method available and since this is discouraged during no-till practices this control method is no longer an option. All in all therefore, current agricultural practices and environmental conditions may intensify the presence and seasonality of this pest species.

This study attempted to develop an early-warning system for black maize beetle incidence in order to lower the risk of unnecessary use of insecticides when insect numbers are very low and ensure effective control measures in years when it reaches economically important levels. An early warning system for the black maize beetle requires knowledge about their spatial and temporal variation over time. Prediction of the black maize beetle through the use of weather variables will contribute to an early warning system. Additionally, knowledge about their preferred habitat and the key factors needed for larval development, such as soil moisture levels, as well as organic matter content in the soil could increase the accuracy of an early warning system for the black maize beetle. This information can also limit survey areas when monitoring population levels.

Eleven years of black maize beetle data indicated highly significant spatial clustering across the study area, not only between locations, but also over years. Hotspots and cold spots tended to recur in the same area with minimal variation, except for one hotspot found on the border of the three provinces which differed in intensity over years (Chapter 2). This indicates that preventative measures for black maize beetle throughout the monitored area could be drastically reduced even for localities found on the border of the Free State, Gauteng and Mpumalanga provinces. However, care should be taken when outbreaks occur (Chapter 2). Hotspots occur within a small spatial range (approximately 90km x 60km). After the five year period from 2004 to 2009, the average seasonal black maize beetle population doubled in size, (5307 to 12186), but only on the border of Gauteng, Free State and Mpumalanga provinces.

When above average black maize beetle flights occur during the onset of warmer temperatures (spring months) and maize mono-cropping is practiced the black maize beetle may be more prone to reach economically important levels. By implementing crop rotation practices using non-host crops such as cowpea and sunflower, black maize beetle populations may be reduced significantly. Another effective tactic, through cultivation practices, is ploughing during September and October months when the most vulnerable pupal stage of the black maize beetle occurs. However, timing and frequent tillage operations are essential for optimal results.

Biological control through the use of GMO (genetically modified organisms) maize commonly known as Bt (*Bacillus thuringiensis*) maize, is another more effective and efficient alternative than cultural control, especially in the areas where maize mono-cropping practices are practiced. This method is species specific and can therefore be used especially when beetle numbers rise. Using transgenic maize is only necessary within the identified hotspot (border of Mpumalanga, Free State and Gauteng provinces) and only during certain years (at five year intervals). This ultimately will lead to significant saving of unnecessary input costs when this trait is released for commercial use and is proved to be specifically effective against the adult stage. However, transgenic control is effective only during the early developmental stages of the insect, it is doubtful if GMO crops will control *H. arator* where the adults are the plant feeders.

Current chemical control options for the control of black maize beetle populations in South African maize production areas are all preventative in nature. This allows for unnecessary use of chemicals during planting, especially when insect numbers are low. Results obtained indicated that preventative chemical control measures should only be used in areas where the Mpumalanga, Free State and Gauteng provinces border each other. This will ultimately lead to saving substantially on input costs.

This area only needs to apply preventative chemicals against black maize beetle populations at five year intervals.

Larval stages of the black maize beetle are controlled naturally by abnormal wet or dry weather conditions. Water logging during wet conditions can also reduce the number of eggs and L1 populations significantly. Therefore, by controlling the identified hotspot area by means of flooding, through the use of pivot systems, during autumn months when oviposition is taking place, black maize beetle populations can be reduced significantly. The procedure is, however, very costly and should be restricted to five year intervals. Further study is needed on this matter.

Natural hosts favoured by *H. arator* include paspalum (*Paspalum dilatatum*) and ryegrass (*Lolium* spp.). Both these species act as reservoirs for ovipositional adults. Black maize beetles' dispersal ability during autumn and spring play a substantial role in infestation of maize fields, especially during epidemic years. Natural hosts surrounding maize fields within the hotspot area may explain the sudden decreases/increases in beetle abundance as was found in this study. By eliminating the surrounding reservoirs, either by expanding the cultivation area into these areas or by burning these areas annually or through chemical control in these areas, will decrease the current organic matter levels found in those areas that are needed for larval development. This needs to be further investigated.

Organic matter in the soil serves as feeding stimulant for young black maize beetle larvae. Current agricultural practices pursue no-till practices. No-till practices alter soil composition and structure resulting in higher organic content and soil moisture. This alteration in soil composition and structure may be more favourable for the development of black maize beetle populations, which in turn may lead to more concentrated and intensified outbreaks within an area. It may also lead to the expansion of currently known black maize beetle hotspots. Further investigation is recommended to monitor potential future black maize beetle outbreaks.

The development of a prediction model for this pest species seemed to be possible since spatial and temporal variation of black maize beetle populations recur in the same area over time (Chapter 2). The use of weather variables such as evapotranspiration, maximum temperature, minimum temperature, maximum humidity, minimum humidity, solar radiation, rainfall and wind speed were used to explain this variation.

All weather variables described above impact either directly or indirectly on soil moisture and soil temperature which are expected to be key factors for the survival of all stages on the black maize beetle. Because of this a prediction model using weather data was developed. A prediction model for black maize beetle populations was identified using a forward regression model at a 10% significance level. Average monthly weather variables, as well as total black maize beetles captured (February to end of April) of the previous year, predicted 62.93% of the black maize beetle flight activity for the following year between 1 February to end of April when light traps were placed within 10km of an active weather station. The number of black maize beetles which is known to damage maize during October to December (main cropping season for maize in South Africa) can be calculated because it is described in the literature as 5% of the population. However, the threshold values for black maize beetle damage to a maize crop are still to be investigated. Through the use of light traps and scouting techniques within black maize beetle damaged maize fields, within the same area, a threshold value can be determined for both light trap captures and maize damage levels caused by black maize beetles. This can then be indicative whether a comparison can be made regarding the number of black maize beetles captured in light traps and the consequent damage to maize. This still needs further investigation. The total number of black maize beetles within a 50km buffer zone was less effectively predicted than when light traps were situated within 10km of an active weather station. This indicated that the nearer a light trap to an active weather station the more accurate the prediction model would be.

According to theory black maize beetles are expected to reach epidemic levels in 32 year cycles. According to this theory another outbreak was expected to occur during 2009. Results indicated that an outbreak did occur during 2009, but was confined to a very small area which was previously described in the literature as the Frankfort district of South Africa. This area amounts to 560 000ha (60km x 90km). The next expected black maize beetle outbreak is expected to occur during 2041.

Key factors for the survival of the black maize beetle include organic matter content in the soil, minimum soil temperature and soil moisture (Chapter 4). The prediction model developed for the black maize beetle indicated that average solar radiation contributed to 26.99% of the total prediction model, while minimum temperature contributed another 15.96%. Solar radiation is energy emitted from the sun, usually in the form of heat. Thus both variables are related to heat and contributed to a total of 42.95% of the 62.93% that is predicted by the model. Therefore minimum temperature has a large impact on the survival of black maize beetle populations, confirming one of the key factors as described in the literature.

All weather variables used were measured above the soil surface. Above surface weather variables either directly or indirectly impact on subsurface variables, such as soil moisture and soil temperature. These subsurface variables are key factors for the survival of black maize beetle populations and may prove to be better sources for predicting population dynamics. This still needs to be investigated.

The prediction model indicated that air humidity and ambient air temperature played a key role towards predicting black maize beetle populations. Further study of soil humidity levels in soil collected from the study area (i.e. Hutton), as well as a sandy soil (i.e. Avalon) from another area may explain the increased survival of the larval stages of black maize beetles in that area. Additionally, determining the percentage organic material in both types of soil forms (Avalon and Hutton) needed for larval development might substantiate or enhance the future prediction of black maize beetle populations.

Black maize beetle larvae favoured Hutton soil collected in the known area of occurrence, which is situated on the border of Mpumalanga, Free State and Gauteng provinces. Soil moisture content favourable for larval development ranged from 40% to 80% for both soil forms. However, larval development in the two soil forms (Hutton and Avalon) differed significantly where the moisture content for Hutton soil ranged from 40% to 80%, whereas in Avalon soil it was limited to a range of 60% to 70%. This indicated that soil collected in the area known for black maize beetle outbreaks had a larger moisture range suitable for larval development than other areas with Avalon soil forms. This information (soil form and soil moisture content) can also be used to calculate the distribution area of black maize beetle populations within a country, thus limiting survey areas of black maize beetles further.

Significant black maize beetle larval mass differences were recorded with different organic matter content. The higher the organic matter content the greater the mass gain of black maize beetle larvae. Feeding larvae needed at least a 70% organic matter content to develop to pre-adults. Larvae favoured Hutton soil, which was rich in organic matter (≥70%). Black maize beetles are active fliers and can therefore fly from soils which are rich in organic matter to nearby maize fields. This indicated that larval development may occur in grass fields, rich in organic material, situated near maize fields.

Highly significant adult black maize beetle mass differences, as well as in the time it took to pre-adults, were recorded for different organic matter content. The higher the organic matter content of soil the greater the pupal and adult mass. In Hutton soil, all larvae reached maturity at 80% to 90% organic matter content. However, in Avalon soil with an organic matter content of 90%, only four larvae in the trial managed to develop into pre-adults. Pre-adult mass was significantly lower in Avalon soil than in Hutton with the same organic matter content. At 100% organic matter content all larvae managed to reach maturity. This indicated that black maize beetle larvae feeding exclusively on organic matter are able to reach adulthood and that no-till practice, especially in the known distribution area of the black maize beetle, may lead to a significant increase in black maize beetle numbers.

Results indicated that with Hutton soil an 80% organic matter content is needed for larvae to reach pre-adults, unless black maize beetle larvae are able to feed on living plant material such as plant roots. This still needs to be investigated further. No-till practices lead to soil compaction at first, but this compaction is usually lifted within a few years after continuous no-till. The effect of soil compaction on larval development of black maize beetle populations also still needs to be investigated.

Highly significant differences in time to pupal and adult emergence were found with variation in organic matter content in different soil forms. It took approximately five days longer for beetles to emerge from a Hutton soil form with an 80% organic matter content compared to the same soil with 100% organic matter content. This indicated that the lower the organic matter content in soil, the longer it will take for black maize beetles to reach maturity unless larvae are able to feed on living plant material. This still needs to be corroborated. Overall therefore, by determining organic matter content in the soil and measuring soil moisture levels, a more effective prediction model for the black maize beetle can be developed.

Summary

Black maize beetle (*Heteronychus arator* Fabricius) (Coleoptera: Scarabaeidae) is economically the most important coleopteran pest that attacks the subterranean part of maize seedlings in South Africa. The sporadic nature of black maize beetles as well as the fact that only preventative chemical control measures exist, lead to unnecessary use of insecticides. Developing an early warning system for black maize beetles can save producers up to R70 000 000 per year on input costs alone.

Three primary factors contributing to the development of an early warning system were looked at. The 1st was by determining the black maize beetles' spatial and temporal variation. The 2nd was to develop a prediction model through the use of weather variables and 3rd to determine what ecological factors (such as soil form, soil moisture level and organic matter content in soil) favour larval development. Two peaks in the annual flight pattern occur during a particular maize production season. The first is during October to December when only 5% of a population flies and the second during February to April, when 95% of the population flies. Black maize beetles are nocturnal and are attracted to artificial light. Ninety nine modified Robinson light traps were placed in quarter degree grids (30km x 30km) throughout the eastern part of the maize production area. Weekly captures for three consecutive months from February of every year were preserved in 70% alcohol and counted. The flight pattern of *H.* arator was in this way monitored for 11 consecutive years. Data were recorded and analysed. Spatial variation indicated that black maize beetles in most years are limited to a small area (540 000ha) located on the borders of the Free State, Mpumalanga and Gauteng provinces. The long term temporal variation of black maize beetle populations according to literature indicated that epidemic outbreaks recur in the same area with 32 year intervals, hence the 32 years cycle theory. The next expected outbreak will be during 2041. Short term temporal variation indicated that populations tend to recur at five year intervals but with varying intensities. Through the use of monthly weather variables as well as black maize beetle captured the previous year (February to end April) a prediction model was developed. This prediction model was able to explain 62.93% of the number of black maize beetles expected to fly during February to end of April. However, the weather station must be within 10km from the sample area. The prediction model developed for black maize beetles indicated that average solar

radiation contributed to 26.99% of the total prediction model while minimum temperature contributed another 15.96%. Solar radiation is energy emitted from the sun, usually in the form of heat. Thus both variables are related to heat and contributed in total to 42.95% of the 62.93% that is predicted by the model. Larval development in two soil forms (Hutton and Avalon) differed significantly, where the favourable moisture content for Hutton soil ranged from 40% to 80%, whereas in Avalon soil it was limited to a range of 60% to 70%. This indicated that soil collected in the area known for black maize beetle outbreaks had a larger moisture range suitable for larval development than other areas known for Avalon soil forms. Significant black maize beetle larval mass differences were recorded with variation in organic matter content. The higher the organic matter content the greater the mass gain of the black maize beetle larvae. Highly significant black maize beetle mass differences were recorded for different organic matter content as well as in the time it took to maturity. The higher the organic matter content of soil the greater the pupal and adult mass. In Hutton soil, all larvae reached maturity at 80% to 90% organic matter content. However, with Avalon soil with an organic matter content of 90% only four larvae managed to develop into adults. Adult mass was significantly lower in Avalon soil than in Hutton with the same organic matter content. At 100% organic matter content all larvae managed to reach maturity. This indicated that black maize beetle larvae feeding exclusively on organic matter are able to reach adulthood and that no-till practice especially in the known distribution area of black maize beetles may lead to a significant increase in black maize beetle numbers. Results indicated that with Hutton soil an 80% organic matter content is needed for larvae to reach maturity unless black maize beetle larvae are able to feed on living plant material such as plant roots. Highly significant differences in time to pupal and adult emergence were found with variation in organic matter content in different soil forms. It took approximately five days longer for beetles to emerge from a Hutton soil form with an 80% organic matter content compared to the same soil with 100% organic matter content. This indicated that the lower the organic matter content in soil, the longer it will take for black maize beetles to reach maturity unless larvae are able to feed on living plant material. By determining organic matter content in the soil and measuring soil moisture levels a more effective prediction model for black maize beetles can be developed.

Opsomming

Swartmieliekewer (*Heteronychus arator* Fabricius) (Coleoptera: Scarabaeidae) is ekonomies die belangrikste kewer plaag wat die ondergrondse dele van mieliesaailinge in Suid Afrika aanval. Die sporadiese aard van swartmieliekewers asook die feit dat slegs voorkomende chemiese beheermaatreëls bestaan, lei tot die onnodige gebruik van insekdoders. Ontwikkeling van 'n tydige waarskuwingstelsel vir swartmieliekewers kan produsente tot R70 000 000 per jaar aan insetkostes spaar. Drie primêre faktore wat bydra tot die ontwikkeling van 'n tydige waarskuwingstelsel is nagevors. Die 1ste was om die ruimtelike en temporale variasie van swartmieliekewers vas te stel. Die 2de was om 'n voorspellingsmodel te ontwikkel deur weerveranderlikes te gebruik en die 3de om vas te stel watter ekologiese faktore (soos grondvorm, grondvog vlak en organiese vlak in grond) larwale ontwikkeling bevoordeel. Twee pieke in die jaarlikse vlugpatroon kom gedurende 'n spesifieke mielieproduksie seisoen voor. Die eerste is gedurende Oktober tot Desember wanneer slegs 5% van die populasie vlieg en die tweede gedurende Februarie tot April, wanneer 95% van die populasie vlieg. Swartmieliekewers vlieg gedurende die nag en word daarom deur kunsmatige lig aangelok. Nege-en-negentig gemodifiseerde Robinson ligvalle was in kwart graad blokke (30kmx30km) geplaas dwarsdeur die oostelike deel van die mielieproduksie area. Weeklikse vangste vir drie aaneenlopende maande vanaf Februarie van elke jaar was gepreserveer in 70% alkohol en getel. Die vlugpatroon van *H. arator* was op hierdie wyse vir 11 agtereenvolgende jare gemonitor. Data was aangeteken en geanaliseer. Ruimtelike variasie het aangetoon dat swartmieliekewers in meeste jare beperk was tot 'n klein area (540 000ha) wat voorkom op die grense van die Vrystaat, Mpumalanga en Gauteng provinsies. Die langtermyn temporale variasie van swartmieliekewer populasies volgens die literatuur het getoon dat epidemiese uitbrake in dieselfde area uitbreek met 32 jaar intervalle, vanwaar die 32 jaar siklus teorie. Die volgende verwagte uitbraak sal gedurende 2041 wees. Korttermyn temporale variasie het aangetoon dat populasie toenames met vyf jaar intervalle voorkom maar met verskillende intensiteite. Deur die gebruik van maandelikse weersveranderlikes asook swartmieliekewer vangstes van die vorige jaar (Februarie tot einde April) is 'n voorspellingsmodel ontwikkel. Die voorspellingsmodel kon tot 62.93% van die aantal swartmieliekewers wat verwag word om te vlieg gedurende Februarie tot einde April

van die volgende jaar voorspel. Die weerstasie moet wel binne 10km van die monster area wees. Die voorspellingsmodel wat ontwikkel is vir swartmieliekewers het getoon dat gemiddelde sonlig uitstraling tot 26.99% van die totale voorspellingsmodel bygedra het, terwyl minimum temperatuur 'n verdere 15.96% bygedra het. Sonlig uitstraling is energie wat deur die son vrygestel word, gewoonlik in die vorm van hitte. Dus, beide veranderlikes is verwant aan hitte en dit het in totaal tot 42.95% van die 62.93% bygedra. Larwale ontwikkeling in twee grondvorms (Hutton and Avalon) het wesenlik verskil waar die gunstige voginhoud vir Hutton grond gestrek het van 40% to 80% terwyl in Avalon grond dit beperk was van 60% tot 70%. Dit het aangetoon dat grond wat van die area wat bekend is vir swartmieliekewer uitbrake versamel is, 'n groter vogreeks geskik vir larwale ontwikkeling het in vergelyke met ander areas bekend vir Avalon grond vorms. Betekenisvolle swartmieliekewer larwale massa verskille was aangeteken met verkillende organiese inhoud. Hoe hoër die organiese inhoud hoe groter die massa toename van die larwes. Hoog betekenisvolle swartmieliekewer massa verskille, asook tyd wat dit geneem het tot volwassenheid, het voorgekom. Hoe hoër die organiese inhoud hoe groter die massatoename van die swartmieliekewers, asook van swartmieliekewer papies. In Hutton grond het alle larwes volwassenheid bereik by 80% tot 90% organiese inhoud. In Avalon grond met 'n organiese inhoud van 90% het egter slegs vier larwes volwassenheid bereik. Kewermassa was wesenlik laer in Avalon grond as in Hutton met dieselfde organiese inhoud. By 100% organiese inhoud het alle larwes volwassenheid bereik. Dit het aangetoon dat swartmieliekewer larwes wat uitsluitlik net op organiese materiaal voed volwassenheid bereik en dat geen-bewerkingspraktyke veral in die area bekend vir swartmieliekewer voorkoms tot 'n wesenlike toename in getalle sal bydra. Resultate het aangetoon dat Hutton grond met 80% organiese inhoud nodig is vir larwale ontwikkeling om volwassenheid te bereik, behalwe as larwes op lewende plantmateriaal soos plant wortels, kan voed. Hoogs wesenlike verskille in tyd tot papie en kewer voorkoms was gevind met variasie in organiese inhoud in grondvorms. Dit het ongeveer vyf dae langer geneem vir kewers om te ontpop in 'n Hutton grondvorm met 'n 80% organiese inhoud in vergelyking met dieselfde grond met 'n 100% organiese inhoud. Dit het aangetoon dat hoe laer die organiese inhoud in die grond hoe langer sal dit neem vir swartmieliekewers om volwassenheid te bereik behalwe as larwes lewende plantmateriaal kan vreet. Deur die organiese inhoud van grond asook die vogvlakke in grond vas te stel kan ‘n meer effektiewe voorspellingsmodel vir swartmieliekewers ontwikkel word.