Comparative diversity of arthropods on conventional and genetically modified Bt soybean plants in field trials in South Africa

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Abstract

Genetically modified soybean expressing Cry1Ac toxins derived from the soil bacterium *Bacillus thuringiensis* (Bt), have been approved for experimental cultivation in South Africa. *Helicoverpa armigera* is the target species of Bt soybean but many other arthropod species may be directly or indirectly exposed to the Bt toxins in soybean fields. Therefore, environmental risk assessments (ERA) which evaluate the risks to non-target arthropods are a compulsory component of the registration process of Bt crops. It is essential to assess the risks that Bt soybean may pose to non-target arthropod species and their community assemblages seeing that they fulfil a variety of ecosystem services such as pollination and pest control. This study had three aims, firstly, to determine whether Bt soybean has any adverse effect on non-target arthropod communities within the soybean agroecosystem, secondly, to use an ecological model to identify high priority species to test in an ERA, and thirdly, to determain the most appropriate sampling methods for arthropods in soybean fields. Arthropod sampling took place in trial plots at five locations within soybean production areas in South Africa during the 2017/18 and 2018/19 seasons. A total of 29 455 individual arthropods were recorded from 370 morphological species over two growing seasons. Results indicate that Bt soybean had no significant effect on the diversity, abundance or community composition of non-target arthropods when compared to non-Bt soybean. The ecological model which was used to prioritize species identified in the soybean agroecosystem, highlighted 31 species that could be considered as priority species, based on their abundance. However, through the use of the model, 10 species were identified by means of a selection matrix and given a rank for maximum potential exposure to the Bt toxin. Five species were given the highest rank and should be included in ERAs. The D-vac, beating sheet and yellow sticky trap sampling methods were compared to determine which method is best for sampling arthropods in soybean fields. The results suggest that the D-vac method was the most efficient for sampling the overall plant-dwelling arthropod community. The beating sheet method was the most effective for sampling Coleoptera and Orthoptera species, while the sticky traps were especially efficient for sampling small flying arthropods such as Thysanoptera, parasitic wasps and Cicadellidae. Since the different methods yielded different results, sampling methods should be used in combination. These results suggest that the D-vac be used for sampling plant-dwelling arthropods and the yellow sticky traps be used to supplement the D-vac method. The results from this study show that Bt soybean expressing Cry1Ac toxins had no effect on non-target arthropod communities in soybean trial plots in South Africa. This study provides a framework for selecting high priority species for monitoring of possible effects that Bt soybean might have on non-target arthropods in the future.

Keywords: Non-target organisms, Bt soybean, sampling methods, ecological model, Cry1Ac proteins

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1 Chapter 1: Introduction

Arthropods are an extremely diverse and abundant group of organisms, therefore it comes as no surprize that they are considered to be some of the most important organisms in the lives of human beings as well as the functioning of natural ecosystems. Biodiversity is an important aspect in both natural and agricultural ecosystems (Bond, 1989). In an agricultural setting, biodiversity of agroecosystems provide ecosystem services that are important for crop production and sustaining the surrounding environment (Jones & Snyder, 2018; Altieri *et al*., 2015; Altieri, 1999). Worldwide, chemical insecticides are used to control insect pests of soybean and other crops. These plant protection methods usually reduce the target pest numbers but also influence the community of non-target organisms.

Chemical insecticides may have adverse effects on many groups of non-target arthropods. However, genetically modified (GM) insect resistant crops that express insecticidal crystalline (Cry) proteins encoded by genes derived from the soil bacterium, *Bacillus thuringiensis* (Bt), are considered to be a safer option for non-target organisms and hold many economic and social benefits (Brookes & Barfoot, 2018; Duan *et al*., 2008). Nevertheless, there have been questions about the potential risks that GM crops might have on the environment. One of the risks commonly associated with the growing of Bt crops is their potential to adversely affect non-target organisms (Romeis *et al*., 2008, 2006). Consequently, to safely and sustainably utilize Bt technology in pest control, the possible risks of GM Bt crops need to be evaluated and studied. Prior to the approval of a Bt crop for commercial production an environmental risk assessment (ERA) needs to be done to evaluate the potential for adverse effects on non-target organism that might occur in the agroecosystem (GMO Act, DAFF 2005).

Because of the high diversity of arthropods in agroecosystems it is often necessary to select appropriate species to serve as representatives for taxonomic groups and ecologically and economically important functions for ERA's in the receiving environment (Romeis *et al*., 2008; Dutton *et al*., 2003). An Ecological model can be

used to select the most important and appropriate test species on a case-by-case basis and in this way improve ERA's of non-target arthropods on Bt crops.

It is important that suitable sampling methods are used to survey non-target arthropod species that occur in the receiving environment. Due to the sheer magnitude of arthropods that occur in local agroecosystems (Botha *et al*., 2015; Perfecto *et al*., 1997) it is impossible to accurately estimate the number of arthropods in a given habitat (Southwood & Henderson, 2000). However, it is essential that these estimates be done in such a way that it ensures proper risk assessment and long-term environmental safety (Meissle & Lang, 2005).

As the human population grows so does the demand for food. This in turn results in the intensification of agricultural practices as well as habitat loss and fragmentation, which are some of the main causes of loss of arthropod diversity (Sánchez-Bayo & Wyckhuys, 2019). Therefore, it is important that we find ways to keep up with the demand but at the same time attempt to protect the environment. Technologies such as GM Bt soybean are relatively target specific and could contribute to reduced insecticide use on this crop. However, ERA's need to be done prior to the crop being approved for commercial production in South Africa. The risk assessment process is hampered by the lack of even the most basic species checklist of soybean arthropods and knowledge gaps regarding suitable sampling methods to use.

An insect resistant genetically modified (IRGM) soybean cultivar MON87701, expressing the Cry1Ac toxins, and cultivar MON87701RR2Y, expressing both the Cry1Ac and cp4 epsps genes (herbicide tolerance) have been developed by Monsanto (St. Louis, Missouri) and was approved for commercial production in Brazil in 2011 (Yu *et al*., 2011). Yu *et al*. (2011) found that both these cultivars exhibit high levels of resistance against *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). This soybean cultivar has been approved for field trials in South Africa and is currently in the risk assessment stage.

Very little is known about the arthropod communities associated with soybean agroecosystems in South Africa. Therefore, the aims of the study were to:

- determine whether Bt soybean plants expressing Cry1Ac toxins have a significant effect on non-target arthropod communities in soybean agroecosystems,
- to use an ecological model for the selection of important arthropods for future non-target risk assessment of Bt soybean in South Africa and
- compare the efficiency of three different sampling methods for sampling arthropods in soybean fields.

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2 Chapter 2: Literature review

2.1 Soybean production in South Africa

Soybean, *Glycine max* (L.) Merr. (Fabaceae), is the most important source vegetable oil in the world covering more than 50% of the world's oilseed production (Van der Merwe *et al*., 2013). It is also a valuable source of protein feed supplements for livestock (Boerma & Specht, 2004). Soybean in South Africa is mainly used for animal feed with 60% of the total produced used for this purpose. Approximately 25% is used for protein and oil consumption and only 15% is consumed by humans in products such as infant formulas, cereals and meat products (Dlamini *et al*., 2014).

Soybean production in South Africa underwent a rough start with farmers experiencing countless production difficulties mainly due to a lack of knowledge of the crop (Dlamini *et al*., 2014). Production only started to gain momentum during the late 1990s, around the same time the GMO Act of 1997 was passed which allows for the commercialization, development and production of transgenic seeds in South Africa (Dlamini *et al*., 2014). This suggests that farmers were able to increase their soybean production due to the availability of high quality, transgenic seeds. In reaction to this increase soybean earned a place on the Industrial Policy Action Plan in 2010 (Dlamini *et al*., 2014). In the 2017/18 season approximately 787 200 ha of soybean was planted in all nine provinces of South Africa, 95% of this total were GM plants (Table 2.1), 37% more than in the 2016/17 season when 573 950 ha were planted (DAFF, 2018).

Table 2.1 Total area of soybean planted in the 2017/18 seasons per province.

Province	**Area planted**	
	2017/18	**2016/17**
Western Cape	800	700
Northern Cape	3 000	3 000
Free State	345 000	240 000
Eastern Cape	2 400	1 850
KwaZulu-Natal	40 000	30 500
Mpumalanga	310 000	241 000
Limpopo	20 000	8 500
Gauteng	30 000	25 400
North West	36 000	23 000
Total	**787 200**	**573 950**

Source: DAFF, 2018

This increase in production was driven by an increasing domestic demand for soybean and farmers being advised to consider planting the crop for the country to produce more, and consequently reduce imports thereof (ISAAA, 2017). In addition, farmers began to realize the value of soybean in crop rotation systems (Van der Merwe *et al*., 2013). It has been found that when maize is rotated with soybean it can increase maize yield by 10 to 20% (Meyer *et al.,* 2018). A study conducted by Crookston *et al.* (1991) showed that when maize and soybean are rotated annually the yields of both crops are significantly higher than when a monoculture system of either crop is used over several years. This can be ascribed to the pest control benefits of a diverse cropping system as well as a possible increase in nutrients in the soil, as soybean are legumes plants that have the ability to fix nitrogen in the soil (Tilman *et al*., 2002).

Furthermore, soybean has the potential to address nutritional problems currently facing people in South Africa. As thousands of South African households live in poverty and face malnutrition due to the rising cost of food, soybean can be utilized as a form of dietary protein that is economical and health promoting helping to curb hunger and malnutrition in these households (Van der Merwe *et al*., 2013).

2.2 Transgenic crops

With the advent of molecular gene technologies, it has become possible for scientists to move novel gene constructs that are coupled with novel promoters into crop plant genomes, creating genetically modified (GM) plants (Sansinenea, 2012). This enables the plant to express novel compounds including insecticidal substances that kill specific organisms when feeding on the transgenic crop, and the ability to tolerate glyphosate applications which allows farmers to control weeds without harming the crop. For the purpose of this study only insect resistant GM crops will be discussed.

2.2.1 Insect resistant genetically modified (IRGM) crops

Bacillus thuringiensis (Bt) is an aerobic, Gram-positive, spore-forming soil bacterium that acts as a facultative bacterial pathogen. This bacterium is easily isolated from a variety of environments including soil, insects, stored-product dust, and leaves of deciduous and coniferous plants (Schnepf *et al*., 1998). Bt has insecticidal properties toward a range of economically important insect pests with a high degree of specificity (Ferry, 2010). These properties come forward when adverse environmental conditions are present causing the bacterium to sporulate and form a spore and parasporal body. The parasporal body contains one or more insecticidal proteins in the form of crystalline inclusions, also known as insecticidal crystal proteins (ICP) or endotoxins (Sansinenea, 2012). An ICP contains crystal (Cry) and cytolytic (Cyt) proteins which are toxic to several insect orders and nematodes (Palma *et al*., 2014). Bt is also able to synthesizes insecticidal proteins in the vegetative growth stage, which are referred to as Vegetative insecticidal proteins (Vips). These proteins are secreted into the environment surrounding the bacterium and have insecticidal activities against coleopteran, lepidopteran and hemipteran pests (Palma *et al*., 2014).

Crops are given insect resistance by the artificial transfer of specific genes from the Bt bacterium that encode for the Cry or Cyt proteins (Douville *et al*., 2005). The mechanism of action of the Bt Cry proteins involves a series of events. Firstly, the protoxins need to become active, for this to happen it must be ingested by an insect (Schnepf *et al*., 1998). For most lepidopterans, protoxins are solubilized under the alkaline conditions of the insect midgut. Differences in the extent of solubilization

sometimes explain differences in the degree of toxicity amongst Cry proteins to lepidopteran larvae (Aronson *et al*., 1991). After solubilisation, protoxins must be processed by insect midgut proteases to become activated toxins, the major proteases of the lepidopteran midgut are trypsin-like or chymotrypsin-like. When the toxins are activated, they need to reach the midgut epithelial cells, where the Cry toxin receptors are located, in order to exert its toxic effects (Peterson *et al*., 2016). This follows the initial receptor-mediated binding which renders the toxin insensitive to proteases and monoclonal antibodies and induces ion channels or nonspecific pores in the target membrane which then induces death by septicaemia (Schnepf *et al*., 1998).

Bt toxins are selective and represent a class of numerous proteins with insecticidal action against pests from various orders. For example, Cry1 and Cry2 proteins are toxic to lepidopteran pests, Cry2A to both lepidopteran and dipteran pests, and Cry3 to coleopteran pests (Malone *et al*., 2009). A few toxins have an activity spectrum that spans over two or three insect orders due to the combination of toxins in a given strain (Sansinenea, 2012). Because of the high degree of specificity of Bt endotoxins for their target organisms that lessens the concern for adverse effects on non-target organisms and its safety to the environment Bt has become a valuable alternative to chemical insecticides worldwide (Sansinenea, 2012).

2.2.2 The importance of IRGM crops

As the global population grows, the amount of arable land decreases. Thus, it is crucial that strategies be implemented that allow for a more sustainable and efficient use of agricultural resources (Li *et al*., 2007). This would ensure that global food production grows along with the population but at the same time does not harm our natural resources and ecosystems (Carzoli *et al*., 2018). Bt crops are an effective tool for controlling target insect pest, they also provide many social, environmental and economic benefits therefore, making considerable contributions to reaching this goal (Brookes, 2019; Brookes & Barfoot, 2018; Wang, 2007). The importance of IRGM crops can be divided into two categories, benefits to the grower and benefits to the environment.

2.2.2.1 Benefits of IRGM crops to the grower

By planting Bt crops growers worldwide have increased their total income. The main reason for this is the fact that Bt crops lower the level of damage caused by insect pests, thus a decrease in yield loss occurs and consequently the total farm income increases (Brookes, 2019; Brookes & Barfoot, 2018). The greatest increase in farm income has been in developing countries, due to a lack of funding available to growers to allow them to apply conventional pest control methods (Brookes & Barfoot, 2014). On global level the gross farm income gains from using IRGM maize and cotton in 2016 were $8.51 billion (Brookes & Barfoot, 2018).

The planting of IRGM crops also saves fuel for growers seeing that less spray runs need to be done. Brookes & Barfoot (2010) concluded that in the global area of insect resistant cotton planting (excluding China and India) a reduction of 132 million ha between the years 1996 and 2009 was being sprayed with insecticides. This resulted in a total of 137.5 million litres of tractor fuel being saved in this period.

2.2.2.2 Benefits of IRGM crops to the environment

One of the most important benefits that Bt crop use holds for the environment is a reduction of insecticide use. According to Brookes & Barfoot (2016) the United States alone reported a 321 million kg reduction in the use of pesticide active ingredients, this is 55% of the total use of pesticides. Furthermore, China and India have also benefitted by planting IRGM cotton and have reduced insecticide active ingredient use with over 211 million kg between 1996 and 2014 (Brookes & Barfoot, 2016).

The specificity of Cry toxins (Wu *et al*., 2008) and the reduction of insecticides used under Bt crop systems (Malone *et al*., 2009) are likely to create a more favourable environment for beneficial arthropods such as pollinators and natural enemies. These beneficial arthropods such as honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), utilize crops and may be exposed to pesticides (O'Callaghan *et al*., 2005). Since Bt crop cultivation results in reduced amounts of insecticides being sprayed by grower's, honey bees have benefitted from the adoption of Bt crops. Furthermore, many studies have found that Cry toxins do not have any adverse effects on *A. mellifera* (Xie *et al*., 2019; Duan *et al*., 2008; Hanley *et al*., 2003). Natural enemies such as predators and

parasitoids seem to only be affected by Bt crops in cases where susceptible herbivores were used as host/prey in laboratory studies (Meissle *et al*., 2005; Vojtech *et al*., 2005; Baur *et al*., 2003). However, in a field study by Yu *et al*. (2014) it was reported that Bt soybean had no adverse effects on the dominant distribution of predators and parasitoids in China. Additionally, it has been found that Bt crops have little to no negative effects on arthropod communities as a whole within the cropping systems (Hernández-Juárez *et al*., 2019; Marques *et al*., 2018; Frizzas *et al*., 2017; Yu *et al*., 2014; Li *et al*., 2007; Dively, 2006). In contrast, insecticidal spray practices have been found to reduce arthropod diversity in agroecosystems (Yu *et al*., 2014).

By planting Bt crops the amount of carbon dioxide released into the atmosphere has been reduced (Brookes & Barfoot, 2010). This is due to reduced tractor runs made for spraying insecticides and the consequential reduction in the amount of tractor fuel being used (Brookes & Barfoot, 2010). For example, 1996-2009 saw a global reduction of 132 million hectares of cotton being sprayed, which resulted in a permanent reduction in carbon dioxide emissions of 378 million Kg (Brookes & Barfoot, 2010).

Overall, since the release of GM crops in 1996 an estimated 174 million ha of natural habitat has been saved due to the increased productivity of GM crops. This is an area equivalent to the size of South Africa (ISAAA, 2017). The amount should increase as more countries start to realize the benefits of planting GM crops. Moreover, the current commercial IRGM crops have reduced the impacts of agriculture on biodiversity, through enhanced adoption of conservation tillage practices, reduction of insecticide use, increasing yields to alleviate pressure to convert additional land into agricultural use as well as increasing farm income (Carpenter, 2011).

2.2.3 Transgenic crops in Africa

The South African Genetically Modified Organism (GMO) Act was passed in December 1997 (GMO Act, DAFF 2005). South Africa was the first African country to approve the commercial production of GM crops (Biosafety South Africa, 2013). In 1998 the first biotech crop was planted and up until now three GM crops have been approved for commercial production, maize, cotton and soybean. These crops have insect pest resistant traits, herbicide tolerance and in some cases both these traits.

South Africa remains on the forefront of biotech adoption in Africa. Over the last 20 years, since the successful commercialization of biotech crops, the adoption of this technology has continued to increase. In 2017 2.73 million hectares of biotech crops were planted, this showed a 2.6% increase from the 2.66 million hectares planted in 2016 (ISAAA, 2017). According to the ISAAA (2017) the average biotech crop adoption rate increased to 93%, and the report concluded that 85% of the total maize, 95% of the total soybean and 100% of the total cotton area planted in 2017 were GM. In Africa only seven countries have approved the commercial production of GM crops, South Africa, Sudan, Nigeria, Ethiopia, Burkina Faso, Egypt and Swaziland.

2.3 Agroecosystems

2.3.1 Introduction

Approximately 40% of the earth's surface has been transformed for agricultural use, which subsequently alters the composition of the worlds plant and animal populations. This alteration is caused by the replacement of the pre-existing wild vegetation by cultivated vegetation cover and the drastic modification of it by grazing of domestic livestock (Tivy, 1990). This creates a kind of man-made ecosystem referred to as an agroecosystem. According to Mongillo and Zierdt-Warshaw (2000) an agroecosystem includes all the biotic and abiotic factors and the interactions that occur between them on land used for agriculture as well as the adjacent areas that provide a habitat for native wildlife. This means that agroecosystems include populations of both wild and introduced species making it a unique system. Agroecosystems differ from wild ecosystems in that they are usually simpler with less biodiversity (Tivy, 1990). However, the most important aspect that sets agroecosystems apart from natural ecosystems is the intervention of man and the specific human-determined function of harvest production which ultimately results in the purposeful reduction of species richness (Swift & Anderson, 1993).

2.3.2 Cropping systems and the general structure thereof

Crop agriculture may involve a variety of special designs depending of the nature of the crop structure. High input monocultures (a single crop species in an area for multiple

years) have increased considerably on a global scale, due to more producers focusing on large-scale market production of their crops (Altieri, 2011).

Monoculture production on a large scale inevitably has a number of negative consequences which stretch over both the agroecosystem and the natural environment. A monoculture system is low in biodiversity and therefore, it is seen as a relatively unstable system, as greater biodiversity may result in greater stability in ecosystems (Tilman *et al*., 2002). Monoculture systems are thus more sensitive to environmental fluctuations due to their lack of trophic complexity (Tivy, 1990).

The lack of sustainability in monoculture production has led many producers to shift to more sustainable polyculture practices, this involves the cultivation of multiple plant species in one area simultaneously (Mongillo & Zierdt-Warshaw, 2000). Polycultures can take on many forms in large-scale and small-scale production, this includes: monocultures with border plantings, intercropping systems such as mixed cropping or strip grouping (Ratnadass *et al*., 2012). This may lead to improved pest control and nutrient cycling as well as water and soil conservation (Altieri, 2011).

2.4 Arthropod diversity and its importance

2.4.1 Arthropod diversity

Arthropods have been found to be the most diverse and abundant group of animals with an estimated species count of between 5 to 10 million (Ødegaard, 2000) with just over a million species being described thus far (Gullan & Cranston, 2005; Stork, 1988). According to Gullan and Cranston (2005) the five largest arthropod orders are: Coleoptera, Diptera, Hymenoptera, Lepidoptera and Hemiptera.

However, a decline in entomofauna is evident. In a recent study by Hallmann *et al*. (2017) a 27-yearlong population monitoring program revealed a 76% decline in flying insect biomass in a number of protected areas in Germany. In Puerto Rico, a biomass loss of 98% and 78% of ground-foraging and canopy-dwelling arthropods was reported over a 36-year period (Lister & Garcia, 2018). It seems that the decline in arthropods are substantially greater than those in birds or plants over the same time-period (Thomas *et al*., 2004). According to Sánchez-Bayo and Wyckhuys (2019) not only

specialists with narrow ecological requirements or restricted habitats are in decline, but also generalist insect species that are common in many countries around the world. This indicates that the cause of insect declines is not tied to particular habitats but affect common traits shared by all insects (Gaston & Fuller, 2007). The honey bee is a good example of an insect that can be found in a variety of habitats, that is not a very specific feeder and is of ecological and economical value but has been declining worldwide. In America the decline in honeybee populations has been estimated at a rate of 0.9% annually for the past six decades (Sánchez-Bayo & Wyckhuys, 2019). Furthermore, one out of six bee species in general have gone regionally extinct (Gullan & Cranston, 2005).

Although the direct cause of insect decline remains a matter of uncertainty, it is speculated that the main probable reasons for the decline are the biotic and abiotic factors discussed below.

In 49.7% of the studies review by Sánchez-Bayo and Wyckhuys, (2019) habitat change was the main driver of insect decline. Habitat change is a direct effect of human activity and is ever increasing as the human population increases. Land is being transformed for food production (agriculture), transportation facilities, to provide residences (urbanization) and for the manufacturing of goods (industrialization). Habitat change seems to affect insect populations from the Coleoptera, Hymenoptera and Lepidoptera most (Sánchez-Bayo & Wyckhuys, 2019).

The second major driver of insect decline seems to be pollution (Sánchez-Bayo & Wyckhuys, 2019). Sources of pollution include sewage and landfill leachates from urbanised areas, industrial chemicals from mining and factories and the fertilisers and pesticides used in agricultural practices, the latter being reported most frequently (in 13% of cases) as the cause of decline in the review by Sánchez-Bayo and Wyckhuys (2019). Pesticides used for insect pest control and fungicides have detrimental effects on insect populations. Herbicides contribute indirectly to the decline of insects by reducing the biodiversity of vegetation. This results in the significant decline or in some cases complete disappearance of insect species that depend on the plants (Marshall *et al*., 2003).

Factors such as parasites, pathogens and invasive species are all biological factors that contribute to the decline in insects worldwide (Sánchez-Bayo & Wyckhuys, 2019). The mite, *Varroa destructor* Anderson and Trueman (Acari: Varroidae), transmits viral infections to honey bees and pose a real concern for the apiculture industry. Although it has been associated with bees historically, the exposure of bees to pesticides weaken their immune system and increase their vulnerability to infections (Long & Krupke, 2016). Insect biological control has helped to mitigate invasive pest worldwide, however unintended ecological impacts have been recorded (Heimpel & Cock, 2018). The human-assisted introduction of exotic species for biological control can contribute to a decline of endemic insects. For example, Boettner *et al*. (2000) found that the introduction of *Compsilura concinnata* (Meigan) (Diptera: Tachinidae) has led to a decline of silk moth populations in New England. However, the practice of biological control has developed and been made safer to implement, reducing the ecological risks that an introduced species might have, by for example avoiding the introduction of generalist feeders (Heimpel & Cock, 2018). Therefore, biological control should not be viewed as a direct threat to insect biodiversity.

Climate change has also been identified as a major contributor to the decline of insects. A change in climate mostly affects the geographic distribution of insects and narrows their distribution range (Sánchez-Bayo & Wyckhuys, 2019). For instance, insects in the tropical regions have more narrow thermal thresholds and are therefore more sensitive to temperature increases.

Each insect species is part of a greater assemblage and its loss will affect the complexities and abundances of other organisms, be it producers such as plants or consumers such as birds (Gullan & Cranston, 2005). Therefore, insect decline is of the utmost importance and cannot be taken lightly. Steps need to be taken to ensure the conservation of insect species as they are substantially important to the overall functioning and stability of ecosystems worldwide (Thomas *et al*., 2004).

2.4.2 The importance of arthropods for ecosystem functioning

The sheer number of arthropods make them highly significant components of the environment and in the lives of human-beings. Some insect species are considered

keystone species seeing that the loss of their ecological function may lead to the collapse of the wider ecosystem or food chain (Gullan & Cranston, 2005).

Ecosystem services are ecological functions or processes that are provided by nature that benefit humans (Breeze *et al*., 2011; Porter *et al*., 2009; Schellhorn *et al*., 2015). These ecosystem services can be classified into four main categories (Kremen & Chaplin-Kramer, 2007; Zhang *et al*., 2007). These are: 1) provisioning services that produce goods such as food and water, 2) regulating services that regulate essential processes such as climate control and food protection, 3) cultural services that provide aesthetic and recreational opportunities such as tourism, and 4) supporting services that form the basis on which all the other services depend, for instance soil production (Fig. 2.1).

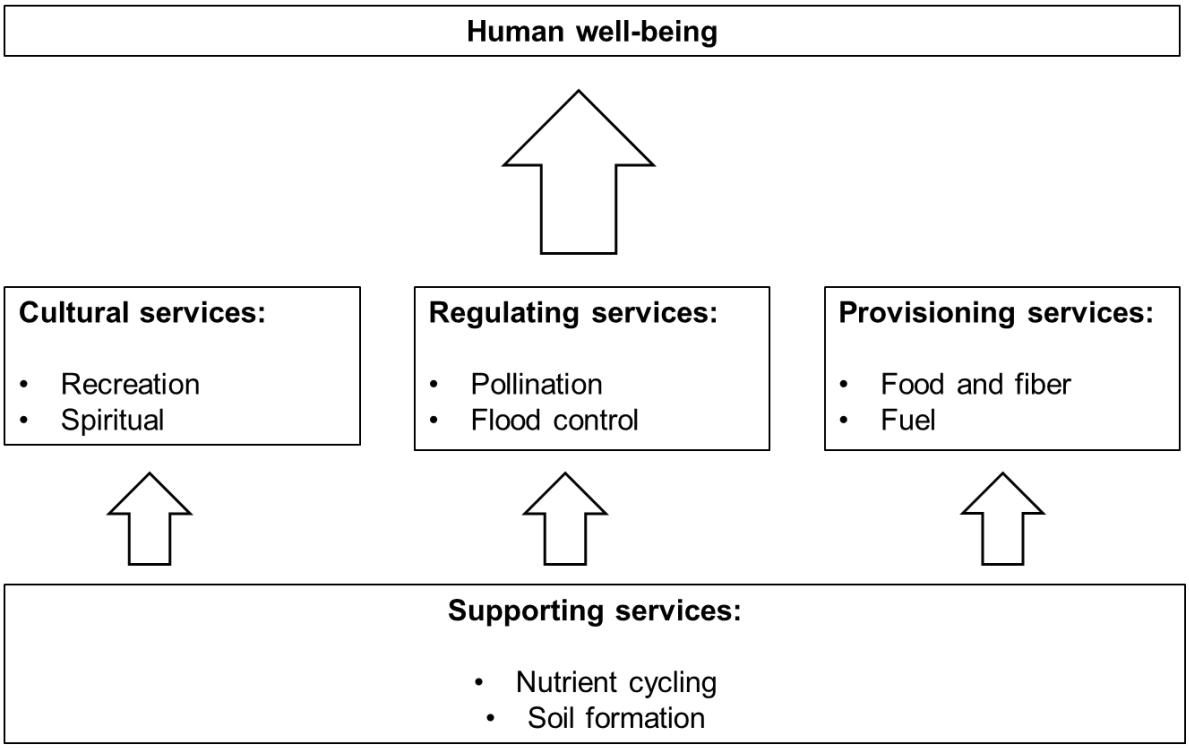

Fig. 2.1. Classification of ecosystem services. Supporting services serve as the bases for the other three classes and they all contribute to human well-being (Zhang *et al*., 2007).

Arthropods are responsible for many important ecosystem services. They are vital for nutrient cycling, for instance the disposal of dung by dung beetles, plant propagation through services such as pollination and seed dispersal by bees and ants, they act as a major food source for many animals and they also regulate and maintain animal community structure through the transmission of diseases to large animals and act as biological pest controlling agents when they predate or parasitize on economically important pests (Gullan & Cranstan, 2000).

One of the most important and well-known ecosystem services provided by arthropods is pollination. It is estimated that one third of human food supply (Jolivet, 1998) and more than 65% of the worlds angiosperm plant species rely directly on insect pollination (Kremen & Chaplin-Kramer, 2007). These pollinators do not only contribute to food security but are also of great economic importance. It is estimated that the value of bee pollination services in the USA alone reach up to 16 billion USD annually (Losey & Vaughan, 2006).

Some arthropods provide pest control services by predating and parasitizing pest species. Globally, pesticide expenditure reaches US$ 30 billion annually, a third of this is on insecticides alone (Kremen & Chaplin-Kramer, 2007). Insect pests are a serious threat to the economy and food security destroying 37% of potential crops in the USA annually (Pimentel *et al.,* 1992). For this reason, arthropods are considered to be of major functional importance for the maintenance of ecosystems and thus the survival of the human population.

2.4.3 Arthropod functional groups

Although all species are unique, there is a degree of similarity among species in terms of their contribution to ecosystem processes (Brussaard, 1998). For instance, some species exert similar functions and could replace each other to some degree when one species disappears from an area (Brussaard, 1998). However, it has been shown that it is critical to maintain multiple species that exert a specific function (Swift & Anderson, 1993). Such groups of species are termed functional groups (Moore *et al*., 1988).

Arthropods can be grouped according to different functional traits. The most common grouping used is according to food habits, for instance, predators, parasitoids, pollinators, detritivores, sap-sucking herbivores and chewing herbivores (Frizzas *et al*., 2017).

Predators may pray on insect pest species in agricultural settings and help to reduce their numbers (Meissle *et al*., 2005). Important predatory arthropods found in agroecosystems are Araneae, some dipterans, hemipterans such as Anthocoridae (*Orius* sp.), Geocoridae and Reduviidae and coleopterans such as Coccinellidae (Naranjo, 2005; Ponsard *et al*., 2002; Zwahlen *et al*., 2000). Parasitoids are arthropods that parasitize on other organisms by for instance, laying eggs on top of or inside of their hosts. Like predators, parasitoids are valuable in agroecosystems as the may parasitize on economically important insect pests and reduce their numbers (Wolfenbarger *et al*., 2008). The most common parasitoids are from the hymenopteran families, Ichneumonidae and Braconidae.

Pollination is the transmission of pollen from the anthers to the stigma of the same plant species (Eardley *et al*., 2010). A pollinator is the agent that transfers the pollen which determines the reproduction success of pollination dependent plants (Shivashankara *et al.,* 2016). Invertebrates provide about 85% of animal pollination to crops with bees being recognised as the most important pollinator species (Breeze *et al*., 2011).

Detritivorous arthropods are mostly associated with the soil, they aid in the degradation of organic materials such as crop residue which improves soil health, and rotting fruits or decaying carcasses (Bitzer *et al*., 2005).

Sap-sucking herbivores and chewing herbivores are arthropods that feed on plant material, for instance aphids and lepidopteran larva. Not all herbivorous arthropods are regarded as pest species seeing that the damage, they cause to the crop may not reach the economic threshold level, meaning that the damage is not economically meaningful. Many herbivorous arthropods may be beneficial to the cropping system by feeding on non-crop plants and thus reduce competition (Wolfenbarger *et al*., 2008).

2.5 Arthropod diversity in agroecosystems

Agriculture directly affects a considerable proportion of insect species. The type and abundance of biodiversity in agriculture differs across agroecosystems when they differ in age, diversity, structure and management practices (Altieri, 1999). Agroecosystems cover large parts of terrestrial land area and subsequently its contribution to biodiversity is critical for successful conservation in the future (Tscharntke *et al*., 2005). According to Southwood and Way (1970) biodiversity of agroecosystems depends on four characteristics: 1) the diversity of the vegetation in and around the agroecosystem, including weeds and crop plants, 2) the permanence of the crops in the agroecosystem, 3) the intensity of the management, for instance how often and to what extent the soil is disturbed, and, lastly 4) the extent of the isolation of the agroecosystem from natural vegetation.

Moreover, the diversity within agroecosystems can be classified based on the role it plays in the system. According to Swift and Anderson (1993) the biodiversity of agroecosystems can be grouped as follows: 1) productive biota: these are the elements chosen by the farmer for instance the crops planted, trees and animals, this can be seen as the planned biodiversity of the system, 2) resource biota: the organisms that contribute to production through, for instance, pollination, decomposition and biological control, these can be seen as the associated biodiversity, they colonize the agroecosystem from the surrounding environment but their survival there is dependent on the management and structure of the system (Fig. 2.2) 3) destructive biota: all organisms that threaten the productivity of the system, insect pests, weeds and pathogens.

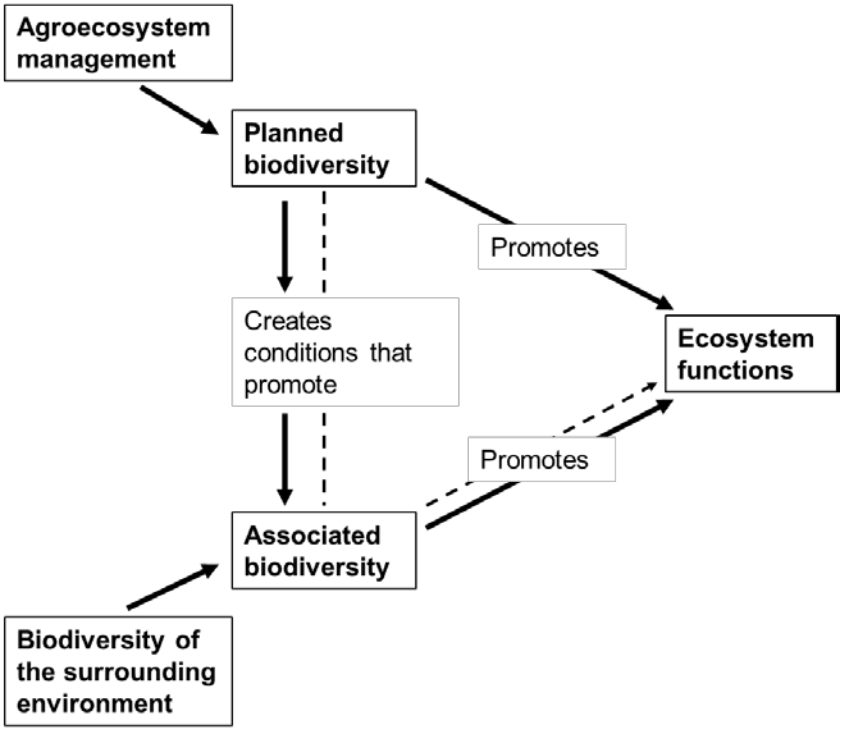

Fig. 2.2. The relationship between the planned biodiversity and associated biodiversity and how they promote ecosystem functions (Altieri, 1999).

In the past much of the focus on biodiversity in agroecosystems has been on the conservation of rare species (Weibull *et al*., 2002). Recently the issue of whether or not an increased biodiversity or species richness enhances ecosystem services such as primary productivity or biological control and pollination has received a lot of attention (Weibull *et al*., 2002). For instance, Hector *et al*. (1999) found that there was an overall linear reduction of average aboveground biomass with loss of plant species. However, many factors impact on the diversity of arthropods in agroecosystems.

2.5.1 **Factors determining arthropod diversity in agroecosystems**

Environmental factors associated with crop fields have an influence on the diversity, abundance and activity of arthropod communities (Altieri & Nicholls, 1999). These factors include microclimate, intra- and interspecific competition, food availability and habitat requirements, all of which are affected by the nature of the cropping system. The most important factors that determine the diversity of arthropods in agroecosystems are the type and diversity of vegetation in and around the agroecosystem, the permanence

of the crop, the type and intensity of management and the extent of isolation of the agroecosystem from natural vegetation (Altieri & Nicholls, 1999). Botha *et al*. (2015) found that an increase in the number of plant species lead to an increase in arthropod species numbers while comparing arthropod and plant community assemblages in maize fields, field margins and natural areas in South Africa. Therefore, it seems that a greater diversity of plants in agroecosystems lead to a greater diversity of arthropods in the system. Siemann *et al.* (1998) also reported that an increase in both plant species richness and functional plant richness resulted in an increased arthropod diversity in old fields situated in east-central Minnesota, USA.

The type of vegetation in the field margin may influence the arthropod community composition in agroecosystems. A study by Meek *et al*. (2002) showed that different mixtures of plant types in the margin influenced the overall composition of arthropod communities with flowery treatments hosting more groups of arthropods. This was probably due to an increase in food resources. Furthermore, the nature of the vegetation may influence the microclimate cropping system and may provide shelter to a number of arthropod species (Altieri, 1999). Crop field margins may also play a vital role in maintaining arthropod biodiversity as it provides arthropod reservoirs from which arthropods colonize the crop during the growing season. It was reported that five times more arthropod species are found in the field margins than in the arable fields during the winter months (Pfiffner & Luka, 2000).

Semi-perennial and perennial crops provide a more stable habitat than annual crops and therefore provide greater support for biodiversity (Stary & Pike, 1999). Annual monocultures, such as maize or soybean fields, seem to be the most difficult environments for biodiversity to persist in, since these systems often lack the necessary resources, are present only for part of the year and are managed by methods that damage the natural vegetation and the natural enemy population in the system (Stary & Pike, 1999). These environments are particularly challenging for relatively immobile arthropod groups such as Collembola, Elateridae and Acari to persist in.

The degree of isolation of the crop from natural vegetation may greatly influence the composition and diversity of non-intentional diversity in the system (Altieri & Nicholls,

1999). Clough *et al*. (2005) found a higher diversity of spiders near crop field edges that were close to natural vegetation compared to field interiors, therefore, wheat fields interspaced by uncultivated field margins may have greater unintentional diversity since the crop centers are closer to field margins and are therefore more accessible to living biota.

Management practices that negatively influence diversity in agroecosystems are mostly practices such as tillage and the application of agro-chemicals such as fertilizers and pesticides (Wardle *et al*., 1999). Agronomic practices such as type and timing of tillage, and mowing have complex effects on the physical, chemical and biological environment of the soil (Kladivko, 2001). Tillage practices have the ability to influence species composition, diversity, and the biomass of arthropods. A study by House and Stinner (1983) in soybean agroecosystems found that ground beetle abundance, diversity and biomass was significantly higher in no-tillage than conventional tillage systems. Furthermore, the study revealed higher densities and diversity of most soil macro-arthropods and higher diversities of foliage-dwelling arthropods in no-tillage systems. These findings were ascribed to the structural diversity of the system provided by weeds and crop residue (House & Stinner, 1983).

Large volumes of pesticides that are commonly used in agroecosystems may affect the field margin flora and fauna along with crop field biodiversity (Marshall & Moonen, 2002). Pesticides have been found to be the cause of decline in moths in rural areas of the U.K. (Hahn *et al*., 2015) and pollinators in Italy as well as beneficial ground-dwelling and foliage-foraging insects (Sánchez-Bayo & Wyckhuys, 2019). Pesticides have detrimental effects on arthropod communities and may lead to large scale die-offs.

2.6 Non-target organisms

2.6.1 What are Non-target organisms?

Non-target organisms (NTO's) are organisms that are not the intended target of the specific plant protection method but are still affected by it. Genetically modified crops with insecticidal activity have been used to control important insect pests (their target species) with great success and many economic and social benefits (Brookes, 2019,

Brookes & Barfoot, 2018, 2016, 2010; Wu *et al*., 2003). Bt crops expressing Cry proteins are selective and have a narrow target species range, decreasing concerns for non-target effects (Malone *et al.,* 2009; Li *et al*., 2007). Nevertheless, concerns have been raised about the potential risks that GM crops might have on the environment. One of the risks commonly associated with the growing of Bt crops is their potential to adversely affect NTO's particularly non-target arthropods (Romeis *et al*., 2008, 2006).

2.6.2 The effects of GM Bt crops on non-target organisms.

2.6.2.1 The effects of GM Bt crops on beneficial NTO's

Beneficial NTO's such as honeybees often feed on the nectar of cotton or the pollen of maize even though these crops are not pollinated by bees (O'Callaghan *et al*., 2005). In this way honey bees are exposed to pesticides that have been applied to the crops (O'Callaghan *et al*., 2005). The adoption of Bt crops has been beneficial to honey bee populations as it has reduced the frequency of pesticide application (Johnson *et al.,* 2010). Studies conducted in Canada, France and the US found no substantial evidence that Bt crops negatively affects honey bees (Johnson *et al.,* 2010). One example of such a study was conducted by Ramirez-Romero *et al.* (2005) where Cry3b proteins were fed to honey bees at concentrations of 1000 times higher than they would typically be exposed to in the wild, even these high dosages of Bt proteins did not have any effects on the honey bees.

Predators and parasitoids are important regulators of insect pest populations but may be affected directly or indirectly by IRGM crops as their survival depends on the supply of host insects. Natural enemies could be affected directly by ingestion of GM pollen, plant tissue or active recombinant protein in the bodies of their prey or hosts (O'Callaghan *et al*., 2005). The indirect effects could result from prey being smaller, sick or less palatable after having fed on GM plants (O'Callaghan *et al*., 2005). This complexity has meant that establishing cause and effect of GM plants on natural enemies has been difficult and the interpretation of results must be done with caution. Another method may be to compare the effects of GM plants on natural enemies to that of the effects of conventional pest control methods (chemical insecticide spray practices) (O'Callaghan *et al*., 2005).

2.6.2.2 The effects of GM Bt crops on arthropod diversity and abundance

Arthropod communities as a whole provide critical services within ecosystems. The loss of these services provided by arthropod communities may have detrimental effects such as the collapse of entire ecosystems, action needs be taken to avert the detrimental loss of arthropod communities (May, 2010). Concerns exist for potential adverse effects of Bt crops to arthropod communities (Romeis *et al*., 2008, 2006). It is therefore important to evaluate the risks of a Bt toxin to arthropod communities in the receiving environment (Andow & Hilbeck, 2004a). Several Bt crops have been approved for cultivation on a commercial scale including soybean, maize, cotton, cowpea, eggplant, poplar, potato, sugarcane and tomato (ISAAA, 2017). A summary of the effects of the most important Bt crops being cultivated on arthropod communities are discussed below.

Bt soybean have been approved for commercially cultivation since 2010 and are currently approved in six countries and cultivated on 24.2 million hectares (ISAAA, 2017). A number of studies on the effects of Bt soybean on arthropod communities have been done. A study by Yu *et al*. (2014) exploring the possible effects of Bt soybean expressing Cry1Ac toxins on arthropod communities under field conditions, found no significant differences of diversity, richness or dominance indices for Bt soybean compared with conventional soybean. Furthermore, the study revealed no negative effects on the dominant distribution of functional groups, including sucking pests, other pests, predators, parasitoids and others except for lepidopteran pests (Yu *et al*., 2014). Thus, no negative effects of Cry1Ac soybean on arthropod communities in soybean fields in China was detected. A field study examining the potential for adverse effects of Bt soybean expressing Cry1Ac and Cry1F proteins on non-target arthropod communities found no significant difference in abundance and diversity of representative non-target arthropods. A community analyses and repeated measures ANOVA indicated that the Bt soybean did not alter the structure of the non-target arthropod communities (Marques *et al*., 2018).

A field study by Meissle and Lang (2005) revealed that Bt maize in Germany had no substantial effects on species richness and abundance of spiders, whereas insecticide

application reduced spider densities. It seems that Bt crops may even increase the abundance of some beneficial insects and consequently better the natural control of certain pest species (Yu *et al*., 2011). Truter *et al*. (2014) found no significant differences in functional guilds, diversity or abundance of arthropods in Bt and non-Bt maize fields over two growing seasons in South Africa. A field experiment conducted over three years in Queenstown, Maryland USA found that maize expressing stacked lepidopteran-active VIP3A and Cry1Ab proteins had no effects on the biodiversity and densities of non-target arthropod communities when compared to non-Bt maize (Dively, 2006). A long-term field plot study in Arizona, USA, in which the effects of Bt cotton producing Cry1Ac toxins was evaluated on 22 taxa of plant-dwelling arthropod natural enemies, found no long-term effects of Bt cotton over multiple generations (Naranjo, 2005). In Spain, Bt maize expressing Cry1Ab toxin was found to have no adverse effects on the abundances of predatory arthropods in the agroecosystem (De la Poza *et al*., 2004). In Mexico the effects of Bt maize expressing Cry1Ab, Vip3Aa20 and mCry3A toxins on three non-target predator species were evaluated and Bt maize was found not to have adverse effects on the abundance and frequency of the predators (Hernández-Juárez *et al*., 2019). In Brazil Frizzas *et al*. (2017) found Bt maize expressing Cry1Ab toxins to have no effects on arthropod communities based on species richness, diversity and evenness indices. The possible effects of Bt maize expressing Cry3Bb1 protein on non-target ground-dwelling (Bhatti *et al*., 2005a) and foliage-dwelling (Bhatti *et al*., 2005b) arthropods were evaluated in Illinois, USA over a three-year period. The studies found no consistent adverse effects on the abundance of any non-target ground- or foliage-dwelling arthropods when compared to the non-Bt isoline.

Bt cotton has been cultivated since 1996 (Tabashnik *et al*., 2013). A short-term (one growing season) field study by Fernandes *et al*. (2007) found that Bt maize expressing Cry1Ab and VIP3A did not cause a reduction in plant-dwelling predators and parasitoids in Brazil. Candolfi *et al*. (2004) found Bt maize expressing Cry1Ab protein to have no adverse effects on the non-target soil- and plant-dwelling arthropod communities within the maize agroecosystem in France over the short-term. In Australia a three-year study comparing the canopy invertebrate community of Bt cotton expressing Cry1Ab, unsprayed and sprayed conventional cotton, found that the diversity of non-target

communities was reduced in the sprayed conventional cotton, and a slight difference occurred between the Bt and unsprayed conventional cotton (Whitehouse *et al*., 2005). However, the most consistent differences between Bt and unsprayed conventional cotton communities were higher numbers of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae in conventional cotton. The effects of Bt cotton expressing Vip3A on non-target beneficial arthropod communities were also evaluated in Australia (Whitehouse *et al*., 2007). The latter study found no major differences in either species richness or diversity of beneficial non-target arthropod communities on conventional and Vip3A cotton. Furthermore, a three-year field study in Georgia USA investigated the effects of Bt cotton on ground-dwelling predatory arthropods and found Bt cotton to have no adverse effects on predator abundance (Torres & Ruberson, 2005).

A three-year field study by Li *et al*. (2007) on Bt rice under paddy field conditions assessed the arthropod guild dominance, family composition, dominance distribution of each guild, individuals of each guild and community indices. The study found no significant differences between Bt rice expressing the Cry1Ab gene and control rice plots in these arthropod community-specific parameters (Li *et al*., 2007). A field study to evaluate the effects of Bt rice expressing Cry1Ab toxins on the aboveground non-target arthropod community during the postharvest season in China found no significant differences among Bt and non-Bt rice plots in all arthropod community-specific parameters (Bai *et al*., 2011).

Overall, declines in insecticide use are associated with the increasing adoption of Bt maize and cotton, and GM crops may have a reduced impact on non-target organisms relative to current pest management practices (Wolfenbarger *et al.*, 2008).

2.7 Environmental risk assessment of non-target arthropods

The Cartagena Protocol on Biosafety (Biosafety Protocol) under the Convention on Biodiversity (CBD) identifies a need for pre-release testing and post-release monitoring of transgenic plants to ensure their environmental safety and sustainable use (Andow & Hilbeck, 2004b). Therefore, before a GM crop is approved for commercial production in South Africa it must undergo vigorous trials and testing to ensure the safety of the organism to the environment, a process called an environmental risk assessment (ERA)

(GMO Act, DAFF 2005). One of the major ecological concerns regarding the environmental risks of IRGM plants is their potential impacts on non-target arthropods (Yu *et al*., 2011). This applies specifically to whether the transgenic crop could possibly affect non-target arthropods in a negative or positive manner and if this will lead to noticeable fluctuation in the population size of the organisms and have major impacts on the natural or agroecosystems (GMO Act, DAFF, 2005).

A risk assessment is a process by which risks are identified and the seriousness of these risks are characterized to ensure appropriate decisions can be made on whether and how to proceed with the technology (Andow & Hilbeck, 2004a). Therefore, it is important that risk assessments are done as efficiently and effectively as possible, by using the best model for non-target risk assessments, to avoid regulatory hampering of the technology (Raybould, 2007). One way to avoid such hampering of the technology is to use a tiered approach to the ERA. This entails a problem formulation, risk hypotheses and then testing (Romeis *et al*., 2008). The process starts with lower-tier tests which usually include laboratory tests, this is then followed by semi-field, glasshouse and field tests which act as the higher-tier tests (Yu *et al*., 2011).

Before any of the tiered tests can be performed it is necessary to select appropriate species for evaluation. These species serve as representatives for taxonomic groups (Romeis *et al*., 2008; Dutton *et al*., 2003). Species that are most likely to be exposed to the Bt toxins should be selected for evaluation, since a risk only exists if a possibility for exposure to the toxin exists (Romeis *et al*., 2008). Furthermore, the selected species should represent different ecological functions and include species that are threatened or endangered and species with cultural value (Yu *et al*., 2011; Romeis *et al*., 2008; Andow & Hilbeck, 2004b). Several models can be used to select the most important and appropriate test species, two of these are: the ecotoxicology model and the ecological model.

The ecotoxicology model for non-target risk assessment aims to evaluate the potential non-target effects of chemicals released in the environment (Andow & Hilbeck, 2004a). This model aims to report on acute responses or mortalities from short-term exposure to a chemical, these responses are simple to evaluate (Andow & Hilbeck, 2004a).

However, these responses disclose little about other ecological impacts on population, community and ecosystem level, also because transgenic plants release a continuous dose of the toxin, it is essential to evaluate long-term exposures that consider the multiple chemical alterations occurring within transgenic plants (Andow & Hilbeck, 2004b). This model also makes us of universal indicator species which are chosen to provide information on the likely effects of the chemicals on a wider range of species. These species are chosen because of their supposed sensitivity to chemical toxins, their extensive availability, ease of culture and genetic uniformity (Andow & Hilbeck, 2004b). This is insufficient for evaluating non-target effects of transgenic plants because these risk assessments need to be done on a case-by-case basis to bring into consideration the specific transgenic plant and environment in which it will be used (Elmegraad & Jagers op Akkerhuis, 2000). Another shortcoming of the selection of universal indicator species is that these species are often not present in the environment where the transgenic plants will be grown (Van Wyk *et al*., 2007).

The ecological model for non-target risk assessment on transgenic crops is a model that takes into consideration the specific transgenic crop as well as the relevant environment (Andow & Hilbeck, 2004b). The ecological model relies on ecological principles to select species to test and specify end points and develop assessment protocols (Andow & Hilbeck, 2004b). This model minimalizes costs through focusing only on a few non-target species it also addresses uncertainties by choosing relevant species that are found within the receiving environment (Andow & Hilbeck, 2004b). The species selection in the ecological model is case specific and follows four steps. 1) Functional groups are established, this is done by taking into consideration the ecological role or function of the organism in the environment. This helps to focus the testing on critical ecological processes and to limit the number of species to be tested, this saves costs and time (Andow & Hilbeck, 2004a). 2) The non-target species found in the relevant environments are then classified into these functional groups, this inclusion of species that occur in the region where the transgenic crop will be planted creates a case-specific set of potential non-target species (Andow & Hilbeck, 2004a). 3) Prioritizing species on the basis of ecological principles, criteria commonly used to prioritize these non-target species are found in

Table 2.2. 4) High-priority species are selected for testing. Andow & Hilbeck (2004a) suggests that a number of species form each functional group be included for testing. The ecological model can thus be tailored for specific environments making it suitable for environmental risk assessments of non-target organisms on transgenic crops.

Once the test species have been selected evaluations through the tiered testing procedure can begin. The lower tier tests serve to identify potential hazards under worst conditions. Often when testing for an effect of Bt toxins protein concentrations that are 10 to 100 times higher than those present in the plant tissue are used. If no adverse effects are observed in this tier it most likely indicates that no risk exists and thus no further evaluations need to be done (Romeis & McLean, 2011). If adverse effects were observed or uncertainties exist higher tier tests should be done (Yu *et al*., 2011). Higher tier tests confirm whether an effect still exists under more realistic circumstances and provide more ecological information (Romeis *et al*., 2008).

Table 2.2. The criteria most commonly used to prioritize non-target species on the basis of ecological principles (Andow & Hilbeck, 2004).

Criteria used to prioritize non-target species in each functional group.	
Maximum possible exposure	The maximum possible exposure of a non-target organism to a transgenic crop. This is based on geographic range, habitat specificity, temporal association with the crop, local abundance and prevalence. Species that have a large geographic range, high abundance and prevalence and have a high temporal overlap with the crop as well as a high specificity to the crop are likely to have greater exposure.
Potential adverse effects	If the species ecologically or economically important, is rare or has symbolic value the potential consequences of an adverse effect from a transgenic plant is considered to be more serious.
Potential exposure	Species that are not exposed directly or indirectly are less likely to be affected by the transgenic crop.

2.8 Biodiversity sampling techniques

Due to the magnitude of arthropods it is impossible to accurately count all the arthropods in a given habitat (Southwood & Henderson, 2000). It is however necessary to estimate the population, this can be done by sampling.

The sampling methods used need to be strictly standardized in order to attain reproducible results (Duelli *et al*., 1999). No single sampling technique is effective for sampling all taxa, consequently the technique used will depend on the purpose of the survey (Samways, 1995). Furthermore, better results might be obtained if a combination of different techniques that are suitable to the specific study are used (Yi *et al.,* 2012).

2.8.1 Beating method

Beating is a method of arthropod sampling that makes use of a beating tray, usually a cloth-covered frame, which is held under a tree or plant whilst beating the overhead vegetation with a stick. This causes the arthropods that are present to fall into the beating tray and can then be collected (Southwood & Henderson, 2000). This method works well in woody habitats.

In agricultural environments a ground cloth method is often used to sample arthropods from crops (Kogan & Pitre, 1980). This method works by forcefully displacing the arthropods from the crop plants by vigorously shaking the plant in order to dislodge the arthropods from the plant. This will result in the organisms falling onto a cloth, which has been spread out on the ground between two rows of the crop and can be collected. This method is not sufficient for arthropods that have quick escape reactions and that can escape by flying away (Kogan & Pitre, 1980).

2.8.2 Suction sampling

This method of sampling makes use of a suction apparatus. A number of different machines have been designed to collect arthropods from vegetation by means of suction. One such machine was developed by Dietrick *et al.* (1961) and is referred to as the D-vac machine, which was the first to become commercially available. Several adapted versions of the suction sampling devices exist (Zou *et al*., 2016). The D-vac method implies the use of a hose which is covered with a mesh sock or bag which

captures the arthropods and prevents them from being sucked into the fan. Suction machines have been used extensively to sample terrestrial arthropods in many different habitats including agricultural crops (Elliott *et al*., 2006).

Despite being bulky and inconvenient for travel and use in remote areas the D-vac machine has been shown to be very effective in arthropod sampling (Yi *et al.,* 2012). Southwood and Henderson (2000) stated that comparisons of the D-vac machine with others have revealed an efficiency of around 50-70%. However, suction sampling does have shortcomings and is not effective in sampling Coleoptera and Linyphiidae (Araneae) in grasslands (Standen, 2000). It has been found to give good results for leafhoppers, small flies, small lepidopterous larvae, nymphs, Hemiptera and Araneae (Chen *et al*., 2006; Meissle & Lang, 2005; Buffington & Redak, 1998).

Although suction devices have shortcomings in sample efficiency and ease of handling, it is still considered to be one of the best techniques for sampling large areas quickly and with sufficient results (Yi *et al*., 2012).

2.8.3 Trap sampling

Many different trap sampling techniques have been developed and used in agroecosystems. Pitfall traps are the most common trap sampling method used in agroecosystems (Duelli *et al*., 1999). It can be used to sample a variety of ground-dwelling arthropods. The method usually consists of an open topped plastic or metal cup that is buried in the with the rim level to the soil surface (Southwood & Henderson, 2000). The cup is then filled with a solution, often ethanol, and left for a predetermined time period. Arthropods that walk on the soil surface fall into the trap and are unable to escape (Southwood & Henderson, 2000). This method is easy to use and low cost but is limited to catching ground-dwelling arthropods.

Sticky traps are a form of fight interception trapping and traps can be colored specifically to attract a certain taxa or groups of arthropods, and this consequently combines flight interception with attraction (Yi *et al.,* 2012). Yellow sticky traps have been shown to be as good or better than other colored cards for most natural enemies (Musser *et al*., 2004). A sticky trap usually consists of a rectangular shaped cardboard

of a specific size that is coated with a sticky glue (Yi *et al.,* 2012). Arthropods settle or impact with the adhesive surface of the trap and are unable to free themselves (Southwood & Henderson, 2000).

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3 Chapter 3: Arthropod species diversity and composition in soybean agroecosystems: a comparison between Bt and non-Bt treatments

Abstract

Genetically modified soybean, *Glycine max* (L.), expressing *Bacillus thuringiensis* (Bt) toxins, could pose a risk to non-target arthropods within the soybean agroecosystem. It is therefore, important to study these possible effects to allow for risk assessments to be done before Bt soybean is release for commercial production in South Africa. Arthropod communities were assessed in Bt- and non-Bt soybean field trials over two growing seasons to determine whether Bt soybean influenced the arthropod communities. Arthropods were collected by means of an adapted D-vac method, a beating sheet method and yellow sticky trap. The arthropod diversity, abundance and community composition was then calculated. The effects of the treatments, locations and sampling times on the arthropod communities were evaluated by means of t-tests and nonmetric multidimensional scaling (nMDS). The results showed that the arthropod community composition was not affected by treatments but was significantly influenced by location and in some cases by sampling time. The results indicate that transgenic Cry1Ac soybean had no effect on the diversity, abundance or community composition of non-target arthropod communities in soybean field plots in the short-term.

Key words: Arthropods, diversity, soybean, agroecosystems, community composition, transgenic crops.

3.1 Introduction

Genetically modified (GM) crops that express Cry proteins derived from soil bacterium, *Bacillus thuringiensis* (Bt), have been planted in many countries since their commercialization in 1996 (ISAAA, 2017). Such crops are widely used to control major lepidopteran and coleopteran pests and can play an important role in integrated pest management (IPM) systems (Romeis *et al.,* 2018; Musser & Shelton, 2003). GM soybean cultivars such as Intacta RR2 Pro (event MON87701), expresses Cry1Ac protein for insect resistance to the lepidopteran pest *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Bt crops in general provide for many social and economic benefits such as increased farm income, decreased environmental pollution due to reduced insecticide spray applications (Brookes, 2019) and target specificity which reduces concerns for possible non-target impacts (Malone & Burgess, 2009; Li *et al*., 2007).

Even though Bt soybean that express Cry1Ac protein is reported to be lepidopteran specific (Yu *et al*., 2011; Höfte & Whiteley, 1989), general concerns exist about the biosafety of Bt crops and the possible effect of these on non-target arthropods. Furthermore, there is a potential for non-target organisms (NTO's) such as non-target arthropod communities (Yu *et al*., 2014), soil organisms (Fan *et al*., 2019) and aquatic organisms (Rosi-Marshall *et al*., 2007) to be exposed to the Bt toxins. Consequently, prior to the approval of a Bt crop for commercial production, an environmental risk assessment (ERA) needs to be done to evaluate the potential for adverse effects on NTO's that might occur within the agroecosystem (Craig *et al*., 2008).

Many non-target arthropods may be beneficial and fulfill important ecosystem services such as pollination and pest control (Hilbeck & Schmidt, 2006) which may be adversely affected if changes in these communities occur (Dutton *et al*., 2002). On the other hand, non-target organisms may become secondary pests that increase in numbers and gradually evolve into key pests when insecticide use is reduced (Yu *et al*., 2011). It is therefore, important to understand the potential impacts of Bt crop cultivation on non-target arthropod populations and to conduct ERAs for insect resistant crops (Ba *et al.,* 2018; Van Wyk *et al*., 2007; Romeis *et al*., 2004). It is essential that the non-target risk

assessments that accompany GM crops be science-based and case-specific, rather than generalizing results from one case to another. It is also important to consider the specific transgene, target organism and the receiving environment (Andow & Hilbeck, 2004).

Several previous laboratory studies using purified protein, and/or leaf feeding bioassays in direct or indirect exposure assays have not demonstrated any adverse effects of Cry1Ac on non-target arthropods (Obrist *et al*., 2006; Lundgren & Wiedenmann, 2005; Ponsard *et al*., 2002). For example, nymphal survival and development rate as well as adult size and weight of *Orius majusculus* (Reuter) (Heteroptera: Anthocoridae) were not affected when fed Bt plant material and pollen (Pons *et al*., 2004). Also, a number of field surveys have determined the safety of Bt toxins across a number of GM events including cotton, maize and rice (Truter *et al*., 2014; Li *et al*., 2007; Lopez *et al*., 2005; Sisterson *et al*., 2004; Musser & Shelton, 2003). Where differences in non-target populations between Bt and non-Bt crops did occur, it could be attributed to a reduction in target pest abundances or the quality thereof as prey or host to predators and parasitoids (Lawo *et al*., 2010; Chen *et al*., 2008; Baur *et al*., 2003; Dutton *et al*., 2002). However, some studies have demonstrated that adverse effects on non-target organisms occurred when exposed to Bt toxins (Kramarz *et al*., 2009; Rosi-Marshall *et al*., 2007; Castaldini *et al*., 2005; Losey *et al*., 1999). For instance, Han *et al*. (2010) found that the feeding behavior of honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), was disturbed during a seven-day oral exposure to cotton pollen from plants expressing Cry1Ac and CpTI toxins.

While it is already commercially available in several countries including, Brazil (Marques *et al*., 2018), China (Yu *et al*., 2014) and Argentina (ISAAA, 2017), the cultivation of Bt soybean in South Africa is currently restricted to experimental areas. Studies that assess the possible effects of Bt soybean on non-target arthropods are required by the registrar of the GMO act (GMO Act, DAFF 2005), prior to approval of GM hybrids for commercial release. Most field studies assessing the potential impacts of Bt crops on NTO's focus only on a limited number of species (Mellet *et al*., 2006; de la Poza *et al*., 2004; Musser & Shelton, 2003), and assessments of the impact of Bt crops on the

environment is hampered by the lack of basic knowledge regarding arthropod diversity in agroecosystems (Truter *et al*., 2014).

In this study the possible effects of Bt soybean which express Cry1Ac proteins on non-target arthropod communities was evaluated over two cropping seasons and compared to that in non-Bt soybean. The diversity and richness of arthropods were quantified in terms of community structure indices and multivariate analyses. This study contributes to knowledge regarding arthropod communities in soybean agroecosystems in South Africa. Additionally, this is the first study investigating the effects of Bt soybean on non-target arthropod communities in Africa.

3.2 Materials and methods

3.2.1 Study sites / General methods

This study was conducted between January and May of the 2017/18 and 2018/19 cropping seasons in confined soybean field trial plots located in four of South Africa's major soybean production provinces (Gauteng, Mpumalanga, Free State and Northern Cape) (Fig. 3.1).

Fig. 3.1 Nearest towns for each study site in four of the soybean production provinces in South Africa. The study sites were, Bethal, Fouriesburg, Bothaville, Jan Kempdorp and Nigel.

During the 2017/18 season, field trials were successfully planted and sampled at all five study localities. During 2018/19 however, the Bothaville study location could not be planted due to drought conditions.

The study consisted of two treatments, a Bt and non-Bt soybean cultivar. The Bt soybean used was Intacta RR2 Pro which is a stacked event with event MON87701 expressing Cry1Ac protein for insect resistance, and MON89788, which provides herbicide tolerance. The target pest of this Bt event is *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). The non-Bt soybean cultivar (GTS 40-3-2) with herbicide tolerance (Roundup Ready) was used as the control treatment.

A graphical presentation of the trial plots is presented in Fig. 3.2. The study design was a randomized complete block. The experiment was replicated five times (study sites) in the 2017/18 season and four times in the 2018/19 season, with six replications (treatment plots) at each site. Treatment plots consisted of six 5-m rows of soybean at a plant rate of 350 000 to 400 000 plants per hectare. During the growing period, crop management was done according to common local agricultural practices.

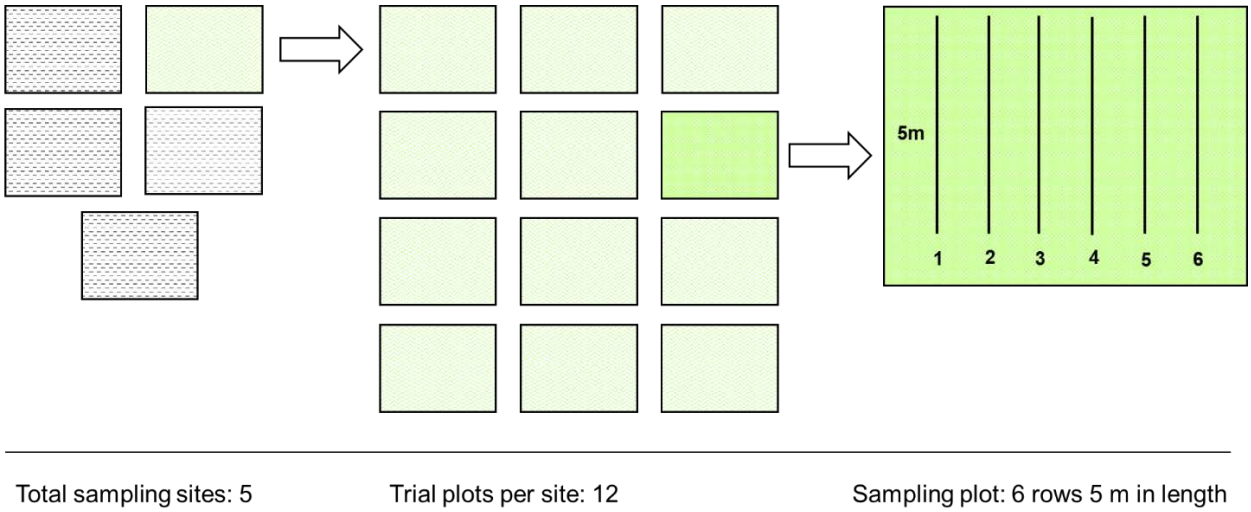

Fig. 3.2 Diagram indicating the experimental layout. The study was done at five sites. Each site contained 12 sampling plots, each comprising six 5-m long rows of soybean.

Fig. 3.3 Examples of treatment plots, each with six 5-m long rows of soybean at a plant rate of 350 000 to 400 000 plants per hectare.

3.2.2 **Arthropod sampling**

Three sampling methods were used in this study. These were the beating sheet method, an adapted D-vac method and yellow sticky traps. Sampling took place during three plant growth stages, i.e., pre-flowering (between V6-V12), flowering (between R1 to R3) and post-flowering (R4 to R7) (Table 3.1). All arthropods collected were preserved in 70% ethanol and transported to the laboratory where the samples were cleaned, and arthropods removed from debris (Fig. 3.5). All specimens were then identified to family level and assigned to morphospecies according to their morphological appearance (Oliver & Beattie, 1996). A photo reference collection of the different morphospecies was compiled to ensure that morphospecies collected at the different sites were the same “species” (Fig. 3.5). As the sampled arthropods were assigned morphospecies status, photos were taken and stored in a computer database according to order and family. This was then consulted to identify the morphospecies at other sites and in the following season.

The number of morphospecies and their abundance per site was determined. The arthropods were then further classified into major functional groups or functional groups

based on their feeding habits. These functional groups were herbivores, predators, parasitoids, pollinators and detritivores.

Table 3.1 Plant growth stages at which the different sampling methods were used. The numbers refer to the sampling occasion that took place during the specific growth stage.

	Plant growth stage		
Method	**Pre-flowering (between V6-V12)**	**Flowering (between R1-R3)**	**Post-flowering (between R4-R7)**
Beating sheet	1		2
D-vac	1	2	3
Sticky trap		1	

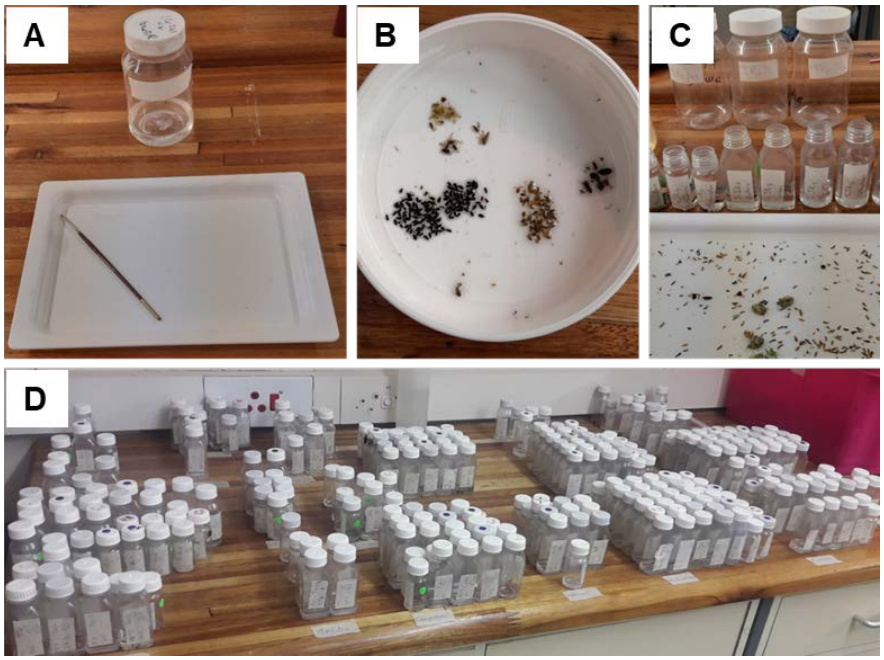

Fig. 3.4 A) D-vac samples were cleaned and arthropods placed into bottles containing 70% ethanol until further sorting. B) Arthropods were then sorted into morphospecies. C) Specimens were then placed in smaller bottles according to morphospecies, sampling site and treatment. D) Bottles containing different morphospecies from different families and orders.

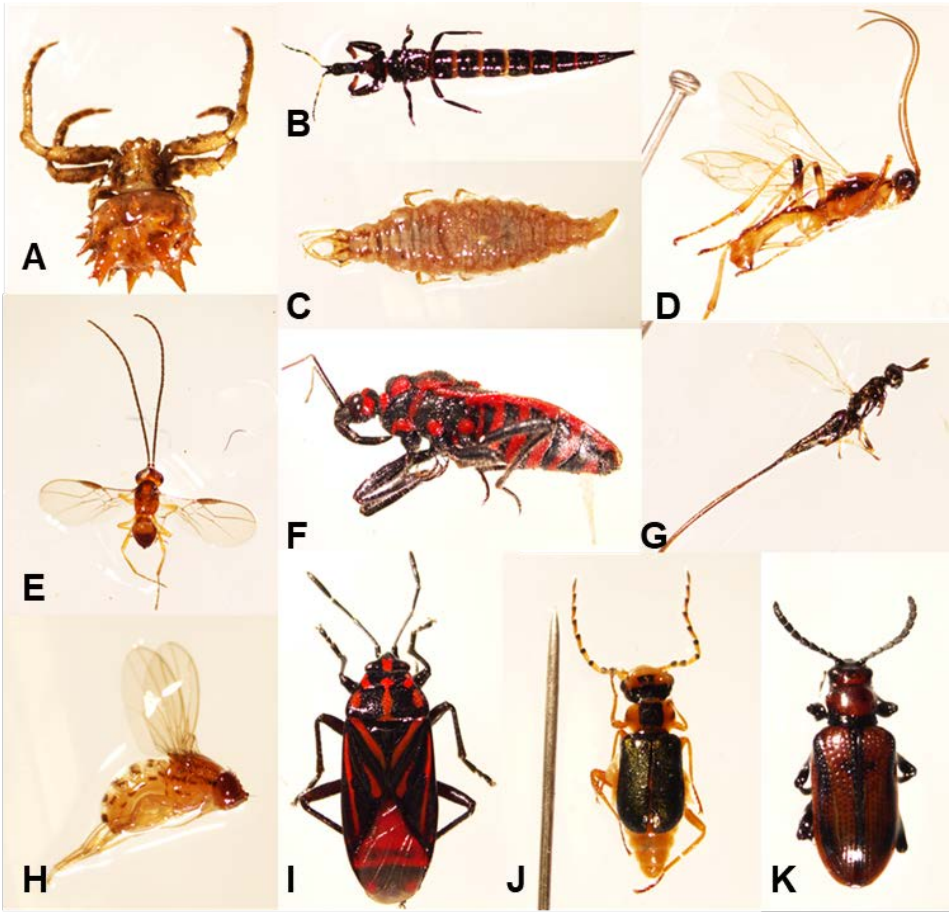

Fig. 3.5 An example of photos used as references for assigning morphospecies. A) Araneidae MS 1, B) Thysanoptera MS 6, C) Chrysopidae MS 1, D) Braconidae MS 14, E) Braconidae MS 24, F) Reduviidae MS 2, G) Chalcidoidea MS 8, H) Drosophilidae MS 1, I) Lygaeidae MS 3, J) Anthicidae MS 3, K) Chrysomelidae MS 2.

3.2.2.1 Beating sheet

This method is commonly used to sample arthropods from row crops (Kogan & Pitre, 1980). A 1-m^2 cloth was placed between the middle two rows of each sample plot (Fig. 3.6). The plants on both sides of the cloth were then vigorously shaken towards the direction of the cloth to dislodge any arthropods which would then drop onto the cloth. All arthropods on the cloth were transferred into 70% ethanol until further identification could take place.

The beating sheet method was used twice at three of the sites (Fouriesburg, Bothaville and Nigel) during the 2017/18 cropping season, resulting in six sampling occasions over two seasons. Sampling took place during two plant growth stages (Table 3.1).

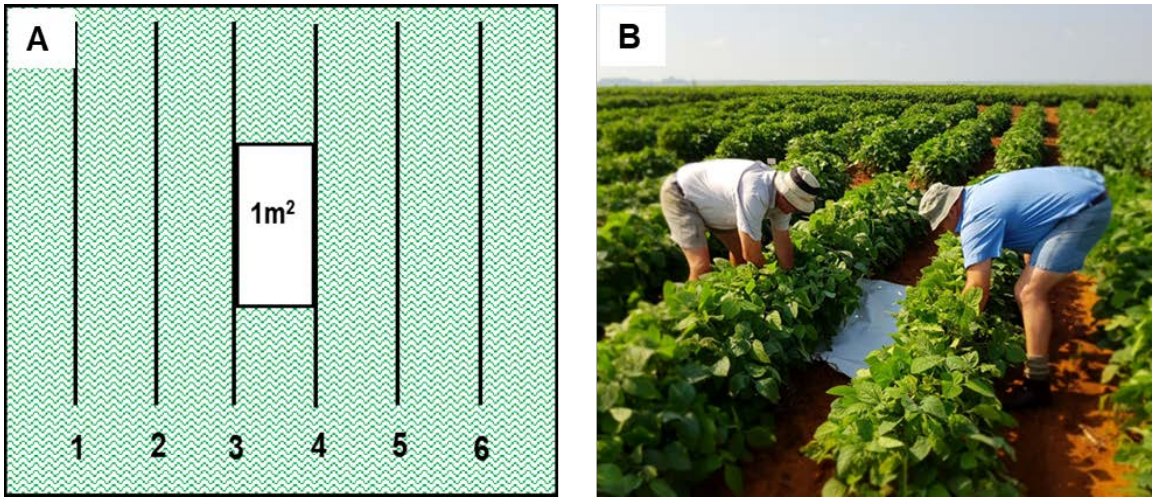

Fig. 3.6 A) Diagram indicating how the sheet was placed between rows three and four of each sampling plot. B) Illustration of the beating sheet method. A white sheet was placed between two rows and the plants were shaken to dislodge arthropods.

3.2.2.2 Suction sampling

Suction sampling of arthropods with an adapted D-Vac method (Dietrick *et al*., 1960) was conducted in rows two and five of each plot at each sampling site during both cropping seasons (Fig. 3.7). Sampling took place during each of the three different plant growth stages (Table 3.1). A 3-m long section of each of rows two and five were sampled on both sides of the row, the nozzle of the D-vac machine was moved slowly over the surface of the plants in upward and zigzag movements to ensure that the upper two thirds of each plant were sampled. The sampling took place three times at each of the five study locations in the 2017/18 season and at four of the sites in the 2018/19 season, resulting in 28 sampling occasions. The samples from each plot were placed in separate plastic bags and frozen for preservation until further analyses could take place.

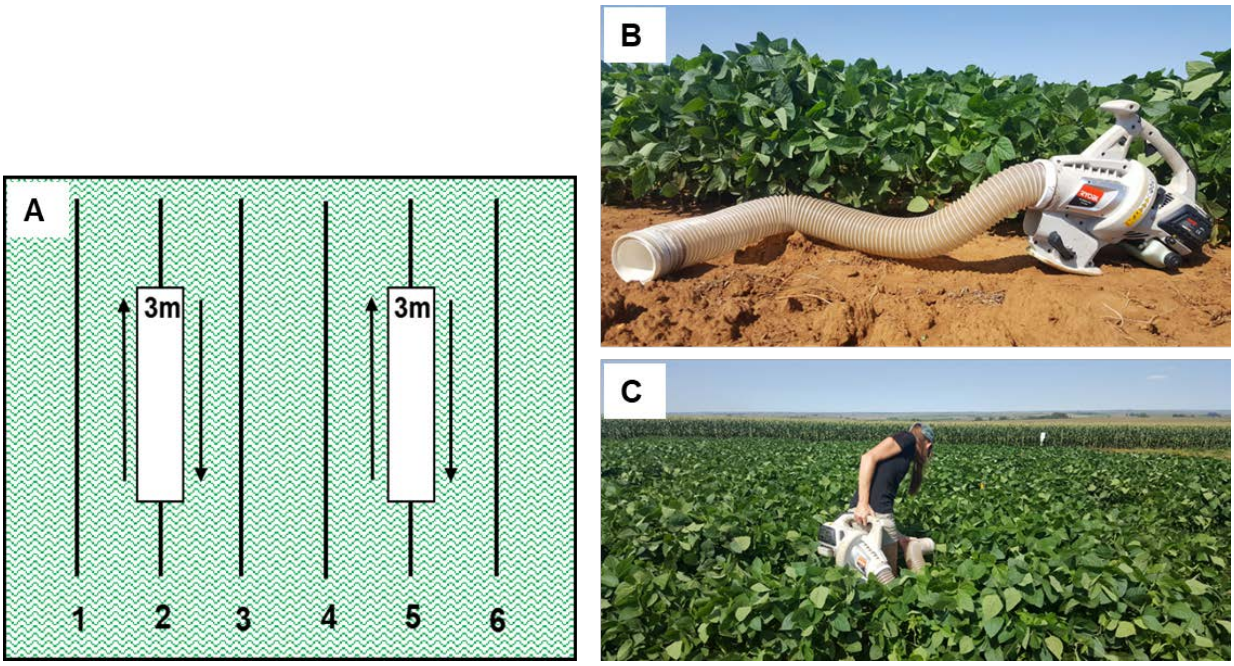

Fig. 3.7 A) Diagram indicating the three-meter-long sections of rows two and five that were sampled on both sides of the row with the D-vac machine. B) The adapted D-vac machine used to sample arthropods. C) D-vac sampling in process.

3.2.2.3 Yellow sticky trap sampling

Two yellow sticky traps were set up in each plot, between rows one and two, and five and six (Fig. 3.8), at each study site in the 2017/18 season, and in four of the study locations in the 2018/19 season, during flowering (Table 3.1). The traps were hung at canopy height and left for a seven-day period after which they were removed and covered with plastic cling-wrap for preservation.

Nineteen morphological groups were counted on each sticky trap since it is difficult to identify small arthropods due to the fact that the specimens get damaged on the glue (Table 3.2).

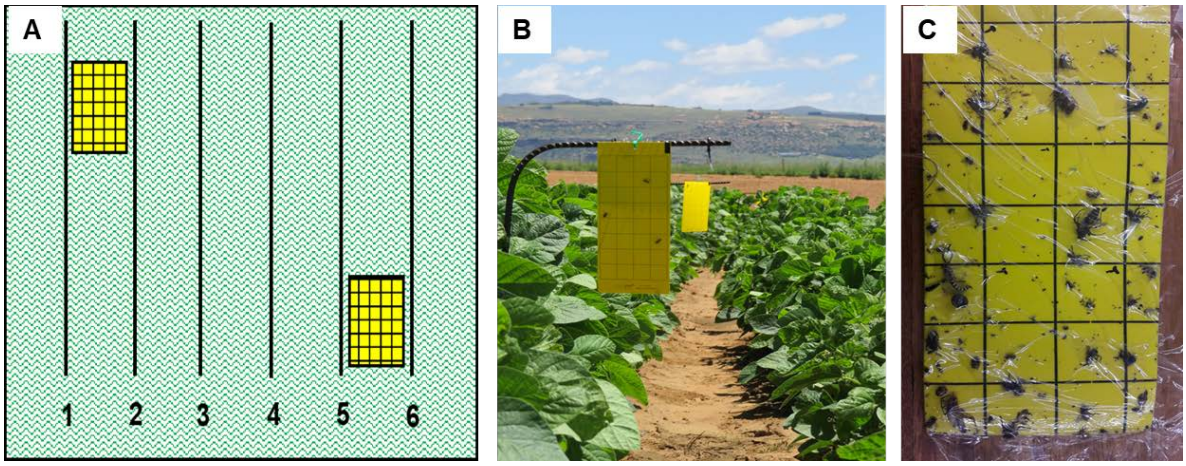

Fig. 3.8 A) Diagram indicating the positions of sticky traps within in each trial plot. B) Sticky traps were hung at canopy height. C) After seven days sticky traps were removed and covered in plastic cling wrap for preservation.

Table 3.2 Morphological groups counted on sticky traps.

Morphological group	**Description**
Araneae	All spiders
Cicadellidae	Leafhoppers
Coreidae	Leaf-footed bugs
Orius spp.	Minute pirate bugs
Miridae and Lygaeidae	Plant and seed bugs
Pentatomidae	Stink and shield bugs
Astylus atromaculatus	Spotted maize beetle
Coccinellidae	Ladybeetles
Chrysomelidae	Leaf beetles
Neuroptera	Lacewings
Asilidae	Robber flies
Syrphidae	Hover flies
Cynthia cardui	Painted lady butterfly
Other lepidopterans	All other lepidopteran adults
Apis mellifera	Honey bee
Large wasps	All large non-parasitic wasps
Parasitic wasps	All parasitic wasps
Other hymenopterans	All other hymenopterans
Thysanoptera	Thrips

3.2.3 **Data analysis**

3.2.3.1 Species diversity

Species diversity and richness indices were used to compare the arthropod communities between the non-Bt and Bt treatments. These indices take both abundance and species richness (S) (the total number of species recorded) into account (Begon *et al.*, 2008) and are used in combination to provide a complete picture of species richness and diversity, since they display different aspects of species diversity (Botha *et al*., 2015). The following indices were used:

Margalef's species richness: **(d) = (S-1)/ln N:** This index (d) is a species richness measure that compensates for the sampling effects by dividing richness (S) by the total number of individuals in a sample (N) (Magurran, 2004).

Simpson Diversity Index: **($\check{D}$) = Σ [n_i (n_i – 1)/N (N – 1)] – 1:** This index describes the probability of individuals from a community to belong to the same species. It is also known as a dominance or evenness measure as it is weighted towards abundances of the most common species rather than species richness (Magurran, 2004). Therefore, the index value will increase with an increase in evenness in a given sample.

Shannon-Wiener Diversity: **(H') = -Σ p_i ln (p_i):** This is the most popular diversity index used in literature and can thus easily be used to compare between studies (Samways, 1984). This index condenses species richness and evenness into a single figure which sometimes make the interpretation difficult since an increase in the index value may be attributed to greater species richness, evenness or both (Botha *et al*., 2015). The Shannon-Wiener index tends to weigh towards species richness and is also dependent on sample size (Magurran, 1988).

Pielou evenness: **(J') = H'/H'max = H'/ln S:** Also known as the Shannon evenness measure, it is derived from the Shannon-Wiener diversity index and it uses the ration of observed diversity to maximum diversity to measure evenness (Pielou, 1975). This index was used to determine evenness of dominance patterns in the dataset. The index values range from 0 to 1.0, with 1.0 indicating complete evenness (Magurran, 2004).

Index values were calculated by means of PRIMER 6 software (Clarke & Gorley, 2006). To test for significant differences in diversity, evenness and richness between the two treatments and the six study sites, *t*-tests were used.

3.2.3.2 Species composition

Arthropod species composition was evaluated by means of non-metric Multidimensional Scaling (nMDS) based on the Bray-Curtis Dissimilarity Index. Analysis was performed in PRIMER 6 software (2012) (Clarke & Gorley, 2006). nMDS is a commonly used multivariate technique in arthropod community composition analysis (Wimp *et al*. 2005), which gives a comprehensive view of abundance, richness and family identity (Shepard, 1962). The Similarity Percentage Analysis (SIMPER) was applied using PAST computer software (Hammer *et al*., 2001) to identify the key species that contributed to the compositional differences between the assemblages of locations and sampling occasions in the nMDS analysis. A Non-Parametric One-Way Analysis of Similarities (ANOSIM) of pair-wise comparisons was used to test for significant differences in species composition between the two treatments (Clarke, 1993). Permutational MANOVA (PERMANOVA) was applied with PRIMER 6 to support the multivariate analysis.

3.3 Results and discussion

3.3.1 Descriptive data for arthropods in soybean trial plots

Overall, 29 455 individual arthropods were recorded over the two growing seasons (17 479 in 2017/18 and 11 976 in 2018/19) by means of the D-vac and beating sheet methods. The numbers of arthropods collected by means of the D-vac and beating sheet were 23 823 and 5 632 respectively. A total of 370 morphospecies from 15 orders were recorded. The species richness and abundance for each study location is provided in Table 3.3. The most dominant orders were Coleoptera, Hemiptera, Diptera, Hymenoptera, Thysanoptera and Lepidoptera. Furthermore, 82 families were identified, of which the most diverse are listed in Table 3.5. The Chalcidoidea and Braconidae made the largest contribution and represented 33 and 28 morphospecies respectively of the total number of species, a combined total of 16.5%. Also, the leaf beetles (Chrysomelidae) and weevils (Curculionidae) made

large contributions with a total of 16 morphospecies each (8.6%). A breakdown of species richness and abundance of each sampling site for the D-vac and beating sheet methods is provided in Table 3.3. The yellow sticky traps yielded a total of 114 927 arthropods over the two cropping seasons (82 402 in 2017/18 and 32 525 in 2018/19) from the 19 morphospecies or groups (Table 3.6). The Thysanoptera were the most abundant with a total of 94 484 (82.21%). The parasitic wasps also made out a large portion of the total abundance with 7 420 (6.45%) individuals.

The arthropods collected with the beating sheet- and D-vac method were separated into five functional groups. A total of 20 290 herbivores, 3 209 predators, 3 060 parasitoids, 1 923 detritivores and 416 pollinators were sampled while 557 individuals could not be assigned to a functional group (Fig. 3.9 a). Herbivores were the most diverse group of arthropods contributing 34% of the total number of morphospecies (Fig. 3.9 b). The predators were second most diverse group (26%).

The overall distribution of morphospecies and individuals in terms of sampling methods and plant growth stages are given in Table 3.7. For the beating sheet method, the first sampling occasion (1: pre-flowering) yielded the highest abundance but the second sampling occasion (2: flowering) had a higher species richness. For the D-vac method in 2017/18 the first sampling occasion yielded the highest abundance and the third sampling occasion (3: post-flowering) yielded the highest species richness. In the 2018/19 cropping season however, the third sampling occasion had the highest abundance as well as the highest species richness. The sticky traps were only set out during one growth stage in both seasons and the 2017/18 cropping season yielded the highest abundance and species richness.

Table 3.3 Species richness and abundance of arthropods sampled by means of beating sheet and D-vac method at five sites over two growing seasons.

Location	Species richness (S)	Abundance (N)
Bethal	192	4 574
Fouriesburg	183	3 002
Nigel	230	6 774
Bothaville	153	9 901
Jan Kempdorp	199	5 204

Table 3.4 Total number of arthropod morphospecies and total abundances for each of the orders identified.

Order	Number of morphospecies	Abundance
Total	370	29 455
Hymenoptera	111	2 544
Coleoptera	78	12 705
Hemiptera	62	6 168
Araneae	41	640
Diptera	37	3 384
Lepidoptera	13	1 711
Orthoptera	8	129
Thysanoptera	6	1 942
Blattodea	3	3
Neuroptera	3	47
Dermaptera	2	6
Psocoptera	2	4
Collembola	2	95
Mantodea	1	1
Ephemeroptera	1	76

Table 3.5 The ten most diverse arthropod families. Families with the same number of morphospecies were given the same ranking.

Rank	Order	Family	Number of morphospecies
1	Hymenoptera	Chalcidoidea	33
2	Hymenoptera	Braconidae	28
3	Coleoptera	Chrysomelidae	16
3	Coleoptera	Curculionidae	16
4	Hemiptera	Lygaeidae	14
5	Araneae	Salticidae	12
6	Hymenoptera	Apidae	11
7	Coleoptera	Coccinellidae	10
8	Coleoptera	Anthicidae	9
8	Hymenoptera	Ichneumonidae	9
8	Diptera	Muscidae	9
8	Araneae	Thomisidae	9
9	Hemiptera	Miridae	8
10	Hemiptera	Cicadellidae	7
10	Hymenoptera	Formicidae	7

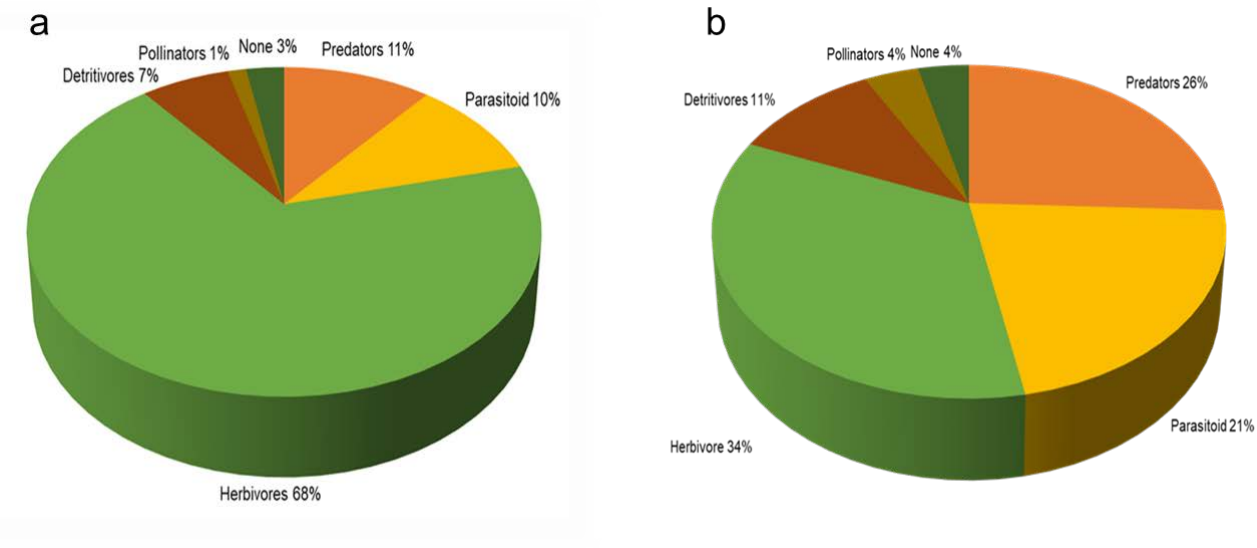

Fig. 3.9 Pie charts showing the functional group composition in terms of (a) individuals and (b) morphospecies identified in each functional group sampled from soybean trial plots by means of the beating sheet and D-vac methods.

Table 3.6 The abundance of each morphological group counted on the yellow sticky traps for each season.

Morphological groups	2017/18	2018/19	Total
Thysanoptera	70 523	23 961	94 484
Parasitic wasps	4 680	2 740	7 420
Cicadellidae	2 066	2 813	4 879
Other Hymenoptera	2 632	452	3 084
Miridae and Lygaeidae	521	575	1 096
Astylus atromaculatus	383	568	951
Orius spp.	302	637	939
Chrysomelidae	224	319	543
Syrphidae	480	11	491
Araneae	182	161	343
Coccinellidae	170	81	251
Large wasps	45	110	155
Other lepidopterans	47	53	100
Asilidae	91	6	97
Apis mellifera	30	11	41
Neuroptera	9	21	30
Coreidae	6	2	8
Nymphalidae	8	0	8
Pentatomidae	3	4	7

Table 3.7 The overall division of morphospecies and individuals in terms of sampling times (1st, 2nd and 3rd sampling) and method of sampling.

		1st sampling		2nd sampling		3rd sampling	
Method	**Season**	**Abundance**	**Diversity**	**Abundance**	**Diversity**	**Abundance**	**Diversity**
Beating	2017/18	4 968	52	664	89	-	-
D-vac	2017/18	5 454	155	3 005	117	3 388	197
	2018/19	2 499	139	3 487	171	5 990	217
Sticky							
traps	2017/18	-	-	82 402	19	-	-
	2018/19	-	-	32 525	18	-	-

3.3.2 Arthropod species diversity patterns in soybean trial plots

Species richness, abundance and community indices (Shannon-Wiener index, Simpson's index, Margalef's index and Pielou evenness index) were calculated for the arthropod community and functional groups and then compared between non-Bt

and Bt treatments. There were no significant differences between the non-Bt and Bt treatments for any of the above-mentioned indices (p>0.05) (Table 3.8, Table 3.9, Table 3.10, Table 3.11, Table 3.12, Table 3.13, Table 3.14, and Table 3.15). When functional groups were considered no significant differences were found between the two treatments for any of the functional groups in terms of species richness, abundance, Shannon-Wiener diversity and Pielou's evenness indices (Table 3.9). Pollinators were not included in this table since only one pollinator was sampled by means of the beating sheet method. The diversity index results for the D-vac method in the 2017/18 and 2018/19 cropping seasons respectively are given in Table 3.10, Table 3.11, Table 3.12 and Table 3.13. Overall, no significant differences were found between the non-Bt and Bt treatments for any of the indices. Only the abundances were taken into account when the arthropod data sampled with the sticky traps were analysed. In both cropping seasons the Bt treatment yielded a higher abundance but no significant differences were found between the two treatments (Table 3.15).

Table 3.8 Total number of species and individuals collected, richness, diversity and evenness indices and significance of the *t*-test (p=0.05) for the treatments non-Bt and Bt soybeans of the beating sheet method in the 2017/18 cropping season.

	Treatment		**p-value**	***t*-value**	**df**
	Non-Bt	**Bt**			
Species richness (S)	80	77	0.85	0.18	10
Abundance (N)	2 989	2 643	0.96	-0.05	10
Margalef richness index (d)	9.87	9.64	0.84	0.20	10
Shannon-Wiener diversity index (H')	1.12	1.37	0.98	-0.01	10
Simpson (1-Lambda)	0.35	0.43	0.94	-0.07	10
Pielou evenness index (J')	0.25	0.31	0.95	-0.05	10

Table 3.9 Total number of species and individuals collected, richness, diversity and evenness indices and significance of the *t*-test (p=0.05) of functional groups for the treatments non-Bt and Bt soybeans of the beating sheet method in the 2017/18 cropping season.

	Species richness (S)					Abundance (N)					Shannon-Wiener (H')					Pielou evenness (J')				
Functional groups	**Non-Bt**	**Bt**	**p-value**	**-value**	**df**	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**
Herbivores	36	38	0.94	0.07	10	2 583	2 197	0.90	0.12	10	0.46	0.64	0.74	0.33	10	0.12	0.17	0.70	0.38	10
Predators	28	25	0.87	0.16	10	133	141	0.92	-0.09	10	2.70	2.70	0.97	0.03	10	0.81	0.84	0.84	0.19	10
Parasitoids	3	4	0.87	0.16	10	3	6	0.97	-0.09	10	1.09	1.32	0.97	0.03	10	1.00	0.95	0.84	0.19	10
Detritivores	8	5	0.40	0.86	10	20	9	0.40	0.87	10	1.69	1.52	0.51	0.67	10	0.81	0.94	0.88	-0.15	3

Table 3.10 Total number of species and individuals collected, richness, diversity and evenness indices and significance of the *t*-test (p=0.05) for the treatments non-Bt and Bt soybeans of the D-vac method in the 2017/18 cropping season.

	Treatment		**p-value**	***t*-value**	**df**
	Non-Bt	**Bt**			
Species richness (S)	221	201	0.20	1.28	28
Abundance (N)	6 370	5477	0.19	1.31	28
Margalef richness index (d)	25.11	23.23	0.22	1.24	28
Shannon-Wiener diversity index (H')	3.47	3.36	0.33	0.97	28
Simpson (1-Lambda)	0.88	0.89	0.93	0.77	28
Pielou evenness index (J')	0.64	0.63	0.86	-0.17	28

Table 3.11 Total number of species and individuals collected, richness, diversity and evenness indices and significance of the *t*-test (p=0.05) of functional groups for the treatments non-Bt and Bt soybeans of the D-vac method in the 2017/18 cropping season.

	Species richness (S)					Abundance (N)					Shannon-Wiener (H')					Pielou evenness (J')				
Functional groups	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**
Herbivores	75	71	0.68	0.41	28	3 416	3 698	0.78	0.28	28	2.35	2.29	0.59	0.53	28	0.54	0.53	0.66	0.43	28
Predators	65	53	0.10	1.66	28	722	506	0.44	0.77	28	3.15	3.08	0.29	1.07	28	0.75	0.77	0.80	-0.24	28
Parasitoids	48	45	0.31	1.02	28	775	641	0.51	0.65	28	2.69	2.44	0.63	0.47	28	0.69	0.64	0.67	0.42	28
Detritivores	22	20	0.43	0.78	28	418	425	0.95	-0.05	28	2.26	2.17	0.93	0.07	28	0.73	0.72	0.29	-1.05	27
Pollinators	6	8	0.68	-0.14	28	134	201	0.47	-0.73	28	0.39	0.24	0.70	-0.38	25	0.21	0.11	0.80	-0.24	28

Table 3.12 Total number of species and individuals collected, richness, diversity and evenness indices and significance of the *t*-test (p=0.05) for the treatments non-Bt and Bt soybeans of the D-vac method in the 2018/19 cropping season.

	Treatment		**p-value**	***t*-value**	**df**
	Non-Bt	**Bt**			
Species richness (S)	222	216	0.85	0.18	22
Abundance (N)	6 910	5 066	0.87	0.15	22
Margalef richness index (d)	24.99	25.20	0.82	0.22	22
Shannon-Wiener diversity index (H')	3.65	4.08	0.74	0.32	22
Simpson (1-Lambda)	0.93	0.96	0.58	0.55	22
Pielou evenness index (J')	0.67	0.75	0.76	-0.30	22

Table 3.13 Total number of species and individuals collected, richness, diversity and evenness indices and significance of the *t*-test (p=0.05) of functional groups for the treatments non-Bt and Bt soybeans of the D-vac method in the 2018/19 cropping season.

	Species richness (S)					Abundance (N)					Shannon-Wiener (H')					Pielou evenness (J')				
Functional groups	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**
Herbivores	84	81	0.40	0.84	22	4 611	2 885	0.20	1.29	22	2.67	3.09	0.40	-0.84	22	0.60	0.70	0.20	-1.30	22
Predators	58	61	0.64	-0.46	22	891	896	0.98	-0.01	22	2.77	2.70	0.94	-0.06	22	0.68	0.65	0.84	0.19	22
Parasitoids	45	45	0.80	-0.25	22	856	779	0.87	0.16	22	2.54	2.78	0.68	-0.41	22	0.66	0.73	0.55	-0.59	21
Detritivores	28	27	1.00	0	22	545	504	0.76	0.29	22	2.48	2.62	0.85	0.18	22	0.74	0.79	0.79	0.26	22
Pollinators	4	7	0.31	-1.02	22	46	34	0.68	0.41	22	0.59	1.02	0.56	-0.58	22	0.42	0.52	0.28	-1.09	22

Table 3.14 Total number of species and individuals collected, richness, diversity and evenness indices and significance of the *t*-test (p=0.05) of functional groups for the Bt and non-Bt soybean plots sampled with both the beating sheet and D-vac methods.

	Species richness (S)					Abundance (N)					Shannon-Wiener (H')					Pielou evenness (J')				
Functional groups	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**	**Non-Bt**	**Bt**	**p-value**	***t*-value**	**df**
Herbivores	108	109	0.48	0.69	64	11 510	8 780	0.47	0.71	64	2.31	2.40	0.92	0.09	64	0.49	0.51	0.92	-0.08	64
Predators	80	75	0.42	0.80	64	1 700	1 509	0.62	0.49	64	3.12	3.05	0.77	0.28	64	0.71	0.70	0.56	-0.58	64
Parasitoids	68	62	0.69	0.38	64	1 634	1 426	0.69	0.38	64	2.96	2.93	0.86	0.17	64	0.70	0.71	0.69	-0.39	51
Detritivores	37	32	0.56	0.57	64	984	939	0.83	0.20	64	2.71	2.66	0.70	0.37	64	0.75	0.76	0.68	-0.40	56
Pollinators	7	12	0.29	-1.04	64	180	236	0.58	-0.54	64	0.46	0.44	0.59	-0.53	64	0.23	0.17	0.14	-1.55	12

Table 3.15 Total abundance collected by means of sticky trap method for the non-Bt and Bt soybean treatments. Significance of the *t*-test (p=0.05).

Season	Index	Treatment		p-value
		Non-Bt	Bt	
2017/18	Abundance (N)	40 285	42 117	0.80
2018/19	Abundance (N)	16 109	16 500	0.92

Arthropod abundances differed over sampling seasons and sampling times (Table 3.7). This could be due to environmental factors such as the drought that persisted over the regions in 2018/19. In 2017/18 the first sampling at the Bothaville site produced large numbers of Melyridae MS1 (*Astylus atromaculatus*) (Coleoptera: Melyridae) (Appendix: Table 1) due to the surrounding maize field that had come into the reproductive stage. With the second and third sampling their numbers had decreased substantially, this possibly caused the high arthropod abundance in the first sampling of 2017/18. If the Melyridae MS1 numbers are removed from the total (3 277), the third sampling occasion would have yielded the highest abundance.

This study evaluated the effects of Bt soybean on arthropod communities. The results show that non-Bt and Bt trial plots did not differ significantly in diversity, abundance or evenness for all three the sampling methods used over two years (Table 3.8; Table 3.10; Table 3.12 and Table 3.15). Thus, the similarity of arthropod communities in the non-Bt and Bt plots were high. Therefore, Bt soybean has no adverse effects on the arthropod communities in soybean trial plots. The overall results were in agreement with those of previous field studies showing that there were little or no change in arthropod community on Bt soybeans compared with non-Bt soybeans (Marques *et al*., 2018; Yu *et al*., 2014).

The specificity of Bt toxins and the reduction of insecticides used under Bt crop systems are likely to create a more favourable environment for beneficial arthropods such as natural enemies of target pests and pollinators (Malone & Burgess, 2009; Brookes, 2019). However, laboratory studies found that the effects of Bt crops on natural enemies may depend on whether the prey or host takes up the toxin or is affected by the toxin

(Romeis *et al*., 2006). Adverse effects on natural enemies have been observed only in studies where susceptible herbivores were used as hosts/prey (Meissle *et al*., 2005; Vojtech *et al*., 2005; Baur *et al*., 2003; Dutton *et al*., 2002; Ponsard *et al*., 2002; Hilbeck *et al*., 1998). Godfray (1994) stated that certain predators and parasitoids live in close relationships with their host/prey and that parasitoids are very sensitive to changes in host quality. Thus, if a natural enemy is a specialist on an herbivore that has a high level of susceptibility to the Bt toxin, the natural enemy numbers are likely to decline in the field because of host absence or low quality of the prey/host (Andow & Hilbeck, 2004). In this study no significant differences were found in the abundance of predators and parasitoids between the two treatments (Table 3.14). This is in accord with those of previous reports on Bt crops. Yu *et al*. (2014) found that Bt soybean had no negative effects on the dominant distribution of functional groups including predators and parasitoids in field plots in China. Another study done in China, on Bt rice expressing Cry1Ab toxins, found no significant differences in the abundances of parasitoids and predators between Bt and non-Bt rice (Li *et al*., 2007). Mellet *et al*. (2006) indicated that Bt cotton had no marked negative impact on ground- or plant-dwelling spiders in South African field conditions. Bt crops have led to a reduction in insecticide use (Brookes, 2019) thus they can contribute to integrated pest management systems with a strong biological control component (Romeis *et al*., 2006).

For a Bt crop to pose a direct toxicity risk to pollinating insects it must present a hazard to the organism and there must be a realistic pathway through which the organism could be exposed to the hazard (Malone & Burgess, 2009). Cry toxins are also expressed in the pollen of transgenic crops (Yao *et al*., 2006; Mattila *et al*., 2005). A variety of pollinators, including *A. mellifera*, solitary bees (Hymenoptera) and flower-visiting flies (Diptera), have been found to visit soybean (Gill & O'ₙeal, 2015), and even reduce the number of soybean flowers that abort (Chiari *et al*., 2005). Villanueva-Gutierrez *et al.* (2014), found pollen of soybean in the honey of *A. mellifera*. This means that a hazard and an exposure pathway do exist. However, a meta-analysis by Duan *et al.* (2008) evaluating 25 separate studies of the effects of Cry toxins on honey bees found no adverse effects on bees. A study examining the effects of dietary transgenic Bt maize pollen on honey bee larvae of 4-5 day old found no significant differences in larval and

pupal mortalities, pupal weight, and haemolymph protein concentrations of newly emerged adults after they were fed various pollens, including non-transgenic, Cry1A(b) and Cry1F maize pollen (Hanley *et al*., 2003). Another study found that the survival of honey bees fed pollen of maize plants expressing Cry1Ab and Cry1Ac was not reduced in adult bees, furthermore the study found that hypopharyngeal gland development of adult bees was not adversely affected when the bees ingested the Cry proteins (Xie *et al*., 2019).

A total of 416 pollinators belonging to 5 families and consisting of 15 morphospecies were collected with the beating sheet and D-vac methods. Of the 15 morphospecies 11 belonged to the Apidae family. The pollinators abundance and diversity did not differ significantly between the Bt non-Bt plots. Therefore, Bt soybean were found to have no negative effects on pollinators in trial plots in South Africa. This is in accord with what was found by Frizzas *et al*. (2017) where pollinators were more abundant in Bt maize than conventional maize in Brazil. This could be due to the specificity of the Cry toxins to the target pests (Lepidoptera) causing the Cry toxins to not affect bees (Frizzas *et al*., 2017).

The effects of genetically modified plants with insecticidal proteins on arthropods have been evaluated in other crops. De la Poza *et al*. (2004) found that Bt maize expressing the Cry1Ab toxin had no significant effects on the abundances of predatory arthropods in Spain. Another study on Bt maize found no significant differences in insect communities based on the richness, diversity and evenness indices (Frizzas *et al*., 2017). Slight differences in arthropod communities were found in conventional and Bt cotton in Australia (Whitehouse *et al*., 2005). A six-year field study on the effects of Bt cotton expressing the Cry1Ac toxin on 22 representative arthropod natural enemies found that the Bt cotton had no chronic long-term effects (Naranjo, 2005a). However, the latter study did find minor reductions in abundances of five of the taxa in the Bt cotton, but a companion study (Naranjo, 2005b), found similar levels of natural control by the natural enemies in Bt and non-Bt cotton. Thus, the function of the natural enemies was not influenced by the Bt cotton. Liu and Lau (2019) found significantly lower species richness in Bt cotton when compared to non-Bt cotton but no significant

differences in abundance and evenness. In Mexico, where the effects of a transgenic maize hybrid expressing Cry1Ab, Vip3Aa20 and mCry3A toxins on three non-target predators were evaluated, a study found that the Bt maize does not have any negative effects on abundance, frequency or change in population density of the three studied predators (Hernández-Juárez *et al*., 2019). In a three-year study on Bt maize no unintended tri-trophic effects of Bt maize on non-target arthropods were found (Dively, 2006).

3.3.3 Arthropod community composition of soybean trial plots

Non-metric multidimensional scaling analyses (nMDS) based on arthropod community composition for non-Bt and Bt treatments, different localities and plant growth stages were performed for general arthropod community composition (ordinates a and b) as well as for functional groups (ordinates c and d) composition for the beating sheet and D-vac sampling methods.

The arthropod species and functional group composition of the arthropods sampled with the beating sheet method are illustrated in Fig. 3.10. Ordinates a and c show the community compositions at the different locations, the locations seem to pair up over the ordinate. When consulting ordinates b and d which show the results for the Bt and non-Bt treatments at different sampling occasions (plant growth stages) it becomes clear that these pairs are the two different treatments sampled at the same locations and on the same occasion. The non-Bt and Bt treatments cluster together on the ordinates (Fig. 3.10 a and c) for each location and sampling time. All four ordinates had a stress value of 0.12 which is relatively low indicating that the 2D image is an accurate resemblance of the 3D image, this was in fact the case with all four the ordinates. The low stress value also indicates that reliance can be placed on the spacing of the plots on the particular ordinates, however it is beneficial to cross-check these results by means of other statistical analyses (Clarke & Warwick, 2001).

The PERMANOVA results (Table 3.16) sustain the nMDS ordinates and indicate that significant differences occurred between the three different locations (a: p=0.047 c: p=0.31). These results also indicate that no significant differences occurred between the

two treatments for both the general species community composition as well as for the composition of functional groups ($p>0.05$) (Table 3.16).

A one-way ANOSIM was then preformed to determine which locations differed from one another (Table 3.17 a and b). These results revealed that Fouriesburg differed from Bothaville and from Nigel significantly for both the general community and functional group composition.

A SIMPER analysis was run between the locations found to differ from one another in the one-way ANOSIM analysis. This comparison revealed that the general species composition from Fouriesburg differed from Bothaville (dissimilarity: 89.64) and from Nigel (dissimilarity: 70.08) (Appendix A: Table 1). Distinctions between Fouriesburg and Bothaville were mainly due to a beetle belonging to the Melyridae family (Melyridae MS1) with a contribution of 53.6%, this beetle was much more abundant at the Bothaville site (mean abundance: 1 090) than at Fouriesburg (mean abundance: 1). Distinctions between Fouriesburg and Nigel were mainly due to an unknown hemipteran species (Hemiptera unknown MS1) and an unknown coleopteran species (Coleoptera unknown MS1) contributing a total of 31.8%, both these morphospecies were more abundant in Nigel (Appendix A: Table 1).

The SIMPER analysis comparing the functional group composition at the locations that differed from one another also showed that the community at Fouriesburg differed from Bothaville as well as from Nigel (Appendix A: Table 2). Distinctions between Fouriesburg and Bothaville were mainly due to species belonging to the herbivore functional group with three of the top five species contributing to the difference being from this group. The herbivore species contributing the most to the distinction between the two locations was Herbivore MS12 with a total contribution of 53.63%. This species was the most abundant at the Bothaville study location (mean abundance: 1 090). Two species that could not be placed into functional groups had the largest contribution to the distinctions between Fouriesburg and Nigel (contribution: 18.33% and 13.52% respectively). No significant differences were found between the two sampling occasions when ANOSIM analysis was performed (Table 3.18). Also, no differences were found between the two treatments for the

different sampling times (Table 3.19).The nMDS results for the D-vac method of the 2017/18 cropping season showed clustering of different locations for the general arthropod (Fig. 3.11 a) and functional group community compositions (Fig. 3.11 b). The PERMANOVA results confirmed that significant differences occurred between the different locations in both cases (Table 3.20). ANOSIM analysis further showed that all the locations differed from one another for both the general community composition (Table 3.21 a) and the functional group composition (Table 3.21 b).

The SIMPER analysis indicated that Melyridae MS1 contributed the most to the distinction between the five study locations for the general species composition (contribution: 18.32) (Appendix A: Table 3). This species had a mean abundance far higher at the Bothaville than at any of the other locations (mean abundance: 547). Fouriesburg, Nigel and Jan Kempdorp all had similar mean abundances for this morphospecies with 3.5, 2.6 and 0.2 respectively. Thysanoptera MS2 and Chrysomelidae MS1 both contributed roughly 5% to the differences between the locations. Thysanoptera MS2 had the highest abundance at Bothaville and Fouriesburg and Chrysomelidae MS1 was most numerous at Bethal. For the functional group composition herbivores had the highest contribution to the differences between the five study location with a total contribution of 41.59% (Appendix A: Table 4). One of the parasitoid species as well as a pollinator and a detritivore species also made notable contributions.

When consulting nMDS ordinates b and d (Fig. 3.11) it seems that the two treatments cluster together for each sampling site and sampling time. However, the stress of these two ordinates were high (0.21). Therefore, it is essential that the PERMANOVA results were consulted in order to make an accurate analysis. The PERMANOVA results showed that the non-Bt and Bt treatments did not differ for both the general community composition ($p= 0.296$) and the functional groups composition ($p= 0.241$) (Table 3.20). The PERMANOVA results also indicated that the sampling times differed from one another over all five sampling locations (Table 3.20). ANOSIM results confirm that

differences occurred between the 1st and 3rd sampling occasions for both the general and functional group community compositions (Table 3.22 a and b).

The SIMPER analysis found Melyridae MS1 to have made the highest contribution to the differences between the two sampling times for the general community compositions (contribution: 21.68%). The abundance of Melyridae MS1 was higher in the first sampling occasions (mean abundance: 339) than in the third sampling occasions (mean abundance: 0.1). In general, the top five contributing morphospecies (excluding Melyridae MS1) had a higher mean abundance in the third sampling occasions (Appendix A: Table 5). For the functional group composition (Appendix A: Table 7) herbivore 45 had the highest contribution (contribution: 21.68%) to the differences between the 1st and 3rd sampling occasions, this morphospecies was more abundant in the first (mean abundance: 339) sampling occasions than in the third (mean abundance: 0.1). The top four contributions were made by herbivores, a parasitoid and a pollinator. No differences were found between the sampling times in a single location for both the general- and functional group community compositions (Appendix A: Table 8). No significant differences were found between the two treatments of different sampling times except between the Bt treatment of the first sampling and the non-Bt treatment of the third sampling time for both general- and functional group community compositions (Table 3.23). This however is negligible as the communities as a whole differed from one another due to plant growth stage (Table 3.7).

The nMDS results for the D-vac method sampled in the 2018/19 cropping season showed less clustering of the different sampling locations than the 2017/18 results (Fig. 3.12 a and c). Jan Kempdorp grouped away from the other locations for both the general- and functional group community compositions (Fig. 3.12). The remaining three sites, Bethal, Fouriesburg and Nigel overlapped somewhat in 2D space. On the remaining two ordinates (Fig. 3.12 b and d), the treatments seem to group together for the different sampling locations and times respectively. PERMANOVA results confirmed that a locality effects occurred, meaning that the four localities differed from one another in terms of general- and functional group community composition (Table 3.24). Furthermore, the PERMANOVA results found no differences in non-Bt and Bt

treatments for both the general- and functional groups community compositions ($p=0.491$ and $p=0.455$ respectively). Differences were found in sampling times over the four treatments ($p<0.05$) (Table 3.24).

All the sampling locations differed from one another for both the general- and functional group compositions (Table 3.25 a and b). The top ten morphospecies contributing to these differences in general community composition were identified by means of SIMPER analyses (Appendix A: Table 9). These results indicated one species of beetle (Melyridae MS1) to have contributed the most to the differences between the four locations (contribution: 13.21%), this contribution was about double that of the morphospecies that contributed the second most (Gelechiidae MS1). For the functional groups SIMPER analysis showed that the top two contributing organisms belonged to the herbivores with the top contributor having a mean abundance of 211 at the Bethal location and contributing 13.21% to the total and the second highest contributor having a mean abundance of 199 at the Jan Kempdorp location (Appendix A: Table 10). Furthermore, predator 42 contributed to the differences with a mean abundance of 69.7 at the Nigel location, this was 7 times higher than at the second most abundant location.

The first and third sampling occasions were found to differed from one another significantly for both the general and functional groups ($p=0.009$ and $p=0.01$ respectively) (Table 3.26). SIMPER analysis showed that Gelechiidae MS1 contributed the most to the distinction between these two sampling times for the general community (contribution: 9.8%) (Appendix A: Table 11). This morphospecies was found only in the third sampling occasions (mean abundance: 149). Furthermore, Melyridae MS1 and Anthicidae MS1 were found in high abundances in the first sampling occasions and Chrysomelidae MS1 and Anthocoridae MS1 were found in overwhelming abundances in the third sampling occasions. The SIMPER analysis for the functional groups revealed that the top three contributors were herbivores with a total contribution of 22.45% (Appendix A: Table 13). Of these three species two occurred in overwhelming numbers in the third sampling occasions (mean abundance: top contributor: 149 and third highest contributor: 34.8). The

second highest contributor occurred in sampling time one 68 times more than in sampling time three. The fourth and fifth highest contributors were predators with a total contribution of 7.72%. No significant differences occurred between the sampling times of single locations (Appendix A: Table 14). Table 3.27 shows the ANOSIM results for the treatments at different sampling occasions, no significant differences occurred. The nMDS results for the sticky trap data show clear distinctions between the sampling locations for both the 2017/18 (Fig. 3.13 a) and 2018/19 (Fig. 3.13 c) cropping seasons. However, no clear distinctions can be made between the non-Bt and Bt treatments for either of the seasons (Fig. 3.13 b and d). PERMANOVA analysis supported the nMDS ordinates indicating that no significant differences occurred between the treatments ($p>0.05$) but between the locations significant differences did occur ($p<0.05$) in both cropping seasons (Table 3.28).

ANOSIM analysis indicated that the locational differences occurred between all five locations in 2017/18 (Table 3.29 A) and between all four locations except between Fouriesburg and Nigel for the 2018/19 cropping season (Table 3.29 B). According to SIMPER analysis the main contributor to the differences between the five sites in 2017/18 was the thrips (Appendix A: Table 15) with a total contribution of 82.01%. The thrips were found in high abundances at all sites (mean abundance above 600), with the lowest mean abundance in Jan Kempdorp (mean abundance: 221), and the highest abundance in Bothaville (mean abundance: 2 610). Furthermore, the parasitic wasps contributed second most (contribution: 6.8%), and the Cicadellidae (contribution: 3.375%) third most to the distinctions between the five locations. For the 2018/19 cropping season the thrips also made the highest contribution to the distinctions between the sites (contribution: 68.26%) (Appendix A: Table 16). The thrips had the highest mean abundance in Jan Kempdorp (mean abundance: 1017), the other three locations did not have mean abundances exceeding 500.

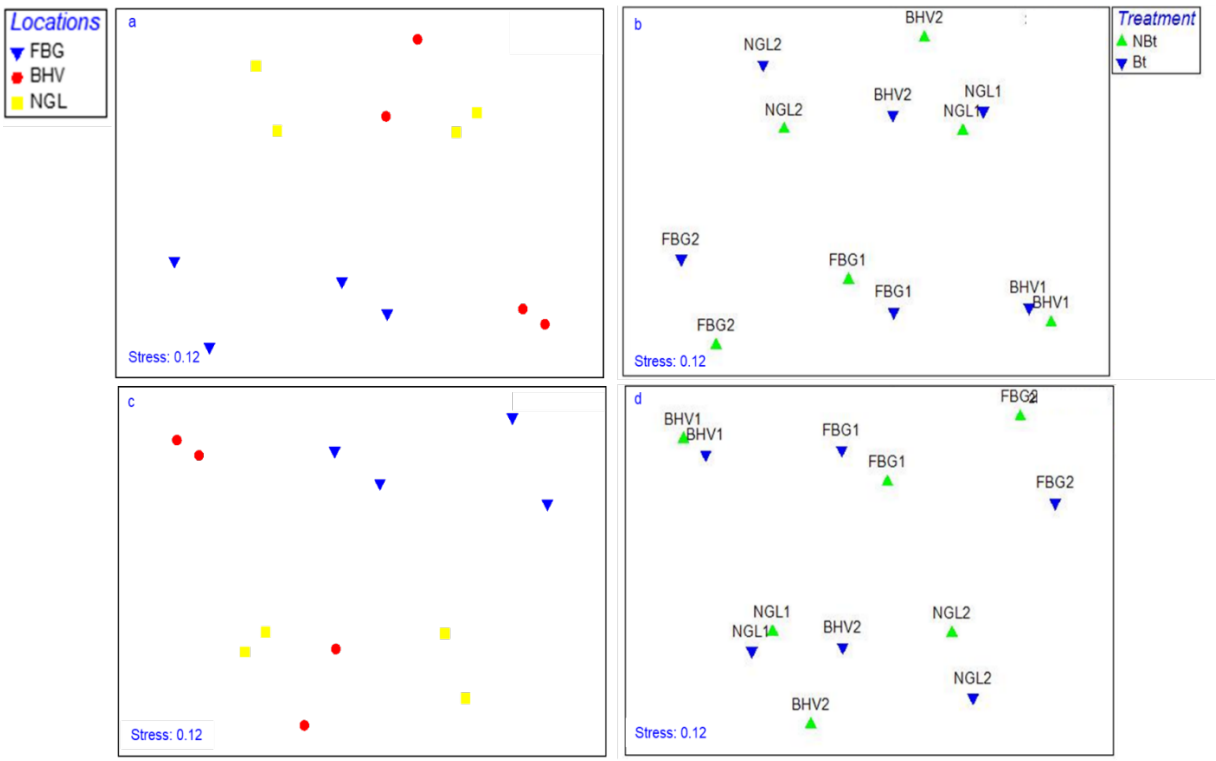

Fig. 3.10 Non-metric multidimensional scaling analyses scatter plots based on arthropod species composition for: (a) general species diversity and different localities (indicated through three letter combinations), (b) general species diversity, different localities and plant growth stage (indicated through numbers behind the letter combinations) (1: pre-flowering, 2: post-flowering), (c) functional groups and different localities and (d) functional groups, different localities and plant growth stage for non-Bt (green) and Bt (blue) treatments for the beating sheet method.

Table 3.16 PERMANOVA results supporting the nMDS ordinates in Fig. 3.10. Values marked in red indicate significant separations at $p<0.05$.

	p-value	
Factors	**Ordinates a & b**	**Ordinates c & d**
Treatments	0.574	0.579
Locations	0.047	0.031
Sampling time	0.080	0.069

Table 3.17 One-way ANOSIM results for the locations of the general arthropod species compositions (A) and the functional group compositions (B) of the beating sheet method. Values marked in red indicate significant differences at p<0.05 (p-values, uncorrected significance; permutation N= 9999).

A	Fouriesburg	Bothaville	Nigel
Fouriesburg	-	-	-
Bothaville	0.030	-	-
Nigel	0.029	0.083	-

B	Fouriesburg	Bothaville	Nigel
Fouriesburg	-	-	-
Bothaville	0.028	-	-
Nigel	0.030	0.087	-

Table 3.18 One-way ANOSIM results for the sampling times of the general arthropod species compositions (A) and the functional group compositions (B) of the beating sheet method. Values marked in red indicate significant differences at p<0.05 (p-values, uncorrected significance; permutation N= 9999).

A	1st sampling	2nd sampling
1st sampling	-	-
2nd sampling	0.134	-

B	1st sampling	2nd sampling
1st sampling	-	-
2nd sampling	0.147	-

Table 3.19 One-way ANOSIM results for the sampling two different treatments at different sampling times (indicated by the numbers 1: pre-flowering, 2: post-flowering) of the general arthropod species compositions (A) and the functional group compositions (B) for the beating sheet method (p-values, uncorrected significance; permutation N= 9999).

A	1Non-Bt	2Non-Bt	1Bt	2Bt
1Non-Bt	-	-	-	-
2Non-Bt	1.000	-	-	-
1Bt	0.803	0.497	-	-
2Bt	0.700	0.601	0.702	-

B	1Non-Bt	2Non-Bt	1Bt	2Bt
1Non-Bt	-	-	-	-
2Non-Bt	1.000	-	-	-
1Bt	0.803	0.490	-	-
2Bt	0.702	0.599	0.696	-

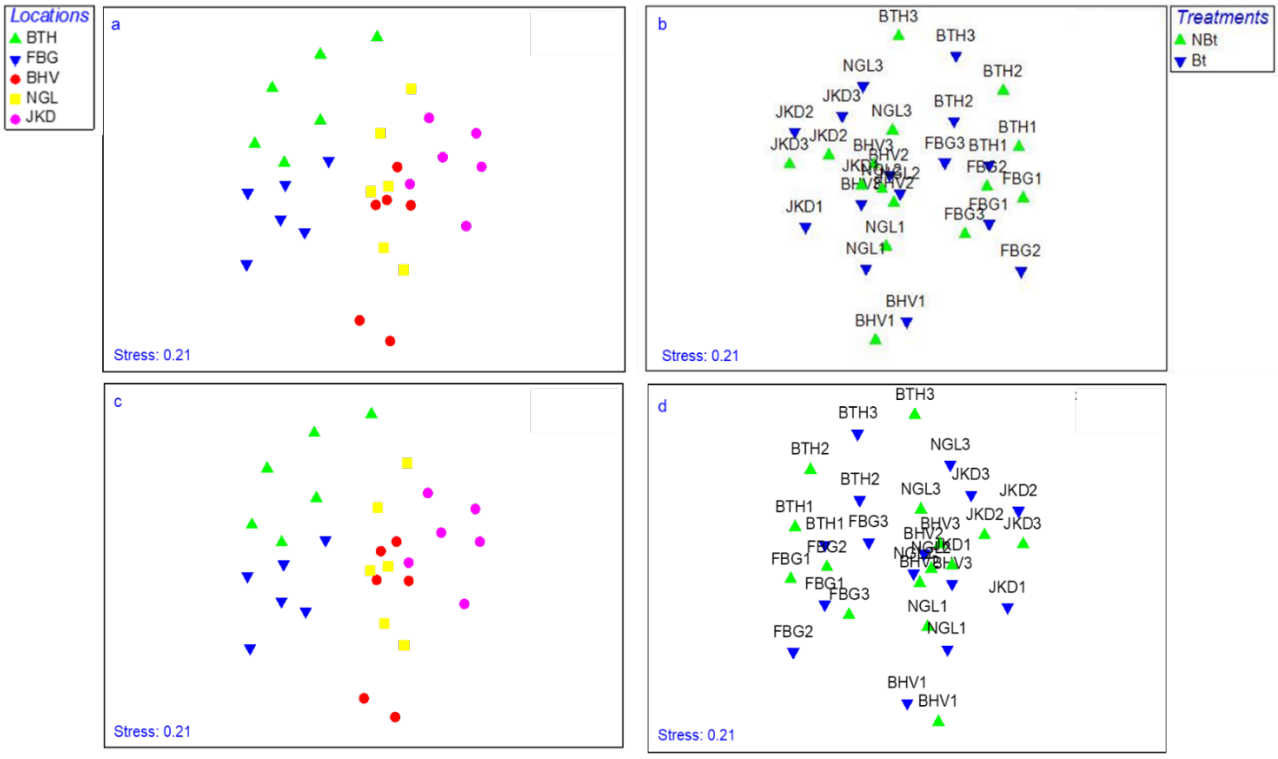

Fig. 3.11 Non-metric multidimensional scaling analyses based on arthropod species composition for: (a) general species diversity and different localities, (b) general species diversity, different localities and plant growth stage, (c) functional groups and different localities and (d) functional groups, different localities and plant growth stage, for non-Bt (green) and Bt (blue) treatments for the D-vac method in the 2017/18 cropping season.

Table 3.20 PERMANOVA results supporting the nMDS ordinates in Fig. 3.11. Values marked in red indicate significant separations at $p<0.05$. 2017/18 cropping season.

	p-value	
Factors	**Ordinates a & b**	**Ordinates c & d**
Treatments	0.296	0.241
Locations	0.001	0.001
Sampling time	0.004	0.002

Table 3.21 One-way ANOSIM results for the locations of the general arthropod species compositions (A) and the functional group compositions (B) of the D-vac method for the 2017/18 cropping season. Values marked in red indicate significant differences at $p<0.05$ (p-values, uncorrected significance; permutation N= 9999).

A	Bethal	Fouriesburg	Bothaville	Nigel	Jan Kempdorp
Bethal	-	-	-	-	-
Fouriesburg	0.010	-	-	-	-
Bothaville	0.006	0.001	-	-	-
Nigel	0.002	0.001	0.044	-	-
Jan Kempdorp	0.001	0.002	0.003	0.005	-

B	Bethal	Fouriesburg	Bothaville	Nigel	Jan Kempdorp
Bethal	-	-	-	-	-
Fouriesburg	0.0106	-	-	-	-
Bothaville	0.0082	0.0027	-	-	-
Nigel	0.0022	0.0019	0.0475	-	-
Jan Kempdorp	0.0026	0.0022	0.0026	0.0048	-

Table 3.22 One-way ANOSIM results for the sampling times of the general arthropod species compositions (A) and the functional group compositions (B) of the D-vac method for the 2017/18 cropping season. Values marked in red indicate significant differences at $p<0.05$ (p-values, uncorrected significance; permutation N= 9999).

A	1st sampling	2nd sampling	3rd sampling	B	1st sampling	2nd sampling	3rd sampling
1st sampling	-	-	-	1st sampling	-	-	-
2nd sampling	0.125	-	-	2nd sampling	0.119	-	-
3rd sampling	0.009	0.061	-	3rd sampling	0.001	0.066	-

Table 3.23 One-way ANOSIM results for the sampling two different treatments at different sampling times (indicated by the numbers 1: pre-flowering, 2: post-flowering) of the general arthropod species compositions (A) and the functional group compositions (B) of the D-vac method for the 2017/18 cropping season. Values marked in red indicate significant differences at $p<0.05$ (p-values, uncorrected significance; permutation N= 9999).

A	1Non-Bt	2Non-Bt	3Non-Bt	1Bt	2Bt	3Bt
1Non-Bt	-	-	-	-	-	-
2Non-Bt	0.847	-	-	-	-	-
3Non-Bt	0.061	0.397	-	-	-	-
1Bt	1.000	0.863	0.022	-	-	-
2Bt	0.219	0.657	0.170	0.079	-	-
3Bt	0.180	0.665	0.974	0.047	0.286	-

B	1Non-Bt	2Non-Bt	3Non-Bt	1Bt	2Bt	3Bt
1Non-Bt	-	-	-	-	-	-
2Non-Bt	0.853	-	-	-	-	-
3Non-Bt	0.062	0.415	-	-	-	-
1Bt	1.000	0.865	0.026	-	-	-
2Bt	0.227	0.653	0.168	0.076	-	-
3Bt	0.181	0.659	0.975	0.050	0.290	-

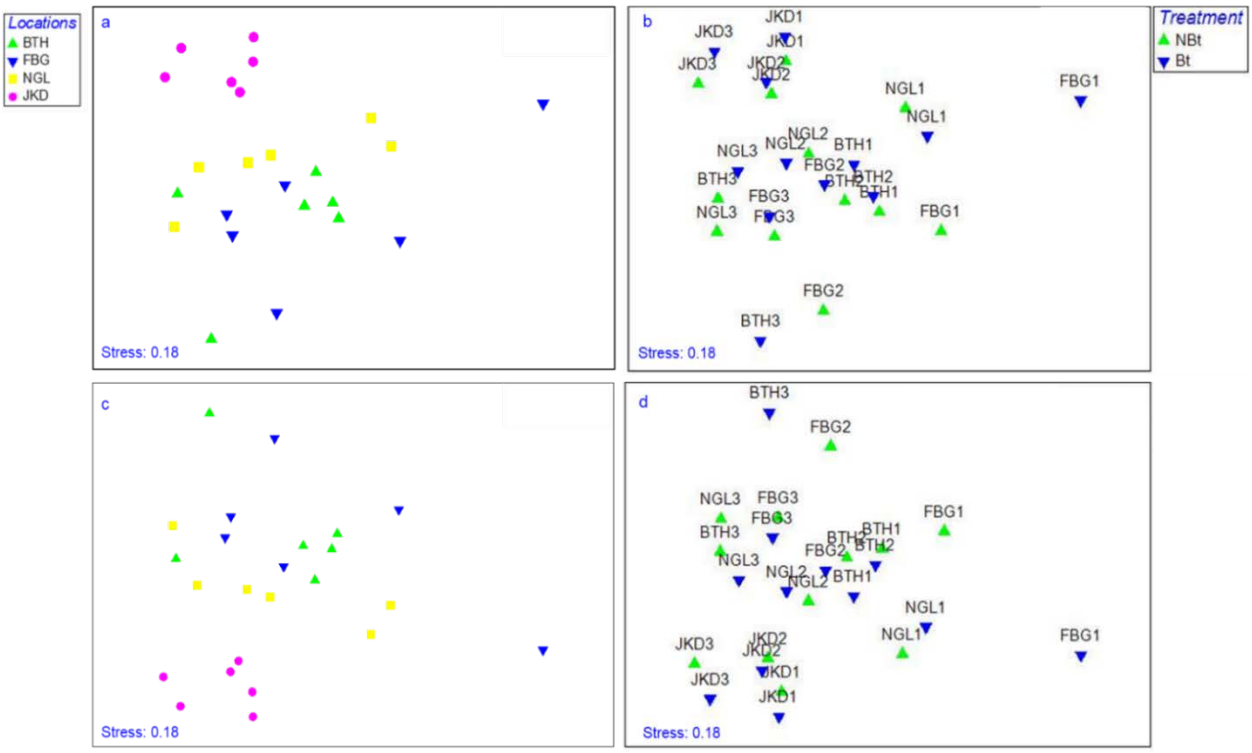

Fig. 3.12 Non-metric multidimensional scaling analyses based on arthropod species composition for: (a) general species diversity and different localities, (b) general species diversity, different localities and plant growth stage, (c) functional groups and different localities and (d) functional groups, different localities and plant growth stage, for non-Bt (green) and Bt (blue) treatments for the D-vac method in the 2018/19 cropping season.

Table 3.24 PERMANOVA results supporting the nMDS ordinates in Fig. 3.12. Values marked in red indicate significant separations at $p<0.05$.

	p-value	
Factors	**Ordinate a & b**	**Ordinate c & d**
Treatments	0.491	0.455
Locations	0.003	0.004
Sampling time	0.012	0.010

Table 3.25 One-way ANOSIM results for the locations of the general arthropod species compositions (A) and the functional group compositions (B) of the D-vac method for the 2018/19 cropping season. Values marked in red indicate significant differences at p<0.05 (p-values, uncorrected significance; permutation N= 9999).

A	Bethal	Fouriesburg	Nigel	Jan Kempdorp
Bethal	-	-	-	-
Fouriesburg	0.026	-	-	-
Nigel	0.024	0.002	-	-
Jan Kempdorp	0.001	0.003	0.003	-

B	Bethal	Fouriesburg	Nigel	Jan Kempdorp
Bethal	-	-	-	-
Fouriesburg	0.0255	-	-	-
Nigel	0.023	0.0025	-	-
Jan Kempdorp	0.003	0.0022	0.0019	-

Table 3.26 One-way ANOSIM results for the sampling times of the general arthropod species compositions (A) and the functional group compositions (B) of the D-vac method for the 2018/19 cropping season. Values marked in red indicate significant differences at p<0.05 (p-values, uncorrected significance; permutation N= 9999).

A	1st sampling	2nd sampling	3rd sampling
1st sampling	-	-	-
2nd sampling	0.706	-	-
3rd sampling	0.009	0.195	-

B	1st sampling	2nd sampling	3rd sampling
1st sampling	-	-	-
2nd sampling	0.709	-	-
3rd sampling	0.010	0.190	-

Table 3.27 One-way ANOSIM results for the sampling two different treatments at different sampling times (indicated by the numbers 1: pre-flowering, 2: post-flowering) of the general arthropod species compositions (A) and the functional group compositions (B) of the D-vac method for the 2018/19 cropping season. Values marked in red indicate significant differences at $p<0.05$ (p-values, uncorrected significance; permutation N= 9999).

A	1Non-Bt	2Non-Bt	3Non-Bt	1Bt	2Bt	3Bt
1Non-Bt	-	-	-	-	-	-
2Non-Bt	0.748	-	-	-	-	-
3Non-Bt	0.366	0.942	-	-	-	-
1Bt	0.555	0.859	0.289	-	-	-
2Bt	0.940	0.889	0.464	0.914	-	-
3Bt	0.367	0.661	0.970	0.277	0.569	-

B	1Non-Bt	2Non-Bt	3Non-Bt	1Bt	2Bt	3Bt
1Non-Bt	-	-	-	-	-	-
2Non-Bt	0.738	-	-	-	-	-
3Non-Bt	0.378	0.939	-	-	-	-
1Bt	0.892	0.862	0.289	-	-	-
2Bt	0.945	0.888	0.455	0.914	-	-
3Bt	0.371	0.655	0.975	0.284	0.573	-

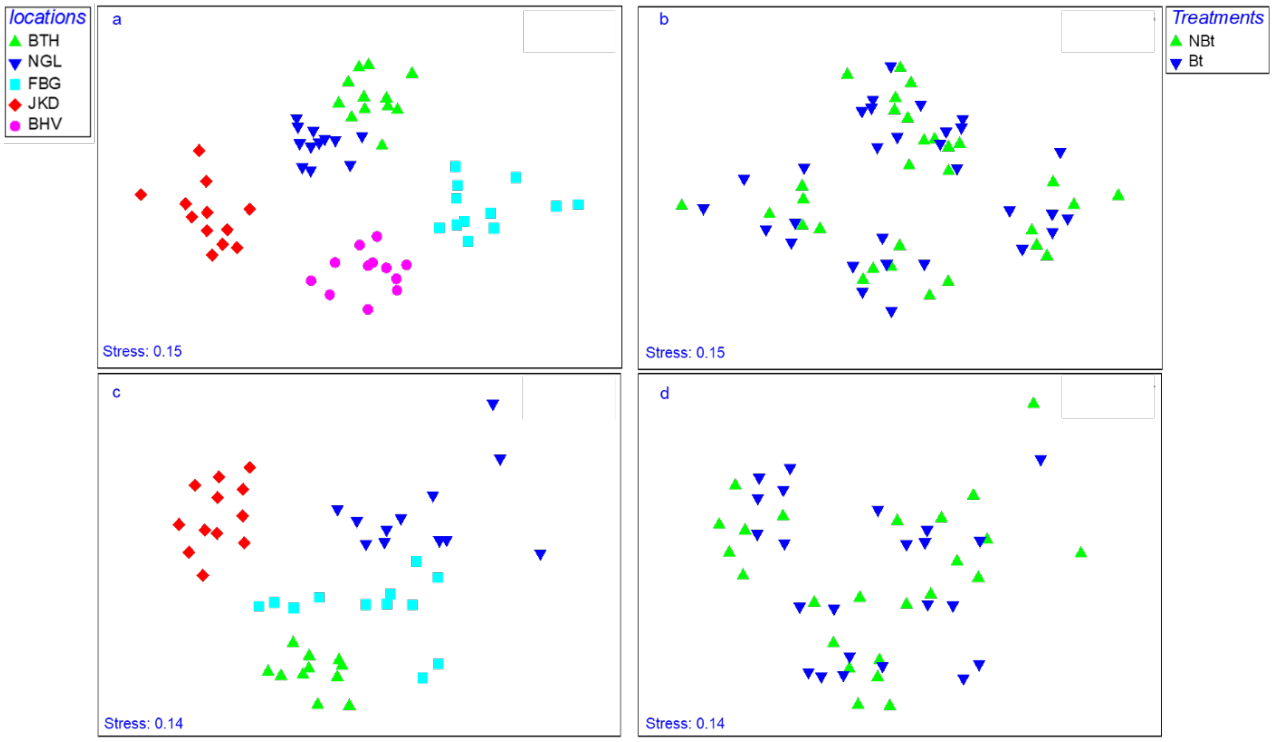

Fig. 3.13 Non-metric multidimensional scaling analyses based on arthropod species composition for: (a) different localities and (b) the two treatments for the 2017/18 cropping season as well as for: (c) different localities and (d) the two treatments for the 2018/19 cropping season.

Table 3.28 PERMANOVA results supporting the nMDS ordinates in Fig. 3.13. Values marked in red indicate significant separations at $p<0.05$.

	p-value	
Factors	**2017/18**	**2018/19**
Treatments	0.284	0.713
Locations	0.001	0.001

Table 3.29 One-way ANOSIM results for the locations of the sticky trap method for the A: 2017/18 and B: 2018/19 cropping season. Values marked in red indicate significant differences at $p<0.05$ (p-values, uncorrected significance; permutation N= 9999).

A	Bethal	Nigel	Fouriesburg	Jan Kempdorp	Bothaville
Bethal	-	-	-	-	-
Nigel	0.003	-	-	-	-
Fouriesburg	0.001	0.001	-	-	-
Jan Kempdorp	0.001	0.001	0.001	-	-
Bothaville	0.001	0.001	0.001	0.001	-

B	Bethal	Nigel	Fouriesburg	Jan Kempdorp
Bethal	-	-	-	-
Nigel	0.001	-	-	-
Fouriesburg	0.004	0.056	-	-
Jan Kempdorp	0.001	0.001	0.001	-

Analysis by nMDS can be used to study the similarity of non-target arthropod community structures in cropping environments, when diversity indices (section: 3.3.2) and multivariate analyses are combined a more comprehensive view of species abundance, diversity and community composition of arthropods in the fields are obtained. Guo *et al*. (2016) used this method to evaluate the effects of Cry1Ie maize on the non-lepidopteran pests.

In this study the nMDS ordination method was used to analyse the relationship between soybean type (treatments), location, sampling time and the non-target arthropod community composition, similar to what was done by Fan *et al*. (2019). The results showed that Bt soybean did not have a significant impact on non-target arthropod communities for all three sampling methods used, which is supported by PERMANOVA analysis. However, location had an impact on the communities within all three sampling methods (Fig. 3.10; Fig. 3.11 and Fig. 3.12 ordinates: a and c) and sampling time had an impact on community structures when the D-vac method was considered (Table 3.20 and Table 3.24). These results were similar to those of Sisterson *et al*. (2004) which found differences in arthropod diversity and abundance between different locations and

Guo *et al*. (2016) which found that maize type had no effect on non-lepidopteran pest communities, but that sampling time did.

The diversity of insects within soybean fields are influenced by the surrounding landscape, species richness of some insect taxa increases when a field is influenced by the surrounding landscape (Gardiner *et al*. 2009). Botha *et al*. (2015) found that arthropod community diversity was correlated with biomes in South Africa. In this study the arthropod community composition differed significantly ($P<0.05$) between the different locations for all sampling methods which indicates that sampling was not done in homogenous environments, thus the sampling sites differed from one another. This is most probably due to the large distances between the locations that encompass different altitudes and climate factors and thus changes in plant communities or biomes surrounding the crops. This is in accordance with the findings of de la Poza *et al*. (2004) who found differences in arthropod communities at two sites due to differences in altitude.

Arthropod community structures varied between sampling times for the D-vac method. Significant differences were found in both years between the first and third sampling occasions (Table 3.22 and Table 3.26). This is to be expected because arthropod populations increase with crop development, as phytophagous insects increase so do their natural enemies (Hernández-Juárez *et* al., 2019). Also, Guo *et al*. (2016) found difference in non-lepidopteran pest species between years and sampling time. This indicates that climatic factors (e.g. temperature and rainfall) had a greater impact on non-target arthropods than the crop type (Bt or non-Bt). A study on the diversity, abundance and composition of soil fauna in Bt maize field plots found that the soil fauna was not influenced by maize type but by year and sampling time (Fan *et al*., 2019).

It has been suggested that the use of small plots (0.05 ha) to compare arthropod diversity and abundance between Bt and non-Bt crops, might underestimate the effects of Bt crops (Sisterson *et al*., 2004). Many studies that have used small plots typically report no effect of Bt crops on non-target arthropods, as was found with this study (Fan *et al*., 2019; Gua *et al*., 2016; Fernandes *et al*., 2007; Li *et al*., 2007; Dively, 2006). This suggest that the effects of Bt crops on arthropod communities might depend on the size

of the Bt crop plantings. However, a number of studies in larger plots also found no significant differences in arthropod communities between Bt and non-Bt crops. Naranjo *et al*. (2005a) found no long-term effects of Bt cotton over multiple generations of nontarget natural enemy taxa. Head *et al*. (2005) found no differences of foliage dwelling generalist predators in large, commercially managed Bt cotton relative to non-Bt cotton. But Liu and Luo (2019) found significant differences in Bt cotton on the composition of insect community but overall abundance was not significantly different between Bt and non-Bt cotton in a large-scale assessment. These results propose that the cultivation of Bt crops had variable effects on arthropods, depending on species and field management. Therefore, it is still uncertain whether or not long-term and large-scale planting of Bt crops will adversely influence non-target arthropod community structure. Arthropod community structures varied between locations and sampling times, but no clear tendencies related to Bt maize were recorded both in terms of abundance and diversity.

3.4 Conclusion

Concerns exist that genetically modified crops that express Bt toxins could present a risk to non-target arthropods. However, these results did not find any significant differences in arthropod communities between Bt and non-Bt soybean in trial plots in South Africa in the short term. Arthropod community structures varied between locations and sampling times, but no clear tendencies related to Bt soybean expressing Cry1Ac proteins were recorded both in terms of abundance and diversity.

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Appendix A

Appendix A: Table 1. Summary of the Similarity Percentage Analysis (SIMPER) showing key species responsible for compositional differences between the three sampling locations found to differ from one another in the ANOSIM analysis of the general species composition for the beating sheet method for the 2017/18 cropping season.

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance (Fouriesburg)	Mean abundance (Bothaville)
Melyridae MS1	48.08	53.63	53.63	1.00	1 090.00
Hemiptera unknown MS1	11.55	12.88	66.51	2.50	42.30
Miridae MS1	1.83	2.04	68.56	0.00	5.00
Thysanoptera MS2	1.82	2.03	70.59	1.75	6.00
Geocoridae MS2	1.55	1.73	72.32	0.00	4.75
Total dissimilarity:	**89.64**				

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance (Fouriesburg)	Mean abundance (Nigel)
Hemiptera unknown MS1	12.85	18.33	18.33	2.50	29.00
Coleoptera unknown MS1	9.47	13.52	31.85	11.50	31.30
Nymphalidae MS1	4.24	6.05	37.90	6.25	0.00
Miridae MS3	3.80	5.42	43.33	0.00	9.50
Coccinellidae MS5	3.08	4.39	47.73	0.00	7.25
Total dissimilarity:	**70.08**				

Appendix A: Table 2. Summary of the Similarity Percentage Analysis (SIMPER) showing key species responsible for compositional differences between the sampling locations found to differ from one another in the ANOSIM analysis of the functional group composition for the beating sheet method.

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance (Fouriesburg)	Mean abundance (Bothaville)
Herbivore 12	48.08	53.63	53.63	1.00	1 090.00
None 3	11.55	12.88	66.51	2.5	42.30
Herbivore 34	1.83	2.04	68.56	0.00	5.00
Herbivore 24	1.82	2.03	70.59	1.75	6.00
Predator 38	1.55	1.73	72.32	0.00	4.75
Total dissimilarity:	**89.64**				

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance (Fouriesburg)	Mean abundance (Nigel)
None 3	12.85	18.33	18.33	2.50	29.00
None 1	9.47	13.52	31.85	11.50	31.30
Herbivore 12	4.24	6.05	37.90	6.25	0.00
Herbivore 36	3.80	5.42	43.33	0.00	9.50
Predator 26	3.08	4.39	47.73	0.00	7.25
Total dissimilarity:	**89.64**				

Appendix A: Table 3. Summary of the Similarity Percentage Analysis (SIMPER) showing key species responsible for compositional differences between the sampling locations found to differ from one another in the ANOSIM analysis of the general community composition for the D-vac method in the 2017/18 cropping season.

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance (Bethal)	Mean abundance (Fouriesburg)	Mean abundance (Bothaville)	Mean abundance (Nigel)	Mean abundance (Jan Kempdorp)
Melyridae MS1	14.64	18.37	18.37	62.80	3.50	547.00	2.67	0.16
Thysanoptera MS2	3.96	4.97	23.34	7.50	24.00	34.20	12.20	13.70
Chrysomelidae MS1	3.68	4.61	27.96	38.70	27.00	6.670	15.70	8.50
Tachinidae MS3	2.94	3.69	31.65	11.20	33.70	6.170	9.67	4.83
Miridae MS3	2.51	3.15	34.80	1.33	1.50	28.20	17.20	9.67
Aphididae MS2	2.38	2.98	37.79	20.20	6.33	12.80	4.17	10.50
Culicidae MS1	2.34	2.93	40.73	19.30	20.00	8.17	4.00	1.17
Pentatomidae MS2	2.08	2.60	43.33	1.17	0.33	6.83	9.17	22.30
Chloropidae MS3	2.05	2.57	45.91	0.33	0.16	17.00	19.20	1.33
Cicadellidae MS2	1.84	2.31	48.22	0.00	0.16	2.33	1.00	28.20
Total dissimilarity:	**79.73**							

Appendix A: Table 4. Summary of the Similarity Percentage Analysis (SIMPER) showing key species responsible for compositional differences between the sampling locations found to differ from one another in the ANOSIM analysis of the functional group community composition for the D-vac method in the 2017/18 cropping season.

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance (Bethal)	Mean abundance (Fouriesburg)	Mean abundance (Bothaville)	Mean abundance (Nigel)	Mean abundance (Jan Kempdorp)
Herbivore 45	14.64	18.37	18.37	62.80	3.50	547.00	2.67	0.16
Herbivore 67	3.96	4.97	23.34	7.50	24.00	34.20	12.20	13.70
Herbivore 1	3.68	4.61	27.96	38.70	27.00	6.67	15.70	8.50
Parasitoid 3	2.94	3.69	31.65	11.20	33.70	6.17	9.67	4.83
Herbivore 84	2.51	3.15	34.80	1.33	1.50	28.20	17.20	9.67
Herbivore 120	2.38	2.98	37.79	20.20	6.33	12.80	4.17	10.50
Pollinator 3	2.34	2.93	40.73	19.30	20.00	8.17	4.00	1.17
Herbivore 76	2.08	2.60	43.33	1.17	0.33	6.83	9.17	22.30
Herbivore 72	2.05	2.57	45.91	0.33	0.16	17.00	19.20	1.33
Herbivore 104	1.84	2.31	48.22	0.00	0.16	2.33	1.00	28.20
Detritivore 20	1.77	2.22	50.45	2.83	22.20	7.17	6.67	2.50
Total dissimilarity:	**79.73**							

Appendix A: Table 5. Summary of the Similarity Percentage Analysis (SIMPER) showing key species responsible for compositional differences between the sampling time found to differ from one another in the ANOSIM analysis of the general community composition for the D-vac method in the 2017/18 cropping season.

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance (1st sampling)	Mean abundance (3rd sampling)
Melyridae MS1	17.86	21.68	21.68	339.00	0.10
Chrysomelidae MS1	5.16	6.26	27.95	9.40	40.30
Thysanoptera MS2	3.36	4.08	32.03	12.80	20.20
Pentatomidae MS2	2.78	3.38	35.41	0.20	18.20
Tachinidae MS3	2.45	2.98	38.39	2.10	18.70
Total dissimilarity:	**82.39**				

Appendix A: Table 6. Non-parametric One-Way Analysis of Similarities (ANOSIM) of pair-wise comparisons of species compositions between different sampling times (plant growth stages) of single locations.

	Bethal 1st sampling	Bethal 2nd sampling	Bethal 3rd sampling
Bethal 1st sampling	-	-	-
Bethal 2nd sampling	1.00	-	-
Bethal 3rd sampling	0.33	0.33	-
	Bothaville 1st sampling	Bothaville 2nd sampling	Bothaville 3rd sampling
Bothaville 1st sampling	-	-	-
Bothaville 2nd sampling	0.33	-	-
Bothaville 3rd sampling	0.33	0.34	
	Jan Kempdorp 1st sampling	Jan Kempdorp 2nd sampling	Jan Kempdorp 3rd sampling
Jan Kempdorp 1st sampling	-	-	-
Jan Kempdorp 2nd sampling	0.33	-	-
Jan Kempdorp 3rd sampling	0.32	0.33	
	Fouriesburg 1st sampling	Fouriesburg 2nd sampling	Fouriesburg 3rd sampling
Fouriesburg 1st sampling	-	-	-
Fouriesburg 2nd sampling	0.32	-	-
Fouriesburg 3rd sampling	0.33	0.33	-
	Nigel 1st sampling	Nigel 2nd sampling	Nigel 3rd sampling
Nigel 1st sampling	-	-	-
Nigel 2nd sampling	0.33	-	-
Nigel 3rd sampling	0.33	0.32	-

Appendix A: Table 7. Summary of the Similarity Percentage Analysis (SIMPER) showing key species responsible for compositional differences between the sampling time found to differ from one another in the ANOSIM analysis of the functional group community composition for the D-vac method in the 2017/18 cropping season.

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance (1st sampling)	Mean abundance (3rd sampling)
Herbivore 45	17.86	21.68	21.68	339.00	0.10
Herbivore 1	5.16	6.26	27.95	9.40	40.30
Herbivore 67	3.36	4.08	32.03	12.80	20.20
Herbivore 76	2.78	3.38	35.41	0.20	18.20
Parasitoid 3	2.45	2.98	38.39	2.10	18.70
Pollinator 3	2.29	2.78	41.17	7.10	14.80
Total dissimilarity:	**82.39**				

Appendix A: Table 8. Non-parametric One-Way Analysis of Similarities (ANOSIM) of pair-wise comparisons of species compositions between different sampling times (plant growth stages) of single locations.

	Bethal 1st sampling	Bethal 2nd sampling	Bethal 3rd sampling
Bethal 1st sampling	-	-	-
Bethal 2nd sampling	1.00	-	-
Bethal 3rd sampling	0.33	0.32	-
	Bothaville 1st sampling	Bothaville 2nd sampling	Bothaville 3rd sampling
Bothaville 1st sampling	-	-	-
Bothaville 2nd sampling	0.32	-	-
Bothaville 3rd sampling	0.33	0.33	-
	Jan Kempdorp 1st sampling	Jan Kempdorp 2nd sampling	Jan Kempdorp 3rd sampling
Jan Kempdorp 1st sampling	-	-	-
Jan Kempdorp 2nd sampling	0.32	-	-
Jan Kempdorp 3rd sampling	0.33	0.33	-
	Fouriesburg 1st sampling	Fouriesburg 2nd sampling	Fouriesburg 3rd sampling
Fouriesburg 1st sampling	-	-	-
Fouriesburg 2nd sampling	0.32	-	-
Fouriesburg 3rd sampling	0.33	0.33	-
	Nigel 1st sampling	Nigel 2nd sampling	Nigel 3rd sampling
Nigel 1st sampling	-	-	-
Nigel 2nd sampling	0.33	-	-
Nigel 3rd sampling	0.33	0.33	-

Appendix A: Table 9. Summary of the Similarity Percentage Analysis (SIMPER) showing key species responsible for compositional differences between the sampling locations found to differ from one another in the ANOSIM analysis of the general community composition for the D-vac method in the 2018/19 cropping season.

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance Bethal	Mean abundance Fouriesburg	Mean abundance Nigel	Mean abundance Jan Kempdorp
Melyridae MS1	11.16	13.21	13.21	211.00	4.67	15.20	0.83
Gelechiidae MS1	5.38	6.37	19.58	0.00	0.33	0.00	199.00
Anthicidae MS1	4.64	5.50	25.08	9.33	0.66	69.70	0.00
Miridae MS3	3.89	4.61	29.70	8.33	3.17	40.70	45.50
Thysanoptera MS2	3.18	3.77	33.47	9.17	16.00	60.70	15.50
Chrysomelidae MS1	3.12	3.70	37.17	16.80	44.30	6.00	1.33
Cicadellidae MS1	2.59	3.07	40.25	25.70	7.00	22.3	17.70
Miridae MS1	2.35	2.78	43.03	1.83	21.30	5.33	25.00
Anthocoridae MS1	2.21	2.62	45.66	9.33	1.17	50.80	7.00
Chalcidoidea MS26	1.88	2.23	47.89	5.33	13.30	30.70	3.00
Total dissimilarity:	**84.45**						

Appendix A: Table 10. Summary of the Similarity Percentage Analysis (SIMPER) showing key species responsible for compositional differences between the sampling locations found to differ from one another in the ANOSIM analysis of the functional group community composition for the D-vac method in the 2018/19 cropping season.

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance Bethal	Mean abundance Fouriesburg	Mean abundance Nigel	Mean abundance Jan Kempdorp
Herbivore 45	11.16	13.21	13.21	211.00	4.67	15.20	0.83
Herbivore 50	5.38	6.37	19.58	0.00	0.33	0.00	199.00
Predator 42	4.64	5.50	25.08	9.33	0.66	69.70	0.00
Herbivore 84	3.89	4.61	29.70	8.33	3.17	40.70	45.50
Herbivore 67	3.18	3.77	33.47	9.17	16.00	60.70	15.50
Herbivore 1	3.12	3.70	37.17	16.80	44.30	6.00	1.33
Herbivore 103	2.59	3.07	40.25	25.70	7.00	22.30	17.70
Herbivore 82	2.35	2.78	43.03	1.83	21.30	5.33	25.00
Predator 82	2.21	2.62	45.66	9.33	1.17	50.80	7.00
Parasitoid 29	1.88	2.23	47.89	5.33	13.30	30.70	3.00
Total dissimilarity:	**84.45**						

Appendix A: Table 11. Summary of the Similarity Percentage Analysis (SIMPER) showing key species responsible for compositional differences between the sampling time found to differ from one another in the ANOSIM analysis of the general community composition for the D-vac method in the 2018/19 cropping season.

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance 1st sampling	Mean abundance 3rd sampling
Gelechiidae MS1	8.60	9.81	9.81	0.00	149.00
Melyridae MS1	6.42	7.32	17.14	68.80	1.25
Chrysomelidae MS1	4.64	5.30	22.45	2.75	34.80
Anthicidae MS1	3.76	4.29	26.74	32.10	9.88
Anthocoridae MS1	3.00	3.42	30.17	4.38	40.60
Total dissimilarity:	**87.65**				

Appendix A: Table 12. Non-parametric One-Way Analysis of Similarities (ANOSIM) of pair-wise comparisons of species compositions between different sampling times (plant growth stages) of single locations for the 2018/19 cropping season.

	Bethal 1st sampling	Bethal 2nd sampling	Bethal 3rd sampling
Bethal 1st sampling	-	-	-
Bethal 2nd sampling	1.00	-	-
Bethal 3rd sampling	0.33	0.32	-
	Jan Kempdorp 1st sampling	Jan Kempdorp 2nd sampling	Jan Kempdorp 3rd sampling
Jan Kempdorp 1st sampling	-	-	-
Jan Kempdorp 2nd sampling	0.66	-	-
Jan Kempdorp 3rd sampling	0.32	0.33	-
	Fouriesburg 1st sampling	Fouriesburg 2nd sampling	Fouriesburg 3rd sampling
Fouriesburg 1st sampling	-	-	-
Fouriesburg 2nd sampling	0.33	-	-
Fouriesburg 3rd sampling	0.33	0.32	-
	Nigel 1st sampling	Nigel 2nd sampling	Nigel 3rd sampling
Nigel 1st sampling	-	-	-
Nigel 2nd sampling	0.33	-	-
Nigel 3rd sampling	0.33	0.33	-

Appendix A: Table 13. Summary of the Similarity Percentage Analysis (SIMPER) showing key species responsible for compositional differences between the sampling time found to differ from one another in the ANOSIM analysis of the functional group community composition for the D-vac method in the 2018/19 cropping season.

Morphospecies	Average Dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance 1st sampling	Mean abundance 3rd sampling
Herbivore 50	8.60	9.81	9.81	0.00	149.00
Herbivore 45	6.42	7.32	17.14	68.80	1.25
Herbivore 1	4.64	5.30	22.45	2.75	34.80
Predator 42	3.76	4.29	26.74	32.10	9.88
Predator 82	3.00	3.42	30.17	4.38	40.60
Total dissimilarity:	**82.39**				

Appendix A: Table 14. Non-parametric One-Way Analysis of Similarities (ANOSIM) of pair-wise comparisons of species compositions between different sampling times (plant growth stages) of single locations for the 2018/19 cropping season.

	Bethal 1st sampling	Bethal 2nd sampling	Bethal 3rd sampling
Bethal 1st sampling	-	-	-
Bethal 2nd sampling	1.00	-	-
Bethal 3rd sampling	0.33	0.34	-
	Jan Kempdorp 1st sampling	Jan Kempdorp 2nd sampling	Jan Kempdorp 3rd sampling
Jan Kempdorp 1st sampling	-	-	-
Jan Kempdorp 2nd sampling	0.66	-	-
Jan Kempdorp 3rd sampling	0.33	0.32	-
	Fouriesburg 1st sampling	Fouriesburg 2nd sampling	Fouriesburg 3rd sampling
Fouriesburg 1st sampling	-	-	-
Fouriesburg 2nd sampling	0.33	-	-
Fouriesburg 3rd sampling	0.33	0.33	-
	Nigel 1st sampling	Nigel 2nd sampling	Nigel 3rd sampling
Nigel 1st sampling	-	-	-
Nigel 2nd sampling	0.33	-	-
Nigel 3rd sampling	0.33	0.33	-

Appendix A: Table 15. Summary of the Similarity Percentage Analysis (SIMPER) showing key species responsible for compositional differences between the sampling locations found to differ from one another in the ANOSIM analysis of the community composition for the sticky trap method in the 2017/18 cropping season.

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance Bethal	Mean abundance Nigel	Mean abundance Fouriesburg	Mean abundance Jan Kempdorp	Mean abundance Bothaville
Thysanoptera	39.25	82.01	82.01	1 100	1 310	630	221	2 610
Parasitic wasps	3.29	6.878	88.89	183.00	61.30	80.50	21.30	43.90
Cicadellidae	1.61	3.375	92.26	7.42	60.80	6.17	45.80	52.10
Other Hymenoptera	1.04	2.173	94.44	38.30	32.80	41.10	62.40	44.80
A.atromaculatus	0.48	1.02	95.46	29.60	1.33	0	0.66	0.33
Syrphidae	0.46	0.96	96.42	1.00	0.66	1.42	1.42	35.50
Miridae/ Lygaeidae	0.45	0.95	97.37	13.20	19.6	2.67	0.25	7.75
Orius majusculus	0.29	0.61	97.99	13.40	5.25	0.08	4.75	1.67
Coccinellidae	0.26	0.56	98.55	0.83	2.00	0	11.00	0.33
Chrysomelidae	0.22	0.46	99.02	3.08	10.90	0.16	4.25	0.25
Araneae	0.15	0.31	99.33	1.17	3.17	0.50	5.42	4.92
Asilidae	0.11	0.23	99.57	0.50	6.08	0.58	0.33	0.08
Large wasps	0.06	0.14	99.71	0.08	0.66	0	2.67	0.33
Other lepidopterans	0.04	0.10	99.81	0.41	0.41	0.08	1.58	1.42
Apis mellifera	0.04	0.09	99.9	0	0.08	0.58	1.50	0.33
Neuroptera	0.01	0.03	99.94	0	0	0	0.66	0.08

Coreidae	0.012	0.02	99.97	0	0	0	0.5	0
Nymphalidae	0.01	0.022	99.99	0.33	0	0.08	0.08	0.16
Pentatomidae	0.01	0.012	100	0	0	0	0.25	0
Total dissimilarity:	**47.86**							

Appendix A: Table 16. Summary of the Similarity Percentage Analysis (SIMPER) showing key species responsible for compositional differences between the sampling locations found to differ from one another in the ANOSIM analysis of the community composition for the sticky trap method in the 2018/19 cropping season.

Morphospecies	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean abundance Bethal	Mean abundance Nigel	Mean abundance Fouriesburg	Mean abundance Jan Kempdorp
Thysanoptera	34.27	68.26	68.26	367	248	316	1 017
Cicadellidae	5.27	10.51	78.77	14.80	13.90	31.50	174
Parasitic wasps	3.79	7.55	86.33	20.30	77.90	72.30	57.80
Astylus	2.02	4.03	90.36	43.00	0.16	3.25	0.91
Orius majusculus	1.15	2.29	92.66	13.80	6.50	24.50	8.25
Miridae/ Lygaeidae	0.99	1.99	94.65	4.50	4.92	8.08	30.40
Chrysomelidae	0.85	1.70	96.36	10.20	0.83	14.50	1.08
Other Hymenoptera	0.77	1.54	97.90	5.50	3.42	5.17	23.60
Spiders	0.34	0.67	98.58	7.33	1.75	2.17	2.17
Large wasps	0.26	0.52	99.10	1.25	0	1.58	6.33
Coccinellidae	0.17	0.35	99.46	0.58	0.08	0.50	5.58
Other lepidopterans	0.12	0.24	99.70	0.16	0.16	0.25	3.83
Neuroptera	0.05	0.10	99.80	0.58	0.08	0.50	0.58
Syrphidae	0.03	0.06	99.87	0.25	0.33	0	0.33
Apis mellifera	0.03	0.06	99.93	0.50	0	0.08	0.33
Asilidae	0.01	0.02	99.96	0.08	0	0.08	0.33
Pentatomidae	0.01	0.02	99.99	0.16	0	0	0.16
Coreidae	0.01	0.01	100	0	0	0	0.16
Nymphalidae	0	0	100	0	0	0	0
Total dissimilarity:	**50.21**						

4 Chapter 4: Using an ecological model to identify non-target arthropod species for risk assessments of GM Bt soybean in South Africa

Abstract

Insect resistant genetically modified soybean have been planted on a commercial scale in several countries since 2011, however, Bt soybean is still in the assessment phase in South Africa. *Helicoverpa armigera* is the target species of Bt soybean but many other arthropod species may be directly or indirectly exposed to Bt toxins. Therefore, it is essential to assess the environmental risks that Bt soybean may hold and to study its effects on non-target arthropod species and their community assemblages which fulfil a variety of ecosystem services such as pollination and pest control. Environmental risk assessments can be improved through the use of an ecological model which can be applied to a specific environment to classify species functionally and prioritize them to identify potential test species. In this study an ecological model approach was followed for identification of non-target arthropod species for ecological risk assessment of Bt soybean. Field surveys were conducted on soybean plots during the 2017/18 and 2018/19 growing seasons. A total of 29 455 arthropod specimens were collected by means of an adapted D-vac method and a beating sheet method and 371 morphospecies were identified. These morphospecies were then grouped into five functional groups and 31 priority species were identified. From these 31 priority species, 10 species which were considered to be of high priority were selected. These 10 species were then further assessed by using a selection matrix to rank their possible maximum potential exposure to Bt toxins in soybean agroecosystems. This study provides a framework for selecting high priority species for monitoring of possible effects of Bt soybean on non-target arthropods in South Africa.

Key words: Environmental risk assessment, non-target arthropods, framework, soybean, ecological model.

4.1 Introduction

Arthropod diversity in agroecosystems is important since these species and their assemblages fulfil a variety of ecosystem services such as pollination and pest control which are vital for crop production and the surrounding environment (Hilbeck *et al*., 2006a; Hooper *et al*., 2005). These ecosystem services may be harmed if changes in the arthropod species assemblages occur (Dutton *et al*., 2003). Arthropod assemblages can however be impacted by a number of factors within an agroecosystem, for example pest management and agricultural management practices (Altieri, 1999).

Genetically modified (GM) crops that express Cry proteins derived from soil bacterium, *Bacillus thuringiensis* (Bt), are successfully used to control several insect pest species, notably lepidopteran pests. Soybean pests that are controlled by means of Bt soybean include *Chrysodeizis includens* (Walker) (Lepidoptera: Noctuidae)*, Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Wu *et al*., 2003; Walker *et al*., 2000). While the majority of studies that addressed the environmental effects of GM crops reported that these crops do not have adverse effects on the environment and specifically on non-target organisms, there are studies that report the contrary. Bt crops have been reported to be target specific, which reduces concerns for non-target effects (Malone *et al*., 2009; Li *et al*., 2007). Not only do GM crops provide an effective alternative for controlling target pests, they also provide many economic, social and environmental benefits such as reducing the use of chemical insecticides and reduced input costs (Brookes, 2019; Brookes & Barfoot, 2018, 2016, 2010; Wang, 2007). Nevertheless, there have been questions about the potential risks that GM crops might have in the environment. One of the risks commonly associated with the growing of insect resistant GM crops is their potential to adversely affect non-target organisms (Romeis *et al*., 2008, 2006). For an organism to be affected by a Bt toxin it has to be exposed to the Cry protein expressed in Bt plants. The two main exposure pathways are: directly, through the consumption of plant tissue of a Bt crop, and indirectly, through consumption of organisms that fed on tissue of Bt crops (Yu *et al*., 2011; Groot & Dicke, 2002). Arthropods are the most diverse group of non-target macro-organisms that may be exposed to GM crops

(Knecht *et al*., 2010) and, contrary to the above, there are studies that report Bt toxins to have adverse effects on non-target organisms (Han *et al*., 2010; Kramarz *et al*., 2009; Rosi-Marshall *et al*., 2007; Losey *et al*., 1999). For example, a laboratory assay by Losey *et al*. (1999) found monarch butterfly, *Danaus plexippus* L. (Lepidoptera: Nymphalidae), larvae to be adversely affected when fed milkweed leaves dusted with Bt maize pollen. Another study that found Bt toxins to have adverse effects on non-target organisms was that of Rosi-Marshall *et al*. (2007). The Lab assay showed that the consumption of Bt maize byproducts adversely reduced growth and increased mortality of non-target stream insects. Consequently, prior to the approval of a Bt crop for commercial release, an environmental risk assessment (ERA) needs to be done to evaluate the potential for adverse effects on non-target organisms that might occur in the agroecosystem (Craig *et al*., 2008).

In South Africa and many other countries, legislation requires that GM crops undergo a pre-commercial ERAs (Nap *et al*., 2003; GMO Act, DAFF, 2005). As a part of this risk assessment an assessment of the potential effects of the GM crop on non-target arthropods species needs to be done. A risk assessment is defined by Andow and Hilbeck (2004a) as a process through which risks are identified, after which the seriousness of the risks is characterized to ensure that appropriate decisions can be made on whether or how to proceed with the technology.

The general principle proposed to assess risks that GM plants may hold for non-target arthropods, is a tiered approach (Romeis *et al*., 2008, 2006; Dutton *et al*., 2003). This procedure starts with laboratory tests (lower-tier), followed by semi-field, glasshouse, and field (higher-tier) tests (Yu *et al*., 2011). Since arthropods have a high diversity in agroecosystems, it is necessary to select appropriate species to serve as representatives of taxonomic groups and ecologically and economically important functions in the receiving environment (Romeis *et al*., 2008; Dutton *et al*., 2003). Furthermore, it is impractical to use high numbers of the arthropod species that occur in specific agroecosystem when assessing potential effects of a GM crop (Yu *et al*., 2011; Knecht *et al*., 2010). The selected species should represent different ecological functions, such as pollinator services, herbivory, decomposition of materials and

predation or parasitism of pest species (Yu *et al*., 2011; Andow & Hilbeck, 2004b). Furthermore, species with cultural value or species that are threatened or endangered should also be considered (Romeis *et al*., 2008). Also, since a risk only exists when the opportunity of exposure to the toxin (hazard) exists, species that are highly likely to be exposed to toxins are most likely to be affected by it and should therefore be selected for evaluation (Yu *et al*., 2011). Several models can be used to select the most important and appropriate test species. Two of these are: the ecotoxicology model and the ecological model.

The ecotoxicology model aims to evaluate the potential non-target effects of chemicals released into the environment (Andow and Hilbeck, 2004b). The strategy with the use of this model is to expose a single chemical to the same battery of universal indicator species, extrapolate estimates of non-target effects, and then make recommendations on how to move forward with the chemical product that was evaluated (Andow & Hilbeck, 2004b). The universal indicator species used in the ecotoxicology model are selected because of their sensitivity to chemical toxins, their extensive availability, ease of culture and genetic uniformity (Chapman, 2002). However, this approach is inappropriate for evaluating non-target effects of transgenic plants because little can be inferred from universal indicator species about the effects of a product on other populations, communities or ecosystem functions (Forbes & Forbes, 1993). This approach is also non-consistent with the need for case-by-case assessment that considers the relevant transgene, crop and cropping environment (Andow & Hilbeck, 2004b), and in some cases the indicator species used often are not present in the environment where the transgenic plants will be grown (Van Wyk *et al*., 2007).

The ecological model on the other hand takes into consideration the specific transgene, crop and cropping environment, and is highly appropriate for risk assessment (Van Wyk *et al*., 2007; Andow & Hilbeck, 2004b). The ecological model relies on ecological principles to select species to test, specify end points and develop assessment protocols for testing (Andow & Hilbeck, 2004b). With this model, costs are minimalized by focusing only on a few relevant non-target species while uncertainties are addressed

by choosing relevant species, e.g. those that are actually found within the receiving environment (Andow & Hilbeck, 2004b).

Once the test species have been selected, they can be evaluated through the tiered testing procedure mentioned earlier. The lower tier or laboratory tests serve to identify potential hazards and measure specific end-points under worst case conditions (Yu *et al*., 2011). For instance, arthropods can be exposed in bioassays to protein concentrations that are 10-100 times higher than those present in plant tissue. A lack of adverse effects at the 1st-tier level might indicate that no risk exists and thus no further testing is needed (Romeis *et al*., 2011). If hazards were detected or uncertainties exist, higher tier tests should be conducted including more complex semi-field or field tests (Yu *et al*., 2011). These tests confirm whether an effect does actually exist under more realistic circumstances. Field tests provide more ecological information and the structure and species diversity of communities in general are assessed as end-points (Romeis *et al*., 2008).

Andow and Hilbeck (2004a) pointed out that there is a need for the strengthening of risk assessments of GM crops worldwide. These assessments are hampered by the lack of even the most basic checklist of species present in the agroecosystems (Van Wyk *et al*., 2007; Truter *et al*., 2014). Therefore, the aim of this study was to use an ecological model to identify priority non-target arthropods species for risk assessment of Bt soybean in South Africa. This study provides a framework for selecting priority species for pre- and post-release monitoring of Bt soybean.

4.2 Materials and Methods

The evaluation of the impact of Bt soybean on the community of non-target arthropods that inhabit soybean fields in South Africa was described in chapter 3. The biodiversity data that were used in the latter chapter were also used in this chapter to select non-target species for ecological risk assessments.

Arthropod sampling took place during the 2017/18 and 2018/19 cropping seasons. Arthropods were sampled by means of a beating sheet and D-vac method as described

in chapter 3. This data were then used in the ecological model suggested by Andow and Hilbeck (2004b) to identify high-priority species.

The methodology of the ecological model follows four steps (Andow & Hilbeck, 2004a, b). These are: 1) establishing functional groups, 2) grouping the non-target arthropod species into the functional groups, 3) prioritizing the species on the basis of ecological principles, and 4) selecting a number of high-priority species to test. This step by step process is then followed by the development of hypotheses that can be tested.

4.2.1 Establishing functional groups

Using functional groups means that ecological functions are considered. This allows for the focus to be on ecological processes and limits the number of species that have to be tested. This avoids inappropriate conclusions to be made that are often associated with the indicator species used in the ecotoxicology model (Andow & Hilbeck, 2004b). Ecological functions can be related to humans, such as secondary pest species, species used to generate income and species of social or cultural value, or they can be ecological such pollination, decomposition or consumers (Andow & Hilbeck, 2004b). For this study, ecological functions were used, and the functional groups selected were predators, pollinators, herbivores, detritivores and parasitoids.

4.2.2 Classifying non-target arthropod species

In the second step, the non-target arthropods sampled from soybean in the receiving environment where the intended transgenic crop is to be released, needs to be identified and classified into the functional groups established in step 1, using available information and expertise. By using species that occur in the region, a case-specific set of potential non-target species is generated (Andow & Hilbeck, 2004b). Many arthropods have unknown functions, therefore it is important to include a category for species of unknown function to ensure that those species are not overlooked.

The arthropods sampled in chapter 3 were identified to family level and then classified to morphospecies level after which they were placed into the five functional groups nl. herbivores, predators, parasitoides, pollinators and detritivores . These five functional groups were chosen since they are easily recognizable. Although most of the species

could be classified into at least one of the groups, a few could not and were given unknown status.

4.2.3 Prioritizing non-target species

For an organism to be affected by a Bt toxin it must be exposed to the toxin. Exposure may occur directly, for instance through feeding on plant material or indirectly through feeding on an organism that fed on transgenic plant material (Andow & Hilbeck, 2004b). The aim of the third step is to identify those species that are most likely to be associated with the crop and therefore most likely to be affected by it, and those species most likely to have a significant role in the agroecosystem which would have the most significant environmental effect if harm is to come to the organism (Hilbeck *et al*., 2006b).

During this step ecological principles are used to prioritize non-target species. Andow and Hilbeck (2004b) suggested that several criteria can be used to prioritize non-target arthropods, including maximum possible exposure and potential adverse effects. Maximum possible exposure of a non-target arthropod species to a transgenic crop is based on its geographical range, habitat specificity, local abundance, prevalence and temporal association with the crop (Andow & Hilbeck, 2004b). Potential adverse effects refer to the potential consequences of an adverse effect on a non-target arthropod species. These are considered to be more serious if the species has ecological or economic significance, is rare, or has symbolic value (Andow & Hilbeck, 2004b).

A selection matrix can be used to support the selection of species for use in a risk assessment analysis. The selection matrix compiled by Van Wyk *et al*. (2007) which was based on a selection matrix developed by Andow and Hilbeck (2004a) was used in this study. The following data were used to rank each species for their maximum potential exposure to Bt toxin: occurrence, abundance, presence and linkage to the soybean agroecosystem, as well as the potential adverse effects that exposure may have on the non-target species (Table 4.3) (Andow & Hilbeck, 2004b; Van Wyk *et al*., 2007). Occurrence, in this context, refers to the presence of a non-target species in the agroecosystem, its geographic range and prevalence. Abundance refers to local abundances and prevalence, while presence refers to the temporal association with the crop. Linkage refers to the habitat specificity and

degree of specialization on the organism on the crop (Andow & Hilbeck, 2004b; Van Wyk *et al*., 2007).

4.2.4 Selecting high-priority species to test

Andow and Hilbeck (2004b) suggested that species with unknown ecological function, species with a high standing biomass, or those found in frequent association with the transgenic crop habitat should be selected for testing. By doing so a scientifically justified precautionary approach is introduced to the risk assessment.

In this study 31 of the 371 species identified in soybean (chapter 3) were selected due to their high abundance and association with the crop. These species were then evaluated by using available expertise and literature. Off the 31 species, 10 were selected and evaluated through the selection matrix and ranked for maximum potential exposure. This group of species consisted of species from each functional group, as suggested by Andow and Hilbeck (2004b). The 10 species were selected based on multiple criteria including abundance, function and association with the crop. This sifting process was necessary due to the large number of morphospecies identified in the study. During this process non-target arthropod species that were recorded in soybean fields were prioritized for their close association with soybean, general occurrence in the soybean growing regions of South Africa and the potential for adverse effects, if a change in their occurrence occurs.

4.3 Results

During the field surveys a total of 29 455 arthropod specimens were recorded (chapter 3). These were classified into 15 orders, 82 families, 371 morphospecies and five functional groups. The Coleoptera were the most abundant order with 12 705 individuals (78 morphospecies) and the Hymenoptera were the most diverse order with 111 morphospecies (Table 4.1).

A number of species were prioritized from each functional group based on their abundances (Table 4.2). In the herbivore group, 127 morphospecies were identified from six orders, of which nine morphospecies were prioritized. In the predator group, 96 morphospecies were identified from nine orders and 10 were prioritized. A total of 79

morphospecies were identified to be parasitoids from the Diptera and Hymenoptera. Of these 79 species, eight were given priority. Seventeen morphospecies were identified to be pollinators, but their abundances were very low and none of them were prioritized. However, *Astylus atromaculatus* Blanch (Coleoptera: Melyridae) (Melyridae MS1) had a high abundance but was not prioritized because it is an invasive species and a pest in South Africa (Midega *et al*., 2007). In the detritivore group, 40 morphospecies, of which four were prioritized were identified from seven orders. The prioritized species from each functional group is shown in Table 4.2.

The priority species listed in Table 4.2 were then evaluated and 10 species that were considered to be important species based on the criteria described above were selected (Fig. 4.1.). These species were evaluated through the selection matrix and ranked for maximum potential exposure to the Bt toxin (Table 4.3). Five morphospecies were given the highest rank (1). These were Anthocoridae MS1 (*Orius* sp.), Geocoridae MS1, Thomisidae MS2 (*Misumenops rubrodecoratus*), Coccinellidae MS5 (*Hippodamia variegata*) and Miridae MS4. Four species were given a rank of 2 and one was given rank 3 (Table 4.3).

Table 4.1 Non-target arthropods recorded in soybean agroecosystems in South Africa.

Order	**Abundance**	**Species richness**	**Number of families**
Coleoptera	12 705	17	78
Hemiptera	6 168	16	63
Diptera	3 384	15	37
Hymenoptera	2 544	11	111
Thysanoptera	1 942	1	6
Lepidoptera	1 711	3	13
Araneae	640	7	41
Orthoptera	129	4	8
Collembola	95	1	2
Ephemeroptera	76	1	1
Neuroptera	47	2	3
Dermaptera	6	1	2
Psocoptera	4	1	2
Blattodea	3	1	3
Mantodea	1	1	1
Total	29 455	371	82

Table 4.2 Priority species identified from each functional group based on abundance.

Functional group	Priority species	Abundance
Herbivores	Chrysomelidae MS1	1 055
	Cicadellidae MS1	729
	Aphididae MS2	624
	Miridae MS1 (Nymphs)	511
	Cicadellidae MS2	375
	Pentatomidae MS2 (*Nezara* sp.)	327
	Nymphalidae MS1 (*Vanessa cardui*)	245
	Chrysomelidae MS14	69
	Noctuidae MS1	51
Predators	Anthicidae MS1	652
	Anthocoridae MS1 (*Orius* sp.)	505
	Geocoridae MS1	197
	Thomisidae MS2	179
	Coccinellidae MS5 (*Hippodamia variegata*)	173
	Anthicidae MS2	148
	Theridiidae MS4	133
	Miridae MS4	103
	Dolichopodidae MS1	79
	Thomisidae MS4 (*Misumenops rubrodecoratus*)	72
Parasitoids	Tachinidae MS3	659
	Chalcidoidea MS26	322
	Chalcidoidea MS9	315
	Braconidae MS26	241
	Braconidae MS8	219
	Chalcidoidea MS3	173
	Tachinidae MS2	76
	Tachinidae MS1	53
Pollinators	None	0
Detritivores	Mycetophilidae MS1	361
	Muscidae MS4	235
	Tenebrionidae MS1	205
	Entomobryidae MS1 (*Seira* sp.)	93

Table 4.3 Selection matrix to rank priority species. Species marked in red received the highest rank.

Species	Maximum potential exposure				Possible adverse effect	
	Occurrence	Abundance	Presence	Linkage	Significance	Rank
Anthocoridae MS1 (*Orius* sp.)	Certain	High	Anytime	Weak	High (important predator)	1
Geocoridae MS1	Certain	High	Anytime	Doubtful	High (important predator)	1
Thomisidae MS2 (*Misumenops rubrodecoratus*)	Certain	High	Anytime	Doubtful	Medium	1
Coccinellidae MS5 (*Hippodamia variegata*)	Certain	High	Anytime	High	High (important predator)	1
Miridae MS4	Certain	Medium	Anytime	Doubtful	High	1
Braconidae MS26	Certain	High	Flowering & post-flowering	Doubtful	Uncertain (parasitoid)	2
Braconidae MS8	Certain	High	Flowering & post-flowering	Doubtful	Uncertain (parasitoid)	2
Tachinidae MS3	Occasional	High	Anytime	Doubtful	Low (uncertain)	2
Nymphalidae MS1 (*Vanessa cardui*)	Certain	Medium	Anytime	Weak	Low (polyphagous)	2
Entomobryidae MS1 (*Seira* sp.)	Certain	Medium	Anytime	Doubtful	Uncertain	3

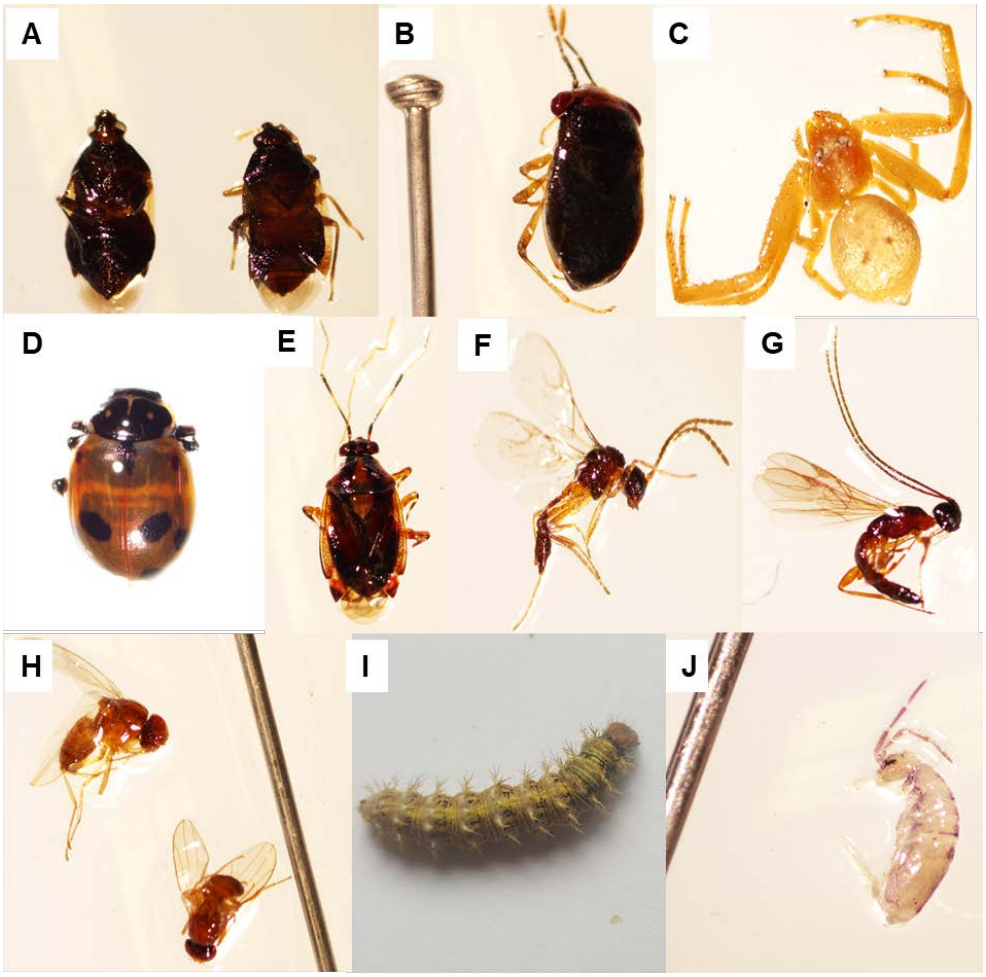

Fig. 4.1 Nine of the 10 species considered to be important and put through the selection matrix to rank them for maximum potential exposure to the Bt toxin. A) Anthocoridae MS1 (*Orius* sp.), B) Geocoridae MS1, C) Thomisidae MS2 (*Misumenops rubrodecoratus*), D) Coccinellidae MS5 (*Hippodamia variegata*), E) Miridae MS4, F) Braconidae MS26, G) Braconidae MS8, H) Tachinidae MS3, I) Nymphalidae MS1 (*Vanessa cardui*), J) Entomobryidae MS1 (*Seira* sp.).

4.4 Discussion

The effects of Bt crops on non-target entomophagous natural enemies of pest species have been a major concern since these organisms often play an important role in the regulation of pest populations through biological control and are therefore considered to be of economic value (Naranjo, 2005; Dutton, 2003). Moreover, this group of organisms may be a good indicator of potential ecological impacts of transgenic plants because they belong to the third trophic level of food chain (Groot & Dicke, 2002) and should therefore be included in non-target risk assessments. In

this study five predator and three parasitoid species were identified for inclusion in future non-target risk assessments of Bt soybean. Of the eight natural enemies identified for inclusion, the five predator species received the highest rank. These predator species were from the Coccinellidae, Anthocoridae, Thomisidae, Miridae and Geocoridae families.

The selection matrix indicated that Coccinellidae MS5, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), received the highest ranking due to its high abundance and common occurrence within the cropping system (Table 4.3). Furthermore, it has a strong linkage with the soybean agroecosystem and is an important predator within agroecosystems in general (Meissle *et al*., 2012). Botha *et al*. (2018) found *H. Variegata* to have a strong associated with maize field environments in South Africa. Predatory Coccinellidae are at risk of indirect exposure to Bt toxins through tritrophic interactions, and direct exposure through feeding on pollen of Bt crops when prey is scarce (Hodek *et al*., 2012). Various Coccinellidae species have been evaluated for effects of Coleoptera-specific Bt proteins in tritrophic interactions (Alvarez-Alfageme *et al*., 2008; Kalushkov & Nedvêd, 2005; Riddick & Barbosa, 1998; Dogan *et al*., 1996) and direct feeding conditions (Duan *et al*., 2002; Lundgren & Wiedenmann, 2002). While the latter studies found no effects, others such as Schmidt *et al*. (2009) reported that the larvae of *Adalia bipunctata* L. (Coleoptera: Coccinellidae) died at significantly higher rates when raised on meal moth eggs coated with a solution containing Bt toxins. The large existing database makes members of the Coccinellidae family appropriate NTO's for evaluations seeing that data can be compared over numerous cases. The Coccinellidae are also valued for their biological control functions (Hodek *et al*., 2012) and have previously been recommended as surrogate species for non-target risk assessments of Bt crops (Romeis *et al*., 2014). Therefore, it is suggested that Coccinellidae MS5 (*Hippodamia variegata*) be included in non-target risk assessments of Bt soybean in South Africa.

Species from the families Anthocoridae and Geocoridae are important predators that have been widely evaluated in risk assessments (Naranjo, 2005; Ponsard *et al*., 2002; Zwahlen *et al*., 2000). Anthocoridae MS1 (*Orius* sp.) and Geocoridae MS1 were identified by means of the selection matrix as high priority species due to their high abundances and important roles as predators (Table 4.3). Anthocoridae,

especially *Orius* spp., are polyphagous predators during both the nymphal and adult stages and feed on thrips, spider mites, aphids and lepidopteran eggs (Chambers & Long, 1992). Geocoridae are important biological control agents in many agroecosystems (Desneux *et al*., 2006). However, since many Geocoridae species are capable of utilizing plant material (Pilcher *et al*., 1997), the potential exists for direct and indirect exposure. Furthermore, few 1st-tier risk assessments of the impacts of Bt toxins on this family have been done (Duan *et al*., 2014). Therefore, this species was given a high rank for inclusion in risk assessments (Table 4.3). Another important predator to which a high ranking was assigned (Table 4.3) was a spider species, Thomisidae MS2 (*Misumenops rubrodecoratus* (Millot) (Araneae: Thomisidae)). This species was commonly found and in high abundances within soybean fields although its linkage with the crop is doubtful. The fifth predator species identified with highest priority was a mirid bug species (Miridae MS4).

Three parasitoid species, Braconidae MS8, Braconidae MS26 and Tachinidae MS3, were considered of lesser importance than the above-mentioned non-target species (Table 4.3). These species could be considered in risk assessments and monitoring programs in regions in which they occur, however their linkage with soybean in unknown or doubtful and their value is also unknown within the soybean agroecosystem. The detritivorous species, Entomobryidae MS1 (*Seira* sp.) (Castaño-Meneses *et al*., 2004) can be considered to be a value unknown species and therefore it is not possible to speculate on the effects that may result should Bt soybean have a negative or positive effect on their populations. It was ranked lower in importance since little is known about it and its linkage with soybean is doubtful seeing that *Seira* sp. occurs in many different habitats (Castaño-Meneses *et al*., 2004).

Nymphalidae MS1, *Vanessa cardui* L. (Lepidoptera: Nymphalidae), has more than 300 host plant species including sunflowers, soybean, green bean, artichoke and canola and rarely becomes a serious pest (du Plessis, 2015). Populations of *V. cardui* will most likely be exposed to Bt toxins since the species was found in relatively high abundances and occurred commonly thorough the season within soybean fields but its linkage with soybean was weak, due to its wide host plant range (Table 4.3). Due to its abundant populations and frequent occurrence the selection matrix indicates that this species has a very high maximum potential

exposure to Bt toxin. It should therefore be considered for evaluation seeing that *V. cardui* is an important pollinator of wildflower species and may thus be an economically and ecologically important species (Johnson, 1997). It does however also have the potential to become an economically important pest. In Iowa *V. cardui* larvae caused serious damage to soybean crops in 1968 and 1976 resulting in growers having to replant their crop (Pedigo, 1975).

Changes in cropping environments such as the introduction of Bt soybean, that aim to suppress the occurrence of primary pests can cause guild rearrangements that may lead to the development of secondary pests (Van Wyk *et al*., 2007). The control of primary pest species by means of Bt crops may be associated with outbreaks of secondary pests that are no longer controlled by the insecticide applications that were previously used to control the primary pests (Catarino *et al*., 2015; Lu *et al*., 2008). The cultivation of Bt crops have been associated with a reduction in insecticide spray applications in many regions (Brookes & Barfoot, 2018; Naranjo, 2009). The target pest of Bt soybean expressing Cry1Ab toxins in South Africa is a Lepidoptera species, *H. armigera*. For this reason, it is essential to assess the potential effects of Bt soybean on other lepidopteran species. The matrix in Table 4.3 suggests that *V. cardui* be assessed because it received the second highest possible ranking and it is unknown how Bt toxins will affect its populations. Therefore, the possibility exists that *V. cardui* could become a secondary pest to Bt soybean as was the case in South Dakota where Bt maize hybrids were found to be susceptible to ear injury by western bean cutworm, *Striacosta albicosta* (Smith) larvae, which was initially not economically important (Catangui & Berg, 2006).

Other non-target Lepidoptera species that feed on soybean may also be affected by guild rearrangements that may occur. It is therefore also important to evaluate the effects of Bt soybean on these species. Other lepidopteran species reported on soybean in South Africa are the groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) (du Plessis, 2003), *Trichoplusia orichalcea* F. (Lepidoptera: Noctuidae), and *Agrotis segetum* (Schiff.) (Lepidoptera: Noctuidae) (Liebenberg, 2012; du Plessis, 2015). These species should be evaluated for non-target effects, for example, secondary pest development.

4.5 Conclusion

GM crops are regulated worldwide, and these regulations often require an environmental risk assessment. ERA's on non-target organisms can be improved by using an ecological model which can be applied to a specific environment in order to classify species functionally and prioritize them to identify potential test species, making the ERA case-specific. This study identified 10 species as important for inclusion in future non-target risk assessments of Bt soybean. Of these 10 species five were predators. The painted lady (*Vanessa cardui*) was given the second highest rank and it is recommended that this species be monitored as the possibility exists for it to develop into a secondary pest. This study provides a framework for the selection of non-target arthropods for risk assessments of the possible effects of Bt soybean expressing Cry1Ac proteins on non-target species in South Africa.

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5 Chapter 5: A comparison of three sampling methods for sampling arthropods in soybean fields

Abstract

The accurate assessment of arthropod communities in agroecosystems is an essential component of pest management and risk assessments. Information collected through sampling contributes to decision making regarding pest control and can be used in ecological studies as well as Ecological Risk Assessments. It is therefore important that suitable sampling methods are chosen to ensure accurate and reliable assessments are made. The aim of this study was to evaluate and compare the efficacy of three sampling methods (beating sheet, adapted D-vac method and sticky traps) for collection of above-ground arthropods in soybean fields. Sampling was done during the 2017/18 cropping season on soybean trial pots at three locations in South Africa's major soybean production regions. The D-vac method was found to be the best method for sampling overall arthropod diversity. The D-vac sampled a higher number of species and individuals than the beating sheet method and was particularly efficient in sampling Diptera, Hymenoptera and Thysanoptera. The beating sheet method sampled comparatively higher abundance of Coleoptera and Orthoptera than the D-vac method. Sticky traps were especially efficient in sampling, Thysanoptera, parasitic wasps and Cicadellidae when compared to the beating sheet and D-vac methods. Since the different sampling methods yielded different results, the use thereof should be determined by the aims of a particular study. For example, to evaluate arthropod communities for non-target risk assessments, the D-vac should be used as it samples the highest diversity of arthropods and the sticky traps be used to supplement the sampling as it was found to be more efficient in sampling parasitoids that are important for non-target risk assessment and biological control studies. Furthermore, the D-vac method was found to be the most sufficient method for sampling the ten priority species identified in chapter 4.

Key words: D-vac, beating sheet, yellow sticky trap, soybean, arthropods, sampling methods.

5.1 Introduction

Biodiversity of agroecosystems provide ecosystem services that are important for crop production and sustaining the surrounding environment (Jones & Snyder, 2018; Altieri *et al*., 2015; Altieri, 1999). Some arthropod species are considered to be keystone species because the loss of their ecological function may lead to the collapse of the wider ecosystem or food chain (Gullan & Cranston, 2000). Integrated Pest Management (IPM) strategies also rely on ecosystem services such as biological control where these services play important roles in the suppression of arthropod pest populations (Romeis *et al*., 2018; Naranjo *et al*., 2015). The diversity of arthropods in agroecosystems can be high (Botha *et al*., 2015; Perfecto *et al*., 1997; Fauvel, 1999) and the communities can consist of arthropods that occupy different crop strata and have different modes of movement and foraging (Schellhorn *et al*., 2014). Sampling for decision making is one of the corner stones of IPM. For example, pest sampling and assessing natural enemy abundances form the basis of decision making in IPM (Blackshaw & Vernon, 2006; Nyrop & Van der Werf, 1994). To make reliable pest management decisions, monitor efficiency of pest management strategies and evaluate the potential insect damage to a crop and potential natural control, it is important to accurately estimate populations of pests and beneficial insects (Musser *et al*., 2004; Kharboutli & Mack, 1993; Marston *et al*., 1976) as an unreliable estimate can lead to inadequate decision making, such as the unnecessary application of control methods that could harm the ecosystem (Jepson & Thacker, 1990).

Accurate assessment of arthropod communities is important for a number of reasons, for example, for large scale ecological studies such as the monitoring of changes in biodiversity over time and for the development of strategies to enhance biodiversity and species conservation (Sánchez-Bayo & Wyckhuys, 2019; Blackshaw & Vernon, 2006). Sampling of pest and beneficial species at a local scale (crop fields) can provide information on which decisions regarding pest management are made (Binns & Nyrop, 1992). Sampling of specific groups or species of arthropods may be required in monitoring programs where pesticides are applied or as part of risk assessment research prior to release of genetically modified (GM) crops with insecticidal traits (e.g. Bt soybean). Identification and monitoring of priority species that may be at risk of harm or adverse effects resulting from crop management

practices such as pesticide applications or off-target effects of GM crops have been highlighted by several studies (Ba *et al*., 2018; Andow & Hilbeck, 2004; Dutton *et al*., 2003).

In South Africa, regulated products such as GM crops are subject to an environmental risk assessment before it can be approved for commercial production as well post release monitoring (GMO Act, DAFF, 2005). As a part of such a risk assessment, the possible impact of the product on non-target arthropods must be evaluated (Andow & Hilbeck, 2004; GMO Act, DAFF, 2005). These non-target risk assessments for GM crops should be case specific, taking in account the specific crop, transgene and receiving environment (Andow & Hilbeck, 2004). Additionally, field experiments are important to detect consequences of new GM crops in the environment (Poppy & Sutherland, 2004; Andow & Hilbeck, 2004). It is thus important that appropriate sampling methods are used to survey the non-target arthropod species that occur in the receiving environment. Due to the sheer magnitude of arthropods that occur in agroecosystems (Botha *et al*., 2015; Perfecto *et al*., 1997) it is impossible to accurately count all the arthropods in a given habitat (Southwood & Henderson, 2000). However, it is essential that these estimates be done in such a way that it ensures proper risk assessment and long-term environmental safety (Meissle & Lang, 2005).

There is a wide variety of arthropod sampling techniques, each with strengths and weaknesses (Zou *et al*., 2016). For sampling of above-ground arthropods in soybean a number of methods have been recommended (Kogan & Pitre, 1980). For instance, sweep nets are used to sample flying insects and those that are more active (Schmidt *et al*., 2008). While sweep net sampling may be very convenient, it is influenced by several factors such as dense foliage which provides resistance to the sweep net movement and variation in handling of the net (Kogan & Pitre, 1980). The beating sheet method has also been recommended by Kogan & Pitre (1980) for sampling of soybean. This method is effective for sampling arthropod species that drop to the ground when disturbed and have slow escape reactions. However, the efficiency of the beating sheet method is influenced by the growth stage of the plant since it is ineffective when plants are small or shed their leaves (Kogan & Pitre, 1980). However, it is possible to achieve consistent results with this method because

the main factor affecting the procedure is the vigor of the shaking (Kogan & Pitre, 1980).

The D-vac method operates by sucking insects off plants and into a collection bag. This method is ideal for sampling smaller arthropods and can also be used to sample fast, agile flying insects (Kogan & Pitre, 1980). Chen *et al*. (2006) suggested that suction sampling provided the benefit of sampling both immature and adult arthropods, and that it was more effective in sampling planthopper populations than yellow sticky traps and Malaise traps

The objective of the study was to compare the effectiveness of three sampling methods (beating sheet, D-vac and sticky traps) for sampling arthropod communities in soybean agroecosystems in South Africa. Recommendations will be made for effective sampling methods for use in monitoring arthropods in soybean agroecosystems in South Africa.

5.2 Materials and methods

5.2.1 Study site

This study was conducted between January and May of the 2017/18 cropping season in soybean trial plots situated at three locations (Fouriesburg, Bothaville and Nigel) in South Africa's major soybean production regions. Each study site (replicate) consisted of 12 trial plots. Each trial plot consisted of six 5-m rows with a plant rate of 350 000 to 400 000 plants per hectare. During the growing period, crop management was done according to local agricultural practices. The study design was a Randomized Complete Block.

5.2.2 Arthropod sampling

Aboveground arthropods were sampled by means of three sampling methods, i.e., a beating sheet, an adapted D-vac and yellow sticky traps. Sampling took place during two different plant growth stages: pre-flowering (between V6-V12) and post-flowering (R4 to R7). Each sample was put into sealable plastic bags and transported to the laboratory where they were frozen for preservation until further analyses could be done. Samples were cleaned and preserved in 70% ethanol. All arthropods were identified to family level and then assigned to morpho-species according to their morphological appearance. It has been shown that this method can provide

estimates of richness similar to methods that make use of species classified by taxonomists (Oliver & Beattie, 1996). A reference collection was compiled in order to assign morpho-species same “species name” over different localities, methods and sampling times. Identification of species was based on that of Picker *et al*. (2004) and Scholtz and Holm (1985). The number of morpho-species and their abundance was determined. The morphospecies were then further classified into major functional groups based on their feeding habits, herbivores, predators, parasitoids, pollinators and detritivores.

5.2.2.1 Beating sheet

The beating sheet method is commonly used to sample arthropods from row crops (Pedigo & Bunting, 1993; Bechinski & Pedigo, 1982; Kogan & Pitre, 1980). A 1 m^2 cloth was placed between the middle two rows of each sample plot. The plants on both sides of the cloth were then vigorously shaken in a direction towards the cloth, to dislodge arthropods which then dropped onto the cloth.

5.2.2.2 D-vac

An adapted D-Vac method was used to sample in rows two and five of each plot. A 3-m long section of each of these two rows were sampled on both sides of the row. This was done by moving the nozzle of the D-vac slowly over the surface of the plants in upward and zigzag movements to ensure that the upper two thirds of each plant were sampled.

5.2.2.3 Yellow sticky traps

Two yellow sticky traps were set up in each plot, between rows one and two, and five and six. The traps were hung at canopy height and left for a seven-day period after which they were removed and covered with plastic cling-wrap for preservation. Nineteen groups of morphospecies were counted on each sticky trap as it is difficult to identify small arthropods due to the fact that the specimens get damaged on the glue (Table 5.4).

5.2.3 **Data analysis**

The aim of this study was to compare the three sampling methods in terms of species richness and abundances and to quantify the variability of the abundance data sampled by means of the different methods. A Venn diagram was created to visualize the number of unique and shared species between the beating sheet and

D-vac methods. The coefficient of variation was calculated as the ratio of the standard deviation to the mean, as a measure of a methods consistency in quantifying arthropod (Rauschen *et al*., 2008).

The species richness and abundances of each arthropod order as well as the five functional groups sampled with the beating sheet and D-vac method was compared by means of *t*-tests. To compare the efficiency of the sticky traps with that of the other two sampling methods, the abundances of 17 morphological groups were compared for all three methods. This was done by means of Spearman rank correlation using Statistica version 13.1.

Sample-based rarefaction curves were constructed as estimates of total species richness, based on all species actually discovered (Sobs), Chao's estimator based on number of rare species (chao1), Chao's estimator using just presence-absence data (chao2) and a Bootstrap estimator based on proportion of quadrats containing each species. This was only done for the beating sheet and D-vac methods. Primer version 6 software was used for this analysis.

5.3 Results

A total of 220 species and 11 875 arthropod specimens were sampled by means of the beating sheet and D-vac methods. Sampling by means of a beating sheet yielded 106 species and 5 671 specimens, while 183 species and 6 204 specimens were sampled by means of the D-vac. Of the total number of species that were sampled, 37 were unique to the beating sheet method and 114 species were unique to the D-vac method, indicating 69 species that were shared between these two sampling methods (Fig. 5.1). The sticky traps captured a total of 61 009 specimens from the 17 selected morphological groups. The majority (89.5%) of specimens on sticky traps were Thysanoptera (54 615 specimens).

The coefficient of variation of the arthropod abundances sampled with the three sampling methods are shown in Table 5.1. The variation in abundance data were similar for the beating sheet and D-vac methods. The beating sheet method did however show less (8.86) variation in the results compared to that sampled by means of the D-vac (10.41). Data collected by means of sticky traps had the lowest coefficient of variation (<1).

Fourteen arthropod orders were identified, with the beating sheet and D-vac yielding 12 and 13 orders, respectively. The D-vac sampled significantly higher species richness and abundances of Hymenoptera, Diptera and Thysanoptera as well as significantly higher abundances of Araneae (Table 5.2). Furthermore, the D-vac method sampled significantly higher species richness and abundances of pollinators, parasitoids and detritivores (Table 5.3).

The accumulation curve estimates indicated that generally insufficient sampling efforts were made for both the beating sheet (Fig. 5.2 a) and D-vac methods (Fig. 5.2 b). However, species accumulation curve estimates compiled from D-vac data over two seasons and altogether 27 sampling occasions (Fig. 5.3) did show sufficient sampling efforts.

The abundances of the 17 morphological groups on sticky traps and those from the D-vac and beating sheet samples are provided in Table 5.4. Spearman rank correlation revealed that no significant correlations occurred between any of the sampling methods for the 17 pre-selected morphological groups (Table 5.5). The sticky traps did not correlate with nether the beating sheet (0.346) nor the D-vac methods (0.553). The beating sheet also did not correlate with the D-vac method (0.922).

When examining which method was the most efficient in sampling the ten species identified as high priority species by means of an ecological model (Chapter 4), it was found that the D-vac method sampled higher abundances of nine out of the ten species (Table 5.6). The only significant difference found was for Thomisidae MS2, for this species the D-vac sampled a significantly higher abundance. The Beating sheet method sampled a higher abundance of Geocoridae MS 1.

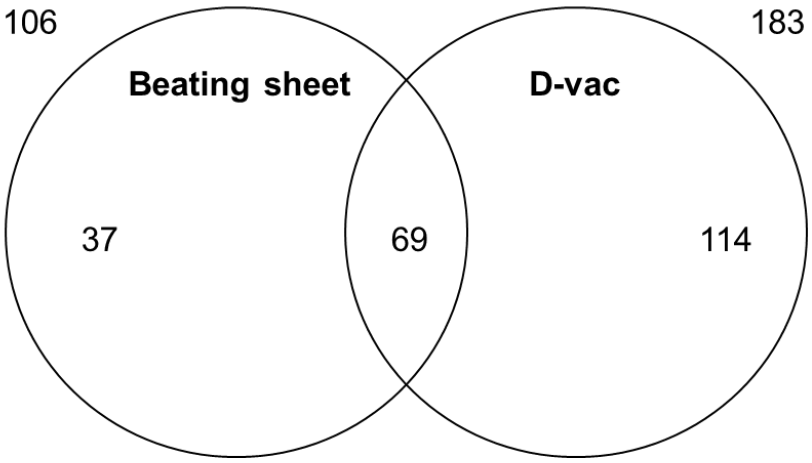

Fig. 5.1 Venn diagram indicating numbers of unique and shared species for two sampling methods, beating sheet and D-vac. The numbers outside the circles indicate the total numbers of arthropods sampled with each method.

Table 5.1 The coefficient of variation for arthropod abundance sampled with the three different methods.

	Standard deviation	Mean	Coefficient of variation
D-vac	173.59	16.67	10.41
Beat sheet	380.62	42.96	8.86
Sticky traps	1054.28	1694.71	0.62

Table 5.2 A comparison of species richness and abundances per arthropod order sampled with by means of the D-vac and beating sheet methods. Values in red indicate significant statistical differences.

Order	Species richness			Abundance		
	Beating sheet	D-vac	p-value	Beating sheet	D-vac	p-value
Hemiptera	31	33	0.209	590	787	0.393
Coleoptera	24	30	1	4 785	3 678	0.763
Araneae	14	18	0.181	74	194	0.004
Hymenoptera	10	54	0.007	22	297	0.031
Diptera	6	26	0.001	46	759	0.007
Lepidoptera	6	7	0.851	74	155	0.244
Orthoptera	5	3	0.355	34	16	0.428
Thysanoptera	3	5	0.002	39	306	0.053
Neuroptera	2	2	0.367	2	6	0.206
Collembola	2	1	1	2	2	1
Dermaptera	2	1	1	2	1	1
Psocoptera	1	0	0.328	1	0	0.328
Ephemeroptera	0	1	0.328	0	2	0.328
Blattodea	0	1	0.328	0	1	0.328

Table 5.3 A comparison of species richness and abundances of each functional group sampled with both the D-vac and Beating sheet methods. Values in red indicate significant statistical differences.

Functional group	Species richness			Abundance		
	Beating sheet	D-vac	p-value	Beating sheet	D-vac	p-value
Herbivores	49	59	0.075	4 819	4 815	0.985
Predator	35	46	0.064	274	586	0.092
Detritivores	10	21	0.004	29	269	0.001
Parasitoids	7	50	0.001	9	449	0.019
Pollinator	1	3	0.004	1	80	0.04

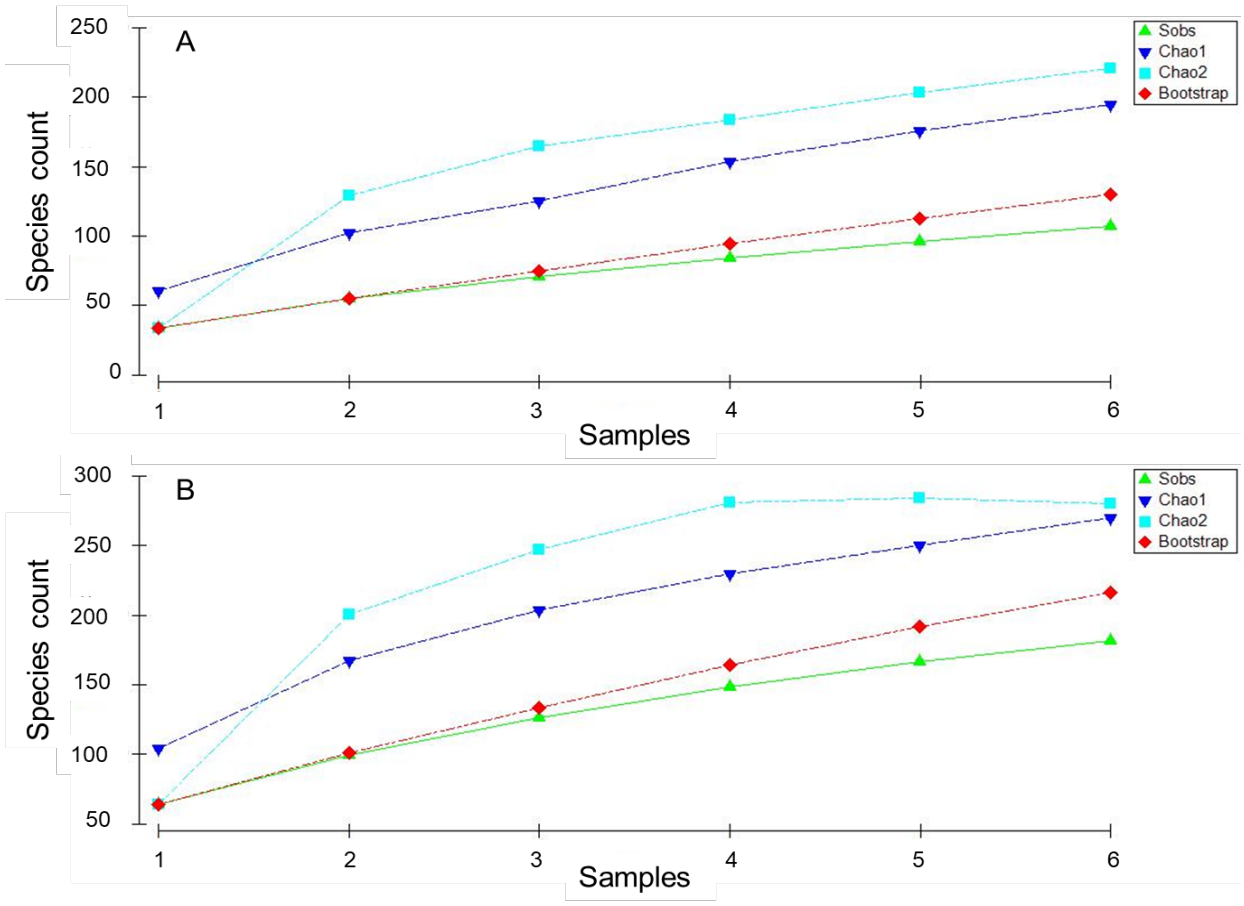

Fig. 5.2 Species accumulation curves generated from 999 permutations for the arthropod species sampled by means of the beating sheet (a) and D-vac (b) methods, species richness estimates based on all species actually discovered (Sobs), Chao's estimator based on number of rare species (chao 1), Chao's estimator using just presence-absence data (chao 2) and a Bootstrap estimator based on proportion of quadrats containing each species.

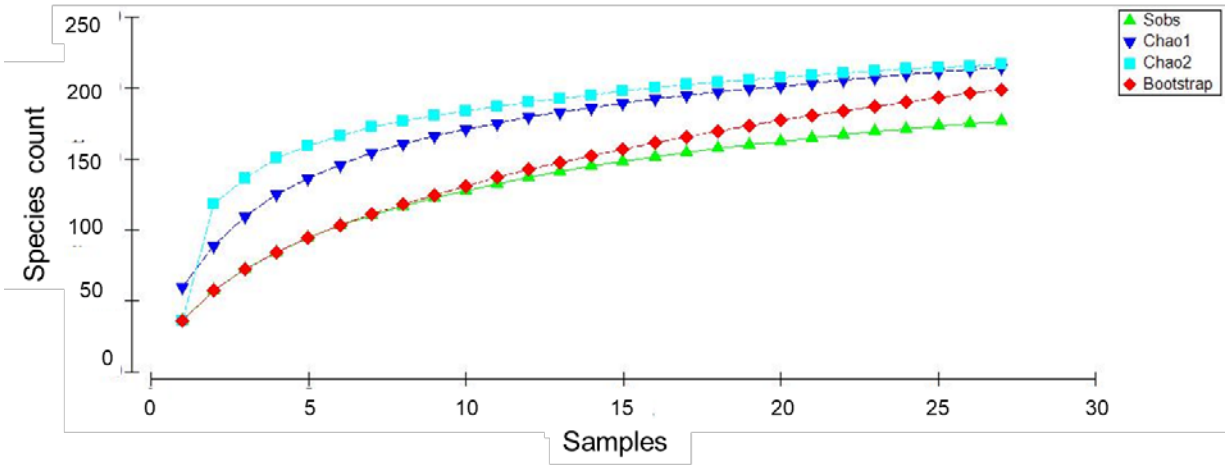

Fig. 5.3 Species accumulation curves for the arthropod species sampled by means of the D-vac method over two seasons with 27 sampling occasions. Species richness estimates based on all species actually discovered (Sobs), Chao's estimator based on number of rare species (chao 1), Chao's estimator using just presence-absence data (chao 2) and a Bootstrap estimator based on proportion of quadrats containing each species.

Table 5.4 Number of individuals of each of the pre-selected morphological groups counted on the sticky traps and compared with the beating sheet and D-vac methods.

	Number of individuals		
Morphological group	**Sticky traps**	**Beating sheet**	**D-vac**
Thysanoptera	54 615	39	306
Parasitic wasps	2 228	21	283
Cicadellidae	1 428	8	164
Other hymenopterans	1 424	1	13
Syrphidae	451	0	3
Miridae and Lygaeidae	360	144	340
Chrysomelidae	136	65	91
Araneae	103	74	194
Orius spp. (Hemiptera: Anthocoridae)	84	2	24
Asilidae	81	0	0
Astylus atromaculatus (Coleoptera: Melyridae)	36	4 367	3 317
Coccinellidae	28	66	122
Other lepidopterans (adults)	23	1	7
Apis mellifera (Hymenoptera: Apidae)	12	0	0
Large wasps	12	0	4
Cynthia cardui (Lepidoptera: Nymphalidae)	3	0	2
Neuroptera	1	2	6

Table 5.5 Spearman rank correlation data comparing the three methods in term of the 17 pre-selected morphospecies groups.

	Sticky traps	Beating sheet	D-vac
Sticky traps	1	-	-
Beating sheet	0.346	1	-
D-vac	0.553	0.922	1

Table 5.6 Comparison of the beating sheet and D-vac method for the 10 species identified as priority species for evaluation with the ecological model (Chapter 4). Values in red indicate significant statistical differences.

Morpho-species	**Abundance**		
	Beating sheet	**D-vac**	**p-value**
Geocoridae MS 1	65	33	0.162
Coccinellidae MS 5	29	74	0.581
Nymphalidae MS 1 (*Cynthia cardui*)	26	77	0.358
Thomisidae MS 2	19	89	0.001
Miridae MS 4	6	10	0.745
Anthocoridae MS 1 (*Orius* sp.)	2	24	0.613
Braconidae MS 26	2	87	0.454
Entomobryidae MS 1	1	2	-
Tachinidae MS 3	0	142	-
Braconidae MS 8	0	1	-

5.4 Discussion

An absolute method for sampling was not included in this study since complexes of arthropods were examined and the use of an absolute sampling method for each of the members of the complex was not feasible. Relative sampling techniques are adequate for experiments where only relative differences between treatments are of interest (Marston *et al*., 1976).

This study found that D-vac, beating sheet and yellow sticky trap methods produced different results. Differences in type and abundance of arthropods occurred between the sampling methods. This is to be expected due to the nature of each sampling method. Yellow sticky taps only provide information on flying arthropods. It can therefore not directly evaluate population densities of arthropod communities, especially as immature arthropods are not capable of flight (Chen *et al*., 2006; Musser *et al*., 2004). Furthermore, sticky traps are time consuming and the identification of certain species are often not possible due to the specimens being

damaged on the glue (Rauschen *et al*., 2008). Yellow sticky traps can however be used to monitor insect pest in crops, for instance Hein & Tollefson (1985) used yellow sticky traps to monitor adult western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) in maize fields. Atakan and Canhilal (2004) found yellow sticky traps efficient in capturing Thysanoptera and leafhopper pests of cotton and found that trap height negatively affected the number of insects captured. Yellow sticky traps have also been found useful to monitor non-target arthropods such as pollinators (Gill & O'neal, 2015). However, Chen *et al*. (2006) suggested that yellow sticky traps only provide information on the dispersal of adult arthropods with the ability to fly and can thus not directly measure population densities of arthropods. The beating sheet method on the other hand is effective for sampling flightless arthropods or those that disperse slowly, for instance Lepidoptera larvae (Kogan & Pitre, 1980). In contrast, the D-vac method was more effective in collecting immature and adult arthropods that may or may not be capable of flight (Chen *et al*., 2006), it is a non-destructive method, making identification easier (Zou *et al*., 2016; Meissle & Lang, 2005). The differences in results produced by different sampling methods will lead to different interpretations of the diversity and abundance data of arthropod species associated with the crop. This was also reported by Rauschen *et al*. (2008), Kharboutle and Mack (1993), Buffington and Redak (1998) and Doxon *et al*. (2011). The Spearman rank correlation data from this study showed no correlation in the abundance or richness of groups between sampling methods. This means that all three methods sampled different types and abundances of the 17 species.

In this study the beating sheet method sampled fewer species and at a lower abundance compared to the D-vac method. Significantly fewer species with the capability of flight (Diptera, Hymenoptera and Thysanoptera) were collected by means of the beating sheet method when compared to the D-vac. This is in accordance with the findings of Chanthy *et al*. (2013) who reported that the numbers of Diptera, Hymenoptera and Thysanoptera collected by means of beating sheets were significantly lower than those collected by means of other sampling methods. Furthermore, the beating sheet was significantly less effective for sampling of highly mobile arthropods such as pollinators and parasitoids, groups that are of importance for non-target risk assessments (Huang *et al*., 2004). The beating sheet also

sampled lower abundances and species richness of lepidopteran larva, which is in contrast with a report by Turnipseed (1974) who found the beating sheet to be significantly more efficient at sampling lepidopteran species such as soybean looper larvae, *Chrysodeizis includens* (Walker) (Lepidoptera: Noctuidae) and green cloverworm larvae, *Plathypena scabra* (F.) (Lepidoptera: Noctuidae). This study therefor suggests arthropod sampling by means of beating sheets is not effective to sample arthropod communities as a whole.

The yellow sticky traps were much more successful in trapping Thysanoptera, Cicadellidae, Syrphidae, Asilidae and Hymenoptera than both the beating sheet and D-vac method. This is due to the efficacy of sticky traps as sampling method for adult insects capable of flight (Chen *et al*., 2006). However, it was ineffective in sampling larger beetles such as *Astylus atromaculatus* Blanchard (Coleoptera: Melyridae) and Coccinellidae (Table 5.4). This is probably due to the glue not being strong enough to hold large insects, or the body shape that may enable the organism to escape more easily (Stephens & Losey, 2004).

The D-vac method was the most effective for sampling overall plant-dwelling arthropod communities since it was successful in sampling a wide variety of species from all functional groups. Furthermore, highly mobile arthropods (Diptera) as well as those that are not (Araneae) occurred in large numbers in D-vac samples. These results are similar to those reported by Buffington and Redak (1998) who indicated that a D-vac could be used effectively to sample Diptera, Hymenoptera and Hemiptera in both juvenile and adult phases of their life cycles. Sanders and Entling (2011) also found the D-vac to efficiently sample Diptera, Hymenoptera and plant-dwelling spiders. Other studies have also shown the effectiveness of the D-vac method for a wide array of arthropod species. The study by Meissle and Lang (2005) also found the D-vac to be effective for sampling plant-dwelling spiders in soybean. The D-vac method was found to be more effective in sampling plant hopper populations than yellow sticky taps and Malaise traps when the most prevalent species was *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) (Chen *et al*., 2006). Turnipseed (1974) found the D-vac to be effective in sampling quick-moving insects such as leafhoppers, whiteflies and parasitoids. Doxon *et al*. (2011) found the D-vac to be effective for sampling Carabidae, Homoptera and Diptera.

In addition, a variety of D-vac samplers are available including a petrol-driven leaf blower-vac (Arida & Heong, 1992: Domingo & Schoenly, 1998). These modifications to the original Dietrick vacuum (1961) are cheaper and easier to handle due to reduced size and weight making them more accessible (Zou *et al*., 2016). Furthermore, the D-vac is capable of providing absolute estimate of arthropod densities when combined with sampling cages in certain environments, it allows for the collection and storage of samples, unlike visual counts and sticky traps (Zou *et al*., 2016). However, the D-vac method has been found not to be suitable for sampling large hymenopterans (Zou *et al*., 2016), this was also found to be the case for this study were the sticky traps sampled a higher abundance of large wasps and *Apis mellifera* L. (Hymenoptera: Apidae) (Table 5.4). A low abundance of pollinators in general (Table 5.3), were sampled with all three methods.

In this study the D-vac method sampled more species and a significantly higher abundance of Araneae than the beating sheet method and a higher abundance than the sticky trap method. This is in contrast with the findings of Meissle and Lang (2005) and Ludy and Lang (2004) who found the beating sheet method to be more effective in sampling plant-dwelling spiders in maize fields. However, both the abovementioned studies found the two methods to yield a relatively similar number of species and abundance of spiders.

Results from this study also show that when the D-vac method is used at least 15 sampling occasions are needed for the species accumulation curve to start reaching a plateau (Fig. 5.2: Fig. 5.3). It is recommended that these evaluations are done over more than one cropping season as arthropod abundances fluctuate from year to year (Rauschen *et al*., 2008; Head *et al*., 2005; Guo *et al*., 2016; Gill & O'neal, 2015). Thus, a sensible strategy for assessing the effects of a pest management strategy on arthropod communities in soybean would be to use at least two techniques over multiple seasons, this study suggests the D-vac method and yellow sticky traps.

In chapter 4 ten species were identified as being important for non-target risk assessments of Bt soybean in South Africa. This study found that the D-vac method was more sufficient in sampling nine out of the ten species, and significantly more sufficient in sampling Thomisidae MS 2 ($p < 0.05$) (Table 5.6). Three of the ten

species were small flying insects from families in the orders Diptera, and Hymenoptera. It is therefore suggested that sticky traps are used as supplementary to the D-vac method when sampling these important species since the sticky traps were found to sample these orders with high efficiency in this study and previous studies (Gill & O'neal, 2015). Furthermore, the remaining species were from the orders Araneae, Lepidoptera, Coleoptera, Hemiptera and Entomobryidae making the D-vac a suitable method for sampling these species.

It is important to utilize a suitable sampling method when risk assessments are done, for instance when assessing the impacts of Bt crops on non-target arthropods. The D-vac was found to be an appropriate method for assessing the impacts of Bt crops on any species of arthropods residing on the surface of the crop plants (Chen *et al*., 2006). When non-target risk assessments of Bt crops are done it is important that predators such as spiders are sampled properly as the risk of indirect effects exist and the important role spiders play as natural enemies of target pests (Ludy & Lang, 2004: Lang, 2003).

Many studies rely on only one method to collect data for non-target risk assessments. Whitehouse *et al*. (2005) made use of suction sampling method to evaluate the effects of Bt cotton on arthropod communities in Australia. Guo *et al*. (2016) used visual counts to evaluate the effects of Bt on non-target lepidopteran species. Visual counts were also used by Yu *et al.* (2014) to evaluate arthropod abundance and diversity in Bt soybean. Li *et al*. (2014) made use of a vacuum-suction machine to evaluate the effects of Bt and non-Bt rice on arthropod abundance and diversity. However, as mentioned earlier different sampling methods were found to produce different results. Thus, studies that assess the possible effects of Bt crops on arthropod communities should use more than one method and chose methods that complement one another to ensure the thorough evaluation of the crop. For instance, Gill and O'neal (2015) suggested that yellow sticky traps could be used to compliment other sampling methods when sampling hymenopteran pollinators and that they were very sufficient in sampling the abundance and diversity of flower-visiting flies, whereas other methods such as sweep nets were found to be ineffective when sampling pollinators. The choice of techniques will depend on, among others, the field conditions and the type of arthropods sampled for instance flying predators or non-target arthropods in general (Kharboutle & Mack, 1993).

5.5 Conclusion

The richness and abundance of species collected in soybean fields by means of D-vac was higher than the beating sheet method. Species identified as priority species for testing with regards to Bt soybean (Chapter 4) were also all collected more effectively with the D-vac method. However, the sticky traps were found to be more successful in sampling parasitoids, Thysanoptera and larger hymenopterans and is therefore recommended as a supplementary method to the D-vac. Therefore, before collecting data careful consideration should be given to the group or species of arthropod that will be monitored and the sampling techniques required to monitor them.

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6 Chapter 6: Conclusion and recommendations

Arthropods are the most diverse and abundant group of organisms on the planet and are important for the overall functioning and stability of ecosystems (Ødegaard, 2000; Wilson, 1987). In an agroecosystem, arthropods provide ecosystem services that are important for crop production and for sustaining the surrounding environment (Jones & Snyder, 2018; Altieri *et al*., 2015 Altieri, 1999). However, it has been reported that entomofauna is declining worldwide (Sánchez-Bayo & Wyckhuys, 2019; Dudley *et al*., 2017). The rate of decline has been estimated at an annual 2.5% loss of biomass worldwide (Sánchez-Bayo & Wyckhuys, 2019). This decline could have detrimental effects on ecosystem services that impact food production such as pollination and biological control (Van der Sluijs & Vaage, 2016; Aizen *et al*., 2009; Dutton *et al*., 2002). One of the many contributing factors to this decline is agricultural intensification (Sánchez-Bayo & Wyckhuys, 2019). Within agriculture many factors including the use of chemical insecticides has been found to have adverse effects on arthropod communities (Elzen, 2001; Leigh *et al*., 1966). Therefore, it is important that sustainable agricultural practices be implemented (Candolfi *et al*., 2000).

Genetically modified crops that express Cry proteins derived from the bacterium *Bacillus thuringiensis* (Bt) were first released for commercial production in 1996 (ISAAA, 2017). Bt crops provide many social and economic benefits including the potential to reduce insecticide spray applications (Brookes, 2019; Brookes & Barfoot; 2018, 2016; Shelton *et al*., 2002). Crops expressing Cry proteins are largely target specific and thus have a narrow target species range, reducing concerns for adverse effects on non-target organisms (Malone & Burgess, 2009; Li *et al*., 2007). Notwithstanding the benefits that Bt crops may provide, concerns still exist about the safety of Bt crops to the environment and non-target organisms (Yu *et al*., 2014; Romeis *et al*., 2008).

Analysis of the potential effects of Bt crops on the environment and other economic and social aspects are needed before the commercialization of the specific crop (Nap *et al*., 2003). Therefore, environmental risk assessments (ERAs) are a compulsory component of pre- and post-release testing of Bt crops to ensure the safety of these crops to the environment (Andow & Hilbeck, 2004). A risk

assessment is a process by which risks are identified and the seriousness of the risks are characterized to ensure that appropriate decision-making takes place on whether or not to proceed with the technology (Andow & Hilbeck, 2004).

A Bt soybean, *Glycine max* (L.), expressing Cry1Ac toxins showing high levels of resistance to *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Yu *et al*., 2011), has been approved for field trials in South Africa and is currently in the risk assessment stage. The study had three aims, firstly, to determine whether Bt soybean plants have a significant effect on non-target arthropod communities in soybean agroecosystems. Secondly, to evaluate the non-target arthropod species found within the soybean agroecosystem by using an ecological model to select high priority species to monitor for possible effects in an ERA. And thirdly to compare sampling methods in order to establish which sampling procedures are most effective for sampling arthropods in soybean agroecosystems.

A comparative risk assessment approach can be used in an ERA for Bt crops. In this approach the Bt crop is compared with its non-Bt equivalent (Conner *et al*., 2003; Nap *et al*., 2003). This approach detects any possible changes in arthropod communities that are the result of the Bt proteins being expressed by the plant (Bradford *et al*., 2005). A similar approach was used in this study to accomplish the first aim, which was to assess the potential effects that Bt soybean might have on arthropod communities in soybean trial plots. The study took place at five locations during two growing seasons in South Africa. The arthropod diversity, abundance and community composition was evaluated by means of three different sampling methods. The results did not show any significant differences in arthropod communities when Bt soybean trial plots were compared to non-Bt soybean trial plots. Thus, these results indicate that Bt soybean expressing Cry1Ac toxins had no effect on the diversity, abundance or community composition of non-target arthropod communities in soybean field plots in the short-term. This study provides information regarding arthropod communities in soybean agroecosystems in South Africa. This is also the first study to investigate the effects of Bt soybean on non-target arthropod communities in Africa and could provide a framework for future risk assessments.

Another approach to ERAs of Bt crops to non-target organisms is to use a tiered approach (Romeis *et al*., 2008; Dutton *et al*., 2003). This approach starts with lower-

tier tests which are usually laboratory studies, followed by higher-tier tests such as glasshouse, semi-field and field tests (Yu *et al*., 2011). Due to the high diversity of arthropods and the impracticality of using high numbers of arthropod species for tests, it is necessary to select appropriate species for evaluation before any of the tiered tests can be performed (Yu *et al*., 2011; Knecht *et al*., 2010; Romeis *et al*., 2008; Dutton *et al*., 2003). Models can be used to select the high priority species for testing (Andow & Hilbeck, 2004).

In order to accomplish the second aim of this study an ecological model was used to prioritize the species identified in soybean agroecosystems for an ERA. An ERA can be improved by using an ecological model which is applied to a specific environment and used to classify species functionally and prioritize them in order to identify the most important potential test species (Andow & Hilbeck, 2004). The ecological model is case-specific, taking into consideration the specific transgene, crop and cropping environment (Van Wyk *et al*., 2007; Andow & Hilbeck, 2004). This model relies on ecological principles to select high priority species to test, uncertainties are addressed since relevant species that actually occur in the receiving environment are selected for testing (Andow & Hilbeck, 2004). In this study a number of species were identified as priority species in five functional groups based on their abundance within the soybean agroecosystem. From these 31 species 10 species were considered to be important species and were evaluated through a selection matrix and ranked for maximum potential exposure to the Bt toxin.

For an organism to be affected by a Bt toxin it must be exposed to the toxin first. This exposure could take place through several pathways, but the two main pathways are through the direct consumption of the Bt plant tissue and through the indirect consumption of the Bt plant tissue by consuming an organism which consumed the plant tissue (Yu *et al*., 2011; Groot & Dicke, 2002). By selecting test species that were actually found within the specific receiving environment and considering ecological principles when evaluating their priority for testing, we can be certain that the test species is exposed in some way to the Bt toxin in the agroecosystem.

There is a need for strengthening the risk assessment process of GM crops worldwide (Andow & Hilbeck, 2004). These assessments are hampered by the lack of a basic species checklist (Truter *et al*., 2014). This study identified 10 important

species found in South African soybean agroecosystems that should be considered when lower- or higher- tier tests are done for ERA's. This study provides a baseline for narrowing down important test species for regulators in South Africa streamlining the ERA process for this crop and ensuring ERA's are done efficiently and effectively by saving time and costs. This study provides a framework for selecting high priority species for monitoring of possible effects of Bt soybean on non-target arthropods in South Africa.

It is important to accurately estimate arthropod populations to ensure reliable pest management decisions are made and to evaluate the potential insect damage to a crops and the potential natural control arthropods may give to a crop (Musser *et al*., 2004; Kharboutli & Mack, 1993). Arthropod diversity in agroecosystems can be high resulting in communities which consist of individuals that occupy different crop strata and display differences in behavior (Schellhorn *et al*., 2014). For this reason, it is important that appropriate sampling methods are used for the specific crop and cropping environment.

Sampling also plays an important role in ERA's ensuring that the non-target arthropod species that occur in the receiving environment are accurately surveyed. This in turn ensures for informed decision making. The aim of this study was to compare the effectiveness of three sampling methods for sampling arthropod communities in soybean agroecosystems in South Africa. A beating-sheet, D-vac and yellow sticky traps were used to sample arthropods over one growing season. The results from this study showed that each sampling method produced different results in terms abundance and species sampled. The D-vac method was the most effective for sampling the overall plant-dwelling arthropod communities of soybean. It has been found that arthropod communities differ from year to year (Rauschen *et al*., 2008; Head *et al*., 2005; Guo *et al*., 2016), thus it is suggested that sampling takes place over more than one cropping season. Furthermore, the species accumulation curve for the D-vac method was found to reach a plateau after 15 sampling occasions, suggesting that less than 15 sampling occasions would give inaccurate estimates of the arthropod community.

No single sampling technique provides accurate estimates of all soybean arthropods throughout the entire season, but analyses suggested that the D-vac method is more

satisfactory than the beating sheet method. For this study each sampling method was better for some species than others. Therefore, before collecting data careful consideration should be given to the group or species of arthropod that will be monitored and the sampling techniques required to monitor them. When considering the 10 high priority species identified for testing in an ERA it was found that the D-vac method was the most appropriate method for sampling most of these species. Therefore, it is recommended that the D-vac method be used to sample for non-target arthropod species for ERA's of Bt soybean crops in South Africa.

Bt soybean expressing Cry1Ac toxins were found to have no significant effects on the non-target arthropod communities of soybean agroecosystems in South Africa. Baseline data gathered during this study was used to identify 10 high priority species for testing in ERA's. Furthermore, it was found that the D-vac method is the most appropriate method to sample these 10 priority species as well as the overall arthropod community of soybean fields. It is however recommended that more than one sampling method be used over multiple seasons to accurately sample the arthropod community of soybean fields. The release of Bt soybean for commercial production in South Africa could benefit the environment by reducing the number of chemical insecticides sprayed helping South Africa to farm in a more environmentally friendly manner.

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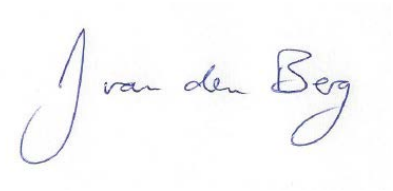
Appendix B: Declaration of language editing

Language editing statement

To whom this may concern,

I, Prof. Johnnie Van den Berg, hereby declare that the thesis titled: “Comparative diversity of arthropods on conventional and genetically modified Bt soybean plants in field trials in South Africa” by Nadine Carol Schutte has been edited for language correctness and spelling by some of the supervisors. No changes were made to the academic content or structure of this work.



Prof. Johnnie Van den Berg Date: 5 November 2019