



Assessment of the pest status, parasitism levels and efficacy of chemical control of *Agrotis segetum* (Lepidoptera: Noctuidae)

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Dissertation accepted in fulfilment of the requirements for the degree *Master of Science in Environmental Sciences with Integrated Pest Management* at the North-West University

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Graduation: October 2025

ACKNOWLEDGEMENTS

“Now all glory to God, who is able, through his mighty power at work within us, to accomplish infinitely more than we might ask or think.” Ephesians 3:20. Thank you Lord, for reminding me that You are my strength and nothing is impossible with You. You provided for me throughout this journey and drew me closer to You.

I want to extend my deepest gratitude to my supervisor, Prof. Johnnie van den Berg, who took a chance on an agricultural student with little background of entomology and pest management. You have not only equipped me with knowledge regarding our field of study, but also important life skills and ways of thinking. Each story you shared showed your passion for learning new things everyday which has installed curiosity within me.

To my co-supervisor, Prof. Hannalene du Plessis, thank you for always thinking ten steps ahead. Your guidance with regards to the set-up of experiments, rearing of the cutworms and smart writing helped me to master this task.

I want to express thanks to the following people at M campus: Leonie, Moses, Bernhard, Tayla, Simoné, Bianca, who either assisted with cleaning, making plans for experiments, cutworm rearing or statistical analysis. A special thanks to Regardt, who was brave enough to face most of the cutworm sampling trips with me. You made each trip interesting and every km worthwhile. Another special thanks to Mishcá, for every encouraging word. Our friendship has been through many phases of life- now we add another.

No number of thank you's will amount to my thankfulness for the support my family has shown throughout these past two years. Thank you, Mom and Dad, for your prayers and motivating speeches when I felt like there was no end in sight. Dad, your perseverance to complete your Masters encouraged me to work every day. Thank you Luanel, for offering up your school holidays and weekends to assist me.

To my boyfriend, Louwrens, you have been my greatest supporter from the start. Thank you for always believing in me. You took every phone call when I was at my wit's end and helped me think of strategies. You kept me calm through the storm. I am blessed with you by my side.

A final word of thanks to GrainSA and the Maize Trust who provided financial support for this study. I am thankful for every encounter with specialists of the industry, who taught me valuable lessons and gave me insight into the field that is Integrated Pest Management. My hope is that I return the favour by sharing what I have learned in my study and contribute to the broader understanding of cutworms.

DECLARATIONS

DECLARATION BY THE CANDIDATE

I, Zunel van Eeden, declare that the work presented in this MSc thesis is my own work, that it has not been submitted for any degree or examination at any other University and that all the sources I have used or cited have been acknowledged by the complete reference.



Signature:

Date: 20/05/2025

DECLARATION AND APPROVAL BY SUPERVISORS

We declare that the work presented in this thesis was carried out by the candidate under our supervision and we approve this submission.

Prof MJ du Plessis

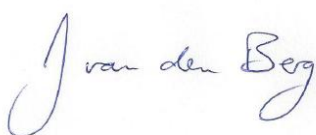
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ABSTRACT

The common cutworm, *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae) is a pest of maize seedlings. During the past four planting seasons in South Africa, cutworms has resurfaced as a threat to maize production. No information existed on the economic impact of this pest in South Africa and since the chemical control of *A. segetum* has largely been described as inefficient, the need existed to investigate factors that may improve its management and affect insecticide efficacy. The overall objective is to improve our understanding of the influence of biotic and abiotic factors in controlling the population of *A. segetum*. The first objective of this study was therefore to conduct a survey among grain farmers to assess the economic impact of this pest, and to determine the possible association between cultivation practices, weed management strategies, chemical control approaches used and the severity of pest infestations. The survey revealed that the economic loss due to cutworm infestation of maize, soybean and sunflower amounted to R44.875 million on the 150 363 ha represented in this survey. Information provided by survey respondents indicated tendencies of higher pest infestation in no-till production systems, and in crop fields where weed control occurred less than three weeks prior to planting. Results further showed that the most affected crop was maize, and it is reported for the first time that this pest also has a significant economic impact on soybean production. Survey results showed that 97.0% of farmers applied insecticides for cutworm control and highlighted that applications were largely not done in accordance with label recommendations. Although pesticide label instructions indicate that insecticides must be applied at high water volumes (300 L/ha) and when the soil is damp, general tendencies recorded from questionnaire responses was that lower than the prescribed water volumes were used and that poor insecticide efficacy was experienced when products were applied under dry soil conditions. The second objective of this study was to establish the natural occurrence and diversity of biological control agents of *A. segetum* within the country's maize production regions. Therefore, cutworm larvae were collected from 14 localities and reared in a laboratory until the emergence of a biocontrol agent, or until they died or moths emerged. Overall cutworm mortality due to parasitism was high (43.6%). Three parasitoid species were identified, i.e., *Macrocentrus collaris* (Hymenoptera: Braconidae), *Gonia bimaculata* (Diptera: Tachinidae) and *Periscepsia carbonaria* (Diptera: Tachinidae). Cutworm mortality due to entomopathogenic viruses, nematodes and fungi were 26.1, 15.7 and 3.2% respectively. Since pesticide label instructions indicate that insecticides must be applied onto soil when the soil is damp, the third objective of this study was to assess the influence of different soil moisture levels on larval behaviour and to relate possible differences in larval behaviour to differences in the efficacy of insecticides. Third- (L3) and 5th-instar (L5) larvae were exposed to field-like soil moisture conditions (dry, damp or wet) in a laboratory and greenhouse setting. Results showed that the behaviour of L3 and L5 larvae differed under different soil moisture conditions. While L3 larvae showed limited movement into the soil, most of the L5 larvae moved to a depth of < 22 mm into the soil, irrespective of soil moisture level. This confirmed the photopositive behaviour of L3 larvae, and tunnelling behaviour of L5 larvae, and showed that soil moisture conditions influence larval behaviour, which may have an indirect effect on insecticide efficacy.

Key words: biological control agents, chemical control, cutworm, cultivation, insecticides, maize seedlings, soil moisture, weeds.

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CHAPTER 1: Literature review and aims of the study

1.1 Introduction

Maize (*Zea mays*), a monoic annual crop of the Poaceae family, is produced worldwide (Erenstein *et al.*, 2022) (Figure 1.1). Maize is among the top three grain crops in the world, the others being wheat (*Triticum aestivum*) and rice (*Oryza sativa*) (Saeed & Saeed, 2020). Apart from humans consuming maize as a source of food, maize is also used as feed for livestock and is the main ingredient in industrial applications such as oils, beverages and biofuels (Wallington *et al.*, 2012; Kaul *et al.*, 2019).



Figure 1.1. Maize produced as a monocrop in KwaZulu-Natal, South Africa (Photo by Z. van Eeden).

In Sub-Saharan Africa (SSA), maize is the most important crop with approximately 40 million hectares cultivated by smallholder farmers (Prasanna *et al.*, 2020). Over 90% of the maize produced in Africa is white maize grown for human consumption. Yellow maize is planted by commercial farmers for industrial use and animal feed (Gouse *et al.* 2005; Ekpa *et al.* 2018). According to Abate *et al.* (2017), maize in SSA provides for the largest per capita calorie consumption of any staple.

The demand for maize as food, animal feed, and industrial raw material is continuously increasing (Badu-Apraku & Fakorede, 2017). By 2050, SSA's demand for maize will

have tripled, which creates an opportunity to reduce the high levels of poverty experienced in developing countries (Cock *et al.*, 2017; Kumela *et al.*, 2019). According to FAO (2021), the average maize production across SSA countries in 2019 was 1.7 metric tons per hectare, which is less than one-third of the global average. These low yields are ascribed to poor soil fertility, drought, insect pests, diseases, weeds, and limited access to fertilizer and improved seed (Gianessi, 2014; Badu-Apraku & Fakorede, 2017; Prasanna *et al.*, 2020; Prasanna *et al.*, 2021).

Insect pests, especially lepidopteran species of the Noctuidae family, threaten maize production in SSA due to their capacity to cause yield losses (Bell & McGeoch, 1996; Van den Berg *et al.*, 2014). Cosmopolitan and African lepidopteran pests are of greater importance as pest species in South Africa than endemic species (Bell & McGeoch, 1996). The maize pest complex in South Africa consists of pests that attack both the above- and belowground parts of plants (Drinkwater, 2017). The most important lepidopteran pests included in the aboveground pest complex are the maize stalk borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), the Spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), African bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Bell & McGeoch, 1996; Calatayud *et al.*, 2014; Erasmus & Van den Berg, 2014; Van den Berg *et al.*, 2014, Drinkwater 2017, Van den Berg *et al.*, 2021).

The most important soil insect pests include the black maize beetle, *Heteronychus arator* (Fabricius) (Coleoptera: Scarabaeidae), false wire worms, *Somaticus* spp. (Coleoptera: Tenebrionidae), and cutworms, *Agrotis* spp. (Lepidoptera: Noctuidae) (Drinkwater, 1980; Drinkwater, 1982; Drinkwater, 1989; Drinkwater *et al.*, 1990; Drinkwater & Janse Van Rensburg, 1992; Drinkwater, 2017). Several cutworm species have been reported as pests of maize and other crops in South Africa. These are *Agrotis subalba* (Walker), the grey cutworm, *Agrotis ipsilon* (Hüfnagel), the black cutworm and *Agrotis longidentifera* (Hampson), the brown cutworm (Bell & McGeoch, 1996; Du Plessis, 2000; Drinkwater, 2017). However, the common cutworm, *Agrotis segetum* (Denis & Schiffermüller) is the species that is most frequently encountered over the entire South African maize production region (Drinkwater, 1980; Du Plessis, 2000; Van den Berg *et al.*, 2014; Drinkwater, 2017).

Although cutworms constitute to a relatively low proportion of lepidopteran pest species worldwide, cutworms have a higher pest status than defoliators in South Africa (Bell & McGeoch, 1996). The pest status of cutworm shifted from the seventh most important pest species in 1983 (Moran, 1983) to the second most important in 1996 (Bell & McGeoch, 1996). Since then, the pest status of cutworms on maize in South Africa has not been re-evaluated, however, incidence reports have increased over the last four years (2021-2024) (GrainSA, 2023; GrainSA 2024a; GrainSA, 2024b; FW, 2024; FW, 2025). However, no scientific research has been done on this pest in South Africa since the publication by Drinkwater (2017).

1.2 The common cutworm, *Agrotis segetum*

1.2.1 Distribution

Agrotis (Ochsenheimer) (Lepidoptera: Noctuidae) is a cosmopolitan genus with approximately 300 species (Medeiros, 2019). The most important *Agrotis* species are *A. ipsilon* (Blair, 1976; Showers, 1987; Frank & Shrewsbury, 2004) and *A. segetum* (Blair, 1976; Bowden *et al.*, 1983; Chandel *et al.*, 2019; Chandel *et al.*, 2022). *Agrotis ipsilon* occurs on all continents, while *A. segetum* is distributed throughout Africa, Europe, the Middle East, and Southeast Asia (Figure 1.2) (Blair, 1976; Bowden *et al.*, 1983; Hülbert & Süss, 1983; Esbjerg & Sigsgaard, 2014; Rodingpuia & Lalthanzara, 2021). In South Africa, *A. segetum* is the only species of economic importance due to its common occurrence throughout the Highveld region and the damage it causes to maize seedlings (Drinkwater, 1980; Du Plessis, 2000; Van den Berg *et al.*, 2014; Drinkwater, 2017).

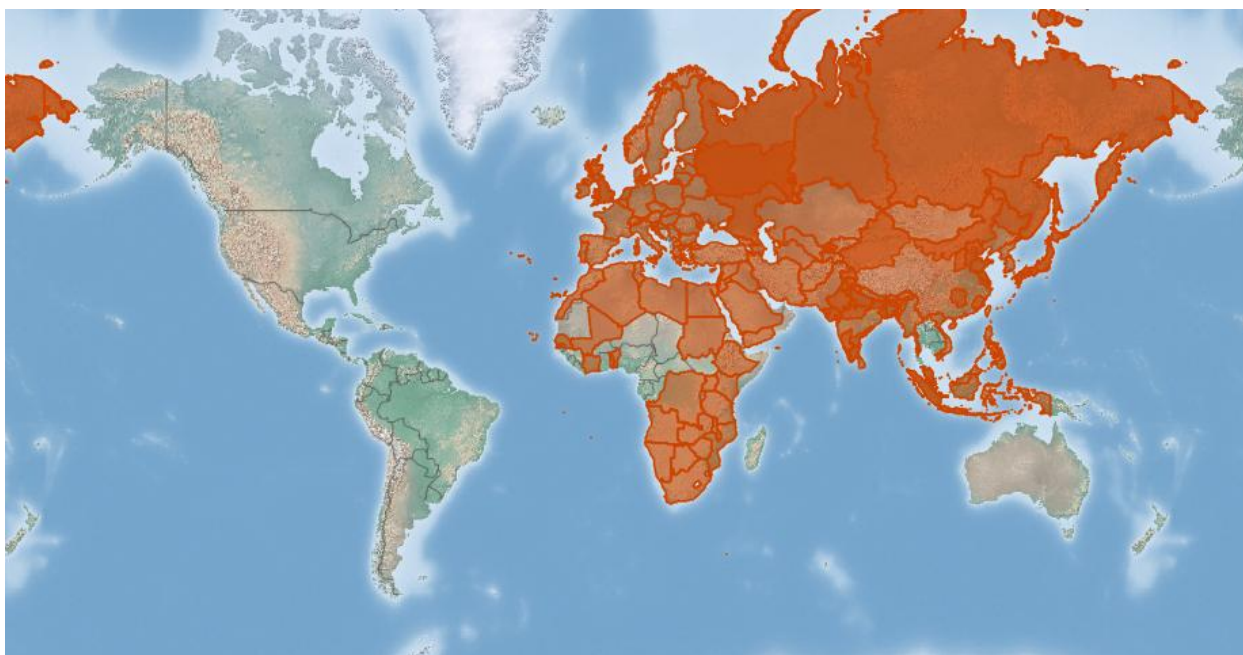


Figure 1.2. World map indicating the distribution of *Agrotis segetum* (Source: CABI, 2021).

1.3 Life stages, biology and ecology of *Agrotis segetum*

The duration of the *A. segetum* life cycle is highly dependent on temperature (Blair, 1976; Bowden *et al.*, 1983; Sherrod *et al.*, 1979; Esbjerg & Sigsgaard, 2019) and therefore depends on where in the world it occurs (Blair, 1975; Bowden *et al.*, 1983; Bues *et al.*, 1989). Blair (1976) studied the mean development period from egg to adult at seven constant temperatures and reported 187 days at 15 °C, 99 days at 18 °C, 61 days at 21 °C, 55 days at 24 °C, 47 days at 27 °C, 49 days at 30 °C and 41 days at 33°C. During the summer season in South Africa, the pest's life cycle is completed in approximately 50 days (Du Plessis, 2000).

1.3.1 Eggs

Newly laid eggs are whitish yellow and become darker as they develop (Chandel *et al.*, 2019) (Figure 1.3). The eggs are dome-shaped, with noticeably sculpted longitudinal ridges and measures 0.54-0.63 mm high with a diameter of 0.51-0.62 mm. The egg stage lasts approximately three to four days (Drinkwater, 2017; Chandel *et al.*, 2019). Nearly 1000 eggs per female moth can be laid, singly or in batches (Drinkwater, 1980; Du Plessis, 2000; Drinkwater, 2017). *Agrotis segetum* eggs are able to withstand moist and cold conditions (Esbjerg, 1992), however, the optimal temperatures for egg production in Denmark are 22 to 23 °C (Esbjerg & Sigsgaard,

2019). Blair (1976) reported optimal egg development of four days between 27 and 33 °C in Zimbabwe. The eggs are usually laid on the soil surface or between the upper soil particles. However, if the soil is wet, large numbers of eggs are laid on the lower portions of vertical objects such as plant stems (Esbjerg & Lauritzen, 2010).



Figure 1.3. Eggs of *Agrotis segetum* laid on *Portulaca oleracea* (Photo by Z. van Eeden).

1.3.2 Larvae

Agrotis segetum larvae are dark grey to brown in colour and hairless, waxy and smooth (Drinkwater, 1980; Du Plessis, 2000) (Figure 1.4). When disturbed, larvae curl into a distinctive “C” shape (Figure 1.5). The larval stage consists of six instars (Blair, 1976; Chandel *et al.*, 2019; Esbjerg & Sigsgaard, 2019) although the development to a ninth instar was reported by Santos and Shields (1998). Newly hatched larvae (L1) are approximately 3 mm long. Larvae in the 2nd-instar (L2) range from 4.4 mm to 5 mm in length, 3rd-instars (L3) are approximately 7 mm long, whereas 4th-, 5th- and 6th-instars (L4, L5, L6) are approximately 14-18 mm, 21-25 mm, and 37-39 mm long, respectively (Chandel *et al.*, 2019).



Figure 1.4. Cutworm larva (Photo by Z. van Eeden).



Figure 1.5. Cutworm larva curling into a characteristic "C" shape (Photo by Z. van Eeden).

The 1st- and 2nd-instars are photopositive and hide under and feed on leaves near the soil surface (Blair, 1975; Drinkwater, 1980; Drinkwater & Janse Van Rensburg, 1992; Du Plessis, 2000, Sidhu *et al.*, 2019). The larvae begin to exhibit a negative response to light a few days after their second molt (Drinkwater, 2017). The switch from positive

to photo negative behaviour occurs between the fourth and fifth day of the 3rd-instar (Blair, 1975). The physiological mechanism for the change in phototaxis from positive to negative is not known (Blair, 1975).

Blair (1975) reported the duration of each instar of *A. segetum* in South Africa, at a constant temperature of 24 °C as follows: 1st- and 2nd-instars is four days, six days in the 3rd-instar, seven days in both 4th- and 5th-instars and 11 days in the 6th-instar. Identification of different larval instars can be made by means of measuring head capsule widths (Reese *et al.*, 1972, Sherrod *et al.*, 1979). Head capsule widths for the different instars reported by Blair (1976) and Chandel *et al.* (2019) are indicated in Table 1.

Table 1.1. Range of the head capsule widths of different *Agrotis segetum* larval instars measured in Blair (1976) and Chandel *et al.* (2019).

Instar	Range (Mean $\pm$ SE) (mm)	Range (Mean $\pm$ SE) (mm)
	Blair (1976)	Chandel <i>et al.</i> (2019)
1	0.26-0.36 (0.32 $\pm$ 0.01)	0.30-0.35 (0.33 $\pm$ 0.02)
2	0.48-0.59 (0.54 $\pm$ 0.03)	0.46-0.50 (0.47 $\pm$ 0.03)
3	0.72-0.92 (0.82 $\pm$ 0.04)	0.68-0.72 (0.70 $\pm$ 0.02)
4	1.04-1.68 (1.34 $\pm$ 0.09)	1.15-1.27 (1.17 $\pm$ 0.03)
5	1.76-2.44 (2.00 $\pm$ 0.11)	1.70-1.81 (1.73 $\pm$ 0.04)
6	2.32-3.36 (2.91 $\pm$ 0.21)	2.41-2.62 (2.50 $\pm$ 0.06)

1.3.3 Pupae

The pupa has a diameter of 4.7-5.8 mm and can reach a length of approximately 15-19 mm (Figure 1.6). The pupal stage may range from 14 to 17 days, depending on temperature (Blair, 1976; Capinera, 2022), and accounts for approximately 40% of the duration of the life cycle (Chandel *et al.*, 2019). Pupae appear dark brown or mahogany in colour and appear almost black in colour before the moths emerge (Chandel *et al.*, 2019). The location of the genital opening, which is located on the 9th abdominal segment in males and on the 8th abdominal segment in females, can be used to distinguish between male and female pupae. Anal openings are found on the tenth abdominal segment in both sexes (Chandel *et al.*, 2022). Female pupae have a shorter

developmental period than male pupae (Blair, 1976). *Agrotis segetum* larvae form earthen cells and pupate inside them beneath the soil surface (Drinkwater, 1980; Du Plessis, 2000; Chandel *et al.*, 2019; Capinera, 2022).



Figure 1.6. Cutworm pupa (Photo by Z. van Eeden).

1.3.4 Moths

With a wingspan of 38–42 mm, *A. segetum* moths are quite large (Chandel *et al.*, 2019). The forewings of male moths are often pale in colour, while the forewings of female moths are typically greyish brown (Drinkwater, 1980; Du Plessis, 2000). *Agrotis* spp. moths can easily be identified using external characters. The legs of *Agrotis* spp. moths have a large number of conspicuous spines along the tibiae and the tarsal segments of all three pairs of legs (Zimmerman, 1958). Specific marks on the wings of moths are also used to distinguish between *Agrotis* species (Drinkwater, 1980; Du Plessis, 2000; Chandel *et al.*, 2019). A semi-circular kidney-shaped marking present on the forewing (Figure 1.7). Moths of *A. segetum* show sexual dimorphism and sexes can be determined by the antennae. Females have filament shaped (setaceous) antennae and the males' antennae are feathery (bipectinate) (Du Plessis, 2000).



Figure 1.7. Female *Agrotis segetum* moth with kidney bean markings on the forewings (Photo by Z. van Eeden).

1.3.5 Overwintering

The number of *A. segetum* generations per year is regulated by the climatic conditions where the pest occurs (Sherrod *et al.*, 1979; Bowden *et al.*, 1983). Bues *et al.* (1989) reported that latitude had an influence on the number of generations produced. Druzhelyubova and Makarova (1963), cited in Bues *et al.* (1989), indicated that in the northern part of the pest's distribution range (lat. 60°), the species produce one generation, two generations between 50° and 45° and partial to complete three or more generations below 45°. In Zimbabwe, Blair (1982) reported no interruption in moth captures during the year, indicating that several generations of *A. segetum* may occur in the Southern hemisphere.

Agrotis larvae overwinter in the top 5 cm of the soil in a quiescent state as fully-grown larvae or pre-pupae (Bowden *et al.*, 1983). In South Africa, overwintering larvae arise from eggs that are laid during autumn and winter (Drinkwater, 1980; Du Plessis, 2000; Drinkwater, 2017). Larvae of different sizes overwinter beneath the soil surface until the start of spring (Drinkwater, 1980; Du Plessis, 2000). Overwintering larvae form pupa in pupa cells during August and September, from which the first-generation moths of the new planting season emerge (Drinkwater, 1980; Du Plessis, 2000). In

other parts of the world, more than one generation of *A. segetum* per year has been reported. In Poland, *A. segetum* produces a first generation, though there is a possibility for an incomplete second generation (Jakubowska & Bocianowski, 2013). Other observations showed that in the northern hemisphere, *A. segetum* has a second partial flight in Denmark (Esbjerg *et al.*, 1980) and in England during 1971 due to higher-than-normal summer temperatures (Bowden *et al.*, 1983). This confirms that the second generation is incomplete. Although there may be more than one generation per year in South Africa (Drinkwater, 1980), the overwintering larvae and larvae from first-generation moths produced in spring cause the greatest damage (Drinkwater, 1980).

1.3.6 Host plants

Cutworms are serious polyphagous pests of many wild plants, weeds, agricultural and horticultural crops, feeding on a wide range of plant species in larval various growth stages (El-Salamouny *et al.*, 2003; Garnis & Dabrowski, 2008; Esbjerg & Lauritzen, 2010; Jakubowska & Bocianowski, 2013; Chandel *et al.*, 2022). A list of host plants of *A. segetum* is provided in Table 2. Cutworms (*Agrotis* spp.) damage different crops in different parts of the world. In India, vegetables attacked by *Agrotis* spp. include radish (*Raphanus sativus*), turnip (*Brassica rapa*), peas (*Pisum sativum*), beans (*Phaseolus vulgaris*), okra (*Abelmoschus esculentus*), beetroot (*Beta vulgaris*), onion (*Allium cepa*), brinjal (*Solanum melongena*), tomato (*Solanum lycopersicum*), capsicum (*Capsicum*), spinach (*Spinacia oleracea*) and fenugreek (*Trigonella foenum-graecum*) (Chandel *et al.*, 2022). In Poland, close-planted crops such as carrots (*Daucus carota*), lettuce (*Lactuca sativa*), celeriac (*Apium graveolens* var. *rapaceum*) and beet are seriously damaged by *A. segetum*, as well as root crops such as potatoes (*Solanum tuberosum*), turnip and parsnip (*Pastinaca sativa*) (Garnis & Dabrowski, 2008). Apart from maize, other economically important plant species damaged by *A. segetum* in South Africa are cotton (*Gossypium hirsutum*), sunflower (*Helianthus annuus*), potato (*Solanum tuberosum*), sorghum (*Sorghum bicolor*), lucerne (*Medicago sativa*), tobacco (*Nicotiana tabacum*) and wheat (*Triticum aestivum*) (Drinkwater, 2017).

Table 1.2. Cultivated and weedy host plants of *Agrotis segetum* (CABI, 2021). *Weed species.

Scientific name	Popular name
<i>Abelmoschus esculentus</i>	okra
<i>Allium porrum</i>	leek
<i>Allium sphaerocephalon</i>	roundhead garlic
<i>Amaranthus</i> *	amaranth
<i>Anethum graveolens</i>	dill
<i>Apium graveolens</i> var. <i>dulce</i>	celery
<i>Arachis hypogaea</i>	groundnut
<i>Asparagus officinalis</i>	asparagus
<i>Atropa belladonna</i>	Deadly nightshade
<i>Avena sativa</i>	oats
<i>Beta vulgaris</i> var. <i>saccharifera</i>	sugarbeet
<i>Boehmeria nivea</i>	ramie
<i>Brassica juncea</i> var. <i>juncea</i>	Indian mustard
<i>Brassica napus</i> var. <i>napobrassica</i>	swede
<i>Brassica napus</i> var. <i>napus</i>	rape
<i>Brassica oleracea</i> var. <i>botrytis</i>	cauliflower
<i>Brassica oleracea</i> var. <i>capitata</i>	cabbage
<i>Brassica rapa</i> subsp. <i>chinensis</i>	chinese cabbage
<i>Brassica rapa</i> subsp. <i>oleifera</i>	turnip rape
<i>Camellia sinensis</i>	tea
<i>Cannabis sativa</i> *	hemp
<i>Capsicum</i>	peppers
<i>Capsicum annuum</i>	Bell pepper
<i>Carum carvi</i>	caraway
<i>Chrysanthemum</i>	daisy
<i>Cicer arietinum</i>	chickpea
<i>Cichorium endivia</i>	endives
<i>Coffea arabica</i>	Arabica coffee
<i>Cucumis melo</i>	melon
<i>Cucurbita pepo</i>	marrow
<i>Cyperus esculentus</i>	Yellow nutsedge
<i>Daucus carota</i>	carrot
<i>Dianthus caryophyllus</i>	carnation
<i>Foeniculum vulgare</i>	fennel
<i>Fragaria vesca</i>	Wild strawberry
<i>Freesia refracta</i>	Common freesia
<i>Gladiolus hybrids</i>	Sword lily
<i>Glycine max</i>	soyabean
<i>Gossypium hirsutum</i>	Bourbon cotton
<i>Guizotia abyssinica</i>	niger
<i>Helianthus annuus</i>	sunflower
<i>Hevea brasiliensis</i>	rubber
<i>Hibiscus cannabinus</i>	kenaf
<i>Hordeum vulgare</i>	barley
<i>Ipomoea batatas</i>	sweet potato

<i>Lactuca sativa</i>	lettuce
<i>Linum</i> sp.	flax
<i>Lupinus luteus</i>	Yellow lupin
<i>Malus sylvestris</i>	Crab-apple tree
<i>Medicago sativa</i>	lucerne
<i>Mentha</i>	mints
<i>Nicotiana rustica</i>	Wild tobacco
<i>Nicotiana tabacum</i>	tobacco
<i>Oryza sativa</i>	rice
<i>Paeonia officinalis</i>	Common paeony
<i>Papaver somniferum</i>	Opium poppy
<i>Petroselinum crispum</i>	parsley
<i>Phyllostachys</i>	Japanese bamboo
<i>Picea sitchensis</i>	Sitka spruce
<i>Pinus sylvestris</i>	Scots pine
<i>Punica granatum</i>	pomegranate
<i>Raphanus sativus</i>	radish
<i>Ribes nigrum</i>	blackcurrant
<i>Ricinus communis</i>	Castor bean
<i>Rubus</i> sp.	blackberry, raspberry
<i>Secale cereale</i>	rye
<i>Sesamum indicum</i>	sesame
<i>Solanum carolinense</i>	horsenettle
<i>Solanum laciniatum</i>	Kangaroo apple
<i>Solanum lycopersicum</i>	tomato
<i>Solanum tuberosum</i>	potato
<i>Spinacia oleracea</i>	spinach
<i>Trifolium</i> sp.	clovers
<i>Trifolium incarnatum</i>	Crimson clover
<i>Trigonella foenum-graecum</i>	fenugreek
<i>Triticum aestivum</i>	wheat
<i>Vitis vinifera</i>	grapevine
<i>Zea mays</i>	maize
<i>Zingiber officinale</i>	ginger

1.4 Damage symptoms

The presence of *A. segetum* larvae in fields of cultivated crops are often only noticed after damage have already occurred (Garnis & Dabrowski, 2008). Sidhu *et al.* (2019) observed that cutworm damage in maize is influenced by the quantity and instar of larvae in accordance with the crop stage. Cutworm larvae commonly feed on available weed species on which larvae develop into larger instars before they attack seedlings of newly emerged crops (Blair, 1975; Chandel *et al.*, 2022). In the American states, Illinois and Ontario, damage to no-till maize was caused by larvae that were already

present at the time of planting, and which originated from eggs laid in fields during early Spring, before maize was planted (Sherrod *et al.*, 1979; Kullik *et al.*, 2005). High cutworm numbers have the potential to drastically reduce the maize plant stand, particularly when large larvae are present at the time of seedling emergence (Showers *et al.*, 1979; Du Plessis, 2000; Chandel *et al.*, 2022). In India, important cash crops like cauliflower and cabbage suffer severe stand losses as a result of cutworm feeding during the early growth stages of the crops (Vashisth *et al.*, 2018). In South Africa, where cutworm infestations are severe, it could even appear as if some of the rows have disappeared as early as four days after seedling emergence of maize (Du Plessis, 2000; Drinkwater, 2017). A cutworm infested maize field is therefore often characterized by a loss in seedling stand (Figure 1.8).



Figure 1.8. Stand loss of maize seedlings cause by *Agrotis segetum* larvae, two to three days after seedling emergence (Photo by Z. van Eeden).

Agrotis segetum moths lay their eggs on young maize seedlings or on the soil surface below seedlings (Drinkwater, 2017). Sherrod *et al.* (1979) observed that oviposition takes place in fields before planting of maize. When neonate larvae hatch from eggs deposited on parts of a plant that is higher than 20 cm from the soil surface, they move to lower parts before the commence feeding (Blair, 1975). As a result of their

photopositive behaviour during these larval stages, *A. segetum* larvae have been reported to feed on the furl leaves of maize seedlings during the day for approximately 10-14 days (Blair, 1975; Bowden *et al.*, 1983).



Figure 1.9. Leaf damage is a symptom caused by feeding of early-instar larvae of *Agrotis segetum* (Photo by Z. van Eeden).

Leaf damage of small cutworm larvae (L1, L2, L3) are identified by its shot-hole appearance (Figure 1.9). Shot hole or window-pane type symptoms can be caused by several other soil insect pests that feed on leaves of seedlings, for example false wireworms (*Somaticus* spp.) (Drinkwater, 2017). In maize, the presence and feeding of small cutworm larvae can be identified as small irregular holes in the leaves of seedlings. Leaf feeding by early instars has minimal impact on the performance of maize seedlings and the later yield of maize crops (Showers *et al.*, 1979; Levine *et al.*, 1983).

Agrotis segetum larval feeding behaviour changes when, in the 3rd-instar, their sensitivity to light changes and they start to hide in the soil rather than remain among leaves of weedy plants (Garnis & Dabrowski, 2008; Jakubowksa & Bocianowski, 2013; Drinkwater, 2017). Cutworms do, however, not cut plants until they reach the 4th-instar (Engelken *et al.*, 1990; Cook *et al.*, 2003; Joshi *et al.*, 2020; Capinera, 2022). Burrows result from larvae tunnelling into the soil and serves as a site of refuge (Blair, 1975).

L4, L5 and L6 larvae emerge from these burrows at night and come to the surface in search of food (Blair, 1975; Bowden *et al.*, 1983). Larvae move from plant to plant, severing seedlings just above or at a short distance below the soil surface (Figure 1.10) (Drinkwater, 1980; Bowden *et al.*, 1983; Du Plessis 2000; Garnis & Dabrowski, 2008; Guo *et al.*, 2015; Chandel *et al.*, 2019; Sidhu *et al.*, 2019; Chandel *et al.*, 2022). Severing of plants is the most common symptom of *Agrotis* spp. damage, and tunnelling into stems occur only on rare occasions (Sherrod *et al.*, 1979; Archer & Musick, 1977).

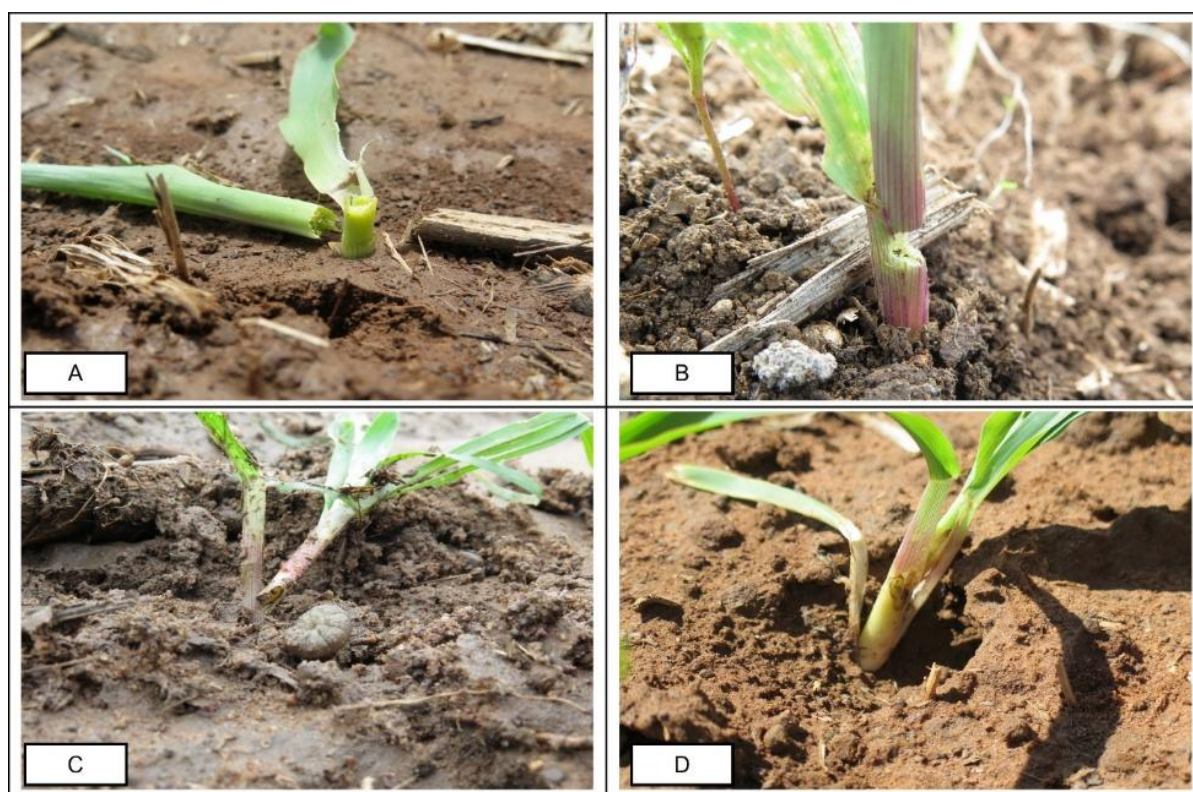


Figure 1.10. Seedlings severed a short distance above (A, B) or below the soil surface (C, D) (Photos by Z. van Eeden).

Cutworms may sometimes drag cut seedlings into tunnels or cracks in the soil where feeding takes place during daytime (Joshi *et al.*, 2020). By emerging from the soil at night and devouring one seedling after another, a single larva can damage a significant number of seedlings (Du Plessis, 2000; Chandel *et al.*, 2022). In many instances, only one plant is consumed, and the consumed plant is partially or entirely pulled underground and consumed (Drinkwater, 2017). Sidhu *et al.* (2019), reported on larval densities and subsequent damage incidences caused by two cutworm species, *A.*

ippsilon and *A. segetum*, in India. In maize, the incidence of damaged seedlings ranged between 10 and 28 %, while larval numbers ranged between 0.33 and 2.67 /m².

Above-ground symptoms of cutworm damage are also sometimes visible as wilting of the central whorl leaf of maize seedlings, followed by wilting of the entire seedling (Drinkwater, 2017). The subsequent plant growth, either vegetative or reproductive, is therefore influenced by larval feeding (Santos & Shields, 1998). The maize seedling's growth stage at the time of attack is important with respect to its ability to survive cutworm injury. A seedling cut below the growing point will not recover from damage. Seedlings injured above the growth point may sometimes regrow, but often does not recover fully (Showers *et al.*, 1979; Santos & Shields, 1998). Although larvae may only feed on a small portion of one seedling before moving on to the next, enough of the seedling's tissue may be destroyed to cause it to wilt and die (Du Plessis, 2000; Chandel *et al.*, 2019; Chandel *et al.*, 2022). In cases when the soil is extremely dry, cutworms tend to sever seedlings below the soil surface (Johnson *et al.*, 1984; Du Plessis, 2000; Chandel *et al.* 2022). By the four-leaf stage, maize seedlings are less prone to damage by larvae (Santos & Shields, 1998; Sidhu *et al.*, 2019). Showers *et al.* (1983) demonstrated that maize at the one-leaf stage is highly susceptible to damage. However, by the four- or six- leaf stage, plant yield is not decreased by larval feeding. Similarly, Levine *et al.* (1983) observed that the reduction in maize yields were due to more newly emerged seedlings being cut than older seedlings. At the four- to six-leaf stage, the seedling's stem is typically too thick to be cut, and when the most damaging larval instars (L4, L5 & L6) are present at the four-leaf stage of seedling development, a clean round hole is eaten into the stem (Drinkwater, 1980; Du Plessis, 2000; Drinkwater, 2017).

The damage inflicted by black maize beetle, *H. arator*, and false wireworm, *Somaticus* spp. to maize seedlings, is similar to that of cutworms, but has a more frayed appearance (Drinkwater, 1980; Du Plessis, 2000). *Heteronychus arator* uses its serrated fore legs when it feeds on stems of maize seedlings, which may result in dead heart symptoms and when the stems are too thick to be severed, younger leaves develop large yellow stripes (Van den Berg *et al.*, 2014; Drinkwater, 2017). *Somaticus* spp. larvae, chew holes into subterranean stems of seedlings and cause the growth point of young plants to die off, leading to stunted growth (Van den Berg *et al.*, 2014; Drinkwater, 2017).

1.5 The effect of abiotic conditions on cutworm ecology and damage potential

In the changing climate faced globally, insect pests are of key interest because their development and damage potential are influenced by abiotic conditions (Esbjerg & Sigsgaard, 2014). The survival of *A. segetum* larvae is strongly influenced by temperature fluctuations and are susceptible to under development and mortality when temperatures reaches very low or very high ranges (Esbjerg & Sigsgaard, 2019). Conditions that favour the likelihood of cutworm attacks have been elucidated by Sherrod *et al.* (1979). Sherrod *et al.* (1979) reported that the damage potential of *A. segetum* may be ascribed to various risk factors and is not limited to factors such as a previous history of cutworm infestation, weed coverage too close to planting, soybean plantations followed by maize, improper field drainage and adjacent permanent vegetation.

1.5.1 Soil moisture and temperature

The damage potential of *A. segetum* is determined firstly by the size of the pest population, which is affected largely by the climate conditions (Allen, 1976; Esbjerg *et al.*, 1986; Esbjerg, 1989; Jakubowska & Bocianowski, 2013). In Europe, several studies confirmed that conditions related to soil moisture and temperature have an impact on *A. segetum* larval survival (Esbjerg & Lauritzen, 2010; Esbjerg & Sigsgaard, 2019). Sunlight and longer than usual periods of severe drought are almost certain to coincide, and early in the growing season, this results in dry soil (Esbjerg & Sigsgaard, 2014). *Agrotis segetum* moths prefer to oviposit on dry soil rather than moist soil (Esbjerg & Lauritzen, 2010). In a recent study by Esbjerg and Sigsgaard (2019) in Germany and Denmark, the developmental time of German and Danish *A. segetum* eggs at certain temperatures were measured and results showed that eggs sampled in Germany hatched after 4.0 days at 26.7°C, whereas eggs from Denmark hatched within 4.8 days. However, at 18°C, the eggs from Germany hatched after 12 days as opposed to the 9.5 days for the eggs sampled in Denmark.

In Denmark, 1st- and 2nd-instars develop slowly at low temperatures of 12 to 15 °C and much more quickly at higher temperatures (25 to 28 °C) (Esbjerg & Sigsgaard, 2019). High and low temperatures exceeding the developmental scopes as mentioned by Esbjerg and Sigsgaard (2019), can possibly lead to increased mortality, particularly in the L1 and L2 stages (Esbjerg & Sigsgaard, 2014; Chandel *et al.*, 2022). Early-instar larvae (L1 and L2) grow rapidly and have an increased probability of survival provided

the soil is sun-exposed, dry, and warm when the eggs hatch (Esbjerg & Vidal, 2003; Esbjerg & Lauritzen, 2010). In Denmark, the proportion of larval survival was reported to be higher in localised dry patches, which were located within close proximity to areas with moist, cool soil (Esbjerg & Sigsgaard, 2014). In contrast, 1st- and 2nd-instar *A. segetum* larvae experience high mortality during early summer in Denmark when the soil is still moist and the soil temperature is low (Esbjerg *et al.*, 1986; Esbjerg, 1989; Esbjerg & Vidal, 2003).

Thus, high and low temperatures critically affect survival as well as development of early-instar larvae in particular. This should also be considered in light of how soil moisture and upper soil temperature interact, since continued soil moisture reduces the temperature of the upper soil layer while high temperatures, especially those brought on by sunshine, causes the upper soil layer to dry up (Esbjerg & Sigsgaard, 2019). L3 larvae are also highly dependent on soil moisture and impacted by low temperatures, however, *A. segetum* larvae's ability to tolerate cold and moist conditions increase when larvae reach the L4, L5 and L6 stages (Esbjerg, 1989). *Agrotis segetum* larvae that are in the L4, L5 and L6 stages occur mostly within the soil during daylight hours (Blair, 1975). Soil moisture content influences the depth at which 6th-instar larvae of *A. segetum* and *A. ipsilon* are able to pupate (Blair, 1975). Pupation takes place within the top 30 mm of the soil and the higher the soil moisture content, the shallower the depth at which pupation occurs (Blair, 1975). Under field conditions, the number of instars appears to be influenced by temperature and humidity and potentially other as yet unidentified factors (Archer *et al.*, 1980; Kullik, 2006; Santos & Shields, 1998).

The wide distribution of *A. segetum* is confirmed to be a result of long-distance migration, confirmed by radar observations in (Wood *et al.*, 2009). Studies have also indicated the strong potential of cutworm moths to migrate. Blair (1978) studied the flight activity of *Agrotis* spp. as the number of 30 minute periods of uninterrupted flights and found that a high percentage of *A. segetum* moths (41%) in a population was able to sustain flight activity, indicating that dispersal flights over long distances are feasible. In a study conducted by Guo *et al.* (2015) on Beihuang Island, in Northern China, 11 years of search light trapping data provided direct evidence that *A. segetum* is a long-distance migrant since no host crops or cutworm larvae were found on this isolated island. Furthermore, the trapping results suggested that the moths completed

a flight of approximately 40-60 km, and that significantly more females were caught than males, suggesting that female moths have a greater flight capability (Guo *et al.*, 2015). Similar results were obtained by Garnis and Dabrowski (2008), who observed *A. segetum* moths to fly approximately 100 km in Warsaw, Poland. Bues *et al.* (1989) reported that *A. segetum* moths are capable of completing migratory flights due to their ability to survive a wide gradient of environmental conditions.

The role of forecasting cutworm damage was developed and implemented in Denmark and Sweden (Esbjerg *et al.*, 1986). Forecasting of cutworms is challenged by varying precipitation quantities/ frequencies and the subsequent heterogeneous soil conditions caused thereby which contributes to an increase in the potential damaging densities of *A. segetum* larvae (Thygesen, 1971 cited by Mikkelsen & Esbjerg, 1981; Esbjerg & Sigsgaard, 2014). Furthermore, Archer and Musick (1977) reported that predicting infestation levels of *A. ipsilon* in a maize field is difficult because it occurs rather sporadically and is often not detected until it has cut many maize seedlings. A study by Jakubowska and Bocianowski (2013) was conducted to evaluate the population size of *A. segetum* by means of pheromone traps during 2005 to 2009 in Poland. Findings indicated that high temperatures and moderate rainfalls were advantageous for moth flights and larval feeding. Bowden *et al.* (1983) observed that the severity of *A. segetum* attacks in England and Wales were highly dependent on the weather, particularly temperature and rainfall, experienced during the development of eggs and the first two larval instars. The risk of *A. segetum* larvae attacking crops is reduced and closely eliminated following a period of cold weather conditions (Esbjerg & Sigsgaard, 2019). The opposite situation applies under warm and dry weather conditions. If it proceeds for a period approximately 8 sunny dry days with an upper soil temperature of 25°C, young larvae may be brought through L1 and L2 phases without any exposure to detrimental coldness or soil moisture (Esbjerg & Sigsgaard, 2019).

A positive link between *A. segetum* attack level and the number of moths caught by means of light traps in Denmark was found by Thygesen (1971) cited by Mikkelsen and Esbjerg (1981). Furthermore he observed that dry weather in summer increased cutworm attack levels (Mikkelsen & Esbjerg, 1981) however, the amount of precipitation in late winter and at the start of spring had a negative influence on attack level. For the summer months June, July and August, there was a stronger negative

influence of the numbers of precipitation days than of the amounts of precipitation (Zethner & Esbjerg, 1978), suggesting that rainfall is the most important climatic factor in relation to cutworm attacks with a strong reducing effect (Mikkelsen & Esbjerg, 1981). The fact that the amount of precipitation has a stronger influence than the number of precipitation days may indicate that the direct effect of rain is weak, while soil moisture plays a more important role. The precipitation in the previous year's autumn had a much weaker influence on cutworm attack levels. A possible explanation for such influence may be the *A. segetum* larvae overwintering, eventually causing increased physiological sensitivity to soil moisture. Increased soil moisture experienced during late winter and early spring in Denmark seems harmful to the overwintering *A. segetum* larvae which lay inactive in the soil (Mikkelsen & Esbjerg, 1981).

1.5.2 Agricultural practices

1.5.2.1 No-till and conventional tillage practices

In the United States, *A. ipsilon* are attracted to no-till fields (Showers *et al.*, 1985; Schoenbohm & Turpin, 1977). *Agrotis ipsilon* occur more frequently in reduced tillage because pre-plant weed infestations are preferred oviposition sites (Sherrod *et al.*, 1979). The presence of large amounts of crop residues on no-till fields increases the survival of larvae because it provides shelter from extreme weather conditions and weeds are a readily available food source (Foster & Ruesink, 1984; Esbjerg, 1990). Foster and Ruesink (1986b) reported that the influence of tillage practices was directly related to the level of *A. ipsilon* infestations and consequent damage (Figure 1.11). The weediness before planting in no-till, disked and conventional fields were high, moderate and low, respectively. In the no-till, disked and conventional fields, an average of 1.9, 1.3 and 0.5 larvae (L4-L6) were recorded infesting 1 to 5 leaf-stage maize seedlings (per 100 seedlings) (Foster & Ruesink, 1986b).

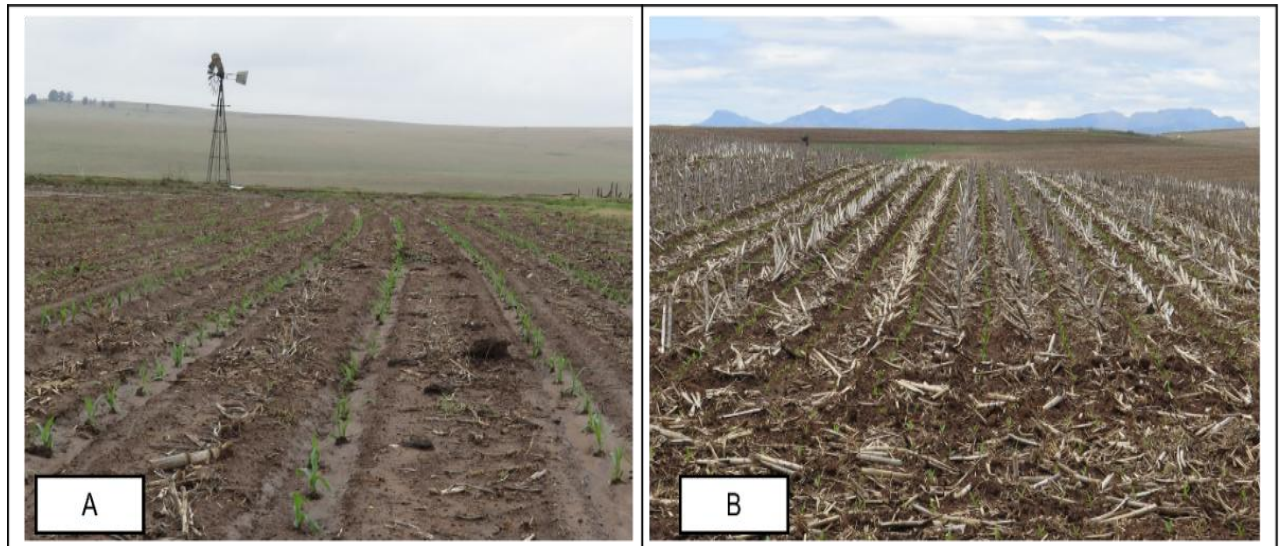


Figure 1.11. Maize fields under conventional tillage (A) and no-till practices (B) (Photos by Z. van Eeden).

From a prediction model (Foster & Ruesink, 1986b), the greatest damage occurred in no-till fields with a yield reduction of nearly 5%, whereas lower yield reduction was experienced in disked fields and the lowest in conventional fields. Mulder and Showers (1983) reported an increase in *A. ipsilon* damage to maize seedlings if weeds, their preferred host, were not present during seedling emergence. Furthermore, weed control performed in a no-till system when maize seedlings are in the four-leaf stage results in significant maize yield loss due to weed competition (Engelken *et al.*, 1990). Similarly, Busching and Turpin (1977) reported that maize planted with no cultivation after seedling emergence (minimum-tillage practices) and the presence of weedy vegetation led to *A. ipsilon* infestations. However, maize planted adjacent to weeds as a form of conservation tillage suffered less damage than maize seedlings in a weed-free field (Mulder & Showers, 1983). Johnson *et al.* (1984) reported that reduced tillage in fields with maize plantings following soybean or wheat and minimal weed management were associated with higher *A. ipsilon* damage.

1.5.2.2 Crop residues

The presence of crop residues in a maize field influences the survival and damage level of *A. segetum* (Figure 1.12). The soil surface in no-till systems is covered by residue from the previous crop (Phillips *et al.*, 1980) and moths are more likely to oviposit on crop residue in no-till than conventionally tilled fields because of this increased crop residue (Busching & Turpin, 1976; Sherrod *et al.*, 1979; Showers *et al.*, 1985). Fourth-instar larvae are unable to survive for longer than 10 days when the only food source is dry or moistened maize stalks (Blair, 1975). Sixth-instar larvae are able to pupate inside maize stalks (residue), due to their ability to withstand adverse conditions, however, the resulting pupae are smaller than those resulting from larvae provided with a fresh food supply (Blair, 1975). Blair (1975) further observed that photonegative larvae (L4, L5), with the exception of the 6th-instar, are unable to survive on crop residues, thus must be surviving on fresh food sources (weeds) to be able to attack newly-emerged seedlings.



Figure 1.12. Maize residues left on fields after harvest (Photo by Z. van Eeden).

1.5.2.3 Presence of weeds

Weeds growing externally to cultivated crops serve as an alternate host for a crop-damaging arthropod for population build-up to then move to adjacent crops (Norris & Kogan, 2000). The presence of weeds lead to increases in the winter population of cutworms that will overwinter. Not only do weeds provide overwintering sites for *A. segetum*, but weeds serve as an early-season food source before the host crop is established, which can lead to population increases (Norris & Kogan, 2000). The availability of weeds as alternative food source for cutworms strongly influences the abundance, survival and subsequent damage caused by cutworm larvae (Foster & Ruesink, 1984; Foster & Ruesink, 1986b; Mulder & Showers, 1983) (Figure 1.13).

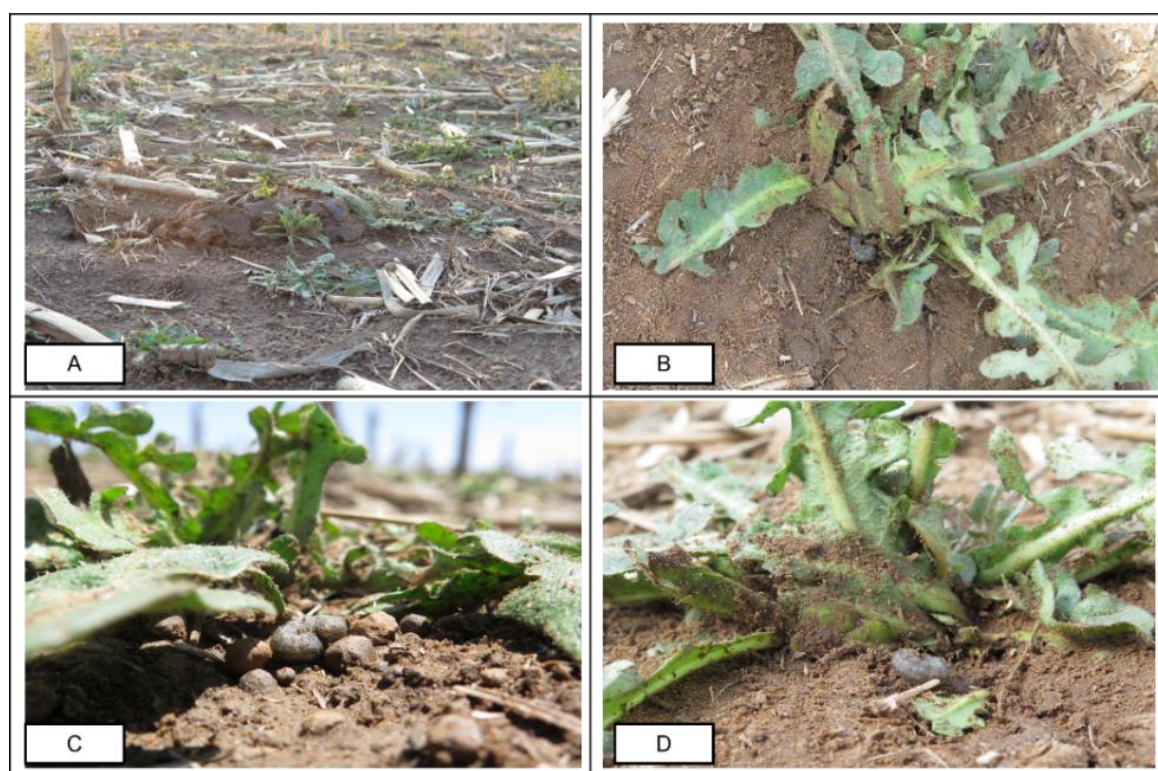


Figure 1.13. Winter weeds present in maize fields after harvest (A) and cutworms found present in these fields (B, C, D) (Photos by Z. van Eeden).

Busching and Turpin (1977) reported higher *A. ipsilon* survival and faster development on various weeds species as opposed to maize. Weeds growing within the crop may alter both the population dynamics of phytophagous pests and may also influence the degree of damage by such pests (Mulder & Showers, 1983). In Washington, United States, redbacked cutworm, *Euxoa ochrogaster* (Guenee) (Lepidoptera: Noctuidae), populations increased by as much as 300-fold when weeds such as *Cirsium arvense*

(Canada thistle) and *Convolvulus arvensis* (field bindweed) were present in *Asparagus officinalis* fields, compared to weed-free fields (Tamaki *et al.*, 1975). Similar interactions were observed in Iowa, United States, where increased incidences of damaged maize seedlings at the two-leaf stage were recorded in maize fields where weed removal took place post emergence (Engelken *et al.*, 1990). Mulder and Showers (1983) also observed that the incidence of damaged seedlings was lower in fields where weeds were present. *Agrotis ipsilon* survival is higher and their development to the pupal stage quicker on several weed species as opposed to maize (Busching & Turpin, 1977; Engelken *et al.*, 1990).

Monnig *et al.* (2007) investigated the effects of herbicide treatments on glyphosate tolerant maize to determine the influence that this practice may have on injury to maize by *A. ipsilon*. In the unsprayed plots, seedling injury was low, and larvae utilised annual winter weeds as their primary food source rather than maize seedlings. The incidence of damaged seedlings recorded in herbicide treated plots, where winter weeds were absent, was low, and larvae could only feed on the developing maize seedlings. Populations established before the main cropping season rely on weeds as a food source. The main damage to newly-emerged crops is caused by larvae, which have established themselves on weeds growing in the field before the planting of a crop (Blair, 1975).

The availability of oviposition sites such as weedy or grassy areas within a maize field is directly related to the level of damage caused by *A. ipsilon* (Busching & Turpin, 1976). Moths prefer to oviposit on densely growing plants, relatively low to the ground as well as plant debris within fields (Esbjerg & Lauritzen, 2010). Sherrod *et al.* (1979) reported that moths typically lay their eggs on weeds, which are the preferred host plants for larvae. Busching and Turpin (1976) reported that ovipositing moths prefer pasture residues to maize residues and bare soil for oviposition. Hence, grasses commonly found within field edges and abandoned pastures provide additional oviposition sites for *A. ipsilon* moths early in the spring before vegetation is abundant (Busching & Turpin, 1976). The latter study further reported that low, densely growing plants and fine-textured plant residues such as dry grass were more attractive for ovipositing moths than taller, less dense vegetation or coarser-textured residues. Agronomic practices that promote the establishment of winter annual or perennial weeds increase the likelihood of their presence and the subsequent damage caused

by cutworm larvae (Sherrod *et al.*, 1979). The timing of weed removal is critical, if weeds are removed after cutworm populations have built up on the weedy vegetation, larvae will be forced to feed on maize seedlings (Mulder & Showers, 1983).

1.5.2.4 Interactions between weeds and natural enemies of cutworm larvae

A number of factors that have changed since the introduction of reduced tillage may have an impact on natural enemies (Foster & Ruesink, 1984). These include higher soil moisture, greater soil compaction, lower spring soil temperatures, and higher abundance of crop residues and weeds (Foster & Ruesink, 1984). Foster and Ruesink (1984) reported that feeding by a braconid wasp, *Meteorus rubens* (Nees) (Hymenoptera: Braconidae) on certain broadleaf weeds associated with reduced tillage, more than doubled its longevity and reproduction compared to starved parasitoids. Weeds significantly increased *M. rubens* survival, density and parasitism of *A. ipsilon* over a period of several days (Foster & Ruesink, 1986a). The reduction of natural enemies by insecticides or tillage operations causes an increase in the number of damaged maize seedlings as a result of increased cutworm pressure (Brust *et al.*, 1985).

1.6 Management of *Agrotis segetum*

Studying soil pests' ecology and behaviour is challenging due to their underground and hidden nature (Toepfer *et al.*, 2014). Accordingly, it is more challenging to manage the pest using traditional methods like soil-applied insecticides (e.g., seed coating, in-furrow liquid or granular applications, or soil insecticides). Soil preparation practises such as rotary hoeing to kill soil pests or expose them to unfavourable conditions and natural enemies (Keller & Zimmerman, 2005). *Agrotis segetum* larvae are among the soil-dwelling insects that continue to challenge the best efforts at pest management. Cultural control measures, insecticide applications, biological control and host plant resistance have been evaluated and used for management of *A. segetum* (Drinkwater & Janse Van Rensburg, 1992; Foster & Ruesink, 1984; Erasmus *et al.*, 2010).

1.6.1 Transgenic crops

Researchers as well as producers have been focused on technological advances as an opportunity to mitigate pest losses and improve food production as a result of the losses incurred by pests (Oerke, 2006). Genetically modified (GM) crops are those whose genomes have been altered by means of genetic engineering in order to

introduce new qualities that are not naturally present in the particular crop species, or to enhance existing traits (Kumar *et al.*, 2020). One such genetic modification is the production of toxic crystalline (Cry) proteins by Bt crops, which are derived from the soil bacterium *Bacillus thuringiensis* (Bt) (Moustafa *et al.*, 2013). These Cry toxins bind to specific receptors in the midgut and derange uptake of nutrients or cause perforation of the gut wall, leading to sepsis and death (Bravo *et al.*, 2004). Over the past 20 years, the production of transgenic crops has significantly increased global agricultural productivity (Kumar *et al.*, 2020). Many commercialised Bt crops have performed well against their target pests and were seen as a replacement for chemical control (Tabashnik *et al.*, 2008; Carriere *et al.*, 2010). In South Africa, Bt maize has the potential to control primary lepidopteran pests such as *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus* effectively (Van den Berg & Van Wyk, 2007; Van Wyk *et al.*, 2009; Kruger *et al.*, 2012). However, since the use of Bt maize, several secondary pests such as *A. segetum*, *Helicoverpa armigera* and *Acantholeucania loreyi* (Duponchel) (Lepidoptera: Noctuidae) have acquired higher levels of economic importance that were previously controlled by the insecticide applications targeting the primary pests (Erasmus *et al.*, 2010). Despite exhibiting varying degrees of susceptibility to Cry proteins, numerous secondary pests can nonetheless cause damage to maize (Van Wyk *et al.*, 2007, Van Wyk *et al.*, 2008). Lambert *et al.* (1996) reported that Cry9Ca was toxic to *A. ipsilon* and *A. segetum* in Belgium. Cry1Ab (Event 176) had no influence on the proportion of plants injured by *A. segetum* in a field assessment of Bt maize in Spain carried out by Eizaguirre *et al.* (2006). *Agrotis segetum* caused more seedling damage in non-Bt fields than in Bt fields (Van Wyk *et al.*, 2007). While transgenic maize expressing Cry1Ab has been shown to have considerable effects on *A. segetum* in a laboratory environment, Erasmus *et al.* (2010) suggested that Bt maize events MON810 and Bt11 will probably be ineffective against *A. segetum* since it is a non-target pest of these two events in field conditions.

1.6.2 Biological control

Biological control provides an important ecosystem service and is an important component of any IPM strategy (Oerke, 2006). In the natural environment, predators, parasitoids, entomopathogenic fungi, - viruses and - nematodes are important bio-control agents (Salim *et al.*, 2016). The presence of natural enemies such as birds,

carabid beetles, spiders and parasitoids have been reported to contribute to suppression of cutworms (House & Alzugaray, 1989; Frank & Shrewsbury, 2004).

1.6.2.1 Entomopathogenic nematodes

The most commonly found entomopathogenic nematode species belong to the families Allantonematidae, Mermithidae, Steinernematidae and Heterorhabditidae (Rahaman *et al.*, 2000). Entomopathogenic nematodes (EPNs) have been used successfully against soil-inhabiting insects in cryptic habitats (Arthurs *et al.* 2004; Trdan *et al.* 2007; Laznik *et al.* 2011). EPNs within the Steinernematidae and Heterorhabditidae family have the capacity to carry and release certain bacteria into the haemolymph of insects, killing the host and facilitating nematode development within the cadaver (Rahaman *et al.*, 2000).

Agrotis segetum larvae is difficult to control due to their soil-dwelling behaviour. The nematodes belonging to the Mermithidae family is less common for use against cutworms (Rahaman *et al.*, 2000). However, in Missouri, United States, the nematode *Hexamermis arvalis* (Poinar & Gyrisco) (Nematoda: Mermithidae) was reported to parasitise *A. ipsilon* in clover and alfalfa fields (Puttler & Thewke, 1971). Puttler *et al.* (1973) inspected the presence of *H. arvalis* in a maize field in Illinois and reported that 64.5% of *A. ipsilon* larvae was parasitised by this EPN. This EPN was also found to only attack the overwintering larvae present in fields during winter (Puttler & Thewke, 1971). Infective juveniles parasitised overwintering 1st- and 2nd-instar larvae, completed their development and emerged from 4th- and 5th-instar in the spring (Puttler & Thewke, 1971). Chen *et al.* (1989) conducted a small plot trial to use *Hexamermis* spp. as a biological control agent against *A. ipsilon* in China and found that the percentage infection of *A. ipsilon* was as high as 63% after a period of five days. Two other EPN species, *Hexamermis taihuensis* (Nematoda: Mermithidae) and *Hexamermis heterocephalis* (Nematoda: Mermithidae), were also reported to parasitise *A. ipsilon* in China (Wang & Wang, 1990). Li *et al.* (1993) studied *Hexamermis agrotis* (Wang) (Nematoda: Mermithidae), a natural enemy of *A. segetum* and found up to 3 to 4 larvae of *H. agrotis* parasitised a single cutworm larva. *Hexamermis agrotis* have a greater ability to parasitise *A. segetum* than *A. ipsilon* (Li *et al.*, 1993). Currently, no research is available for the use of Mermithidae against *A. segetum* in South Africa.

The entomopathogenic nematode *Steinernema carpocapsae* (Rhabditidae: Steinernematidae) is a promising biological control agent against *A. segetum* (Ebrahimi *et al.*, 2019). Host size is a significant factor in the pathogenicity process of infective juveniles (IJs) as it affects the penetration into the host body and host location capabilities of IJs (Bastidas *et al.*, 2014). Ebssa and Koppenhöfer (2012) investigated the pathogenicity of EPNs against different larval instars of another cutworm species, *A. ipsilon*, and reported that the 4th- and 5th-instars were the most susceptible developmental stages. Goudarzi *et al.* (2015) also reported that the 5th-instar larvae of *A. segetum* were more susceptible to *S. carpocapsae* than other instars.

Unlu *et al.* (2007) evaluated the effect of Turkish isolates of *Steinernema feltiae*, *Steinernema weiseri*, and *Steinernema carpocapsae* against *A. segetum* and reported a higher efficacy of *S. carpocapsae* (75% mortality) compared to other species. *Agrotis segetum* larvae were also reported to be highly susceptible to *Steinernema websteri*, with an 84% mortality rate after inoculation with 100 IJs inoculated in dry sand (Gokce *et al.*, 2013). Similarly, *Steinernema kraussei* caused 98% mortality 7 days post inoculation with 500 IJs (Gokce *et al.*, 2013). Chandel *et al.* (2009) reported 100% mortality of L3 and L4 of *A. segetum* at inoculation levels of 10 - 40 IJs, due to co-infection with *Heterorhabditus bacteriophora* (Rhabditidae: Heterorhabditidae). Fetoh *et al.* (2009) tested an Egyptian strain of *H. bacteriophora* against L4 *A. ipsilon* larvae and reported that *H. bacteriophora* was highly virulent against this pest. *Steinernema carpocapsae*, *Steinernema abbasi*, *Steinernema glaseri*, and two *Heterorhabditus indica* strains caused 100% mortality of final-instar *A. ipsilon* larvae in laboratory assays (Hussaini *et al.*, 2005).

When larvae develop into late-instars, the effectiveness of most biological control agents, including EPNs, decrease (Laznik & Trdan, 2015). EPN's are also compatible with chemical control strategies and can be tank-mixed with a variety of chemical herbicides, fungicides, and insecticides (including bacterial or fungal products) (Koppenhöfer & Grewal, 2005). For example, Oxamyl, an organophosphate, enhanced the synergistic effectiveness of *S. carpocapsae* against *A. segetum*, but only in fumigated soil (Ishibashi, 1993). This effect is likely due to the nematodes' enhanced nictation behaviour (Ishibashi, 1993). The application of *S. feltiae* in an *A. segetum*-infested lettuce crop resulted in a higher yield when compared to an endosulfan treatment (Lössbroek & Theunissen, 1985).

1.6.2.2 Predators

Cutworms have a high reproduction rate but the presence of natural enemies such as birds, carabid beetles, spiders and parasitoids help to keep populations low (House & Alzugaray, 1989; Frank & Shrewsbury, 2004). Carabid beetles have been found to prey on the eggs, larvae and pupae of *A. ipsilon* (Best & Beegle, 1977; House & Alzugaray, 1989). Predators of *Agrotis* spp. that were reported to be common in DPR Korea, East Asia, included *Anisodactylus signatus* (Panzer) (Coleoptera: Carabidae), *Harpalus simplicidens* (Schauberger) (Coleoptera: Carabidae) and *Harpalus chalcatus* (Bates) (Coleoptera: Carabidae), *Amara congrua* (Morawitz) (Coleoptera: Carabidae) and *Harpalus (Zangoharpalus) tinctulus luteicornoides* (Breit) (Coleoptera: Carabidae) in cabbage fields and peach plantations in China (Toepfer *et al.*, 2014).

1.6.2.3 Parasitoids

Parasitoids are insects that spend a significant portion of their life in a parasitic relationship, attached to, or within a single host (Salim *et al.*, 2016). Parasitoids are important natural enemies of *Agrotis* spp. Most parasitoid species are highly specialised, and they attack only a particular host life stage. The different life history strategies of parasitoids can be described as follows: egg parasitoids, egg-larval parasitoids, larval parasitoids, larval-pupal parasitoids and pupal parasitoids (Pennacchio & Strand, 2006).

One of these parasitoids, *Apanteles telengai* (Tobias) (Hymenoptera: Braconidae), has been recorded as a primary larval endoparasitoid of *A. segetum* in central Asia and southern Spain (Caballero *et al.*, 1993). In a study conducted by Caballero *et al.* (1993), it was reported that *A. telengai* was able to parasitise all larval instars of *A. segetum* and temperatures between 25 and 30 °C appeared to be optimal for the parasitoid survival. Although parasitized larvae do not die quickly, the damage they caused is reduced. Parasitism rates of up to 27% of *A. ipsilon* have been reported in maize fields (Levine & Clement, 1981). Sajap *et al.* (1978) reported that damage to maize seedlings caused by parasitised *A. ipsilon* larvae is less compared to that of non-parasitised larvae. For example, parasitism by *Microplitis kewleyi* (Muesebeck) (Hymenoptera: Braconidae) can reduce the ability of *A. ipsilon* larvae to damage plants (Schoenbohm & Turpin, 1977; Sajap *et al.*, 1978). Schoenbohm and Turpin (1977) reported that 36% less damage to maize at the three-leaf stage when larvae were parasitised by *Meteorus leviventris* (Wesmael) (Hymenoptera: Braconidae).

A list of parasitoids reported to parasitise *Agrotis* spp. across the world is presented in Table 1.3.

Table 1.3. List of parasitoids associated with *Agrotis* sp. across the world.

Parasitoid	Country/region	Reference
<i>Aleiodes gasteratus</i> (Hymenoptera: Braconidae)	Spain	Caballero <i>et al.</i> (1989)
<i>Apanteles telengai</i> (Hymenoptera: Braconidae)	Spain, Asia	Caballero <i>et al.</i> (1993)
<i>Macorcentrus collaris</i> (Hymenoptera: Braconidae)	Spain	Caballero <i>et al.</i> (1989)
<i>Zelet</i> sp. (Hymenoptera: Braconidae)	Spain	Caballero <i>et al.</i> (1989)
<i>Amblyteles</i> sp. (Hymenoptera: Ichneumonidae)	Spain	Caballero <i>et al.</i> (1989)
<i>Campoletis annulata</i> (Hymenoptera: Ichneumonidae)	Spain	Caballero <i>et al.</i> (1989)
<i>Gonia bimaculata</i> (Diptera: Tachinidae)	Spain, Turkey	Caballero <i>et al.</i> (1989), Gözüağık <i>et al.</i> (2009)
<i>Linnaemyia</i> sp. (Diptera: Tachinidae)	Spain	Caballero <i>et al.</i> (1989)
<i>Peleteria rubescens</i> (Diptera: Tachinidae)	Spain	Caballero <i>et al.</i> (1989)
<i>Periscepsia carbonaria</i> (Diptera: Tachinidae)	Spain, Asia, Turkey	Caballero <i>et al.</i> (1989), Salim <i>et al.</i> (2016)
<i>Apanteles</i> sp. (Hymenoptera: Braconidae)	Turkey	Gözüağık <i>et al.</i> (2009)
<i>Bracon hebetor</i> (Hymenoptera: Braconidae)	Turkey	Gözüağık <i>et al.</i> (2009)
<i>Conomorium patulum</i> (Hymenoptera: Pteromalidae)	Turkey	Gözüağık <i>et al.</i> (2009)
<i>Meteorus rubens</i> (Hymenoptera: Braconidae)	Turkey, USA, Egypt	Foster & Ruesink (1984), El-Heneidy & Hassanein (1987)
<i>Pimpla turionellae</i> (Hymenoptera: Ichneumonidae)	Europe	Salim <i>et al.</i> (2016)
<i>Apanteles ruficrus</i> (Hymenoptera: Braconidae)	Egypt	El-Heneidy & Hassanein (1987)
<i>Microplitis kewleyi</i> (Hymenoptera: Braconidae)	USA	Schoenbohm & Turpin (1977), Sajap <i>et al.</i> (1978)
<i>Meteorus leviventris</i> (Hymenoptera: Braconidae)	USA	Schoenbohm & Turpin (1977)
<i>Bonnetia comta</i> (Diptera: Tachinidae)	USA	Levine & Clement (1981)
<i>Microplitis mediator</i> (Hymenoptera: Braconidae)	Asia, Europe	Khan & Ozer (1988)
<i>Tachina magnicornis</i> (Diptera: Tachinidae)	Turkey	Kara & Tschorsnig (2003)
<i>Linnaemya comta</i> (Diptera: Tachinidae)	Turkey	Kara & Tschorsnig (2003)

Sajap *et al.* (1978) reported that damage to maize seedlings at the two-leaf stage were reduced by 68% if *A. ipsilon* were parasitised by *M. kewleyi*. *Agrotis ipsilon* larvae parasitised by *Bonnetia comta* (Wiedemann) (Diptera: Tachinidae) cut 53% fewer maize seedlings than non-parasitised larvae (Levine & Clement, 1981). El-Heneidy and Hassanein (1987) investigated the level of parasitism over three successive seasons (1983-1986) in Egypt and observed an average of 22.5% parasitism of which *Apanteles ruficrus* (Haliday) (Hymenoptera: Braconidae) was responsible for 10.3% and the remaining 12.2% was a result of *M. rubens*.

1.6.2.4 Entomopathogens

1.6.2.4.1 Entomopathogenic fungi (EPFs)

Entomopathogenic fungi (EPFs) cause disease in insects and play an important role in integrated pest management (El-Hawary & Abd El-Salam, 2009; Gabarty *et al.*, 2014; Yangchan *et al.*, 2022). In Northern Europe, Steenberg and Ogaard (2000) found *Tolypocladium cylindrosporium* (Hypocreales: Ophiocordycipitaceae) as a rare fungal pathogen of overwintering *A. segetum* larvae, causing 50-100% mortality. Sherlock (1983) conducted a study to determine the incidence of natural diseases in *A. segetum* in England and Wales and found fungal infection of mature larvae and prepupae by *Entomophthora megasperma* (Entomophthorales: Entomophthoraceae) that formed either conidiophores or resting spores. Another pathogen, *Fusarium solani* (Hypocreales: Nectriaceae), was isolated from *A. segetum* pupae that resembled a white dense mycelial mass that almost completely replaced the internal tissues of the pupa (Sherlock, 1983). Other studies evaluated EPF's against *A. ipsilon*. El-hawary and Abd El-Salam (2009) observed that *A. ipsilon* larvae treated with an EPF product, Priority, *Paecilomyces fumosoroseus* (Hypocreales: Cordycipitaceae) at a concentration of 1×10^9 spores caused 100% larval mortality within 5.3 days. According to Gabarty *et al.* (2014), two EPFs were found to be pathogenic on *A. ipsilon* larvae. After seven days, the formation of white spores indicated the growth of *Beauveria bassiana* (Hypocreales: Clavicipitaceae), and after 15 days, the formation of green spores on the cadaver of prepupa indicated the growth of *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae). Similarly, Ahmed *et al.* (2022) evaluated *B. bassiana* isolates against 6th-instar *A. ipsilon* larvae and observed 100% mortality after 14 days.

1.6.2.4.2 Entomopathogenic viruses (EPV's)

Another promising approach for the biological control of *Agrotis* spp. is the application of baculoviruses to infect this insect pests (Bourner *et al.*, 1992). The *Agrotis* baculoviruses complex describes such an example of baculoviruses that co-infect and interact in host larvae. Both *A. segetum* and *A. ipsilon* larvae can become cross-infected by these viruses and have shown a different susceptibility to the different *Agrotis* baculoviruses (Ignoffo & Garcia, 1979; El-Salamouny *et al.*, 2003; Bourner & Cory, 2004). Several baculoviruses, both nucleopolyhedroviruses (NPVs) and granuloviruses (GVs), have been isolated from *A. segetum* (Allaway & Payne, 1983) and *A. ipsilon* (Boughton *et al.*, 1999). At least four different baculoviruses were characterised from larvae of *A. segetum* (Agse) and *A. ipsilon* (Agip), i.e., the AgseNPV-A (Jakubowska *et al.*, 2005), AgseNPV-B and AgseGV (Allaway & Payne, 1983; Jakubowska *et al.*, 2006) and AgipNPV (Boughton *et al.*, 1999). All show control potential in the field (Bourner *et al.*, 1992). *Agrotis segetum* NPV was pathogenic for eight species of noctuids within the cutworm complex, i.e., *A. segetum*, *A. ipsilon*, *Agrotis exclamationis* (Linnaeus) (Lepidoptera: Noctuidae), *Agrotis puta* (Hübner) (Lepidoptera: Noctuidae), *Noctua comes* (Hübner) (Lepidoptera: Noctuidae), *Peridroma saucia* (Hübner) (Lepidoptera: Noctuidae), *Xestia sexstrigata* (Haworth) (Lepidoptera: Noctuidae), and *Xestia xanthographa* (Denis & Schiffermüller) (Lepidoptera: Noctuidae) (Bourner & Cory, 2004).

1.6.3 Cultural control

Several cultural control strategies may be implemented to reduce damage by cutworm larvae (Kullik, 2006). Cultural control refers to any method that involves altering the environment in order to make it less conducive to pests (Dent, 2002). These techniques limit a pest's capacity to invade, live, establish, find mating partners, and reproduce (Dent, 2002).

1.6.3.1 Weed management

Weeds serve as an alternate host for developing cutworms during the off-crop season (Drinkwater, 2017). Cutworm larvae and moths prefer to feed and lay eggs on weeds, however, when weeds are not present, numerous host plant species act as an alternative. The removal of weeds, the preferred host of this pest, too close to the planting season in spring may enhance cutworm damage as they migrate to alternative host plants. i.e., cultivated crops (Mulder & Showers, 1983). A serious problem may

occur when weeds within a crop are controlled which are supporting a pest arthropod population. Following the destruction and removal of weeds, these insects that had been living on the weeds migrate to, and attack the crop (Norris & Kogan, 2000).

Levine *et al.* (1981) reported that *A. ipsilon* larvae survived tillage operations before maize planting and subsequently attacked maize seedlings after emergence. However, the timing of tillage operations or herbicide applications may influence the extent of *A. ipsilon* damage that occur on emerging maize seedlings (Mulder & Showers, 1983; Showers *et al.*, 1985). For instance, low numbers of maize seedlings were cut by larvae when tillage operations and herbicide applications were performed 8 to 14 days prior to planting, suggesting larvae starved within the timeframe. However, a greater number of seedlings were cut when tillage operations were performed 2 days prior, or on the same day of planting. Higher incidences of damaged seedlings have been reported when herbicide applications are done 2 days prior, at planting, or 2 days after planting. Maize seedlings emerging from the latter management option provided a food source for larvae before weeds die off. Engelken *et al.* (1990) suggested that weeds left until maize seedlings were at the two-leaf stage provided larvae (L3-L6) with food. The use of pre-emergence or early post-emergence herbicides, or cultivation to destroy weeds when maize seedlings emerged (4-day old) and *A. ipsilon* larvae are 5th- to 6th-instars, can result in significant maize seedling damage (Engelken *et al.*, 1990). Larvae developed faster in the presence of weeds and reached pupation by the time maize seedlings were at the four-leaf stage. Weed removal at the two-leaf stage reduced seedling damage by 35% (Engelken *et al.*, 1990). The opposite is true for smaller larvae (L1, L2). The cutting of maize seedlings increased when weeds were removed at the two-leaf stage rather than at emergence stage. The increased seedling damage by these instars are presumably because larvae used weeds for food and shelter (Engelken *et al.*, 1990). For this reason, fields must be kept clear of weeds 35 days (4-6 weeks) before planting in order eradicate *A. segetum* by means of starvation (Blair, 1975; Drinkwater & Walters, 1976; Drinkwater, 1980).

Additionally, larvae are brought to the surface during the removal of winter weeds, leaving them exposed to unfavourable environmental conditions and vulnerable to attacks from birds and frost. By removing weeds from fields, *A. segetum* moths and larvae are deprived of their preferred oviposition and feeding sites (Drinkwater & Janse

Van Rensburg, 1992). Furthermore, it is suggested that producers should begin cutworm control strategies in the spring and monitor moth flights to predict cutworm activity (Engelken *et al.*, 1990). Preplant herbicides should also be properly timed (Showers *et al.*, 1985). Herbicide treatments up to a week before planting do not hinder survival and development of cutworm larvae. A minimum of 14 days prior to planting would allow the implementation of pre-emergence weed control practices to minimise *A. ipsilon* damage to maize seedlings in a no-till cropping system (Showers *et al.*, 1985).

1.6.4 Chemical control

Insecticides are classed according to their mode of action (MoA), that refers to the target site of the pest, for example, respiration and midgut, growth and development and nerve function, on which the insecticide acts on (IRAC, 2024). When choosing and formulating an insecticide, factors such as the insecticide's biological efficacy in controlling cutworm, the cutworm's susceptibility, the techniques used during application, human safety and toxicity to non-target organisms (including residual activities) need to be taken into account (Joshi *et al.*, 2020). The development of an integrated pest management (IPM) of cutworms is challenged by the selection of highly efficient insecticides and appropriate application methods (He *et al.*, 2019).

The application of insecticides is the most common method used for control of *Agrotis* spp. throughout the world. In Zimbabwe, Blair (1991) tested four concentrations of a pyrethroid, lambdacyhalothrin, on 3rd and 4th instar *A. segetum* larvae and found that increased concentrations above the recommended dosage did not increase larval mortality but had a repelling and anti-feeding effect on larvae. He *et al.* (2019) tested the sublethal effect of anthranilic diamide, chlorantraniliprole, on nutritional physiology, enzyme properties and population parameters of *A. ipsilon*. Results suggested that low concentrations of chlorantraniliprole can decrease the duration of the developmental period, normal eating and digestion and fertility. While the efficacy of corrective insecticide applications may vary, there seems to be significant developments in the use of seed treatments against this pest (Liu *et al.*, 2016; Allen *et al.*, 2018; Joshi *et al.*, 2020; Aquino *et al.*, 2022). Kullik *et al.* (2011) investigated the relationship between Pioneer Hi-Bred 38P04, a Bt maize expressing the Cry1Fa2 gene, and the effectiveness of clothianidin as a seed treatment on *A. ipsilon*. The results showed that clothianidin reduced larval weight gains additively and increased

larval mortality on Bt maize. *Agrotis ipsilon* larvae feeding on Bt maize alone and non-Bt maize seedlings treated with clothianidin experienced higher mortality and lower weight gains. The efficacy of seed treatment of soybean was studied by Aquino *et al.* (2022) who reported that thiamethoxam, cyantraniliprole + thiamethoxam, thiamethoxam + lambda-cyhalothrin, imidacloprid, imidacloprid + thiodicarb and fipronil provided effective control of *A. ipsilon* under field conditions.

The need for improved insecticide resistance management (IRM) has been addressed by IRAC (2024). *Agrotis ipsilon* have been frequently exposed to high concentrations of conventional chemical insecticides without active ingredient rotation and has developed resistance to organophosphates, carbamates, and pyrethroids (Xu *et al.* 2016). Various studies aimed at developing environmentally friendly control methods for the management of *A. segetum* are being conducted (Goudarzi *et al.* 2015; Rodingpuja & Lalthanzara, 2021). A new range of formulations have been developed to reduce the risk of resistance development (Sparks *et al.*, 2019). Nanoformulations may achieve this purpose (Yan *et al.*, 2021). Awad *et al.* (2022) used some nanoformulations against the larvae of *A. ipsilon* and found that it was more effective than other formulations. This statement is supported by Sabry and Hussein (2023), who tested the toxicity of conventional and nanoformulations of etofenprox, imidacloprid and indoxacarb against 4th instar larvae of *A. ipsilon*. The nanoetofenprox, nanoimidacloprid and nanoindoxacarb was 5-times, 4-times and 7-times more effective than the normal etofenprox, imidacloprid and indoxacarb, respectively, indicating that nanoformulations used instead of conventional formulations may reduce the amount of insecticides used per treatment/ application and its toxicity on non-target organisms.

1.5.4.1 Influence of larval behaviour on chemical control efficacy

The efficacy of insecticide applications is influenced by larval behaviour and environmental conditions like varying soil moisture levels and the presence of weeds during the winter season (Drinkwater, 1980; Van den Berg & Du Plessis, 2022). Other studies reported that producers often spray their fields with the recommended insecticides, but they did so when the cutworms were already in the late larval stages (Garnis & Dabrowski, 2008; Jakubowksa & Bocianowski, 2013). Furthermore, it is often difficult to effectively use insecticides against soil pests due to problems of distribution of the active ingredients in soil, adsorption or rapid break down, and the

impracticality of applying contact insecticides. Thus, insecticides often provide less control in the soil than above ground (Toepfer *et al.*, 2014).

1.7 Problem statement

Cutworms, particularly *A. segetum* pose a significant threat to maize cultivation in South Africa's Highveld region (Van den Berg *et al.*, 2014). *Agrotis segetum* also affect various other crops such as cotton, sunflower, lucerne, tobacco, and wheat (Drinkwater 2017). The presence of this polyphagous pest is often only noticed after damage have been inflicted (Garnis & Dabrowski, 2008), which may lead to severe stand losses (Du Plessis, 2000), prompting costly replanting efforts for producers. Despite numerous control strategies attempted in the past, managing cutworm populations remains challenging due to their soil-dwelling behaviour. Current control methods primarily rely on timely insecticide applications targeting the immature stages of the insect (Gregg *et al.*, 2018), but efficacy can be hindered by factors like larval behaviour, environmental conditions, and insecticide application timing. Although traditional practices like weed removal and field cultivation were once recommended to mitigate cutworm populations (Blair, 1975; Drinkwater & Walters, 1976; Drinkwater, 1980), the shift towards herbicide-tolerant crops and reduced tillage practices has altered the pest management landscape, exacerbating the problem. Furthermore, Bt maize, which controls other target lepidopteran pest species, is also not effective against this non-target pest (Erasmus *et al.*, 2010).

Insecticide application, while commonly employed, has shown varying levels of success in controlling cutworm populations, with reports over the four previous cropping seasons indicating control failures across the maize production region of South Africa. While there is speculation regarding insecticide resistance (GrainSA, 2023; GrainSA 2024a; GrainSA, 2024b; FW, 2024; FW, 2025), other factors such as larval behaviour and environmental conditions, including soil moisture levels and weed presence, may play a significant role in the efficacy of cutworm control (Drinkwater, 1980, Van den Berg & Du Plessis, 2022). The timing of insecticide application is critical, as cutworm larvae tend to remain below the soil surface to avoid daylight exposure, limiting their contact with applied insecticides. Despite efforts to understand the interplay between soil moisture, larval behaviour, and insecticide efficacy, gaps in knowledge persist, complicating effective control strategies for this persistent

agricultural insect pest. No information is available on the levels of parasitism of this pest in South Africa

1.8 Objectives

1.8.1 Main objective

The overall objective of this study was to improve the understanding of the influence of biotic and abiotic factors in controlling the population of *Agrotis segetum*.

1.8.2 Specific objectives

The objectives are to:

1. Conduct a survey of damage caused by *Agrotis segetum*, tillage practices, weed presence, insecticide use patterns and chemical control practices used against this pest in the grain production region of South Africa.
2. Determine the occurrence and parasitism levels of biological control agents of *Agrotis segetum* present in grain production regions of South Africa.
3. Determine the effect of different soil moisture conditions on *Agrotis segetum* larval behaviour.

The results of this study are presented in the form of chapters with the following titles:

Chapter 2: Damage assessment and practices used to manage *Agrotis segetum* (Lepidoptera: Noctuidae) in maize, soybean and sunflower in South Africa.

Chapter 3: Biological control agents and levels of parasitism of *Agrotis segetum* in grain production regions of South Africa.

Chapter 4: Effect of soil moisture on behaviour of *Agrotis segetum*.

Chapter 5: Conclusion and recommendations.

1.9 References

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CHAPTER 2: Damage assessment and practices used to manage *Agrotis segetum* (Lepidoptera: Noctuidae) in maize, soybean and sunflower in South Africa

Abstract

Over the past four planting seasons, producers reported an increase in pest status of cutworm, *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae) in South Africa. Chemical control failure was often reported and ascribed to possible insecticide resistance. However, tillage practices, pre-planting weed management strategies, insecticide application methods, environmental conditions and the soil-dwelling behaviour of this pest may influence the efficacy of insecticides. It is therefore important to establish if certain agricultural practices are associated with the increased pest status of cutworm and to estimate the economic impact of this pest. A survey was done among farmers within the maize and soybean production region, to assess the economic impact of this pest and if pest incidence was associated with specific cultivation practices, weed management strategies and the amount and type of insecticides applied. Some farmers also reported on sunflower. This study showed that the overall economic impact of cutworm damage over the 150 363 ha that was surveyed, was R44.87 million. There was a tendency that no-till farmers experienced greater damage than conventional farmers, due to greater residue cover and presence of weeds shortly before, or at the time of planting. The incidence of cutworm damage was higher in fields where weed management was done between one and three weeks before planting, compared to the recommended four weeks before planting. Maize was the most affected crop compared to soybean and sunflower, but this study shows, for the first time in South Africa, significant losses to soybean. Results further show a general tendency of poor levels of chemical control and that insecticides were not applied according to label instructions. Further research is needed to determine the role of farming practices on the incidence and severity of *A. segetum* in grain production regions of South Africa.

Key words: cutworm damage, insecticide application, tillage practices, weed management

2.1 Introduction

Of the approximately 300 *Agrotis* species, only *Agrotis ipsilon* (Hüfnagel) and *Agrotis segetum* (Denis & Schiffermüller) are considered economically important (Rings & Musick, 1976; Blair, 1976; Bowden *et al.*, 1983; Showers, 1987; Frank & Shrewsbury, 2004; Chandel *et al.*, 2019; Chandel *et al.*, 2022). In South Africa, *A. segetum* commonly occurs throughout the Highveld region where it causes economically important damage to maize seedlings (Drinkwater, 1980; Du Plessis, 2000; Van den Berg *et al.*, 2014; Drinkwater, 2017). During South Africa's autumn and winter months, cutworm eggs are laid on the soil surface as well as on weeds and crop residues left in fields after harvest. Larvae of different sizes survive the winter by feeding on weeds and hiding within the soil and underneath weeds or crop residues (Drinkwater, 2017).

The availability of winter weeds as food strongly influences the survival, abundance and subsequent damage caused by cutworm larvae (Foster & Ruesink, 1984; Foster & Ruesink, 1986b; Mulder & Showers, 1983). Weeds not only serve as a food source during winter months but also sustain large numbers of larvae of all instars until crops are planted in spring (Blair, 1975; Chandel *et al.*, 2022). The presence and impact of cutworms in crop fields are influenced by tillage practices (Drinkwater, 1980). Crop residues in no-till fields present suitable oviposition sites for moths in the period before the planting season commences (Busching & Turpin, 1976; Sherrod *et al.*, 1979; Phillips *et al.*, 1980; Showers *et al.*, 1985). Larval survival is also increased by the presence of weeds and crop residues because it serves as a shelter from harsh weather conditions (Blair, 1975; Foster & Ruesink, 1984; Engelken *et al.*, 1990). Conventional tillage systems, characterised by fields with exposed soil and minimal presence of weeds and crop residues, typically experience lower densities of cutworm larvae due to reduced shelter and food availability (Foster & Ruesink, 1986b). In contrast, where reduced tillage is practiced, for example where discs are used, moderate levels of infestation may be experienced while no-till fields often harbour the highest densities of cutworms (Schoenbohm & Turpin, 1977; Sherrod *et al.*, 1979; Showers *et al.*, 1985).

In South Africa, insecticide applications is the most widely utilised control strategy for cutworms. Cutworm control failure has often been reported in South Africa's maize-producing region since the pest status of cutworm started to increase from the 2021/22 growing season onwards, leading to concerns regarding the possibility of insecticide

resistance (GrainSA, 2023; GrainSA 2024a; GrainSA, 2024b; FW, 2024; FW, 2025). However, the efficacy of insecticide applications is influenced by a wide range of factors such as larval behaviour and size, environmental conditions, the presence of weedy host plants before planting, and soil moisture conditions during the time of insecticide application (Drinkwater, 1980; Van den Berg & Du Plessis, 2022), which influence the suitability of the habitat (crop field) for insect pest survival.

The reasons for the increased pest status of *A. segetum* in South Africa are unknown. However, changing practices such as reduced winter cultivation related to no-tillage practices, planting of cover crops, and the timing of pre-plant weed control may affect the numbers of larvae that are present in fields when the crop is planted. Since weeds play an important role in larval survival and pest status of cutworms, cultivation of fields to remove weeds has been recommended as a cutworm management tool (Drinkwater, 1980). Previous recommendations were that crop fields must be kept clear of weeds for 35 days (4-6 weeks) before planting to kill larvae by means of starvation and exposing them to natural enemies as well as harsh environmental conditions (Blair, 1975; Drinkwater & Walters, 1976; Drinkwater, 1980).

The aims of this survey were to assess the severity of cutworm damage to maize, soybean and sunflower, to assess whether certain cultivation and weed management practices could be associated with increased pest damage, and to determine the levels of insecticide use, and insecticide use practices for control of *A. segetum*.

2.2 Materials and Methods

2.2.1 Study area

A questionnaire survey was conducted in different geographical areas within the main maize production region of the country, where soybean and sunflower are also cultivated. A large proportion of this region can be considered to be semi-arid. The maize production area was subdivided into three regions (Figure 2.1), based on the general environmental conditions and soil type in these areas (Haarhoff *et al.* 2020). The regions are (i) the Western region (35% of total production), (ii) Eastern region (45%), and (iii) KwaZulu-Natal region (10%). The Western and Eastern regions are on the South African inland plateau (1500 - 1800 m a.s.l.) (Haarhoff *et al.*, 2020) and are characterised by hot summers and very cold winters. The KwaZulu-Natal (KZN) region is characterised by long hot summers and mild winters and has an average annual

rainfall ranging between 500 and 800 mm (Adisa *et al.*, 2018). Climatic conditions in the KZN region are similar to those where maize is produced in the Eastern Cape (EC) province and for the purposes of this study, the data from these two regions were grouped under EC+KZN. The number of respondents to questionnaires were as follows: Western (13), Eastern (41) and EC + KZN (11).

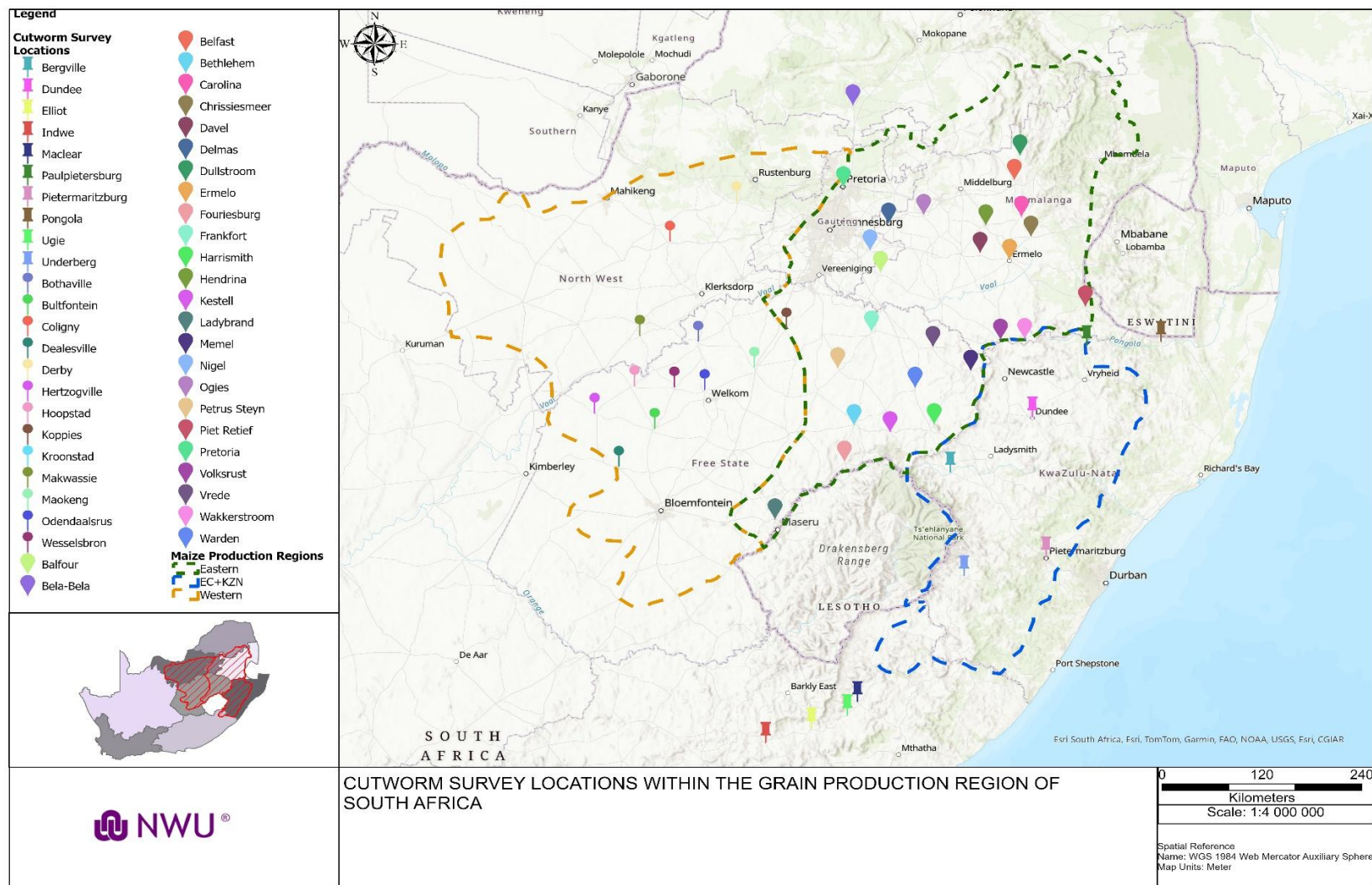


Figure 2.1. A map illustrating the Western (orange), Eastern (green) and EC+KZN (blue) grain production regions in which the cutworm survey was done (Map created by Michael Cloete).

The questionnaire (Appendix A) was created to collect information about agronomic parameters such as cultivation practices, insecticide application methods and the extent of damage related to cutworm infestations for the 2023/2024 season. The questionnaire was provided via a dedicated website, and individual interviews were also done. The original aim was to collect at least 100 responses, but only 65 were submitted.

To provide easy access to the online questionnaire, a QR code (Figure 2.2) was developed and distributed to possible respondents. Since the questionnaire was available in both paper and digital (Survey Monkey) formats, it could be used to do face-to-face interviews in locations with limited internet connection.



Figure 2.2. The QR code distributed to potential respondents to facilitate easy access to the questionnaire.

The contact network of GrainSA was also used to distribute the questionnaire to producers from various regions, ensuring that different geographical areas and farming techniques were represented. While the internet-based version saved time, personal interviews provided clearer responses and improved data quality control. Agrochemical consultants and researchers assisted during the process and provided technical assistance to explain questions without influencing producers' answers.

The wording of questions in relation to possible answers were carefully considered. Respondents were asked for their assessment of cutworm damage based on the cultivation practices, weed management and insecticide application strategies they implemented.

A quality check of the feedback provided on the questionnaires was done. This was based on the objectivity, reliability and validity of certain answers. To check the completeness and correctness of the information provided on the questionnaires, data, quality and plausibility control measures were employed. These consisted of:

1. *A completeness check*: Certain questions within each section were defined as mandatory since the answers were directly related to critical aspects of cutworm management.
2. *A quality check*: Several quality control methods were used to check that data values were correct. Questions regarding quantitative data were formulated in such a way that respondents were provided with a visual scale with a minimum-maximum range. Qualitative data were only collected for certain categories.
3. *A plausibility check*: Data values had to be logical. For example, the planting date had to be after the date of first rainfall. In the case of missing data, those respondents' answers were excluded from calculations.

The survey focused on three themes, i.e., (1) cutworm damage assessment, (2) insecticide application practices, and (3) cultivation and weed management practices.

1. Cutworm damage assessment:

This section focussed on the degree of cutworm damage experienced on crop fields. Producers were requested to provide information on the severity of the infestations, the estimated surface areas that were affected, as well as the areas that had to be replanted. The surface areas impacted by cutworm and the number of hectares that had to be replanted provide an indication of the economic impact of cutworm infestations. The different types of crops damaged by cutworm was also recorded.

2. Insecticide application practices:

Information regarding insecticide application is necessary to determine if producers followed label instructions, which active ingredients were used, at what dosage rates these were applied, and how many insecticide applications were made. The time of day at which insecticide applications were made were recorded to establish whether there was a possible association between poor insecticide performance and time of application, since insecticide efficacy may be influenced by environmental conditions.

3. Cultivation and weed management practices:

Information regarding cultivation and weed management practices were requested to assess whether these practices have an influence on the occurrence of cutworm and severity of damage.

2.3 Data analysis

Questionnaire data were summarised in Excel. Where relevant, the percentages that were calculated were based on the total number of responses to a particular question. The respondents that did not respond to a particular question were excluded from the calculation of percentage values for that question. When a respondent selected more than one answer or gave more than one method to a question, percentages were calculated for each group or similar answers.

2.4 Results

A summary of the questionnaire data is provided below. All the respondents (n = 65) were male, which, in South Africa, is the norm on commercial farms. Sufficient data were recorded on cutworm damage and cultivation practices of maize, soybean and sunflower. One respondent provided information regarding cutworm damage experienced on cotton fields. For this reason, only data regarding maize, soybean and sunflower are presented in the tables below. Other crops that were reported to be attacked were dry beans (*Phaseolus vulgaris*), potato (*Solanum tuberosum*), and ground nut (*Arachis hypogaea*).

2.4.1 Cutworm damage assessment

A summary of cutworm damage data is provided in Table 2.1. Of the 65 respondents, 89.1% indicated that they experienced cutworm damage during the 2023/2024 planting season.

Overall, 79.9% respondents reported that they experienced high incidences of cutworm damage in maize fields, followed by 41.5% for soybean. Stand losses ranged between 30.8 and 81.8% for maize, and 30.8 and 48.8% for soybean. In the Eastern region, stand loss of sunflower was low (2.4%). Overall, 36.3% of the respondents indicated that it was necessary to replant their crops due to cutworm damage. In EC+KZN region, 54.5% of respondents had to replant their fields. In the Eastern and

Western regions, 39.0% and 15.4% of respondents respectively indicated that it was necessary to replant.

Table 2.1. Mean percentage respondents indicating cutworm damage to crops during the 2023/2024 planting season in the three grain production regions of South Africa.

Question	Region			
	Eastern	Western	EC+KZN	Mean
Number of respondents	41	13	11	
Did you experience cutworm damage?	%	%	%	%
Yes	90.2	76.9	100.0	89.1
No	9.8	23.1	-	10.9
Were affected fields under dryland or irrigation?				
Dryland	92.7	100.0	63.6	85.4
Irrigation	2.4	-	9.1	3.8
Both	4.9	-	27.3	10.7
On which crops was cutworm damage experienced?				
Maize	85.4	53.8	100.0	79.7
Soybean	56.1	23.1	45.5	41.5
Sunflower	4.9	23.1	-	9.3
On which crops were stand loss experienced?				
Maize	73.2	30.8	81.8	61.9
Soybean	48.8	30.8	36.4	38.6
Sunflower	2.4	15.4	-	5.9
Was it necessary to replant?				
Yes	39.0	15.4	54.5	36.3
No	61.0	84.6	45.5	63.7

- Indicates no respondents provided information for the relevant question

The areas (ha) planted to the different crops and the areas that had to be replanted due to cutworm damage are provided in Table 2.2. Twelve percent of the area planted to maize was affected by cutworms, followed by 12.9% for soybean and 19.4% for sunflower. Of these affected areas, 3.9% and 4.5% had to be replanted for maize and

soybean respectively. No respondents indicated that they had to replant sunflower. The production region in which the incidence of cutworm damage to maize was the highest, was the Eastern region where 15.6% of the maize area and 22.1% of the soybean area was affected.

Table 2.2. Mean areas (ha) of crops planted, affected and replanted during the 2023/2024 planting season due to *Agrotis segetum* damage per production region.

Region	Planted	Affected	Replanted
Maize (ha)			
Eastern	45 155	7057	2457
Western	31 950	1905	200
KZN	24 461	3221	1303
Total Maize	101 566	12 183	3960
Soybean (ha)			
Eastern	19 388	4279	888
Western	14 760	400	150
KZN	12 846	1375	1064
Total Soybean	46 994	6054	2102
Sunflower (ha)			
Eastern	415	0	0
Western	1388	350	0
KZN	-	-	-
Total Sunflower	1803	350	0

- No data collected since sunflower is not produced in this region

In the Eastern (92.7%) and Western region (100.0%), most respondents reported that they cultivated crops under dryland conditions. In the EC+KZN region, 63.6% of the respondents cultivated their crops under dryland farming conditions, while 27.3% practised irrigation. Data on the date of the first rain event that was sufficient for planting to commence, as well as the date of planting of maize and soybean per region are provided in Figure 2.3. In the Eastern region, most respondents indicated that the period between 22 and 28 September 2023 was the week in which sufficient rain fell, and that most of the planting was done between 22 September and 10 November 2023. In the Western region, planting was done over a long period, from 22 October to 8 December 2023. In the KZN region, most respondents indicated the first sufficient

rain to allow for planting fell between 13 and 19 October 2023. Most of the planting in this region was done between 13 October and 9 November 2023.

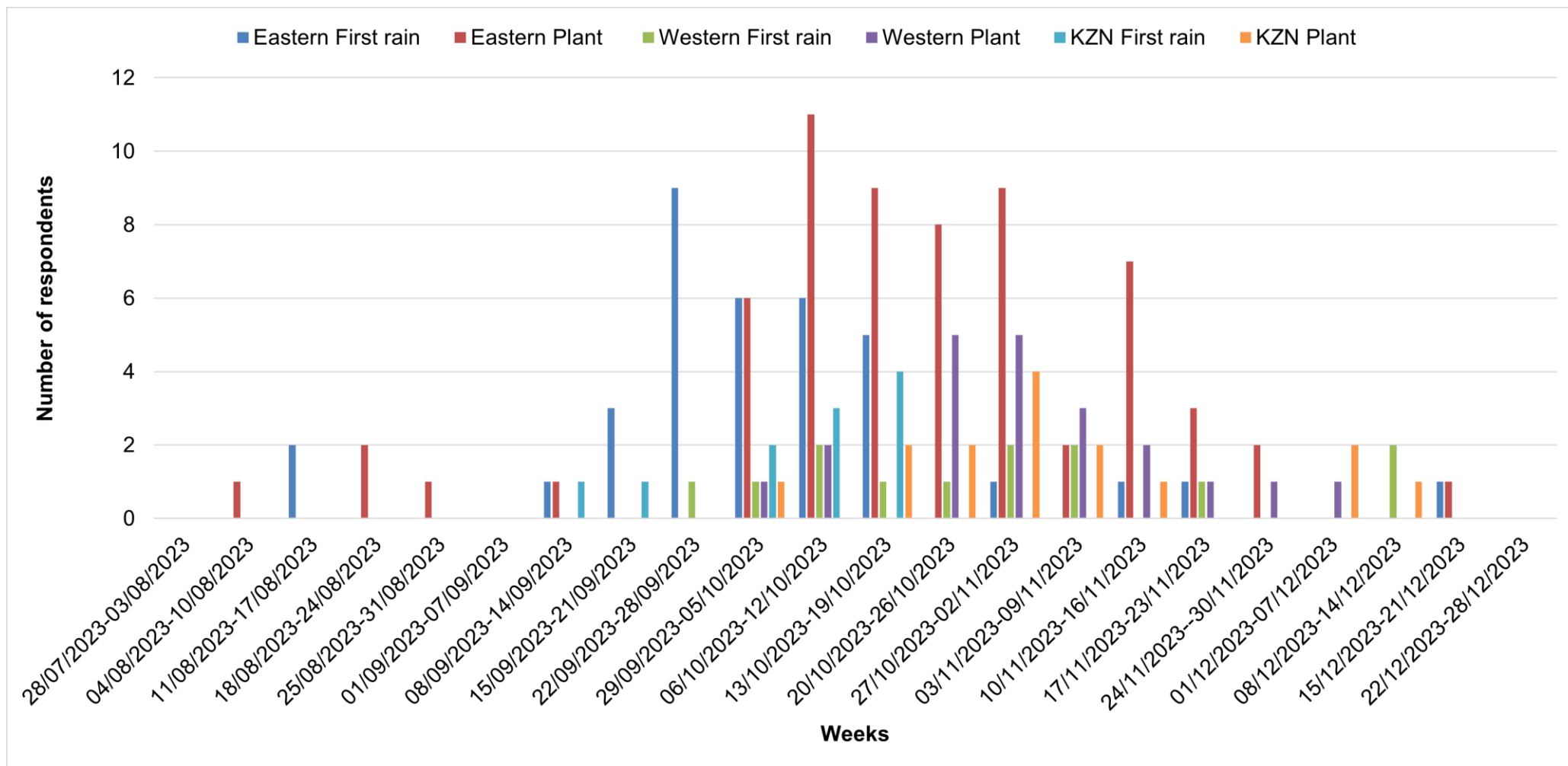


Figure 2.3. The dates of first sufficient rain for planting, and planting date per region during the 2023/2024 season.

2.4.2 Insecticide application practices

A summary of data on insecticide application practices is provided in Table 2.3. The incidence of respondents in the different regions that applied insecticides ranged between 90.9% in the Western region and 100% in the Eastern and EC+KZN regions (overall mean 97.0%). All respondents that made insecticide applications did so with tractor-mounted sprayers. Most respondents applied insecticides at planting (64.4%), while 36.0% indicated that they made applications shortly after planting. In the EC+KZN region, most respondents (87.5%) applied insecticide after planting.

Overall, 94.8% of respondents indicated that the target site of insecticide applications was on the soil surface during planting (Table 2.3). In the Western region, 100.0% of respondents indicated that they applied insecticides onto the soil surface with planting, whereas in the Eastern region, both foliar and in-furrow applications were done by a few respondents (6.1%). Overall, 83.8% of respondents indicated that insecticides were applied during the late-morning and afternoon (10:00-16:00).

Overall, most respondents (70.5%) indicated that the soil moisture during the first insecticide application was wet (damp) (Table 2.3). Overall, 66.3% responded 'yes' when asked if they considered the insecticide applications to be effective. In the Western region, 90.9% of respondents considered applications to be effective.

While 52 respondents indicated that they applied insecticides, only 16 (30.7%) applied a follow-up application. The number of days between the first and follow-up applications ranged between 7 and 30 days (data not shown).

Information on the different insecticides that were used for cutworm control is provided in Table 2.4. Between 58.0 and 63.5% of respondents applied lambdacyhalothrin for cutworm control in sunflower, soybean and maize.

Table 2.3. Mean percentage respondents indicating insecticide use and application practices per region during the 2023/2024 planting season of South Africa.

Information	Region			
	Eastern	Western	EC+KZN	Mean
Number of respondents	33	11	8	
Did you apply insecticides specifically for cutworm control?	%	%	%	%
Yes	100.0	90.9	100.0	97.0
No	-	9.1	-	3.0
Application method				
Tractor sprayer	100.0	90.9	100.0	97.0
Applications were done...				
As a seed treatment	6.1	-	25.0	10.4
Before planting	27.3	18.2	62.5	36.0
With planting	72.7	45.5	75.0	64.4
After planting	36.4	45.5	87.5	56.4
What was the target-site of the insecticide application(s)?				
In-furrow	6.1	-	37.5	14.5
On soil surface with plant	97.0	100.0	87.5	94.8
Foliar	6.1	-	25.0	10.4
During which time of the day were applications done?				
Morning (06:00-10:00)	72.7	54.5	75.0	67.4
Afternoon (10:00-16:00)	87.9	63.6	100.0	83.8
Evening (16:00-23:00)	39.4	63.6	50.0	51.0
The soil moisture during first insecticide application was...				
Dry	15.2	36.4	12.5	21.3
Wet (damp)	81.8	54.5	75.0	70.5
Very wet	3.0	9.1	12.5	8.2
In your opinion, was the application effective?				
Yes	45.5	90.9	62.5	66.3
No	54.5	9.1	37.5	33.7

- Indicates no respondents provided information for the related question

Table 2.4. Percentage respondents indicating which insecticides were used for *Agrotis segetum* control in maize, soybean and sunflower during the 2023/2024 planting season per region. – indicates that no respondents provided information for the relevant question.

Insecticides applied (class)*	Region			
	Eastern	Western	EC+KZN	Mean
Maize: number of respondents	32	8	8	
lambdacyhalothrin (pyrethroid)	78.1	50.0	62.5	63.5
gammacyhalothrin (pyrethroid)	6.3	25.0	-	10.4
alphacypermethrin (pyrethroid)	3.1	-	-	1.0
indoxacarb (oxadiazine)	3.1	-	12.5	5.2
betacypermethrin (pyrethroid)	-	-	12.5	4.2
esfenvalerate (pyrethroid)	9.4	50.0	-	19.8
cypermethrin (pyrethroid)	-	-	12.5	4.2
chlorantraniliprole (anthranilic diamide)	-	-	12.5	4.2
deltamethrin (pyrethroid)	-	-	-	-
thiamethoxam (neonicotinoid)	-	-	-	-
Soybean: number of respondents	21	6	5	
lambdacyhalothrin (pyrethroid)	81.0	66.7	40.0	62.5
gammacyhalothrin (pyrethroid)	4.8	-	-	1.6
chlorantraniliprole (anthranilic diamide)	-	-	20.0	6.7
indoxacarb (oxadiazine)	-	-	20.0	6.7
alphacypermethrin (pyrethroid)	4.8	-	-	1.6
esfenvalerate (pyrethroid)	14.3	33.3	-	15.9
deltamethrin (pyrethroid)	-	-	20.0	6.7
thiamethoxam (neonicotinoid)	-	-	20.0	6.7
Sunflower: number of respondents	2	4	-	
lambdacyhalothrin (pyrethroid)	100.0	75.0	-	58.3
esfenvalerate (pyrethroid)	-	25.0	-	8.3

*class= insecticide class according to IRAC (2024).

Insecticide labels provide clear instructions regarding water volumes and dosage rates as well as adjuvants that may be required for insecticide applications (Agri-Intel, 2023). The insecticide label information on pyrethroid products for cutworm control in South Africa reads as follows: *“Cutworms tend to feed sub-surface when the soil surface is dry. Damage to seedlings is usually not visible until the plants start to wither. When planting in dry soil, or when the soil shortly after planting rapidly desiccates, poor control of cutworms can be expected, since the pest does not come into contact with the product applied to the soil surface. Follow-up application will not necessarily ensure control, unless the soil surface is moist.”* For row-treatments, label instructions are as follows: *“Apply post-emergence to the crop as soon as the pest is noticed, in at least 3 litres water per 100-meter row. Apply in a band at least 30 cm wide over the row. Ensure that soil is moist right to the surface at the time of application. Later infestation may require a second application. For overall ground applications, 300 L of water should be used per hectare.”*

In this study, the water volume used varied between 75 and 200 L/ha, while dosage rates ranged between 35 and 500 ml/ha (data not shown). On average, respondents applied twice the recommended insecticide dosage rate, with one respondent applying 11 times the recommended dosage. In response to the question whether buffers were added to insecticide tank mixes, responses were equally divided with 50.0% indicating that they added a buffer while the other 50.0% did not.

Seed planted in commercial farming systems in South Africa are purchased from commercial companies and the standard practice is that seed treatment against diseases and beetle pests are applied by the company. Since it was not possible to assess which crop hybrids were planted and which seed treatments were used, only general data are provided. Of the 65 respondents, 52.3% reported that their seed was treated with insecticides. The active ingredients of seed treatments that were reported in this study, and which are not registered for cutworm control, were the following: fludioxonil, mefenoxam, azoxystrobin, thiabendazole, imidacloprid, thiamethoxam, clothianidin, prothioconazole and lipo-chito oligosaccharide.

2.4.3 Cultivation and weed management practices

A summary of data on cultivation and weed management practices are provided in Table 2.5. Overall, 55.8% of respondents indicated that they made use of conventional

clean cultivation. Fewer respondents followed no-till practices (29.5%) and practiced conservation agriculture (13.1%). In the Western region, the incidence of respondents that used conventional clean cultivation was high (85.7%). In the Eastern region, no-till practices were more common (40.9%).

Respondents were asked to indicate the estimated residue cover (%) on the fields at the time of planting. A guideline based on the photo comparison-method to estimate crop residue cover (Shelton *et al.*, 1995) was provided to respondents in the survey questionnaire (Table 2.6). Overall, most respondents (47.1%) indicated that crop residue cover at the time of planting was between 26-50%. The incidence of respondents that reported crop high residue cover (76-90%) was low (4.5%) in the Eastern region.

Table 2.6. A photo comparison-method used to estimate crop residue cover (adapted from Shelton *et al.*, 1995).

Option	Maize	Soybean
0%	Fields were bare	Fields were bare
1-25%		
26-50%		
51-75%		
76-90%		

The overall percentage of respondents that applied mechanical weed control was 54.7% and those that made use of chemical burndown practices was 52.4%. The number of days prior to planting on fields where mechanical control was done, ranged between 1 and 58 days, while the number of days between planting and herbicide application ranged between 1 and 42 days.

Table 2.5. Cultivation and weed management-related data for the 2023/2024 planting season per region.

Information	Region			
	Eastern	Western	EC+KZN	Mean
Number of respondents	22	7	6	
Which cultivation method was used?	%	%	%	%
Conservation agriculture	22.7	-	16.7	13.1
Conventional clean cultivation	31.8	85.7	50.0	55.8
No-till	40.9	14.3	33.3	29.5
The estimated (%) residue cover on the field during planting was...				
0% (bare fields)	-	-	-	-
1-25%	13.6	28.6	-	14.1
26-50%	31.8	42.9	66.7	47.1
51-75%	40.9	28.6	33.3	34.3
76-90%	4.5	-	-	1.5
What type of weed management was applied before planting?				
Mechanical	54.5	42.9	66.7	54.7
Herbicide with plant	4.5	-	-	1.5
Preplant burndown	50.0	57.1	50.0	52.4
None	4.5	-	-	1.5

- Indicates no respondents provided information for the related question

Of the 53.9% respondents that indicated that they did winter weed cultivation for cutworm control, 5.7% and 62.9% ploughed and disked their fields respectively (data not shown). Fifty percent of respondents indicated that they planted Bt maize while 52.9% indicated they planted only herbicide tolerant maize hybrids.

The most dominant weed species in crop fields were *Conyza* spp. (54.5% of respondents), Grass spp. (48.5%) and *Amaranthus* spp. (33.3%) (Table 2.7). The specific grass species present in affected fields were not indicated.

Table 2.7. Dominant weed species in affected crop fields ranked by 33 respondents.

Ranking of dominant weed species			
Region	Nr. 1	Nr. 2	Nr. 3
Eastern	<i>Amaranthus</i> sp.	Grass	Not indicated
	<i>Pseudognaphalium</i> sp.	<i>Conyza</i> sp.	Not indicated
	Grass	<i>Conyza</i> sp.	<i>Xanthium</i> sp.
	<i>Eriocephalus</i> sp.	<i>Senecio</i> sp.	Not indicated
	<i>Conyza</i> sp.	<i>Senecio</i> sp.	Grass
	<i>Conyza</i> sp.	<i>Amaranthus</i> sp.	<i>Senecio</i> sp.
	<i>Amaranthus</i> sp.	<i>Portulaca</i> sp.	Grass
	<i>Conyza</i> sp.	<i>Senecio</i> sp.	Not indicated
	<i>Senecio</i> sp.	Not indicated	Not indicated
	<i>Tagetes</i> sp.	<i>Cyperus esculentus</i>	<i>Amaranthus</i> sp.
	<i>Conyza</i> sp.	<i>Ipomoea</i> sp.	<i>Tagetes</i> sp.
	<i>Conyza</i> sp.	<i>Ipomoea</i> sp.	<i>Amaranthus</i> sp.
	<i>Conyza</i> sp.	<i>Pseudognaphalium</i> sp.	Not indicated
	<i>Conyza</i> sp.	<i>Amaranthus</i> sp.	<i>Bidens</i> sp.
	Grass	Not indicated	Not indicated
	<i>Conyza</i> sp.	Not indicated	Not indicated
	<i>Portulaca</i> sp.	<i>Amaranthus</i> sp.	<i>Cyperus esculentus</i>
	<i>Chenopodium</i> sp.	<i>Amaranthus</i> sp.	Not indicated
	Grass	<i>Conyza</i> sp.	<i>Cyperus esculentus</i>
	<i>Cyperus esculentus</i>	<i>Ramanas</i> sp.	<i>Chenopodium</i> sp.
	<i>Conyza</i> sp.	<i>Conyza</i> sp.	<i>Conyza</i> sp.
EC+KZN	Grass	Grass	<i>Conyza</i> sp.
	<i>Portulaca</i> sp.	<i>Rapistrum</i> sp.	<i>Cyperus esculentus</i>
	<i>Parthenium</i>	<i>Amaranthus</i>	<i>Conyza</i> sp.
	<i>Conyza</i> sp.	<i>Conyza</i> sp.	Grass
	<i>Conyza</i> sp.	<i>Bidens</i> sp.	<i>Cyperus esculentus</i>
Western	<i>Tribulus</i> sp.	<i>Portulaca</i> sp.	Grass
	<i>Portulaca</i> sp.	<i>Amaranthus</i> sp.	<i>Cyperus esculentus</i>
	Grass	<i>Bidens</i> sp.	<i>Datura</i> sp.
	Grass	<i>Conyza</i> sp.	<i>Taraxacum</i> sp.
	Grass	<i>Cyperus esculentus</i>	<i>Commelina</i> sp.
	Grass	<i>Portulaca</i> sp.	<i>Emex australis</i>
	<i>Amaranthus</i> sp.	Grass	Grass

2.4.4 Tendencies

The questionnaire data was further interpreted to determine possible trends and potential links between farming practices and cutworm damage. To ensure reliable assumptions, only data from respondents who completed the questionnaire in full and reported > 60 hectares of damage to maize or > 50 hectares to soybean crops during the 2023/2024 planting season, were included. In the section below, information provided on the separate topics above were summarized for the qualifying 20 maize and 12 soybean farmers, and grouped to focus on three major associations per region: (1) cutworm damage in relation to tillage practices, (2) cutworm damage in relation to target insecticide application site, time of insecticide application and active ingredient sprayed, and (3) cutworm damage in relation to weed presence. The highest incidence of cutworm damage in maize fields occurred in the Eastern region (80.0%), followed by EC+KZN (15.0%) and the Western regions (5.0%). For soybeans, the most affected fields occurred in the Eastern region (91.7%), followed by EC + KZN (8.3%).

1. Cutworm damage in relation to tillage practices

Overall, 35.0% of these respondents practiced no-till, 35.0% conventional clean cultivation of maize fields, while 30.0% followed conservation agriculture practices. Most respondents relied on disking and only 10.0% used ploughing for winter cultivation practices. The crop residue cover left on fields at the time of planting ranged between 17 and 72.0%. Mechanical weed control was used by 60.0% of the respondents but 50.0% reported that they also made use of chemical burndown of weeds. The number of days that expired between pre-plant weed control and planting of maize ranged from 1 to 58 days (mean = 14 days).

Most of the affected soybean fields were in the Eastern region and cultivated under dryland conditions (91.7%). No-till and conservation agricultural practices were employed by 50.0% and 33.3% of these respondents, respectively. Only 16.7% used conventional clean cultivation practices and 72.7% used disking for winter weed cultivation. The percentage of crop residues left on fields during the planting process ranged between 20 and 72.0%. The weed management practices were dominated by chemical burndown (58.3%) and mechanical control (50.0%) and some respondents applied herbicides during planting (12.0%). The number of days that expired between

winter weed management and planting date of soybean ranged from 0 to 58 days (mean = 15 days).

2. Cutworm damage in relation to target site, time of insecticide application and active ingredient sprayed

The majority of respondents (80.0%) indicated that they applied insecticides when the soil was wet (damp) and only 10.0% applied on dry soil and 5.0% on wet soil. Nearly all applications were made onto the soil surface during planting but in-furrow applications with planting were also done by 10.0% of respondents. Most respondents (90.0%) applied insecticides with planting, followed by 60.0% that applied insecticides after planting. The most applied insecticides were lambdacyhalothrin (60.0%) and esfenvalerate (20.0%). Only 55% of respondents perceived these insecticide applications to be ineffective.

In the case of soybean, most respondents (91.7%) indicated that they applied insecticides when the soil was wet (damp) and all applied insecticides onto the soil surface during planting. Most respondents (91.7%) applied insecticides with planting and the most frequently applied insecticide was lambdacyhalothrin. Most respondents (66.7%) reported that the insecticide applications were effective.

3. Cutworm damage in relation to weedy host plants

The most dominant weed species in maize and soybean were *Conyza* sp. and Grass spp. Other frequently observed weeds included *Cyperus esculentus* and *Amaranthus* sp. Less common weeds were *Ipomoea* sp., *Tagetes* sp., and *Chenopodium* sp.

2.5 Discussion

Although the number of respondents to this survey was low, the results represent data collected from a total surface area of 150 363 ha, of which 101 566 ha was under cultivation of maize and 46 994 ha under soybean. Because of the small area reported for sunflower and cotton in this study, the quantitative results are not further discussed because it is not representative of these industries in South Africa. Cognisance should be taken of the fact that the response rate was low, and it is highly likely that those that responded to the survey, were ones that experienced cutworm damage during the 2023/2024 planting season. The results of this study can, therefore, not be considered

as representative of the whole grain production region of South Africa but can be used as an indication of the extent of damage cutworms can cause, if left unmanaged.

2.5.1 Assessment of the economic impact of cutworm damage

This study showed that cutworms are an important pest of especially maize and soybean in South Africa. Results showed that 12.0% of the maize surface area under cultivation by the 65 respondents was affected by cutworm and that 3.9% had to be replanted. Overall, respondents reported stand loss on 12 183 ha in maize fields and 6 054 ha in soybean fields. In total, 6 062 ha of maize and soybean had to be replanted. Although Prinsloo and Uys (2015) reported cutworm as an occasional pest of many crops in South Africa, including soybean and sunflower, this study reports for the first time that soybean and sunflower are also greatly affected by cutworms. *Agrotis segetum* was also previously reported to be a major pest of maize seedlings (Drinkwater, 1980; Du Plessis, 2000; Van den Berg *et al.*, 2014; Drinkwater, 2017). This study does, however, facilitate the estimation of the economic losses associated with cutworm damage to maize and soybean in South Africa.

In South Africa, Du Plessis (2000), Van den Berg *et al.* (2014) and Drinkwater (2017) all reported that cutworm damage can be of such an extent that it necessitates replanting of maize seedlings. Based on recent and available data from GrainSA (2025a, 2025b) and BFAP (2024), an estimated cost of replanting was calculated (Table 2.8).

Table 2.8. Estimated cost for replanting of maize and soybean in the Eastern, Western and EC+KZN grain production regions. Calculations were based on available input cost data from GrainSA (2025a, 2025b) and BFAP (2024).

Region	Eastern		Western		EC + KZN	
	Maize	Soybean	Maize	Soybean	Maize	Soybean
Replanted area (ha)	2457	888	200	150	1303	1604
Replant expenditures per ha						
Seed	R3 880,91	R1 625,76	R1 998,88	R1 671,82	R3 709,00	R1 760,00
Fuel	R1 419,70	R941,60	R1 696,75	R1 583,07	R1 479,00	R897,00
Insecticide	R703,17	R824,16	R821,60	R1 591,77	R931,00	R407,00
Total	R6 003,78	R3 391,52	R4 517,23	R4 846,66	R6 119,00	R3064,00
Total replanting cost						
ha replanted x cost/ha	R14 751 287,46	R3 011 669,76	R903 446,00	R726 999,00	R7 973 057,00	R3 260 096,00

The economic impact of replanting of maize and soybean after cutworm-inflicted stand losses on the surface areas indicated in this study illustrates the importance of this pest. The overall economic losses due to replanting during the 2023/24 season was estimated at R 30 626 555,22. These losses do not include direct expenses in terms of insecticide application costs. If the assumption is that at least a single insecticide application was made with planting to prevent cutworm damage, it could be estimated that the overall cost of only a single insecticide application on the total maize and soybean affected area of 18 587 ha, would have amounted to R14 249 083,33. It is therefore estimated that the overall economic loss experienced on the total surface area of 150 363 ha represented in this study, was R44 875 638,55.

Although the incidence of cutworm-damaged plants and stand loss were not quantified at field level in this study, such results were reported for cutworms from other regions of the world. In India, Sidhu *et al.* (2019) reported the incidence of damaged maize seedlings caused by *Agrotis* spp. ranged between 10.0 and 28.0%. Another cutworm species, *A. ipsilon*, was reported as a major pest of soybean in India (Biswas, 2013) as well as in South America where stand loss of up to 100% may occur (Aquino *et al.*, 2022). In Illinois (USA), Levine *et al.* (1983) reported stand loss between 39 and 100.0% due to *A. ipsilon* attack. In Kenya, up to 50.0% of stand loss of sunflowers were experienced and a single larva was reported to damage two to five seedlings per night (Khaemba & Mutinga, 1982).

2.5.2 Insecticide application practices

In this study, 97.0% of respondents applied insecticides in an attempt to control cutworms, but 33.7% did not experience effective control. Poor control may be ascribed to application techniques, environmental conditions and larval behaviour. Lambdacyhalothrin was the most used pyrethroid. In South Africa, most of the products registered for cutworm control are pyrethroids (Agri-Intel, 2023). However, this study showed that respondents also applied non-registered insecticides of other classes for cutworm control, for example, thiamethoxam (neonicotinoid), chlorantraniliprole (anthranilic diamide) and indoxacarb (oxadiazine). Studies in other countries have suggested that the effectiveness of pyrethroids is variable. In Zimbabwe, Blair (1991) found that increased concentrations (8 times the recommended dosage) of lambdacyhalothrin did not increase mortality rates of

cutworms but rather had a repellent than lethal effect on 3rd and 4th instar *A. segetum* larvae.

In Egypt, other insecticides were reported to be more effective than pyrethroids against 4th- and 5th-instar larvae of *A. ipsilon* (Abd-El-Aziz *et al.*, 2019; Massoud *et al.*, 2024). Ab-El-Aziz *et al.* (2019) reported that lambdacyhalothrin was effective and killed 88.2% of *A. ipsilon* larvae in potato fields while chlorantraniliprole and lambdacyhalothrin + thiamethoxam tank mixtures killed > 91% of larvae. Massoud *et al.* (2024) reported that the efficacy of cypermethrin was low (21.61% mortality), compared to profenofos, alphacypermethrin and indoxacarb which resulted in the death of >71% of larvae. The poor control observed by respondents after pyrethroid applications cannot be explained. Many factors influence the efficacy of insecticides at field-level. These factors include larval behaviour and size, environmental conditions, the presence of weeds and soil moisture conditions at the time of insecticide application (Drinkwater, 1980; Van den Berg & Du Plessis, 2022).

Seed treatment insecticides, imidacloprid, thiamethoxam or clothianidin (systemic neonicotinoids), were used by 52.3% of respondents in this study. These insecticides are routinely used for control of coleopteran pests of maize and soybean in South Africa and are not registered for cutworm control. Studies in other countries reported effective control *A. ipsilon* and another lepidopteran pest of maize seedlings, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) with seed treatments (Zhang *et al.*, 2019; Oliveira *et al.*, 2022; Chinwada *et al.*, 2023; Utono & Adamu, 2023). In China, a greenhouse study revealed that cyantraniliprole (diamide) used as a seed treatment was highly effective against 4th-instar *A. ipsilon* larvae, causing >92% mortality. Furthermore, Zhang *et al.* (2019) reported that clothianidin provided some control (44.2 - 59.6%) of *A. ipsilon*, whereas cyantraniliprole was more efficient (80.6 - 87.2%). In Brazil, Oliveira *et al.* (2022) assessed chlorantraniliprole, cyantraniliprole and thiodicarb + imidacloprid (carbamate + systemic insecticide) as seed treatments against *S. frugiperda* and reported >80% of 5th-instar larvae were killed.

This study showed that respondents did not always adhere to pesticide label recommendations. For example, the water volume used was between 75 and 200 L/ha, which is lower than the general recommended water volume which, depending on the label, is between 200 and 500 L/ha. Likewise, depending on the insecticide, the

recommended dosage is between 35 and 70 ml/ha, but respondents indicated they applied between 35 and 500 ml/ha, with majority of respondents applying dosages higher than recommended. The use of lower than recommended water volumes could also have contributed to poor control.

Insecticide labels also indicate the ideal time of the day, and the preferred soil moisture conditions when insecticides should be applied. For cutworm control, the general recommendation is that soil must be prepared, and that the top 3 cm must be moist at the time of application. In this study, most respondents indicated that soil was wet (damp) when insecticides were applied onto the soil surface with planting.

Respondents further indicated that the time at which applications were made was mostly during between 10:00 and 16:00. According to Haarhoff *et al.* (2020) and Adisa *et al.* (2018), the Western, Eastern and EC+KZN regions in South Africa are characterised by hot summers. Studies have confirmed that UV light significantly accelerates pyrethroid breakdown (Hussain *et al.*, 1990; Katagi, 1991; Djouaka *et al.*, 2018). In Europe, Hussain *et al.* (1990) reported that more than 90.0% of deltamethrin degraded within six hours when exposed to simulated sunlight at 35°C. Similarly, in West Africa, Djouaka *et al.* (2018) evaluated the degradation of lambdacyhalothrin in lettuce and cabbage fields and found that 50.0% degraded within 20 minutes of exposure to direct sunlight, and after 6 hours, no residues were detected. Additionally, in Japan, Katagi (1991) reported that UV radiation accelerated pyrethroid degradation on soil surfaces and that soil type and characteristics also affected break down rate. This suggests that the efficacy of insecticide applications made during the warm/hot period of the day, could be adversely affected by the high soil temperatures that are experienced in the grain production regions of South Africa. For example, a study conducted in agricultural soils at Bloemfontein, South Africa, reported that the maximum soil surface temperature at 15:00 can be as high as 34.17 °C (Mengistu *et al.*, 2018).

Larval size and behaviour may further influence the efficacy of insecticide applications. The first three instars of *A. segetum* are photopositive and feed on leaves of weeds and maize seedlings (Blair, 1975; Showers *et al.*, 1979; Bowden *et al.*, 1983; Levine *et al.*, 1983). However, their behaviour changes towards the end of the third instar when they become photonegative (Blair, 1975; Garnis & Dabrowski, 2008;

Jakobowksa & Bocianowski, 2013; Drinkwater, 2017). From the late 3rd-instar onwards, larvae start to hide in the soil during the day and emerge at night in search of food (Blair, 1975; Drinkwater, 1980; Bowden *et al.*, 1983; Du Plessis 2000; Garnis & Dabrowski, 2008; Guo *et al.*, 2015; Chandel *et al.*, 2019; Sidhu, 2019; Chandel *et al.*, 2022). In Egypt, Massoud *et al.* (2024) reported that *A. ipsilon* tend to hide deeper in the soil during the day, evading direct contact with soil-applied insecticides.

One possible contributor to the poor control achieved after insecticide application could be that soil was too wet during application time, and that this could have influenced larval behaviour. In this study, most respondents indicated that soil was wet at the time of insecticide application. The effect of soil moisture on efficacy of insecticide applications against *A. segetum* is unknown, but it could influence larval behaviour which could determine the degree to which larvae make contact with soil-applied insecticides when they appear aboveground during nighttime. In Europe, several studies reported the effect of soil moisture on *A. segetum* larval survival. *Agrotis* larvae that are in the L4, L5 and L6 stages have an increased tolerance for high soil moisture levels (Esbjerg, 1989; Esbjerg & Lauritzen, 2010; Esbjerg & Sigsgaard, 2019). However, L1 to L3 larvae are less tolerant to high soil moisture levels (Esbjerg *et al.*, 1986; Esbjerg, 1989; Esbjerg & Vidal, 2003). Other authors reported that in cases when the soil is extremely dry, cutworms tend to damage seedlings below the soil surface (Johnson *et al.*, 1984; Du Plessis, 2000) where they do not come into contact with soil-applied insecticides.

2.5.3 Cultivation and weed management practices

In this study, respondents' tillage practices varied, but there was a tendency that increased cutworm pest status was associated with no-till and conservation agriculture practices. Respondents who indicated their fields were covered with 26-50% crop residue cover were also those that experienced greater cutworm damage. These findings are consistent with previous studies. In the United States, *A. ipsilon* are more attracted to no-till fields due to the availability of winter weeds as oviposition sites and the shelter provided by crop residues (Showers *et al.*, 1985; Schoenbohm & Turpin, 1977; Sherrod *et al.*, 1979). A study conducted in the United States reported that moths prefer to lay eggs in residue-covered fields over conventionally tilled fields with bare soil (Busching & Turpin, 1976; Showers *et al.*, 1985). Additionally, crop residues may enhance larval survival by sheltering them from adverse environmental conditions

(Foster & Ruesink, 1984). However, Blair (1975) reported that L4-L6 larvae cannot survive on dry or moist maize stalks for more than 10 days and that they use winter weeds to survive until maize seedlings become available to feed on. This suggests that cutworm populations in no-till fields may persist primarily due to the presence of weeds that provide an alternative food supply prior to crop emergence. As a result, fields with high residue cover and a high abundance of weeds shortly before planting, are likely to provide suitable conditions for cutworm population establishment, resulting in high pest pressure. These findings emphasise the significance of combining tillage techniques with weed control strategies to reduce cutworm infestations in conservation and no-till systems.

In this study, the most prevalent weeds were *Conyza* spp. (54.5%), Grass species (48.5%) and *Amaranthus* spp. (33.3%). In the United States, it was reported that the availability of weeds as alternative food sources for cutworms strongly influences their abundance, survival and subsequent damage caused by larvae (Mulder & Showers, 1983; Foster & Ruesink, 1984; Foster & Ruesink, 1986b; Norris & Kogan, 2000; Monnig *et al.*, 2007). The presence of dominating weed species in fields where cutworm larvae did damage to crops indicate that these plants may have supplied food and shelter prior to and during crop emergence. This agrees with the findings of Busching and Turpin (1976), and Engelken *et al.* (1990), who observed increased development rates and survival of larvae on weed species compared with survival on maize seedlings. Furthermore, the high prevalence of *Conyza* spp. and grasses in crop fields may have provided attractive oviposition sites for moths in the period prior to planting. Studies in the United States has shown that moths prefer to lay eggs on low-growing weeds and in grassy areas rather than on bare soil (Busching & Turpin, 1976; Esbjerg & Lauritzen, 2010).

Respondents in this study made use of chemical burndown and mechanical weed management practices which they applied at an average of 14 days before planting. The absence of weedy host plants over this 2-week period due to mechanical control could therefore have been too short to starve larvae to death before maize and soybean seedlings emerged from the soil. Furthermore, in the case of weed burndown by means of herbicides, weed suitability as larval food source slowly decline over a 2-week period and would also not be sufficient to let larvae starve to death before crop seedlings emerge. In South Africa, recommendations to mitigate the risk of cutworm

infestations are that weeds should be removed from fields at least 4-6 weeks before planting, in order to starve larvae (Drinkwater & Van Rensburg, 1992) and expose them to natural mortality factors such as predation from birds and environmental stresses such as heat or frost (Blair, 1975; Drinkwater & Walters, 1976; Drinkwater, 1980). This highlights the importance of pre-plant weed management scheduling in cutworm control since weed removal too close to planting and seedling emergence may exacerbate damage (Mulder & Showers, 1983; Sherrod *et al.*, 1979).

Another factor which could indirectly add to the risk of cutworm damage is the planting of herbicide tolerant maize and soybean hybrids, which were planted by 52.9% of respondents in this study. Glyphosate tolerant soybean and maize varieties were introduced into South Africa in 2001 and 2003, respectively, providing farmers with the benefits of easy weed control and flexible management strategies in terms of timing of herbicide applications (Gouse *et al.*, 2005). The adoption of herbicide tolerant maize and soybean hybrids in South Africa increased to approximately 95% and 75% in 2018 (Masehela *et al.*, 2021). Because pre-plant burn-down can be done at any time, this resulted in changing weed management practices (Gouse *et al.*, 2005). This study showed that the period between pre-plant burndown and planting ranged between one and 48 days (mean = 14 days). This high incidence of herbicide tolerant maize and soybean in South Africa and the use of pre-plant burndown by 52.4% of respondents in this study, suggest that the duration of the period between pre-plant weed control and planting does not meet the 35-day requirement to ensure starvation of larvae.

Although 50% of respondents in this study indicated that they planted Bt maize, this technology does not provide any protection against damage caused by *A. segetum* larvae (Erasmus *et al.*, 2010). In South Africa, the target pests of Bt maize are the stem borers, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and the Spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) (Van den Berg *et al.*, 2014).

2.6 Conclusion and recommendations

This study showed that *A. segetum* damage results in significant economic losses in maize and soybean production systems in South Africa. Although not conclusive, it could be suggested that the increased pest status of *A. segetum* may be due to a shift from conventional to no-tillage methods, improper timing of weed management practices before planting, inappropriate use of insecticides, and application thereof

under unfavourable conditions. Further studies should be conducted to elucidate the relationships between weed control practices and cutworm incidence on crop fields.

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APPENDIX A: Cutworm damage survey



Cutworm Damage survey

Disclaimer

Purpose of the Questionnaire:

The primary objective of this questionnaire is to collect data on various agricultural management practices and their potential influence on the prevalence and control of cutworm (*Agrotis segetum*) infestations. The insights gained from this survey will be instrumental in enhancing our understanding of the factors that contribute to cutworm presence in crops and will aid in the development of more effective pest management strategies.

Voluntary Participation:

Your participation in this questionnaire is entirely voluntary. You have the freedom to partake in this survey, and you may withdraw at any point without any consequences.

Confidentiality Assurance:

We are committed to maintaining the highest level of confidentiality. All personal information provided will be kept strictly confidential and will be used solely for the purposes of this research. We guarantee that no personal details will be disclosed or shared with any third parties.

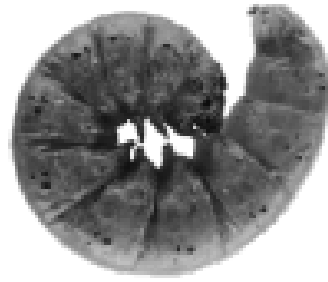
Data Use and Analysis:

The collected data will be subjected to thorough analysis to identify potential correlations between agricultural management practices and the intensity of cutworm infestations. The aggregate findings may be used to inform research publications or presentations for the institutions involved and the agricultural press; however, no individual data will be identifiable in such reports.

Consent:

By proceeding with this questionnaire, you acknowledge that you have read and understood this disclaimer, and you agree to participate under the terms described herein. Your responses will contribute valuable information to our research and could potentially lead to improved pest control recommendations for the agricultural community.

We appreciate your time and valuable contributions to this study.



Cutworm Damage survey

General questions

*** 1. Role**

- ☐ Agent (crop advisor)
- ☐ Farmer
- ☐ Farm manager
- ☐ Other (please specify)

*** 2. Location of affected farm**

GPS Coordinates
(optional)

City/Town

Province

*** 3. Did you experience cutworm damage during the 2023/2024 season?**

- ☐ Yes
- ☐ No

*** 4. Are the affected fields under dryland or irrigation?**

- ☐ Dryland
- ☐ Irrigation
- ☐ Other (please specify)

* 5. Date of first rain (after winter) sufficient enough for planting/ cultivation

Date

Date

DD/MM/YYYY

6. On which crops did you experience cutworm damage?

☐ Maize

☐ Soybean

☐ Sunflower

☐ Other (please specify)

* 7. What was the date of planting for the selected crops (even if no damage was observed)

Maize

Date

DD/MM/YYYY

Soybean

Date

DD/MM/YYYY

Sunflower

Date

DD/MM/YYYY

8. Total area planted (ha)

Maize

Soybean

Sunflower

9. Total area Affected by cutworm (ha)

Maize

Soybean

Sunflower

10. On which crops did you experience stand losses because of cutworm?

- ☐ Maize
☐ Soybean
☐ Sunflower

11. Did you have to replant? If so, how many ha?

Maize	
Soybean	
Sunflower	



Cutworm Damage survey

Cutworm Control strategy

* 12. Did you apply insecticides specifically for cutworm control?

- ☐ Yes
☐ No

* 13. If Insecticides were applied, select from the following options. Applications were done...

- ☐ As a seed treatment
☐ Before planting
☐ With planting
☐ After planting

14. In total, how many spray applications were applied for cutworm control?

15. If yes, specify the insecticide(s) applied to the respective crop(s)

Maize	
Soybean	
Sunflower	

16. If yes, what dosage was applied (ml/ha)

Maize	
Soybean	
Sunflower	

17. If yes, what volume (water/ha)?

Maize	
Soybean	
Sunflower	

18. If yes, was a buffer added?

Buffer added yes or no	
Maize	
Soybean	
Sunflower	

* 19. How would you describe the soil moisture during first insecticide spray application?

- ☐ Very wet
- ☐ Wet (damp)
- ☐ Dry
- ☐ Very dry

* 20. What was the target-site of the insecticide application(s)?

- ☐ In-furrow
- ☐ On soil surface with plant
- ☐ Foliar
- ☐ Other (please specify)

* 21. How were the insecticides applied on the following crops?

	Method of application
Maize	
Soybean	
Sunflower	

Other (please specify)

* 22. During which time of the day were applications done?

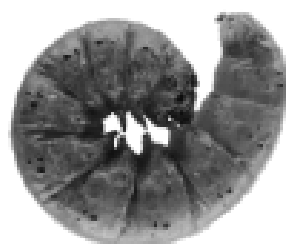
- ☐ Morning (06:00-10:00)
- ☐ Afternoon (10:00-16:00)
- ☐ Evening (16:00-23:00)
- ☐ Other (please specify)

23. In your opinion, was the application effective?

- ☐ Yes
- ☐ No

* 24. Was the seed treated with one of the following insecticides?

- ☐ Imidacloprid
- ☐ Thiamethoxam
- ☐ Clothianadin
- ☐ Other (please specify)



Cutworm Damage survey

Cultivation practices

* 25. Which cultivation method is mainly used on these fields?

- ☐ No-till
- ☐ Conventional clean cultivation
- ☐ Conservation agriculture
- ☐ Regenerative agricultural practices
- ☐ Other (please specify)

* 26. If you planted maize, which type of hybrid was planted?

- ☐ Stack (double/triple-gene hybrid)
- ☐ Roundup-Ready only
- ☐ Other (please specify)

* 27. Was any mechanical cultivation applied (ploughing/ disking) for weed control before planting?

	Ploughing	Disking
Maize	☐	☐
Soybean	☐	☐
Sunflower	☐	☐

Other (please specify)

Keep this scale in mind for the next question

Option	Maize	Soyabean
0%	Fields were bare	Fields were bare
1-25%		
26-50%		
51-75%		
76-90%		

* 28. Provide an estimate (%) of the residue cover on the field during planting.

Totally clean

completely covered with crop residues

☐

* 29. Prioritize the three most dominant weed species in the problem fields.

Number 1:

Number 2:

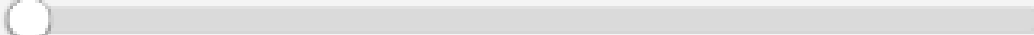
Number 3:

* 30. What type of weed management was applied before planting?

- ☐ Pre-plant burndown
☐ Mechanical (disking/ploughing, etc.)
☐ Other (please specify)

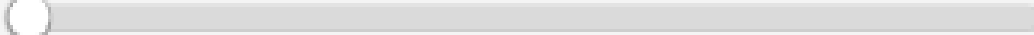
31. If you made use of pre-plant burndown, how many days before planting?

0 100

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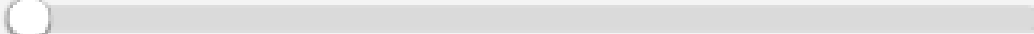
* 32. How many days lapsed from your mechanical weed control practice, to date of planting?

0 100

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33. If you applied any herbicides after planting, how many days after planting?

0 100

A horizontal slider bar with a circular handle at the 0 position. The bar is light gray and spans from 0 to 100.

CHAPTER 3: Biological control agents and levels of parasitism of *Agrotis segetum* (Lepidoptera: Noctuidae) in grain production regions of South Africa

Abstract

The management of *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae) is challenging since larvae spend most of their life cycle hiding underneath weeds or within the soil. Given that producers reported poor control of this pest over the past four cropping seasons, despite the use of insecticides, the need for alternative control strategies exists. Many species of parasitoids, entomopathogenic fungi (EPFs), viruses (EPVs) and nematodes (EPNs), have been reported from around the world where this pest occurs. However, little is known about the occurrence and impact of these biological control agents in South Africa. To determine which *Agrotis* sp. was the dominant *Agrotis* species in crop fields and assess their incidence and diversity, 14 cutworm populations were obtained from different geographical regions, reared in a laboratory and monitored for parasitism and other mortality factors. This study revealed that parasitism was an important mortality factor (43.6%), with *Macrocentrus collaris* (Spinola) (Hymenoptera: Braconidae) being the most common (39.2%), followed by *Gonia bimaculata* (Wiedemann) (Diptera: Tachinidae) (3.2%) and *Periscepsia carbonaria* (Panzer) (Diptera: Tachinidae) (1.2%). Entomopathogenic viruses were responsible for 26.1% of larval mortalities, while entomopathogenic nematodes (Mermithidae) and -fungi caused 15.7% and 3.2% of mortalities, respectively. The incidence of parasitism, particularly by *M. collaris*, suggest that parasitoids could play a role in the suppression of *A. segetum* populations.

Key words: cutworms, biological control, parasitoids, entomopathogenic fungi, entomopathogenic viruses, entomopathogenic nematodes

3.1 Introduction

Cutworms (*Agrotis* spp.) are polyphagous and cause damage to crops such as maize (*Zea mays*), potatoes (*Solanum tuberosum*), tomatoes (*Solanum lycopersicum*), turnips (*Brassica rapa*) and many other host plants (CABI, 2021). The two *Agrotis* species that are considered the most important, are *Agrotis ipsilon* (Hüfnagel) (Lepidoptera: Noctuidae) which is widely spread across all continents, and *Agrotis segetum* (Denis & Schiffermüller) which occurs in Africa, Europe, the Middle East and Southeast Asia (Blair, 1976; Bowden *et al.*, 1983; Hülbert & Süss, 1983; Esbjerg & Sigsgaard, 2014; Rodingpuia & Lalthanzara, 2021). *Agrotis segetum* is the dominant cutworm species and most important pest of maize seedlings in South Africa (Drinkwater, 1980; Du Plessis, 2000; Van den Berg *et al.*, 2014; Drinkwater, 2017).

Throughout the winter, *A. segetum* moths are active and lay their eggs on winter weeds which provide food and shelter for larvae. From the 4th-instar onwards, larvae hide in the soil during the day and emerge to feed at night, while the first three instars are photopositive and feed on weeds above the soil surface during the day (Blair, 1975, Drinkwater & Janse Van Rensburg, 1992). Cutworms do not go into diapause but survive the cold winter months as slow-developing larvae that feed on winter weeds until spring (Bowden *et al.*, 1983; Drinkwater, 1980; Drinkwater, 2017). These overwintering larvae attack emerging maize seedlings during spring and early summer (Drinkwater, 2017).

Although the use of synthetic chemical compounds is the most common tool to control *Agrotis* spp. populations, maize producers have often reported insecticides to be ineffective. Cultural control strategies, such as the removal of host plants (weeds) from fields before planting have been recommended by Drinkwater (1980).

Predators, parasitoids, entomopathogenic fungi, viruses and nematodes are promising biological control agents of cutworms (Wennmann *et al.*, 2015; Salim *et al.*, 2016; Staude *et al.*, 2020). Predators such as carabid beetles have been reported to reduce cutworm numbers in crop fields in the USA as they prey on the eggs, larvae and pupae of these pests (Best & Beegle, 1977; House & Alzugaray, 1989; Frank & Shrewsbury, 2004). Entomopathogenic fungi (EPFs) have been reported to cause mortality of cutworms in Northern Europe (Steenberg & Ogaard, 2000), England and Wales (Sherlock, 1983). Studies under laboratory conditions have shown that the

application of commercial formulations of EPFs have potential as control agents due to the 100% mortality of cutworms observed 5 to 14 days after application (El-Hawary & Abd El-Salam, 2009; Ahmed *et al.*, 2022). Similarly, entomopathogenic viruses (EPVs) can infect cutworms and cause mortality (Bourner *et al.*, 1992). Several baculoviruses, both nucleopolyhedroviruses (NPVs) and granuloviruses (GVs) have been isolated from *A. segetum* and *A. ipsilon* (Allaway & Payne, 1983; Boughton *et al.*, 1999; Jakubowska *et al.*, 2006) and effective control has been demonstrated under laboratory (El-Salamouny *et al.*, 2003) and field conditions (Bourner *et al.*, 1992; Bourner & Cory, 2004; Wennmann *et al.*, 2015). Cutworms are highly susceptible to entomopathogenic nematodes (EPNs) (Morris & Converse, 1991). Nematodes of the Mermithidae family have been reported to parasitise cutworm larvae in the USA (Puttler & Thewke, 1971; Puttler *et al.*, 1973) and China (Chen *et al.*, 1989; Wang & Wang, 1990; Li *et al.*, 1993). Infection by EPNs of the Heterorhabditidae and Steinernematidae families have also been shown to cause mortality of cutworms (Hussaini *et al.*, 2005; Unlu *et al.*, 2007; Fetoh *et al.*, 2009; Ebssa & Koppenhöfer, 2012; Gokce *et al.*, 2013; Goudarzi *et al.*, 2015; Ebrahimi *et al.*, 2019).

Over 50 species of parasitoids have been recorded for *Agrotis* spp. worldwide, mainly on the larvae (Schoenbohm & Turpin, 1977; Sajap *et al.*, 1978; Levine & Clement, 1981; Foster & Ruesink, 1984; El-Heneidy & Hassanein, 1987; Khan & Ozer, 1988; Caballero *et al.*, 1989; Caballero *et al.*, 1993; Kara & Tschorsnig, 2003; Gözüağık *et al.*, 2009; Salim *et al.*, 2016). In Spain, for example, the most important of these were *Periscepsia carbonaria* (Panz) (Diptera: Tachinidae) and *Macrocentrus collaris* (Spinola) (Hymenoptera: Braconidae) (Caballero *et al.*, 1989). The tachinid larval-pupal parasitoid, *Gonia bimaculata* (Wiedemann) (Diptera: Tachinidae) have also been recorded in Spain and Turkey (Caballero *et al.*, 1989; Gözüağık *et al.*, 2009). In other parts of the world, cutworm infestations and their damage are significantly reduced when levels of parasitism are high (Schoenbohm & Turpin, 1977; Sajap *et al.*, 1978; Caballero *et al.*, 1993). Numerous hymenopteran and dipteran parasitoid species have been reported to be successful biological control agents of cutworms in the USA (Schoenbohm & Turpin, 1977; Sajap *et al.*, 1978; Levine & Clement, 1981; Foster & Ruesink, 1984), Egypt (El-Heneidy & Hassanein, 1987; El-Hawagry, 2018), Spain (Caballero *et al.*, 1989; Caballero *et al.*, 1993), Turkey (Kara & Tschorsnig, 2003; Gözüağık *et al.*, 2009; Lakin *et al.*, 2016; Lutovinovas *et al.*, 2018), Europe and

Asia (Khan & Ozer, 1988; Cerretti & Tschorsnig, 2010; Lee & Han, 2010; Salim *et al.*, 2016; Hou *et al.*, 2018). In southern Africa, parasitoid-host associations have been recorded for 66 Lepidoptera families (Staude *et al.*, 2020). However, only four records of unidentified Braconidae species that parasitise *A. segetum* were reported by Staude *et al.* (2020).

Little information exists regarding which *Agrotis* species dominates in maize fields and the occurrence of biological control agents in South Africa. Drinkwater (2017) listed the following cutworm species to occur in maize fields: *Agrotis subalba* (Walker), the grey cutworm, *A. ipsilon*, the black cutworm, *Agrotis longidentifera* (Hampson), the brown cutworm and the common cutworm, *A. segetum*. The role of natural enemies in the suppression of cutworms in South Africa has, however, not been investigated before. Therefore, the aim of this study was to conduct a survey of the diversity and incidence of biological control agents and to determine which *Agrotis* spp. occur in grain crop fields in South Africa.

3.2 Materials and Methods

3.2.1 Collection sites

Larvae of *Agrotis* spp. populations were collected from maize, soybean and sunflower fields at the localities listed in Table 3.1. The fields were distributed throughout the maize production region (Figure 3.1). The maize production region was classified into three regions (Chapter 2, Figure 2.1) based on their overall environmental conditions and soil type (Haarhoff *et al.*, 2020). The regions are: (i) Western (35% of total production), (ii) Eastern (45%), and (iii) KwaZulu-Natal (KZN) (10%). The Western and Eastern areas are located on the South African inland plateau (1500 - 1800 m a.s.l.) (Haarhoff *et al.*, 2020), with hot summers and very cold winters. The KZN region is known for long hot summers and mild winters, with an average annual rainfall of 500-800 mm (Adisa *et al.*, 2018).

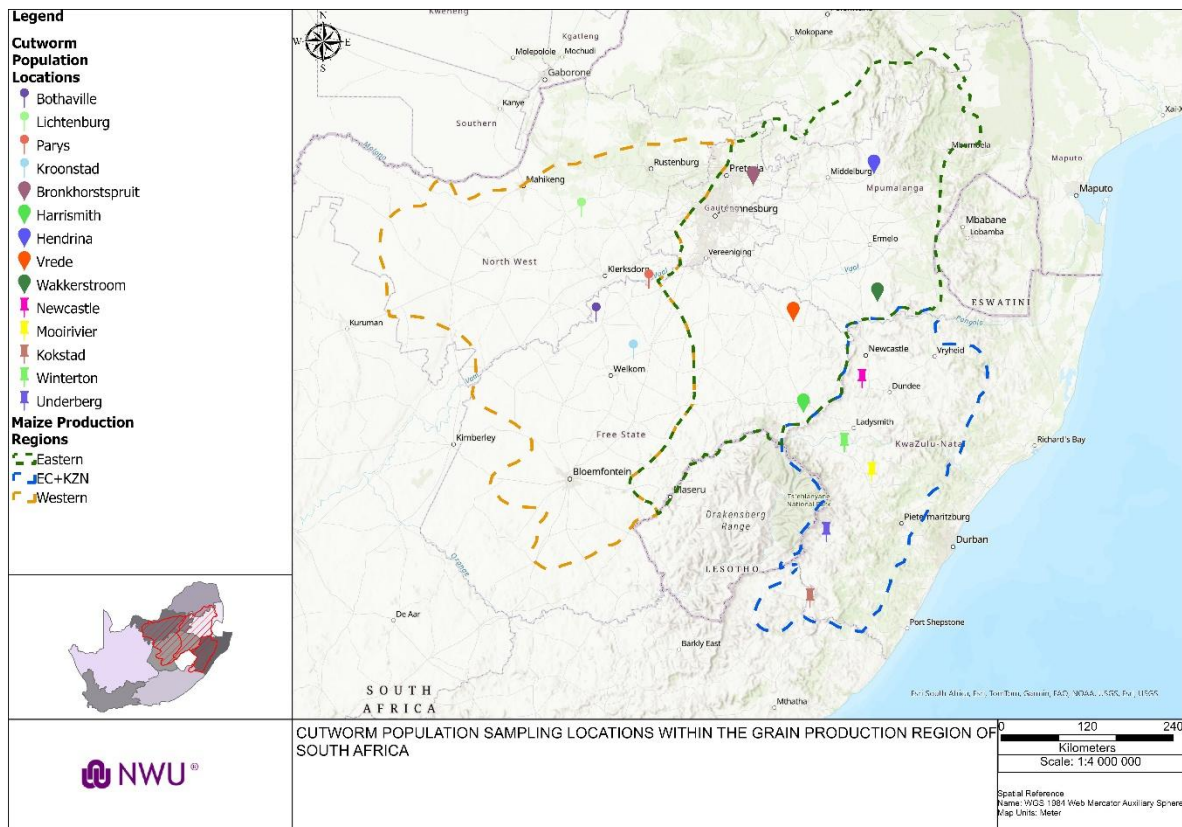


Figure 3.1. Map indicating the localities at which cutworm populations were collected in the grain production region of South Africa (Map created by Michael Cloete).

Larvae were sampled in October, November, January and February during the 2023/24 and 2024/25 cropping seasons. At each location, an assessment of seedling stand loss was made to identify areas within fields where the likelihood was high to find cutworm larvae. Insecticides were applied at all the collection sites prior to larval collection, except Kokstad, Mooirivier, Underberg, and Harrismith. This was because collections were only made at localities where producers reported cutworm infestations and because they did not postpone insecticide applications for a few days until larvae could be collected. If any evidence of cutworm damage to maize seedlings, weeds or cover crop plants were evident, the surrounding topsoil (3-10 cm) was carefully searched, and any observed larvae were collected. Larvae were placed inside plastic containers aerated with plastic mesh-infused lids (380 mm x 270 mm x 140 mm) and provided with weedy plants retrieved from the collection sites as a temporary food source. Different quantities of larvae were collected from each collection site and then transported to the Entomology laboratory at the North-West

University, Potchefstroom, South Africa. Larvae were in transit for the minimum time feasible (< 36 hrs).

Table 3.1. Localities at which *Agrotis segetum* larvae were collected during 2023/24 and 2024/25.

Region	Nearest town	Geographical reference	Date of collection
2023/2024			
Eastern	Hendrina	25°44'10.2"S; 30°02'05.0"E	20/11/2023
	Wakkerstroom	27°10'17.2"S; 30°04'43.1"E	10/11/2023
	Bronkhorstspuit	25°51'55.1"S; 28°31'19.2"E	29/01/2024
Western	Parys	27°01'29.6"S; 27°12'47.9"E	25/10/2023
	Kroonstad	27°48'18.1"S; 27°01'10.9"E	29/11/2023
KwaZulu-Natal	Underberg	29°48'29.7"S 29°26'17.4"E	18/11/2023
	Winterton	28°50'12.1"S; 29°39'51.4"E	09/11/2023
2024/2025			
Eastern	Bothaville	27°23'29.6"S; 26°33'10.9"E	06/11/2024
	Harrismith	28°24'03.6"S; 29°09'03.0"E	22/10/2024
	Vrede	27°23'13.5"S; 29°01'25.6"E	14/11/2024
KwaZulu-Natal	Kokstad	30°31'23.7"S; 29°14'02.2"E	14/11/2024
	Newcastle	28°07'40.8"S; 29°53'08.6"E	14/11/2024
	Moorivier	29°09'14.9"S; 30°00'26.0"E	18/10/2024
Western	Lichtenburg	26°13'11.6"S; 26°22'05.2"E	12/02/2025

3.2.2 Rearing conditions and causes of mortality of cutworm larvae

After the larvae arrived at the laboratory, they were removed from the plant material and transferred into individual plastic containers with aerated mesh-infused lids (50 mm x 50 mm x 50 mm). Larvae were reared on artificial diet inside these containers until they either pupated, parasitoids emerged or died. Stonefly *Heliothis* Premix Diet (Ward's Natural Science Establishment, LLC, USA) was provided as food for larvae. To prevent starvation of larvae, food was provided ad libitum. The containers were kept in an insect rearing room at 28 ± 1 °C, $65 \pm 5\%$ humidity and a 14L:10D photoperiod.

Larvae were inspected every second day and those that appeared sick or parasitised were removed and kept separately to identify the possible causes of mortality (Figure 3.2). Parasitoids that emerged were kept separately for each larva. Pupae were kept inside containers until moths or parasitoids emerged. Parasitoids were identified to species level where possible. The number of parasitoid pupae that were present in each container was determined for one of the parasitoid species (Figure 3.2B) while the numbers of cocoons per container was determined for another. The mean number of parasitoids that emerged per larvae was then calculated. Entomopathogenic nematodes that emerged from larvae were placed in 70% ethanol. The number of EPN per cutworm larva was recorded (Figure 3.2D). Dead larvae that had a dark brown appearance and from which no parasitoids emerged were considered to have died from an EPV infection (Figure 3.2A). Dead larvae covered with white or green spores were considered to have died from an EPF infection (Figure 3.2C). The number of dead larvae exhibiting symptoms EPVs or EPFs were also recorded. If the cause of larval death could not be identified, these were indicated as unknown.



Figure 3.2. Causal agents responsible for larval mortality, i.e., A) entomopathogenic virus, B) entomopathogenic fungi, C) larval-pupal parasitoid, D) entomopathogenic nematodes (Photos by Zunel van Eeden).

Parasitoids that emerged were identified by means of the taxonomic keys provided by Hou et al. (2019) for *Gonia* spp., BOLD (2014) for *M. collaris*, and Andersen (2022) and NBIC (2022) for *P. carbonaria*.

3.2.3 *Agrotis* species identification

The numbers of moths that emerged in oviposition containers of each collection site was counted and a selection of moths from each site was removed from containers and preserved for species identification/confirmation (Figure 3.3). Between 18 and 90% of the moths of each site was preserved for further identification, using the



guidelines described by Drinkwater (2017).

Figure 3.3. *Agrotis segetum* moths kept for identification purposes (Photos by Zunel van Eeden). A-C show colour variations of moths that emerged from pupae during this study.

3.3 Data analysis

Descriptive analyses were used. The percentage contribution of each of the mortality factors to overall larval mortality at each of the sampling localities were determined in Excel.

3.4 Results

3.4.1 *Agrotis* species confirmation

All the moths that were identified were *A. segetum* (Figure 3.3). The total number of moths that emerged from the pupae of larvae collected at each of the sites are provided in Table 3.2.

3.4.2 Incidence of larval mortality

A total of 2723 larvae were collected from all the sites. The mean mortality of larvae from all sampling localities was 63.2% (n = 1663). Larval mortality ranged from 41.6

to 92.1% for the different localities (Table 3.2). The overall mean incidence of larval parasitism was 43.6%. The overall mean incidence of larvae that died due to EPF and EPV infections were 3.2% and 26.1%, respectively (Figure 3.4). The overall mean incidence of mortality due to EPNs were 15.7% while 11.4% of larvae died of unknown causes.

Table 3.2. Overall mortality of *Agrotis segetum* larvae collected at different sites during the 2023/24 and 2024/25 growing seasons.

Collection site	Number of larvae collected	Number of moths emerged	Overall larval mortality (%)
Lichtenburg	89	52	41.6
Kokstad	108	42	43.5
Hendrina	63	21	46.0
Newcastle	383	119	46.2
Wakkerstroom	189	108	46.6
Harrismith	384	163	50.8
Moorivier	143	52	62.9
Kroonstad	593	101	63.4
Underberg	201	39	68.7
Vrede	37	10	73.0
Bronkhorstspuit	56	38	80.4
Parys	48	3	83.3
Winterton	391	125	86.7
Bothaville	38	5	92.1

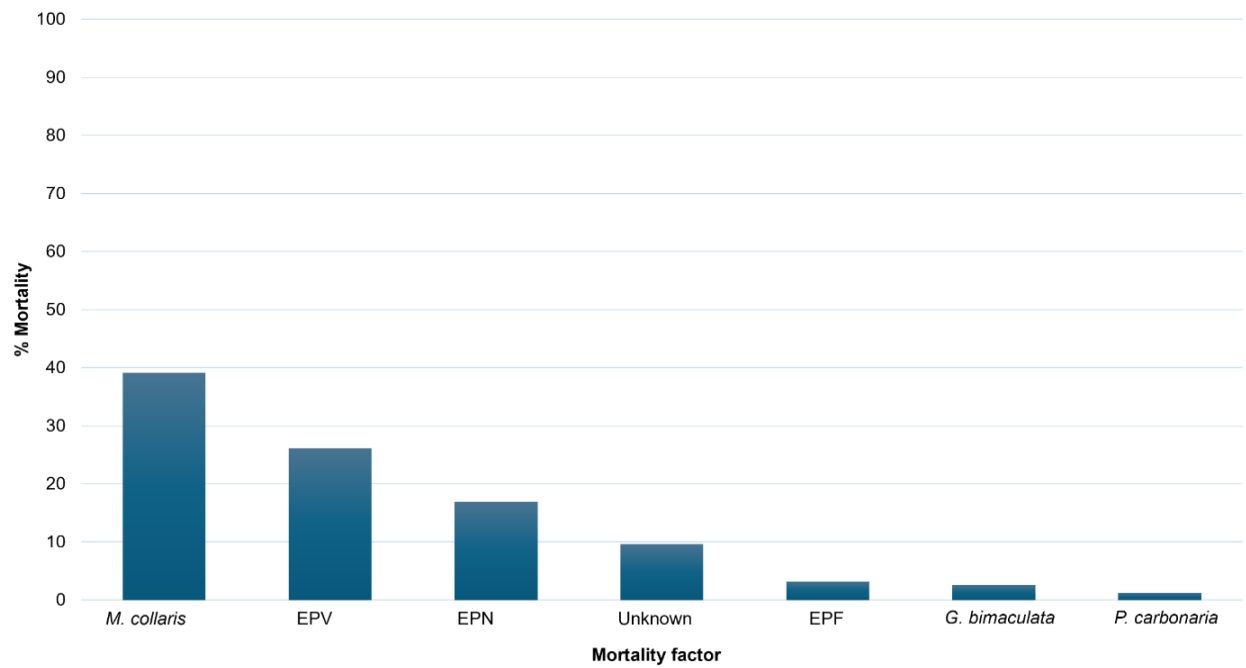


Figure 3.4. Percentage contribution of each biological control agent to overall mortality of *Agrotis segetum* larvae and pupae collected at all sites during 2023/2024 and 2024/2025 in South Africa. EPF = entomopathogenic fungi, EPV = entomopathogenic virus, EPN = entomopathogenic nematode. *P. carbonaria* = *Periscepsia carbonaria*; *G. bimaculata* = *Gonia bimaculata* and *M. collaris* = *Macrocentrus collaris*.

3.4.3 Biological control agents of *Agrotis segetum*

The percentage contribution of different biocontrol agents to overall mortality of *Agrotis segetum* larvae and pupae collected from 14 localities in South Africa during 2023/24 and 2024/25 is provided in Figure 3.8. Three parasitoid species were encountered during this study, two that emerged from larvae and one that emerged from pupae. These species were *Periscepsia carbonaria* (Panzer) (Diptera: Tachinidae) (Figure 3.5C, 3.5D), *Gonia bimaculata* (Wiedemann) (Diptera: Tachinidae) (Figure 3.5E, 3.5F) and *Macrocentrus collaris* (Spinola) (Hymenoptera: Braconidae) (Figure 3.5A, 3.5B). An EPN species belonging to the Mermithidae family was also identified from larvae.

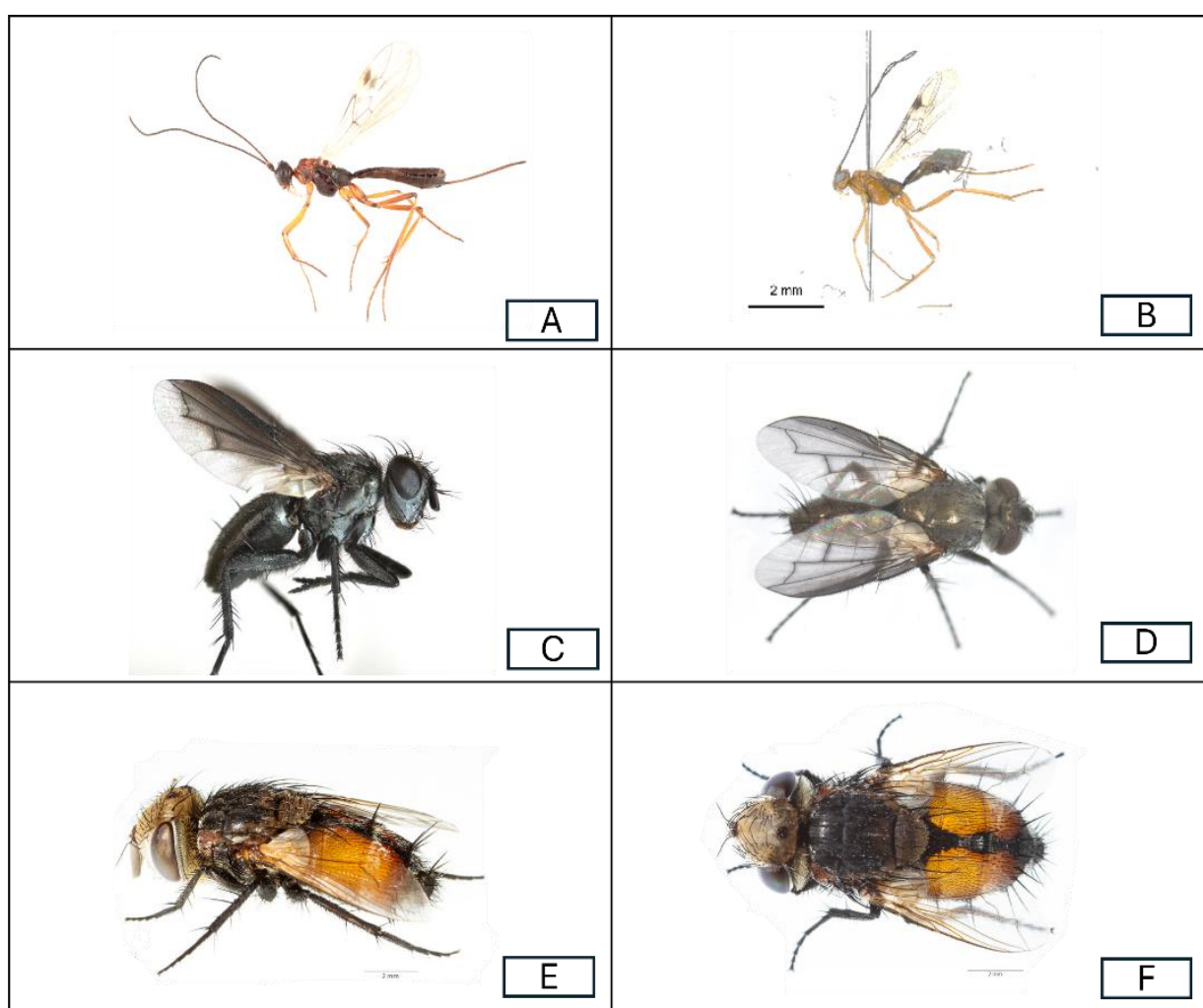


Figure 3.5. Parasitoids responsible for larval mortality, i.e., *Macrocentrus collaris* (A, B), *Periscepsia carbonaria* (C, D) and *Gonia bimaculata* (E, F). Sources: A, B (BOLD, 2014), C (Andersen, 2022), D (NBIC, 2022), E, F (Camacho, 2020).

Macrocentrus collaris occurred at all sites (Figure 3.8) and was responsible for the highest overall incidence of larval mortality (39.2%). The highest incidence of parasitism by *M. collaris* at a single site was at Kroonstad (75.5%) and the lowest at Harrismith (4.6%). The number of parasitoids that emerged per cutworm larva ranged between 20 and 130 (mean = 68).

Periscepsia carbonaria (Figure 3.6) was recorded at three of the fourteen sites, i.e., Wakkerstroom, Parys and Winterton, and was responsible for 1.2% of the overall larval mortality (Figure 3.8). The highest incidence of parasitism by *P. carbonaria* was at Parys (10.0%) and the lowest at Winterton (1.5%). The number of parasitoids that emerged per larva ranged from 1 to 4 (mean = 2).

Gonia bimaculata (Figure 3.7) was recorded at five of the fourteen sites, i.e., Wakkerstroom, Parys, Kroonstad, Bronkhorstspuit and Lichtenburg and contributed to 3.2% of the overall larval mortality (Figure 3.8). The highest incidence of parasitism was recorded at Bronkhorstspuit (22.2%) and the lowest at Kroonstad (0.5%).



Figure 3.6. *Periscepsia carbonaria* and its pupa (Photo by Zunel van Eeden).

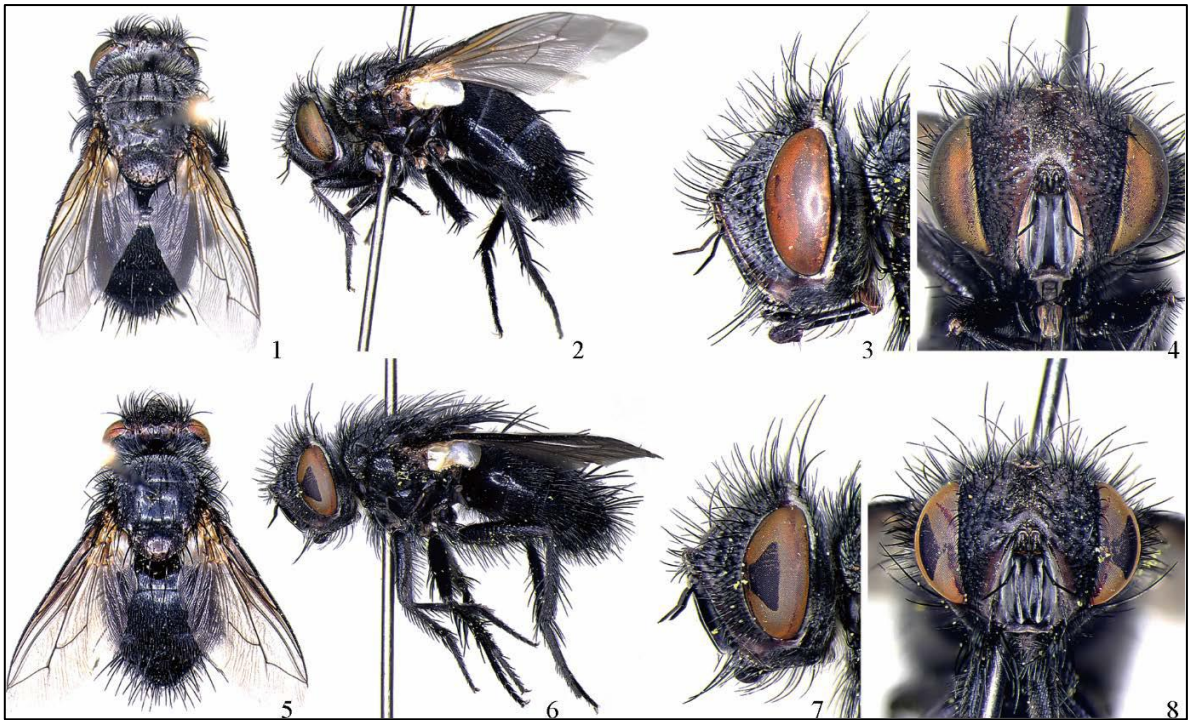


Figure 3.7. Photos of *Gonia bimaculata* (Source: Hou *et al.*, 2018).

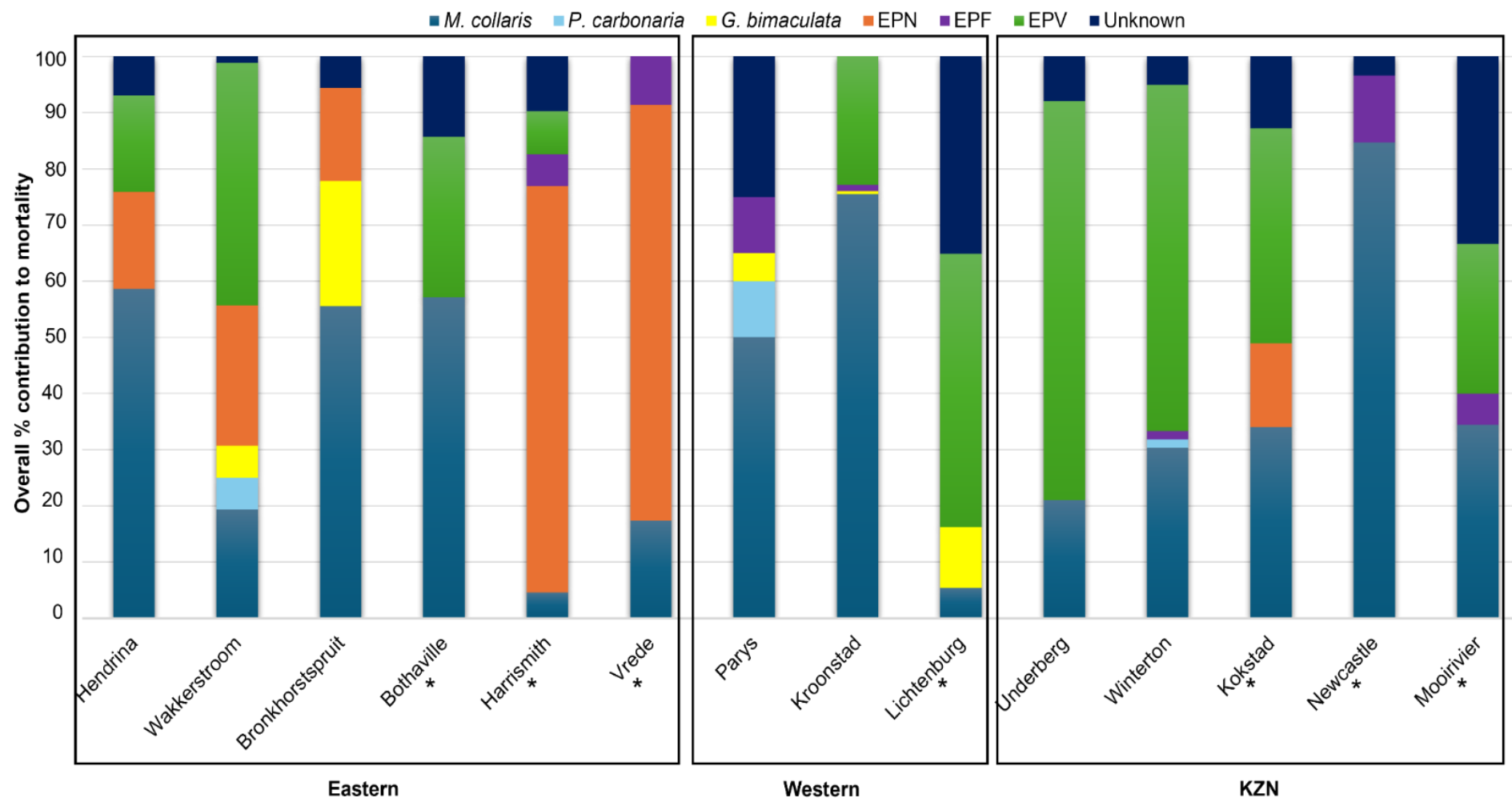


Figure 3.8. Percentage contribution of different biocontrol agents to overall mortality of *Agrotis segetum* larvae and pupae collected from maize production regions in South Africa during 2023/24, and 2024/25. Sites at which sampling was done during the 2024/25 season are indicated by an asterisk. *P. carbonaria* = *Periscepsia carbonaria*; *G. bimaculata* = *Gonia bimaculata* and *M. collaris* = *Macrocentrus collaris*.

The mermithid nematode contributed 15.7% to the overall parasitism. This nematode occurred at six of the 14 sites (Figure 3.8) with the highest incidence of parasitism recorded at Vrede (73.9%) and the lowest at Kokstad (14.9%). The number of EPNs that emerged per cutworm larva ranged from 1 to 41 (mean = 5).

EPFs were recorded from seven of the 14 collection sites (Figure 3.8), and overall incidence of infected larvae was 3.2%. The highest incidence of infection was recorded at Newcastle (11.9%) and the lowest at Kroonstad (1.1%).

EPV infections caused 26.1% of the overall incidence of larval mortalities. Larvae with EPV infections were recorded from 10 of the 14 collection sites (Figure 3.8) with the highest incidence of EPV infected larvae being recorded at Underberg (71.0%) and the lowest at Harrismith (7.7%). A total of 11.4% of the overall number of larvae died due to unknown factors.

Table 3.3. Biological control agents and their respective contribution (%) to mortality of *Agrotis segetum* larvae collected from 14 localities in the maize production areas of South Africa. *EPN = entomopathogenic nematodes, EPF = entomopathogenic fungi, EPV = entomopathogenic virus

Locality	<i>Macrocentrus collaris</i>	<i>Periscepsia carbonaria</i>	<i>Gonia bimaculata</i>	EPN*	EPF*	EPV*	Unknown
Hendrina	58.6	0.0	0.0	17.2	0.0	17.2	6.9
Wakkerstroom	19.3	5.7	5.7	25.0	0.0	43.2	1.1
Bronkhorstspuit	55.6	0.0	22.2	16.7	0.0	0.0	5.6
Bothaville	57.1	0.0	0.0	0.0	0.0	28.6	14.3
Harrismith	4.6	0.0	0.0	72.3	5.6	7.7	9.7
Vrede	17.4	0.0	0.0	73.9	8.7	0.0	0.0
Parys	50.0	10.0	5.0	0.0	10.0	0.0	25.0
Kroonstad	75.5	0.0	0.5	0.0	1.1	22.9	0.0
Lichtenburg	5.4	0.0	0.0	0.0	0.0	48.6	45.9
Underberg	21.0	0.0	0.0	0.0	0.0	71.0	8.0
Winterton	30.4	1.5	0.0	0.0	1.5	61.7	5.0
Kokstad	34.0	0.0	0.0	14.9	0.0	38.3	12.8
Newcastle	84.7	0.0	0.0	0.0	11.9	0.0	3.4
Moorivier	34.4	0.0	0.0	0.0	5.6	26.7	33.3
Mean	39.2	1.2	3.2	15.7	3.2	26.1	11.4

3.5 Discussion

All the moths that were identified during this study were confirmed as *A. segetum*, based on the characteristic kidney-bean shaped markings present on their wings (Figure 3.3A, 3.3B, 3.3C). This agrees with observations made by Du Plessis (2000) and Drinkwater (2017) that, although other *Agrotis* spp. may occur in grain crop fields in South Africa, *A. segetum* was the only species of economic importance.

Macrocentrus collaris, which was the most abundant parasitoid (39.2%) recorded across all the entire maize production region in this study (Figure 3.8). This parasitoid also recorded by Ragimov and Rustamova (1977) in Russia where it was abundant and widespread on *A. segetum* in cotton fields. Caballero *et al.* (1989) also recorded *M. collaris* as a parasitoid of cutworms in Spain.

Macrocentrus spp. are 4 to 10 mm in length (ovipositor excluded), with long legs and have a delicate appearance. These species are all konobiont endoparasitoids of lepidopteran larvae. Although a few of these species attack noctuids such as cutworm that feed on roots or inside stems of plants, they are most frequently observed to attack Pyralidae and Tortricidae that feed in shoots or rolled leaves (Shaw & Huddleston, 1991). Hosts of *Macrocentrus* spp. are easily attacked during movement between plants and the middle-instars are largely targeted. Oviposition takes place into any part of the haemocoel of the overwintering host. The parasitoid larvae feed on the host before emerging from it to complete their feeding externally and moult into the final instar. Individual, long, brownish cocoons are spun and arranged within a communally spun outer envelope on the surface of the larva. To emerge, each *Macrocentrus* sp. larva removes a tidy, round cap off the cocoon's extremity (Shaw & Huddleston, 1991).

The second most abundant parasitoid was *G. bimaculata* (3.2%) (Figure 3.8). This parasitoid has also been reported to parasitise *A. segetum* in Egypt, Italy, Korea, China, Spain and Turkey, (Caballero *et al.*, 1989, Gözüaık *et al.*, 2009; Lee & Han, 2010; El-Hawagry, 2018; Hou *et al.*, 2018).

Other tachinids that have also been reported as parasitoids of *Agrotis* spp., are *Gonia chinensis* (Wiedemann) (Diptera: Tachinidae), *Gonia ornata* (Meigen) and *Gonia divisa* (Meigen) (Diptera: Noctuidae) in China and Italy (Cerretti & Tschorsnig, 2010; Lee & Han, 2010), *Bonnetia comta* (Fallen) (Diptera: Tachinidae) in the USA, Spain

and Turkey (Levine & Clement, 1981; Caballero *et al.*, 1989; Kara & Tschorsnig, 2003; Stireman *et al.*, 2006) and *Tachina magnicornis* (Zetterstedt) (Diptera: Tachinidae) in Turkey and Italy (Kara & Tschorsnig, 2002; Cerretti & Tschorsnig, 2010). *Exorista larvarum* (Linnaeus) (Diptera: Tachinidae), *Spallanzania hebes* (Fallen) (Diptera: Tachinidae), *Peleteria rubescens* (Robinaeaeu-Desvoidy) (Diptera: Tachinidae) and *Phryxe vulgaris* (Fallen) (Diptera: Tachinidae) were recorded as parasitoids of *Agrotis* spp. in Italy (Cerretti & Tschorsnig, 2010).

Gonia spp. incubates microtype eggs in which ova are ingested by hosts as they feed. These eggs hatch within the gut of the host larva, and the emerging first-instar parasitoid larva burrows into the hemocoel (Stireman *et al.*, 2006). *Gonia bimaculata* seem to be monophagous on *Agrotis* species since no records were found in the literature of it parasitizing other lepidopteran hosts.

In the current study, the least abundant parasitoid (1.2%) was the tachinid fly, *P. carbonaria* (Table 3.3). *Periscepsia carbonaria* was also found by Ragimov and Rustamova (1977) as a parasitoid of *A. segetum* in cotton fields in Russia. Multiparasitism of up to four parasitoids emerging from a single host was recorded by Ragimov and Rustamova (1977). This is similar to observations made during this study where three to four parasitoids emerged from each parasitised *A. segetum* larva. *Periscepsia carbonaria* has also been confirmed as a parasitoid of *A. segetum* in other countries such as Pakistan (Khan, 1989; Khan, 2002), Turkey (Kara & Tschorsnig, 2003; Lekin *et al.*, 2016; Lutovinovas *et al.*, 2018), Spain (Caballero *et al.*, 1989) and India (Kumari & Chandla, 2010, Chandel *et al.*, 2022).

According to Bayram and Kılınçer (1987), larvae from *P. carbonaria* eggs are laid externally on *A. segetum* larvae, after which they infest the host during their first instar. The parasitoid larvae move freely in the hemocoel for five to six days before forming a respiratory funnel through its integument. It progresses through two more larval instars after which the parasitoid larvae leave the host to pupate. Khan (2002) reported that *P. carbonaria* parasitises *A. segetum* and *A. ipsilon* larvae and that older larvae (L4-L6) were less parasitised compared to younger larvae (L2-L3). Similar to *G. bimaculata*, *P. carbonaria* also seem to be monophagous on *Agrotis* spp. since no records were found in the literature of it parasitizing other lepidopteran hosts.

The second highest overall incidence of mortality was caused by entomopathogenic viruses (26.1%). Although it is not known by which viruses the cutworms in this study were infected with, other studies reported that cutworms are susceptible to EPVs. For example, Bourner (1992) evaluated a nucleopolyhedrovirus (AsNPV) and a granulosis virus (AsGV) in both spray and bait formulations against *A. segetum* and reported that AsNPV caused higher and more rapid mortality than AsGV. El-Salamouny *et al.* (2003) tested two NPVs, AgipNPV and AgseNPV, against *A. segetum* and *A. ipsilon* and reported that both viruses infected the two species and caused mortalities of between 50 and 90%. Similarly, Bourney and Cory (2004) reported that an NPV, AgseNPV, caused 50% mortality of *A. segetum* and that AgseNPV was susceptible to seven other species of noctuids within the cutworm complex. In Pakistan, Zethner *et al.* (1987) observed that the damage to maize caused by *A. segetum* and *A. ipsilon* was reduced by 64-85% when sprayed with a granulosis virus of *A. segetum* (AsGV).

The incidence of larval mortalities due to the mermithid nematode was low (15.7%) in comparison with other studies. Puttler *et al.* (1973) in Illinois (USA) reported that 64.5% larvae of an *A. ipsilon* population collected from maize fields were parasitised by a *Hexamermis arvalis* (Poinar & Gyrisco) (Nematoda: Mermithidae). Furthermore, in China, Chen *et al.* (1989) reported parasitism of up to 63% of *A. ipsilon* by *Hexamermis* spp. In the present study, a single *A. segetum* larva was often parasitised by more than one nematode, which is similar to results obtained by Li *et al.* (1993) who found up to three or four larvae of *Hexamermis agrotis* (Nematoda: Mermithidae) to parasitise a single *A. segetum* larva.

The overall incidence of mortality caused by EPFs was low (3.2%). In Northern Europe, Steenberg and Ogaard (2000) ascribed 50 to 100% mortality of overwintering *A. segetum* larvae to infection by a rare fungal pathogen, *Tolypocladium cylindrosporium* (Hypocreales: Ophiocordycipitaceae). Other authors have also reported EPFs as significant biological control agents of *Agrotis* spp. In Europe, Sherlock (1983) reported two naturally occurring fungal pathogens, *Entomophthora megasperma* (Entomophthorales: Entomophthoraceae) and *Fusarium solani* (Hypocreales: Nectriaceae), to infect mature *A. segetum* larvae, prepupae and pupae. Studies conducted in Egypt reported 100% larval mortality of *A. ipsilon* after treatment with an EPF product (Priority, *Paecilomyces fumosoroseus* (Hypocreales: Cordycipitaceae)) (El-Hawary & Abd El-Salam, 2009), 50% mortality of

A. ipsilon larvae caused by *B. bassiana* and *M. anisopliae* (Gabarty *et al.*, 2014) and 100% mortality by *B. bassiana* (Ahmed *et al.*, 2022).

Although parasitoids can parasitise *Agrotis* larvae during all phases of their life cycle, the effectiveness and consequences of parasitism fluctuate depending on larval instar. Studies have shown that, under laboratory conditions, parasitism rates can be as high as 87.5% in 1st-instars and 100% in 3rd-instars, with early instars (L1–L3) being the most vulnerable (Caballero *et al.*, 1993). In contrast, later instars show a significant reduction in susceptibility, with parasitism ranging from 34% in the 5th-instar to 4% in 6th-instar larvae (Caballero *et al.*, 1993). Because the 3rd- and 4th-instars provide optimum conditions for larval development and emergence, parasitoids tend to favour these stages. Since L1-L3 larvae are largely active during the day (Blair, 1975) and parasitoids are associated with flowering weeds which provide nectar as food (Foster & Ruesink, 1984), the assumption could be made that the larvae collected during this study were parasitized during winter months, or at least several weeks before planting. Although parasitism in the late instars is less common, when it does occur, parasitoids emerge from the late 5th- or early 6th-instar, when it stops feeding and ultimately perish (Schoenbohm & Turpin, 1977). This suggests a close relationship between parasitoid and host development, with increased parasitism rates that occur when environmental conditions, such as higher temperatures during the planting season, occur (Caballero *et al.*, 1993).

The development of parasitoids within *Agrotis* larvae results in reduced feeding activity and altered behaviour (Sajap *et al.*, 1978). Initially, they behave exactly like non-parasitised larvae, remaining active and feeding normally until just before the emergence of parasitoids (Sajap *et al.*, 1978). However, the feeding period is greatly shortened by parasitism, and on average, parasitised larvae still feed for an average period of 4.5 days as opposed to 10.8 days for non-parasitised larvae. According to Sajap *et al.* (1978), parasitised larvae damage an average of 4.4 plants, whereas non-parasitised larvae may damage 13.9 plants. According to Schoenbohm and Turpin (1977), parasitism restricts feeding by 6th-instar larvae, which normally consumes 77-86% of a larva's total food consumption during its life cycle. This is ascribed to the cessation of feeding and decreasing larval activity. Similar to healthy pupating larvae, parasitised larvae tunnel into the soil before parasitoid emergence. When, in the case of *M. collaris*, parasitoid larvae emerge, they build cocoons close to the host's body,

which prevents the host from pupating and ultimately results in its death (Sajap *et al.*, 1978).

It is important to note that nearly all the larvae collected in this study were sampled from fields on which one to three insecticide applications have been applied for cutworm control, prior to sampling. However, no information is available on the level of exposure of the larvae to insecticides.

Compared to *Agrotis* spp. parasitoid diversity reported in other countries (Schoenbohm & Turpin, 1977; Sajap *et al.*, 1978; Levine & Clement, 1981; Foster & Ruesink, 1984; El-Heneidy & Hassanein, 1987; El-Hawagry, 2018; Caballero *et al.*, 1989; Caballero *et al.*, 1993; Kara & Tschorsnig, 2003; Gözüağık *et al.*, 2009; Lekin *et al.*, 2016; Lutovinovas *et al.*, 2018; Khan & Ozer, 1988; Cerretti & Tschorsnig, 2010; Lee & Han, 2010; Salim *et al.*, 2016; Hou *et al.*, 2018), the diversity in South Africa seems to be low (Staude *et al.*, 2020).

3.6 Conclusion and recommendations

This study showed that several species of parasitoids, EPNs, EPFs and EPVs that attack or infect *A. segetum* occur in South Africa, and that these agents are responsible for high levels of mortality. However, despite the high incidence of parasitism recorded during this study, their impact is still insufficient to prohibit the economic damage to crops. Further studies should include more localities within the grain production regions of South Africa and focus on the environmental conditions and cultivation practices that might have an influence on the abundance of biological control agents.

3.7 References

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CHAPTER 4: Effect of soil moisture on behaviour of *Agrotis segetum*

Abstract

Chemical control of *Agrotis segetum* (Denis & Schifferrmüller) (Lepidoptera: Noctuidae) has been described as poor over the past four cropping seasons in South Africa. The poor control has been ascribed to possible insect resistance and environmental conditions. Previous studies on other cutworm species showed that insecticide efficacy was influenced by numerous factors such as larval biology and behaviour, species, soil type, soil moisture, adsorption, insecticide properties, method of application, formulation and climatic conditions. More recently, insecticides such as pyrethroids have been used for the control of cutworms. Although pyrethroids are used globally, a lack of comprehensive information regarding their usage against cutworm and its efficacy exists. For the contact action of pyrethroids to be effective against cutworm larvae, it is essential that larvae come into contact with the applied product on the soil surface when they move in search of food during nighttime. In South Africa, no information exists regarding the influence of soil moisture conditions on the behaviour of *A. segetum* and the effect that it could have on insecticide efficacy. Two laboratory assays were conducted, the one to observe the nocturnal activity of 5th-instar larvae at different soil moisture levels and the other to determine the influence of soil moisture conditions on the tunnelling behaviour of 3rd- and 5th-instar larvae. This study showed that the duration of larval tunnelling activity and on-surface movement was longer in dry and damp conditions, as opposed to the short and limited activity in wet conditions. This study showed that 3rd instars did not move into the soil, as opposed the 5th-instars which occurred 20-30 mm below the soil surface. A review based on studies of other cutworm species indicated that insecticide efficacy is influenced by more than one factor. Further research is needed to determine the effect of environmental conditions on insecticide efficacy against *A. segetum*.

Key words: chemical control, cutworms, larval behaviour, moisture, soil, tunnelling

4.1 Introduction

Lepidopteran pests of the Noctuidae family are an important threat to maize production and attainable yields in South Africa (Bell & McGeoch, 1996; Van den Berg *et al.*, 2014). The most important aboveground lepidopteran pests are the maize stalk borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), the Spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), African bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Bell & McGeoch, 1996; Calatayud *et al.*, 2014; Erasmus & Van den Berg, 2014; Van den Berg *et al.*, 2014, Drinkwater, 2017, Van den Berg *et al.*, 2021). The common cutworm, *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae), however, occurs belowground (Drinkwater, 1980; Du Plessis, 2000; Van den Berg *et al.*, 2014; Drinkwater, 2017).

Variations in temperature, precipitation, and soil moisture can have both direct and indirect effects on insect pests (Kocsis & Hufnagel, 2011; Skendžić *et al.*, 2021; Saha & Dutta, 2025). Directly, the reproduction, development, survival and distribution of insect pests are affected, whereas indirectly, their interaction within the environment is affected (Woiwod 1997; Nyamukondiwa *et al.*, 2022). In other studies, the direct and indirect effects of soil moisture on the ecology and behaviour of cutworm species have been reported. In Canada, the dark-sided cutworm, *Euxoa messoria* (Harris) (Lepidoptera: Noctuidae) is attracted to dry soil and tunnels early in the 3rd instar, whereas the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), does not tunnel until later in the 3rd instar and is attracted to moist soil (Harris & Svec, 1968a, 1968b, 1968c; Harris, 1972). However, the variegated cutworm, *Peridroma saucia* (Hübner) (Lepidoptera: Noctuidae) also prefers moist soil and only tunnels late in the 5th instar. In Canada, Jacobson and Blakeley (1957) reported that larval movement and feeding of the pale western cutworm, *Agrotis orthogonia* (Morrison) (Lepidoptera: Noctuidae), occurred in the interface between dry and moist soil. Furthermore, larvae can absorb moisture from the soil and the plants on which they feed. In the United States, Manis (1936) reported that *A. ipsilon* moths refrain from depositing their eggs in moist soil and that dry soil increases the duration of the larval stages. The optimum soil moisture for successful larval development was between 25 to 30%, however, prepupae and pupae were able to develop successfully in soil with a moisture content of up to 58%.

In Europe, the growth and survival of larvae are strongly influenced by the complex interactions between soil moisture and temperature. While high soil moisture has a cooling effect on soil temperature, high temperature, on the other hand, dries out the upper soil surface (Esbjerg & Sigsgaard, 2014; Esbjerg & Sigsgaard, 2019). *Agrotis segetum* moths prefer to oviposit on dry rather than moist soil (Esbjerg & Lauritzen, 2010). High mortality of 1st and 2nd-instar *A. segetum* larvae is experienced in moist soil and at low soil temperatures (Esbjerg *et al.*, 1986; Esbjerg, 1989; Esbjerg & Vidal, 2003). Larvae in the 3rd-instar are also dependent on soil moisture, however, from the 4th instar onwards, *A. segetum* larval tolerance to cold and moist conditions increase (Esbjerg, 1989; Esbjerg & Sigsgaard, 2019). The depth of pupation in the soil is also influenced by soil type and moisture levels (Blair, 1975). Pupation takes place within the top 30-50 mm of soil and the higher the moisture content, the shallower the depth at which pupation occurs (Blair, 1975). In South Africa, during very wet conditions, *A. segetum* larvae come to the soil surface and may also exhibit climbing behaviour on plant material (Drinkwater, 1980; Van den Berg & Du Plessis, 2022).

Abiotic conditions do not only influence pest pressure and damage potential but also insecticide efficacy (Saha & Dutta, 2025). The efficacy of insecticides registered for the control of cutworm in other countries was reported to be influenced by soil moisture, along with other factors such as soil type, larval behaviour, method of application and formulation (Harris & Svec 1968a, 1968b, 1968c; Harris, 1972). Furthermore, insecticides efficacy against cutworms may also be influenced by the distribution of the active ingredients in soil profile, adsorption or rapid break down due to sunlight (Leistra & Green, 1990), and the impracticality of applying contact insecticides (Harris & Svec 1968a, 1968b, 1968c, 1970). Although the requirements for the effectiveness of insecticides differ, many principles remain the same (Leistra & Green, 1990). Pyrethroids have become a predominant class of insecticide used against insect pests, especially lepidopteran larvae, as a substitute for highly toxic organophosphates insecticides (Gajendiran & Abraham, 2018). Pyrethroids are particularly effective due to their strong lipophilicity and low water solubility, which allows rapid adsorption to soil particles (Leistra & Green, 1990). These properties limit the leaching in soils, which is ideal for cutworm control, since they spend most of their life cycle just below the soil surface. Currently used pyrethroids are oil-based emulsifiable concentration formulations that retain the pyrethroid in its liquid form and

enhance their persistence in soils (Saha & Dutta, 2025). Insecticide labels from agrochemical companies provide clear instructions regarding the environmental conditions under which these products should be applied to ensure optimal efficacy (Agri-Intel, 2023, IRAC, 2024). For instance, the general label recommendations for *A. segetum* are that soils must be prepared, and the top 30 mm must be moist at the time of insecticide application.

Failure of cutworm control has been reported over the past four cropping seasons across the maize production region of South Africa, with speculations of insecticide resistance. However, chemical control of cutworms is complicated by the behaviour and biology of larvae, as well as environmental factors such as temperature, fluctuating soil moisture levels, the presence of weeds during winter, and varying conditions across fields (Drinkwater, 1980; Jakubowska & Walczak, 2008; Van den Berg & Du Plessis, 2022). In South Africa, no information exists regarding the influence of soil moisture conditions on cutworm behaviour and subsequent efficacy of soil applied insecticides. Therefore, the aim of this study was to conduct assays to determine whether different soil moisture levels influence larval behaviour of *A. segetum* and review information on factors that influence insecticide efficacy against other cutworm species.

4.2 Materials and Methods

4.2.1 Collection of *Agrotis segetum* population

An *A. segetum* population was collected from infested maize and sunflower fields at Lichtenburg (26°13'11.6"S; 26°22'05.2"E) in January during the 2024/25 planting season. The topsoil (5-10 cm) beneath injured maize and sunflower seedlings within rows were separated by hand and larvae were collected. Approximately 200 larvae were collected in order to establish a rearing colony. Larvae were placed inside transparent plastic containers aerated with plastic mesh-infused lids (380 mm x 270 mm x 140 mm) and provided weedy plants growing at the site as a temporary food source. Larvae were in transit for the minimum time feasible (< 36 hrs) and transported to the Entomology laboratory at the North-West University, Potchefstroom, South Africa.

4.2.2 Rearing conditions

Larvae were transferred from the temporary containers to individual clean transparent plastic containers with aerated plastic mesh-infused lids (50 mm x 50 mm x 50 mm). Stonefly *Heliethis* Premix Diet (Ward's Natural Science Establishment, LLC) was prepared with water added to the premix powder in a 3:1 ratio and provided as food to larvae. The containers were kept in a rearing room at 28 ± 1 °C, $65 \pm 5\%$ humidity and a 14L:10D photoperiod. Fresh diet was added to the rearing containers ad libitum and the larvae were transferred to clean containers when needed. Once pupae formed, they were removed from the rearing containers and placed in an open Petri dish (90 mm x 90 mm x 10 mm) inside an oviposition container aerated with a cotton sleeve-infused lid (420 mm x 420 mm x 200 mm). As the moths emerged, the male and female moths were free to mate. A 10% sugar solution provided in a small bottle with a cotton plug, served as an energy source for the moths. Three wax paper sheets (300 mm x 300 mm) were placed into the container for oviposition. Oviposition containers were examined daily, and the eggs which remained adhered to the wax paper were removed. Eggs that were deposited on the side and lid of the plastic containers were carefully removed with a fine paintbrush. Eggs were placed into separate transparent plastic containers aerated with steel mesh-infused lids and kept in the rearing room. Larvae that hatched from these eggs were put into transparent plastic containers aerated with plastic mesh-infused lids (50 mm x 50 mm x 50 mm). These larvae were reared to the third (L3) and fifth-instars (L5) and used in the respective experiments.

Two experiments were conducted. In the one experiment, the nocturnal behaviour of larvae was recorded by means of GoPro cameras over a 12-hour period, and in the other, the influence of different soil moisture treatments on the tunnelling depth of larvae was determined.

4.2.3 Soil classification

The soil used for this study was collected from the M-campus at the North-West University. A soil analysis conducted at Eco-Analytica laboratory (Building G23A, Ground floor, Potchefstroom Main campus) confirmed that the soil was a sandy soil. Sandy soils have a coarse texture with particles with a diameter of 0.05 to 2.0 mm, is well aerated and loose, allowing a high drainage rate (Brady & Weil, 2017).

4.2.4 Determination of soil moisture conditions

Precipitation (irrigation) is measured as a depth of water (e.g. mm of rain). Volumetric water content (θ_v) is the ratio of the volume of water (V_w) occupied in the pore space of a volume of dry soil (V_t) expressed as a fraction or percentage (Formula 1) (Brady & Weil, 2017). For this reason, θ_v was used to simulate different soil moisture conditions that result from the absence or presence of rainfall or irrigation. The three soil moisture conditions used in this study were: 1) *Dry*, no additional water was added to the soil to imply un-irrigated fields or prolonged dry spells with no rainfall, 2) *Damp*, a certain amount of water was added to soil until field capacity was reached to represent an irrigated or rainfed field with optimal moisture, and 3) *Wet*, water was added until soil was drenched to simulate a saturated field directly after rainfall or excessive irrigation. For sandy soils, the standard θ_v ranges (% by volume) for each soil moisture condition are as follows: dry (2-5%), field capacity (10-20%) and saturated (25-35%) (FAO, 2006).

Formula 1. Calculation of volumetric water content,

$$\theta_v = \frac{V_w}{V_t} \times 100$$

Where:

θ_v = Volumetric water content,

V_w = Volume of water added (ml),

V_t = Volume of soil (cm³).

Since 1 cm³ of water is 1 ml, soil volume can be directly converted to ml for the use of uniform units.

Table 4.1. Volumetric water content (θ_v) for each treatment for each experiment.

Treatment		Water added (ml)	Soil volume (ml)	θ_v (%)
Container	Dry	0	17 680	0
	Damp	2500	17 680	14.1
	Wet	5000	17 680	28.3
Test tube	Dry	0	43.75	0
	Damp	8	43.75	18.3
	Wet	12	43.75	27.4

4.2.5 Experiment 1: Nocturnal behaviour of larvae

Observations of larval behaviour under three different soil moisture treatments were made from 17:00 to 05:00. To do this, larvae were placed in containers (680 mm x 520 mm x 260 mm) filled with soil to a depth of 50 mm. At 17:00, a single L5 larva was placed onto the soil surface in the centre of each container and left to roam around till 05:00 the following morning. GoPro cameras were mounted at the top of each container, together with an infrared light so that movement could be recorded throughout the night (Figure 4.1). The video material of each GoPro was reviewed to describe larval behaviour at each soil moisture treatment. Observations focused on the time it took for larvae to tunnel into the soil, larval movement above and below the soil surface, and any other responses to different soil moisture conditions.

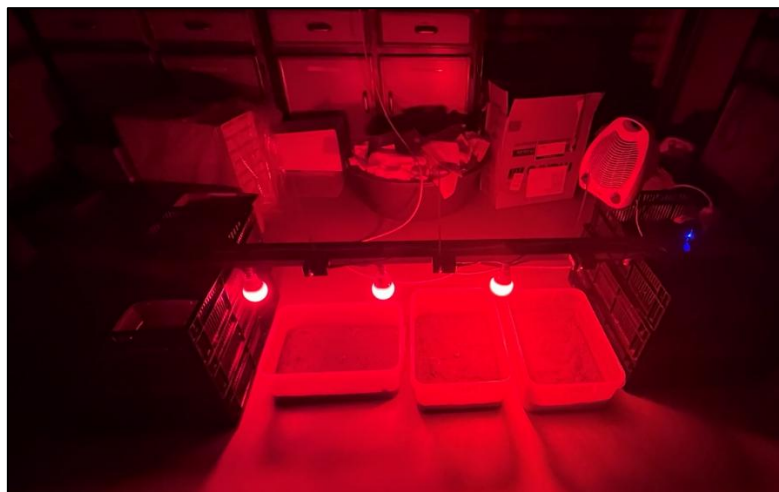


Figure 4.1. Experimental setup for observation of 5th-instar larval behaviour at three soil moisture conditions (Photo by Zunel van Eeden).

4.2.6 Experiment 2: The influence of different soil moisture treatments on the tunnelling depth of larvae

Two laboratory assays were conducted to determine the influence of soil moisture on the tunnelling depth of cutworm larvae. In the one assay, L3 larvae were used and in the other, L5. The three soil moisture conditions listed in Table 4.1 were used as the treatments. Each treatment was replicated 54 times.

The study was conducted in glass test tubes to facilitate easy observation of larval depth. Test tubes (25 mm x 25 mm x 200 mm), were filled with soil to a depth of 160 mm. For each treatment, water was added to achieve the desired θ_v within the top 50 mm of the soil (Table 4.1). A single L3 or L5 larva was inoculated onto the soil surface of each test tube at 17:00 after which the tubes were covered with a mesh material to prevent the escape. Test tubes were placed in a dark room for 24 hours. The depth (mm) that larvae had tunnelled into the soil was measured after 24 hours.



Figure 4.2. Experimental setup with test tubes to observe tunnelling behaviour of cutworms at three soil moisture conditions (Photo by Zunel van Eeden).

4.3 Data analysis

Observations on the time that larvae spent burrowing in soil or walking on the soil surface was summarised in Excel. ANOVAs were used to determine whether differences existed between treatments and Turkey HSD tests were used to indicate significant differences between the depths that larvae moved into the soils of the different treatments. All statistical analyses were performed with TIBCO® Statistica™ software version 14.0.1.25 (TIBCO, 2020).

4.4 Results

4.4.1 Experiment 1: Nocturnal behaviour of larvae

The behaviour of larvae varied in the three soil moisture conditions (Figure 4.3). In dry soil, the larva was highly active throughout the night. The larva emerged and entered the soil repeatedly, and at different entry points within the container. For a duration of 122 minutes, the larva extensively moved around in the container, from corner to corner, in a seeking manner. Tunnelling and on-surface movement continued intermittently.

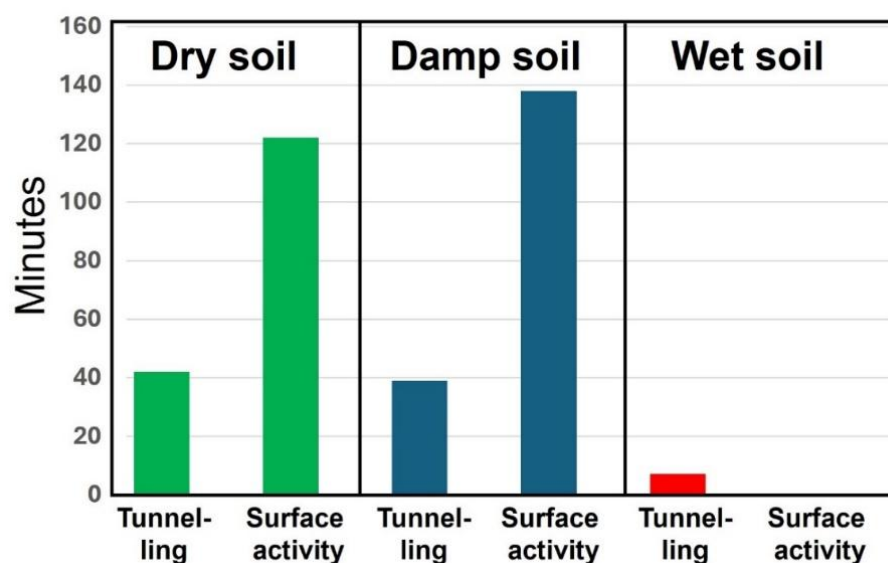


Figure 4.3. Time (minutes) spent by *Agrotis segetum* larvae tunnelling within or crawling on top of the surface of dry, damp or wet soil.

In the container with damp soil, the larva struggled for 39 minutes before it successfully tunnelled into the ground. For a total period of 138 minutes the larva emerged intermittently to move along the edges of the container.

Under wet soil conditions, the larva took six minutes to tunnel into the soil. The larva remained below the soil for the duration of the observation period.

4.4.2 Experiment 2: The influence of different soil moisture treatments on the tunnelling depth of larvae

L3-larvae showed minimal tunnelling activity at all three soil moisture conditions. In dry soil, no L3-larvae tunnelled below the soil surface (Figure 4.4A). In the damp and wet soil, the mean depths at which larvae occurred below soil surface were 15 mm and 16

mm, respectively. Larvae tunnelled significantly ($p = 0.040$) deeper into damp and wet soil than dry soil (Figure 4.4A). This significant difference is however, ascribed to the fact that none of the L3 larvae tunnelled into the dry soil. In the damp soil, only six L3 larvae tunnelled into the soil, with depths ranging from 10 to 20 mm. In the wet soil treatment, only six L3 larvae tunnelled into the soil, with some going as deep as 30 mm.

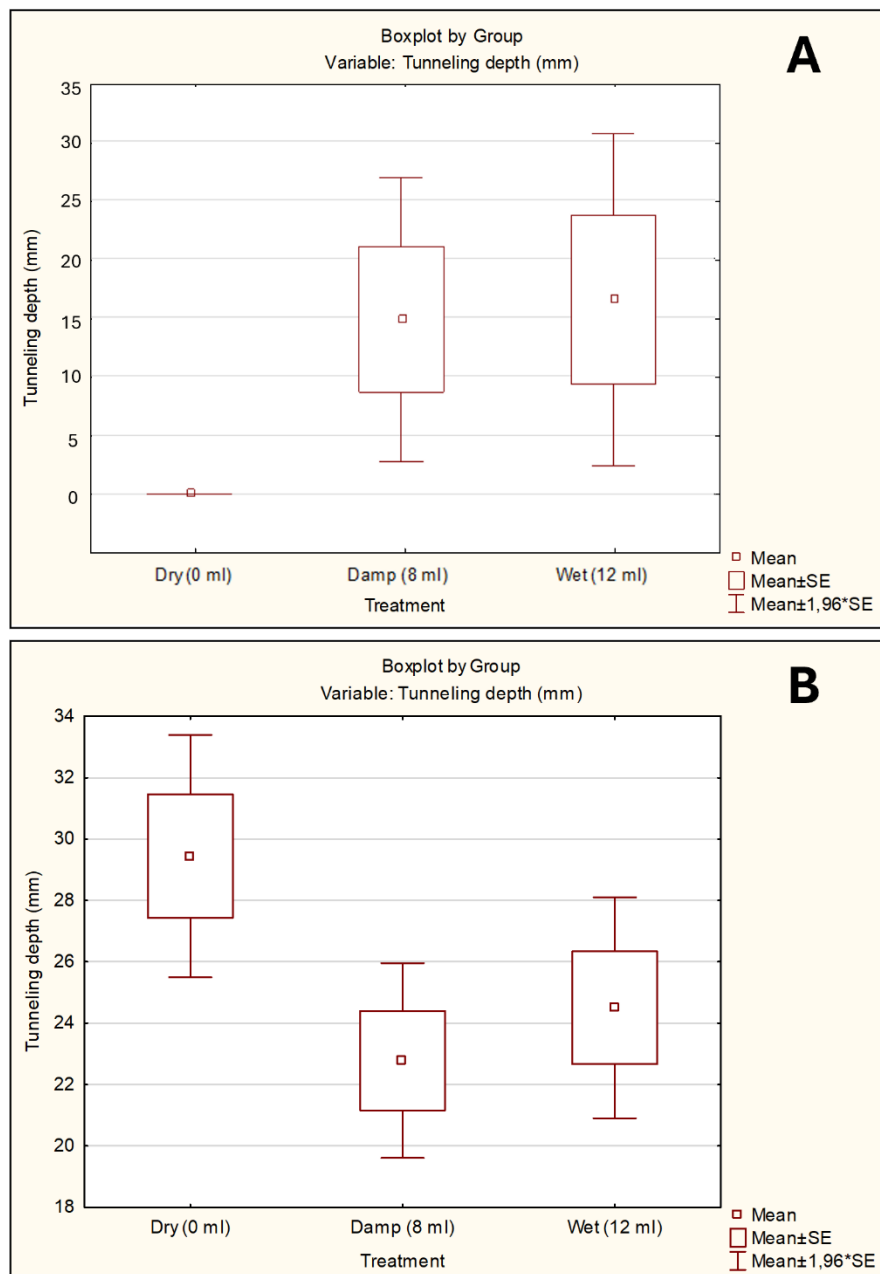


Figure 4.4. Mean distance (mm) of third-instar (A) and fifth-instar (B) *Agrotis segetum* larvae below the surface of dry, damp or wet soil.

In the dry soil, most L5 larvae (90.7%) tunnelled into the soil to a mean depth of 29.4 mm. In the damp and wet soils, 96.2 and 90.7% of larvae tunnelled into the soil. The mean depths at which L5-larvae occurred in damp and wet soils were 22.8 and 24.5 mm respectively. Some larvae in the wet soil occurred up to a depth of 60 mm.

4.5 Discussion

This study showed that L5 larvae spend much less time above the soil surface when soil is wet, than under dry or damp conditions. Larvae under damp and dry conditions spent between 122 and 138 minutes walking the soil surface. This study shows for the first time, the surface activity of *A. segetum* larvae under different soil moisture conditions in South Africa. In Canada, Jacobson and Blakeley (1957) reported that larval movement and feeding of *A. orthogonia* was influenced by the soil moisture gradient, with these activities usually occurring at the interface between dry and moist soil. When there is a gradient from dry to moist conditions from the surface downward, the larvae move to that level in the soil that provides the preferred environmental conditions. Other studies in Europe and the United States have reported that burrows result from the tunnelling behaviour of larvae and serves as a site of refuge from which the larvae emerge at night and search the surface for food (Blair, 1975; Bowden *et al.*, 1983). The intermittent tunnelling and on-surface larval activity in the damp and dry treatments suggests that larvae were searching for food. Although this study did not include available food resources, other studies in South Africa, India and the United States have reported that a single larva can damage several seedlings when it emerges from the soil at night (Du Plessis, 2000; Chandel *et al.*, 2022) and in many cases, a single seedling is severed and partially or entirely pulled beneath the surface for consumption (Drinkwater, 2017).

This study showed that the tunnelling behaviour of L3 and L5 *A. segetum* larvae differ under different soil moisture conditions. While L3 larvae showed limited tunnelling into the soil, most of the L5 larvae tunnelled to a depth of < 22 mm into the soil, irrespective of soil moisture level. This difference in behaviour between the L3 and L5 instar larvae is confirmed by other studies in other parts of the world that revealed characteristic changes in behaviour of *A. segetum* larvae during its development and the influence of soil moisture. According to Blair (1975), 1st to 3rd instar *A. segetum* larvae is photopositive and occur aboveground but start to exhibit a negative response to sunlight between the fourth and fifth day in the 3rd instar. The physiological mechanism

for the change in phototaxis is not known (Blair, 1975). In Europe, Esbjerg (1989) reported that L3 larvae are highly dependent on soil moisture, which confirms the fact that L3 larvae in this study evaded tunnelling into the soil, especially the damp and wet treatments. From the L3 onwards, *A. segetum* larvae start to hide in the soil rather than above the soil surface (Garnis & Dabrowski, 2008; Jakubowska & Bocianowski, 2013; Drinkwater, 2017). The few L3 larvae that did tunnel into the soil in this study must have just completed its moult and was between the fourth and fifth day of the 3rd instar and changed to photonegative behaviour.

Esbjerg (1989) further reported that *A. segetum* larvae's ability to tolerate moist conditions increase when they reach the 4th, 5th and 6th instars, which was confirmed by the observation that the L5 larvae in this study tunnelled into soil, irrespective of soil moisture level. Other studies have reported that cutworm species habits and their reaction to soil moisture may vary. In Canada, Harris and Svec (1968c) reported on the tunnelling (burrowing) behaviour of three cutworm species, i.e., black cutworm, *Agrotis ipsilon* (Hüfnagel), dark-sided cutworm, *Euxoa messoria* (Harris) (Lepidoptera: Noctuidae) and variegated cutworm, *Peridroma saucia* (Hübner) (Lepidoptera: Noctuidae). *Euxoa messoria* started to tunnel at the beginning of L3, *A. ipsilon* in the latter part of L3, whereas *P. saucia* only started to tunnel in L5. Furthermore, *E. messoria* prefers dry soil, but do tunnel in moist soil, whereas *P. saucia* and *A. ipsilon* prefers moist soil and the latter species will only tunnel into dry soils after extensive time was spent on the soil surface.

A review of the literature showed yielded valuable information that suggests insecticide efficacy against cutworms are influenced by numerous factors. No recent studies on this topic were found. In this study, the larval behaviour of *A. segetum* changed according to soil moisture and larval stage. Similarly, Harris and Svec (1968a, 1968b) reported that larval tolerance of *A. ipsilon* and *E. messoria* to insecticides increase with larval stage, which makes control efforts against larger larvae (L4-L6) less effective. For example, a concentration of 0.1% chlorpyrifos caused 100% mortality of 1st-instar *A. ipsilon*, but 0% mortality of the 5th and 6th-instar. This high tolerance of larger larvae to contact insecticides was ascribed to poor penetration of the integument by the insecticide. Harris and Svec (1968a) therefore suggested that insecticide applications should be made early in the season to target small larvae (L1-L3), regardless of planting time.

According to Harris and Svec (1968c), varying insecticide rates was required to control *P. saucia*, *A. ipsilon* and *E. messoria* because of larval behaviour differences. Each species burrows at different instars, i.e., *E. messoria* early in L3, *A. ipsilon* later in L3 and *P. saucia* only in L5. Furthermore, each species has its own soil moisture preference with *E. messoria* preferring dry soil, whereas *A. ipsilon* and *P. saucia* prefer moist soil. For this reason, Harris and Svec (1968c) suggested that an insecticide that is effective as a contact insecticide to one species, may be less effective to another species as a soil insecticide.

Cultivation practices influence insecticide efficacy by influencing soil moisture, soil type and organic content (Holland, 2004). In studies of *A. ipsilon* and *E. messoria*, Harris and Svec (1968a, 1968b) reported that in sandy soils, which dry quickly, chlorpyrifos was more effective when incorporated into the soil whereas in the sandy loam and muck soils, which retain moisture better, surface applications of chlorpyrifos were four times more effective than when the insecticide was incorporated into the soil.

Another limiting factor is adsorption. The physiochemical properties of insecticides influence their effectiveness as a soil treatment (Harris, 1972). Although an insecticide may be a highly toxic contact insecticide, its toxicity as a soil insecticide may differ due to differing levels of adsorption by the soil. For example, Harris (1972) found that methomyl was the most toxic direct contact insecticide, but the least effective in the soil. Furthermore, Harris (1972) observed that other highly effective contact insecticides such as parathion (organophosphate) and DDT (organochlorine), were strongly adsorbed by the soil, and therefore less active as a soil treatment, whereas chlorpyrifos, aldrin (organochlorine) and trichloronate (organophosphate) were less adsorbed by the soil, thus effective as soil insecticides.

The method of insecticide application and soil moisture is another influencing factor (Harris & Svec, 1968a, 1968b, 1968c; Harris & Svec, 1970; Leistra & Green, 1990). In Chapter 2, farmers reported that they applied insecticides onto the soil surface with planting between 10:00 and 16:00 during the day. According to Harris and Svec (1970), most insecticides are adsorbed and become inactivated in dry soil while they are desorbed and activated in moist soil. To confirm, Harris and Svec (1970) tested the toxicity of six emulsifiable concentrate (EC) formulations against L4 and L5 *E.*

messoria larvae using field-moist or airdry conditions for surface and incorporated applications. Results showed that insecticides were less effective in dry soil, especially when applied onto the surface. Soil-surface applications were complicated by the deactivation of insecticides in dry soil and the rapid degradation of short-residual compounds on the surface. Harris and Svec (1968a) also reported that chlorpyrifos (EC) was more effective when incorporated into the soil than when applied onto the soil surface, since surface applications became inactive when the soil dried. The incorporation of the EC into the soil helped to maintain consistent soil moisture levels, which kept the insecticide active. It was concluded that to obtain cutworm control, insecticides had to be incorporated into the top 5 cm of the soil and that applications must be made while the soil is moist to extend the activity of the insecticide. Leistra and Green (1990) also reported that the distribution of insecticides should be matched to the spatial pattern of the insect pest. Surface applications are exposed to sunlight that might cause photochemical transformation and rapid activity loss. In the case of cutworms, which occur in the soil at shallow depths and attack crop plants below the soil surface, Leistra and Green (1990) suggested that effective control may be achieved by the incorporation of insecticides into the soil. Insecticide labels from agrochemical companies in South Africa provide instructions regarding the environmental conditions under which insecticides should be applied to ensure optimal efficacy (Agri-Intel, 2023). For *A. segetum*, labels indicate that soils must be prepared, and the top 30 mm must be moist at the time of insecticide application.

According to Harris and Svec (1968c), product formulation is also an influencing factor. Dusts are most effective when emergency control is needed, whereas emulsifiable concentrations are the most consistent type of formulation whether applied as a pre-planting treatment onto the soil surface or incorporated into the soil. No dust formulations of insecticides aimed at cutworm control are however registered in South Africa.

Leistra and Green (1990) listed the factors that influence the efficacy of soil-applied insecticides as follows: properties of the insecticide, application method, properties of the soil, climatic conditions and the behaviour of the pest. From this review, it is clear that cutworm control is challenged by more than one factor and that insecticide resistance may not be the reason for poor cutworm control as experienced over the past four planting seasons in South Africa.

4.6 Conclusion and recommendations

This study showed that soil moisture conditions have an influence on larval activity at night, which may have an indirect effect on the contact-time with surface applied insecticides. Furthermore, this study showed that large larvae are more tolerant of wet soils, which highlights the importance of applying insecticides based on the biology and behaviour of cutworms. Studies should be conducted to determine the degree of which the efficacy of soil-applied or incorporated insecticides is influenced by soil moisture and *A. segetum* behaviour.

4.7 References

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CHAPTER 5: Conclusion and recommendations

5.1 General conclusion, recommendations and future studies

Maize (*Zea mays* L.) (Poaceae) is among the three most important staple crops produced globally (Erenstein *et al.*, 2022). The demand for maize is continually rising as the human population increases, which puts farmers under pressure to produce greater yields. Various factors can contribute to low yields, of which insect pests, especially lepidopteran species of the Noctuidae family, are the most important. Among these lepidopteran pests is the common cutworm, *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae). In South Africa, cutworms have been ranked as the second most important insect pest in 1996 (Bell & McGeoch, 1996), but from 2021 onwards, incidence reports have increased as cutworms re-emerged as a major constraint to maize production in the country. No research has been done on this pest in South Africa after the publication by Drinkwater and Van Rensburg (1992), and only review information was provided by Drinkwater (1980), Du Plessis (2000) and Drinkwater (2017). This study is the first that assessed the economic impact of this pest in South Africa and to provide any information on biological control agents of this pest.

Agrotis segetum reduces attainable yields at the start of planting season by severing maize seedlings, often to such an extent that the seedling is chewed off entirely and unable to recover. If stand loss is high, this leaves farmers with no other option than to replant, which is many a times an unforeseen financial expense (Du Plessis, 2000; Drinkwater, 2017). This study showed that *A. segetum* continues to cause significant damage to maize seedlings and for the first time, stand losses to soybean and sunflower seedlings were reported. The damage observed to other host plants is coherent with the species' polyphagous behaviour (CABI, 2021) and highlights the possibility of cutworm infestations in soybean and sunflower fields in South Africa.

In the semi-arid production regions of South Africa, farmers are shifting away from traditional conventional tillage practices and increasingly adopting tillage practices such as no-till and conservation agriculture (Sithole *et al.*, 2016). The benefits this presents include reduced soil erosion, soil compaction and fuel consumption, higher soil organic matter, improved water quality and soil moisture, and increased yield (Holland, 2004; Oerke, 2006). However, along with the benefits come disadvantages.

For instance, the populations of insect pests may be higher in no-till than in conventional tillage systems because of a more favourable habitat (Phillips *et al.*, 1980; Capinera, 2005; Rowen *et al.*, 2020). Weed species composition and presence is favoured by different tillage practices (Pant *et al.*, 2021). In South Africa, the establishment of cutworm populations are associated with winter weeds (Drinkwater & Van Rensburg, 1992). Previously, Drinkwater (1980) stressed the importance of clearing fields of weeds at least four to six weeks prior to planting to reduce habitat suitability for oviposition, larval development and survival. This study showed that cutworm infestations were mostly related to no-tillage systems and fields where pre-plant weed management practices were implemented between one to three weeks before planting. The possibility also exists that the increased planting of genetically modified herbicide tolerant maize and soybean hybrids in South Africa over the last decade resulted in changing weed management practices, and that duration of the period between the time that pre-plant weed control is done and planting, does not meet the previously recommended 35-day requirement to ensure starvation of larvae before crop seedlings emerge. Although weed management practices were implemented, this study reiterates that the timing thereof, in accordance with the pest's life cycle, is critical.

The role weeds play with regards to the presence of natural enemies have been reported in the United States (Foster & Ruesink, 1984, Foster & Ruesink 1986a, 1986b; Capinera, 2005; Pretorius *et al.*, 2018). This study revealed the abundance of weed species in grain production systems as a result of reduced tillage and conservation agriculture. Although the direct relationship between weed diversity and the diversity of cutworm and natural enemies were not assessed in this study, it is well known that plant species diversity may influence the natural occurrence of biological control agents, specifically parasitoids (Foster & Ruesink, 1984, Foster & Ruesink 1986a, 1986b; Capinera, 2005; Pretorius *et al.*, 2018). Previous studies regarding the presence of cutworm parasitoids in South Africa reported a limited number of parasitoid species, none of which were identified (Staude *et al.*, 2020). In this study, parasitoids responsible for approximately 43.6% of larval mortalities were identified as *Macrocentrus collaris* (Spinola) (Hymenoptera: Braconidae), *Gonia bimaculata* (Wiedemann) (Diptera: Tachinidae) and *Periscepsia carbonaria* (Panzer) (Diptera: Tachinidae). These parasitoid species have also been reported as biocontrol agents

of *Agrotis* spp. in Spain and Turkey (Caballero *et al.*, 1989; Gözüaık *et al.*, 2009). In the United States, black cutworm infestations and their subsequent damage were significantly reduced when parasitism rates were high (Schoenbohm & Turpin, 1977; Sajap *et al.*, 1978). The natural occurrence of these parasitoid species presents a possibility for biological control strategies. However, during the rearing of cutworms in this study, it was observed that although parasitised, larvae continued to feed at a slower rate up until death. Thus, parasitoids may contribute to the reduction of pest pressure, but the effects of parasitism come into effect too late after damage to seedlings have already occurred.

Chemical control is the most widely used tool to manage cutworms. Upon investigation of the chemical control practices used in grain production systems of South Africa, it was shown that farmers resorted to the application of higher dosage rates in an attempt to control cutworms, but pest populations remained unmanaged and continued to inflict damage to seedlings. Control failure, as experienced over the past four cropping seasons, was partly ascribed to possible insecticide resistance (GrainSA, 2023, GrainSA 2024a, GrainSA 2024b, FW, 2024, FW, 2025). However, this study showed that more than one factor influences the effectiveness of insecticides. These include larval size at the time of insecticide application, larval behaviour (tunnelling) with regards to soil moisture, soil moisture at the time of application, and the time of day of application. These findings are consistent with previous studies (Drinkwater, 1980; Leistra & Green, 1990; Van den Berg & Du Plessis, 2022). Neglecting knowledge about the pest's biology and its behaviour within the soil environment as well as the behaviour of different insecticides in different soil conditions may result in the misuse of insecticides, as evidenced by the inconsistent adherence to label recommendations and the application of unregistered insecticides found in this study.

5.1.1. General conclusion

The findings of this study showcase the limitations of relying on a single control strategy and highlights the importance of an integrated management approach to effectively manage *A. segetum*. It is therefore recommended to implement weed management practices as early as five weeks before planting, to create a less favourable habitat for cutworm establishment, development and survival. Furthermore, it is important for farmers and the agrochemical industry representatives to make

informed decisions regarding the method and timing of insecticide applications, based on the ecology of cutworm larvae and the preferred soil moisture conditions for insecticide efficacy.

5.1.2 Recommendations and future studies

Further research should be conducted to establish the economic threshold levels for *A. segetum* in grain production systems in order to minimise pesticide use, to determine how cutworm population buildup is influenced by shifts in the cultivation of herbicide tolerant crops and adoption of reduced tillage practices and the timing of weed management practices. It is further suggested that a study be conducted to investigate the effect of timing of insecticide applications, as well as different methods of application (e.g., overall spray applications, in-furrow applications with planting, and on-row applications after planting) against early and late instars under field conditions, and to investigate possible strategies through which the abundant biological control agents of *A. segetum* can be utilized to manage this pest.

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APPENDIX B: Ethical clearance



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ETHICS APPROVAL LETTER OF STUDY

Based on the review by the Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC), the Committee hereby clears your study as no ethical risk. This implies that the FNASREC grants permission that, provided the general conditions specified below are met, the study may be initiated, using the ethics number below.

Study title: Assessment of the pest status, parasitism levels and efficacy of chemical control of *Agrotis segetum* (Lepidoptera: Noctuidae)

Study Leader/Supervisor: Prof J Van Den Berg

Student: Ms Z Van Eden

Ethics number:

N	W	U	-	0	1	2	6	8	-	2	3	-	A	9
Institution			Study Number						Year		Status			

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single

Commencement date: 25/05/2023

Expiry date: 04/12/2027

Risk Category:

No risk

General conditions:

The following general terms and conditions apply:

- The commencement date indicates the date when the study may be started.
- In the interest of ethical responsibility, the NWU-SCRE and FNASREC reserves the right to:
 - request access to any information or data at any time during the course or after completion of the study;
 - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;
 - withdraw or postpone approval if:
 - any unethical principles or practices of the study are revealed or suspected;
 - it becomes apparent that any relevant information was withheld from the FNASREC or that information has been false or misrepresented;
- submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and

accurately; and / or

- new institutional rules, national legislation or international conventions deem it necessary.
- FNASREC can be contacted for further information or any report templates via

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The FNASREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the FNASREC or the NWU-SCRE for any further enquiries or requests for assistance.

Yours sincerely

Prof Nomali Ngobese

Chairperson Faculty of Natural and Agricultural Sciences Ethics Committee (FNAS-REC)