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Diversity and Abundance of Arthropods on Conventional Sugarcane under Field Conditions in South Africa

by

Roshay Smith

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Supervisor: Prof. Gustav Bouwer

Co-supervisor: Dr Lawrence Malinga

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DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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Roshay Smith

16th day of September 2024 at Bloemfontein, Free State.

ABSTRACT

Insect diversity and abundance are often the base for formulating strategies that involve the appropriate application of pest control methods, considering the ecosystem services provided by insects. Therefore, the aim of this study was to provide recent baseline data on the diversity and abundance of insects in conventional sugarcane based on two sugarcane fields in KwaZulu-Natal. Three sampling methods, namely pitfall, sticky and water pan traps, were used to sample insects in rain-fed and irrigated sugarcane in Gingindlovu and Pongola from March to October 2022. This study collected 12 493 insects belonging to 14 insect orders and 88 families in rain-fed sugarcane and 22 309 insects belonging to 14 orders and 94 families in irrigated sugarcane. Significant differences in the diversity indices were found between the sampling methods and the sampling periods. This study provides recent baseline data on the diversity and abundance of insects in sugarcane.

Keywords: Diversity indices, sampling methods, insect diversity

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"No matter what he does, every person on earth plays a central role in the history of the world. And normally, he doesn't know it" – Paulo Coelho, The Alchemist

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LIST OF ABBREVIATIONS

SASRI	South African Sugarcane Research Institute
ANOSIM	Analysis of Similarities
NMDS	Non-metric Multidimensional Scaling
WP	Water Pan Traps
ST	Sticky Traps
PF	Pitfall Traps
KW	Kruskal-Wallis
PCR	Polymerase Chain Reaction

CHAPTER ONE - INTRODUCTION

1.1 General introduction

Arthropods represent more than half of the global described biodiversity (Bukowski et al., 2022), and form part of many food webs providing ecosystem services and serving as ecosystem engineers (Rosenberg et al., 2023). Since there has been a notable decline in arthropods (Seibold et al., 2019, Sánchez-Bayo and Wyckhuys, 2021), conserving biodiversity has become a prominent subject of research within both natural and agricultural ecosystems wherein biodiversity influences the productivity of that system (Fritz et al., 2011). Agricultural intensification is one of the lead causes of arthropod decline (Blaise et al., 2022). Agricultural production systems are associated with several practices which are detrimental to arthropod communities (Vera-aviles et al., 2020, Blaise et al., 2022). These practices can damage arthropod habitats, disrupt life cycles or cause direct increases in mortality (Sánchez-Bayo, 2021, Jasrotia et al., 2023). The investigation of the inherent biodiversity of agroecosystems is therefore important in ecology and conservation disciplines, as biodiversity maintenance is vital for ecologically sustainable productivity in agriculture (Fritz et al., 2011).

Sugarcane (*Saccharum* sp.) is one of the most vital crops produced globally due to its daily household uses (Zulu, Sibanda and Tlali, 2019) and its major contribution to the national economy of the producing country (Mashoko, Mbohwa and Thomas, 2010). Sugarcane is also an essential source of energy material that can further sustainable development (Prabowo et al., 2021). In South Africa, sugarcane is primarily planted in the KwaZulu-Natal and Mpumalanga provinces, where 95% is under rain-fed conditions (Thibane et al., 2023). Sugarcane is an intensely managed system commonly cultivated as a monoculture (Chi et al., 2020), where procedures such as burn harvesting, chemical control (Prabowo et al., 2021), and other crop management practices have negative effects on biodiversity conservation (Sajjad et al., 2012, Chi et al., 2020, Prabowo et al., 2021). The influence of agricultural intensification on biological diversity is particularly concerning, as intensive management in agriculture is known to be the main driver of global biodiversity loss (Attwood et al., 2008).

Arthropods display a swift reaction to changes in the environment as opposed to vertebrates and therefore, offer prompt detection of ecological alterations (Botha et al., 2015). Insects are helpful bio-indicators of disturbance and add to our knowledge of biodiversity in general because they are found in most habitats (Botha et al., 2015). Also, providing baseline data is essential for understanding the variations in biodiversity over time and assessing the advancements made to conserve biodiversity (Rochette et al., 2019). To accurately study insect biodiversity, collection methods should be carefully chosen to sample the most representative data of an area (Li et al., 2023). Using a range of sampling techniques can provide more diverse data as opposed to using one sampling method (Missa et al., 2009).

1.2 Study Justification

Over 40 years ago, Leslie (1981) conducted a study on the macro-arthropod community of sugarcane fields in four sugarcane industry regions in KwaZulu-Natal, South Africa, as part of a project to assess the predators of the sugarcane pest *Eldana saccharina* Walker. They collected samples using three sampling methods, namely, vacuum sampling, hand sampling and a "fumigant tent", where a plastic tent was put over a sugarcane stool, and fumigant was released beneath it for a suitable time. Sampling was conducted every month for eight months and insects were identified to order level. They recorded 20 taxa with 86-95 % of arthropods belonging to seven taxa, namely, Hymenoptera, Araneida, Hemiptera, Isoptera, Blattaria, Orthoptera and Coleoptera.

Additionally, Beje (1998) investigated the effect of intercropping beans on *E. saccharina* [Lepidoptera: Pyralidae] arthropod predator populations in sugarcane and reported on the epigeal and foliage associated arthropods in sugarcane in La Mercy, KwaZulu-Natal. They collected insects monthly for a year from April 1996 to May 1997, using three sampling methods, namely, pitfall traps, sticky traps and vacuum sampling. They collected insects in sugarcane alone, in a sugarcane-bean intercrop and on the roadway. The pitfall and sticky traps were left out continuously for 96 hours. Vacuum sampling was conducted from 10 am to 11 am after removing pitfall and sticky traps, using 3 m sections as sampling units. Insects were identified to the family level, with only Formicidae identified to the species level.

Since then, there has been no updated data on the diversity and abundance of insects in commercial sugarcane under field conditions. Long-term changes in weather patterns, economic uncertainties, and social pressure to meet food demands have led farmers to adopt new crop cultivation practices, which includes extensive tillage, crop residue burning, and the use of high external inputs (Jasrotia et al., 2023). These new practices may have an impact on recent insect population dynamics (de Oliveira et al., 2021; Prabowo et al., 2021). Furthermore, both previous studies, giving a comprehensive account of the insects present in the sugarcane system, were not the primary focus of the studies, but rather just a part of the study to identify the potential predators of *E. saccharina*. Several other assessments have been conducted on the diversity and community structure of insects in sugarcane in countries such as Indonesia (Rubiana and Meilin, 2020), India (Selvi and Dayana, 2015), and the United States of America (Atencio, Goebel and Miranda, 2019). However, a knowledge gap exists on the current diversity and abundance of insects associated with conventional sugarcane in South Africa. It is practically impossible to make conclusions about the effects that current processes have on temporal dynamics without knowledge of historical and/or contemporary patterns.

1.3 Aim and Objectives

This study aimed to provide recent baseline data on the diversity and abundance of insects in conventional sugarcane using pitfall, sticky and water pan traps. Therefore, the objectives of this study were:

1. To provide a list at order and family level of insects present in the two conventional sugarcane fields for comparison with earlier surveys.
2. To assess different insect sampling methods in sampling insects in irrigated and rain-fed sugarcane fields with regard to the value of each method for revealing insect orders and families occurring in the sugarcane habitats.
3. To determine how insect assemblage composition varies between sampling methods in irrigated and rain-fed sugarcane fields.
4. To determine the diversity and abundance of insects in irrigated and rain-fed conventional sugarcane in KwaZulu-Natal for comparison with earlier studies and to create a baseline for future studies.

CHAPTER TWO - LITERATURE REVIEW

2.1 Sugarcane Production

2.1.1 Sugarcane Production in South Africa

Sugarcane crops cover about 28.3 million hectares of land in more than 90 countries (Zulu, Sibanda and Tlali, 2019). Out of the 120 sugar-producing countries, South Africa is the 15th largest sugarcane-producing country worldwide (Mohlala et al., 2016) and the largest in sub-Saharan Africa, accounting for 23% of the total production in this region (Hess et al., 2016). In 2015, South Africa produced an average of 19.9 million tons of sugarcane, which produced approximately 2.2 million tons of sugar (Dlamini, 2021). However, production has decreased, with the industry producing 18.22 million tons of sugarcane in the 2021/2022 season (Jones et al., 2021). This decline can be attributed to various factors, including climatic conditions (Jones et al., 2021). The sugarcane industry in South Africa plays a major role in the national economy (Mashoko, Mbohwa and Thomas, 2010) and the livelihoods of local farmers, primarily in the province of KwaZulu Natal (Cockburn et al., 2014). Sugarcane contributes 14% to the gross domestic product and 0.5% of tax revenue as well as 0.3% to the national salaries and wages bill (SASA 2019/2020).

KwaZulu-Natal is the country's leading sugarcane-producing region, followed by Mpumalanga and the Eastern Cape (Dlamini, 2021). Sugarcane cultivation depends on weather patterns, particularly how the climate changes during the growth stages of the plant (Riajaya, 2020). In northern KwaZulu-Natal and Mpumalanga, sugarcane is mainly irrigated due to lower annual average rainfall of 700mm and 500mm, respectively (Jones, Singels and Ruane, 2015). In other parts of KwaZulu-Natal, such as the Midlands, Zululand, South, and North Coasts, where the rainfall ranges between 800 and 1 200mm (Jones, Singels and Ruane, 2015), sugarcane is primarily produced under rain-fed conditions (SASA 2019/2020). Too much rainwater during planting can inhibit germination; therefore, sugarcane is ideally planted during the dry months (Riajaya, 2020). During the vegetative stage, sugarcane requires ideal rainfall to promote fast stem growth, stalk elongation, and internode development (Riajaya, 2020). During the maturation stage, dryer conditions are required to improve sugarcane quality and decrease the water content in the plant tissues (Riajaya, 2020). The sugarcane variety selected for planting is primarily based on the harvest cycle, soil type, the time of the year for harvest, and the distance from the mill (SASRI 2019).

2.1.2 Sugarcane Cultivation and Development

Land preparation before planting a new crop promotes good plant emergence and succeeding root growth (Otim et al., 2019). This is generally done through the application of tillage practices (de Oliveira et al., 2021).

Conventional tillage practices comprise subsoiling, harrowing, and ploughing operations (Barbosa et al., 2019). Sugarcane can be planted mechanically or by hand (Otim et al., 2019). Unlike other grass crops, where seed forms part of the production cycle, commercial sugarcane is commonly vegetatively propagated through setts planted and cultivated, where new shoots grow during germination (Pierre, Rae and Bonnett, 2014; Showler, 2016). Setts are fragments cut from stalks containing more than one node with buds and are sometimes referred to as "seed cane" (Shezi, 2017). Depending on the climate, topography, and farm management, sugarcane is harvested annually or every two years between April and December (Ramburan, 2011). After sugarcane is harvested, new shoots can grow from the underground portion of the harvested strikes (Bhatt et al., 2021; Xu et al., 2021). This practice is known as ratooning (Xu et al., 2021), and it plays a significant role in sugarcane production as it reduces production costs and early field management and speeds up plant growth (Bhatt et al., 2021). A single planting can be harvested for 20 successive ratoons depending on environmental stress factors such as weed competition, insect attack, air temperature, soil, and the specific sugarcane cultivar, which can restrict ratoons to only a few seasons (Showler, 2016). In South Africa, sugarcane burning and mulching are common harvesting procedures (Otim et al., 2019). Burning before harvesting eliminates excess residue, making harvesting, handling, and milling easier (Otim et al., 2019).

2.2 Arthropod Diversity and Abundance in Agroecosystems

2.2.1 Arthropod Biodiversity in Ecosystems

Biodiversity encompasses all species of plants, animals, and small organisms and their relationship within and with an ecosystem (Abbas et al., 2013). Additionally, Lingbeek et al. (2017) state that biodiversity comprises the composition and abundance of species present in a certain area and the different ecological roles species play in fulfilling ecosystem services. Every species in an ecosystem forms part of the processes and movements of energy, elements, and materials within that ecosystem (Kazemi, Klug and Kamkar, 2018). Biodiversity is, therefore, a key element in the functioning (Abbas et al., 2013) and intrinsic value of an agroecosystem, which are both crucial for producing sustainable agricultural systems (Truter, Van Hamburg and Van Den Berg, 2014). The biodiversity displayed by natural ecosystems enables these systems to endure and recover from major disturbances (Jankielsohn, 2018). Despite the benefits of biologically diverse ecosystems, agricultural intensification has increased biodiversity loss (Amprako et al., 2020). This decrease in biodiversity is also coupled with a decline in species richness, abundance, and biomass (Hausmann et al., 2020).

Biodiversity is evaluated at three levels, namely, genetic, species, and ecosystem diversity (Feest, Aldred and Jedamzik, 2010, Verma, 2016). The most used measure of biodiversity is species diversity, which strongly correlates with the other two levels (Chiarucci, Bacaro and Scheiner, 2011).

Species diversity describes the variation in species within an area (Verma, 2016), and it consists of richness, evenness, and disparity (Daly, Baetens and De Baets, 2018). Disparity refers to the degree of similarity between species (Daly, Baetens and De Baets, 2018). Species richness describes the number of species in a population (Morris et al., 2014). Evenness refers to the distribution of the abundance among species present in a community (Wilsey and Potvin, 2000; Okpiliya, 2012). If the species in a community is present in equal proportions with no one species dominating, that community is described as even (Daly, Baetens and De Baets, 2018). The higher the evenness value, the more equally the species in a community are distributed (Morris et al., 2014). Evenness influences ecosystem stability and functioning (Chiarucci, Bacaro and Scheiner, 2011) as an ecosystem with higher evenness increases the depiction of each species' functional trait (Daly, Baetens and De Baets, 2018).

Agroecosystems with a high level of biodiversity are known to be more stable and resilient (Dimitrova et al., 2020) as more species are present to fulfil various ecosystem roles (Jankielsohn, 2018; Crowley et al., 2023). Arthropods are a major element of biodiversity in land ecosystems (Ebeling et al., 2018), and they play an integral part in both natural and agricultural ecosystems as they provide a range of ecosystem services such as pollination and nutrient cycling (Jankielsohn, 2018, Crowley et al., 2023). Arthropod abundance and diversity within crops are mainly affected by the agricultural practices implemented and can increase or decrease insect diversity (Adams et al., 2017; Ikemoto et al., 2021). Therefore, assessing arthropods in agricultural fields is an important element for ensuring the implementation of sustainable crop management regimes (Ikemoto et al., 2021).

The two components of diversity are the equal distribution of individuals between groups and the abundance of species groups (Vera-aviles et al., 2020). The evaluation of arthropod diversity is essential for identifying applicable assessment methods for the estimation, observation, and management of biodiversity in agriculture, as well as applying beneficial ecological concepts in agriculture (Dimitrova et al., 2020). In general, the arthropod diversity found within agroecosystems comprises pests, pollinators, and insects of non-economic importance (Atencio, Goebel and Miranda, 2019).

Arthropod diversity in agroecosystems is influenced by several factors relating to the crop-adjacent environment, crop management and the crop landscape (Domínguez et al., 2018). The diversity of arthropods present within crop plants is influenced by the time the crop has been grown in a certain region, the proximity of other appropriate habitats, and how intensely human economic trade is occurring (Chen and Bernal, 2011). Agricultural crop domestication and selection have decreased plant intrinsic defences, and improved yield and quality have been prioritised (Mitchell et al., 2016). Therefore, arthropod pests are more successful on crops than wild crop cultivars (Chen and Bernal, 2011).

The increase in crop pest species has necessitated the increased application of chemical pesticides for crop protection (Mitchell et al., 2016). However, the increased use of chemical pesticides, in turn, affects the natural biodiversity of many agricultural systems (Brühl and Zaller, 2019) and drastically disrupts the food webs existing within these systems (Follett, Bruin and Desneux, 2020). Furthermore, this food web disruption leads to lost linkages, generally keeping insect pest populations at acceptable levels (Follett, Bruin and Desneux, 2020). Jankielsohn (2018) reported that most field studies have shown that generalist predators can drastically decrease pest populations in cultivated lands. For example, ground beetles are the most common generalist predators found in agricultural lands and can successfully control important crop pests like aphids and several others.

However, due to overreliance on chemical pesticides, the efficacy of these generalist predators is reduced as they also succumb to the pesticides (Jankielsohn, 2018). Food web disruption also compromises essential ecosystem services, such as pollination and nutrient cycling, delivered by arthropods in the wild (Moonen and Bàrberi, 2008). However, pesticides are often misused and employed in fields without considering the health and environmental impacts they may have (del-Val, Ramírez and Astier, 2021). Therefore, while pesticide use may benefit crop production, it remains one of the main catalysts of biodiversity loss and environmental degradation (Addison, Baauw and Groenewald, 2013).

Another factor that may influence arthropod communities in crops is monoculture farming. Monocultures result from agricultural intensification and cause low biodiversity levels in agroecosystems (Boutin, Martin and Baril, 2009; Rivera-Pedroza et al., 2019). They are predominantly employed in the cultivation of crops such as sugarcane as it reduces costs and increases efficiency (Gao et al., 2019). However, monocultures can be associated with a range of negative results, such as decreased soil quality, hydrological function, and landscape diversity (Putra et al., 2020). Landscape diversity aids in the maintenance of ecosystem services as it provides beneficial insects with alternative habitats in agroecosystems (Ali et al., 2022). Brandmeier et al. (2021) determined that an increase in crop diversity and a decrease in management intensity was associated with a positive influence on arthropod abundance and diversity, especially in the case of pollinators. Additionally, this phenomenon occurred without compromising yield (Brandmeier et al., 2021). Ghazali et al. (2016) found an increased arthropod order richness in polyculture oil palm smallholdings compared to monocultures. They concluded that polyculture farming supports land arthropod diversity, possibly due to an increase in habitat heterogeneity. The abundance of arthropods is influenced not only by the number of plant species present in a system but also by the identity of the plant species present, as demonstrated by a study conducted by Haddad et al. (2001).

In a later study conducted by Haddad et al. (2009), an increase in plant species richness was associated with an increase in arthropod predators and phytophages. Interestingly, the trophic structure shifted from arthropod predator-dominated to arthropod herbivore-dominated when plant species richness was decreased (Haddad et al., 2009).

2.2.2 Arthropod Diversity and Abundance in Sugarcane

According to a review conducted by El Chami et al. (2020), literature dealing with the impact of sugarcane production on biodiversity is scant. El Chami et al. (2020) observed that authors limited their assessment to the species richness of certain groups of animals, including ants, spiders, and earthworms, and found reduced communities compared to other land use types. Furthermore, different management practices in sugarcane were also associated with different numbers of these communities (El Chami, Daccache and El Moujabber, 2020). Sugarcane is often cultivated in big monoculture plantations, which are more inclined to generalist species as opposed to specialist species, which are not persistent, by this means decreasing biodiversity within this system (Lukhele et al., 2021). Siqueira et al. (2016) conducted a study on the diversity of soil macrofauna under sugarcane monoculture and two other different natural vegetation types. Compared to the other two vegetation types, they reported that sugarcane had the lowest diversity index value at the start of the growth season, while it had the highest diversity index value at the end of the study. This led to the conclusion that soil macrofauna undergoes selection at the beginning of sugarcane growth, and only individuals who can adapt to the climatic conditions and management practices are able to survive (Siqueira et al., 2016). Sugarcane burning has also been noted to influence the arthropods associated with this crop (Sajjad et al., 2012; Sunarto, 2020). Sajjad et al. (2012) found that sugarcane trash burning decreased spider populations by 95%, ladybird beetle populations by 85%, ant populations by 61%, and sowbug populations by 96%, five days following trash burning. In a study conducted by Sunarto (2020), higher arthropod abundance was associated with unburned sugarcane land compared to burned sugarcane land. A study by Siqueira et al. (2016), found that straw burning initially assisted arthropod taxa such as Formicidae, which are adapted to extreme changes in the sugarcane system.

Ranjith et al. (2022) conducted a study on the diversity and composition of arthropods in the sugarcane ecosystem in Tamil Nadu. Results indicated the presence of nine arthropod orders, of which Hymenoptera had the highest abundance. Similar results were found by de Oliveira et al. (2021) when looking at the diversity of soil arthropods in sugarcane in Brazil. This dominating abundance of Hymenoptera was also found in work done by Santos et al. (2017) when examining edaphic arthropods associated with organic and conventional sugarcane. Additionally, organic sugarcane had a higher insect abundance, whereas conventional sugarcane had a higher species richness (Santos, Naranjo-Guevara and Fernandes, 2017).

Several studies investigating arthropod abundance and diversity in sugarcane focus on soil-dwelling arthropods (Shakir and Ahmed, 2014; Santos, Naranjo-Guevara and Fernandes, 2017: Kaur and Sangha, 2020, de Oliveira et al., 2021) and pest insects (Leslie, 2003, Way and Goebel, 2007, Goebel and Sallam, 2011). However, there is limited literature that discusses the diversity and abundance of flying and crawling insects concurrently, especially in South Africa.

Sugarcane is prone to insect attacks on its stems and leaves (Prabowo et al., 2021) as well as to an extensive range of diseases caused by bacteria, viruses, and fungi (Goebel and Sallam, 2011). This can be attributed to the fact that sugarcane is an annual crop presenting idyllic micro-climates for harbouring several different arthropods (Sajjad et al., 2012). Wilson (2019) reported on several hemipterans considered pests on sugarcane in North America. Among the identified species were the Sugarcane Aphid, *Melanaphis sacchari*, and Yellow Sugarcane Aphid, *Sipha flav,* which also occur on sugarcane in South Africa. The primary lepidopteran insect pests of sugarcane are in the genera *Chilo, Sesamia, Diatraea*, and *Eldana* (Bi Péné et al., 2018).

2.3 Factors Influencing Insect Diversity and Abundance in Agroecosystems

2.3.1 Water Management

Irrigation is one practice that was brought about because of agricultural intensification (González-Estébanez et al., 2011). Other practices include increases in mechanization, chemical input, the extension of monocultures, and agricultural fields (González-Estébanez et al., 2011). For profitable commercial production, irrigation is necessary for certain countries, such as Swaziland (Carr and Knox, 2011). Yet, in countries such as South Africa, it is often an addition to irregular rainfall (Carr and Knox, 2011). Insect herbivore performance and development are often directly influenced by host plant water availability (Sconiers and Eubanks, 2017). Insect herbivores respond to water-stressed plants differently, ranging from population drops to pest outbreaks (Carvajal Acosta, Agrawal and Mooney, 2022). Phytophagous insects can perceive drought-stressed plants through chemo- and olfactory receptors (Sconiers, Rowland and Eubanks, 2020). In drought-stressed plants, stress-related compounds containing nitrogen may be increased, resulting in food with higher nutritional quality for insects (Sconiers and Eubanks 2017). However, water scarcity may also negatively influence the growth and development of arthropods (Pérez-Fuertes et al., 2015) and drought-stressed plants may deter insect herbivores as they have lower water content and potential (Sconiers and Eubanks, 2017). For instance, aphids feeding on plants under water stress have been observed to grow slower and be smaller in size. Thus, the increased availability of water through irrigation in fields, especially throughout water-scarce months, may present more favourable environmental conditions as well as higher quality food sources for phytophagous insects and their predators because of bottom-up effects (Pérez-Fuertes et al., 2015)

However, the water requirement from plants for insects differs between insect groups. For example, aphids need plants with higher water potential to enable efficient feeding on plant phloem cells (Sconiers and Eubanks, 2017). Chewing insect herbivores require plants with higher water content for effective protein absorption (Sconiers and Eubanks, 2017).

Several studies (González-Estébanez et al., 2011; Pérez-Fuertes et al., 2015; González-Zamora et al., 2021) have been conducted on the effects of irrigation on arthropod development and activity. Pérez-Fuertes et al. (2015) conducted a study to assess the effects irrigation had on arthropod communities in cereal agroecosystems. The study focused on six specific groups of insects with different ecological needs, namely, Aphididae, Aphidiinae, Coccinellidae, Formicidae, Heteroptera, and Syrphidae. The results indicated that Aphididae and Coccinellidae had a higher abundance in irrigated fields compared to dryland fields, while the other four groups did not show a significant difference in abundance between dry and irrigated fields. The study also concluded that irrigated fields showed increased insect abundance, diversity, and species richness compared to dry fields. While the irrigated fields may have shown higher diversity and abundance of some insect groups, this alteration in the agroecosystem may negatively influence insects adapted for dryer conditions.

In a study conducted by González-Estébanez et al. (2011) on the effect of irrigation and landscape heterogeneity on butterfly diversity in farmlands, increased butterfly diversity was observed in irrigated crops compared to dry cereal-steppe landscapes. On the other hand, cereal-steppe landscapes showed to have a higher abundance of butterflies. González-Zamora et al. (2021) investigated how the rational use of water can influence pest and arthropod abundance in olive orchards by comparing two different irrigation schemes. The two irrigation strategies were no limitation of irrigation (control) and regulated deficit irrigation. The results from González-Zamora et al. (2021) indicate that more arthropods were found in the control field as opposed to the other treatment. However, the irrigation treatment did not have a noteworthy influence on the abundance of some groups of beneficial insects like Neuroptera, Ichneumonoidea, and Chalcidoidea.

Sugarcane can endure some moisture stress even though the crop has a high water requirement ranging between 1500 to 2500mm per season (Gunarathna et al., 2018). The irrigation methods used in sugarcane are generally surface, overhead, or drip irrigation (Carr and Knox 2011). The type of irrigation method employed depends on, among others, the crop's physical characteristics and economic and social factors (Gunarathna et al., 2018). The most commonly employed method in sugarcane cultivation is surface irrigation, which is simple and cost-effective (Gunarathna et al., 2018). Sugarcane requires ideal moisture conditions for various metabolic and physiological activities to transpire nutrients and water through the roots into the plants (Bhatt et al., 2022).

Drought conditions may decrease leaf water potential, resulting in the downregulation of photosynthesis genes and carbon dioxide accessibility (Bhatt et al., 2022). Therefore, it is important for sugarcane growth, yield, and quality to have sufficient water available (Bhatt et al., 2022). However, according to González-Estébanez et al. (2011), irrigation schemes may negatively affect the environment in numerous ways, including, among others, soil salinization and increased chemical compound input. It is noted that the data available on the effect of irrigation schemes on biodiversity is limited (González-Estébanez et al. 2011).

2.3.2 Crop Growth Stage

The development stage of plants can influence insect population dynamics. For instance, certain species of aphids alternate their feeding site and reproduction rates with the developmental stage of crops such as barley, oat, or wheat (Batyrshina et al., 2020). Also, young seedlings of winter wheat are preferable for aphids to feed on, as well as mature flowering spring wheat (Batyrshina et al., 2020). In Spain, crops such as maize are planted from March to July and harvested from September to early December (Clemente-Orta et al., 2022). This means several maize fields with different phenologies may be found within the same area.

Therefore, phytophages and their natural enemies can move across the area and select the most preferable phenology of maize fields for feeding and reproduction (Clemente-Orta et al., 2022). Clemente-Orta et al. (2022) looked at how the planting period influences different groups of arthropods in maize fields. The results showed that insect abundance differed between certain growth stages of maize. The maximum number of phytophagous insects was found during the early growth stage, while the other groups of studied insects had the highest numbers during maize plant pollination. Phytophagous insects may be more abundant during the early stages of plant growth as they prefer softer leaves to feed on (Clemente-Orta et al., 2022).

In rice crops, the tillering stage is known to have the highest arthropod abundance (Dominik et al., 2018). Bambaradeniya and Edirisinghe (2008) stated that arthropod predator numbers increase in rice crops during the ripening stage, which may be due to their spatial habitat expansion relating to the rice plant structure and prey abundance. Additionally, the rice plant has a thick growth (Bambaradeniya and Edirisinghe, 2008), which presents more resources like shelter and food (Ali et al., 2022). After the crops are harvested, the arthropod population is expected to decrease as the vegetation the arthropod inhabited is decreased or completely removed (Bambaradeniya and Edirisinghe 2008). Hasibuan et al. (2022) conducted a study on the impact of soil fertilization on arthropod abundance and diversity in soybean crops. The results showed that arthropod abundance increases with the growth of the soybean crop. It was concluded that arthropod population numbers are directly related to plant performance (Hasibuan et al., 2022).

There are five stages in the development of sugarcane crops, namely, germination, tillering, stem elongation, maturing, and flowering (Dlamini, 2021). Maneerat and Suasa-ard (2015) conducted a study on the population trends of sugarcane moth borers and their larval parasitoids. Results showed that these insect populations increased during the tillering initiation phase and continued to fluctuate through the rest of the growth stages (Maneerat and Suasa-ard 2015). According to Cherry et al. (2017), soil arthropod pest numbers are lower in newly planted sugarcane because of the disking and insecticide application that accompanies planting.

2.3.3 Seasonality

Arthropod species distributions may fluctuate in a year because of long-term changes in weather patterns and the availability of food resources, which is directly linked to their abundance and diversity (Silva et al., 2015). This is because arthropods are ectotherms that tend to be thermophilic, reaching maximum activity levels during the warmer months of the year (Fitzgerald et al., 2021). Insect seasonality can also be influenced by physical conditions such as favourable temperatures and humidity for flying and reproduction (Ribeiro and Freitas, 2011).

Kaur and Sangha (2020) investigated the seasonal abundance of soil arthropod diversity in high-input sugarcane production systems at different depths. It was found that lower temperatures during post-monsoon and monsoon seasons, combined with higher relative humidity, were associated with a higher abundance of soil arthropods. Similar findings were reported by Sharma and Paewez (2017) who sampled soil arthropods from grassland and teak plantation fields in India, and by Begum et al. (2014), who studied soil microarthropod communities in the Mid-hills of Nepal. Contrastingly, Majeed et al. (2020) reported peak species richness and evenness for spring and lowest abundance for winter for arthropods collected around wetlands in Pakistan.

Seasonality, however, has varying effects on different arthropod species. Mavasa et al. (2022) conducted a study in Mpumalanga, South Africa, to evaluate the patterns of seasonal changes in surface-active arthropods using pitfall traps. The results showed that ants had similar species richness and abundance during winter and summer, whereas spiders and beetles showed higher species richness and abundance during summer. This can also be observed from families within the same order; for instance, in a study conducted by Liu et al. (2013), Carabidae displayed increased numbers during autumn, whereas Curculionidae reached its peak in spring. Arthropods also display seasonal specialization; for example, pollinators are more active throughout spring in temperate regions (Fitzgerald et al., 2021).

2.3.4 Soil Characteristics

Several studies have confirmed that soil type influences arthropod abundance and community composition (Zaller et al., 2014). Soils high in nitrogen can house plant species with higher nutritional value for chewing and sucking phytophagous arthropods (Showler, 2016). According to studies done in the USA and Indonesia, the early harmful effects of soil preparation practices on epigeal arthropods are transient as sugarcane is a semi-perennial crop, making this ecosystem somewhat stable for soil arthropods (de Oliveira et al., 2021). The primary factor influencing soil insects is mechanical disturbances due to soil ploughing (de Oliveira et al., 2021). Cherry (2003) suggested that few ploughing operations are needed as sugarcane is a long-term crop with planting occurring every three to five years. In Africa, high inputs of nitrogen fertilizer add to the attack of *Eldana saccharina* and in South Africa, only moderate applications of nitrogen fertilizer are suggested, especially during drought periods (Showler, 2016).

2.4 Insect Diversity and Abundance Sampling Techniques

2.4.1 Sampling for Insect Diversity and Abundance

Insect species diversity and community composition can be determined through sampling and monitoring programmes. Efficiency, repeatability, and representation are key qualities that insect assemblage sampling methods should have as they are generally applied to environmental monitoring (Castro et al., 2017). Moreover, to produce monitoring and biodiversity lists, sampling methods must allow for the achievement of the study objectives (Castro et al., 2017). Arthropod community abundance and diversity are key elements when assessing short-term impacts agricultural practices may have on an agroecosystem (Dimitrova et al. 2020), simultaneously providing information on the changes an ecosystem has undergone over a long period (Dimitrova et al., 2020). Sampling effort (Gaspar et al., 2014) and appropriate sampling methods are the basis for establishing insect diversity in any specific area (Olea et al., 2018). Both sampling efforts and methods are dependent on whether the aim is to create an inventory of diversity or to monitor diversity (Gaspar et al., 2014). Generally, a species inventory aims to provide the most complete representation of the taxonomy and ecology of taxa present in a given site (Missa et al., 2009). Biological monitoring is aimed at periodic sampling over time to establish changes in diversity or changes in the habitat that may influence diversity (Gaspar et al., 2014).

Sampling methods are categorized into three groups. The first is active sampling, which involves physically collecting insects, such as visual counts (Missa et al., 2009), sweep-netting, and beating (Metspalu et al., 2015). Secondly, passive sampling involves an attractive component such as coloured pan traps and pheromone traps (Missa et al., 2009).

The last category is passive sampling without an attractive component, which incepts arthropod movement, such as malaise and pitfall traps (Missa et al., 2009). The use of passive or active sampling methods is dependent on the aims of a study and the applicability of the chosen methods (Yi et al., 2012). For example, crops such as maize can grow up to 3 m in height and have solid stems with large leaves; thus, active sampling methods would be difficult (Matsukura, Yoshida and Matsumura, 2011).

In general, using more than one sampling method can improve the measure of the diversity of insects present in a sampling field (Kent, Peele and Sherry, 2019). Researchers are constantly assessing the capture efficiency of sampling methods and how this can be improved (Yi et al., 2012). According to Montgomery et al. (2021), the previously mentioned methods can be used to measure abundance, diversity, and species composition. Furthermore, developing a standardized methodology offers many advantages, including making research less complicated for individual researchers, as it allows for synchronized research over larger geographical or temporal scales (Brown and Matthews, 2016). Standardization of collection methods allows for comparison between studies and enhances their importance in ecological monitoring (Montgomery et al., 2021).

Using a combination of sampling methods can result in finding more individuals of a certain species and lower the number of singletons (Tourinho et al., 2014). Thus, ecological traits and habitat conditions of targeted arthropods are important elements to consider when choosing sampling methods as well as the setup and costs tied to the chosen methods (Yi et al. 2012). Arthropod sampling methods should be efficient, repeatable, and descriptive, as they are generally employed in environmental monitoring (Castro et al., 2017). However, the effectivity of each sampling method may be influenced by several factors such as the sampling location, vegetation, resource availability for arthropods, etc. (Castro et al., 2017).

Various sampling methods function in different ways, simultaneously targeting differing assemblages (Kitching, Li and Stork, 2001) by collecting a range of taxa (Missa et al., 2009). Sampling methods such as pitfall traps are effective and frequently used for surveying ground-dwelling arthropods (Yi et al. 2012; Ahmed and Petrovskii 2019; Kaur and Sangha 2020). Pitfall traps are cost-effective and easily installed in the field (Hohbein and Conway, 2018). The pitfall trap consists of a container sunk into the ground with the top of the container level with the soil surface and traps insects falling into it (Montgomery et al., 2021). Sticky traps comprise a card with strong adhesive glue that traps insects landing on or crawling over it. These traps are an effective method for monitoring arthropods as they are inexpensive, attract a range of insects (Matsukura, Yoshida and Matsumura, 2011) and do not require professional training (Dimitrova et al., 2020). Sticky traps can also be used for continual sampling (Dimitrova et al., 2020), thereby decreasing the time spent sampling (Musser, Nyrop and Shelton, 2004).

Sticky cards are available in a variety of different colours targeting different taxa (Yi et al. 2012), while yellow sticky cards are a good option for attracting natural enemies (Musser, Nyrop and Shelton, 2004). The fundamental structure of a pan trap consists of a tray filled with water and an additive, normally liquid soap, to break the surface tension of the water (Campbell and Hanula, 2007). This method is easily replicated, cost-effective, and commonly used to sample pollinators (González, Salvo and Valladares, 2020).

2.4.2 Pitfall Traps

Pitfall traps are used for sampling ground-dwelling arthropods (Brown and Matthews, 2016); they are cost-effective and easy to set up in sampling fields (Hohbein and Conway, 2018). Pitfall traps are usually glass, plastic, or metal containers sunk into the ground with the top of the container level with the soil surface (Montgomery et al., 2021). The containers are normally filled with a trapping solution to prevent insects from escaping or damaging other insects (Montgomery et al., 2021).

Trapping solutions include saltwater, ethylene glycol, and diluted formaldehyde (Yi et al., 2012). Furthermore, Montgomery et al. (2021) suggested the use of clear trapping containers as colour influences the taxonomic composition of insects caught in the traps.

The shape, material, and size of the trap influence the composition of insects that will be caught in the trap (Yi et al., 2012). González et al. (2020) compared the captures of three sampling methods in terms of taxonomic and functional diversity. The results indicated that pitfall traps had the lowest number of species and individuals, and it was noted that this was because of the reduced capture surface and location of the traps in the field (González, Salvo and Valladares, 2020). Regardless of the low arthropod numbers in pitfall traps, the different community members collected using this method complemented the flight interception and yellow pan traps and justified the use of this method in insect community studies (González, Salvo and Valladares, 2020).

2.4.3 Water Pan Traps

Pan traps are a passive sampling method (Gonzalez et al., 2020), which usually consists of trays filled with trapping liquid, such as water mixed with detergent (Montgomery et al., 2021). Due to pan traps being effective without requiring specialized equipment or highly skilled personnel, this sampling method has been employed in many pollinator assemblage studies (Vrdoljak and Samways, 2012). According to Yi et al. (2012), a diverse range of insects, especially flower-visiting flies and hymenopterans, can be sampled using pan traps. The colour of the tray generally influences the types of insects it attracts (Saunders and Luck, 2013), as insects perceive the coloured tray as a food resource (Montgomery et al., 2021). Vrdoljak and Samways (2012) found that yellow and white pan traps produced high measures of species richness.

Saunders and Luck (2013) conducted a study on pollinator abundance using different pan-trap colours. The results showed that the highest number of insects were found in yellow pan traps, whereas the blue and white traps had similarly low numbers (Saunders and Luck, 2013).

2.4.4 Sticky Traps

Sticky traps have been extensively used in arthropod sampling (Yi et al., 2012) and generally consist of cardboard coated with strong sticky glue on its surface to trap insects coming into contact with it (Kent, Peele and Sherry, 2019). These traps can be classified as non-attractive, meaning they are transparent and unscented, or attractive, meaning they have attractive colours, odours, and shapes (Dimitrova et al., 2020). Generally, they are hung to catch flying insects, but they can also be attached to branches or tree trunks to trap crawling insects (Kent, Peele and Sherry, 2019), taking into account the height of the sticky trap, as this influences the insects caught (Yi et al., 2012) This low-cost sampling method can have numerous replicates in a sampling area; however, removing insect specimens from the glue can be difficult without damaging the specimens (Yi et al., 2012).

2.5 Diversity Assessment

2.5.1 Statistical Indices

Diversity can be measured at three levels: within a community, which is α-diversity; between communities, which is β-diversity; and at a landscape level, which is γ-diversity (Thukral, 2017). Diversity can be quantified using diversity indices (Mendes et al., 2008; Daly, Baetens and De Baets, 2018). Species abundance is also a contributor to diversity; therefore, diversity indices generally also comprise the relative abundance of species (Morris et al., 2014). A diversity index indicates the number of different types, such as species, present in a dataset while also considering the evenness of the individuals between the types (Okpiliya, 2012). An index is chosen based on the context of a study, whether it is evaluating the effects of anthropogenic pressures on ecosystem biodiversity or estimating the biodiversity level of an ecosystem for protection purposes (Guisande et al., 2017; Daly, Baetens and De Baets, 2018). Indices such as Shannon-Weiner, Margalef, and Simpson are commonly used to evaluate α-diversity, whereas Sorensen and Steinhaus' coefficients of similarity are used to evaluate β-diversity (Travlos et al., 2018). Once the form of diversity to measure is chosen, quantifying diversity is still challenging, as using a single index does not sufficiently encapsulate the concept (Ramya et al., 2021).

The Shannon-Weiner and the Simpson index are the two most common indices used to measure biodiversity as they incorporate both species richness and evenness into one number (Mendes et al., 2008). The Shannon-Wiener index measures α-diversity and is based on a combination of species composition and abundance (Barrantes and Sandoval, 2009). Abundance quantifies the number of individuals of the same species (Travlos et al., 2018).

This index also considers the percentages of each species present in the ecosystem being investigated instead of just the number of species present (Konopiński, 2020). Tarno et al. (2016) and Erdiansyah et al. (2021) used the following criteria for interpreting the Shannon-Wiener index values: <1, between 1 and 3, and >3 each indicating low, medium, and high levels of diversity and individual distribution of each species. Ghosh and Biswas (2015) used a range of 0 to 5 in a study conducted for quantitative and biological assessment of the aquatic health status of a lake ecosystem using macroinvertebrates and diversity indices. Whereas Sonico (2022) investigated insect diversity in an organic rice farm, and Travlos et al. (2018) studied weed community composition and structure both studies reported an index range between 1.5 and 3.5, rarely exceeding 4. Nonetheless, in all three instances, a higher index value is attributed to a higher diversity (Ghosh and Biswas 2015; Travlos et al. 2018; Sonico 2022). Siqueira et al. (2016) noted that a lower Shannon-diversity index value indicates that the study community is mostly dominated by one specific species. Previous studies used the Shannon-Weiner diversity index as a comparative measure between communities, areas, or timescales (Razzak, Awwal and Zulfiker, 2022; Akbari et al., 2023).

The Simpson and Shannon-Wiener indices differ in how they are interpreted and their theoretical basis (Morris et al., 2014). The Simpson dominance index displays species dominance and shows the probability of two individuals of the same species being chosen at random (Kim et al., 2017). The index value ranges between zero and one, with higher values indicating lower diversity (Kim et al., 2017).

Taxon sampling curves such as accumulation and rarefaction curves are also commonly encountered in biodiversity research as such studies generally involve big data sets regarding the number of individuals, samples, and species sampled (Buddle et al., 2005). Species accumulation curves assess whether sufficient sampling has been conducted at a specific site by showing the increase in the number of species with an increase in sampling effort (Thompson and Thompson, 2007; Chiarucci et al., 2008). Accumulation curves roughly reaching an asymptote show sufficient sampling has been conducted, while a curve that distinctly rises towards its end indicates more species may be collected with more sampling effort (Chiarucci et al., 2008). Rarefaction curves are constructed by an algorithm repetitively resampling individuals or samples from the total group in the collection (Buddle et al., 2005). Rarefaction eliminates the effect of sampling differences among collections of different sizes (Collins and Simberloff, 2009). It also standardizes the estimates of the number of species per study unit to corresponding sample sizes, granting significant statistical comparisons of species richness (Buddle et al., 2005).

CHAPTER THREE - MATERIALS AND METHODS

3.1 Study Area Description

Existing sugarcane fields located at the South African Sugarcane Research Institute's (SASRI) research stations, namely Gingindlovu Research Farm (29°01'43''S 31°35'32''E) 92 m above sea level and Pongola Research Farm (27°25'19.00"S 31°35'35.86"E) 380 m above sea level in KwaZulu Natal, South Africa were used for this study. In Gingindlovu, the sugarcane was rain-fed and harvested every 15 months. The cultivar N27 was planted in Gingindlovu in its 4th ratoon and was last harvested in November 2021. This cultivar performs optimally in heavy clay soils, is vigorous against drought, and has a high sucrose yield (Dlamini, 2021). Temperature and rainfall data were collected from weather stations located at each of the research stations and obtained from the SASRI Weather Web. Maximum temperatures during sampling in Gingindlovu fluctuated between 22°C and 32°C. The weekly average maximum temperature during sampling from March to May 2022 was 27 °C; from June to July 2022 was 25 °C and from September to October 2022 was 28 °C. Maximum rainfall in Gingindlovu during sampling in 2022 occurred during the months of March to May, and minimum rainfall from June to July. The weekly average rainfall during sampling was 49 mm, 1 mm and 12 mm for March to May, June to July and October to September 2022, respectively.

In Pongola, the sugarcane was under dripper irrigation, and harvesting was done annually. The cultivar planted in Pongola was N41 in its 8th ratoon, and it was last harvested in late November 2021. The N41 cultivar has a good ratooning ability that improves yield with each ratoon, and it performs well under irrigated conditions and sandy soils. The maximum temperatures during sampling in Pongola, fluctuated between 22°C and 33°C. The weekly average maximum temperature during sampling from March to May was 27 °C; from June to July was 24 °C and from September to October was 30 °C. Maximum rainfall in Pongola during sampling occurred from March to May 2022 and the lowest from June to July 2022. The weekly average rainfall during sampling was 19 mm, 6 mm and 7 mm for March to May, June to July and October to September 2022, respectively.

3.2 Sampling Design

The rain-fed field comprised 30 plots that consisted of six rows of sugarcane that were 8 m long with an inter-row spacing of 1.2 m (Figure 3.1A). The irrigated field comprised 25 plots, each with seven rows of sugarcane that were 10 m long with an inter-row spacing of 1.2 m (Figure 3.1B). Since both fields were located next to a road and were boundary plots to natural vegetation, they had border rows on each side.

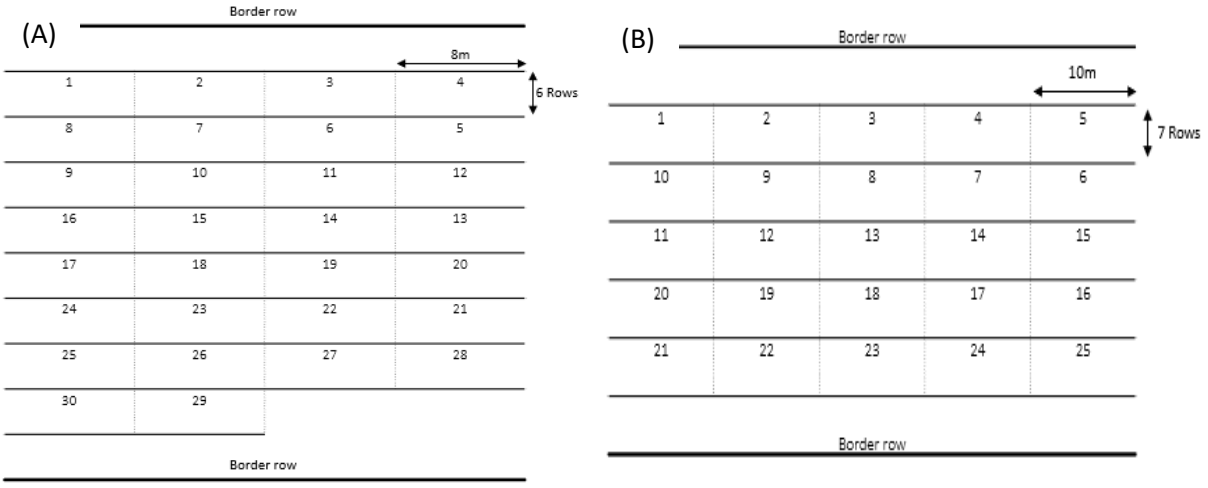

Figure 3.1: Diagrammatic representation of the (A) rain-fed and (B) irrigated sugarcane fields in Gingindlovu and Pongola with the numbers indicating the plots within each field.

3.3 Insect Sampling

Sampling was done following the common procedure of resampling (Banu and Merlin Dayana, 2019; Mahendran et al., 2021). Sampling was conducted at three sampling periods of seven weeks each. The first sampling period was between March and May 2022, the second between June and July 2022, and the last sampling period between September and October 2022. Only insects were considered during this study. To capture a complete representation of insects present in the system, insect sampling was done using pitfall, water pan, and sticky traps adapted from the methods used by Westmacott (2007).

3.3.1 Pitfall Traps

Glass tubes with dimensions of 15 cm long, 5.5 cm in diameter, and a volume of 340 ml were used as pitfall traps. Each trap was placed in plastic irrigation tubing that was 16 cm long and 6 cm in diameter, permanently sunk into the ground with its top level with the soil surface. An ethanol-glycerol mixture was prepared using 70% (v/v) ethanol (Enterprise Solvents (Pty) Ltd, Pretoria, South Africa) and 99% (v/v) glycerol (Merck Life Science (Pty) Ltd, Durban, South Africa) in a 70:30 ratio. Each glass tube was filled with 100 ml of the ethanol-glycerol mixture, serving as a trapping medium and a preservative. The location of the pitfalls was marked with red and white tape and wooden spikes. One pitfall trap was placed at the centre of each plot in both fields (Figure 3.2). Gingindlovu comprised 30 plots; therefore, 30 pitfall traps were distributed in Gingindlovu, and Pongola comprised 25 plots; therefore, 25 pitfall traps were distributed in Pongola. During collection, the contents of each glass tube were transferred to a 340 ml plastic jar marked with the trap's field position and location. The traps were collected and replaced weekly.

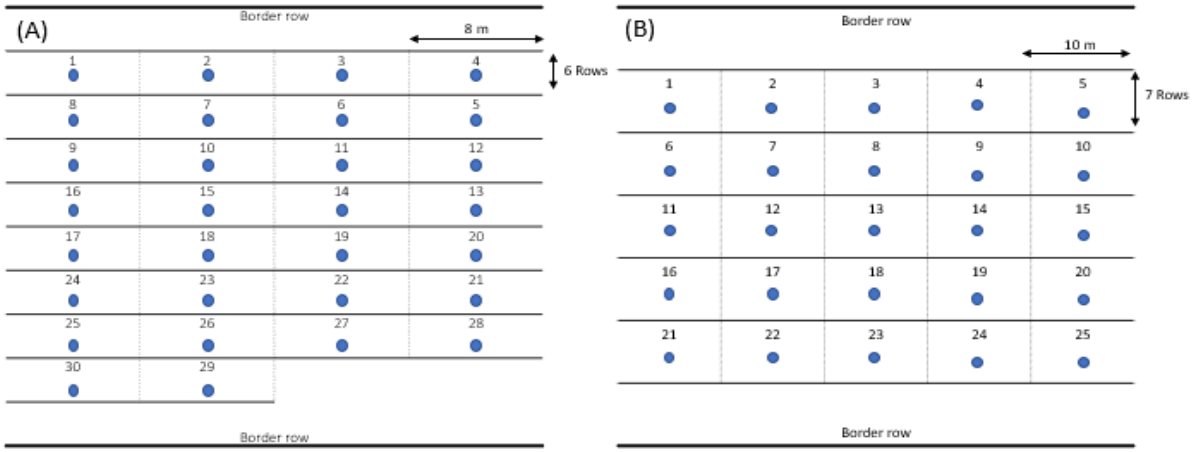

Figure 3.2: Diagrammatic representation of pitfall trap placement in the rain-fed (A) and irrigated (B) sugarcane fields. The pitfall traps were placed at the centre of each plot, represented by the blue dots above.

3.3.2 Sticky Traps

Five large yellow sticky traps (Large yellow sticky cards, 125 mm x 78 mm; Insect Science (Pty) Ltd., Tzaneen, South Africa) with double-sided sticky tape were used to capture flying insects. Five 1.5 m poles were installed in each field (Figure 3.3). One sticky trap was attached to the top of each pole, level with the top of the sugarcane leaves. During collection, sticky traps were removed and placed in plastic zip-lock bags (215 mm x 230 mm; Sanigene Systems & Solutions, Durban, South Africa), which were cut open on the side for easy insertion. The sticky traps were collected and replaced weekly.

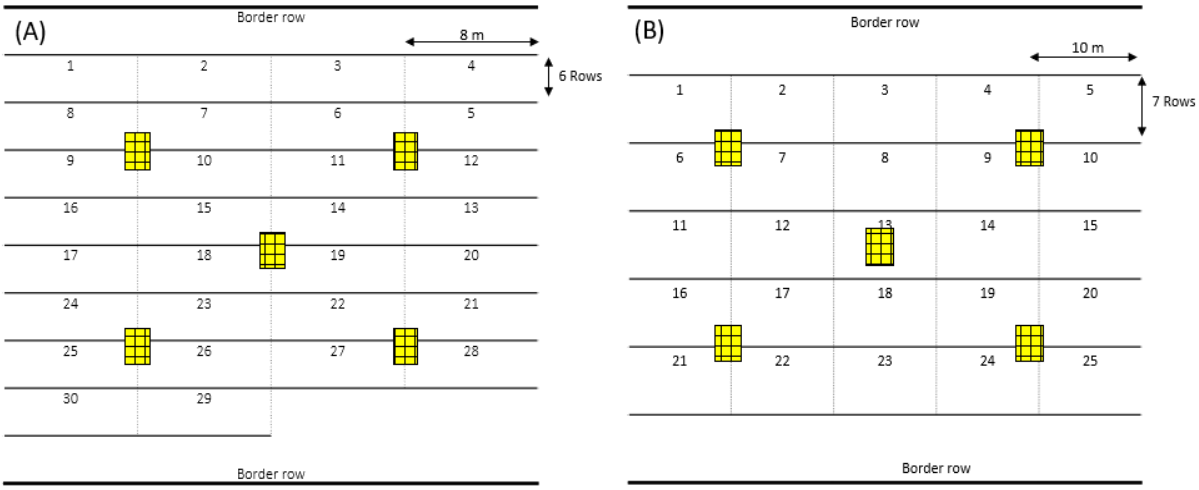

Figure 3.3: Diagrammatic representation of sticky trap placement in the rain-fed (A) and irrigated (B) sugarcane fields. Five sticky traps were attached to installed poles in both fields in the locations indicated on the diagram by the yellow cards.

3.3.3 Water Pan Traps

In each field, five-round 5 L pans served as water pan traps (Water Trap, Insect Science (Pty) Ltd., Tzaneen, South Africa). The pans, with dimensions of 180 mm radius and 60 mm height, were filled with 1.5 L of a soapy solution of 1 % (v/v) detergent (Sunlight dishwashing liquid) and tap water. The dishwashing liquid breaks the surface tension of the water, thus trapping the insect in the water. The water pan traps were placed on the ground at the same field position as the yellow sticky trap poles (Figure 3.3). During collection, the trap contents were transferred to a 2 L plastic jar marked with the trap's field position. The water pan traps were collected and replaced weekly.

3.4 Insect Sorting and Identification

After collection, specimens were transported to the SASRI Entomology laboratory, where they were to be sorted. The insects from the pitfall and water pan traps were carefully removed using tweezers and transferred to different vials filled with 99% (v/v) ethanol. The vials were labelled according to each trap's location, collection date, and sampling method used. The collected sticky traps were placed in the freezer until further processing. Sampled insects were observed under a dissection microscope (Nikon SMZ1000, Zeiss), counted, and identified based on morphological characteristics to the family level. Borror and Delong's Introduction to the Study of Insects (Triplehorn and Johnson, 2005) was used for identification, and the samples were classified into morphospecies. Thereafter, insects were placed in a reference collection at the SASRI Entomology Department to be used to compare unidentified specimens. Any unidentified specimens were catalogued and identified using molecular barcoding by DNA sequencing. In cases where molecular identification was unsuccessful, specimens were sent from SASRI to the National Collection of Insects of the Agricultural Research Council in Pretoria, South Africa, for taxonomic identification.

3.4.1 DNA Extraction

For insects smaller than 5 mm, DNA extraction methods adapted from Rugman-Jones et al. (2014) were used. Individual insects were removed from a vial containing 99% (v/v) ethanol, placed on folded tissue paper, and allowed to dry for ±5 min. For carnivorous insects, the head, thorax, and legs were used for DNA extractions. Meanwhile, the whole body was used for non-carnivorous insects. Insect material was then placed in a 1.5 ml microcentrifuge tube in 100 µl TNES buffer, comprising 50 mM Tris pH 7.5, 400 mM NaCl, 20 mM EDTA, and 0.5 (w/v) SDS. Insect material was ground using a crushing rod until an almost homogenous mixture was achieved.

Thereafter, 2 µl Proteinase K (10 mg/ml) was added to the tubes and incubated at 37 °C overnight (maximum of 18 hours). After incubation, 28 µl of 5 M NaCl was added to the tube and vortexed for 15 seconds. Tubes were placed in the centrifuge and spun at 12 298 rcf for 15 min using the Eppendorf Centrifuge 5417C (Eppendorf 5417C Digital Centrifuge, Merck Chemicals (Pty) Ltd), and the supernatant was transferred to a clean 1.5 ml microcentrifuge tube. DNA was precipitated from the supernatant by adding 1 volume of ice-cold ethanol and incubating at -20 °C for 1 hour. After incubation, the DNA was pelleted by placing the tubes in a centrifuge and spinning for 10 min at 12 298 x *g*, removing the supernatant by pipetting, and washing the pellet with ice-cold 70 % (v/v) ethanol. The tube was then spun in a centrifuge at 12 298 x *g* for 5 min. Thereafter, the supernatant was removed, and the pellet was left to air dry. Finally, the pellet was resuspended in 20 µl sterile water.

For DNA extractions of insects larger than 5 mm, the GeneJET Genomic DNA Purification Kit (Thermo Scientific) was used according to the manufacturer's instructions with minor modifications. The genomic DNA purification protocol for Mammalian Tissue and Rodent Tails was followed. Individual insects were ground in 1.5 ml microcentrifuge tubes using a crushing rod instead of placing the samples in liquid nitrogen and grinding with a mortar and pestle. The samples were incubated at 37 °C overnight instead of at 56 °C for 3 hours, which was recommended by the manufacturer's instructions. Additionally, DNA was eluted in 40 µl Elution buffer instead of the 200 µl Elution buffer recommended by the manufacturer's instructions.

3.4.2 Polymerase Chain Reaction (PCR) Amplification and DNA Sequencing

PCR amplification was done using PCRBIO Ultra Polymerase (PCRBiosystems) with ± 50 ng DNA template. A specific sequence was used for species identification, which is approximately 650 bp in the mitochondrial cytochrome c oxidase subunit I (COI) coding region (Folmer et al., 1994; Becker, König and Hoppe, 2021). The primers used were 10 µM LCO 1490 (5’ TAA ACT TCA GGG TGA CCA AAA AAT CA 3’) and 10 µM HCO 2198 (5’ GGT CAA CAA ATC ATA AAG ATA TTG 3’).

PCR reactions were carried out in 0.2 ml MicroAmp 8-Tube Strip tubes (Applied Biosystems, Thermo-Fisher Scientific, USA) containing 1.25 µl 10 µM LCO 1490, 1.25 µl 10 µM HCO 2198, 5 µl 5x Ultra Buffer, 0.25 µl PCR BIO Ultra Tag 5U/µl, 3 µl DNA template, and 14.25 µl nuclease-free water (Ambion, Thermo-Fischer Scientific, USA) in a final volume of 25 µl. PCR amplification was carried out using a Veriti 96 Well Thermal Cycler (Applied Biosystems, CA, USA). The PCR cycling conditions were as follows: 94°C for 3 min, followed by 35 cycles of 94 °C for 30 sec, 43 °C for 30 sec, and 72 °C for 30 sec, followed by a final extension of 72 °C for 10 min (Yashiro and Sanada-Morimura, 2021).

The PCR products were electrophoretically separated on a 1.2% (w/v) agarose (SeaKem LE Agarose Gel) in 0.5x TBE buffer, stained with 6 µl gel stain (SYBR Safe DNA gel stain, Invitrogen, Thermo Scientific) and viewed with a UV transilluminator to confirm that the correct size amplicon (658 bp) was obtained. Under a UV light, the amplicon was excised using an Xtracta lab gadget, a disposable gel extraction tool. The gel was cut by inserting the Xtracta lab gadget over the area of the amplicon. The amplicon was dispensed from the Xtracta lab gadget into 1.5 ml microcentrifuge tubes by squeezing the bulb of the gadget. The amplicon was purified using the Zymoclean Gel DNA Recovery kit (Zymo Research, USA) according to the manufacturer's instructions with minimal modifications. The purified products were eluted in 30 µl elution buffer. Using 1.2 µl of the template, the eluted DNA was measured using a NanoDrop spectrophotometer (NanonDrop One, Thermo Scientific).

DNA sequencing was conducted using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) at SASRI's Biotechnology Laboratory. The template amount used for successful DNA sequencing ranged between 20 ng and 150 ng. Sequencing reactions were conducted using the LCO 1490 primer with thermocycling conditions as follows: 96 °C for 1 min, followed by 25 cycles of 96 °C for 10 sec, 50 °C for 5 sec, and 60 °C for 4 min. Sequencing products were purified using the BigDye XTerminator Purification Kit (Applied Biosystems) according to the manufacturer's instructions. DNA sequences were analysed by capillary electrophoresis using the ABI3500 Genetic Analyser following standard operating protocols.

The sequences were edited and run through the Barcode of Life DataSystems (BOLD) database Identification Engine (Ratnasingham and Hebert, 2007). The system matched the input sequence to sequences already in the database, and insects were identified based on COI matches from previous entries. Insects were identified to either family, genus or species level based on the taxonomic level of the COI match available on the database. If a COI match was not obtained using BOLD insect similarities were also determined against known sequences in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST) algorithm (https://www.ncbi.nlm.nih.gov/BLAST/, (Altschul et al., 1990, Altschul, 1997, Ye, McGinnis and Madden, 2006)).

3.5 Data Analysis

The number of individuals for each morphospecies was used as a measure of abundance for the analyses (González, Salvo and Valladares, 2020). Tables were constructed from the total arthropod abundance for each order, which were then used to calculate the percentage of each order present within each sampling method. All statistical analyses were conducted using the statistical program R Studio 2023.06.0 Build 421 with the underlying R engine (R Core Team, 2023) and associated packages. Venn diagrams were constructed (R package: VennDiagram (Chen, 2022)) to show the number of species shared among sampling methods within fields.

To determine the differences in species diversity, three diversity indices, namely, the Shannon-Wiener diversity index, Simpson's diversity index, and Pielou's evenness index were calculated (R package: vegan (Oksanen et al., 2022)) for each sampling period and field utilizing the total weekly number of individuals of each morphospecies. The total weekly number of individuals for each species was square root transformed to balance the contribution of dominant and rare species prior to the calculation of the diversity indices (Adao et al., 2022). Species richness was also calculated by counting the total number of species present each week (Python, 2010).

Shannon (1948), Simpson (1949) and Pielou (1966) stated the following formulas for calculating the Shannon-Weiner, Simpson, and Pielou's Evenness indices. The formula for the Shannon-Weiner diversity index (*H*) is as follows: $H = -\sum Pi\ (lnPi)$, where *H* denotes the index of the species diversity at a specific site, *Pi* is the proportion of the total sample belonging to the i^{th} species and ln denotes the natural logarithm of the proportion of *Pi*. A value of *H* less than 1 indicates that the diversity is low, a value ranging between 1 and 3 indicates moderate diversity, and a value greater than 3 indicates high diversity (Paudel and Tiwari, 2022). The formula for the Simpson diversity index (*D*) is as follows: $D = 1 - \{\sum ni(ni-1)/N(N-1)\}$, where ni is the number of individuals of the i^{th} species, and N is the number of individuals in the total species population. The Simpson index value ranges from 0 to 1, with greater values indicating greater diversity, 0 indicating no diversity, and 1 indicating infinite diversity (Dilebo et al., 2023). The formula for Pielou's measure of evenness (*J*) is as follows: $J = \frac{H}{\ln(S)}$, where J denotes Pielou's evenness, H is the Shannon-Weiner diversity index, and S is the total number of individuals in the sample across all samples in the collection (Razzak, Awwal and Zulfiker, 2022).

The diversity indices were compared between the three sampling periods within each field using the Kruskal-Wallis test (Theodorsson-Norheim, 1986). The results from the diversity indices were also used to construct tables for each sampling period and boxplots (R package: ggplot2 (Wickham, 2016)) for each sampling method and field. Sampling methods were compared in terms of the evenness, diversity indices and species richness using the Kruskal-Wallis test. If a significant difference existed between the groups, Dunn's post hoc tests (R package: dunn.test (Dinno, 2017)) were used with Bonferroni correction to establish between which groups the significant difference occurred.

Raw abundance data were square root transformed before being used to visualize species composition within fields and across methods using nonmetric multidimensional scaling (NMDS) analysis in a two-dimensional scale based on the Bray-Curtis dissimilarity distances (R package: vegan) for both species abundance and identity (Holland, 2008). NMDS is a powerful method used to depict samples as points in a low dimensional space in a manner that the relative distances between all points represent the same rank order as the relative dissimilarity of the samples (Ando, Utsumi and Ohgushi, 2010). The Bray-Curtis ordination method detects gradients without the assumption of linear relationships between variables, and provides an ordination based on a distance or dissimilarity matrix (del-Val, Ramírez and Astier, 2021). Non-parametric analysis of similarities (ANOSIM) (R package: vegan) was used to test for a statistical difference in species composition between the three methods within irrigated and rain-fed sugarcane fields using the Bray-Curtis dissimilarity distances (Thompson et al., 2021).

CHAPTER FOUR - RESULTS

4.1 Insects in Irrigated Sugarcane

4.1.1 Abundance of Insects

A total of 22 309 insects belonging to 14 orders and 94 families and 309 morphospecies were collected in irrigated sugarcane (Table 4.1). The most abundant orders were Diptera (40.836%), Hemiptera (29.383%) and Hymenoptera (15.456%). The families Cicadellidae (Hemiptera), belonging to eight morphospecies and Hybotidae (Diptera), belonging to one morphospecies, showed the highest abundance of insects, making up 20.808% and 18.275% of the total, respectively.

Table 4.1: Number of insect orders, families and morphospecies collected from irrigated sugarcane from March 2022 to October 2022.

Order (∑ individuals; %)	Total Families	Total morphospecies per order	Number of individuals per family (∑ individuals, %)
Blattodea (231; 1.035)	2	5	Blattellidae (45; 0.202), Blattidae (186; 0.834)
Coleoptera (1532; 6.867)	20	58	Anthicidae (4; 0.018), Buprestidae (3; 0.013), Cantharidae (1; 0.004), Carabidae (8; 0.036), Chrysomelidae (64; 0.287), Coccinellidae (3; 0.013), Curculionidae (438; 1.963), Dermestidae (3; 0.013), Dytiscidae (3; 0.013), Elateridae (51; 0.229), Hybosoridae (7; 0.031), Lycidae (1; 0.004), Mordellidae (10; 0.045), Nitidulidae (469; 2.102), Phalacridae (1; 0.004), Scarabaeidae (4; 0.018), Silvanidae (18; 0.081), Smicripidae (6; 0.027), Staphylinidae (428; 1.919), Tenebrionidae (10; 0.045)
Collembola (295; 1.322)	1	2	Entomobryidae (295; 1.322)
Dermaptera (30; 0.134)	1	4	Forficulidae (30; 0.134)

Table 4.1 continued: Number of insect orders, families and morphospecies collected from irrigated sugarcane from March 2022 to October 2022.

Order (∑ individuals; %)	Total Families	Total morphospecies per order	Number of individuals per family (∑ individuals, %)
Diptera (9110; 40.836)	25	99	Calliphoridae (71; 0.318), Cecidomyiidae (10; 0.045), Chloropidae (11; 0.049), Culicidae (209; 0.937), Diopsidae (24; 0.108), Dolichopodidae (59; 0.264), Drosophilidae (854; 3.828), Ephydridae (185; 0.829), Heleomyzidae (5; 0.022), Hybotidae (4077; 18.275), Lauxaniidae (603; 2.703), Muscidae (346; 1.551), Mycetophylidae (85; 0.381), Phoridae (782; 3.505), Pipunculidae (35; 0.157), Sarcophagidae (175; 0.784), Sciaridae (393; 1.762), Sepsidae (17; 0.076), Simulidae (68; 0.305), Sphaeroceridae (5; 0.022), Syrphidae (408; 1.829), Tabanidae (210; 0.941), Tachinidae (82; 0.368), Tephritidae (310; 1.390), Tipulidae (86; 0.385)
Hemiptera (6555; 29.383)	14	36	Aleyrodidae (4; 0.018), Aphididae (54; 0.242), Berytidae (1; 0.004), Cercopidae (10; 0.045), Cicadellidae (4642; 20.808), Cixiidae (105; 0.471), Cydnidae (26; 0.117), Delphacidae (1509; 6.764), Lygaeidae (167; 0.749), Miridae (19; 0.085), Nabidae (4; 0.018), Nepidae (1; 0.004), Pentatomidae (2; 0.009), Reduviidae (11; 0.049)
Hymenoptera (3448; 15.456)	13	80	Apidae (12;0.054), Braconidae (256; 1.148), Chalcididae (33; 0.148), Diapriidae (9; 0.040), Eumenidae (16; 0.072), Formicidae (2764; 12.390), Ichneumonidae (179; 0.802), Pompilidae (63; 0.282), Pteromalidae (5; 0.022), Scelionidae (8: 0.036), Sphecidae (91; 0.408), Vespidae (11; 0.049)
Lepidoptera (151; 0.677)	6	8	Arctiidae (3; 0.013), Crambidae (101; 0.453), Geometridae (1; 0.004), Noctuiidae (1; 0.004), Nymphalidae (43; 0.193), Pyralidae (2; 0.009)
Mantodea (4; 0.018)	1	1	Mantidae (4; 0.018)
Neuroptera (13; 0.058)	1	1	Chrysopidae (13; 0.058)

Table 4.1 continued: Number of insect orders, families and morphospecies collected from irrigated sugarcane from March 2022 to October 2022.

Order (∑ individuals; %)	Total Families	Total morphospecies per order	Number of individuals per family (∑ individuals, %)
Orthoptera (287; 1.286)	5	**7**	Acrididae (5; 0.022), Anostostomatidae (7; 0.031), Gryllidae (270; 1.210), Tetrigidae (1; 0.004), Tettigonidae (4; 0.018)
Psocoptera (265; 1.188)	3	3	Caeciliusidae (262; 1.174), Lachesillidae (1; 0.004), Psocidae (2; 0.009)
Siphonaptera (2; 0.009)	1	2	Ceratophyllidae (2; 0.009)
Thysanoptera (386; 1.730)	1	3	Thripidae (386; 1.730)
Total	**94**	**309**	**22 309**

4.1.2 Abundance of Insects Collected Using Different Sampling Methods

A total of 8 217 insects were collected using pitfall traps, 10 754 using yellow sticky traps, and 3 338 using water pan traps in irrigated sugarcane (Table 4.2). The insects from the pitfall traps represented 13 orders and 169 morphospecies. The largest proportion of individuals belonged to the orders Hymenoptera (26.226%) and Hemiptera (25.910%), followed by Diptera (20.251%) and Coleoptera (17.52%). The family Formicidae contributed to Hymenoptera as the dominant order, with an abundance proportion of 25.362% in the pitfall traps. The orders Dermaptera, Lepidoptera, Psocoptera, Siphonaptera, and Thysanoptera each showed fewer than 40 individuals.

The sticky traps collected insects belonging to 10 insect orders and 102 morphospecies (Table 4.2). Sticky traps collected the highest abundance of Diptera (60.526%), followed by Hemiptera (27.441%). Neuroptera and Orthoptera were the least abundant orders in the sticky traps, with a total of less than 20 individuals collected for each. The large number of individuals collected from the family Hybotidae (36.507%) contributed to Diptera being the dominating order from the sticky trap collections. In the order Hemiptera, the family Cicadellidae showed the highest abundance.

The water pan trap traps in irrigated sugarcane showed insect individuals belonging to eight arthropod orders and 103 morphospecies. Water pan traps collected the highest abundance of Hemiptera (44.188%), followed by Diptera (28.071%) and Hymenoptera (24.146%). Blattodea, Neuroptera, and Orthoptera were the least abundant orders, with a total number of less than 20 individuals for each.

The family with the highest abundance within the order Hemiptera was Cicadellidae (32.924%), followed by Delphacidae (10.336%) in the water pan trap samples. Within the order Diptera, the family Drosophilidae showed the highest number of individuals (5.422%).

Table 4.2: Order abundance and abundance percentage of insects collected in irrigated sugarcane using three sampling methods, namely, pitfall traps, sticky traps, and water pan traps.

	Sampling Method					
	Pitfall Trap		**Sticky Trap**		**Water Pan**	
Order	**Number of individuals**	**%**	**Number of individuals**	**%**	**Number of individuals**	**%**
Blattodea	151	1.838	68	0.632	12	0.359
Coleoptera	1 440	17.525	55	0.511	37	1.108
Collembola	295	3.590	-	-	-	-
Dermaptera	28	0.341	-	-	2	0.060
Diptera	1 664	20.251	6 509	60.526	937	28.071
Hemiptera	2 129	25.910	2 951	27.441	1 475	44.188
Hymenoptera	2 155	26.226	487	4.529	806	24.146
Lepidoptera	31	0.377	55	0.511	65	1.947
Mantodea	4	0.049	-	-	-	-
Neuroptera	-	-	12	0.112	1	0.030
Orthoptera	278	3.383	6	0.056	3	0.090
Pscocoptera	36	0.438	229	2.129	-	-
Siphonaptera	2	0.024	-	-	-	-
Thysanoptera	4	0.049	382	3.552	-	-
Total	**8 217**	**100**	**10 754**	**100**	**3 338**	**100**

4.1.3 Comparing Various Diversity Indices Within Different Sampling Methods Across Three Sampling Periods

The Kruskal-Wallis (KW) tests showed no significant difference in the species richness and diversity indices between the three sampling periods for pitfall traps (Table 4.3). In the sticky traps, the species richness and diversity indices all show a significant difference between the three sampling periods ($p < 0.05$). In the water pan traps, there was no difference in the species richness, Shannon-Wiener and Simpson indices; however, there was a difference in Pielou's evenness between the three sampling periods ($H = 13.514$, df = 2, $p = 0.001$)

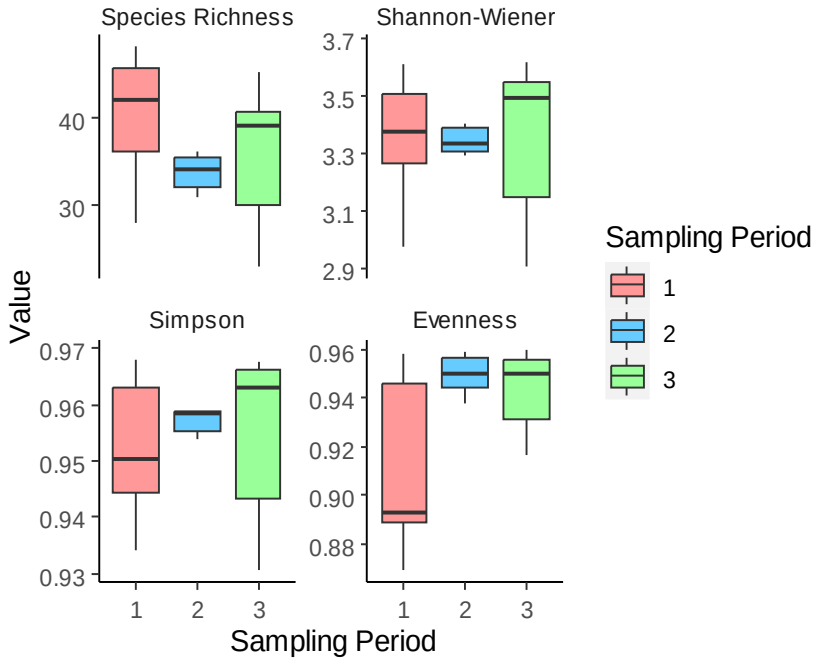

Figure 4.1: Insect diversity indices and species richness for insects collected using pitfall traps in irrigated sugarcane across three sampling periods from March to October 2022. In each boxplot, the first box from the left is for sampling period one, the second box for sampling period two and the third for sampling period three. Box plots display the first and third quartiles and the median, maximum, and minimum observed values within each dataset.

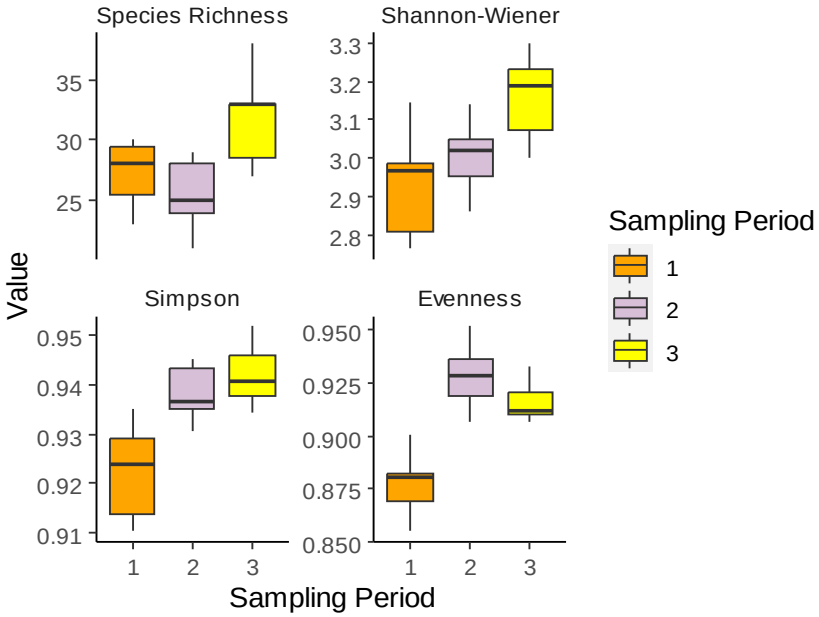

Figure 4.2: Insect diversity indices and species richness for insects collected using sticky traps in irrigated sugarcane across three sampling periods from March to October 2022. In each boxplot, the first box from the left is for sampling period one, the second box for sampling period two and the third for sampling period three. Box plots display the first and third quartiles and the median, maximum, and minimum observed values within each dataset.

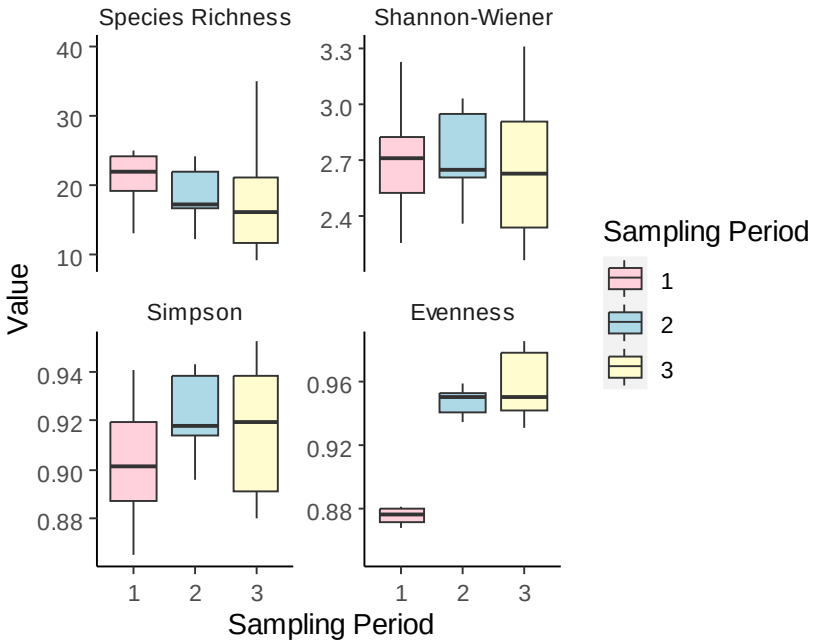

Figure 4.3: Insect diversity indices and species richness for insects sampled using water pan traps in irrigated sugarcane across three sampling periods from March to October 2022. In each boxplot, the first box from the left is for sampling period one, the second box for sampling period two and the third for sampling period three. Box plots display the first and third quartiles and the median, maximum, and minimum observed values within each dataset.

Table 4.3: Kruskal-Wallis comparison of diversity indices and species richness across three sampling periods within each sampling method of insects collected in irrigated sugarcane from March - October 2022.

Sampling method	Index Compared Within Sampling method	Kruskal-Wallis *H*	df	Kruskal-Wallis *p*-value
Pitfall traps	Species Richness	2.323	2	0.313
	Shannon-Wiener Index	0.141		0.932
	Simpson's Index	0.052		0.974
	Pielou's Evenness	3.792		0.150
Sticky traps	Species Richness	6.052	2	**0.049**
	Shannon-Wiener Index	9.625		**0.008**
	Simpson's Index	11.807		**0.003**
	Pielou's Evenness	14.345		**0.001**
Water pan traps	Species Richness	2.576	2	0.276
	Shannon-Wiener Index	0.320		0.853
	Simpson's Index	1.492		0.474
	Pielou's Evenness	13.514		**0.001**

4.1.4 Insect Diversity and Community Composition

There was a significant difference in the species richness and diversity indices between the three sampling methods ($p < 0.001$) (Table 4.4). Across all comparisons, the lowest median index values were obtained from the water pan traps, while the highest median index values were obtained from the pitfall traps (Figure 4.3). Subsequent Dunn's post hoc tests confirm a significant difference in Pielou's evenness specifically between the pitfall and water pan traps. The post-hoc test showed a significant difference in the Shannon-Wiener index between the pitfall, sticky and water pan traps (Table 4.4). The Simpson index showed a significant difference between the water pan trap and pitfall trap comparison and the sticky trap and water pan trap comparison. Pielou's evenness index showed a significant difference between the water pan and pitfall traps.

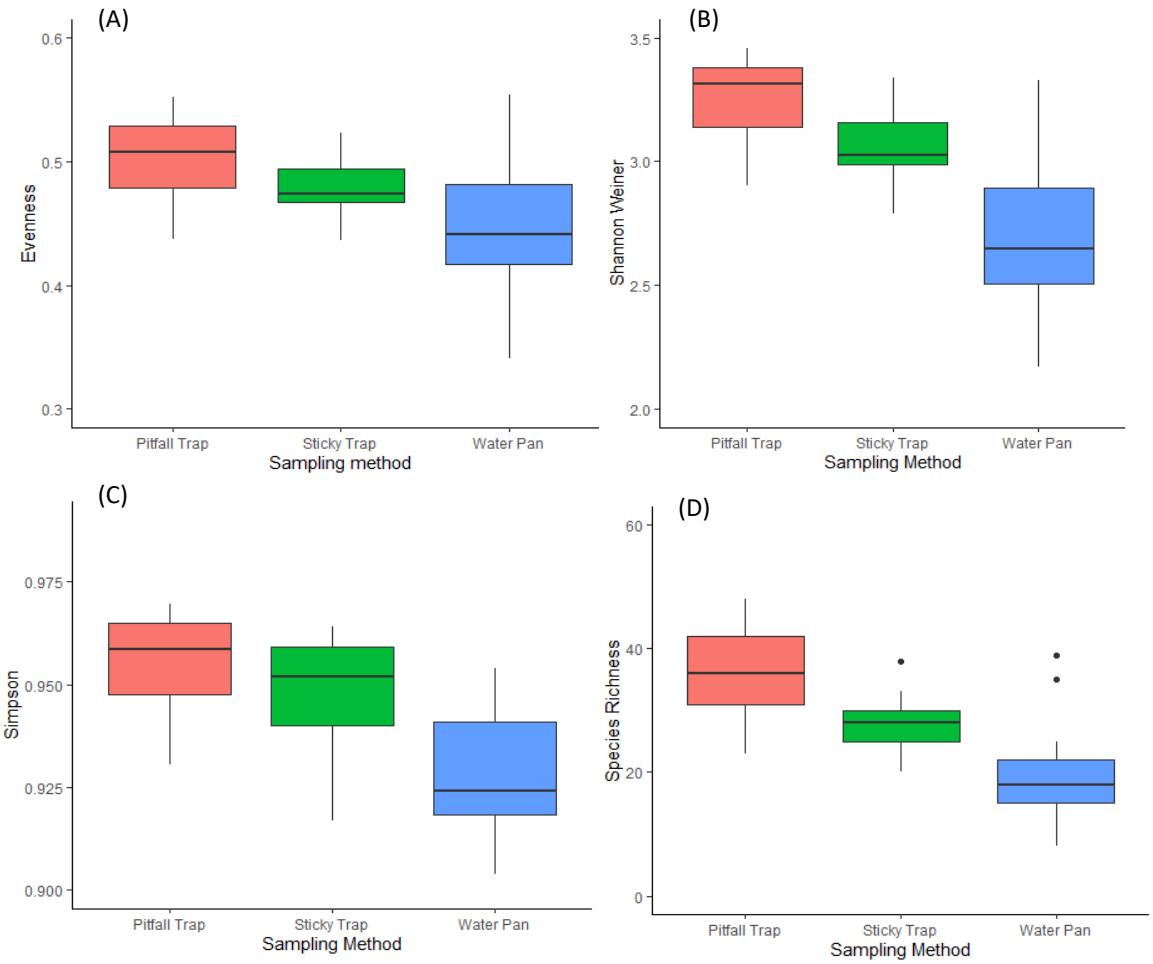

Figure 4.4: Boxplots for insect diversity indices for each trap type for insects collected in irrigated sugarcane during March – October 2022. Box plots display the first and third quartiles and the median, maximum, and minimum observed values within each dataset. Pielou's evenness (A), Shannon-Weiner index (B), Simpson's diversity index (C), and Species Richness (D). The dots show the outliers in the datasets.

Table 4.4: Kruskal-Wallis comparison of diversity indices and species richness between three sampling methods of insects collected in irrigated sugarcane. Dunn's post hoc test results are given under significant differences. Pitfall traps are abbreviated as PF, sticky traps as ST and water pan traps as WP.

Index compared	Kruskal-Wallis *H*	Kruskal-Wallis *p*-value	df	Significant difference
Pielou's Evenness	16.2781	< 0.001	2	WP vs PF
Shannon-Wiener	35.551	< 0.001	2	WP vs PF WP vs ST ST vs PF
Simpson's Index	30.542	< 0.001	2	WP vs ST WP vs PF
Species richness	35.2171	< 0.001	2	WP vs PF WP vs ST ST vs PF

An NMDS plot (stress=0.14) based on the Bray-Curtis dissimilarity matrix for the irrigated sugarcane displays the clustering of samples from each sampling method (Figure 4.4). This shows that there are three assemblage compositions based on the three sampling methods used to collect insects. The stress value indicates whether the ordination is a good representation of the distances seen among the samples (Holland, 2008). The general stress limit for an acceptable representation is 0.2 or lower (Dexter, Rollwagen-Bollens and Bollens, 2018); therefore, the results in the NMDS plot showed an acceptable representation of the distances among samples. The ANOSIM test showed a significant difference in the community composition of insects collected using the three different sampling methods (R=0.9241, p < 0.001).

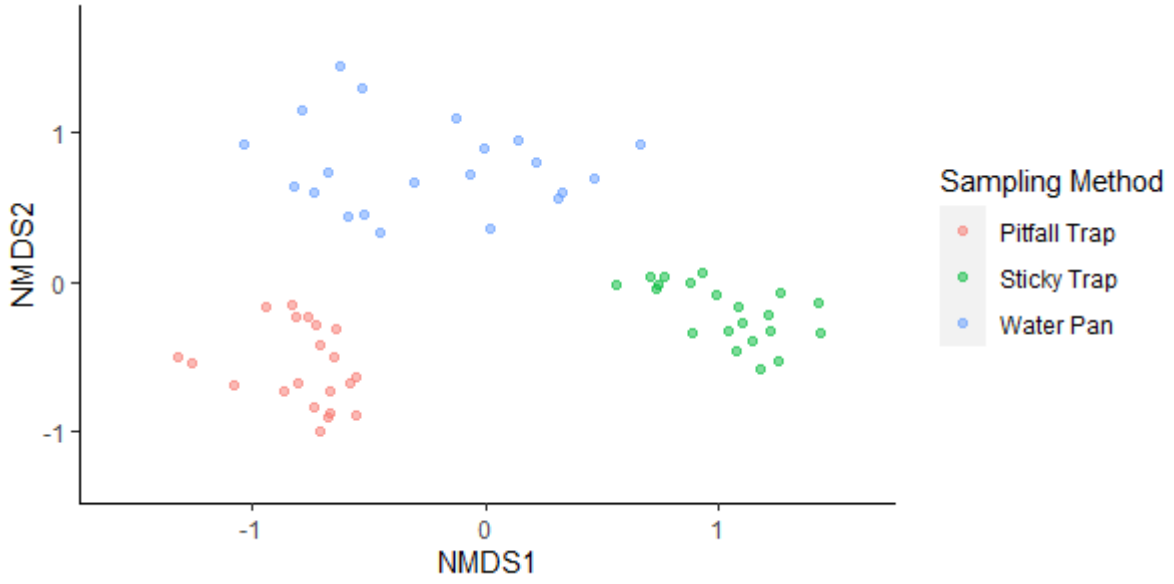

Figure 4.5: NMDS analysis of insect community similarity among the different sampling method groups in the irrigated sugarcane based on the Bray-Curtis dissimilarity matrix.

From the collected insects, 20 morphospecies (hereafter referred to as species) (7.52%) were collected in all three trap types (Figure 4.6) in the irrigated field. The highest proportion of species was collected using pitfall traps (45.1%), and the lowest proportion of species was found using sticky traps (11.3%) (Figure 4.6). The largest proportion of shared species was collected between the sticky traps and water pan traps (13.5%), and the lowest proportion between water pan traps and pitfall traps (5.64%).

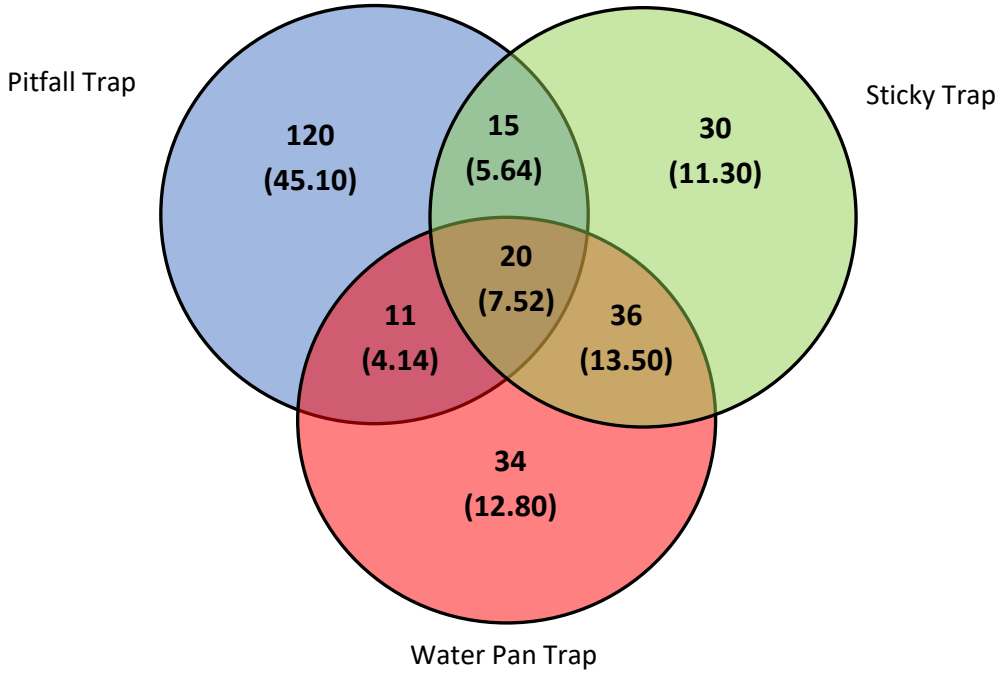

Figure 4.6: The number of species and percentage of total species (in brackets) collected exclusively within each trap and shared in the different traps in the irrigated sugarcane.

4.2 Insects in Rain-Fed Sugarcane

4.2.1 Abundance of Insects

Overall, 12 493 insects belonging to 14 insect orders, 88 families and 284 morphospecies were collected during sampling from the rain-fed sugarcane field (Table 4.5). Hymenoptera (29.192%) showed the highest abundance of insects with 14 insect families, followed by Coleoptera (19.843%) with 17 insect families and Diptera (16.881%) with 20 insect families. The most abundant families were Formicidae (Hymenoptera), with 23.669% and Gryllidae (Orthoptera) with 11.711%. Orders Isoptera, Neuroptera, Mantodea, Pscocoptera and Thysanoptera were the least abundant, with proportions lower than 0.1% each.

Table 4.5: Total number of insects collected in rain-fed sugarcane from March 2022 to October 2022 according, to order, family and morphospecies.

Order (∑ individuals; %)	Total Families	Total morphospecies per order	Number of individuals per family (∑ individuals, %)
Blattodea (266; 2.129)	2	9	Blattellidae (157; 1.257), Blattidae (109; 0.872)
Coleoptera (2479; 19.843)	17	62	Anthicidae (15; 0.120), Buprestidae (8; 0.064), Cantharidae (1; 0.008), Carabidae (37; 0.296), Chrysomelidae (302; 2.417), Coccinellidae (6; 0.048), Curculionidae (484; 3.874), Dermestidae (137; 1.097), Derodontiidae (1; 0.008), Hydarenidae (9; 0.072), Latridiidae (39; 0.312), Lycidae (1; 0.008), Meloidae (5; 0.040), Mordellidae (17; 0.136), Nitidulidae (649; 5.195), Scarabaeidae (49; 0.392), Staphylinidae (719; 5.755)
Collembola (793; 6.348)	2	3	Entomobryidae (780; 6.243), Sminthuridae (13; 0.104)
Dermaptera (35; 0.280)	2	2	Anisolabidae (22; 0.176), Labiduridae (13; 0.104)
Diptera (2109; 16.881)	20	72	Calliphoridae (18; 0.144), Chloropidae (22; 0.176), Culicidae (21; 0.168), Dolichopodidae (362; 2.898), Drosophilidae (141; 1.129), Ephydridae (167; 1.337), Hybotidae (24; 0.192), Lauxaniidae (26; 0.208), Muscidae (150; 1.201), Mycetophilidae (10; 0.080), Phoridae (182; 1.457), Pipunculidae (24; 0.192), Sarcophagidae (41; 0.328), Sciaridae (148; 1.185), Sepsidae (85; 0.680), Simulidae (45; 0.360), Syrphidae (348; 2.786), Tachinidae (54; 0.432), Tephritidae (237; 1.897), Tipulidae (4; 0.032)
Hemiptera (1168; 9.349)	13	35	Aleyrodidae (224; 1.793), Aphididae (313; 2.505), Cercopidae (12; 0.096), Cicadellidae (346; 2.770), Cixiidae (103; 0.824), Cydnidae (2; 0.016), Delphacidae (64; 0.512), Lygaeidae (17; 0.136), Membracidae (5; 0.040), Miridae (71; 0.568), Pentatomidae (1; 0.008), Reduviidae (4; 0.032), Tingidae (6; 0.048)

Table 4.5 continued: Total number of insects collected in rain-fed sugarcane from March 2022 to October 2022 according, to order, family and morphospecies.

Order (∑ individuals; %)	Total Families	Total morphospecies per order	Number of individuals per family (∑ individuals, %)
Hymenoptera (3648; 29.192)	14	72	Apidae (25; 0.200), Braconidae (50; 0.400), Chalcididae (115; 0.921), Eumenidae (11; 0.088), Eupelmidae (114; 0.913), Formicidae (2957; 23.669), Ichneumonidae (5; 0.040), Mutillidae (1; 0.008), Perilampidae (1; 0.008), Pompilidae (97; 0.776), Scelionidae (157; 1.257), Scoliidae (1; 0.008), Sphecidae (82; 0.656), Vespidae (31; 0.248)
Isoptera (1; 0.008)	1	1	Termitidae (1; 0.008)
Lepidoptera (475; 3.802)	5	8	Arctiidae (3; 0.024), Crambidae (161; 1.289), Geometridae (1; 0.008), Nymphalidae (278; 2.225), Pyralidae (32; 0.256)
Mantodea (2; 0.016)	1	1	Mantidae (2; 0.016)
Neuroptera (11; 0.088)	2	2	Chrysopidae (9; 0.072), Mantispidae (2; 0.016)
Orthroptera (1481; 11.855)	6	10	Acrididae (7; 0.056), Anostostomatidae (5; 0.040), Gryllidae (1463; 11.711), Gryllotalpidae (1; 0.008), Tetrigidae (1; 0.008), Tettigonidae (4; 0.032)
Psocoptera (16; 0.128)	2	4	Caeciliusidae (5; 0.04), Pscocidae (11; 0.088)
Thysanoptera (10; 0.080)	1	3	Thripidae (10; 0.080)
Total	**88**	**284**	**12 493**

4.2.2 Abundance of Insects Collected Using Different Sampling Methods

Insects were collected using pitfall traps, yellow sticky traps, and water pan traps with insect totals of 8 370, 2 530 and 1 593, respectively (Table 4.6). From the pitfall traps, the highest number of individuals belonged to the order Hymenoptera (37.010%), followed by Coleoptera (25.591%) and Orthoptera (17.563%). Orders Dermaptera, Isoptera, Lepidoptera, Neuroptera, Mantodea, Psocoptera and Thysanoptera had a total insect abundance of less than 50 individuals in the pitfall traps (Table 4.6).

Within the order Hymenoptera, the family Formicidae was the most abundant (34.256%), which included two species of ants, *Monomorium junodi* and *Pheidole megacephala*, in the pitfall trap samples. The most abundant family within Coleoptera was Staphylinidae (8.578%), followed by Nitidulidae (7.754%) and Curculionidae (5.639%), which included the species *Xyleborus monographus.*

From the sticky trap samples, the order Diptera (37.905%) showed the highest insect abundance, followed by Hemiptera (26.047%), Lepidoptera (12.174%) and Coleoptera (11.858%). Orders Neuroptera, Orthoptera, Pscocoptera, and Thysanoptera showed a total abundance of less than eight individuals each (Table 4.8). Within the order Diptera, the family Syrphidae (12.767%) show the highest abundance in the sticky traps for rain-fed sugarcane. The most abundant family within Hemiptera was Aphididae (10.672%); within Lepidoptera, it was Nymphalidae (10.949%); and within Coleoptera, it was Chrysomelidae (11.225%). The water pan trap samples consisted of insects belonging to eight insect orders and 100 morphospecies. Most insects belonged to the order Diptera (44.947%), followed by Hymenoptera (27.495%), Hemiptera (13.371%) and Lepidoptera (10.295%) (Table 4.6). Less than 50 individuals were collected from orders Coleoptera, Blattodea, Neuroptera and Orthoptera (Table 4.6).

Table 4.6: Order abundance and abundance percentage of insects collected in rain-fed sugarcane using three sampling methods, namely pitfall traps, sticky traps, and water pan traps.

	Sampling Method					
	Pitfall Trap		**Sticky Trap**		**Water Pan Trap**	
Order	**Number of individuals**	**%**	**Number of individuals**	**%**	**Number of individuals**	**%**
Blattodea	163	1.947	86	3.399	17	1.067
Coleoptera	2142	25.591	300	11.858	37	2.323
Collembola	793	9.474	-	-	-	-
Dermaptera	35	0.418	-	-	-	-
Diptera	347	5.185	1179	37.905	715	44.947
Hemiptera	296	3.536	461	26.047	213	13.371
Hymenoptera	3100	36.010	173	7.708	438	27.495
Isoptera	1	0.012	-	-	-	-
Lepidoptera	3	0.036	308	12.174	164	10.295
Mantodea	2	0.024	-	-	-	-
Neuroptera	1	0.012	9	0.356	1	0.063
Orthoptera	1470	17.563	4	0.158	7	0.439
Pscocoptera	13	0.155	3	0.119	-	-
Thysanoptera	3	0.036	7	0.277	-	-
Total	**8370**	**100**	**2530**	**100**	**1593**	**100**

4.2.3 Comparing the Diversity Indices Within Different Sampling Methods Across Three Sampling Periods in Rain-Fed Sugarcane

The species richness ranged between 20 and 41 across the three sampling periods in the pitfall traps (Figure 4.7), 9 and 32 in the sticky traps (Figure 4.8) and 3 and 33 in the water pan traps (Figure 4.9). The Shannon-Wiener index varied between 2.65 and 3.36 in the pitfall traps, between 2.17 and 3.32 in the sticky traps and between 1.10 and 3.32 in the water pan traps. The range of Simpson's index was 0.90 to 0.96 in pitfall traps, 0.88 to 0.96 in sticky traps, and 0.67 to 0.96 in water pan traps. Pielou's evenness index varied between 0.84 and 0.95 in the pitfall traps, 0.92 and 0.99 in the sticky traps and 0.91 and 1 in the water pan traps. The Kruskal-Wallis test showed a significant difference in the diversity indices and species richness between the three sampling periods for the pitfall and sticky traps with $p < 0.05$. In the pitfall traps, the median for species richness was highest (42) for sampling period 1 compared to sampling period 2 (34) and sampling period 3 (39). The median for the Shannon-Wiener index was highest for sampling period 3 (3.495) compared to sampling period 1 (3.377) and sampling period 2 (3.334). For the water pan traps, there was a significant difference in the species richness (H = 8.577, df = 2, p = 0.014), Shannon-Wiener (H = 8.905, df = 2, p = 0.012) and Simpson indices (H = 8.779, df = 2, p = 0.012) across the three sampling periods. However, there was no significant difference in Pielou's evenness between the three sampling periods (H = 1.179, df = 2, p = 0.555).

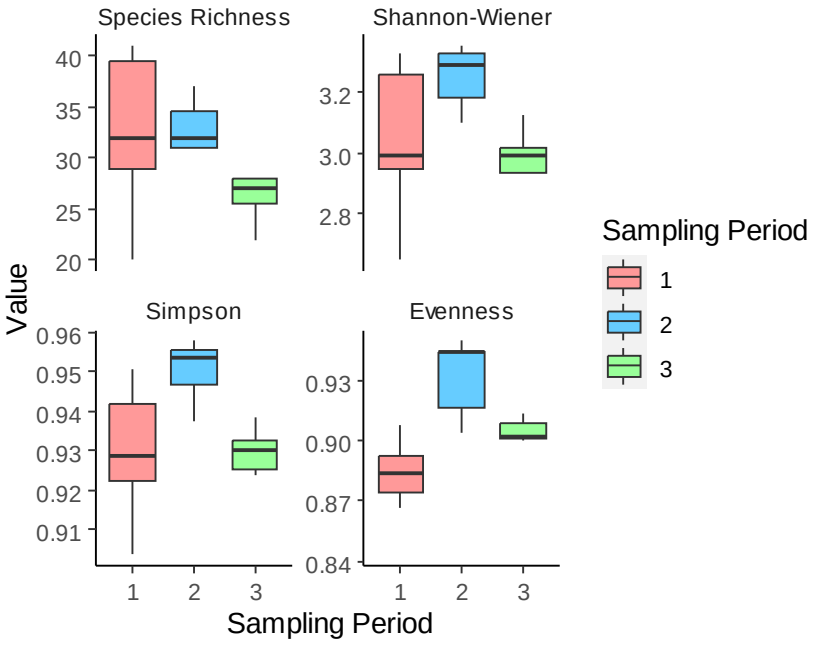

Figure 4.7: Insect diversity indices and species richness for insects sampled using pitfall traps in rain-fed sugarcane across three sampling periods from March to October 2022. In each boxplot, the first box from the left is for sampling period one, the second box for sampling period two and the third for sampling period three. Box plots display the first and third quartiles, the median, outliers, maximum, and minimum observed values within each dataset.

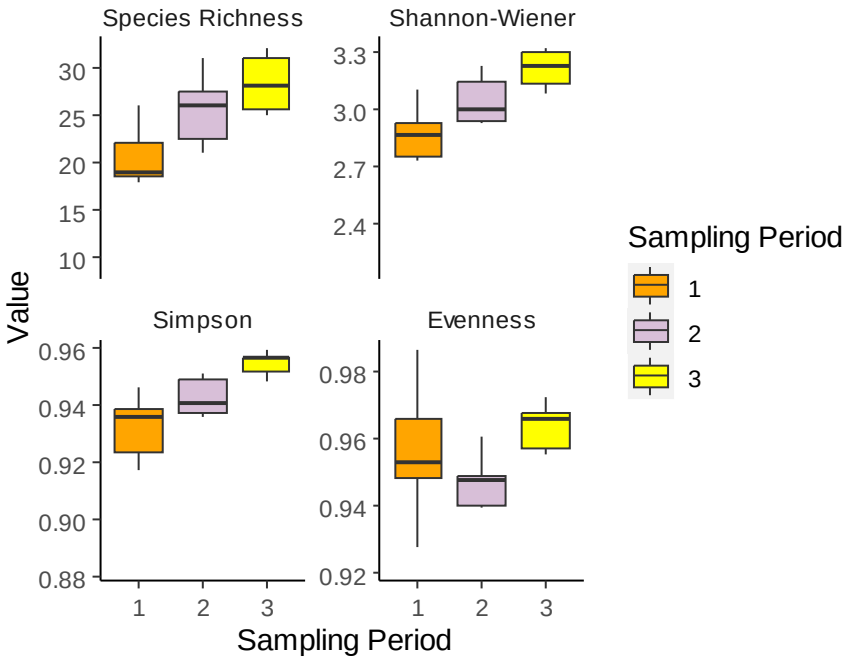

Figure 4.8: Insect diversity indices and species richness for insects sampled using sticky traps in rain-fed sugarcane across three sampling periods from March to October 2022. In each boxplot, the first box from the left is for sampling period one, the second box for sampling period two and the third for sampling period three. Box plots display the first and third quartiles, the median, outliers, maximum, and minimum observed values within each dataset.

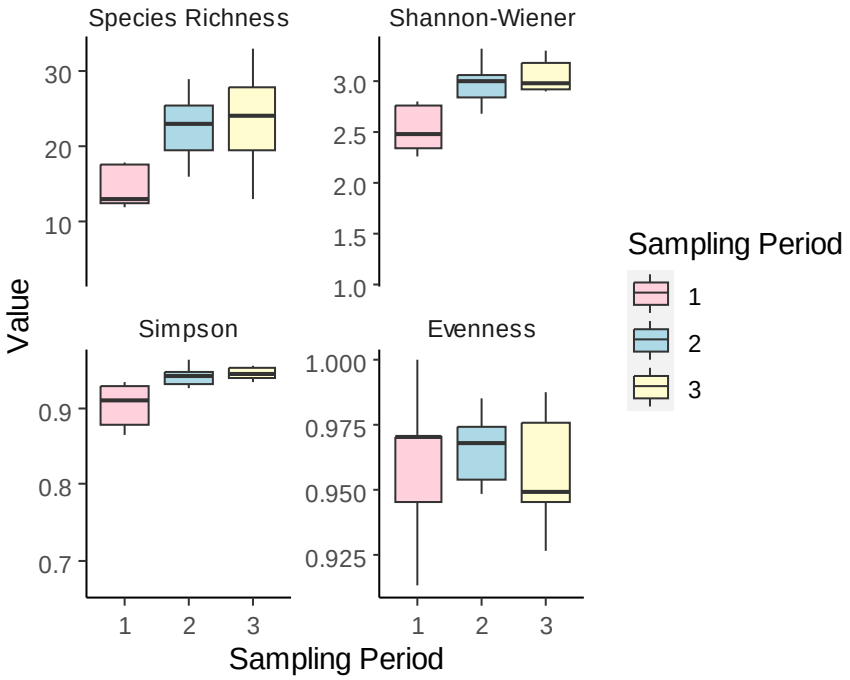

Figure 4.9: Insect diversity indices and species richness for insects sampled using water pan traps in rain-fed sugarcane across three sampling periods from March to October 2022. In each boxplot, the first box from the left is for sampling period one, the second box for sampling period two and the third for sampling period three. Box plots display the first and third quartiles, the median, outliers, maximum, and minimum observed values within each dataset

Table 4.7: Kruskal-Wallis test results comparing the diversity indices and species richness across sampling periods within each sampling method from insects collected in rain-fed sugarcane.

Sampling method	Index Compared	Kruskal-Wallis *H*	df	Kruskal-Wallis *p*-value
Pitfall traps	Species Richness	7.333	2	**0.026**
	Shannon-Wiener Index	8.156		**0.017**
	Simpson's Index	10.516		**0.005**
	Pielou's Evenness	13.455		**0.001**
Sticky traps	Species Richness	10.008	2	**0.007**
	Shannon-Wiener Index	11.874		**0.003**
	Simpson's Index	13.054		**0.001**
	Pielou's Evenness	7.221		**0.027**
Water pans	Species Richness	8.577	2	**0.014**
	Shannon-Wiener Index	8.905		**0.012**
	Simpson's Index	8.779		**0.012**
	Pielou's Evenness	0.141		0.932

4.2.4 Insect Diversity and Community Composition

There was a significant difference in the Shannon-Weiner index, evenness index and species richness between the three sampling methods ($p < 0.05$) (Table 4.10). There was no significant difference in Simpson's index between the sampling methods ($p = 0.14$). The sticky traps show the highest median for Pielou's evenness compared to pitfall and water pan traps (Figure 4.10). Dunn's post hoc tests show a significant difference in Pielou's evenness, specifically in the comparison of the pitfall and water pan traps and the pitfall and sticky traps. The pitfall traps show the highest median value for the Shannon-Wiener index as well as the highest median value for species richness (Figure 4.10). Dunn's post hoc test shows a significant difference in the Shannon-Wiener index between the pitfall and water pan traps. Species richness was significantly different when pitfall and water pans were compared, as well as when pitfall and sticky traps were compared (Table 4.8). An NMDS plot (stress=0.10) based on the Bray-Curtis dissimilarity matrix shows a clear clustering of samples from each sampling method group, indicating a significant difference among the different sampling methods (Figure 4.11). The clusters of the sticky traps and water pan traps appear to be closer in relation to the distances between the clusters of the other sampling method pairs. The ANOSIM test confirmed that there was a significant difference in the community composition of insects collected using the three different sampling methods (R=0.9791, $p < 0.001$).

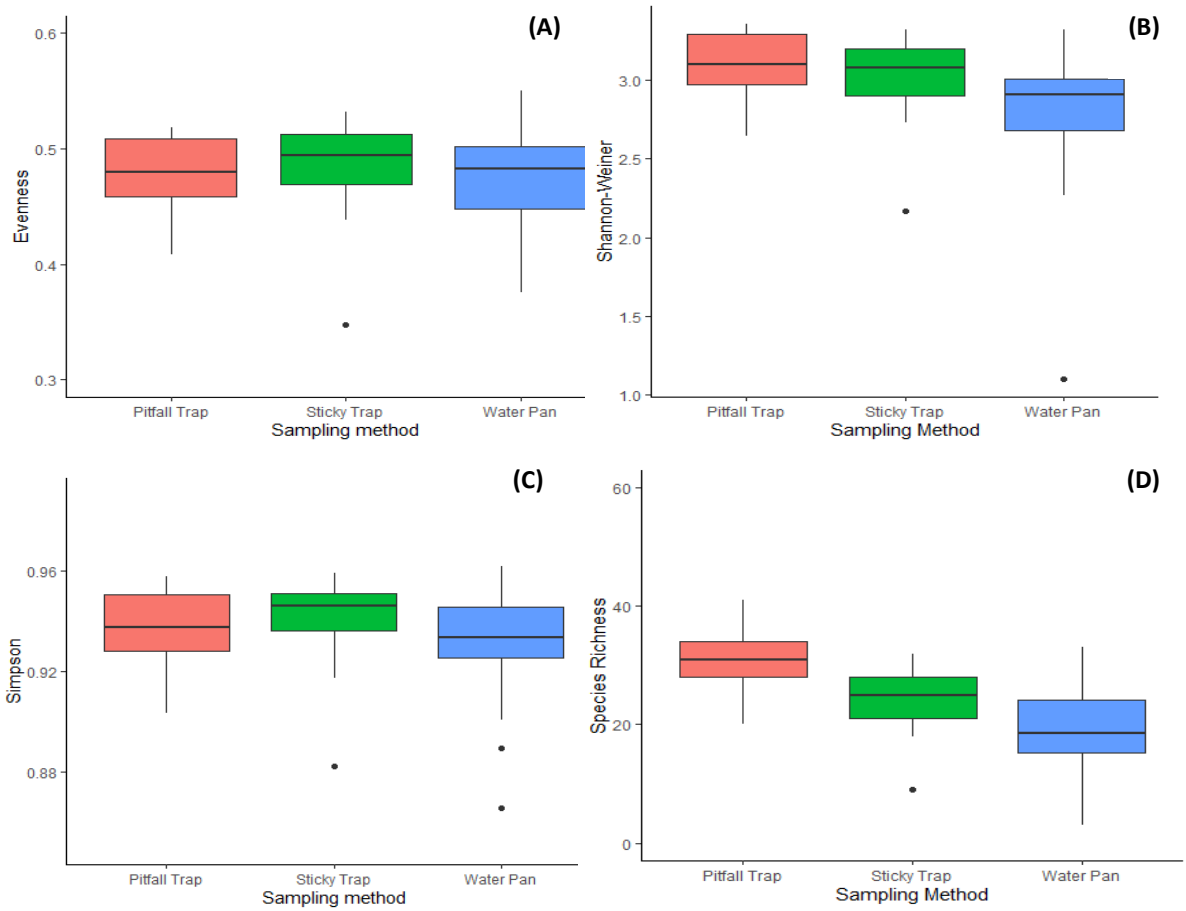

Figure 4.10: Insect diversity indices for each trap type for insects collected in rain-fed sugarcane in Gingindlovu during March – October 2022. Box plots display the first and third quartiles and the median, maximum, and minimum observed values within each dataset. Pielou's evenness (A), Shannon-Weiner index (B), Simpson's diversity index (C), and Species Richness (D). The dots indicate outliers in the data set.

Table 4.8: Kruskal-Wallis comparison of diversity indices and species richness between three sampling methods of insects collected in rain-fed sugarcane. Dunn's post hoc test results are given under significant differences. Pitfall traps are abbreviated as PF, sticky traps as ST and water pan traps as WP.

Index compared	**Kruskal-Wallis *H***	**Kruskal-Wallis *p*-value**	**df**	**Significant difference**
Pielou's Evenness	33.525	< 0.001	2	WP vs PF PF vs ST
Shannon-Wiener	8.1913	0.02	2	WP vs PF
Simpson's Index	3.9943	0.14	2	
Species richness	23.4332	< 0.001	2	WP vs PF ST vs PF

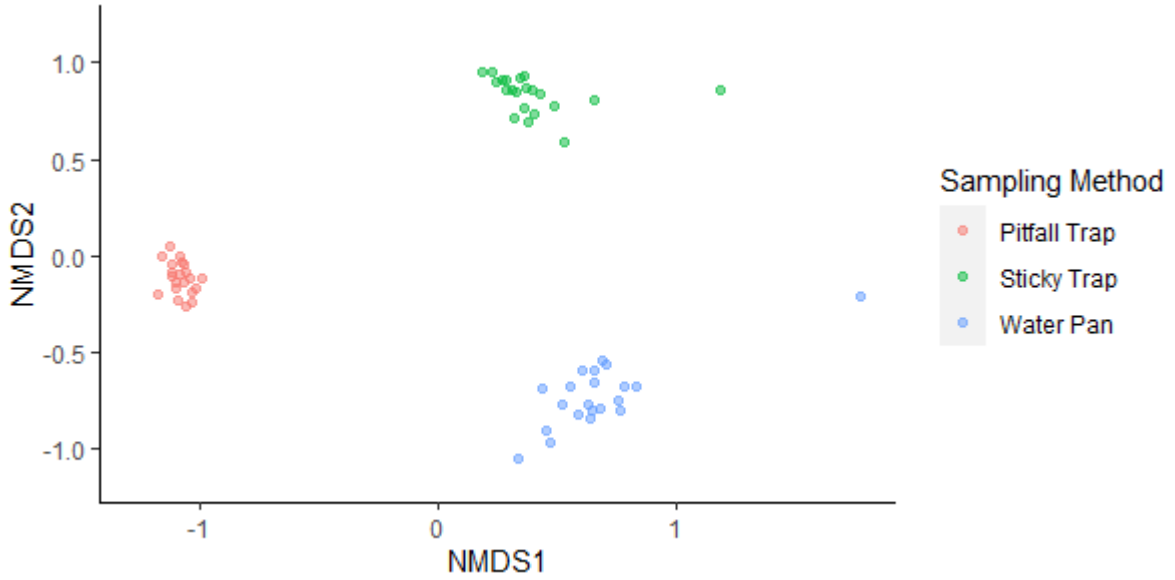

Figure 4.11: NMDS analysis of insect community similarity among the different sampling method groups in the rain-fed sugarcane based on the Bray–Curtis dissimilarity matrix.

The three sampling methods had a total of 10 (3.82%) species in common (Figure 4.12). A total of 180 species were exclusively collected using pitfall traps, 45 species using sticky traps, and 32 using water pan traps. The largest proportion of species was collected using the pitfall traps (41.2%), whereas the water pan traps collected the lowest proportion of species (12.2%) (Figure 4.12). The sticky traps and water pan traps had the largest proportion of species in common (15.6%), while sticky traps and pitfall traps had the least species in common (4.2%).

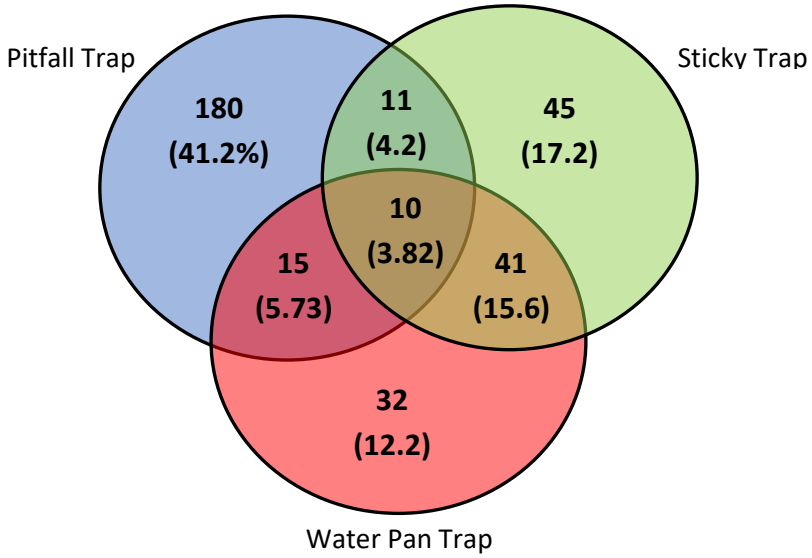

Figure 4.12: Number of species and percentage of total species (in brackets) collected exclusively with each trap and shared in the different traps in the rain-fed sugarcane.

CHAPTER FIVE - DISCUSSION

5.1 Abundance of Insects

The present study collected more than 12 000 insects in rain-fed sugarcane and more than 22 000 in irrigated sugarcane across three sampling periods. These numbers would suggest that there is a vast abundance of insects; however, a reference is needed to make this comparison. Ranjith, Bajya and Ramya (2022) found 1 029 insects in a 1 acre sugarcane field in India after four months of sampling. Innocent and Dayana (2012) recorded 2 660 insects in Indian sugarcane over a period of three months. Banu and Merlin Dayana (2019) recorded 984 insects in one sugarcane field in India across five months of sampling. Ahmed et al. (2004) found a total of 11 720 insects in sugarcane in Pakistan sampled from March to August. These authors used different sampling methods and sampling periods in different geographic locations. Therefore, direct comparisons between the mentioned studies and the present study cannot be made; however, this presents a background for the abundance of insects associated with sugarcane systems in general.

The order Diptera shows the overall highest abundance of individuals (40.836%) belonging to 25 different families in the irrigated sugarcane, with the dominant families being Hybotidae and Drosophilidae. Innocent and Merlin Dayana (2012) assessed insect diversity in sugarcane in South India, and a higher abundance for Diptera (62%) was recorded; however, insects belonged to only four dipteran species, while in the present study, 25 dipteran families were recorded. Factors such as the soil and climate conditions greatly influence the number of insects in sugarcane (Ávila et al., 2023). For instance, Diptera are largely influenced by soil humidity and the quantity of organic matter (De Bruyn et al., 2001) because certain life stages of Diptera are sensitive to dry soil conditions (Menta and Remelli, 2020). Soil characteristics such as high water content in the present irrigated sugarcane system may have provided suitable habitats for Diptera and explain the dominant abundance of Diptera in the irrigated field. The sugarcane floor in the current irrigated field was covered with sugarcane stalks and foliage since this variety is associated with increased lodging. The lodged sugarcane may have provided favourable micro-climate conditions such as shelter, moisture, protection, and food for more insects. Lodging is the dislocation of vertical stems, which generally occurs as a result of wind, rain and soil (Berry et al., 2003). This is a characteristic of high-yielding varieties in wet soil conditions and wet leaf canopy (Singh et al., 2002). The lodging in the current irrigated sugarcane system could be seen as a form of green chop mulching (Showler, 2023) or green cane trash blanketing (Manwaring, Wallace and Weaver, 2018), where sugarcane leaf tissue is left as a mat on the soil surface after harvesting. However, in the case of the present irrigated system, the mulch layer was created during crop growth and provided insects with more micro-habitats, which may have led to increased dipteran abundance in this field.

In the rain-fed sugarcane, insect abundance was dominated by Hymenoptera (29.192%) belonging to 14 insect families. Studies done by de Oliveira et al. (2021) in Brazil and Mahendran et al. (2021) in India also found Hymenoptera as the dominant insect order with 21.9% and 21.41% abundance proportions, respectively. This shows that Hymenoptera is commonly the dominant insect order in sugarcane fields; however, it is not always the norm shown by the irrigated sugarcane in the present study and the cited studies.

5.3 Abundance of Insects Collected Using Different Sampling Methods

Pitfall traps in the irrigated sugarcane were dominated by insects belonging to the order Hymenoptera. This order comprises pollinators, predators, and parasitoids, and they are useful biodiversity indicators since their abundance and richness regulate the diversity of other arthropods (Abhishek et al., 2020). The species from the Formicidae family dominated the insects in the pitfall traps in the irrigated sugarcane and the rain-fed sugarcane in the present study. This agrees with the findings of several other studies on sugarcane. Vanolli et al. (2021) reported that 67% of individuals collected in sugarcane in Brazil using pitfall traps were from this family, and Siqueira et al. (2016) reported a proportion of 55% using pitfall traps in the same country. In a study conducted in Panama, Formicidae (39.41%) was the most abundant family when assessing entomofauna associated with irrigated sugarcane using pitfall and bottle traps (Atencio, Goebel and Miranda, 2019). In a study done by Beje (1998) in La Mercy, KwaZulu-Natal, South Africa, at the SASEX (now SASRI) sugarcane field station, and Leslie (1981), Formicidae was identified as the dominant family in pitfall trap samples. This suggests that the family Formicidae is commonly collected in high abundance from pitfall trap samples in sugarcane.

In the pitfall traps in the current rain-fed sugarcane, two species of ants, *M. junodi* and *P. megacephala,* were also identified. Santos, Naranjo-Guevara and Fernandes (2017) found ants of the genus *Pheidole* were super-abundant when looking at both conventional (53.02%) and organic (32.80%) sugarcane systems in Brazil. In Tanzania, *P. megacephala* was found to be feeding on the eggs and larvae of *E. saccharina* (Waiyaki, 1971), which is the major sugarcane pest in South Africa (Zhou, 2016). This species has also been identified as a control agent of the sugarcane stem borer, *Chilo sacchariphagus* (Goebel et al., 1999); however, this species is linked to the spread of sugarcane smut disease (Grillo Ravelo and Saucedo Castillo, 1985) and may present as a potential agricultural pest due to its mutualistic relationship with Hemiptera (Wetterer, 2007). These factors make *P. megacephala* unsuitable as a biocontrol agent in sugarcane; however, they still play a role in the predator/prey population dynamics of the sugarcane system, and their abundance is important to take note of. Overall, as expected, mostly ground-dwelling arthropods were caught using the pitfall traps, making this sampling method best for studies aimed at ground-dwelling insects such as ants.

In the sticky traps in the irrigated sugarcane system, the order Diptera showed the highest abundance. The large number of individuals collected from the family Hybotidae contributed to Diptera being the dominant order. Hybotidae are small predaceous flies which spend the majority of their time at the tips of leaves, waiting to pounce on prey (Jaume-Schinkel et al., 2022). This explains why this family was so common in the sticky trap, as the placement of these traps was level with the sugarcane canopy, in contact with the tips of the sugarcane leaves. A study conducted by Kumar and Pal (2022) in the Terai region in Northern West Bengal to determine the arthropod diversity in sugarcane reported that coleopteran species were the most dominant (36%), followed by hemipterans when sampling using sweep nets, light traps, and yellow sticky traps. Coleoptera was, however, not the dominant order in sticky trap samples of the present irrigated sugarcane system and showed an abundance proportion of 0.511% in this sampling method.

In the sticky trap samples from the rain-fed sugarcane, the dominant order was Diptera, with the major families being Syrphidae and Tephritidae. Hoverflies or flower flies (Diptera: Syprhidae) are foragers in crops, providing ecosystem services such as pollination and biological control (Li, Wyckhuys and Wu, 2023). The larvae of members of this family are predators of primary aphids as well as other agricultural pests, including thrips, spider mites and lepidopteran larvae (Pekas et al., 2020; Li, Wyckhuys and Wu, 2023). *Eupeodes*, a genus in the Syrphidae family, has been shown to aid in the reduction of the populations of sugarcane aphids, which is an important agricultural pest in Africa (Dlamini and Mhlongo, 2022).

The water pan trap samples in irrigated sugarcane showed insect individuals belonging to eight arthropod orders and 103 morphospecies, with Hemiptera (44.188%) being the dominant order. The water pan traps in the rain-fed sugarcane trapped insects belonging to eight insect orders, with the most insects belonging to the order Diptera (44.947%). Dolichopodidae contributed to this high abundance of Diptera, with a proportion of 21.343%. Dolichopodid species, such as *Chrysotus gramineus,* are commonly sampled using pan traps and are found in most biotopes, creating dense populations (Gelbič and Olejníček, 2011).

5.4 Insect Diversity and Species Richness Across Three Sampling Periods

Diversity, richness, dominance, and evenness indices are important elements of diversity studies and provide astute quantitative data. In the irrigated sugarcane, there was no significant difference in the species richness and diversity indices between the three sampling s in the pitfall trap samples. In contrast, in the sticky trap samples, there was a significant difference in the species richness and diversity indices between the three sampling periods.

Statistical comparisons using the Kruskal-Wallis test were important in the present study as it highlighted the significant changes in diversity indices across the three sampling periods in the sugarcane systems for different sampling methods.

The Shannon-Wiener and the Simpson index are the two most common indices used to measure biodiversity as they incorporate species richness and evenness into one number (Mendes et al., 2008). A higher Shannon-Wiener value indicates greater diversity (Ghosh and Biswas, 2015); therefore, the highest diversity of insects for the sticky traps was recorded during sampling period three, with the median for the Shannon-Wiener index being 3.189 in the irrigated sugarcane. The Shannon-Wiener diversity index is primarily influenced by the presence of rare species and highlights the species richness factor of diversity, whereas the Simpson diversity index is primarily influenced by the presence of dominant species and highlights the evenness factor of diversity (Morris et al., 2014, Roswell, Dushoff and Winfree, 2021, de Vries, Kraak and Verdonschot, 2023). This trend is evidently shown in the sticky trap samples as increased Shannon-Wiener values are seen with increased species richness values in sampling period three in the irrigated sugarcane. Similarly, increased Simpson's index values are observed with increased evenness. A higher median for Pielou's evenness is observed in sampling period two in the sticky traps. This indicated that the individuals were more evenly spread among the species in the sticky trap samples in sampling period two compared to the other sampling periods (Morris et al., 2014).

The meteorological data for the irrigated sugarcane field shows that sampling period three overall had higher temperatures compared to the other sampling periods. Among other biotic and abiotic factors, insect diversity and abundance are largely influenced by temperature and rainfall. Catching probability increases with higher temperatures and dry weather due to the increasing activity of arthropods (Fürst et al., 2023). Therefore, a higher number of species were collected in the sticky traps during this sampling period, leading to higher diversity values in the irrigated sugarcane system.

In the rain-fed sugarcane, there was a significant difference in all the diversity indices between the three sampling periods in the pitfall and sticky trap samples. The median Shannon-Wiener index was highest during sampling period two in the pitfall traps, sampling period three in the sticky traps and sampling period two in the water pan traps.

Sampling period three in the rain-fed sugarcane was also associated with overall higher temperatures which could explain the difference in diversity between the sampling periods. In the water pan trap samples in this field, there was a significant difference in the Shannon-Wiener index, Simpson's index and Species richness, but not in Pielou's evenness between the three sampling periods.

Studies on arthropods in sugarcane focused more on the seasonal abundance of arthropods as opposed to the seasonal diversity of insects. For example, a previous study in South African sugarcane reported a higher abundance of arthropods during low temperature, rainfall and sunshine hours (Leslie, 1981). Kaur and Sangha (2020), reported higher populations of soil arthropods in post monsoon (autumn) seasons and lower populations in summer in India. This occurred as soil arthropods migrate to the deeper soil profile layers in response to rising temperatures. Abundance does have an influence on diversity indices, but it is not the primary factor indicating diversity. Therefore, information from the previous studies (Leslie, 1981; Kaur and Sangha, 2020) cannot be compared to the indices in the present study since a higher abundance of insects does not necessarily equate to a higher diversity. However, this still presents a picture of insect responses to changes in ambient temperatures in sugarcane and can relate to the diversity observed in the present sugarcane system across the different sampling periods. Seasonal insect abundance was not within the scope of the present study; however, interestingly, the cited studies (Leslie, 1981; Kaur and Sangha, 2020) report a decrease in insect abundance during warmer temperatures, and the present study shows an increase in diversity indices during warmer temperatures. This presents opportunities for further studies on insect diversity and abundance across different seasons.

5.5 Insect Diversity and Community Composition Collected Using Different Sampling Methods

There was a significant difference in the diversity of insects collected using the three sampling methods in the irrigated sugarcane. The trends of the diversity indices were similar across all comparisons, with the pitfall traps showing the highest values of indices and species richness and the water pan traps showing the lowest. There was a significant difference in the Shannon-Wiener index, Pielou's evenness and species richness between the sampling methods in the rain-fed sugarcane. The highest median for the Shannon-Wiener index shows that the pitfall traps collected the highest diversity of insects. Singh et al. (2023) conducted an insect diversity study in three sugarcane fields where they found the highest Shannon diversity index value to be 3.10 in one field, followed by 3.04 and 3.03 in the other fields. This study calculated a single value for the Shannon-Diversity index, whereas the present study is reporting the medians. The median gives an accurate representation of the typical value in a dataset. Therefore, the values from the study done by Singh et al. (2023) can be considered in relation to the medians in the present study and show higher values of the Shannon-Wiener index in all the sampling methods in the rain-fed sugarcane. In a study done by Kaur and Sangha (2020), at a one inch soil depth, soil arthropods showed a maximum Shannon-Wiener index of 0.67 in one field and 0.64 in another field. The cited study differs from the present as the authors included spiders in the calculation of the indices, and arthropods were collected from soil samples. The value for Shannon-Wiener in the present study was, however, still higher, and the cited study provides a reference for soil insects in sugarcane.

Arthropod sampling methods have different trapping efficiency, which is subject to the arthropod's biology, behaviour, and trap design (Montgomery et al., 2021). Sticky and pan traps are typically used to sample flying insects, especially in agricultural systems (Saunders and Luck, 2013; McCravy, 2018; Kent, Peele and Sherry, 2019). This explains the largest proportion of shared species between the two traps. Although both traps are efficient in catching flying insects, the number of species shared between the two sampling methods may be influenced by the variation in trap design (Manning, Perry and Bahlai, 2022), species specialisation (Umair Sial et al., 2022), and placement in the sugarcane field. Yellow sticky traps are also used to sample sucking insects such as aphids, whiteflies, thrips, and leafhoppers (Umair Sial et al., 2022).

Only 7.52% of the species in the irrigated sugarcane and 3.82% of species in the rain-fed sugarcane were common in all three trap types, with the pitfall and water pan traps having the least species in common in both fields. This low similarity is because pitfall traps are commonly used to sample ground-dwelling arthropods, such as carabid beetles (McCravy, 2018), while water pan traps are used to monitor mainly flying insects in the orders Diptera and Hymenoptera but are also employed to sample thrips and some beetles and grasshoppers (Montgomery et al., 2021). The pitfall and sticky traps in the irrigated sugarcane had 15 species in common. This may be explained as some soil insects spend their lives migrating between the ground and above ground portions of plants (Wolters, 2001). For instance, many early stages of dipteran families are first found in the soil, particularly soil high in organic matter, which includes families Tipulidae, Dolichopodidae, Mycetophildae and many others (McColloch and Hayes, 1922). Therefore, some insects were observed in all three trap types.

The NMDS indicated a significant difference in the community composition of insects collected using the three different sampling methods in the rain-fed sugarcane as well as in the irrigated sugarcane. This information correlates with the information in the Venn diagrams, where the large proportions of individuals were unique to one trap type. A study comparing insect sampling methods was conducted by González, Salvo and Valladares (2020), where a pair of methods were deemed complimentary when the composition of insect communities collected with each trap type was different, thereby employing both methods would yield a better representation of the samples. From this information, the sampling methods in the current study can be deemed as complimentary as the arthropod community composition differed across sampling methods. The results from González, Salvo and Valladares (2020) and the current study showed the importance of employing a combination of sampling methods to study diverse arthropod communities.

CHAPTER SIX - CONCLUSION AND RECOMMENDATIONS

This study provides recent baseline data on the diversity and abundance of insects in conventional sugarcane based on two sugarcane fields in KwaZulu-Natal, which were sampled using three sampling methods. The results from this study contribute to the understanding of the insect communities associated with sugarcane cultivation in the two regions of KwaZulu-Natal by providing a list of insect families found in sugarcane. It is important to note that the aim was not to compare the two sugarcane systems but rather to report on the findings in each separately since they were not in the same geographic location. Observations in this study show that the three sampling methods collected a vast diversity of insects in sugarcane habitats and emphasize the need for employing diverse sampling techniques to comprehensively assess arthropod diversity in such environments. Furthermore, the sampling methods used in the current study were time and labour efficient and would be recommended for use in sugarcane insect collection studies where these factors are some of the constraints. The sampling methods used in the current study were only passive sampling methods, which means sedentary insects were less likely to be sampled. Further studies using a combination of passive and active sampling methods could provide more insight into insect communities in sugarcane in KwaZulu-Natal. The sampling methods used complemented each other as each collected different insect communities. Pitfall traps proved to be a good sampling method for Hymenoptera and Coleoptera in both sugarcane fields. Sticky traps were effective for sampling the orders Diptera and Hemiptera in both fields. Water pan traps favoured the order Diptera, capturing the highest number of Diptera in the rain-fed field and the second highest number of Diptera in the irrigated field. The statistical analysis in this study focused on calculating the diversity of insects for each sampling method, as this was aligned with the aims of this study. Further studies can explore calculating diversity indices for each insect order, as this would facilitate comparisons with previous studies. Although the Shannon-Wiener and Simpson indices both measure diversity, reporting on both indices gave a more comprehensive representation of the diversity in the sugarcane systems. Further research on the continued monitoring of insect populations across different temporal scales within sugarcane will also provide valuable insights into long-term trends and potential insect responses to environmental changes and farming practices.

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