Efficacy of selected insecticides for control of stem borers in maize

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Abstract

Lepidopteran stem borers are important pests of cereal crops in sub-Saharan Africa. The stem borer species *Busseola fusca*, *Chilo partellus* and *Sesamia calamistis* are the most widespread and important stem borer species, damaging maize throughout sub-Saharan Africa. Bt maize was planted in South Africa for the first time during the 1998/99 growing season for control of stem borers. The first Bt maize resistant *B. fusca* in South Africa was recorded during the 2006/07 growing season and resistance has since spread to many areas in the country. As a result, renewed interests in insecticides for stem borer control exist. Neonate *B. fusca* and *C. partellus* larvae feed inside the whorls of maize plants and neonate *S. calamistis* larvae feed on the leaf sheath for a short time before penetrating the stem directly from behind leaf sheaths. The efficacy of 14 insecticide treatments was evaluated for control of these three borer species under greenhouse and field conditions. These evaluations were done in 9 greenhouse and 2 field trials. The insecticides evaluated were benfuracarb, benfuracarb in combination with lambda-cyhalothrin, bifenthrin, chlorantraniliprole in combination with lambda-cyhalothrin, chlorfenapyr, chlorpyrifos in combination with lambda-cyhalothrin, gamma-cyhalothrin, indoxacarb in combination with lambda-cyhalothrin, lambda-cyhalothrin 50, lambda-cyhalothrin 106, lufenuron in combination with lambda-cyhalothrin, nuvaluron in combination with lambda-cyhalothrin, spinetoram and spinosad. All these insecticides provided effective control of *B. fusca*, *C. partellus* and *S. calamistis* under greenhouse and field conditions.

**Keywords:** *Busseola fusca*, chemical control, *Chilo partellus*, insecticides, maize pests, *Sesamia calamistis*
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Chapter 1

Introduction and literature review

1.1. Introduction

The increase in world population causes food to be in great demand (Walker and Hodson, 1976). Maize (Zea mays) is one of the most important food sources for humans in sub-Saharan Africa. This cereal crop provides food and an income to more than 300 million people in Africa (Tefera et al., 2011). In South Africa maize has been one of the most important crops since the 1950’s (Van Rensburg et al., 1987).

South African farmers produced 11.8 million tons of maize in 2012 on 3.1 million hectares (FAOSTAT, 2012). Maize can only be successfully cultivated in certain areas of South Africa due to a decreasing gradient in temperature and rainfall from the eastern to western parts (Van Rensburg et al., 1988a, Gbetiboou and Hassan, 2005). Ninety percent of maize in South Africa is therefore produced in the Highveld region (Fig 1.1), with higher precipitation in these eastern parts (Fig. 1.2) (Walker and Schulze, 2008).

Figure 1.1: Regions where 90% of the maize in South Africa is cultivated (Walker and Schulze, 2008).
In earlier years maize was cultivated in South Africa at plant populations varying from 12 to 40 000 plants per hectare according to spacing between rows and between plants (Van Rensburg et al., 1988a). In more recent times maize is cultivated at plant populations of up to 80 000 plants per hectare (Du Plessis, 2003).

Maize is continually attacked by insect pests which pose economical threats to farmers (Van den Berg et al., 2001; Moolman et al., 2013). These insect pest species include various stem borer species with *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) probably being the most widespread and damaging to maize in Africa (Walker and Hodson, 1976).

### 1.2. Maize stem borers in South Africa

The latest surveys show that there are 136 species of stem borers present in East and southern Africa, with 20 of these species that are of economic importance (Moolman et al., 2013; Ong’amo et al., 2013). *Busseola fusca*, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) are considered the most important borers in southern Africa on maize, sorghum and sugar cane (Van Wyk et al., 2007). *Busseola fusca* and *S. calamistis* were present in South Africa before the extensive
cultivation of maize, sorghum and other crops commenced. After the introduction of extensive cropping systems, some of these species became pests and are now considered to be the most important pests of maize and sorghum (Ong’amo et al., 2013). As early as 1920 annual maize yield losses of up to 10% was ascribed to *B. fusca* (Mally, 1920).

Larvae that feed in the whorl of young maize plants are able to cause dead heart symptoms by damaging the growth point of young plants to such an extent that it cannot grow any further (Tilahun and Azerefegne, 2013). Penetration of the stem by larvae causes disruption in the transportation of nutrients and minerals in the maize plant and decreases the functionality of the plant (Van Rensburg et al., 1988a; Tilahun and Azerefegne, 2013). This damage results in yield loss and second generation larvae may attack the ears of the plant causing even more damage. The second generation moths generally attack plants 90-100 days after the first generation moth attack (Walker and Hodson, 1976). Second generation larvae of *B. fusca* may cause yield losses of up to 100% in maize (Tilahun and Azerefegne, 2013).

![Damage caused by *Busseola fusca* larvae in the whorl of a maize plant.](image)

**Figure 1.3:** Damage caused by *Busseola fusca* larvae in the whorl of a maize plant.

Larvae of *B. fusca* are also able to migrate from one plant to another especially when infestation levels are high. The distances between plants influence the migratory success of *B. fusca* as well as the damage caused by this species (Van Rensburg et al., 1988b; Van den Berg et al., 1991). Injuriousness is also influenced by the growth rate of the cultivar planted.
Slower growing cultivars will be damaged more extensively (Van Rensburg et al., 1988b). Secondary damage (on other parts of the plant except the whorl) is more important than primary damage and the number of larvae per plant can therefore not be used to predict yield losses (Van Rensburg et al., 1988a). The variation that occurs in larval infestation levels are mostly due to different planting dates of maize and the number of moths that are present in the environment (Van Rensburg et al., 1987).

Records show that *C. partellus* can cause up to 50% of annual maize yield losses due to their infestation in sub-Saharan countries, India as well as in South East Asia (Sharma and Sharma, 1987; Sharma et al., 2010). This species was described as one of the most damaging species to maize in these parts of the world (Duale, 1999; Khan et al., 2000; Kfir et al., 2002). *Chilo partellus* often re-infests in the same crop through second and even third generation larvae (Van den Berg and Van Rensburg, 1993). The pest status of this species varies considerably between years (Van den Berg and Van der Westhuizen, 1995). Larvae of *C. partellus* are often found feeding behind the leaf sheaths of maize and sorghum in contrast to *B. fusca* which feeds largely on the whorl leaves during early larval stages (Van den Berg and Van Rensburg, 1996).

The rapid reproduction rate of *C. partellus* has an effect on the degree of damage caused by this species. The time of infestation is of more economic importance than the level of infestation because it determines the extent of damage and subsequent yield loss (Van Rensburg and Van den Berg, 1992a).

Like other stem borer species, *S. calamistis* is able to cause ‘dead heart’ by damaging the growth point of the plant to such extent that it is not able to grow any further (Sithole, 1989). Larvae do not leave any feeding lesions on leaves but holes can be seen where penetration took place into the stem. In West Africa, yield losses of up to 100% have been recorded where *S. calamistis* was found in mixed populations with *B. fusca* (Gounou and Shulthess, 2004). In South Africa, the highest infestation levels are found late in the season (Waladde et al., 2001). In the Eastern Cape province of South Africa, *S. calamistis* infestation levels of up to 75% with as much as 13 larvae per plant had been reported (Waladde et al., 2001).
1.3. *Busseola fusca*

*Busseola fusca* larvae are commonly found on maize plants, and this species was also the main pest of grain sorghum until the 1970’s when *C. partellus* became a bigger threat to grain sorghum (Van den Berg and Van Rensburg, 1991).

1.3.1. Biology and identification

Eggs of *B. fusca* are round in shape with the poles slightly flattened and can be found in clusters of 10-80 eggs per batch beneath the leaf sheaths of maize (Figure 1.4a). Female moths lay between 100 and 800 eggs during their short life span (Unnithan, 1987; Van Rensburg et al., 1987; Kruger et al., 2012; Calatayud et al., 2014). Eggs are laid on grain sorghum and other related species, but maize is preferred due to the higher nutritional value in the plant, less deleterious secondary metabolites and thicker stems for the insect to tunnel into (Haile and Hofsvang, 2002). Brownish larvae hatch from eggs (Figure 1.4b) and climb to the whorl of the maize plant where they feed on the whorl leaves (Walker and Hodson, 1976; Kfir, 1997). Larvae leave the whorl after approximately 10-14 days after which they tunnel into the stem of the plant (Walker and Hodson, 1976; Kfir, 1997). All larval instars are known to migrate to neighbouring plants throughout the larval stage (Van Rensburg et al., 1987; Calatayud et al., 2014). Within five weeks after hatch, up to 70% of larvae will migrate to other plants (Van Rensburg et al., 1988b). After this migration of larvae up to 67% of them occur as single individuals per plant (Van Rensburg et al., 1987). The larval stage of *B. fusca* lasts between 31 and 50 days (Onyango and Ochieng’-Odero, 1994; Ratnadass et al., 2001; Kruger et al., 2012; Calatayud et al., 2014) and is at least six weeks when conditions are favourable (Van Rensburg and Van Rensburg, 1993). The larval stage consists of seven to eight instars with the minimum number of instars being six (Unnithan, 1987; Calatayud et al., 2014).

Larvae pupate after completion of the larval stage (Figure 1.4c). Adult moths emerge from the pupae approximately 14 days later and are ready to mate (Figure 1.4d) (Songa et al., 2001). Moths are brown with a wingspan of 25-35 mm. These moths live 5-9 days and eggs are laid two to 4 days after adults emerge from the pupae (Harris and Nwanze, 1992). The life cycle of *B. fusca* takes eight to 10 weeks to complete when conditions are optimal (Songa et al., 2001).
Figure 1.4: Life cycle of *Busseola fusca*: a) eggs; b) larva; c) pupa and d) adult.

Larvae enter diapause during the winter inside the stubble of maize plants for three to five months after which they pupate when conditions are suitable again (Walker and Hodson, 1976). These diapause larvae can be found below the soil surface during the cold winters of the Highveld region of South Africa (Van Rensburg *et al.*, 1987). This is, however, not the case in areas with warmer, more tropic climates. According to Smithers (1960) larvae in diapause can be found up to 60 cm above soil surface in countries such as Zimbabwe. Diapause of larvae is terminated when they physically come into contact with water (Okuda, 1990).
1.3.2. History and distribution

*Busseola fusca* is a lepidopteran species indigenous to Africa and the first studies regarding this species date back to 1920 when it was already identified as a pest of cereal crops (Mally, 1920). This species occurs throughout sub-Saharan Africa but is not found in Zanzibar and Madagascar (Le Ru *et al*., 2006; Calatayud *et al*., 2014). *Busseola fusca* was described scientifically for the first time by Fuller in 1901 as *Sesamia fusca* (Fuller, 1899-1900; Calatayud *et al*., 2014). Hampson described this species one year later under the same name *Sesamia fusca* (Hampson, 1902; Calatayud *et al*., 2014). *Sesamia fusca* was morpho-taxonomically revised in 1953 and placed under the genus *Busseola* (Tams and Bowden, 1953; Calatayud *et al*., 2014). In the eastern parts of Africa, *B. fusca* have been found to pose threats to crops in areas with high altitudes (Mally, 1905) and in most agroecosystems including the semi-arid and arid lowlands to the mountain forests in the highlands of east Africa (Ndemah *et al*., 2001; Ong’amo *et al*., 2006a; Ong’amo *et al*., 2006b; Le Ru *et al*., 2006; Ndemah *et al*., 2006). Central African countries have been found to have similar distributions of this species throughout most altitudes (Cardwell *et al*., 1997). *Busseola fusca* also occurs in most agroecosystems throughout South Africa. These habitats where this species can be found includes areas with relatively low altitudes, coastal areas and areas that are in the mountain areas of altitudes up to 2,131 m a.s.l. (Ebenebe *et al*., 1999; Krüger *et al*., 2008).

Genetically Modified (GM) maize was introduced to South Africa during the 1998/99 season and an approximate area of 50 000 hectares of Bt maize was planted to reduce damage
caused by lepidopteran pests (Van Rensburg, 2001). During the 2004/05 season, B. fusca caused alarming damage to Bt maize plants (Van Rensburg, 2007). The next year another resistant population was recorded approximately 60 km from the area where it was first recorded (Kruger et al., 2009). Resistance to the Bt protein may force farmers to revert to using chemicals to control this pest in the future.

1.3.3. Moth flight patterns

Precautions to control stem borers more effectively can be taken using early indications of the first flight of stem borer moths (Van Rensburg, 1992). The number of moths that are present at a specific locality 3-5 weeks after emergence of a maize crop can be used as a good indication of infestation levels during that particular growing season (Van Rensburg et al., 1985; Van Rensburg et al., 1987). Moth traps are used to quantify flight patterns. Although light traps are effective and can warn farmers of a possible lepidoptera pest infestations, there are some practical limitations in the execution of this method when used for long periods of time. Moth numbers caught in light traps may be influenced by climatic events such as the intensity of moon light which should be taken into consideration (Van Rensburg, 1992). Sex pheromone traps can be used as an alternative method to attract and monitor moth numbers. Control actions should be taken for B. fusca when the mean number of moths captured in three sex pheromone traps exceeds two, at a specific locality per trap in one week (Revington, 1987). This is, however, not an economic threshold but rather an action threshold.

Flight patterns of B. fusca differ between regions. This could be due to differences in climate in different areas (Walters, 1979). There are three moth flights in the warmer central part of South Africa were flights are spaced approximately nine weeks apart (Van Rensburg et al., 1985). The first moth flight in Potchefstroom in the North-West province, commonly take place between the months of October and December, followed by the second flight during January and February and the third flight occurring during late February until middle May (Van Rensburg et al., 1985). The second moth flight is considerably larger than the first flight with regards to the number of moths present. The three flights can be distinguished from one another by a drastic decline in moths between flights although there are seldom periods of time where no moths are present after the second flight (Van Rensburg et al., 1985).
1.3.4. Wild host plants

Busseola fusca is not limited to maize and can successfully reproduce on other wild grass species belonging the Poaceae, Cyperaceae and Typhaceae families (Khan et al., 1997). These host plants serve as a reservoir where B. fusca and other borer species survive when crops are not available (Haile and Hofsvang, 2002; Moolman et al., 2014). There are more than 30 different wild grass species in Africa that can be infested by stem borers including cultivated crops (Khan et al., 1997) (Table 1). Some of the wild host species have been found to have higher infestation levels than crops (Le Ru et al., 2006).

This could be due to various reasons including the fact that some of these species do not consist of thick enough stems for the larvae to penetrate. Two of these species are Napier- and Sudan grass and can therefore be used as trap crops for the the control of certain lepidopteran pests (Van den Berg et al., 2001).

1.4. Chilo partellus

Similar to B. fusca, C. partellus is important on cereal crops (Bate and Van Rensburg, 1992; Van den Berg and Viljoen, 2007; Slabbert and Van den Berg, 2009). Chilo partellus, commonly known as the spotted stem borer, is exotic to Africa and originates from the continent of Asia (Tams, 1932). Chilo partellus can be found in mixed populations with B. fusca in maize and sorghum (Bate and Van Rensburg, 1992). Although C. partellus is not indigenous to Africa, it is still one of the most damaging species of stem borers that occur in this region.

1.4.1. Biology and identification

Chilo partellus moths are able to produce an average of 343 eggs per moth (Ofomata et al., 2000). Eggs are oval with a creamy white colour and approximately 0.8 mm in length (Figure 1.6a) (Panchal and Kachole, 2013). Larvae hatch from the eggs 4-8 days after oviposition (Panchal and Kachole, 2013) and pupate in 28-35 days. Final instar larvae (Figure 1.6b) are 25-30 mm long and rows of dark spots can be seen on the body (Panchal and Kachole, 2013). Pupae are long cylindrical forms that are dark brown in colour (Figure 1.6c), with those of the males smaller than the females (Panchal and Kachole, 2013). Adults (Figure 1.6d) emerge from pupae 5-12 days after pupation (Panchal and Kachole, 2013). The moths have a pale brown colour with an approximate wingspan of 20-30 mm. These moths live for 3-8 days during which they mate and lay eggs (Panchal and Kachole, 2013). The life cycle of C.
partellus takes between 25-50 days to complete which is in some cases up to three times faster than the life cycle of B. fusca (Kfir, 1997; Panchal and Kachole, 2013). This could cause C. partellus to be more competitive than B. fusca. As in the case of B. fusca, C. partellus also goes into diapause during colder winter times, although the occurrence of a mere rest-phase has also been reported (Kfir, 1991).

![Figure 1.6: Life cycle of Chilo partellus. a) eggs; b) larva; c) pupa and d) adult.](image)

### 1.4.2. History and distribution

Until the late 1970’s, only 10% of the stem borer population in maize was C. partellus and this species was not a big threat, in contrast to B. fusca (Van Rensburg et al., 1988c). However, during the 1990’s, 90% of the mixed population with B. fusca, consisted of C. partellus. Before the release of Bt maize in South Africa, Bate and Van Rensburg (1992) argued that the increase in geographical range of C. partellus may result in a threat, complicating chemical control of this species. However, in more recent times C. partellus have not been observed as a threat to maize producers in South Africa because of its susceptibility to the Bt toxin present in Bt maize (Van den Berg et al., 2013). This species does not occur in the cooler eastern and southern parts of the Highveld but thrive in warmer parts in the north-western areas of South Africa. According to Van Hamburg (1979) this species also thrives in more coastal areas of...
Natal (now KwaZulu Natal province) and the lower parts of the Transvaal (now Mpumalanga and Gauteng provinces).

1.4.3.  Moth flight patterns

Pheromone traps can be used to monitor the flight patterns of *C. partellus*. Moth flight patterns can provide useful information with regard to the best time of insecticide application to suppress the number of individuals of this species (Kfir *et al.*, 2002). There is, however, limited information on the relationship between moth catches and the infestation levels in maize and sorghum fields (Kfir *et al.*, 2002). Numbers of moths captured in traps can therefore not be used to determine the economic threshold.

Van Hamburg (1979) studied changes in *C. partellus* adult populations during the grain sorghum growing season by means of light traps and found it to be present throughout the growing season, with a few individuals also active during winter months. There are two distinct moth flights during spring and in late summer. The first peak occurs during September to October and the second from February until the beginning of May (Van Hamburg, 1979).

1.4.4.  Wild host plants

*Chilo partellus* does not only attack sorghum but can survive on other host plants such as wild grass species which enables this species to survive periods when crops are not available (Haile and Hofsvang, 2002) making complete control impossible (Van den Berg and Van Rensburg, 1991). A list of host plants that occur in South Africa is provided in Table 1.1. According to Moolman *et al.* (2014) *C. partellus* has been recorded on wild plant species that are part of the Poaceae and Typhaceae families on the following species: *Arunda donax*, *Coix lacryma-jobi*, *Pennisetum purpureum*, *Sorghum bicolor* and *Sorghum halepense* (Poaceae) family and *Typha capensis* (Typhaceae) family (Moolman *et al.*, 2014). *Chilo partellus* does not occur on any host plants of the family Cyperaceae (Moolman *et al.*, 2014).
1.5. **Sesamia calamistis**

*Sesamia calamistis* is an economically important stem borer species that may cause serious damage if crop management is not performed effectively (Van den Berg and Drinkwater, 2000).

1.5.1. **Biology and identification**

Unlike other stem borer species, larvae of *S. calamistis* do not enter into diapause, but develop throughout the year, even during the dry season (Harris, 1962; Van den Berg and Drinkwater, 2000). Eggs (Figure 1.7a) are laid behind the leaf sheaths of the host plant, in batches of approximately 20 eggs per batch (Ingram, 1958). A single female moth lays an average of 500 eggs which hatch within 6-9 days depending on abiotic factors. Ingram (1958) reported neonate *S. calamistis* larvae to feed for approximately one week in the whorl of the plant after which they penetrate the stem (Ingram, 1958). However, Van den Berg and Van Wyk (2007) reported the vast majority of neonate larvae to feed on the leaf sheath before directly penetrating the stem of the maize plant. Larvae remain inside the stem or ears of the host plant until they pupate. The larval stage is shorter than that of *B. fusca* and is approximately 35-36 days when kept at 28 °C with relative humidity of 65-70% (Songa *et al.*, 2001). *Sesamia calamistis* remains in the pupal stage (Figure 1.7c) for 10-12 days after which the adult moth emerges and are ready to mate (Figure 1.7d) (Sithole, 1989). The life cycle of *S. calamistis* takes 53-54 days to complete when kept at 28 °C and a relative humidity of 65-70% (Songa *et al.*, 2001).
1.5.2. **History and distribution**

*Sesamia calamistis* occurs across Africa and it damages different crops particularly in sub-Saharan Africa where they thrive in warmer coastal areas (Harris, 1989). This species is not economically important on crops in the eastern and southern parts of Africa (Harris, 1962; Overholt and Maes, 2000), although it is the stem borer species with the widest distribution of all stem borer species on the African continent (Van den Berg and Van Wyk, 2007). This species was reported in 1958 to occur in Kenya from sea level to altitudes of up to 1400 m (Ingram, 1958), but Nye (1960) reported it at altitudes of up to 2 400 m in East Africa and mostly near areas with high water supplies. In South Africa, *S. calamistis* is only regarded as a pest in the certain areas of the Western Cape province. It did, however, became a threat to maize and other crops especially in irrigated fields during the 1990's in the Highveld region with an average altitude of below 1300 m above sea level (Van den Berg and Drinkwater, 2000).
1.5.3.  Moth flight patterns

The moth flight patterns of *S. calamistis* are erratic with no definite peaks in numbers of moths although the number increases when conditions are favourable for this species (Harris, 1962). This is due to the fact that *S. calamistis* does not go into a state of diapause but rather develop continuously even when conditions are not favourable for development (Harris, 1962).

1.5.4.  Wild host plants

*Sesamia calamistis* occurs on a number of different plant species with maize, sorghum and sugar cane, the host plants with the highest economic value. Host plants of *S. calamistis* are listed in Table 1.1.

Table 1.1: Wild host plants of *Busseola fusca*, *Chilo partellus* and *Sesamia calamistis* in South Africa and Mozambique (Moolman *et al.*, 2014).

<table>
<thead>
<tr>
<th>Wild host plant</th>
<th>Family</th>
<th><em>B. fusca</em></th>
<th><em>C. partellus</em></th>
<th><em>S. calamistis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Arunda donax</td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Carex distans</td>
<td>Cyperaceae</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>Cenchrus ciliaris</td>
<td>Poaceae</td>
<td>-</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Coix lacryma-jobi</td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Cymbopogon nardus</td>
<td>Poaceae</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Echinochloa haploclada</td>
<td>Poaceae</td>
<td>-</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Echinochloa pyramidalis</td>
<td>Poaceae</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eleusine jaegeri</td>
<td>Poaceae</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>Eriochloa fatmensis</td>
<td>Poaceae</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>Hyparrhenia cymbaria</td>
<td>Poaceae</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hyparrhenia filipendula</td>
<td>Poaceae</td>
<td>-</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
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<td>Poaceae</td>
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<td>X</td>
<td>-</td>
</tr>
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<td>Hyparrhenia rufa</td>
<td>Poaceae</td>
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<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Hyparrhenia tamba</td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Panicum deustum</td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Panicum maximum</td>
<td>Poaceae</td>
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<td>X</td>
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<td>Paspalum urvillei</td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td></td>
<td></td>
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<tr>
<td>----------------------</td>
<td>-----------</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><em>Pennisetum purpureum</em></td>
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<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><em>Pennisetum sphacelatum</em></td>
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<td>-</td>
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</tr>
<tr>
<td><em>Phragmites australis</em></td>
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<td>-</td>
<td>X</td>
<td>X</td>
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<tr>
<td><em>Rottboellia cochinchinensis</em></td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td><em>Setaria incrassata</em></td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td><em>Setaria sphacelata</em></td>
<td>Poaceae</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Setaria verticillata</em></td>
<td>Poaceae</td>
<td>-</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td><em>Sorghum arundinaceum</em></td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em></td>
<td>Poaceae</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Sorghum halepense</em></td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td><em>Sorghum sudanense</em></td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td><em>Sorghum versicolor</em></td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td><em>Sporobolus pyramidalis</em></td>
<td>Poaceae</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Sporobolus marginatus</em></td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td><em>Tripsacum laxum</em></td>
<td>Poaceae</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Typha capensis</em></td>
<td>Typhaceae</td>
<td>-</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><em>Zea mays</em></td>
<td>Poaceae</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

1.6. Genetically modified maize in South Africa

Bt maize was developed by isolating certain genes of the bacterium *Bacillus thuringiensis* (Bt) and inserting it into the genome of the maize plant in order for the plant to express insecticidal proteins. This was done to protect plants against damage caused by lepidopteran pests. Endotoxins are consumed by target insects which feed on maize plants (Mugo *et al.*, 2011). These endotoxins, referred to as Cry1 proteins, were found to be the most effective Cry proteins in killing lepidopteran larvae. Initially this Cry1 transgene was used in maize to control *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) (Archer *et al.*, 2001) and *Diatraea grandiosella* (Dyar) (Lepidoptera: Pyralidae) (Ostlie *et al.*, 1997) in North America. It was later also introduced into South Africa for the control of *B. fusca* and *C. partellus* (Van Rensburg, 1999).
Bt maize was planted in South Africa for the first time during the 1998/99 growing season, making this country the first to use Bt maize at a commercial scale on the African continent (Van Rensburg, 2001). Bt maize planted during the 1998/99 growing season was yellow maize which is mainly used as animal feed in South Africa (Gouse et al., 2005). White Bt maize, mainly for human consumption, was introduced during the 2001/02 growing season (Gouse et al., 2005). In certain areas in South Africa, 100% of farmers plant Bt maize. Although the seed is more expensive, the effect it has on lepidopteran pests is of great economic benefit to farmers. Planting Bt maize resulted in higher incomes for farmers compared to farmers that planted non-GM maize crops because of savings on pesticides and higher yields due to less damage caused by pests (Van den Berg et al., 2013). Significant increases in maize yields of commercial farmers were recorded during the 2000/01 season where up to 10% of smaller farmers recorded increases of up to 32% because of the introduction of Bt maize. However, farmers that planted GM crops had similar yields as farmers that planted non-GM crops during seasons with lower lepidopteran pest pressure (Gouse et al., 2006).

The introduction of new GM plant species to a specific area has, however, some disadvantages. Scientists believe that GM plants that kill specific insect pest species may cause other non-target species to become more abundant (Truter et al., 2014). The increase in numbers of non-target species may cause these organisms to become secondary pests in the agro-ecosystem. The balance in biodiversity is very important especially in agro-ecosystems because of the impact different species have on the functionality of the agronomic system as well as the surrounding ecosystems (Truter et al., 2014). All insect species have a specific function in an agronomic system and provides certain ecosystem services. Disturbances in the number of species and number of organisms may lead to decreases in landscape functionality. Risk assessments should therefore be carried out before the introduction of GM crops into an environment in order to assess the possible effects it may have on the whole insect community. These assessments should also include the possibility that resistance may occur over time and how this evolutionary effect can be delayed (Zhao et al., 2003). There are four main ways that can be used to delay resistance development of insects to Bt crops, namely to make sure that the expression of the Bt-gene provides for a concentration of the toxin that will kill all susceptible individuals in the population, to provide a refuge area where susceptible individuals survive, to use different types of toxins and varieties in a single field, and to use GM plants that are able to produce more than one type of toxin (Zhao et al., 2003).
1.7. Resistance of stem borers to Bt maize

Target pests becoming resistant to the Bt protein poses a threat to the continued success of Bt maize. Field-evolved resistance is a term used to describe the decrease in susceptibility of a specific population to a toxin because of exposure to the specific toxin under field conditions (Tabashnik, 1994; Tabashnik et al., 2009; Van den Berg et al., 2013). The continuous usage of GM crops that produce the same Bt toxin may cause lepidopteran pests to evolve and become resistant to the Bt protein. Resistance against the Bt toxin by target pests (excluding Busseola fusca) have already been recorded in studies under field- and laboratory conditions even before the release of the GM crop, Bt maize (Tabashnik, 1994). The risks of resistance evolution by target pests include a decrease in financial income to Bt maize producers, increased usage of ecological harmful insecticides, and the negative effect it may have on the decision of some countries to adopt GM crops (Frisvold and Reeves, 2010). These risks are recognised and specific regulatory requirements are set and monitored by an IRM (Insect Resistant Management) program for growers, producers and the public to benefit from (Frisvold and Reeves, 2010).

The IRM strategy is used in South Africa is referred to as the high-dose/refuge strategy (Van den Berg et al., 2013). This strategy is based on two important measures that will be ineffective if not applied. These measures include that farmers plant a large area of maize plants that express a high dose of the Bt toxin and an area that is planted with non-Bt maize (refuge area). The high dose area that produces the Bt toxin is important to kill as many as possible of the target pest while the refuge area is used to produce large numbers of susceptible individuals. Heterozygous (RS types) individuals are more difficult to control because they carry an allele that makes them resistant to the Bt toxin. Homozygous (SS types) individuals are not resistant to the Bt toxin and the refuge area is used to increase the number of homozygous SS individuals in the population. A high number of homozygous individuals will increase the chance of mating between a homozygous individual and a heterozygous individual which will cause the offspring to remain susceptible to the Bt toxin. The offspring that is not resistant to the Bt toxin will complete their life cycle if they feed on non-Bt maize and will again produce offspring that is not resistant to Bt maize. This will not stop resistance development but is set in place to delay the process (Van den Berg et al., 2013). It is important to apply insecticides that are effective and to use a variety of chemical formulations in a chemical control strategy to contribute to delay resistance development to the chemical compounds (Yu, 2008).

In South Africa there are certain standards that must be met regarding the size of the refuge area. These requirements state that a refuge area must consist of at least 20% of the maize field if insecticides will be applied during the growing season, otherwise 5% of the maize field.
should consist of non-Bt maize but no additional insecticides may be applied throughout the season (Kruger et al., 2011; Van den Berg et al., 2013). This IRM strategy is used all over the world where Bt maize is planted to postpone resistance development of target insects to this toxin. This strategy will only be effective if a series of assumptions are true which include: the gene that encodes for resistance against the Bt toxin is recessively inherited; that resistant alleles are rare in a population and mating will take place randomly between individuals that are susceptible and resistant; the refuge area produces a high number of individuals that are susceptible to the Bt toxin and the Bt plants expresses a high dose of toxins that will kill a very high proportion of individuals (Bourguet, 2004; Tabashnik et al., 2009; Van den Berg et al., 2013).

Resistance of *B. fusca* to the Bt toxin in crops is currently a threat in South Africa and reports of resistant populations are more frequently found in new localities (Van den Berg et al., 2013). The Cry1Ab gene is thus not an effective method to control this lepidopteran pest any longer in many localities in South Africa.

Bt maize was genetically modified to express either the Cry1 or Cry2 genes to control Lepidoptera (Van den Berg et al., 2013). There was only one event available for commercial farmers in South Africa after the introduction of MON810 in 1998 until 2006. During 2006, Bt11 was introduced which is a different product but expressed the same Cry1ab protein as the MON810 event (Van den Berg et al., 2013). The resistance development of *B. fusca* during the growing season of 2004/05 lead to new events tested to control these lepidopteran pests in South Africa (Van den Berg et al., 2013). MON89034 is a pyramided event, containing two different Cry toxins, namely Cry1A.105 and Cry2Ab2 proteins (Van den Berg et al., 2013). It was planted commercially for the first time in South Africa during the 2012/13 growing season for the specific reason to control *B. fusca* that became resistant to the MON810 event (Van den Berg et al., 2013). Up to date, there have been no reports of field resistance to the MON89034 event.

### 1.8. Insecticides

Insecticide application has been practiced for more than two millennia in countries including China, India, Greece and Egypt to prevent insect infestation or to reduce the numbers of insects in crops (Isman, 2006). In Europe and North-America evidence of insecticide usage dating more than 150 years back have been found (Isman, 2006). Insecticides can be organised into different groups. There are three main ways to classify insecticides, namely according to the mode of entry of the insecticide into the insects' body, the chemical
composition of the insecticide, and the mode of action of the chemical group (Isman, 2006). Insecticides can also be grouped according to how the remedy is taken up by insect pests. These groups include contact insecticides which enter the body of the insect when the insect comes into contact with the remedy. Insects can therefore move over the treated area of the plant or the spray can be applied directly onto the target pest (Gerolt, 1969). Stomach insecticides on the other hand should be ingested by the insect through plant material (Toppozada et al., 1964). Systemic insecticides which are taken up by plants are ingested by pests that feed on plant material (Drinkwater et al., 1979). After ingestion of plant material these chemicals then serve as stomach insecticides. Fumigants are used to control insect pests by gaseous vapours that are absorbed through spiracles (Isman, 2006). Insect pest damage can also be reduced by chemical compounds that do not necessarily reduce the number of individuals in a population, for example, insect growth regulators (Shimizu et al., 1997).

Some insecticides may have more than one of the above mentioned characteristics. For example, the active ingredient benfuracarb has systemic- and contact insecticidal properties (Tomlin, 2009). The insecticides that are registered in South Africa and can be legally applied currently by farmers in South Africa for the control of the stem borer species *B. fusca*, *C. partellus* and *S. calamistis* are listed in Table 1.2.
### Table 1.2

Chemical formulations currently registered in South Africa for control of *Busseola fusca*, *Chilo partellus* and *Sesamia calamistis* (Van Zyl, 2013).

<table>
<thead>
<tr>
<th>Chemical subgroup</th>
<th>Pesticide active ingredient (a.i.)</th>
<th>Species a.i. registered against</th>
<th>Type of formulation</th>
<th>Mode of action in/on plant</th>
<th>Mode of action in/on insect</th>
</tr>
</thead>
<tbody>
<tr>
<td>carbamate +</td>
<td>benfuracarb + alpha-cypermethrin</td>
<td>Bf, Cp</td>
<td>EC / SC</td>
<td>S + NS</td>
<td>C, S</td>
</tr>
<tr>
<td>pyrethroid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>carbamate +</td>
<td>benfuracarb + cypermethrin</td>
<td>Bf, Cp</td>
<td>EC</td>
<td>S + NS</td>
<td>C, S</td>
</tr>
<tr>
<td>pyrethroid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>carbamate +</td>
<td>benfuracarb + esfenvalerate</td>
<td>Bf, Cp</td>
<td>EC</td>
<td>S + NS</td>
<td>C, S</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>benfuracarb + lambda-cyhalothrin</td>
<td>Bf, Cp</td>
<td>EC</td>
<td>S + NS</td>
<td>C, S, Re</td>
</tr>
<tr>
<td>pyrethroid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>carbamate</td>
<td>benfuracarb/fenvalerate</td>
<td>Bf, Cp</td>
<td>EC</td>
<td>S + NS</td>
<td>C, S</td>
</tr>
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<td>/pyrethroid</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>beta-cyfluthrin</td>
<td>Bf, Cp</td>
<td>SC</td>
<td>NS</td>
<td>C, S</td>
</tr>
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<td>chlorpyrifos/lambdacypsyhalothrin</td>
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<td>EC</td>
<td>NS</td>
<td>C, S, R, Re</td>
</tr>
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<td>NS</td>
<td>C, S</td>
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<tr>
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<td>esfenvalerate</td>
<td>Bf, Cp</td>
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<td>oxadiazine</td>
<td>indoxacarb</td>
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<td>NS</td>
<td>C, I, AF</td>
</tr>
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<td>GR</td>
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<td>EC</td>
<td>S</td>
<td>C, S</td>
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<td>carbaryl</td>
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<td>GR</td>
<td>SS</td>
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<td>EC</td>
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<td>C, S</td>
</tr>
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<td>thiodicarb</td>
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<td>SC</td>
<td>S</td>
<td>S, Sc</td>
</tr>
<tr>
<td>pyrethroid</td>
<td>zetacypermethrin</td>
<td>Bf</td>
<td>EW</td>
<td>NS</td>
<td>C, I</td>
</tr>
</tbody>
</table>

**Key to table 1.2** (Van Zyl, 2013):

- EC – Emulsifiable concentrate: A liquid, homogeneous formulation to be applied as an emulsion after dilution in water.
- GR – Granule: A free-flowing solid product of a defined granule size range, ready for use.
- WG – Water dispersible granule: A formulation consisting of granules to be applied after disintegration and dispersion in water.
- EW – Emulsion, oil in water: A fluid, heterogeneous formulation consisting of a dispersion of fine globules of pesticide in an organic liquid in a continuous water phase.
- Bf – *Busseola fusca*
- Cp – *Chilo partellus*
- Sc – *Sesamia calamistis*
- C – Contact action
- S – Stomach action
- Re – Repellent
- R – Respiratory action
- AF – Anti-feeding
- I – Ingestion
- NS – Non-systemic
- SS – Slightly systemic
- Sc – Slightly contact

### 1.9. Mode of action of insecticides

There is a wide variety of chemical products available on the market to kill different insect pests that damage crops. Insecticides that are available have different modes of action and some can be mixed with others to increase the efficacy of the agent. Chemical control of stem borers is limited because complete control is very seldom achieved and small farmers cannot afford these chemicals in order to control pests (Midega *et al*., 2005).

The Insecticide Resistance Action Committee (IRAC) developed a scheme to classify various modes of actions (MoA) of insecticides (IRAC, 2015). The aim of this scheme is to provide farmers with information regarding active ingredients in order to reduce resistance development of insect pests by using rotation of insecticides with different modes of action throughout the growing season (IRAC, 2015).

The mode of action of insecticides is what happens at a cellular level to an organism when it is exposed to a certain chemical compound (IRAC, 2015). There is a wide variety of chemical compounds used to control insect pests, some may even have the same mode of action but are different chemical compounds. According to IRAC, chemical compounds are grouped in a main group and primary site of action and further divided into chemical sub-groups or examples of active ingredients as presented in Table 1.3 (IRAC, 2015).
Table 1.3: Main groups of chemical compounds based on primary site of action and chemical sub-groups (IRAC, 2015).

<table>
<thead>
<tr>
<th>Main group</th>
<th>Primary target site of action</th>
<th>Chemical sub-group(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acetylcholinesterase (AChE) inhibitors</td>
<td>Nerve and muscle</td>
<td>1A Carbamates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1B Organophosphates</td>
</tr>
<tr>
<td>2. GABA-gated chloride channel antagonists</td>
<td>Nerve and muscle</td>
<td>2A Cyclodiene organochlorines</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2B Phenylpyrazoles (Fiproles)</td>
</tr>
<tr>
<td>3. Sodium channel modulators</td>
<td>Nerve and muscle</td>
<td>3A Pyrethroids and Pyrethrins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3B DDT and Methoxychlor</td>
</tr>
<tr>
<td>4. Nicotinic acetylcholine receptor (nAChR) agonists</td>
<td>Nerve and muscle</td>
<td>4A Neonicotinoids</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4B Nicotine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4C Sulfoxaflor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4D Butenolides</td>
</tr>
<tr>
<td>5. Nicotinic acetylcholine receptor (nAChR) allosteric activators</td>
<td>Nerve and muscle</td>
<td>5 Spinosyns</td>
</tr>
<tr>
<td>6. Chloride channel activators</td>
<td>Nerve and muscle</td>
<td>6 Avermectins and Milbemycins</td>
</tr>
<tr>
<td>7. Juvenile hormone mimics</td>
<td>Growth regulation</td>
<td>7A Juvenile hormone analogues</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7B Fenoxycarb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7C Pyriproxyfen</td>
</tr>
<tr>
<td>8*. Miscellaneous nonspecific (multi-site) inhibitors</td>
<td>Non-specific</td>
<td>8A Alkyl halides</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8B Chloropicrin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8C Sulfuryl fluoride</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8D Borates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8E Tartar emetic</td>
</tr>
<tr>
<td>9. Modulators of Chordotonal Organs</td>
<td>Nerve and muscle</td>
<td>9B Pymetrozine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9C Flonicamid</td>
</tr>
<tr>
<td>10. Mite growth inhibitors</td>
<td>Growth regulation</td>
<td>10A Clofentezine, Hexythiazox and Diflovidazin</td>
</tr>
<tr>
<td>Main group</td>
<td>Primary target site of action</td>
<td>Chemical sub-group(s)</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------</td>
<td>-----------------------------</td>
<td>-----------------------------------------------------------</td>
</tr>
<tr>
<td>10B Etoxazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Microbial disruptors of insect midgut membranes</td>
<td>Midgut</td>
<td>11A <em>Bacillus thuringiensis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11B <em>Bacillus sphaericus</em></td>
</tr>
<tr>
<td>12. Inhibitors of mitochondrial ATP synthase</td>
<td>Respiration</td>
<td>12A Diafenthiuron</td>
</tr>
<tr>
<td>Energy metabolism</td>
<td></td>
<td>12B Organotin miticides</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12C Propargite</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12D Tetradifon</td>
</tr>
<tr>
<td>13*. Uncouplers of oxidative phosphorylation via disruption of the proton gradient</td>
<td>Respiration</td>
<td>13 *Chlorfenapyr, DNOC and Sulfluramid</td>
</tr>
<tr>
<td>14. Nicotinic acetylcholine receptor (nAChR) channel blockers</td>
<td>Nerve and muscle</td>
<td>14 Nereistoxin analogues</td>
</tr>
<tr>
<td>15. Inhibitors of chitin biosynthesis, type 0</td>
<td>Growth regulation</td>
<td>15 Benzoylureas</td>
</tr>
<tr>
<td>16. Inhibitors of chitin biosynthesis, type 1</td>
<td>Growth regulation</td>
<td>16 Buprofezin</td>
</tr>
<tr>
<td>17. Moulting disruptor, Dipteran</td>
<td>Growth regulation</td>
<td>17 Cyromazine</td>
</tr>
<tr>
<td>18. Ecdysone receptor agonists</td>
<td>Growth regulation</td>
<td>18 Diacylhydrazines</td>
</tr>
<tr>
<td>19. Octopamine receptor agonists</td>
<td>Nerve and muscle</td>
<td>19 Amitraz</td>
</tr>
<tr>
<td>20. Mitochondrial complex III electron transport inhibitors</td>
<td>Respiration</td>
<td>20A Hydramethylnon</td>
</tr>
<tr>
<td>Energy metabolism</td>
<td></td>
<td>20B Acequinocycl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20C Fluacrypyrim</td>
</tr>
<tr>
<td>21. Mitochondrial complex I electron transport inhibitors</td>
<td>Respiration</td>
<td>21A METI acaricides and insecticides</td>
</tr>
<tr>
<td>Energy metabolism</td>
<td></td>
<td>21B Rotenone</td>
</tr>
<tr>
<td>22. Voltage-dependent sodium channel blockers</td>
<td>Nerve and muscle</td>
<td>22A Indoxacarb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22B Metaflumizone</td>
</tr>
<tr>
<td>23. Inhibitors of acetyl CoA carboxylase. Lipid synthesis</td>
<td>Growth regulation</td>
<td>23 Tetronic and Tetramic acid derivatives</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24. Mitochondrial complex IV electron transport inhibitors</td>
<td>Respiration</td>
<td>24A Phosphine</td>
</tr>
<tr>
<td>Energy metabolism</td>
<td></td>
<td>24B Cyanide</td>
</tr>
</tbody>
</table>
### Key to table 1.3:

* number refers to a sub-group within the main group

*Groups 8, 13 and UN do not share a common target site and can be rotated at any time if there are no signs of cross-resistance.

Some of the sub-groups are examples of active ingredients while other consists of various active ingredients that belong to that sub-group (IRAC, 2015). Sub-groups containing insecticides relevant to this study will be discussed below:

### 1.9.1. Carbamates

The first time that carbamates were successfully used was in 1956 with the active ingredient, carbaryl (Fukuto, 1990). The active ingredient benfuralcarb is an example of a carbamate and has a systemic and contact action. The mode of action of a carbamate is to inhibit the enzyme, cholinesterase (ChE) in the insect’s body that is vital for its survival. There are also carbamates available that are able to inhibit the enzyme, aliesterase, to a large extent. Carbamates are able to reversibly inhibit neuropathy target esterase (Sogorb and Vilanova, 2002).
1.9.2. Pyrethroids

Pyrethroids have a stomach and contact mode of action (Tomlin, 2009). Pyrethrums that occur naturally have not been used often in the past in crops because it is expensive and it rapidly breaks down in sunlight. Pyrethroids have been synthesised and enhanced over the last three decades and is very effective on a wide variety of insect pest species (Tomlin, 2009). It is, however, also very toxic to beneficial insects. The peripheral and central nervous system of an insect is affected when it is exposed to a pyrethroid. Paralysis is caused by nerve cells which are stimulated to produce high quantities of discharges in the sodium channel. The sodium ions do not enter the channel and excitation is inhibited. Examples of pyrethroids are bifenthrin and lambda-cyhalothrin.

1.9.3. Diamides

Exposure of an insecticide with a diamide as active ingredient, will lead to the activation of ryanodine receptors (Lahm et al., 2005). These receptors regulate the flow of calcium inside the body of an insect. The deficiency of calcium in the body causes muscle paralysis and lethargy (Tomlin, 2009). The insect is killed when it ingests the chemical compound or come into direct contact with it, and it therefore has a stomach and contact mode of action. The active ingredient chlorantraniliprole is an example of a diamide.

1.9.4. Arylpyrroles

Chlorfenapyr is an active ingredient that is the only member of the arylpyrrole group. This unique insecticide is taken up by the insect through either ingestion or by coming into physical contact with it (stomach and contact mode of action). This insecticide inhibits the process of oxidative phosphorylation. Oxidative phosphorylation is a process in the insect's body that forms an energy source, adenosine triphosphate (ATP), that is crucial for the survival of the insect (Tomlin, 2009).

1.9.5. Organophosphates

Organophosphates have a stomach and contact mode of action. Organophosphates are chemically very unstable and non-persistent and are therefore used on crops that are consumed by people (Rosenstock et al., 1991). When the insect consume or come into contact
with organophosphates, its muscles will start twitching involuntarily which is followed by paralysis. This is caused by the irreversible binding with a very important enzyme of the nervous system of the insect called cholinesterase (ChE). The irreversible binding on ChE causes acetylcholine (ACh) to accumulate at the synapses (Tomlin, 2009). The active ingredient, chlorpyrifos, is an example of an organophosphate.

1.9.6. Oxadiazines

The active ingredient, indoxacarb, was introduced to the market for the first time in 2000 and is an example of an oxadiazine insecticide. This compound is activated in insects through amidases and esterases, but mammals are able to break this substance down (Tomlin, 2009). This product is therefore safer for humans than organophosphates. The vast majority of Lepidoptera species can be controlled by this product as well as certain insects belonging to the Coleoptera and Diptera (Tomlin, 2009). Indoxacarb blocks the sodium channel by introducing the N-decarbomethoxylated metabolite (Wing et al., 1998). This active ingredient is activated when an insect ingest or comes into contact with it (Tomlin, 2009).

1.9.7. Benzoylureas

The sub-group benzoylurea inhibits the biosynthesis of chitin, the most essential element in the cuticle of insects. For moulting insects, the lower concentration of chitin in the body of the insect leads to an exoskeleton that is soft and weak. Appendages and reproductive organs are also deformed (Matsumura, 2010). Benzoylurea is especially efficient for the control of Lepidoptera. This sub-group is considered to have a low toxicity level to mammals but may be very toxic to aquatic arthropods, although it is not very soluble in water and have a low potential of leaching in the soil (Tomlin, 2009). Lufenuron and nuvaluron are examples of this sub group and is mainly effective when consumed by the pests but also have some contact characteristics (stomach and contact action) (Tomlin, 2009).

1.9.8. Spinosyns

Spinosyn insecticides are a unique sub-group and are produced by gram-positive soil microbes (Kirst, 2010). Spinosyns was available on the market for the first time in 1995 in the form of spinosad. Spinosad and spinetoram are the only two active ingredients that is part of this sub-group (Kirst, 2010). Spinetoram was introduced in 2007 and is considered to be even
more lethal to a wider variety of insect pests than spinosad. Spinosyns are selective allostatic modulators that act on the macrocyclic lactone site. Therefore these compounds act on different target proteins (Kirst, 2010). Spinosyns binds to a specific receptor that slightly modifies the ACh binding site and continuously activates acetylcholine receptors. It causes hyper-excitation and after a while contractive paralysis. Spinosyns are considered in IPM because it is not known to be toxic to beneficial arthropods, but it is toxic to honeybees (Tomlin, 2009). Spinosyns are fairly safe for mammals except in very high quantities. This insecticide is considered to be the safest agricultural remedy to humans according to the Environmental Protection Agency (EPA) of the United States (Environmental Protection Agency) (Liu and Li, 2004). Spinosyns are effective when the substance is ingested by an insect or when the insect comes into contact with this chemical (Tomlin, 2009).

1.10. Application of insecticides

There are different approaches regarding the chemical control of stem borers in South Africa (Van Rensburg, 1990). The efficacy of insecticides is influenced by many factors, for example timing of insecticide application, nozzle type and droplet size, addition of adjuvants, mixing of insecticides and method and direction of application.

1.10.1. Timing of insecticide application

There are two main insecticide application strategies, namely preventative and curative (Morales-Rodriguez and Peck, 2009). The timing of insecticide application for the control of stem borers have been studied by various authors and some differ from one another (Van Rensburg et al., 1988d; Van Rensburg, 1990; Van Rensburg and Van den Berg, 1992b). These differences in results obtained in different studies are largely ascribed to different planting dates and different localities of study. Du Plessis and Lea (1943) recommended that the best time for insecticide application is when 33% of plants show stem borer damage, while Heenop (1963) suggested that chemicals should be applied when only five percent of plants has been infested. Heenop (1963) also suggested that it may even be of economic importance to apply insecticides when the maize plants reach a height of 30 cm before any damage is visible to any of the plants as a preventative treatment to control the first generation larvae.
1.10.2. Nozzle type and droplet size

There are different types of nozzles readily available on the market and the different types are used to apply pesticides to control insects, weeds or diseases. Some of these nozzles include: flat fan nozzles, turbo teejet nozzles, floodjet nozzles, air induction nozzles, even flat fan nozzles, hollow cone nozzles and full cone nozzles (Janse van Vuuren, 2013). Nozzles have three main functions during application of pesticides. These functions are regulation of the flow rate of the chemical applied, transformation of the pesticide into droplets to form a certain pattern, and assisting in guiding the chemical to the target area (Gil and Sinfort, 2005).

Identifying the correct nozzle and pressure at which the insecticide should be applied is very important. This ensures that the correct concentration of insecticide is applied to the plant at a constant rate. Spray drift can also be minimized when applying insecticides with a larger nozzle which increases the droplet size (Nuyttens et al., 2007).

1.10.3. Addition of adjuvants

Surfactants are used to improve the physico-chemical characteristics of the carrier of an insecticide by means of changing the properties of the carrier in various ways (Slabbert and Van den Berg, 2009). Changing the physical and chemical properties of the carrier of the insecticide by reducing the surface tension of the carrier will ensure that insecticides will move further down the whorl of a maize plant, reaching more larvae (Slabbert and Van den Berg, 2009). Surfactants have been used for a long time as wetting-, spreading-, emulsifying- and sticking agents in order to improve the efficacy of the insecticide with some even having some types of insecticidal property (Liu and Stansly, 2000).

1.10.4. Mixing of insecticides

It is important to prepare insecticides for application as specified by the label for maximum efficacy. Pesticides that have a sub-lethal level of toxicity to target pests, but still kill natural enemies of the pests, may cause target pests to thrive, resulting in even higher yield losses (Kipkoech et al., 2010).
1.10.5. Method and direction of application

The method used when applying insecticides as well as the position in which the nozzles are directed is of utmost importance. The mode of action of insecticides must also be kept in mind when applying it to the crop, for instance, it will be of no use if a contact insecticide is sprayed on the lower parts of a plant like some systemic insecticides that have acropetal characteristics. When controlling stem borers, chemicals should be applied into the whorls of plants while larvae are still young (Van den Berg and Van Rensburg, 1996).

The position of the larvae on the plant must be considered when applying insecticides. The larvae of *C. partellus* and *B. fusca* occur on different parts of the plant and the method of control should apply to the position they occur. Before tunnelling into the stem, *C. partellus* occurs between the leaf sheath and the stem while *B. fusca* larvae are largely found in the whorls of maize plants until tasseling occur (Van den Berg and Van Rensburg, 1996). Van den Berg and Van Rensburg (1996) speculated that it might be more effective to apply insecticides towards the sides of the plants when *C. partellus* is present and from the top (inside the whorl) in the case of *B. fusca*. Slabbert and Van den Berg (2009) did, however, show that the application of insecticides to the sides of plants does not improve the efficacy of chemical control of *C. partellus* and that the most effective method to reduce stem borer damage is the application of chemicals in the whorl of the maize plant. This is also not always effective because the chemicals do not always reach the larvae inside the whorl (Slabbert and Van den Berg, 2009).

The time and method used to control these stem borer pests can be very complex especially when mixed populations of *B. fusca*, *C. partellus* and *S. calamistis* are found in the same crop. The biological differences between the different species can make it difficult because of the period of larval development; intra-seasonal infestation levels and the degree of damage caused are all different with each species (Van Hamburg, 1980; Van Rensburg *et al.*, 1988c; Van den Berg and Van Rensburg, 1991). Because of these differences between species, other chemical application methods should be used when controlling mixed populations and populations consisting of only one species on maize (Van den Berg and Van Rensburg, 1996).

1.11. Toxicity and safety of insecticides

Toxicity of a substance can be defined as the poisonous potential of a chemical under specific conditions under laboratory conditions (Pedigo, 2002). Toxicological studies are performed on new active ingredients in order to verify if the substance is safe enough for human use if
applied to crops (Pedigo, 2002). The mode of action of the insecticide determines how a person that has been affected by such a substance will respond. There are two main types of poisonings in humans. These types include acute- and chronic poisoning. Acute poisoning refers to when a human have been affected by a single dose of a pesticide that may cause serious illness or death (Ranjbar et al., 2005). Chronic poisoning on the other hand refers to the long-term exposure of chemicals at very low levels. Although symptoms are not usually visible during the early stages of exposure, laboratory studies on animals have shown that exposure to certain pesticides over a long period of time may cause diseases like cancer, damage the genetic material of mammals and birth defects when pregnant individuals are exposed to these substances (Pedigo, 2002).

Human safety is of utmost importance especially with people becoming more concerned about their health when consuming certain foods (Wilcock et al., 2004). The International Programme on Chemical Safety provides a set of standards that can be used by governments and local authorities were they prescribe the maximum residue limit (MRL) (International Programme on Chemical Safety, 2009). The MRL is the maximum amount of residue of a particular substance that is considered to be safe for human use (International Programme on Chemical Safety, 2009).

Therefore toxicologists have to keep in mind the “no residue” concept when evaluating these chemicals. According to Van Zyl (2013) all pesticides are toxic and should be handled accordingly. The toxicity of pesticides is tested on mammals (rats for example) under laboratory conditions to estimate the LD$_{50}$. The LD$_{50}$ of a pesticide is the quantity of the active ingredient in milligrams that will kill 50\% of a random selected animal population per kilogram of the body weight of the individual (Van Zyl, 2013). Chemicals can be absorbed orally by physically ingesting a substance or by coming into contact with skin. Therefore, there are two LD$_{50}$ values according to how the substance is taken in by the body. Pesticides are ranked according to their level of toxicity in hazardous classes. The different hazardous classes as described by the World Health Organisation are indicated in Table 1.4 (World Health Organisation, 2009).
Table 1.4: Classification of pesticide according to the level of hazard (World Health Organisation, 2009).

<table>
<thead>
<tr>
<th>WHO class</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; for the rat (mg/kg body weight)</th>
<th>Oral LD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Dermal LD&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia - Extremely hazardous</td>
<td></td>
<td>&lt; 5</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>Ib - Highly hazardous</td>
<td></td>
<td>5 - 50</td>
<td>50 - 200</td>
</tr>
<tr>
<td>II - Moderately hazardous</td>
<td></td>
<td>50 - 2 000</td>
<td>200 - 2 000</td>
</tr>
<tr>
<td>III - Slightly hazardous</td>
<td></td>
<td>&gt; 2 000</td>
<td>&gt; 2 000</td>
</tr>
<tr>
<td>U - Unlikely to present acute hazard</td>
<td></td>
<td>&gt; 5 000</td>
<td></td>
</tr>
</tbody>
</table>

Labels of pesticides should indicate to which class a particular chemical belongs in order to warn operators who handle these chemicals. There are also pictograms and hazard statements (Table 1.5) that must appear on the colour coded label according to the classification of hazard to ensure that illiterate people understand what safety equipment should be used when handling and applying the different classes of hazardous substances (Rother, 2008).

Table 1.5: Hazard statement, band colour and pictogram that must appear on labels of pesticides according to the hazard class (FAO, 1995).

<table>
<thead>
<tr>
<th>WHO hazard class</th>
<th>Information to appear on label</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard statement</td>
</tr>
<tr>
<td>Ia - Extremely hazardous</td>
<td>VERY TOXIC</td>
</tr>
<tr>
<td>Ib - Highly hazardous</td>
<td>TOXIC</td>
</tr>
<tr>
<td>II - Moderately hazardous</td>
<td>HARMFUL</td>
</tr>
<tr>
<td>III - Slightly hazardous</td>
<td>CAUTION</td>
</tr>
<tr>
<td>U - Unlikely to present acute hazard</td>
<td>CAUTION</td>
</tr>
</tbody>
</table>

Operators working with chemicals that are considered to be highly poisonous (group 1a and 1b) should wear protective eyewear, special protective clothing, rubber gloves as well as
boots, headgear and a respirator in order to protect themselves and to prevent coming into contact with or to inhale these remedies (Figure 1.8).

Figure 1.8: Protective clothing used during the application of agrochemicals.

Protective clothing prevents uptake of agrochemicals through the skin, by inhaling substances or through absorption through the nose, eyes or ears (Ogg et al., 2012). The absorption rate of agrochemicals on different areas of the human body differs. Figure 1.9 shows the absorption rate of different body areas as compared to that of the forearm when the latter is standardised as 1.0 according to Ogg et al. (2012).
The genital area has the highest potential of absorbing agrochemicals through the skin, followed by the head area. The potential for absorption by the genital area is 11.8 times higher than that of the forearm and the ear canal and forehead, 5.4 and 4.2 times higher, respectively (Ogg et al., 2012).

Agrochemicals should be stored as prescribed by law and care should be taken that it is out of reach of children. The Poison Control Centre in the USA received more than 90 000 calls in the year 2010. Three percent of exposure to all poisonous substances was caused by pesticides to children under the age of five and pesticide exposure to adults were as high as 6% in the USA (Ogg et al., 2012). Pesticides caused 4% of children’s deaths in the USA during 2010 (Ogg et al., 2012).

Figure 1.9: Absorption rates of agrochemicals in the human body in comparison to the forearm (Ogg et al., 2012).
1.12. Conclusion

*Busseola fusca* developed field resistance against the Cry1Ab toxin produced by Bt maize in South Africa. As a result, renewed interests in the use of insecticides for stem borer control exist. The efficacy of foliage-applied agrochemicals is enhanced by surfactants and wetting agents. Optimal control by insecticides is of utmost importance to maize producers in South Africa, because chemical control costs forms a significant part of their input costs. Not only are high input costs a concern, but sub-optimum control of stem borers will result in unnecessary profit loss.

1.13. General objective

The general objective of the study was to evaluate the efficacy and most effective time of application of insecticides for control of three stem borer species: *B. fusca*, *C. partellus* and *S. calamistis* in maize.

1.14. Specific objectives

The specific objectives were to:

- Evaluate insecticides for control of *Busseola fusca*, *Chilo partellus* and *Sesamia calamistis* under confined enclosure conditions, and to
- Evaluate insecticides for the control of *B. fusca* under field conditions.

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

- **Chapter 2**: Greenhouse evaluation of selected insecticides for control of *Busseola fusca*, *Chilo partellus* and *Sesamia calamistis*
- **Chapter 3**: Field evaluation of selected insecticides for control of *Busseola fusca*
- **Chapter 4**: Conclusion and recommendations
1.15. References


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1.16. Appendix 1

Table 1.6: Active ingredients and formulations of the insecticides listed and used on maize in South Africa.

<table>
<thead>
<tr>
<th>Active/s</th>
<th>Formulation type</th>
</tr>
</thead>
<tbody>
<tr>
<td>alpha-cypermethrin</td>
<td>FS, EC, EW, SC</td>
</tr>
<tr>
<td>benfuracarb</td>
<td>EC</td>
</tr>
<tr>
<td>benfuracarb/fenvalerate</td>
<td>EC</td>
</tr>
<tr>
<td>benfuracarb/lambda-cyhalothrin</td>
<td>EC</td>
</tr>
<tr>
<td>beta-cyfluthrin</td>
<td>EC, GR, SC</td>
</tr>
<tr>
<td>beta-cypermethrin</td>
<td>EC</td>
</tr>
<tr>
<td>carbaryl</td>
<td>GR</td>
</tr>
<tr>
<td>carbofuran</td>
<td>GR</td>
</tr>
<tr>
<td>carbosulfan</td>
<td>EC</td>
</tr>
<tr>
<td>chlorpyrifos</td>
<td>CS, EC, WG</td>
</tr>
<tr>
<td>chlorpyrifos/cypermethrin</td>
<td>EC</td>
</tr>
<tr>
<td>chlorpyrifos/lambda-cyhalothrin</td>
<td>EC</td>
</tr>
<tr>
<td>cyfluthrin</td>
<td>EC</td>
</tr>
<tr>
<td>cypermethrin</td>
<td>EC</td>
</tr>
<tr>
<td>deltamethrin</td>
<td>EC</td>
</tr>
<tr>
<td>esfenvalerate</td>
<td>EC</td>
</tr>
<tr>
<td>fenvlareate</td>
<td>EC</td>
</tr>
<tr>
<td>indoxacarb</td>
<td>EC, SC</td>
</tr>
<tr>
<td>lambda-cyhalothrin</td>
<td>CS, EC</td>
</tr>
<tr>
<td>thiodicarb</td>
<td>SC</td>
</tr>
<tr>
<td>trichlorfon</td>
<td>GR</td>
</tr>
<tr>
<td>zeta-cypermethrin</td>
<td>EW</td>
</tr>
<tr>
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<td>EC, SC</td>
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<tr>
<td>benfuracarb</td>
<td>EC</td>
</tr>
<tr>
<td>carbofuran</td>
<td>GR</td>
</tr>
<tr>
<td>cypermethrin</td>
<td>EC</td>
</tr>
<tr>
<td>gamma-cyhalothrin</td>
<td>CS</td>
</tr>
</tbody>
</table>

FS – Flowable concentrate for seed treatment

EC – Emulsifiable concentrate

EW – Emulsion, oil in water

SC – Suspension concentrate

GR – Granule
WG – Water dispersible granule

CS – Capsule suspension
Chapter 2

Greenhouse and field evaluation of insecticides for control of *Busseola fusca*, *Chilo partellus* and *Sesamia calamistis*

2.1. Introduction

There are more than 10 000 insect species that damage food crops in the world (Dhaliwal *et al.*, 2010). It is estimated that an average of 31% of the global maize production was lost during the 2001/03 seasons due to pests (insects, weeds and diseases) (Oerke, 2006). Control of stem borers on grain crops in Africa is important because they can cause up to 100% yield loss in maize, millet and sorghum (Van den Berg and Nur, 1998). These cereal crops are considered as staple foods in some African countries making the control of these pests very important (Van den Berg and Nur, 1998).

Before the approval of Bt maize in South Africa, insecticides were intensively applied to control *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) among others (Van den Berg and Van der Westhuizen, 1995). After the introduction of Bt maize, insecticide usage for the control of stem borers on maize was reduced (Gouse *et al.*, 2005). Insecticides are however applied on the non-Bt maize refuges planted next to Bt maize as part of a strategy to delay resistance evolution of *B. fusca*. Farmers on the eastern Highveld region of South Africa also apply insecticides preventatively on both Bt maize and the non-Bt maize refuges (Kruger *et al.*, 2011; Van den Berg *et al.*, 2013). The pesticides purchased and used in Africa was reported in 2003, to be 3% of the global total (Fig. 2.1), with South Africa being the main user with 60% of this African usage (Naidoo and Buckley, 2003). Money spent on insecticides globally is estimated at US$ 33.59 billion (Yu, 2008).

Before the introduction of Bt maize in South Africa, insecticides for the control of stem borers on maize were as high as US$ 7 million in years with high numbers of stem borers but an average of US$ 2.5 million was reported in years with average infestation levels of these pests (Van den Berg and Nur, 1998).
During 2011, more than 500 pesticides were registered and available on the South African market (Quinn et al., 2011). The last data published on the amount of pesticides produced in South Africa, was in 2002. The quantity liquid formulation insecticides was 10 000 kℓ (Quinn et al., 2011) and 2 800 t of solid insecticides (Statistics South Africa, 2003). Forty-three percent of the liquid insecticides manufactured, were organophosphates which are highly toxic to mammals (Statistics South Africa, 2003).

Many studies on chemical control of stem borer species were done in South Africa towards the end of the previous millenium (Van Rensburg and Walters, 1978; Van Rensburg, 1990; Van Rensburg and Van den Berg, 1992b; Van den Berg and Van der Westhuizen, 1995). These addressed the timing of insecticide application, the efficacy of different insecticides, and the methods of application. The most effective time for application can be predicted by using sex pheromone traps to determine when moth numbers in a population increases (Van Rensburg et al., 1985).

In a study on the control of mixed populations of *B. fusca* and *C. partellus* on grain sorghum and maize in Potchefstroom (South Africa), an endosulfan + deltamethrin mixture controlled both *B. fusca* and *C. partellus* effectively when applied at the early growth stages in maize and later growth stages in grain sorghum (Van Rensburg and Van den Berg, 1992a). The organochlorine, endusulfan, is however no longer available for use in South Africa because of its high toxicity to humans, causing neuro-, hepato- and immuno-toxicity (Kannan et al., 2000). Another trial performed on the control of *C. partellus* on grain sorghum included the mixtures...
of deltamethrin and endosulfan, as well as benfuracarb and fenvalerate and deltamethrin, endosulfan, triazophos, profenophos and demeton-S-methyl applied alone (Van den Berg and Van Rensburg, 1992). All these insecticides controlled *C. partellus* effectively on grain sorghum, but the highest yield increases were observed in treatments where benfuracarb + fenvalerate and endosulfan + fenvalerate mixtures and demeton-S-methyl were applied (Van den Berg and Van Rensburg, 1992). From these, only the benfuracarb + fenvalerate mixture is still available on the South African market for the control of stem borer species on maize (Van Zyl, 2013). Benfuracarb is a carbamate with a systemic mode of action while fenvalerate is a pyrethroid that has a contact mode of action. Van den Berg and Van Rensburg (1992) suggested that a systemic active ingredient should be combined with an active ingredient with a contact mode of action to improve control of stem borer species behind the leaf sheaths of plants. This combination with a systemic and a contact insecticide will ensure that the treatment is effective for a longer period of time which is very important for stem borer control (Van den Berg and Van Rensburg, 1992). A more recent study performed on *C. partellus* included treatments of lambda-cyhalothrin, polythrin, deltamethrin, alpha-cypermethrin and esfenvalerate applied with a surfactant or an adjuvant added (Slabbert and Van den Berg, 2009). All these treatments, with or without a surfactant or an adjuvant added, controlled *C. partellus* effectively. Lambda-cyhalothrin in combination with organo-trisiloxane showed a significant higher control of *C. partellus* than esfenvalerate in combination with an adjuvant and was the only significant difference between all treatments included in this trial (Slabbert and Van den Berg, 2009).

The addition of surfactants can also help to increase the efficacy of insecticides. Surfactants such as organo-trisiloxane transport water further downwards into the whorls of maize plants whereas without this additive, it did not move as far down (Van den Berg and Viljoen, 2007). This could be very important especially when the larvae are already deep inside the whorl of the maize plant where the larvae of both *B. fusca* and *C. partellus* tend to feed (Slabbert and Van den Berg, 2009).

Different control strategies should be applied for the three stem borers of *B. fusca, C. partellus* and *S. calamistis* because of the differences in larval behaviour. Larvae of *B. fusca* and *C. partellus* tend to migrate to the whorl of the maize plant after hatching (Walker and Hodson, 1976; Kfir, 1997) while only some of the *S. calamistis* climb to the whorl while others penetrate directly into the stem (Van den Berg and Van Wyk, 2007). Because *S. calamistis* does not usually feed inside the whorl it makes it much more difficult to control this species and insecticides that are applied to the whorl of the maize plant will not be as effective as for the other two species (Van den Berg and Van Wyk, 2007).
The aim of the study was to evaluate the efficacy of selected insecticides against *B. fusca*, *C. partellus* and *S. calamistis*.

2.2. Materials and Methods

Confined enclosures

*Plant tunnel*

The confined enclosure at the North-West University, Potchefstroom is used routinely for growing of maize plants for research purposes. The plant growth tunnel is covered with green, light defused plastic with 75% light transmission on top and 40% grey shade netting on the lower part of the sides up to a height of 1.25 m from ground level, as well as at the back of the tunnel (Fig. 2.2). Temperature and humidity were not regulated, but environmental conditions such as rain, hail and wind were eliminated. Planting of maize in this enclosure was done in pots.

*Commercial greenhouse*

The confined enclosure at the ARC-Grain Crops Institute, Potchefstroom is a commercial greenhouse, where planting of maize was done in the soil and not in pots (Fig. 2.3).

2.2.1. Efficacy of selected insecticides applied 7 days after inoculation with stemborer larvae

Non-Bt maize (Phb30Y83) was planted in 4L pots and kept in a tunnel at the North-West University. Pots were watered every second day.

The experimental design was a randomised block with 15 treatments, each with five replicates of five plants. The insecticides applied included active ingredients currently registered for *B. fusca* control on maize, as well as active ingredients not currently registered for this purpose (Table 2.1).

Maize plants were artificially inoculated with 10-15 neonate *B. fusca* or *C. partellus* larvae four to six weeks after plant emergence using a bazooka applicator (Fig. 2.4). Neonate larvae were thoroughly mixed with maize ear grits after hatching. The grits containing the larvae was dispensed into a bottle and a quantity was released by three trigger releases into a Petri dish
and the larvae counted. Calibration was done by either adding grits if too many larvae were released or applying more trigger releases per plant if too few larvae were inoculated by a single trigger release.

Treatments consisted of spray applications of insecticides and insecticide combinations (mixtures) (Table 2.1). Pots were removed from the tunnel and insecticide treatments applied 7 days after inoculation of plants. Insecticide sprays were applied with a CO$_2$-pressurised sprayer, using a hollow cone nozzle and the spray directed into the whorls of maize plants (Fig. 2.5). Plants were returned to the tunnel approximately an hour after application to allow for the spray application to dry on the plants. Insecticide efficacy was compared to a control treatment (water).

Another trial was planted with non-Bt maize (Phb30Y83) in the commercial greenhouse with an inter-row spacing of 90 cm and an intra-row spacing of 15 cm for the evaluation of insecticides (Table 2.1) against _S. calamistis_. In this case, plants were not removed from the greenhouse but insecticide sprays applied to plants inside the enclosure. The experimental design was a randomized block with 15 treatments and three replicates per treatment. Each replicate contained 10 maize plants. Treatments were applied as described above, but with large plastic covers between the treatments blocks to prevent insecticide drift.

For all trials, plants were cut off at soil level, dissected and the number of surviving larvae determined per plant, 7 days after application of treatments.

2.2.2 Efficacy of selected insecticides applied 14 days after inoculation with stem borer larvae

Non-Bt maize (Phb30Y83) was planted in the commercial greenhouse as described above (see 2.2.1). The treatments and methodology were also the same as described above (see 2.2.1), but application of insecticides was done 14 days after inoculation of plants with neonate larvae. Neonate larvae of _B. fusca_, _C. partellus_ and _S. calamistis_ were inoculated into whorls of maize plants in three separate trials.

Plants were dissected and surviving larvae were counted 7 days after application of treatments.
2.2.3 Efficacy of selected insecticides against stem borers that hatched 7 – 10 days post application of insecticides

Non-Bt maize (Phb30Y83) was planted in the commercial greenhouse as described above (see 2.2.1). The treatments and methodology were also the same as described above (see 2.2.1). In this experiment, application of insecticides was done 7 days prior to inoculation of plants with neonate larvae of *B. fusca*, *C. partellus* and *S. calamistis*.

![Plant growth tunnel in which trials were done at the North-West University, Potchefstroom.](image)

**Figure 2.2:** Plant growth tunnel in which trials were done at the North-West University, Potchefstroom.
Figure 2.3: Commercial greenhouse at the ARC-GCI.

Figure 2.4: Bazooka applicator and grits used for the inoculation of larvae.
2.2.4 Field trials

The non-Bt maize cultivar Phb30Y83 was planted during the 2014/2015 season in two field trials, at Potchefstroom (26°43’59”S; 27°04’49”E) (a) and Buffelsvallei (26°29’38”S; 26°36’01”E) (b), respectively (Fig. 2.6).
Trials were planted at an intra-row spacing of 90 cm and an inter-row spacing of 30 cm, equivalent to approximately 37 000 plants ha$^{-1}$. Treatment plots consisted of a single row of 10 maize plants each. Maize plants were inoculated with 10-15 neonate *B. fusca* larvae, four to six weeks after plant emergence.

The experimental design was a randomized block with 15 treatments and three replicates per treatment. Treatment plots were 2 m apart and one maize row was left unsprayed between adjacent treatment plots to avoid insecticide drift effects. Three rows were also left untreated on each side of the field, as well as the first 10 plants in each treatment row, to eliminate border effects. The treatments applied are provided table 2.1. Treatments were applied 7 days after artificial inoculation of plants with neonate larvae by means of a CO$_2$-pressurised sprayer, using a hollow cone nozzle and the spray directed into the whorls of the maize plants. Each treatment was further applied in 2 or 3 litres of water per 100 m$^{-1}$ row length according to the specific treatment used. Plants were removed, cut open and the number of surviving larvae counted 7 days after application of treatments.
2.2.5. Statistical analyses

Efficacy of control was calculated according to Abbott’s formula (Abbott, 1925). The corrected percentage efficacy (Abbott, 1925) of each treatment was compared by means of one way ANOVA, followed by Tukey HSD test to determine significant differences between treatments using STATISTICA version 12 (StatSoft, Inc., 2013).

2.3. Results

2.3.1.1. Efficacy of selected insecticides applied 7 days after inoculation with Busseola fusca larvae

There were no significant differences in efficacy of control of B. fusca larvae 7 days after inoculation between any of the treatments, except for the mixture of chlorantraniliprole + lambda-cyhalothrin, that provided only 44% corrected efficacy of control (Fig. 2.7). Mortality was significantly higher in the benfuracarb and bifenthrin treatment plots as well as the plots where the mixtures: benfuracarb + lambda-cyhalothrin; chlorpyrifos + lambda-cyhalothrin and indoxacarb + lambda-cyhalothrin were applied. It did, however, not differ from the control provided by chlorfenapyr, gamma-cyhalothrin, lambda-cyhalothrin 50, lambda-cyhalothrin 106, lufenuron + lambda-cyhalothrin 106, novaluron + lambda-cyhalothrin 106, spinetoram and spinosad. Benfuracarb as well as the mixture of indoxacarb and lambda-cyhalothrin, both provided a corrected percentage efficacy of 96%, but this high level of efficacy was not significantly different from that achieved by the other treatments (except for chlorantraniliprole + lambda-cyhalothrin).

2.3.1.2. Efficacy of selected insecticides applied 14 days after inoculation with Busseola fusca larvae

Twelve of the insecticides and insecticide mixtures were highly effective in controlling B. fusca larvae 14 days after inoculation (Fig. 2.8) with no significant difference in their respective corrected percentage efficacies. Although not significantly different from the other 10 treatments, benfuracarb and the mixture of indoxacarb and lambda-cyhalothrin, provided the highest corrected percentage efficacy of 92% and 91% respectively. The lowest corrected percentage efficacy (44%) was achieved by the chlorantraniliprole + lambda-cyhalothrin mixture. It did, however, not differ from the efficacy of control of chlorfenapyr and lambda-cyhalothrin 50. There was also no difference in efficacy of control between the chlorfenapyr, chlorpyrifos + lambda-cyhalothrin, gamma-cyhalothrin, lambda-cyhalothrin 50 and lambda-cyhalothrin 106 treatments.
2.3.1.3. Efficacy of selected insecticides against *Busseola fusca* larvae that hatched 7 – 10 days post application of insecticides

The highest percentage corrected efficacy of 77% was obtained with the chlorantraniliprole + lambda-cyhalothrin mixture and the lowest with lambda-cyhalothrin 106 (35%) (Fig. 2.9). The differences between the corrected percentage efficacies of the treatments were high but no statistical differences were observed between treatments. This is ascribed to the high variation in the data obtained between the treatments for this trial.

2.3.2.1. Efficacy of selected insecticides applied 7 days after inoculation with *Chilo partellus* larvae

All insecticides provided good control of *C. partellus*, with no significant difference between their corrected percentage efficacies, except for chlorfenapyr which provided poorer control (Fig. 2.10). The corrected percentage efficacy of this insecticide was, 94%, which can also be regarded as very good. The efficacy of control, differed significantly from that obtained with benfuracarb, the combination of benfuracarb and lambda-cyhalothrin, bifenthrin, the combination of chlorpyrifos and lambda-cyhalothrin, gamma-cyhalothrin, the combination of lufenuron and lambda-cyhalothrin, spinetoram and spinosad.

2.3.2.2. Efficacy of selected insecticides applied 14 days after inoculation with *Chilo partellus* larvae

All treatments were effective in controlling *C. partellus*, but the lowest corrected efficacy was obtained with the chlorantraniliprole + lambda-cyhalothrin mixture (Fig. 2.11). The corrected percentage efficacy of benfuracarb, the combination of benfuracarb and lambda-cyhalothrin, lambda-cyhalothrin, the combination of lufenuron and lambda-cyhalothrin, the combination of novaluron and lambda-cyhalothrin, spinetoram and spinosad, was significantly higher than that of the chlorantraniliprole + lambda-cyhalothrin mixture.

2.3.2.3. Efficacy of selected insecticides against *Chilo partellus* larvae that hatched 7 – 10 days post application of insecticides

Low corrected percentage efficacy was obtained for all insecticide treatments when infestation of plants was done 7 days after application of insecticides (Fig. 2.12). Gamma-cyhalothrin (58% corrected percentage efficacy) provided significantly better control of *C. partellus* than the novaluron + lambda-cyhalothrin 106 mixture and spinosad, while the low corrected efficacy
of control provided by spinosad (18%) also differed from that of bifenthrin and the mixture of chlorantraniliprole + lambda-cyhalothrin.

2.3.3.1. Efficacy of selected insecticides applied 7 days after inoculation with *Sesamia calamistis* larvae

All insecticide treatments controlled *S. calamistis* effectively and equally well (Fig. 2.13), except for chlorfenapyr with which significantly lower corrected control was achieved (59%), compared to all other treatments.

2.3.3.2. Efficacy of selected insecticides applied 14 days after inoculation with *Sesamia calamistis* larvae

This trial showed similar results to the abovementioned trial (2.3.3.1), where all treatments, except chlorfenapyr (58% efficacy) controlled the larvae well (Fig. 2.14). The highest corrected percentage efficacy was obtained with benfuracarb with 99% control.

2.3.3.3. Efficacy of selected insecticides against *Sesamia calamistis* larvae that hatched 7 – 10 days post application of insecticides

The corrected percentage efficacy of treatments applied preventatively ranged between 36 and 100% (Fig. 2.15). Gamma-cyhalothrin, the indoxacarb + lambda-cyhalothrin mixture, and lambda-cyhalothrin controlled *S. calamistis* significantly poorer compared to the lufenuron + lambda-cyhalothrin mixture, novaluron + lambda-cyhalothrin mixture, spinetoram and spinosad. There was no significant difference in corrected percentage efficacy between the latter four treatments with control of 90% and higher being achieved. The benfuracarb + lambda-cyhalothrin mixture, chlorantraniliprole + lambda-cyhalothrin mixture and chlorfenapyr were less effective in controlling *S calamistis* than the lufenuron + lambda-cyhalothrin and novaluron + lambda-cyhalothrin mixtures in this trial. Control by the chlorantraniliprole + lambda-cyhalothrin mixture was also significantly poorer than that of spinosad.

2.3.4.1 Field trial 1

Corrected percentage efficacy of control by the insecticide treatments ranged from 52.6% (lambda-cyhalothrin 50) to 86.7% (spinetoram) (Fig. 2.16). There was, however, no significant
difference in corrected percentage efficacy of control between any of the treatments applied, but large variation did occur. All treatments therefore effectively controlled *B. fusca*.

### 2.3.4.2. Field trial 2

All treatments were effective in controlling *B. fusca* larvae 7 days after inoculation in this field trial. A higher percentage efficacy was recorded in the second field trial (Fig. 2.17), compared to the first trial (Fig. 2.16).

Chlorfenapyr controlled *B. fusca* larvae less effective than all other treatments except for benfuranacarb and gamma-cyhalothrin with which similar control was recorded. The lowest corrected percentage efficacy in this trial was 91%.

### 2.4. Application time

There was no significant difference in mean corrected percentage efficacy between insecticides applied 7 and 14 days after artificial inoculation of stem borer larvae to maize seedlings. The efficacy of control of stem borer larvae was significantly less when insecticides were applied 7 days before artificial inoculation compared to insecticide applications after inoculation of larvae.

### 2.5. Discussion

The percentage control obtained in this study ranged between 44 and 96% with the respective treatments when applied as curative control 7 and 14 days after *B. fusca* larvae were artificially inoculated onto plants. The mixture of chlorantraniliprole + lambda-cyhalothrin provided the poorest control of *B. fusca* larvae in both trials. Chlorantraniliprole is primarily used for the control of various chewing lepidopteran species (Tomlin, 2009), and is not registered for the control of *B. fusca* on maize in South Africa (Van Zyl, 2013). The chlorantraniliprole + lambda-cyhalothrin mixture is only registered for the control of *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (tuber moth) on potatoes in South Africa (Van Zyl, 2013). When applied preventatively, this mixture controlled *B. fusca* larvae effectively. High efficacy of control was, however, also achieved with lambda-cyhalothrin when applied as an individual treatment and not in combination with other insecticides in a mixture. It therefore seems as if the efficacy of lambda-cyhalothrin was reduced when it was used in combination with
chlorantraniliprole as a curative control treatment. When this mixture was used as a preventative application, it was however effective in controlling *B. fusca*. It can be speculated that the lambda-cyhalothrin (contact insecticide) did not reach the larvae inside the whorl when applied curatively, but that contact with neonates happened when applied preventatively. If this is the reason for the lower percentage control achieved, it can be overcome by adding a wetting agent to the mixture to improve the efficacy. Good control of *C. partellus* larvae was achieved with application 7 days after inoculation with chlorantraniliprole + lambda-cyhalothrin mixture, however, when applied 14 days after artificial inoculation, control was lower compared to the other treatments.

The efficacy of control by chlorfenapyr for the stem borer species evaluated was generally lower compared to the other treatments. It is in contrast to the results reported by Argentine *et al.* (2000). They found chlorfenapyr to be highly effective against a variety of lepidopteran species including *Heliothis virescens* (Noctuidae), *Spodoptera exigua* (Noctuidae), *Plutella xylostella* (Plutellidae) and *Trichoplusia ni* (Noctuidae). Chlorfenapyr is effective in controlling *H. virescens* up to 14 days after application of sprays (Argentine *et al.*, 2000). Although lepidopterans, none of these species are stem borers which occur inside maize whorls, out of easy reach of insecticides. Chlorfenapyr is most effective when the insect comes into contact with the chemical or ingests it where it then inhibits the production of ATP that is crucial for the survival of the insect (Liu *et al.*, 2013). Chlorfenapyr is not registered in South Africa for control of stem borers (Van Zyl, 2013). It is registered for the control of red spider mite, thrips, weevils, diamondback moth, large white cabbage moth, banded fruit weevil and tuber moth on various crops, but not on maize (Van Zyl, 2013). Higher efficacy of control of stem borer larvae was, however, expected because chlorfenapyr have been found to have a long residual activity on crops (Argentine *et al.* 2000).

Benfuracarb was highly effective against all three borer species when applied 7 and 14 days after inoculation of neonate larvae onto plants. The efficacy was, however, lower when inoculation was done 7 days after application. This result cannot be explained because benfuracarb has a systemic mode of action (Tomlin, 2009). Although benfuracarb is not registered for control of the *S. calamistis* (Van Zyl, 2013), it showed promising results in controlling this pest if applied as a curative control measure 7 and 14 days after inoculation. Benfuracarb was applied to the foliar parts of the plant, but it can also be applied as a soil treatment for the roots to absorb and to transport it acropetally (Umetsu *et al.*, 1985; Tomlin, 2009). Once absorbed, the active ingredient is inside the plant and chewing insects can then be controlled when feeding on the plant material where it acts as a stomach insecticide (Osaki *et al.*, 1992b). Benfuracarb is a carbamate with systemic properties and have been used to successfully control various species on various crops when applied to the roots or applied to
the stem of the crop (Osaki et al., 1992a, b). Application to the lower parts of the plant has shown to successfully control another lepidopteran species, diamondback moth (Osaki et al., 1992a).

The lambda-cyhalothrin in the benfuracarb + lambda-cyhalothrin mixture complements benfuracarb that has a strong systemic action. This increased efficacy is ascribed to it being a non-systemic pyrethroid with a strong contact and stomach mode of action. Control by the benfuracarb + lambda-cyhalothrin mixture, was, however, not improved when compared to control by benfuracarb alone against all three stemborer species under confined enclosure as well as under field conditions.

The indoxacarb + lambda-cyhalothrin mixture was effective in controlling all three borer species under confined enclosure as well as field conditions. This combination of insecticides is not registered for the control of stem borers in South Africa (Van Zyl, 2013).

The benzoylureas and spinosyns controlled all three stem borer species effectively when applied as a curative treatment, although not registered for the control of stem borers on maize in South Africa (Van Zyl, 2013). These insecticides are more environmentally friendly and safer for human use than many of the other insecticides that are registered for the control of stem borers in maize (Besard et al., 2011; Pete et al., 2012; Athanassiou and Kavallieratos, 2014). When the pyrethroid lambda-cyhalothrin is added benzoylurea treatments, the dangers to humans increases and it is also more toxic to beneficial arthropods (Dewetto et al., 2007; Desneux et al., 2007).

The four pyrethroids that were used during the trials were bifenthrin, gamma-cyhalothrin and two formulations of lambda-cyhalothrin. Gamma-cyhalothrin and lambda-cyhalothrin are registered for control of all three stem borer species on maize in South Africa (Van Zyl, 2013). Bifenthrin is, however, not registered but all pyretoirids evaluated controlled these lepidopteran pests equally well. Gamma-cyhalothrin is known to degrade rapidly in the presence of sunlight (He et al., 2008). The photostability of synthetic pyrethroids are, however, improving (He et al., 2008). This is achieved by using extraction methods that are highly effective and high resolution detection techniques. These methods enable scientists to identify multiple trace photoproducts and photodegradation pathways in synthetic pyrethroids (Fernandez-Alvarez et al. 2007).

The chlorpyrifos + lambda-cyhalothrin mixture also controlled all three species effectively, although it is only registered for the control of B. fusca and C. partellus (Van Zyl, 2013). Previous studies have shown that certain insecticides may have synergistic effects when used in combination. These studies proved that an insecticides with a pyrethroid as an active
ingredient can have synergistic effects with an insecticide with a non-pyrethroid active ingredient (All et al., 1977; Robertson and Smith, 1984). Effective control of stem borers was achieved in this study when lambda-cyhalothrin was applied in mixtures with benfuracarb, chlorpyrifos, indoxacarb, lufenuron and novaluron. Synergism was, however, not observed in this study because the efficacy of all insecticides applied in mixtures was not compared to the levels of control achieved when they were applied alone, also. Van den Berg and Van Rensburg (1993) combined pyrethroids with various insecticides and evaluated it for control of *C. partellus* as mixtures or applied alone. The results of this study with regard to insecticide mixtures where a pyrethroid was added are in agreement with that of Van den Berg and Van Rensburg (1993). They concluded that *C. partellus* can be controlled effectively by a persistent chemical and/or a mixture of active ingredients that have a synergistic effect on one another.

All insecticides controlled *S. calamistis* effectively when applied curatively, except for chlorphenapyr in the confined enclosures. Chlorfenapyr was, however, reported to control the lepidopteran species, *P. xylostella* (Sun et al., 2011) and *S. exigua* (Mascarenhas et al., 1998) effectively, and is considered to have relatively long residual activity (Argentine et al., 2000). This active ingredient has not been registered for the control of stem borer species on maize in South Africa (Van Zyl, 2013).

The timing of insecticide application is of great importance and a few days deviation from the optimum time of application may result in a marked difference in efficacy (Van Rensburg and Van den Berg, 1992b). It was also clear from the current study where more effective control was achieved with curative than with preventative insecticide applications.

Van Rensburg (1990) stressed the importance of correct timing of insecticide application for control of *B. fusca* and suggested that insecticides should be applied when 10% of plants exhibit shot hole damage to whorl leaves. Heenop (1974) recommended a second successive insecticide application to be applied two weeks after the first application, because larvae that hatch more than a week later may survive on the maize plant because of a sub-lethal residual concentration of the insecticide that remains. The successive application two weeks later will ensure that these surviving larvae are also killed. The lower efficacy of control achieved in this study can therefore also be explained by the sub-lethal residual concentrations which could have been present when larvae were inoculated a week after insecticide application was done.

The lufenuron + lambda-cyhalothrin mixture was also effective in controlling all three stem borer species. An advantage of lufenuron is that it is a growth regulator with a long residual activity of up to 42 days after application on grapes (Likas and Tsiropoulos, 2011). This long residual activity was, however, not reported on tomatoes where the residue of this agrochemical dissipated up to 96%, 15 days after application (Malhat et al., 2012). This active
ingredient with its long residual action, especially in a combination with an insecticide with a quick knock down action like a synthetic pyrethroid may be a good combination to investigate further for stemborer control. The lufenuron + lambda-cyhalothrin mixture evaluated is, however, not yet registered for the control of any insect pest on any crop in South Africa (Van Zyl, 2013).

The spinosyns, spinosad and spinetoram effectively controlled all three lepidopteran species. Spinetoram was reported to have a higher corrected percentage efficacy than spinosad, because it is more active than spinosad and has a longer period of control (Sparks et al., 2008). This agrochemical is effective in controlling a variety of lepidopteran species (Sparks et al., 2008) and is stable over a long period (Vassilakos et al., 2015). The latter authors have found that this chemical can be effective for more than eight months when applied to stored grain (Vassilakos et al., 2015). However, spinetoram has been shown to lose its insecticidal toxicity within 14 days after application when exposed to warm, dry field conditions (Yee et al., 2007).

The differences in efficacy of insecticides in the two field trials may be ascribed to abiotic factors that differed during the two field trials. The first field trial was conducted during November and December which was at the peak of the rainy season with high prevailing temperatures. The second trial was conducted during March when conditions were dry and lower temperatures prevailed. It is known that the residual effect and efficacy of insecticides can be influenced by abiotic factors such as relative humidity and exposure to light (Rust, 1995) and may therefore provide an explanation for the difference in efficacy of insecticides between the two field trials.
2.6. References


2.1. Appendix 2

**Figure 2.7:** Corrected percentage efficacy of various insecticides applied 7 days after artificial inoculation for the control of *Busseola fusca.*

**Treatments:**
1. benfuracarb  
2. benfuracarb + lambda-cyhalothrin  
3. bifenthrin  
4. chlorantraniliprole + lambda-cyhalothrin  
5. chlorfenapyr  
6. chlorpyrifos + lambda-cyhalothrin  
7. gamma-cyhalothrin  
8. indoxacarb + lambda-cyhalothrin  
9. lambda-cyhalothrin 50  
10. lambda-cyhalothrin 106  
11. lufenuron + lambda-cyhalothrin 106  
12. novaluron + lambda-cyhalothrin 106  
13. spinetoram  
14. spinosad
Figure 2.8: Corrected percentage efficacy of various insecticides applied 14 days after artificial inoculation for the control of *Busseola fusca*.
Figure 2.9: Corrected percentage efficacy of various insecticides applied 7 days prior artificial inoculation for the control of Busseola fusca.

Treatments:
1: benfuracarb
2: benfuracarb + lambda-cyhalothrin
3: bifenthrin
4: chlorantraniliprole + lambda-cyhalothrin
5: chlorfenapyr
6: chlorpyrifos + lambda-cyhalothrin
7: gamma-cyhalothrin
8: indoxacarb + lambda-cyhalothrin
9: lambda-cyhalothrin 50
10: lambda-cyhalothrin 106
11: lufenuron + lambda-cyhalothrin 106
12: novaluron + lambda-cyhalothrin 106
13: spinetoram
14: spinosad
**Figure 2.10:** Corrected percentage efficacy of various insecticides applied 7 days after artificial inoculation for the control of *Chilo partellus.*
Figure 2.11: Corrected percentage efficacy of various insecticides applied 14 days after artificial inoculation for the control of *Chilo partellus*.
Figure 2.12: Corrected percentage efficacy of various insecticides applied 7 days prior artificial inoculation for the control of *Chilo partellus*.

*Treatments:*
1: benfuracarb
2: benfuracarb + lambda-cyhalothrin
3: bifenthrin
4: chlorantraniliprole + lambda-cyhalothrin
5: chlorfenapyr
6: chlorpyrifos + lambda-cyhalothrin
7: gamma-cyhalothrin
8: indoxacarb + lambda-cyhalothrin
9: lambda-cyhalothrin 50
10: lambda-cyhalothrin 106
11: lufenuron + lambda-cyhalothrin 106
12: novaluron + lambda-cyhalothrin 106
13: spinetoram
14: spinosad

F<sub>13,28</sub> = 3.6
HSD (P < 0.01)
Figure 2.13: Corrected percentage efficacy of various insecticides applied 7 days after artificial inoculation for the control of *Sesamia calamistis.*
Figure 2.14: Corrected percentage efficacy of various insecticides applied 14 days after artificial inoculation for the control of *Sesamia calamistis.*
Figure 2.15: Corrected percentage efficacy of various insecticides applied 7 days prior artificial inoculation for the control of *Sesamia calamistis*. 

**Treatments:**
1: benfuracarb
2: benfuracarb + lambda-cyhalothrin
3: bifenthrin
4: chlorantraniliprole + lambda-cyhalothrin
5: chlorfenapyr
6: chlorpyrifos + lambda-cyhalothrin
7: gamma-cyhalothrin
8: indoxacarb + lambda-cyhalothrin
9: lambda-cyhalothrin 50
10: lambda-cyhalothrin 106
11: lufenuron + lambda-cyhalothrin 106
12: novaluron + lambda-cyhalothrin 106
13: spinetoram
14: spinosad
Figure 2.16: Corrected percentage efficacy of various insecticides applied 7 days after artificial inoculation for the control of *Busseola fusca* (field trial 1).
Figure 2.17: Corrected percentage efficacy of various insecticides applied 7 days after artificial inoculation for the control of *Busseola fusca* (field trial 2).

**Treatments:**
1: benfuracarb
2: benfuracarb + lambda-cyhalothrin
3: bifenthrin
4. chlorantraniliprole + lambda-cyhalothrin
5: chlorfenapyr
6: chlorpyrifos + lambda-cyhalothrin
7: gamma-cyhalothrin
8: indoxacarb + lambda-cyhalothrin
9: lambda-cyhalothrin 50
10: lambda-cyhalothrin 106
11: lufenuron + lambda-cyhalothrin 106
12: novaluron + lambda-cyhalothrin 106
13: spinetoram
14: spinosad
Figure 2.18: Average corrected percentage efficacy of all treatments on all three stemborer species of the three different application times. d.a.i. = days after inoculation; d.b.i. = days before inoculation
Table 2.1: Formulations and dosage rates of insecticides evaluated for efficacy against *Busseola fusca, Chilo partellus* and *Sesamia calamistis*.

<table>
<thead>
<tr>
<th>Treatments (Active ingredient)</th>
<th>Formulation (g L(^{-1}))</th>
<th>Dosage rate applied (g a.i. 100L(^{-1}) H(_2)O)</th>
<th>Dosage rate applied (g a.i. ha(^{-1}))</th>
<th>Registered for stem borer control on maize</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. water (control)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2. benfuracarb</td>
<td>200</td>
<td>66</td>
<td>200</td>
<td>Bf, Cp</td>
</tr>
<tr>
<td>3. benfuracarb + lambda-cyhalothrin</td>
<td>200 + 6</td>
<td>66 + 1.98</td>
<td>200 + 6</td>
<td>Bf, Cp</td>
</tr>
<tr>
<td>4. bifenthrin</td>
<td>100</td>
<td>10</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>5. chlorantraniliprole + lambda-cyhalothrin</td>
<td>100 + 50</td>
<td>16.5 + 8.25</td>
<td>45 + 22.5</td>
<td>-</td>
</tr>
<tr>
<td>6. chlorfenapyr</td>
<td>360</td>
<td>360</td>
<td>72</td>
<td>-</td>
</tr>
<tr>
<td>7. chlorpyrifos + lambda-cyhalothrin</td>
<td>338 + 12</td>
<td>55.77 + 1.98</td>
<td>169 + 6</td>
<td>Bf, Cp</td>
</tr>
<tr>
<td>8. gamma-cyhalothrin</td>
<td>60</td>
<td>0.9</td>
<td>3</td>
<td>Bf, Cp, Sc</td>
</tr>
<tr>
<td>9. indoxacarb + lambda-cyhalothrin</td>
<td>150 + 106</td>
<td>67.5 + 2.12</td>
<td>450 + 6.36</td>
<td>Bf, Cp</td>
</tr>
<tr>
<td>10. lambda-cyhalothrin</td>
<td>50</td>
<td>2</td>
<td>6</td>
<td>Bf, Cp, Sc</td>
</tr>
<tr>
<td>11. lambda-cyhalothrin</td>
<td>106</td>
<td>2.12</td>
<td>6.36</td>
<td>Bf, Cp, Sc</td>
</tr>
<tr>
<td>12. lufenuron + lambda-cyhalothrin</td>
<td>50 + 106</td>
<td>11.5 + 2.12</td>
<td>35 + 6.36</td>
<td>-</td>
</tr>
<tr>
<td>13. novaluron + lambda-cyhalothrin</td>
<td>100 + 106</td>
<td>11.5 + 2.12</td>
<td>35 + 6.36</td>
<td>-</td>
</tr>
<tr>
<td>14. spinetoram</td>
<td>75</td>
<td>4.88</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>15. spinosad</td>
<td>480</td>
<td>31.2</td>
<td>96</td>
<td>-</td>
</tr>
</tbody>
</table>

Bf – *Busseola fusca*
Cp – *Chilo partellus*
Sc – *Sesamia calamistis*
Chapter 3

Conclusion and recommendations

3.1. Discussion

Before the introduction of Bt maize in South Africa during the 1998/99 maize production season, stem borers were mainly controlled by means of chemical insecticides. There is currently a renewed interest in insecticides for stem borer control in maize as a result of resistance development by *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) to Bt maize in South Africa. Twenty-three active ingredients of insecticides are currently registered for stem borer control on maize in South Africa (Van Zyl, 2013). These chemicals include active ingredients that are used alone or in combination with other active ingredients. Some active ingredients that are mixed with other active ingredients may have a synergistic effect on each other and therefore may be more effective in controlling pests than when used separately (Van den Berg and Van Rensburg, 1993). Before any insecticides are mixed together it is important to determine if these chemicals are compatible. This is done by following directions on the label of the pesticide (Sarwar, 2015). The label of the chemical indicates what chemicals may be mixed with the content of that specific container (Sarwar, 2015).

There are two main insecticide application strategies, namely preventative and curative (Morales-Rodriguez and Peck, 2009). Insecticides evaluated in the present study were for experimental purposes and not all insecticides as well as insecticide mixtures evaluated in this study are currently registered for stem borer control in South Africa. However, all insecticides evaluated during this study were effective in controlling *B. fusca*, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). Two of the insecticides, namely chlorantraniliprole (here used in a mixture with lambda-cyhalothrin), and chlorfenapyr which was less effective in controlling *B. fusca* in the greenhouse trials in which insecticides were applied both 7 and 14 days after inoculation as well as one of the field trials, are not registered for stem borer control on maize in South Africa. Although this chemical is not registered for the control of these stem borers on maize, it have been found to be effective controlling the lepidopteran species, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on maize in the USA (Hardke et al., 2011). The preventative treatment of chlorantraniliprole and lambda-cyhalothrin controlled *B. fusca* and *C. partellus* effectively when the treatment was applied 7-10 days before inoculation of larvae. This may be an indication that the curative application did not reach the larvae in the whorls to control them as effective as the preventative application. A wetting agent or adjuvant added to the
mixture may improve the efficacy. However, previous studies conducted on cauliflower in India showed that the active ingredient chlorantraniliprole applied, rapidly breaks down in field and laboratory conditions in only 7 days to 0.01 mg/kg when applied at a rate of 700 g.a.i./ha (Kar et al., 2013). When the two active ingredients are combined it seems to provide improved control over a longer period of time. Taking into account the abovementioned it may then possibly be ascribed to the control provided lambda-cyhalothrin rather than to chlorantraniliprole. The combination of chlorantraniliprole and lambda-cyhalothrin has also been evaluated in Brazil to control Maruca vitrata (Lepidoptera: Crambidae) on soybeans (Grigolli et al., 2015). It was found to effectively control M. vitrata within one day of application and 79% of the population up to 14 days after application (Grigolli et al., 2015). The prospect of preventative control identified for this mixture found in the current study confirmed results reported by Grigolli et al. (2015). These authors (Grigolli et al., 2015) also reported chlorpyrifos to have an even higher efficacy in controlling M. vitrata 14 days after application than the mixture of chlorantraniliprole and lambda-cyhalothrin, flubendiamide, teflubenzuron and methomyl. The mixture of chlorpyrifos and lambda-cyhalothrin was, however, found to be less effective when applied before the application of B. fusca and C. partellus although there are no significant differences between these two treatments for the respective trials.

The efficacy of control by chlorfenapyr for the three stem borer species evaluated in this study was generally lower compared to the other treatments. It is in contrast to results reported by Argentine et al. (2002) of almost 100% control of another lepidopteran species, S. exigua in the USA on sugar beet. The feeding behaviour of the two species is, however, totally different with S. exigua feeding exposing it to insecticides applied on the leaves, while stem borers which are more concealed from insecticide applications. The systemic insecticide, benfuracarb was applied alone as well as in a mixture with lambda-cyhalothrin. These two treatments showed very good control of all three species in most trials. This high control in stem borers seen in this study was also found in another study where benfuracarb was applied in a granular form to control P. xylostella on brassicas in Japan (Yasudomi et al., 1990). The benfuracarb + lambda-cyhalothrin mixture controlled S. calamistis less effective when applied 7 days prior to artificial inoculation compared to the lufenuron + lambda-cyhalothrin and novaluron + lambda-cyhalothrin mixtures. However, there was no difference in efficacy of control between these mixtures for all other curative and preventative trials on B. fusca, C. partellus and S. calamistis reported in this study. This result therefore needs verification. Benfuracarb applied as a soil treatment and absorbed by the roots of a plant, can be transported acropetally to the foliar parts where larvae feed (Umetsu et al., 1985), and should therefore be effective in controlling stem borer larvae. The contact action of a pyrethroid added in a mixture with benfuracarb, should therefore add to efficacy of control. Larvae that
escape the contact action of the pyrethroid should then be killed by the systemic action of benfluracarb in the plant when feeding.

Due to high toxicity levels of certain organophosphates many have been banned from the market (Liu et al., 2005), leaving a gap in the market to be filled by new synthetic pyrethroids that are synthesised (Liu et al., 2005). The pyrethroid, bifenthrin, is effective in controlling the rice stem borer *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae) in China (Chen and Klein, 2012). It also controlled *B. fusca*, *C. partellus* and *S. calamistis* effectively in this study. Gamma-cyhalothrin is as effective as lambda-cyhalothrin to control the rice stem borers *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) and *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae) (Reay-Jones et al., 2007), and was also found to be effective in controlling *B. fusca*, *C. partellus* and *S. calamistis* in this study. An interesting phenomenon observed during this study, was that gamma-cyhalothrin showed a high efficacy of control of *C. partellus* when applied 7-10 prior to artificial inoculation of larvae. The lower UV levels in the confined areas may have extended the residual activity. The different concentrations of lambda-cyhalothrin used in this study as pyrethroid treatments, also controlled the three stem borer species, namely *B. fusca*, *C. partellus* and *S. calamistis* effectively, with the exception of the preventative application 7-10 days before inoculation of *S. calamistis* larvae. It may be ascribed to the short residual action of pyrethroids in general. Synthetic pyrethroids rapidly brake down when they are exposed to sunlight (He et al., 2008). High efficacy of control of *B. fusca* and *C. partellus* by lambda-cyhalothrin was also reported from Nigeria (Adamu et al., 2015).

Indoxacarb and lambda-cyhalothrin are both registered for the control of *B. fusca* and *C. partellus* on maize in South Africa (Van Zyl, 2013). The indoxacarb + lambda-cyhalothrin mixture is, however, not registered. Certain combinations of active ingredients can be more toxic to insects (Khan et al., 2013) and the combination of the two chemical groups will delay resistance development (Hemingway and Ranson, 2000). However, adverse effects may also occur when mixing two or more pesticides together (Hernández et al., 2013). Antagonistic active ingredients either stimulate the metabolism of one another or interfere with the absorption of the chemical (Hernández et al., 2013). The combination of DDT and parathion can be used as an example of an antagonistic mixture because DDT is an inducer of Cytochrome P450 whereas parathion inhibits Cytochrome P450 (Hernández et al., 2013). When two active ingredients are mixed together and the toxicity of the compound increases to more than the added effect of each insecticide when applied separately, these active ingredients have a synergistic effect (Khan et al., 2013). If the toxicity of the two or more substances reduces the toxicity of the compound it is called antagonists (Khan et al., 2013). The Indoxacarb + lambda-cyhalothrin mixture was less effective as a preventative application
7-10 days before inoculation of *S. calamistis* larvae compared to the lufenuron + lambda-cyhalothrin and novaluron + lambda-cyhalothrin mixtures, spinetoram and spinosad. The active ingredient of indoxacarb is not registered for the control of *S. calamistis* in South Africa.

Lufenuron and novaluron are two of the newer insecticides available on the market and have a growth regulatory effect on insects (Likas and Tsiropoulos, 2011). These growth regulators inhibit the biosynthesis of chitin in the body of the insect that makes it impossible for the insect to moult and eventually kills the insect (Matsumura, 2010). The toxicity of these two active ingredients to mammals is low with regards to most active ingredients available on the market (Khay *et al.*, 2008) making these insecticides more widely acceptable to people. The combination of these two active ingredients with pyrethroids increases their toxicity to mammals and therefore also to humans. The lufenuron + lambda-cyhalothrin and novaluron + lambda-cyhalothrin mixtures were both found to be highly effective in controlling all three stem borer species under field and greenhouse conditions in this study. The high efficacy of the mixtures of these benzoylureas with lambda-cyhalothrin indicated that the active ingredients are compatible as a mixture and that these compounds may have an additive effect or even a synergistic response to one another. If the application rates of the two insecticides can be lowered without sacrificing efficacy of control, it will be a synergistic effect (Wraight and Ramos, 2005).

The two spinosyns that were used during this study included spinetoram and spinosad. Both active ingredients are not registered for the control of stem borers on maize in South Africa. Spinosyn products derived from the soil bacterium *Saccharopolyspora spinosa*. Spinosyns are produced as a secondary metabolite when *S. spinosa* undergoes aerobic fermentation (Thompson *et al.*, 2000). The active ingredients of spinosad and spinetoram are considered as natural insecticides and they are not as dangerous for humans and the environment as most other insecticides available on the market (Thompson *et al.*, 2000). Spinosad have been proven to have no carcinogenic, teratogenic, mutagenic or neurotoxic properties against humans (Thompson *et al.*, 2000). Previous trials showed that the LD\(_{50}\) of spinosad to rats is 3783-5000 mg/kg\(^{-1}\) where lambda-cyhalothrin was as little as 56 mg/kg\(^{-1}\) (Thompson *et al.*, 2000). In the environment these active ingredients are rapidly broken down mostly by sunlight or microbes to non-harmful elements including nitrogen, oxygen, hydrogen and nitrogen (Thompson *et al.*, 2000). According to the latter author, spinosad is broken down by sunlight in 1.6-16 days when applied to the foliar parts of plants depending on the intensity of the sunlight, the crop it is applied to and the rate at which it is applied. Spinosad breaks down rapidly (Huang and Subramanyam, 2007) and it is not as lethal as spinetoram (Sparks *et al.*, 2008). Spinosyns kill a variety of insect pests including lepidopteran species (Cleveland *et al.*, 2001; Sparks *et al.*, 2008). Spinosad was the first spinosyn that was available on the market.
in 1995 with the even more lethal spinetoram introduced to the market in 2007 (Kirst, 2010). The two spinosyns that were included during this trial showed very good control of all three stem borer species under greenhouse and field conditions. Similar results were reported when spinosad was applied to maize for the control of *Sesamia cretica* (Lederer) (Lepidoptera: Noctuidae) under field conditions in Egypt (Osman *et al.*, 2014). It effectively controlled first to fifth instar larvae on maize (Osman *et al.*, 2014). Spinetoram was also found to be effective in controlling the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Belay *et al.*, 2012).

These results highlight the importance of the timing of application when treatments are applied. Extensive studies have been done on the timing of insecticide applications in order to reduce the damage caused by stem borers on maize (Du Plessis and Lea, 1943; Heenop, 1963; Van Rensburg *et al.*, 1988d; Van Rensburg, 1990; Van Rensburg and Van den Berg, 1992). Variation in the data obtained from the latter authors can be ascribed to the different study localities and differences in abiotic factors that may have occurred in the different areas although all suggested that insecticides should be applied after the first signs of damage on the maize plant.

### 3.2. Recommendations to industry

All insecticides currently registered for stem borer control in South Africa controlled all three stem borer species effectively. Treatments should be applied to the foliar parts of the plant when 10% (which is the threshold level) of plants show visible damage on the whorl leaves in order to control the larvae that are already feeding on these leaves (Van Rensburg, 1990). Timing of insecticide application is important. Most insecticides are rapidly broken down by UV light which contributes to preventative treatments usually being not effective. Therefore a successive application two weeks after the first application is very important to control most of the stem borer larvae. Moth catches by pheromone traps can also be used to predict when the insecticide application should be done. When the mean number of moths caught in three pheromone traps exceed the number of two moths per trap in a single week insecticides should be applied. The first infestation after the first peak in moth activity is very important because this is infestation causes the most damage (Van Rensburg *et al.*, 1988). It have been estimated that 35-41 grams of the yield can be lost per plant if the larvae are not treated with insecticides during the second and the third week after plant emergence (Van Rensburg *et al.*, 1988).
In order to delay resistance it is very important to rotate not only the active ingredient of the insecticide but also the chemical group that is applied (Ahmad et al., 2009). An example can be lambda-cyhalothrin (pyrethroid) can be applied for the first application while the second application could be indoxacarb (oxadiazine). Unfortunately there are only pyrethroids that are registered for the control of *S. calamistis* on maize and therefore no rotation in chemical classes can be recommended. This species is also more difficult to control on maize because many of the larvae feed behind the leaf sheaths before penetrating the stem without moving to the whorl (Van den Berg and Van Wyk, 2007).

### 3.3. Future research

The reason why the efficacy of control of the chlorantraniliprole + lambda-cyhalothrin mixture as well as chlorfenapyr was lower than the other insecticides should be investigated further. The question whether the high efficacy of control provided by the indoxacarb + lambda-cyhalothrin mixture can be ascribed to an additive effect or synergistic response may determine if the application rate of the two active ingredients could be lowered when applied as a mixture. Novaluron and lufenuron are less harmful to mammals than most other insecticides, but the adding of a pyrethroid into a mixture, makes it much less safe for human use. It is therefore suggested that these two benzoylureas should be evaluated on their own in future studies to determine their efficacy when applied alone.
3.4. References


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