THE BIO-ECOLOGY OF THE GRASS LEAF MINER, *AGROMYZA OCULARIS* (DIPTERA: AGROMYZIDAE), ON WHEAT AND BARLEY IN THE NORTHERN CAPE PROVINCE, SOUTH AFRICA

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    'The earth is the Lord's, and everything in it, the world, and all who live in it; for he founded it upon the seas and established it upon the waters.' (Psalm 24:1-2)

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ABSTRACT

The grass leaf miner fly, *Agromyza ocularis* (Spencer) (Diptera: Agromyzidae) was first described from specimens collected at Ceres (1959), Giant's Castle (1961) and the Maseru (1963) district. These specimens were collected with a sweep net from grass and no information was recorded on the host range and biology of the species. During 2000, *A. ocularis* was recorded on irrigated wheat in the Prieska district, Northern Cape and since then the species has spread to many wheat and barley fields in the Prieska and Douglas area.

Two distinct types of injury are caused by *A. ocularis*, namely feeding punctures and leaf mining. The adult female cause's circular punctures on the upper leaf surface of the host plant with her ovipositor, the ovipositor puncture can provide a feeding or egg-laying site. Mines are caused by the larvae feeding on tissue inside the leaf. The mine commences at the egg laying site where the larvae hatch. The mines are usually linear in the direction of the stem attachment or apex of the leaf. The area mined is dead, necrotic tissue and could not be revived through plant growth compensation. Photosynthesis rate by the leaves is therefore diminished.

A mature larva cuts a slit in the leaf epidermis, escapes from the mine, drops to the ground and burrows into the damp soil adjacent to the plant and pupates. The pupal stage can last 23 days at 25°C under laboratory conditions. A pupal diapause stage which lasts for ±10 months in the laboratory can occur. The function of this stage is not clear, but it could be a mechanism to survive through periods when food resources are not readily available and extreme high temperatures occur. The lifespan of the adult fly is 30 days at 25°C, also under laboratory conditions. This can lead to overlapping generations of flies in a field and increase of damage to the crop. The flies were found to be active in the field when mean daily temperatures ranged between 10°C and 30°C and high relative humidity prevailed due to

irrigation. This could be the optimal conditions the flies need to survive and thrive.

A number of alternative host plants for *A. ocularis* were identified, *i.e. Phalaris minor* (small canary grass), *Bromus catharticus* (rescue grass), *Lolium perenne* (perennial rye grass) and *Avena fatua* (common wild oats). These grasses were recorded on the edges of the wheat fields during the wheat growing season, but not during the peak of the summer or in the natural vegetation surrounding the fields.

The appearance of the flies in the cultivated fields differed by 14 days between 2008 and 2009 growing seasons. In spite of this difference, the crops in both years were older than plant growth stage (GS) 5 (Joubert scale) and the plants had already entered the stem elongation phase when the leaf miner outbreak occurred. It thus seems as if the appearance of flies is not linked to the specific plant growth stage, but rather to climatic conditions.

At plant GS 5 the number of tillers per plant is already set and the plants' energy is utilized to initiate the number of heads per plant and the number of florets per head. If the plant is heavily damaged by this time, the number of heads and florets per plant will be influenced. The leaf miners commence oviposition on the lower older leaves of the plant, which are then obviously mined first. The rest of the leaves are attacked as they develop.

In 2008, 100% of both barley and wheat plants sampled in the Douglas area were damaged by leaf miners when evaluated at GS 5. This damage intensity continued in all subsequent investigations. The number of damaged tillers per wheat plant increased from 48% - 63% between GS 5 and GS 17, the latter being the stage immediately prior to flowering. The number of damaged wheat leaves per tiller increased from 33% - 58% between GS 5 and 17, whilst indices ranging from 10% to 100% of leaf area damage were recorded. This amply demonstrates the severity of damage that is caused by the leaf

miner. The damage varies, however, between fields and between years and could be due to specific spatial and temporal parameters. Damage to the crop and its yield is thus expected. However, insecticide trials conducted under similar conditions during 2008, demonstrated no difference in yield, albeit that a number of larvae were still present per tiller. The plant therefore seems to be able to compensate for the damage inflicted by the leaf miner. If the optimal conditions under which the crop is produced. *i.e.* sufficient water and fertiliser, is considered, this could be possible.

The testing of different insecticides in field trials between 2007 and 2008 provided variable results, with a double dosage of Unimectin® resulting in 80% reduction in larval numbers on barley, which is the only reduction figure which is according to the pesticide registration application (Act 36 of 1947). In 2008 only Abamectin® double dosage met these standards and was the most successful in larval reduction, resulting in figures of 53% - 85% on barley. In spite of all these variables no significant decrease or increase in yield could be measured on any of the treatments in any of the years, suggesting that the plants could absorb the damage levels through compensatory growth.

Key words: *Agromyza ocularis, grass leaf miner, biology, alternative host range, damage intensity, insecticides, Unimectin®, Abamectin®, growth compensation*

Uittreksel

Die gras blaarmynervlieg, *Agromyza ocularis* (Spencer) (Diptera: Agromyzidae) se eerste beskrywing was gebaseer op eksemplare wat by Ceres (1959), Giant Castle (1961) en Maseru (1963) versamel is. Hierdie eksemplare was met veenette vanaf gras versamel en geen inligting oor gasheerplante of biologie is ingewin nie. Gedurende 2000, is *A. ocularis* op besproeiing aangeplante koring in die Prieska distrik van die Noord Kaap aangeteken en sedertdien het die spesie na verskeie koring- en garslande in die Prieska en Douglas gebied versprei.

Twee kenmerkende tipes skade word deur *A. ocularis* veroorsaak, naamlik voedingswonde en blaarmyning. Die volwasse wyfie produseer sirkelvormige gate op die blaaroppervlak met haar ovipositor, wat voedings- of eierleggingsplekke verskaf en wat ’n kenmerkende kollerige voorkoms op die blaar veroorsaak. Myne in die blaar word deur die larwes, wat die binneste blaarweefsel vreet, veroorsaak. Die myn ontstaan op die eierleggings plek waar die larwes uitbroei. Die myne is gewoonlik liniêr op die blaar in die rigting van die stam of die blaarpunt. Die gemynde area is dooie, nekrotiese weefsel en kan nie by wyse van kompensasie groei deur die plant herstel word nie. Fotosintese tempo van die blare word gevolglik op hierdie manier verminder.

’n Volgroeide larf maak ’n snit in die blaar, ontsnap uit die myn, val af grond-toe en boor in die klam grond in naby die plant en pupeer. Teen 25^{o}C onder laboratorium toestande kan die papiestadium tot 23 dae duur. ’n Papie diapause fase is teenwoordig wat vir ±10 maande in die laboratorium volgehou het. Die doel van hierdie fase is onduidelik, maar dit kan wel ’n meganisme van oorlewing wees gedurende periodes van voedselskaarste en uiterse hoë temperature. Die lewensduur van die volwasse vlieg is 30 dae teen 25°C onder laboratoium toestande. Hierdie kan aanleiding gee tot oorvleuelende generasies van die vlieg in ’n land en ’n gepaardgaande verergering van gewasskade gewasse vererger. Die vlieë was aktief in die

veld by gemiddelde daaglikse temperature van tussen 10°C en 30°C, terwyl hoë relatiewe humiditeit as gevolg van besproeiing geheers het. Hierdie kan moontlik die optimale omstandighede wees wat die vlieg benodig vir oorlewing en voortplanting.

'n Aantal alternatiewe gasheerplante is vir *A. ocularis* geïdentifiseer, *nl*. *Phalaris minor*, *Bromus catharticus, Lolium perenne* en *Avena fatua*. Hierdie grasse was op die rande van die koringlande, gedurende die koring groeiseisoen aangeteken. Hierdie plante was egter afwesig gedurende die piek van die somer of in die omliggende natuurlike plantegroei.

Die verskyning van die vliee in die bewerkte lande het met 14 dae tussen groeiseisoene 2008 en 2009 verskil. Ten spyte hiervan was die gewasse in albei jare alreeds ouer as plant groeistaduim (GS) 5 (Joubert skaal) en die plante was alreeds in die stam verlengingsfase toe die blaarmyner uitbraak plaasgevind het. Aan die hand hiervan wil dit voorkom of die verskyning van die vliee nie met plant groeistaduims gekoppel kan word nie, maar eerder met klimaatsomstandighede.

Tydens plant GS 5 is die getal helmstokke van die plant reeds gevorm en die energie van die plant word gebruik om die aantal koppe per plant en aantal blomme per kop te inisieer. Indien die plant op hierdie staduim alreeds baie swaar beskadig is sal die aantal koppe en blomme per plant beïnvloed word. Blaarmyner eierlegging neem op die ouer blare wat laer af aan die plant geleë is in aanvang, wat dan logiesgesproke ook eerste gemyn word. Die oorblywende blare word daarna aangeval soos hulle ontwikkel.

In 2008 is bevind dat 100% van beide gars- en koringplante deur die blaarmyner teen GS 5 beskadig is. Hierdie skadeintensiteit het herhaaldelik in alle latere ondersoeke voorgekom. Die aantal helmstokke per koringplant wat beskadig is het van 48% -63% tussen GS 5 en GS 17, laasgenoemde synde die stadium net voor blom, toegeneem. Die skade aan die koringblare per

helmstok het van 53% - 58% tussen GS 5 en GS 17 toegeneem, terwyl indekse, wat gewissel het van tussen 10% tot 100% blaaroppervlakskade, aangeteken is. Die skade varieer egter tussen verskillende lande en verskillende oesjare en kan dus toegeskryf word aan spesifieke ruimtelike en tydsgebonde veranderlikes. Alhoewel insektisiede toetse onder soortgelyke omstandighede gedurende 2008 uitgevoer was, was daar geen verskil aan die opbrengs nie, ten spyte van die teenwoordigheid van ’n aantal larwes per helmstok. Dit blyk dus dat die plant kan kompenseer vir die skade wat deur die blaarmyner aangerig is. Indien die die optimale bewerkingpraktyke van voldoende water en kunsmis waaronder hierdie gewasse geproduseer word, in ag geneem word, is so ’n verskynsel ’n moontlikheid.

Die toetsing van verskillende insektisiedes tydens die veldproewe van 2007 tot 2008 verskaf variërende resultate.’n Dubbel dosis Unimectin® veroorsaak 80% vermindering in die getal larwes op gars en voldoen sodoende aan plaagdoder registrateur se regulasies (Wet 36 van 1947). In 2008 het slegs Abamectin® dubbel dosis aan hierdie standaarde voldoen en het ’n vermindering van 53% - 85% in larfgetalle op gars tot gevolg gehad. Ten spyte van al hierdie variasies het geen noemenswaardige verlaging of verhoging in opbrengs in enige van die toetse of jaartalle voorgekom nie. Dit dui aan dat die plante die skade as te ware absorbeer by wyse van kompensasie groei.

Sleutel woorde: *Agomyza ocularis, gras blaarmynervlieg, biologie, alternatiewe gasheerplante, skadeintensiteit, insektisiedes, Unimectin®, Abamectin®, groeikompensasie*

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1. The origin of Agromyzidae

The habit of insect larvae to feed or "mine" inside the leaf of its host plant occurs in at least 51 families of holometabolous insects worldwide. These families are found in the orders Coleoptera, Diptera, Lepidoptera and Hymenoptera with 10,000 described species as leaf miners (Connor & Taverner 1997). Leaf mining occurs in nine families of the Diptera order, with Agromyzidae as the largest leaf mining lineage (Connor & Taverner 1997).

Agromyzidae is distributed throughout the world, from the tropics to the high arctic (Spencer 1973). The majority of pest species are limited to a single zoogeographical region and overlapping cases are a result of recent introduction. *Liriomyza brassicae* (Riley) is the only cosmopolitan species that is widely distributed (Spencer 1973).

The evolution of the Agromyzidae is closely linked to their degree of host specificity (Oldroyd 1964). According to fossil evidence, the leaf mining habit evolved during the Mesozoic era which was later than feeding types such as galling, boring and margin feeding (Sinclair & Hughes 2008). According to Oldroyd (1964) the family existed by the late Cretaceous time, and assumes that Agromyzidae and angiosperms evolved parallel. Fossil deposits from the Miocene time indicate a great evolutionary explosion when many species in the leaf mining genera evolved in association with their host (Oldroyd 1964).

The majority of Agromyzidae has a restricted host range (Oldroyd 1964) and is mostly oligophagous or monophagous with 95% of species feeding on a single plant family (Scheffer *et al.* 2007). Agromyzidae is mostly known as the leaf miner family because of the feeding habits of the larvae (Spencer 1973). The larvae chew a cavity between the epidermal layers known as a mine, while feeding on the parenchyma with both epidermal layers remaining intact for food and shelter (Oldroyd 1964). Because leaf mining insects tunnel inside leaves and feed on specific tissue layers, they may be able to avoid plant defenses, both structural and chemical, that occur on the exterior of the leaf or in the tissue layers not encountered while feeding (Connor & Taverner 1997). All Agromyzidae are plant feeders (Connor & Taverner 1997), with mining occurring in bark or stem, but mostly in leaves (Needham *et al*. 1928). Agromyzidae larvae's feeding habits differ regarding the specific plant tissue that is targeted for feeding, for example leaves, fruit, stem, root or seeds (Spencer 1973).

Leaf galls also occur but can be seen as highly specialized mines which induce the host to provide additional tissue (Sinclair & Hughes 2008). Their galls provide both a food resource and a habitat that can provide more protection against predators and parasitoids (Sanver & Hawkins 2000). A gall forming insect does not need to mine because nourishment is stimulated by the presence and activity of the insect (Sinclair & Hughes 2008).

Specialization has been established through an evolutionary process that is not entirely clear, but could be explained by plant populations becoming isolated and that Agromyzid populations were simply present during this evolutionary change. These host associations that occur in Agromyzidae have been experimentally confirmed with the females of *Phytomyza matricariae* (Hendal). This fly usually feeds on *Tanacetum vulgare* (Family: Asteraceae) but was offered a broad non-host range to choose for oviposition. No eggs were deposited in the non-host plants, though when larvae were implanted in these plants most of the lifecycles could be completed (Spencer 1973).

The larvae are unable to move between plants and in most cases complete instar transformation in the same leaf, therefore the females have the responsibility of finding the correct host and maintaining inherited host-specificity. Agromyzid females have been documented to pierce the leaf or stem tissue with their ovipositors and drinking the exuding sap. This habit is a mechanism of finding the correct host by means of olfactory cues, ingesting specific proteins and feeding on the available carbohydrates (Spencer 1973). Agromyzid flies can have a restricted host range and some species are oligophagous, attacking plant families such as Poaceae that consist of important cereals such as rice, wheat and barley (Watson & Dallwitz 2003). Three Agromyzid genera, *i.e*. *Cerodontha*, *Liriomyza* and *Phytomyza* are conspicuous polyphagous leaf miners with, *Liriomyza* exhibiting the widest host range (Scheffer *et al.* 2007).

2. Agromyzidae as agricultural pests

Agromyzidae is one of the most important crop pest families (Spencer 1973) and have been recorded to attack 150 cultivated plant species around the world (Thapa & Thapa 2004). The various species can cause economical damage to a broad range of host plants under both field and greenhouse conditions (Hossain & Poehling 2006). The earliest accounts of damage to cultivated plants were only a few isolated cases during the nineteenth century, although many species were described during this period (Spencer 1973). Only after World War I, Agromyzidae became better known as a primary agricultural pest (Spencer 1973).

Liriomyza is the most documented genus in the family Agromyzidae containing many pest species, albeit that very confusing morphological similarities and overlapping host ranges occur in the genus (Weintraub & Horowitz 1995). Damage to crops vary with species and range from the destruction of young seedlings, vectoring diseases, reducing crop yields, to causing "sunburn" of the fruit and reduction in aesthetic value of ornamental plants (Shiao 2004). An example is *Liriomyza huidobrensis* (Blanchard), the pea leaf miner, which is an important pest in Europe, Israel, Sri Lanka, Philippines, South Africa and Indonesia, especially on potatoes (Ridland 2004; Weintraub & Horowitz 1995). In South Africa it is known as the potato leaf miner. Another example is *Liriomyza trifollii* (Burgess) which is known as the American serpentine leaf miner and which is the most serious pest of vegetable and horticultural crops (Lee *et al.* 2005).

Leaf miners attacking grasses or cereal crops are not well studied (Scheirs *et al*. 2001). Grasses have a low nutrient content with rare active feeding deterrents and structural defenses (Scheirs *et al.* 2001). Agromyzidae feeding on cultivated cereals are found throughout the world and belong to five genera e.g. *Agromyza*, *Liriomyza*, *Pseudonapomyza*, *Phytomyza* and *Cerodontha*. A small degree of generic range overlap can occur, but continents usually have their own species (Spencer 1973).

Fluctuating populations and sporadic outbreaks can occur in Agromyzidae feeding on cereals (Spencer 1973). According to Spencer (1973) plant damage by grass leaf miners must be considered as actual or potential pests especially when large populations attack young plants.

During 1986/87 insect pests of spring wheat was documented in central Saudi Arabia. The two insect species ranked as major pests of wheat was the greenbug, *Schizaphis graminum* (Rondani) and a wheat leaf miner, *Agromyza* sp. (El-Hag & El-Meleigi 1991a). According to El-Hag and El-Meleigi (1991b) *Agromyza megalopsis* (Hering), *A. intermittens* (Becker) and *A. prespana* (Spencer) were reported on wheat and barley in Hungary and *A. megalopsis* in Iraq. In Poland Agromyzidae has been observed in cereal crops causing a reduction in the number of grains in the kernel (Walckzak 1995). The economic injury level based on yield loss and spray cost, according to Iwasaki (1999), were estimated at 12% and 16% for spring and winter wheat respectively. In Bulgaria, *A. megalopsis* cause yield decreases in barley of 26.8% (Khristov 2000).

3. Economic important leaf miners of South Africa

Although numerous Agromyzid species occur in South Africa (Spencer 1961, 1963, 1965) only the pea leaf miner, *Phytomyza horticola* (Goureau) and Petunia leaf miner, *P. atricormis* (Meigen), which are introduced species, are pests of economic importance to cultivated crops (Annecke & Moran 1982). The latest introduction, *Liriomyza huidobrensis,* was documented in the Western Cape in 1999 for the first time when enormous damage was caused to the potato crop (Scheffer *et al*. 2001). *L. trifolii* was first identified in South Africa during 1983 and is not an important economic pest on potatoes but it does attack tomato plants (Van Burick 2003). Both *L. huidobrensis* and *L. trifolii* are also potential pests of peanuts (Van Rooyen 2001).

Agromyza ocularis (Spencer) is an indigenous grass leaf miner in South Africa (Fig. 1). It was described in 1959 from material collected in the Ceres area of the Western Cape Province, South Africa (Spencer 1961). Sampling was conducted with a sweep net from numerous grasses, but no host plants were recorded. It was also collected in 1961 and 1963 from grasses in the Giant's Castle district close to the Drakensberg Mountains in the KwaZulu-Natal Province and close to Maseru in Lesotho (Spencer 1963, 1965). During 1992, infestation by this leaf miner was recorded on wheat in South Africa for the first time. This was in the Bergville district of KwaZulu-Natal and it persisted for three wheat seasons, after which it seems to have been naturally controlled (Prinsloo 2001).

During 2000, leaf miners were reported from two irrigated wheat fields in the Prieska district of the Northern Cape Province, while an infestation was also recorded on a single field in the Eastern Free State (Prinsloo 2001). Material sampled at these sites was identified by Dr. Michael von Tschirnhaus at the Bielefeld University in Germany. Since 2000, the infestation by these species increased annually in wheat fields in the Prieska and Douglas areas and in

2006 it was also reported on barley in the same area ([1]André Coetzee, personal communication). Since heavy infestations occurred on some fields and nothing has being published on the host range, biology and ecology of this species, it was decided to study these aspects so that a decision could be made regarding optimal control strategies.

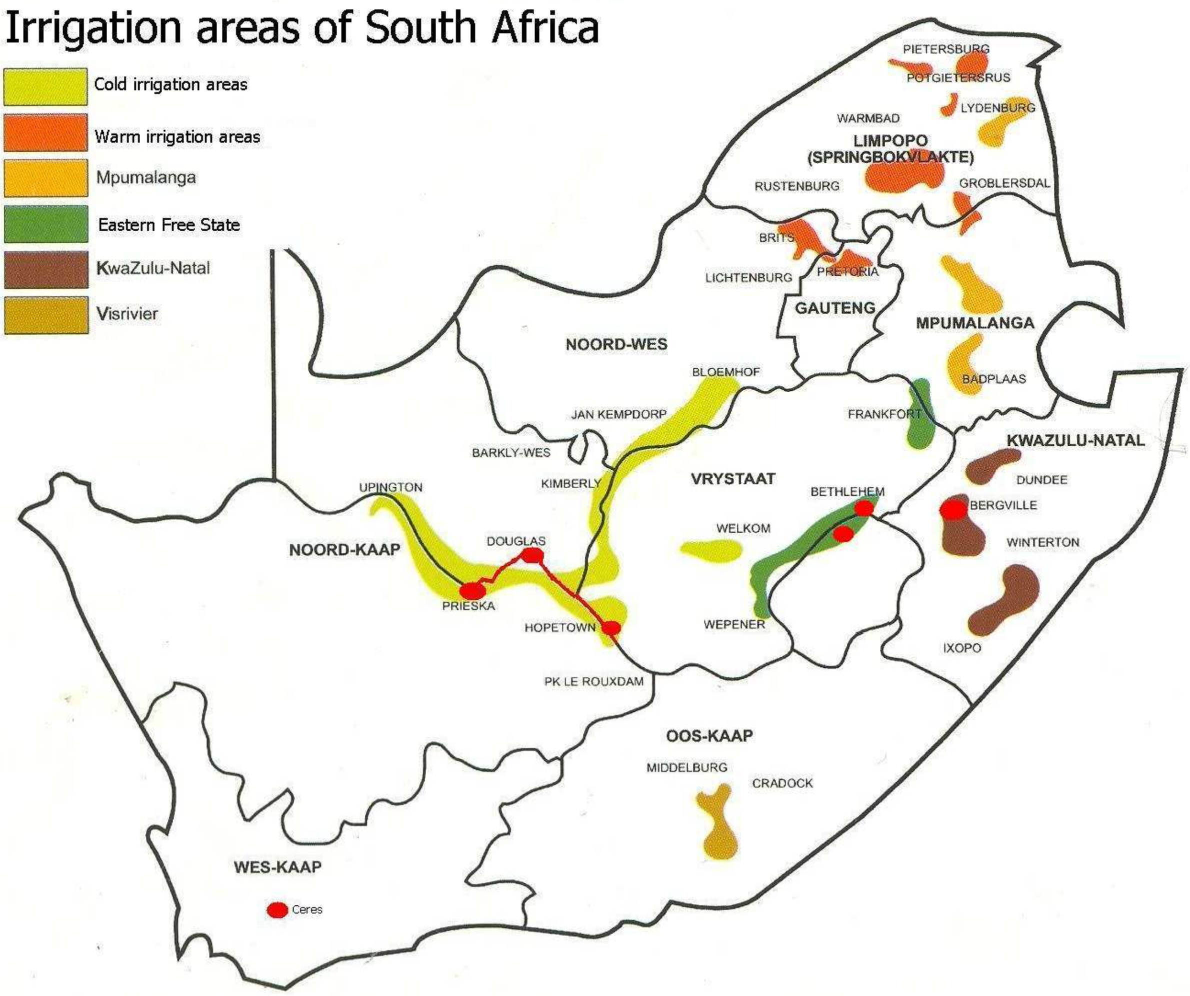

Figure 1. Distribution of *Agromyza ocularis* in South Africa (in red) in relation to the irrigation areas (as documented by the ARC-Small Grain Institute 2007).

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[1] André Coetzee, GWK (Pty) Ltd. in Douglas, Northern Cape, South Africa.

4. Objectives of the study

The following aspects will be studied:

- A taxonomic overview of *Agromyza ocularis* for comparison with other Agromyzidae pests.
- Biology of *Agromyza ocularis*

  The biology of this species must be studied i) to understand the driving forces behind the current change in its behaviour, ii) to understand the mechanisms that allow the species to reach and maintain outbreaks density iii) to effectively manage the outbreaks.

- Damage levels of *Agromyza ocularis*

  The damage threshold must be determined in order to determine whether this species does in fact cause economical damage. It is important to bear in mind that once leaf tissue is destroyed it cannot recover in the particular growth season on the crop and the initial damage incurred during the infestation stage is permanent.

- Chemical control of *Agromyza ocularis*

  There is registered chemical for this species in South Africa. It is therefore important that the correct control measures be determined before further dispersal and spread occurs.

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WEINTRAUB, P.G. and HOROWITZ, A.R. 1995. The newest leafminer pest in Israel, *Liriomyza huidobrensis. Phytoparasitica* **23**: 177-184.

CHAPTER 2

IDENTIFICATION OF *AGROMYZA OCULARIS*

1. Introduction

Agromyzidae is a world-wide distributed family of which 2500 species are known (Spencer 1973). *Liriomyza trifolii* and *Liriomyza huidobrensis* are both economically important cosmopolitan species. Both these species are therefore also present in South Africa with *L. huidobrensis* being a recent invader (Lochner 2000). Therefore when leaf mines were found on the wheat in the Prieska district in 2000, an assumption was made that the leaf miners were either the American leaf miner (*L. trifolii*), or the Potato leaf miner (*L. huidobrensis*) or a new invader. Specimens were collected and send to Dr. M. von Tschirnhaus for identification. The specimens were identified as, *Agromyza ocularis* Spencer, the grass leaf miner ([2]Von Tschirnhaus, personal communication). *Agromyza ocularis* was not a new invader but is an endemic species of South Africa (Spencer 1961).

Economically important *Liriomyza* species have been confused with one another due to their morphological similarities and overlapping host ranges (Parrella 1982). Therefore the male genitalia are one of the most important taxonomic characters in adult male Agromyzidae and are used by leading specialists (Spencer 1973; Shiao 2004). The male genitalia of Agromyzidae are strongly developed. The aedeagus is the most important structure which is usually concealed below the apical abdominal sclerite (Spencer 1973). Although this is the most important taxonomic character, it is only a specialist that can identify it accurately and therefore it cannot be used by farmers to determine whether *Agromyza ocularis* is present in the crop.

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[2] M. Von Tschirnhaus Faculty of Biology, University of Bielefeld, Germany.

Even though *L. huidobrensis* and *L. trifolii* are in a different genus than *A. ocularis* confusion still exists amongst farmers and extension officers concerning the identification of the flies. In order for farmers to identify the leaf miner accurately, other approaches have to be used and an identification guideline has to be created to recognize the difference between the three mentioned species:

- Leaf miners are often identified by means of the pattern of the mine which the larvae create (Smith 1989). By identifying the leaf mine pattern on the leaves of the host plant, the species might be readily recognized in the field without having to wait to rear immature through to the adult stages (Stegmaier 1967; Scholtz & Holm 1985; Borror *et al.* 1989). Blotch and serpentine leaf mines are most common, with blotch leaf mines irregularly rounded producing large blotches or meandering tunnels, whilst serpentine leaf mines are 'snake-like' and wind across the leaf surface with the mine growing wider as the larva grows (Stegmaier 1967; Cranshaw *et al*. 2009).

- Identification can also be achieved by defining the host plant of the leaf miner. This provides an indirect route for recognising the species, since different species often have different hosts; however, assumptions made in this manner require morphological verification.

- A morphological diagnosis separating the two *Liriomyza* species and *A. ocularis* from one another on the basis of certain characteristics can be compiled to aid farmers.

2. Material and methods

Specimens of *Agomuza ocularis* were collected in wheat fields at Prieska, (22° 33′ E 29° 32′ S Northern Cape Province) in October 2000. A sample was sent to Dr. M. von Tschirnhaus at the Faculty of Biology, University of Bielefeld, Germany for identification and he based his identification on Spencer (1961). The holotype of *Agromyza ocularis* was received on loan from the Natal Museum, Pietermaritzburg, South Africa which created the opportunity to also compare the sample specimens with the type. Examination was carried out at 6.3 magnifications with an Olympus SZ microscope. The original description of Spencer (1961) was used for comparison and eventual identity verification. In addition, Agromyzidae characters were defined and the description of *Liriomyza huidobrensis* and *Liriomyza trifolii* was done according to the European and Mediterranean Plant Protection Organization Standards (Anon 2005).

3. Descriptions

Family: Agromyzidae

Agromyzidae is a family of small blackish or yellow marked flies which are 2.0-5.0 mm in length with clear wings (Anon 2005) (Figs 1 & 2).

According to Anon (2005), who cited Spencer (1987), the following combination of characters is important when defining the family:

- Vibrissae present.
- 1-7 frontal bristles present.
- Costal break present at the apex of sub-costa (Sc).
- Cell cup small.
- A_1 vein not reaching wing margin.
- Pregenital sclerites of male with a simple (fused) tergal complex (tergites 6-8) with only two spiracles between tergite 5 and the genital segment.
- Anterior part of abdominal segment 7 in female forming an oviscape.

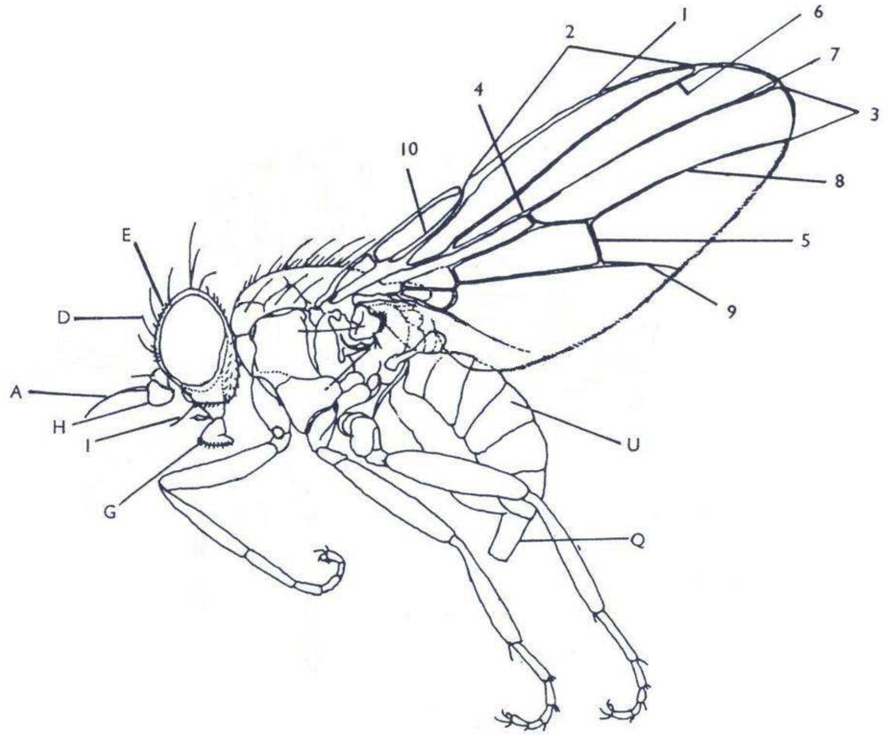

Figure 1. **A lateral view of an *Agromyza* sp. (after Spencer 1973):** **A** = arista, **D** = orbital bristles, **E =** orbital setulae, **G** = proboscis, **H** = third antennal segment, **I** = vibrissae, **Q** = ovipositor sheath, **U** = tergites.

1 = costa, **2** = second costal section, **3** = fourth costal section, **4** = first cross-vein, **5** = second cross-vein, **6** = R_1, **7** = R_{4+5}, **8** = M_{1+2}, **9** = M_{3+4}, **10** = subcosta.

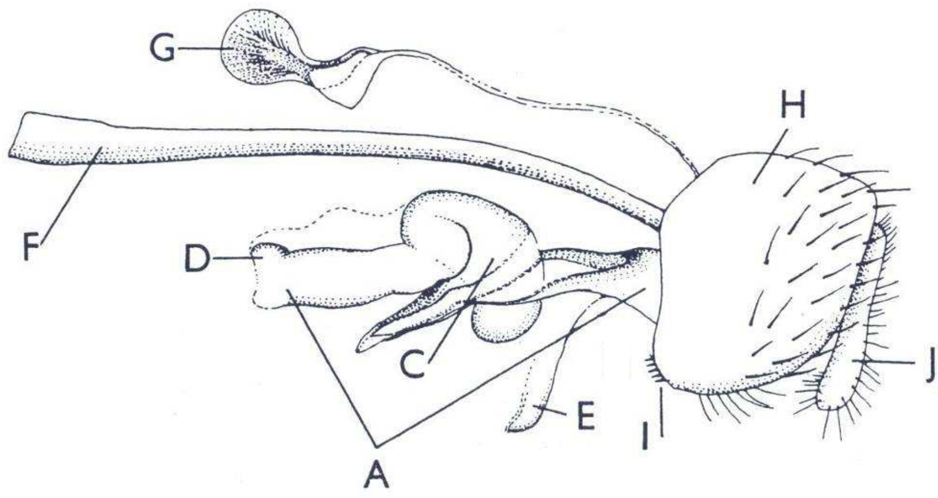

Figure 2. Male genitalia of *Agromyza* sp. (after Spencer 1973):
A = aedeagus, **C** = mesophallus, **D** = distiphallus, **E** = hypophallus, **F** = aedeagal apodeme, **G** = ejaculatory apodeme, **H** = epandrium, **I** = surstylus, **J** = cercus.

3.1. *Liriomyza huidobrensis* Blanchard, 1962.

L. huidobrensis is a South American species (commonly known as the potato leaf miner) that was recorded in South Africa in December 1999. This leaf miner species is widely distributed in the country and is especially well-known from the Sandveld, Ceres, the Gametoosvallei, Dendron, Vivo and the Pretoria regions (Van Burick 2003).

3.1.1. Damage and host preference

This leaf miner species is polyphagous and develops in a wide variety of vegetables and flower crops, as well as weeds in the field (Weintraub & Horowitz 1995; Van Burick 2003). It is known to cause yield losses of up to 70% on potato crops in South Africa (Picker *et al*. 2002; Visser 2005). According to Spencer (1973), a local host preference can be expected to start developing for *L. huidobrensis*.

3.1.2. Feeding punctures

The adult female produces circular punctures on the upper leaf surface which provides both feeding and egg-laying sites. These feeding punctures are similar in the different species of Agromyzidae (Anon 2005; Visser 2005).

3.1.3. Leaf mines

The larval feeding tunnels of *L. huidobrensis* are tightly coiled and irregularly shaped. The leaf midribs and lateral leaf veins are usually the starting point of a tunnel and the tunnel becomes wider as the larvae mature (Fig. 3) (Watson & Dallwitz 2003). The larvae can also tunnel into stems causing the entire tiller to die off (Visser 2005).

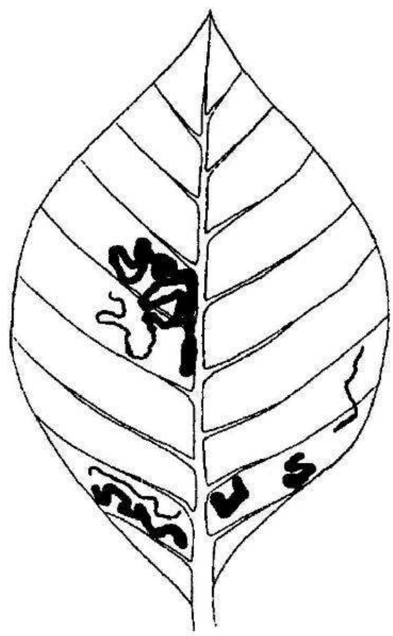

Figure 3. Characteristic pattern of leaf mines of *Liriomyza huidobrensis* Blanchard (Anon 2005).

3.1.4. Larvae and pupae

The larvae are cream coloured, but in the final stage a yellow patch develops anterior-dorsally (Anon 2005). According to Spencer (1973) each posterior spiracle is elliptical with 6 – 9 pores along the margin. The pupae are formed on the leaves of the host, but usually become dislodged and fall to the ground (Visser 2005). The pupae are orange to dark brown in colour (Anon 2005; Visser 2005).

3.1.5. Adult

The fly has variable yellow and black colour patterns (Ridland 2004; Visser2005). *Liriomyza huidobrensis* is generally darker than *L. trifolii* (Ridland 2004) and this is especially evident on the ventral side which is conspicuously dark (Visser 2005). The male genitalia are a single curving spine (Spencer 1973).

3.2. *Liriomyza trifolii* Burgess, 1880

This species is a leaf miner of American origin and was unknown outside the New World until the mid-1970s (Dove 1985). *Liriomyza trifolii* (popularly referred to as the American leaf miner) was recorded in the Krugersdorp region in 1983 (Van Burick 2003).

3.2.1. Damage and host preference

L. trifolii is polyphagous, and severely damages the leaves of plants (Spencer 1973). The fly is a problem species on peas, tomatoes, beans, potatoes and variety of other vegetable types (Picker *et al*. 2002).

3.2.2. Feeding punctures

The adult female produces punctures that result in a stipple effect on the upper leaf surface and which provides feeding and egg-laying sites (Dove 1985).

3.2.3. Leaf mines

L. trifolii has its frass on either side of the tunnel with granular material formed towards the end of the mine (Watson & Dallwitz 2003). A slight zigzag form of a linear type. The leaf midrib and veins are also attacked (Fig. 4) (Hafez *et al*. 1970). Only one larva per mine is present. The length of the mines average ±3.3 cm (Hafez *et al*. 1970).

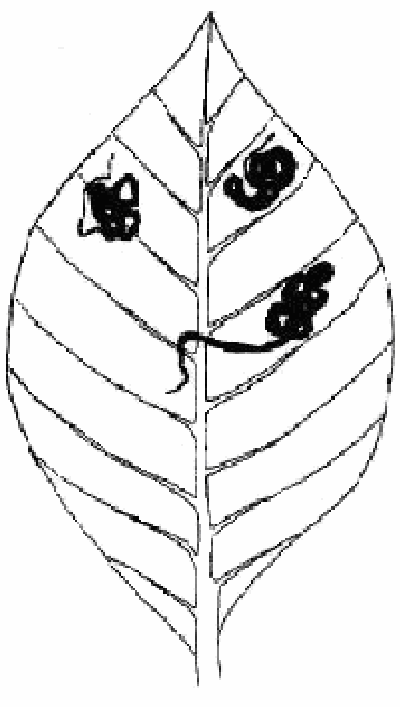

Figure 4. Leaf mines of *Liriomyza trifolii* Burgess (Anon 2005).

3.2.4. Larvae and pupae

The larva is yellow-green in colour and the anterior spiracles are on the last abdominal segments and each has six orifices (Hafez *et al.* 1970). The anterior and posterior spiracles are sessile (Hafez *et al*. 1970). The pupa is yellow-orange in colour (Anon 2005).

3.2.5. Adult

L. trifolii has distinctive colour patterns of yellow and black with four divided tergites (Shiao 2004). The fly is slightly smaller with a darker yellow colour than *L. huidobrensis,* especially on the ventral side (Visser 2005).

3.3. *Agromyza ocularis* Spencer, 1961

Agromyza ocularis is an endemic species in South Africa first recorded in the Ceres district (Spencer 1961). In later years it was also sampled in the Eastern Free State and Lesotho. More recently, in 2000, the species was recorded from the irrigated wheat and barley fields of the Northern Cape (Prinsloo 2001).

3.3.1. Damage and host preference

A. ocularis is a grass leaf miner ([3]Von Tschirnhaus, personal communication). This species has been recorded to feed on wheat and barley. Agromyzidae feeding on Gramineae show an extensive degree of oligophagy *e.g.* feeding on cultivated cereals, including rice, maize, wheat, rye, barley and oats (Spencer 1973). (The host range of *A.*

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[3] M. Von Tschirnhaus, 2007. Faculty of Biology, University of Bielefeld, Germany.

ocularis is discussed in Chapter 3 and the damage impact in Chapter 4.)

3.3.2. Feeding punctures

Similar to Agromyzidae species mentioned above.

3.3.3. Leaf mines

Because of structure of the leaves of its host plant, the grass leaf miner species has limited space and foliage available to feed on. The mine begins where the egg is oviposited and the larva hatches. The mines are usually linear, either towards the stem where the leaf is attached or towards the apical tip of the leaf (Fig. 5). No specific distinction is made towards a certain area of the leaf. When the apex or the stem attachment of the leaf is reached, the larvae will forage further by turning towards available tissue on the leaf blade. Frass is black in colour and granular and can be found anywhere in the mine.

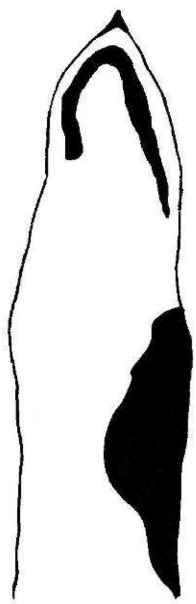


Figure 5. Leaf mines of *Agromyza ocularis* Spencer (J. Adendorff 2010).

3.3.4. Larvae and pupae

The larva is yellow in colour and the alimentary canal takes on a black colour as the larva feeds and is visible through the body wall. The mature larva cuts a circular slit in the mined leaf prior to pupation and emerges from the mine. The larva drops from the mine, borrows into the soil and pupates. The pupa is a light brown colour.

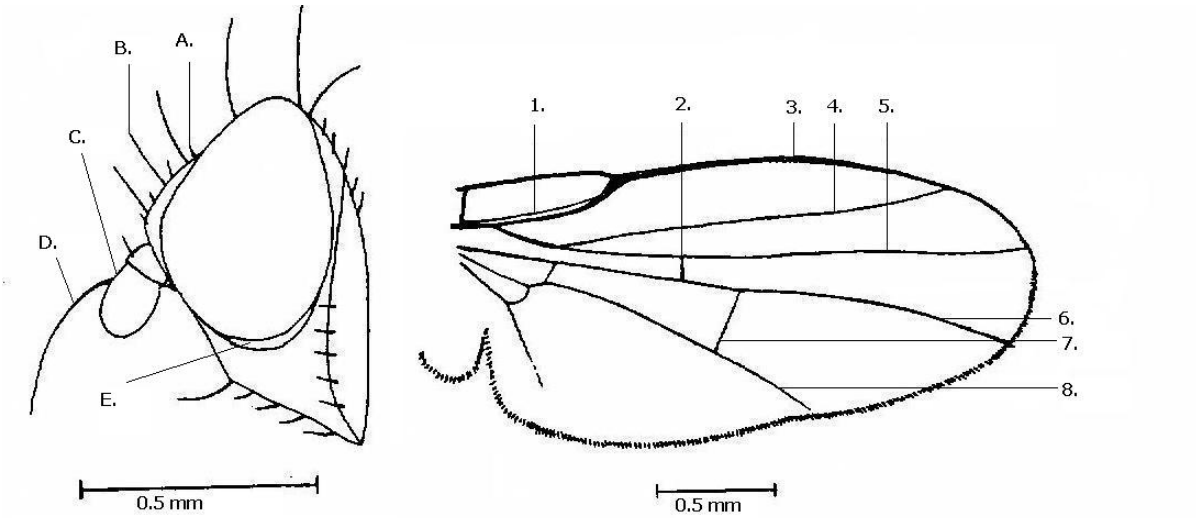
3.3.5. Adult

Description of male [Based on Spencer (1961)]

Measurements. Overall length: 1.9 - 2.9mm; length of head and thorax combined: 1.3 - 1.7mm; length of thorax and scutellum combined: 1.9 - 2.3mm; wing length: 2.2 – 3.0mm.

Head (Fig. 6a): Frons only slightly wider than eye, broadly projecting above eye in lower half; one strong orbital setulae directed upwards, three weaker equal orbitals directed predominantly inwards; orbital setulae fine, sparse, largely upright; jowls broad, deeply extended at rear, lower margin straight; cheeks forming distinct ring below eye; eye conspicuously slanting; third antennal segment slightly longer than broad, rounded at end; arista fine, short, bare; face with well-defined central keel.
Mesonotum: Four distinct post-sutural dorso-central bristles, decreasing greatly in size; fourth less than one-third the length of first, midway between third and suture; pre-scutellars well-developed, stronger than third dorsocentral brisles; acrostichals coarse, in eight rows.
Legs: Mid-tibiae with two short postero-dorsal bristles.
Wing (Figs 6b & 7): Length 3mm; costa extending only to vein r4+5, first cross-vein at distal third of discal cell, last segment of m4 in ratio 22:26 with penultimate.

Colour: An entirely black species; frons matt, ocellar triangle and orbits scarcely shining; antennae black; mesonotum and abdomen shining black, ovipositor shining distally, more matt on basal half; wings clear, veins black; squamae whitish, margins pale brown, fringe silvery; halteres white.



a. b.

Figure 6. ***Agromyza ocularis*** **(Spencer 1961)**

a. Head A = orbital setulae, **B** = orbital bristles, **C =** third antennal segment, **D** = arista, **E** = cheek.

b. Wing 1 = subcosta, **2** = first cross-vein, **3** = costa, **4** = R_1, **5** = R_{4+5}, **6** = M_{1+2}, **7** = second cross-vein, **8** = M_{3+4}.

Figure 7. ***Agromyza ocularis*** **Wing (Photo: J. Adendorff 2010).**

Genitalia: Spencer (1961) provides a figure of the male genitalia of this species (Fig. 8) which shows a strong incurved spine at the lower edge of the epandrium was present. The male genitalia of the species investigated during this study were identical (Fig. 9).

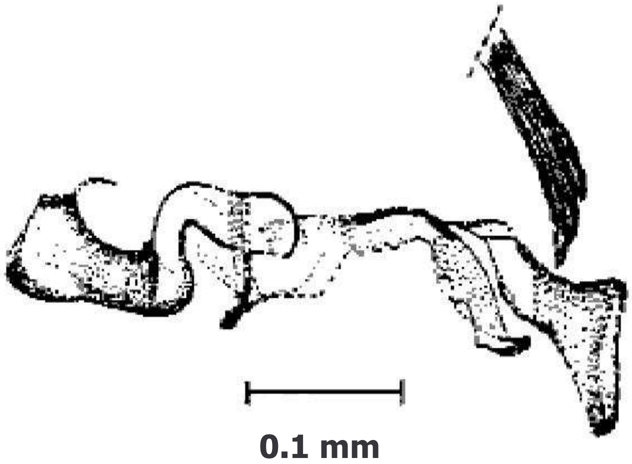


Figure 8. **Male genitalia of *Agromyza ocularis* according to Spencer (1961).**

Figure 9. **Male genitalia of *Agromyza ocularis* from the study areas in the Northern Cape (Photo: J. Adendorff 2010).**

4. Identification summary of economical important Agromyzidae species in South Africa

All available information regarding the identification of the Agromyzidae species important in agriculture in South Africa is provided in Tables 1 & 2. This information should allow farmers to rapidly and accurately identify the relevant species in the field.

Table 1. Leaf mining characteristics of *Liriomyza huidobrensis, Liriomyza trifolii* and *Agromyza ocularis* (Agromyzidae).

Leaf mining characteristics			
Appearance	**Species**	**Host Plant**	**Mining**
	*Liriomyza huidobrensis*	Polyphagous; vegetable-, flower crops and weeds	Tunnels tightly coiled and irregularly shaped. The midribs and lateral leaf veins are usually the starting point of a tunnel. Larvae prefer the midrib area, moving outwards to the leaf margins.
	*Liriomyza trifolii*	Polyphagous; a variety of vegetables *e.g.* peas, tomatoes, beans and potatoes	A slight zigzag form of a linear type. No preference towards an area on a leaf but do not favour the midrib.
	*Agromyza ocularis*	Oligophagy; Graminae that include cultivated crops *e.g.* rice, maize, wheat, rye, barley and oats	Mines are usually linear towards the stem attachment area or apex of leaf. There is no specific preference for any area on the leaf.

Table 2. Morphological characteristics of *Liriomyza huidobrensis, Liriomyza. trifolii* and *Agromyza ocularis* (Agromyzidae).

	Morphological characteristics					
Photo	**Species**	**Third antennal segment**	**Frons & orbits**	**Femur**	**Mesonotum**	**Wing length**
	*Liriomyza huidobrensis*	Slightly enlarged; usually darkened	Frons yellow; generally more orange; upper orbits slightly darkening towards the upper orbits	Yellow, viarably darkened with black striations	Matt black	1.7 - 2.25mm
	*Liriomyza trifolii*	Small, yellow	Yellow	Yellow, occasionally with slight brownish striations	Matt black with grey undertone	1.3 - 1.7mm
	*Agromyza ocularis*	Slightly enlarged; black	Black	Brown with black striations	Black with a brown undertone	2.2 – 3.0mm

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CHAPTER 3

BIOLOGY OF *AGROMYZA OCULARIS*

1. Introduction

Agromyza ocularis, a grass leaf miner, was recorded and described by Spencer in 1961. This was a pure taxonomic description of a new species and at that stage no host plant records or biological data were recorded. During 2000 *A. ocularis* was reported to be present in an irrigated wheat field in the Prieska district ([4]Goddy Prinsloo, personal communication). The flies had also been recorded on wheat and barley crops along the Gariep (Orange) River, reaching population levels which could be damaging to the crop ([5]André Coetzee, personal communication). This information may be an indication that the species is becoming a major concern for the farmers in the area.

Insects become pests as the result of a wide variety of factors and often an understanding of these factors will lead to improved systems of management (Kumar 1984). Intensification of agriculture has created fluctuating agricultural ecosystems which influence the quality of the habitat and result in new and/or greater pest problems (Thomas & Waage 1996). The habitat quality is determined by abiotic and biotic factors and population abundance is a reflection of how well the particular niche area meets the needs of a species (Cornelissen & Stiling 2009). In cases of species reaching pest status, manipulation of these factors can be implemented to manage the outbreaks. Determination of the habitat quality and the biology and mechanisms maintaining the pest status of the species are important factors to investigate.

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[4] Goddy Prinsloo, ARC-Small Grain Institute, Bethlehem, South Africa.

[5] André Coetzee, GWK (Pty) Ltd. in Douglas, Northern Cape, South Africa.

Temperature is one of the most important variables that have an effect on insects reaching pest status. Especially in temperate regions an increase in temperature will not only enhance population size and development tempo, but could also give rise to multiple generations, both of which could intensify the pest status of an insect species. Leaf miner lifecycles, specifically, can be as short as three weeks (Black *et al.* 2003) and an increase in population densities can occur (Johns & Hughes 2002) with warmer temperature.

Agromyza ocularis was recorded in several different climatic regions in South Africa and is therefore adapted to survive under different climatic conditions. Areas where the flies were originally collected include the Ceres district (Spencer 1961) in the Western Cape, which falls in a Mediterranean type climatic zone. Here temperature averages at 29,9°C maximum during mid-summer in January and February and drops to an average minimum of 2,4°C during mid-winter in July. The rainfall average is 1088mm in the winter, and on the high mountain peaks snow can occur (Ceres Tourism Bureau 2010). *Agromyza ocularis* was also recorded in the Giants Castle area of the Drakensberg (Spencer 1963) and the Maseru district (Spencer 1965). Mountain peaks in the Drakensberg exceeding 3000 meters result in mild climate conditions. Here summer temperatures hardly rise above 30°C and summer rainfall occurs. The winter months of June to August is dry with maximum temperatures not exceeding 20°C, while a minimum temperature drop below zero. Snow occurs regularly during the winter (South African Weather Services 2010). The Northern Cape region, where *A. ocularis* was found on wheat in 2000, is a dry Savanna region with temperatures on the extreme side, reaching maximums from 34°C– 40°C in summer and drops below zero in winter. Two major rivers, the Vaal River and Gariep (Orange) River unite in this area and create the opportunity for agricultural production under irrigation. About 140 000 ha of land in this area are being utilized to produce different crops, which include annuals such as maize, wheat, barley, groundnuts, cotton and lucerne. More specifically, about 51 000 ha of wheat and 8000 ha of barley are grown on an annual basis (Anon 2009). To determine the reasons behind the successful establishment and progress of *A. ocularis* as a pest in this region it is necessary to study the influences of temperature on its development in the context of these crop habitats.

It is general knowledge that environmental stress conditions such as drought (=low moisture and high temperature levels) and nutrient deficiencies could directly affect plant quality and indirectly the success of phytophagous insects (such as leaf miners) on the plant. The larval performance of a grass leaf miner, *A. nigripis* Meigen, was recorded to be the most active under wet soil conditions with intermediate nutrient supply, which support the hypotheses that vigorously growing plants are beneficial to herbivores more favourable (De Bruyn *et al*. 2002). This would probably be the case with the grass leaf miner *A. ocularis,* since mentioned optimal conditions are also present during the wheat and barley growing season in the Northern Cape. High yield potential is possible under irrigation in this area, (7.5t/ha for wheat and 6-8t/ha for barley) with high seeding and fertiliser rates applied accordingly (ARC-Small Grain Institute, 2009), which possibly creates a perfect optimum environment for leaf miners to thrive in. In an oligophagous grass miner, *Chromatomyia nigra* (Meigen), females were recorded to maximise their fitness by selecting high-quality host plants for feeding, with the expected outcome that more eggs can then be laid (Scheirs *et al.* 2000). Due to the optimal conditions under irrigation, wheat and barley plants in monocultural fields definitely provide higher quality than the natural grasses growing under stressed conditions in this region and these conditions could therefore also be preferred by *A. ocularis* to maximise their fitness in the area.

A study of the biology of *A. ocularis* will generate information on the reasons why *A. ocularis* became such a successful phytophage on wheat and barley. By studying the life span of this species, its demographics, duration of activity and negative influence on the crop can be determined (Norris *et al*. 2003). To achieve this, the following biological aspects should be studied:

1.1 Oviposition, including time of oviposition, number of eggs laid and the period from oviposition to hatch. This information could indicate an oviposition preference for plant variety or age of host leaves (Facknath & Wright 2007), an estimation of when larval damage is estimated to commence and if plants could defend themselves through egg extrusion (Videla & Valladares 2007).

1.2 The larvae are the damaging stage of *A. ocularis* and it is therefore important to determine if mining occurs throughout the complete larval period or only when younger instars are present (Rott & Godfray 2000). By studying the relationship between larval development and temperature the effect of temperature on the progression of damage can be determined. El-Hag & El-Meleigi (1991), for example, determined that on wheat the larval stage can cause total leaf area damage of up to 11% during the seedling stage.

1.3 The influence of temperature on the duration of the pupal stage must be determined, since this will determine how many generations can be completed in a crop season, which, in turn, could influence severity of infestation.

1.4 The presence of diapause and the environmental stimuli that influence diapause must be determined, since this could have an influence on the initiation of infestation. *Agromyza megalopsis* (Hering), in Bulgaria, for instance, pupates and overwinters in the soil (Khristov 2000). *Agromyza nigripes* (Meigen), a blotch leafminer of wheat, was recorded in the pupal stage at the end of the wheat growing season with 99% of the pupa recorded to be in a state of diapause (El-Serwy 1999).

1.5 To determine the host range of *A. ocularis* after wheat harvest could provide additional information on survival. Alternate or natural host plants of *A. ocularis* are not known and should therefore be investigated.

1.6 The aim of this study was to investigate certain biological aspects which could hopefully answer the question why *A. ocularis* became a successful pest on barley and wheat in the Northern Cape Province. The following aspects were studied:

- Population growth during three plant growth stages (GS) of wheat and barley in the 2008/09 season.
- The different life stages.
- Diapause
- The host range during the off-season in order to determine survival between crop seasons.

2. Material and methods

2.1 Population growth

Observations to determine population growth of *A. ocularis* was conducted in two fields in the Douglas area during 2008 and 2009. Field A (Gwerna S 29° 21.090′, E 23° 08.589′) and field B (Nuwejaarskraal S 29° 23.782′, E 23° 08.613′) were planted with barley and wheat respectively (Fig. 1). Field A and B were respectively situated ±200m and 3km from the Gariep (Orange) River. Both fields are irrigated by centre pivot systems and the two crops are produced annually. During summer maize were planted between 1 December and 20 December. After harvesting the maize in June the stubble on field B was burned and the field disked, before planting wheat. On field A maize stubble after harvesting was left on the surface and superficial tilling was conducted before planting barley. Wheat and barley were planted between 25 June and 20 July. No herbicides, fungicides or insecticides were applied during 2008 and 2009.

Figure 1. Aerial photo of Northern Cape with pin points of experimental field A (Gwerna) and field B(Nuwejaarskraal) (Google Earth 15/03/2010).

The presence and abundance of *A. ocularis* in fields A and B was monitored at growth stage 5, 10 and 14 between 16 September 2008 and 14 October 2008. During 2009 presence and abundance was monitored from 10 September 2009 – 14 October 2009 at GS 11, 15 and 20 in the same fields. *A. ocularis* was recorded on 14 August 2008 and 26 August 2009 in both fields A and B. The wheat and barley growth stages were documented according to the Joubert scale for growth point stages (Fig. 2).

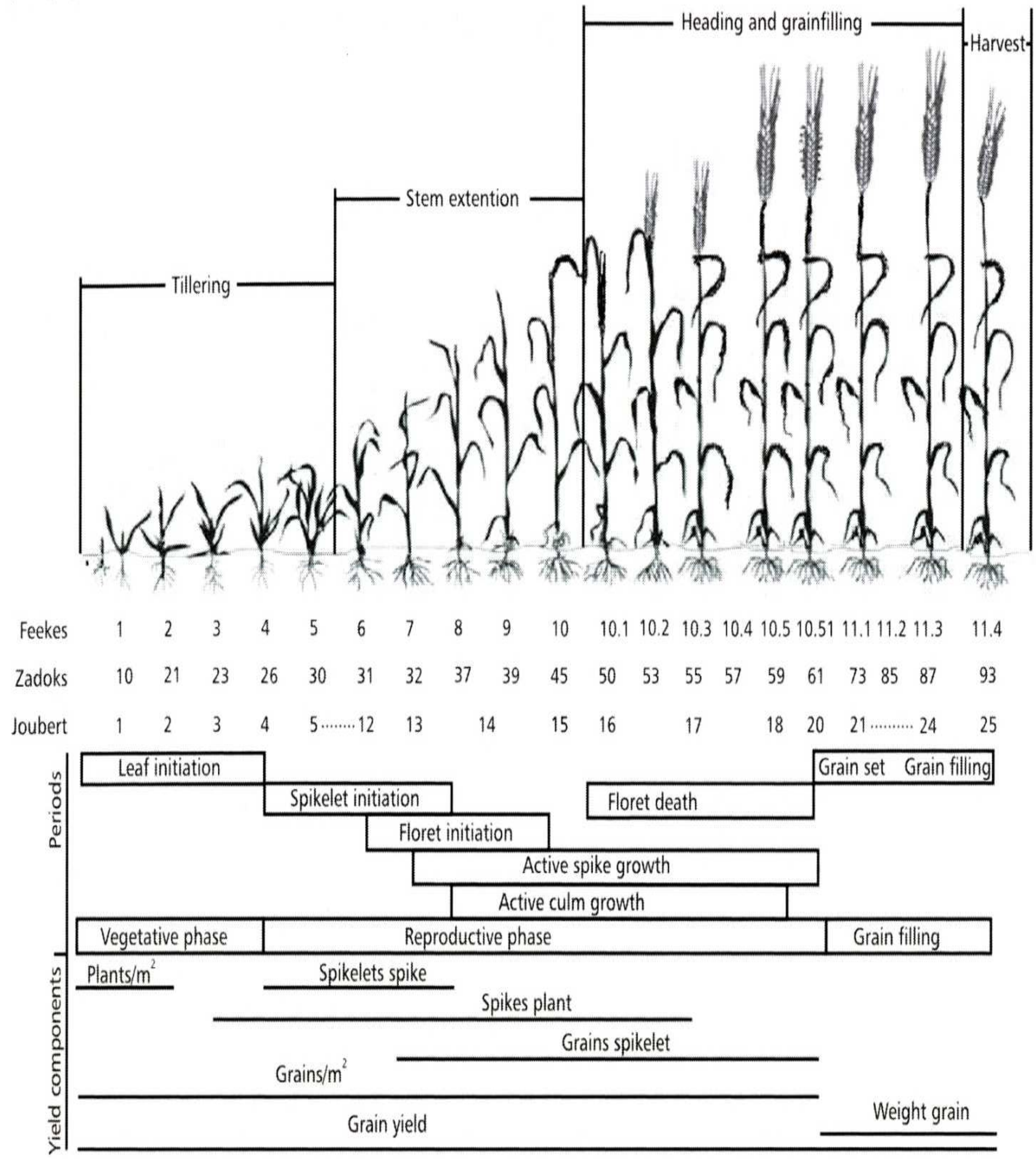

Figure 2. Different growth stage scales of wheat (ARC-Small Grain Institute 2009).

Leaf miner population increase was determined by walking a transect (Fig. 3) through the two fields. Every fifty steps, fifty tillers at the right foot of the person walking the transect were examined, and the number of mines and larvae were recorded. This was repeated twenty times in each field.

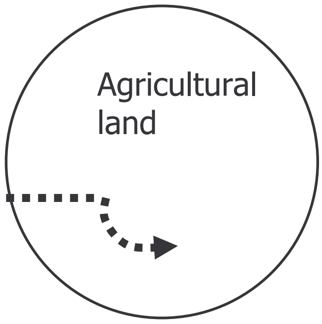

Figure 3. Transect that was walked through barley and wheat fields

2.2. Stages of development

Attempts were made to rear *A. ocularis* in the laboratory. During 2007 growth season, specimens were sampled in the field by clipping wheat and barley leaves containing larvae and transporting the material to the laboratory. The leaves were placed on damp cotton wool in plastic containers at room temperature (±25°C) until pupation set in. The adult flies hatching from these pupae were caged on wheat plants of different growth stages in the laboratory and plants were examined daily for mines and larvae.

Adult flies were also sampled in field A and field B during the 2008 season, placed in cages with sugar water and transported to the laboratory. They were then transferred to cages with wheat plants. Both these rearing attempts were unsuccessful and no larvae were found on the plants.

Due to these failures another method was implemented in order to obtain plants in which eggs had been deposited. In 2009 three pots containing single barley plants and three pots containing single wheat plants were placed in fields A and B respectively so that flies could deposit eggs in the leaves. After two weeks in the field the plants were removed from the field and placed inside netted cages under growth lights (Grow Lux®) with the photoperiod of 12L:12D and constant temperature of 25 °C. The plants were then examined daily in the morning and afternoon for larval activity. The period from the appearance of the first larvae through to adult eclosion from the pupae was recorded.

2.3 Diapause

Not all the pupae sampled from field A and B in 2007 for rearing purposes hatched successfully, with ±50% remaining in the pupal stage. These pupae were maintained in the laboratory on filter paper in a Petri-dish at 25°C. They were maintained in order to determine eventual eclosion, or to investigate the possible emergence of parasitoids.

A suction trap (Fig. 5) was set up between the barley field (field A) and the adjoining Gariep (Orange) River bed. The suction trap, 1,8 m high, consisted out of a metal cylinder containing a wire gauze funnel. Air was sucked through the cylinder by means of an electrical fan and all insects passing above the cylinder was sucked into the funnel and collected in a 500 ml sampling bottle, containing 70% ethanol and glycerol, at the end of the funnel. The suction trap was run 24 hours daily from 30 June 2008 to 28 November 2009. The sample bottles were replaced on a weekly basis and samples were analyzed for the presence *of A. ocularis* through the year.

Figure 5. Suction trap with cylinder funnel (left) and the plastic sample bottle with 70% ethanol and glycerol (right) (Photo: J. Adendorff, 2009).

2.3.1 Search for host plants and pupae

Sampling was conducted during 2008/09 at a third field site for potential alternate host plants during the off-season and to determine if the flies reside as pupae in the soil. This site, Remhoogte (S 29° 29.74′ E 23° 1.344′), was chosen because *A. ocularis* damage was first recorded here during 2000 with repeated outbreaks occurring through to 2009. At this site (Fig. 4), wheat is planted annually after residue from the previous maize crop has been burned.

Soil samples, to determine the presence and abundance of pupae in the soil, were taken. In field C (Fig. 4), samples were collected from each of the four sampling sites and in fields A & B in the Douglas area one sample was collected on ten different occasions between May and October in 2008 and 2009. A total six samples were taken inside wheat and barley fields and six outside these fields at each of the sampling occasions. A sample consisted of the first 5cm of top soil sampled over an area of 30 X 30cm. This soil was sifted and the number of pupae present per sample recorded.

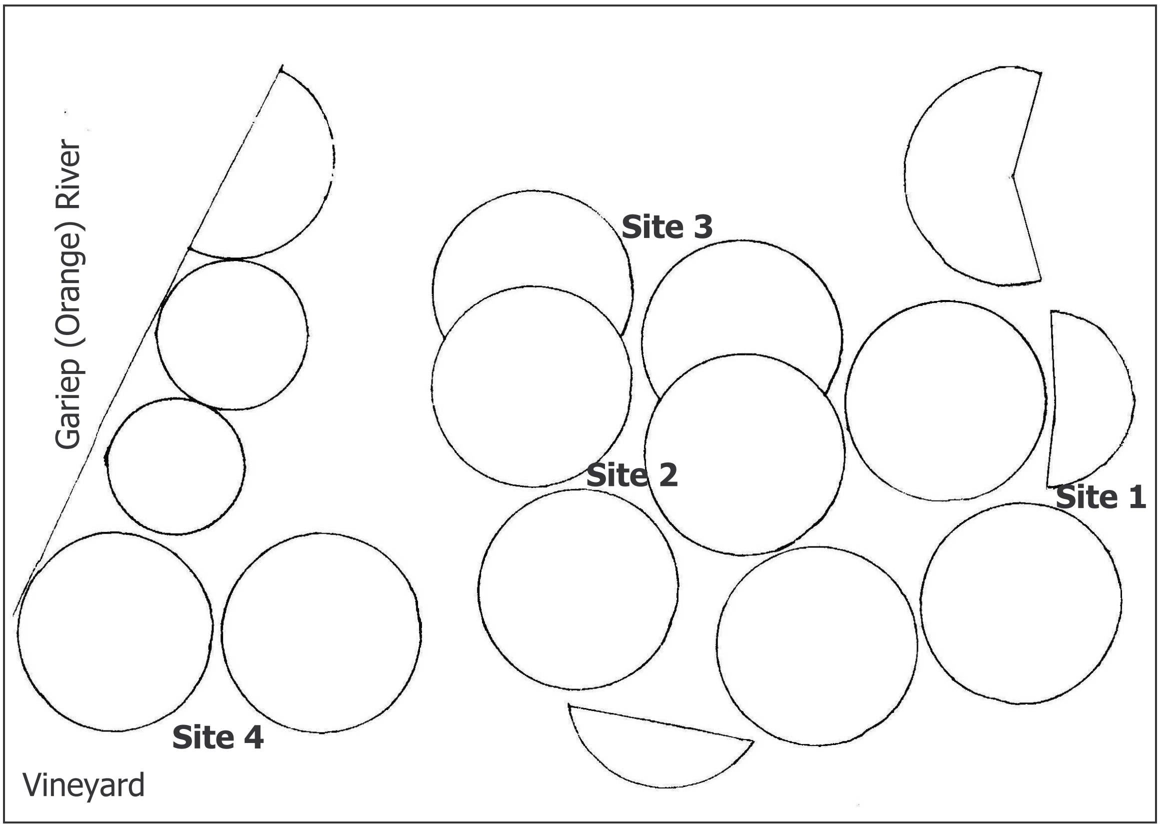

Figure 4. Map of Remhoogte indicating the four survey sites of active searching and sweeping, sampled during 2008/09. The circles are the pivot outlines where wheat is planted under irrigation.

2.4 Potential host range

During the summer months, maize fields and green natural grasses ± 500 m around these fields were inspected for larvae in the leaves. This was conducted at the three localities (field A, field B and field C). These inspections were conducted on a monthly basis from May till November 2008/09 in order to determine possible alternative host plants. The alternative host plants recorded was identified according to van Oudtshoorn (2006).

2.5 Data analysis

Data was analyzed by GraphPad software (GraphPad Software 1998). Comparisons were made between the number of larvae in different growth stages of wheat and barley by applying the chi-square goodness-of-fit test (5% significance level). Data from each year were analyzed separately.

3. Results and discussion

3.1 Population growth

The number of larvae differed significantly between growth stages of wheat in 2008 (Chi-square=9.509; P<0.01). The high numbers of larvae at GS 5 and 17 could be an indication of two successive generations that occur in these fields (Fig. 6).

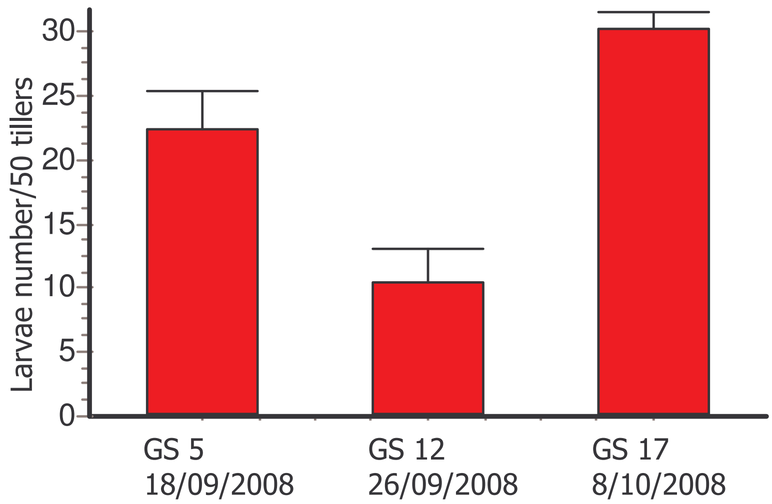

Figure 6. Mean number of larvae per 50 tillers (±se) sampled on wheat in the Douglas area in 2008.

During 2008 the number of larvae present at the different growth stages in barley differed significantly (Chi-square=5.45; P<0.01) (Fig. 7). Here, once again, high numbers were present at GS 5 and 17 which could be an indication of two successive generations.

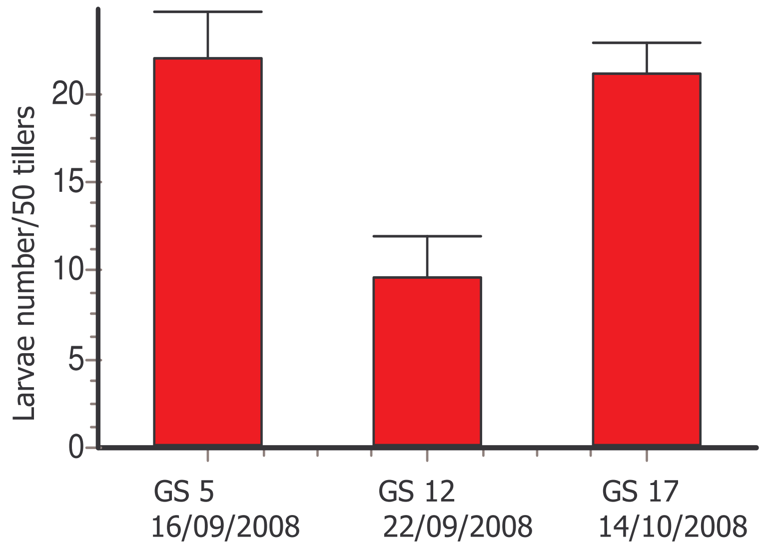

Figure 7. Mean number of larvae per 50 tillers (±se) sampled on barley in the Douglas area in 2008.

The population growth of *A. ocularis* is rapid with high larval numbers present within a month following the first appearance of the flies (Figs 6 & 7). It is highly likely that two generations occurred on both wheat and barley in 2008. Mean daily temperatures during the occurrence of the flies ranged between 10ºC and 27ºC but could not be correlated with the different generations (Fig. 8).

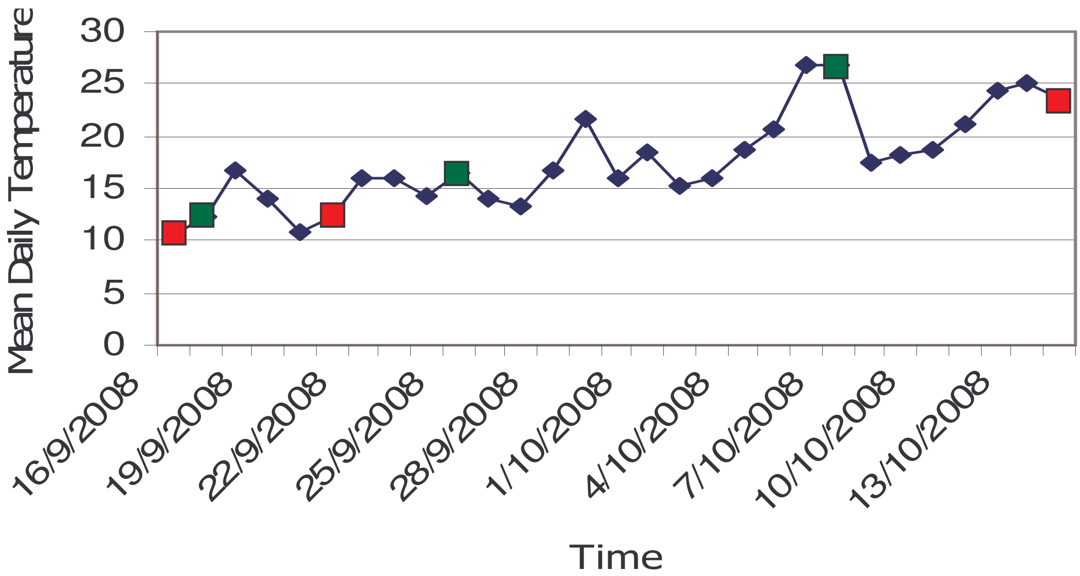

Figure 8. Mean daily temperature from 16/9/2008 to 13/10/2008.
(■= barley field inspection) (■= wheat field inspection).

During 2009 in both the wheat and barley fields there was a significant difference between the numbers of larvae present. A different between growth stages 11 and 15; 15 and 20 in wheat (Chi-square=16.591; $P < 0.001$) and growth stages 11 and 20; 15 and 20 in barley (Chi-square=26.321; $P>0.001$) (Figs 9 & 10). In wheat larval numbers were low at GS 11 and 20 which could be and indication that only one generation occurred, while in barley high numbers occurred only at GS 20 again indicating the presence of only one generation (Figs 9 & 10).

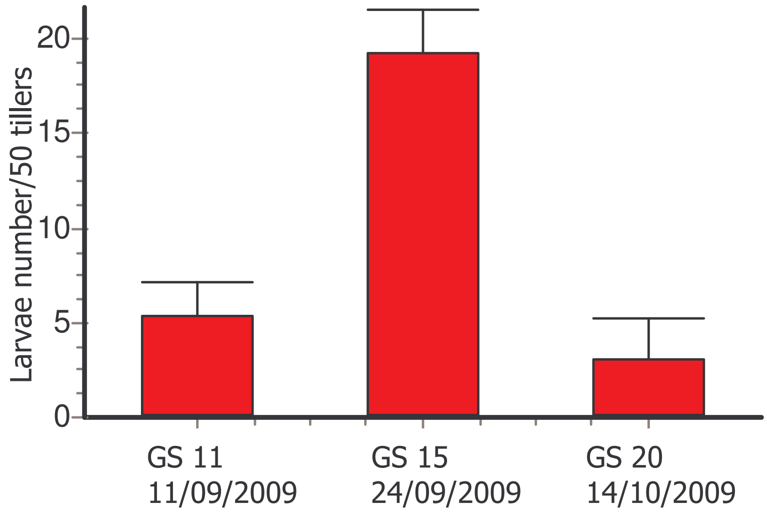

Figure 9. Mean number of larvae per 50 tillers (±se) sampled on wheat in the Douglas area in 2009.

The reason for the presence of only one generation in 2009 (Figs 9 & 10) could be because the flies emerged two weeks later than in 2008. Again no correlation could be made between temperature and increase population size in 2009 (Fig. 11). Evidently more population dynamics and weather data is needed before any patterns on the establishment and growth of the population can be made.

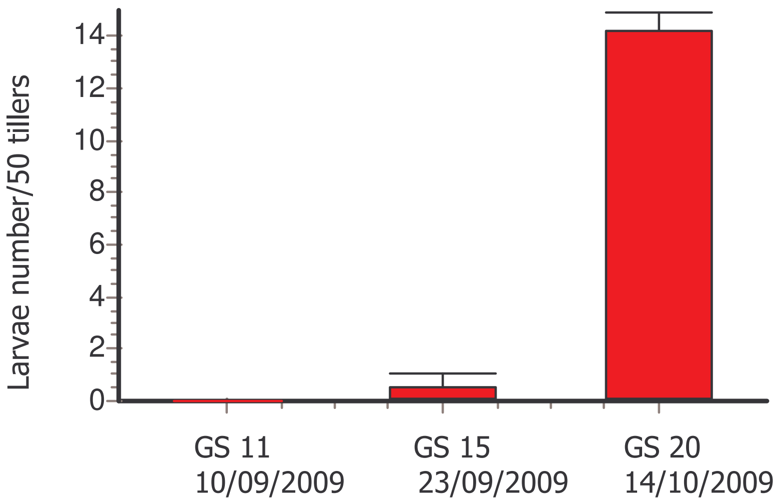

Figure 10. Mean number of larvae per 50 tillers (±se) sampled on barley in the Douglas area in 2009.

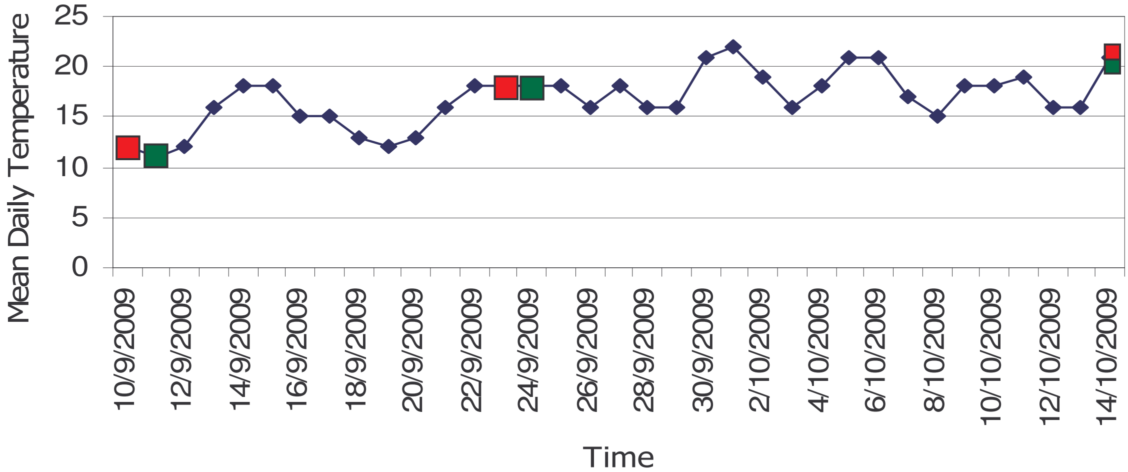

Figure 11. Mean daily temperature from 16/9/2008 to 13/10/2008 (■= barley field inspection) (■= wheat field inspection).

Dense plant growth which block off the sunlight (such as you find in crop monocultures) might have an influence on the population growth of *A. ocularis*. Wheat in this particular field was planted in rows with ± 30 cm inter row spacing, which allowed sunlight to reach the larva and flies. Barley, however, is densely sown with very little spacing between plants which creates a dense canopy that does not allow much sunlight through to the soil surface. Denser barley canopies are a benefit against heat. However lower temperatures in the barley canopy foliage might stunt the growth of larvae.

Flies in general are r-selected strategists that rapidly produce offspring and therefore those that develop on plants can rapidly colonize agroecosystems (Norris *et al.* 2003). This study showed a carrying capacity can be reached resulting in S curve, while the insect utilizes all available resources. This can lead to high economic yield loss.

3.2 Stages of development

According to Prinsloo (2001) there are four stages in the *A. ocularis* lifecycle *i.e.* egg, larva, pupa and adult fly. The development time from the first mining activity until adult eclosion is 27 days and the longevity of the adult was 30 days at 25°C (Table 1). The pre-and post-oviposition period was however not determined and the lifecycle time from egg to egg is not known.

Table 1. Preliminary lifecycle of *Agromyza ocularis* on wheat and barley at 25°C in the laboratory.

Stage	N	Mean duration of days (±se)
Larval - pupae	17	4 (0.4926)
Pupae – adult	10	23 (1.476)
Adult	10	30 (1.054)
Total		57

The female fly has an elongated ovipositor with which the egg is deposited into the leaf tissue (Needham *et al.* 1928). Only one egg is laid in the puncture where the ovipositor is inserted (Fig. 12), but many eggs can be laid in a leaf. Leaves were dissected and eggs were recorded to be a translucent white colour. They are, however, very difficult to spot without dissecting the leaf. According to Prinsloo (2001) the egg are mostly laid at the apex of the leaf, but, as observed, eggs can also be laid anywhere along the leaf sheath. Due to difficulty in determining when an egg was laid, it was not possible to determine the exact duration from egg laying to larval hatching.

Figure 12. Adult female fly puncturing the leaf surface with her ovipositor (Photo: B. Muller, 2007)

Development duration from larvae to pupae was four days at 25°C (Table 1). When the egg hatch the larva immediately starts feeding on the mesophyll (Scheirs *et al.* 2001). The larva is cylindrical in shape, vermiform, legless and yellow in colour (Fig. 13).

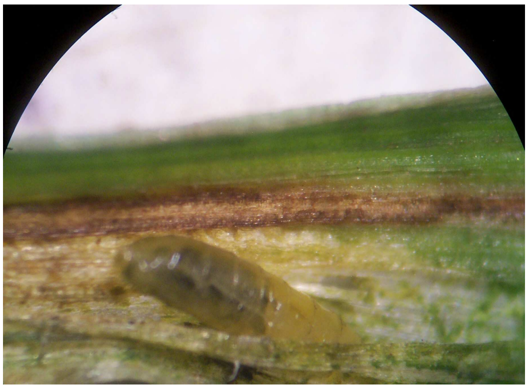

Figure 13. A larva emerging from the leaf (Photo: J. Adendorff, 2008).

The larvae tunnel feeds continuously in the leaf, whilst moving forward. This behaviour that creates straight and narrow mines are common for larvae mining in grasses (Fig. 14) and is in contrast to the blotch-like mines of leaf miner larvae in broad leaves (Stiling & Strong 1981). The larva usually feed linear towards the apex or downwards towards the leaf stem. After continued feeding the mines can become a single yellow blotch (Fig. 14). Several larvae can feed together in a single leaf (Fig. 15).

Figure 14. Wheat leaf with a straight narrow mine becoming a single yellow blotch (Photo: J. Adendorff, 2009).

Figure 15. Single wheat leaf with three larvae feeding in the mines (Photo: J. Adendorff, 2009).

During larval feeding activity, the upper epidermal layer of the mine elevated and the larva can be observed with the naked eye, or felt by sliding the leaf between the fingers (Fig. 16).

Figure 16. A larva observed in the mine (Photo: G. Prinsloo, 2006).

Larvae cannot move around between leaves and therefore stay in a single leaf to complete their larval cycle. The larva does not pupate inside the leaf as is the case of the most leaf miner species, but does so away from the leaf in the soil. The mature larva cuts a slit in the upper surface of the mined leaf, emerges onto the leaf surface, drops from the plant, burrows into the soil and pupates.

The pupation period can last from 20-24 days at 25°C (Table 1). If an adult fly does not eclose within two months, it could possibly have entered into diapause. This happened with seven of the pupae during the study that stayed in diapause for a year. Diptera pupa is typically coarctate, with the cuticle of the final larval instar forming a hardened exterior 'shell' (Borror *et al*. 1989). This is also the case with *A. ocularis*, whose pupae are light brown and approximately 2 mm in length (Fig. 17).

Figure 17. The coarctate pupa of *Agromyza ocularis* (Photo: J. Adendorff, 2009).

The adult leaf miner (Fig. 18) is a small black fly with clear shiny wings (Spencer 1961). An adult fly can live for about 30 days (Table 1). The adult can therefore pierce a lot of leaves of the host plant and can oviposit many eggs over an extended period resulting in overlapping generations.

Figure 18. Adults of *Agromyza ocularis* (Photo: T. Viljoen, 2008 (left); J. Adendorff, 2009 (right)).

3.3 Diapause

The pupae that were retained because they did not eclose in 2007 showed extended diapause for a year until July 2008 under controlled laboratory conditions. Based on these results diapause does occur in *A. ocularis*. It can be concluded that adults eclosing from diapausing pupae starts the new population when host plants are present in late winter and spring of the following wheat growth season. Studies have shown that leaf miners usually overwinter in the pupal stage (Chen & Kang 2005).

Samples from the extensive suction trap survey yielded *A. ocularis* (Fig. 19) only on 25 August 2008, 1 September 2008, 15 September 2008 and 27 October 2008. In 2009 a single specimen was recorded on 11 May 2009 and its presence could not be explained. The suction trap did not contain the number of specimens that was expected, even during the outbreak season. The reason could be that the insects are very small and are mostly localized in the immediate vicinity of the plants they feed on. They probably, therefore, do not fly around at heights that are higher than their host plants and they shelter among the leaves. This could imply that they will be missed by the suction trap that stands above the plants.

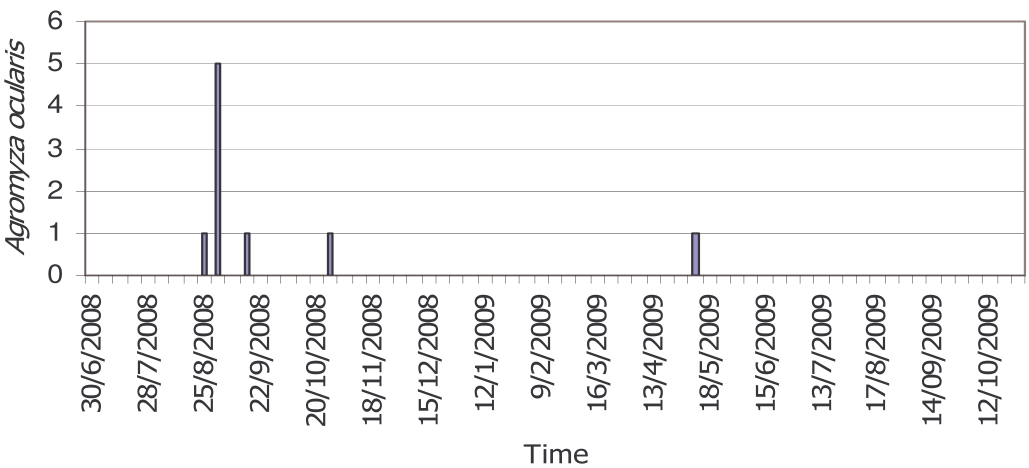

Figure 19. Quantitative record of *A. ocularis* specimens suction trapped from 30 June 2008 to 26 October 2009 in the Douglas area.

3.3.1 Search for host plants and pupae

The sifted soil taken from inside the agricultural fields and in the surrounding natural grass veld in 2008 did not contain any pupae. However, in 2009 the soil samples were taken directly next to the plants in which leaf mining activities were present instead of between the plants in the rows as in 2008. Due to this alteration concerning the soil sampling methods pupae were recorded in the planted fields after completion of the first generation (Table 2.). Nothing was recorded in the surrounding natural grass. The pupae were also only recorded during the wheat and barley season with no pupae recorded during the off-season.

Pupa were recorded in the soil shortly after the life stage of the fly was completed on the plant, clearly indicating that the larvae do not move far from their host plant to pupate. This soil was also very wet suggesting that larval survival prior to pupation is dependent on moist soil conditions. This could also be the reason why no pupae could be found in the natural grass veld during 2008/09 where much drier soil conditions prevailed and where the grass tussocks were spread quite a vast distance apart from one another. This suggests that the adult flies and pupa are probably more concentrated in the agricultural fields where more favorable environmental conditions are present.

Table 2. Number of pupae recorded from soil samples from wheat and barley plants at the different growth stages in the Douglas area in 2009.

Growth stages 2009	**Field A Barley**	**Field B Wheat**	**Field C Wheat**
11	3	1	0
15	20	16	6
20	18	33	10

3.4 Alternative host range

Besides wheat and barley, *A. ocularis* were also recorded to utilize few grass species under cultivated conditions. These included *Phalaris minor* (small canary grass), *Bromus catharticus* (rescue grass), *Lolium perenne* (perennial rye grass) and *Avena fatua* (common wild oats) (Fig. 20). This alternative host range could only be established during the wheat growing season and are all Poaceae species, indicating an oligophagous trophic strategy for the leaf miner. No larvae or adults were recorded during the wheat off-season even though some of the other plant species were present. The range of hosts that were recorded was not present in the natural veld and is commonly available under irrigated conditions in the agricultural fields of the Northern Cape.

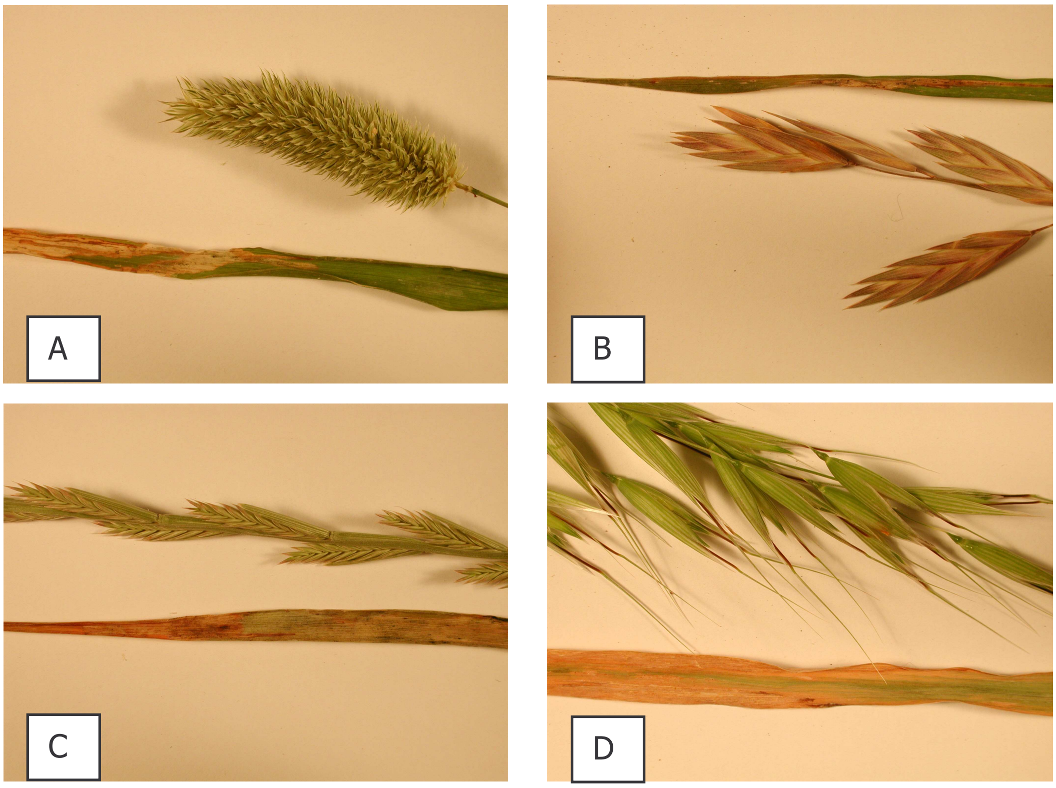

Figure 20. Natural grasses that were recorded in the study area a. *Phalaris minor* (small canary grass), b. *Bromus catharticus* (rescue grass), c. *Lolium perenne* (perennial rye grass) and d. *Avena fatua* (common wild oats) (Photos: J. Adendorff, 2009).

Small canary grass (Fig. 20 a) is a problem weed in the grain crop pivots of the Northern Cape. It grows in disturbed places, but seldom in the veld. It flowers from September to January (van Oudtshoorn 2006). Rescue grass (Fig. 20 b) also occurs as a weed in winter wheat areas, but not in natural grazing areas. It does occur naturally along rivers. This species flowers from October to April (van Oudtshoorn 2006).

The perennial rye grass (Fig. 20 c) is a winter grass species that flowers from October to December and grows under irrigated conditions in virtually any soil type and can also establish as a weed in irrigated wheat (van Oudtshoorn 2006). Common wild oats (Fig 20 d.) has become a serious weed problem in the Northern Cape where winter wheat is cultivated.

All alternative host plants that could be positively determined were present from September to November, which implies that there is no food available for *A. ocularis* during the off-season, which could be one of the reasons why *A. ocularis* has a dormant period.

4. Conclusion

There was no correlation between the rapid population increase of *A. ocularis* and temperature. The survey in 2008 indicated that more than one generation per season could possibly occur under field conditions. This might be related to early in the season adult eclosion which could be trigged by a combination of temperature and relative humidity.

The life cycle of *A. ocularis* can last for at least two months, which could result in damage to the crop over an extended period. A period of diapause does exist, but the exact stimuli for this condition to occur are not known. By being able to go into diapauses the fly could survive periods of low food availability during the off-season in the field. The only possible alternate host plants that could be found were mostly growing during the wheat/barley growth season.

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CHAPTER 4

DAMAGE FREQUENCY BY *AGROMYZA OCULARIS* IN WHEAT AND BARLEY FIELDS

1. Introduction

In the year 2000, *Agromyza ocularis* Spencer, an indigenous leaf miner fly, was reported from two irrigated wheat fields in the Prieska district, while an infestation was also recorded in one field in the Eastern Free State (Prinsloo 2001). This fly causes two distinct types of injury to wheat and barley plants, *i.e.* feeding punctures and leaf mining. The focus of this study will be on the larval activity of this fly.

1.1 Feeding and oviposition punctures

Feeding punctures are induced by the adult female ovipositor through the upper leaf surface (Parrella *et al.* 1985) causing a stippling appearance on the leaf (Fig. 1a). Both adult flies (male and female) then feeds on the fluids leaking from the wounds (Marino *et al.* 1993).

Figure 1 a. Feeding punctures of adult female leaf miner fly; b. A larva tunnelling in a leave. (Photos: J. Adendorff 2009).

The effects of the feeding punctures on the leaf surfaces of a host plant that are caused by the ovipositioning activities of the female can also reduce photosynthetic and mesophyll conductance processes up to 75%, which, in turn, can result in significant yield loss (Parrella *et al.* 1985).

1.2 Leaf mining damage

Most damage to the leaves is caused by the larvae (Fig. 1b) that tunnel feed between the upper and lower surfaces of the leaf (Xu *et al*. 2007). Leaf mining may damage large proportions of a leaf which can cause wilting of succulent leaves. The plant is unable to compensate and this will eventually result in a yield decrease (Xu *et al*. 2007). As already mentioned grass leaf miner larvae feed mostly on the mesophyll and a food quality comparison of the damaged and undamaged leaf parts showed that the nutrient content is indeed lower in the mined leaf parts, indicating that the selective feeding of the larvae remove the most nutritious tissues (Kimmer & Potter 1987; Scheirs *et al*. 2001).

According to Iwasaki (1999) the estimated yield loss can be between 12% and 16% for spring and winter wheat with artificial defoliation. In Poland, Agromyzidae larval damage in cereal crops causes reduction in the number of grains and so called '1000 kernel weight' (Walczak 1995). Massor (1989) reported that in barley maximum larval abundance can occur at plant growth stage (GS) 21 to GS 23 (translated to Joubert scale). Alternatively he found two peaks on wheat, firstly at the end of GS 15, and the secondly at GS 21 (Massor 1989). *Agromyza megalopsis* (Hering) caused a yield decrease of 26.8% in the barley fields of Bulgaria (Khristov 2000).

1.3 Aim of this study

Since *A. ocularis* was only reported to attack cereal crops in South Africa since 2000, the extent of the damage caused by this leaf miner fly has not yet been quantified. The aim of this study is to describe and quantify the damage that can be caused by the larval stage of this grass leaf miner on wheat and barley crops under field conditions. The following questions were posed:

- What percentage of plants are damaged per season?
- What number of tillers are damaged per plant?
- Which leaves of a plant are damaged first and does progressing damage occur?
- What are the number of leaves damaged per tiller?
- What is the percentage of leaf area damaged?

2. Material and methods

2.1 The study area

Field observations to determine the different aspects of damage on wheat and barley were conducted in two different fields in 2008 and 2009. Field A (Gwerna S 29° 21.090′, E O 23° 08.589′) and field B (Nuwejaarskraal S 29° 23.782′, E O 23° 08.613) were planted with barley and wheat respectively (Fig. 2). Both fields are irrigated by centre pivot systems and two consecutive crops are produced annually. During summer maize was planted between 1 December and 20 December. After harvesting the maize in June, the stubble on field B was burned and the field disked, while stubble on field A was left on the surface, superficially tilled and planted with barley and field B with wheat. No herbicides, fungicides or insecticides were sprayed on these fields during the 2008/09 season. *A. ocularis* is more abundant than other insects in the wheat and barley irrigation fields of this area ([6]André Coetzee, personal communication).

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[6] André Coetzee, GWK (Pty) Ltd. in Douglas, Northern Cape, South Africa.

Figure 2. Aerial photo of the Northern Cape region with pin points of experimental fields A (Gwerna) and B (Nuwejaarskraal) (Google Earth 15/03/2010).

2.2 Leaf miner damage

Agromyza ocularis activity was first noticed on 14 August 2008 and 26 August 2009. Fields were monitored for *Agromyza ocularis* at GS 5, 12 and 17 in 2008 and at GS 11, 15 and 20 in 2009.

During observations in 2008 and 2009 the plant growth stages (Fig. 3) were documented and 50 plants were randomly selected, dug out, bagged in the field and examined in the laboratory the same day. The following datasets were recorded:

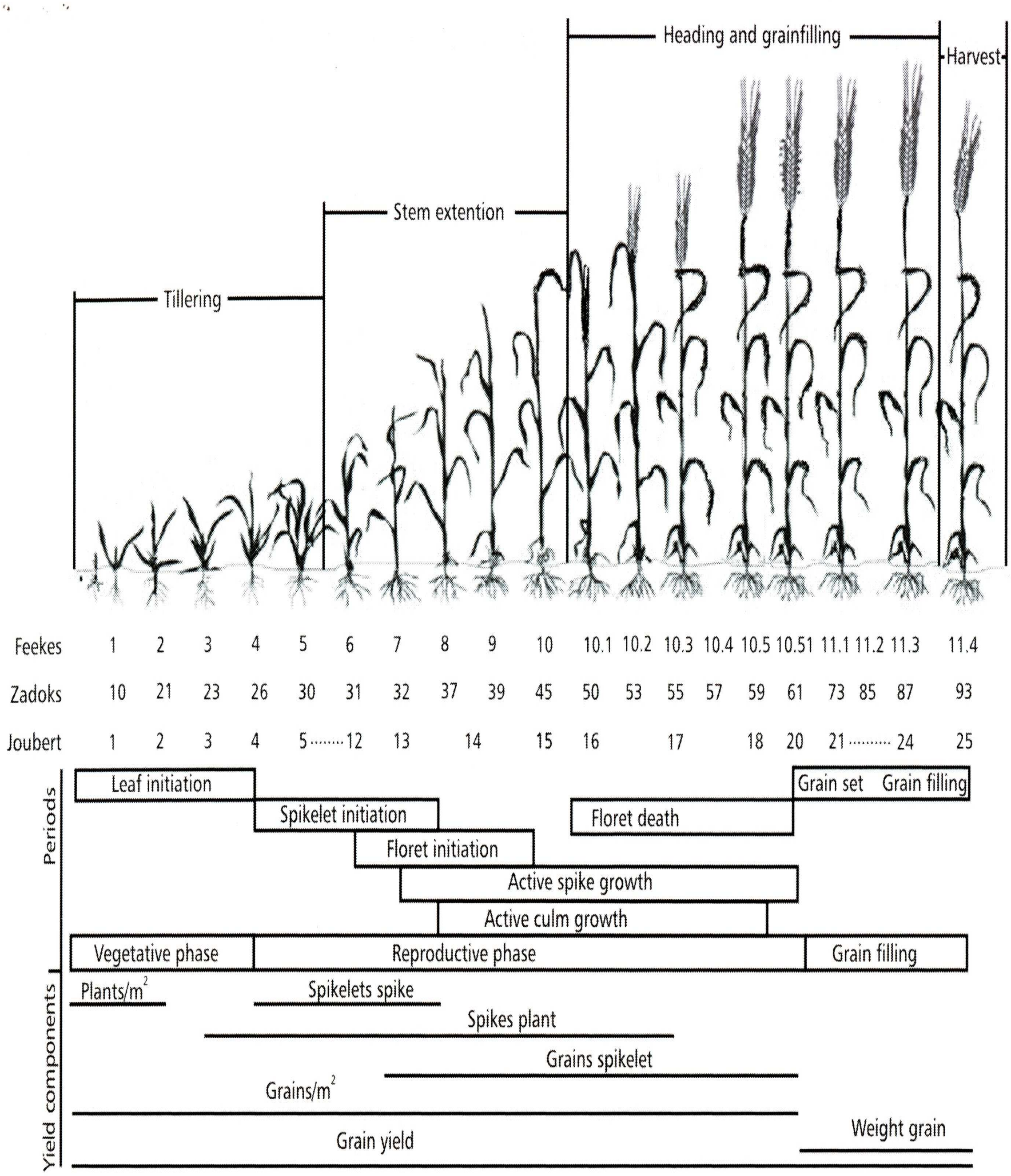

Figure 3. Different growth stage scales of wheat (ARC-Small Grain Institute 2008).

- Percentage of plants damaged.
- The total number of tillers per plant were quantified and noted, as were the number of damaged tillers. From this data the percentage of damaged tillers per plant was calculated. To get a picture of the severity of damaged tillers, the data of both the barley and wheat plants were then

pooled for all the growth stages and ranked into percentage damage categories ranging between 0 and 100% at 10% increments.

- The damage pattern on a tiller was described by quantifying the number of leaves and numbering these by starting from the oldest leaf at the bottom and continuing upwards on the plant (Fig. 4). From this the percentage feeding damage per numbered leaf was subsequently determined. This was done by determining the number of mines present per leaf. Each damaged leaf was then documented and the number of mines present on each leave recorded. The damaged leaves were also ranked according to a damage category, ranging between 0 % and 100% at 10% increments in order to determine the percentage of leaves damaged per tiller.

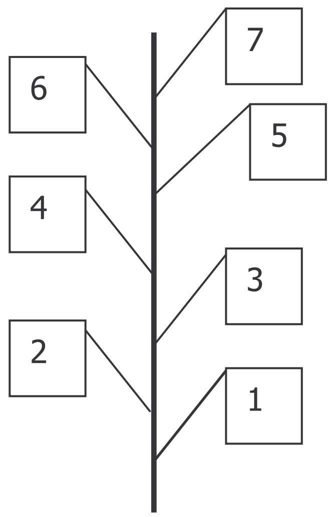

Figure 4. The numbering system for the leaves on a barley or wheat tiller.

- The length of both a wheat and barley leaf was on average 24 cm. Based on this, leaves were then rated from 0 % (no damage) to 100 % (total leaf area damage). Ultimately damage indices determined at 10% increments form 0% to 100% were determined and noted.

2.3 Data analysis

Data was analyzed by using GraphPad software (GraphPad Software 1998). Data from each year was analyzed separately. Comparisons were made by means of the chi-square goodness-of-fit test (5% significance) between the wheat and barley growth stages of the number of tillers damaged per plant, and the percentage of leaf damage per plant.

3. Results and discussion

3.1. Percentage of plants damaged

In 2008, all the randomly selected plants had leaf miner damage (Table 1). During this season the leaf miner was abundant and widely distributed throughout both the wheat and barley fields.

Table 1. The percentage plants damaged by the grass leaf miner in 2008 and 2009 during the different growth stages of barley and wheat in the Douglas area.

Percentage of plants damaged		
Plant type	**Growth stage**	**2008**
Wheat	5	100%
	12	100%
	17	100%
Barley	5	100%
	12	100%
	17	100%
Plant type	**Growth stage**	**2009**
Wheat	11	52%
	15	78%
	20	84%
Barley	11	22%
	15	54%
	20	96%

In 2009, the leaf miner damage gradually increased throughout the season, but fewer plants were damaged than in 2008 (Table 1). The flies emerged about two weeks later in 2009 than the previous year and reached pest status at a later growth stage. There were no significant temperature (Fig. 5) peaks during this period that could be correlated with the emegence of the insects. It therefore seems that soil temperature could be more important in triggering emergence of the insects than plant growth stages and atmospheric temperature.

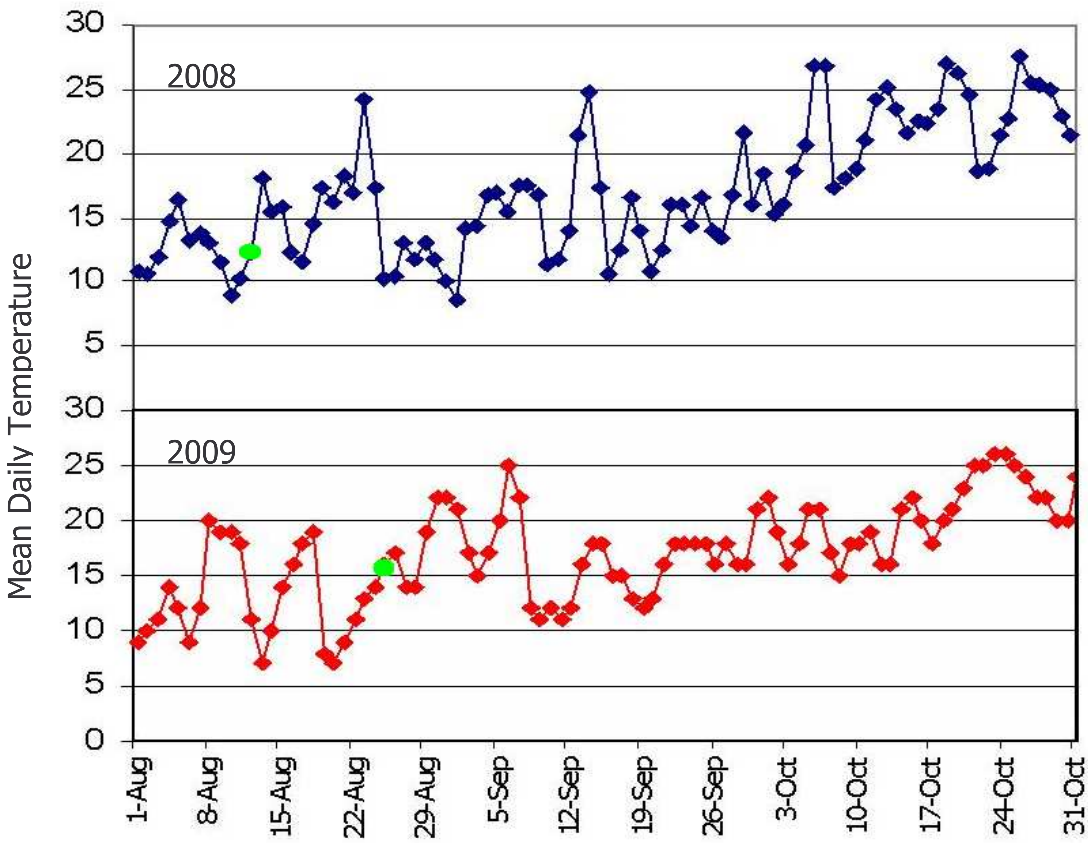

Figure 5. Mean daily temperature from August to October in 2008 and 2009 in the Douglas area, indicating 14 August 2008 and 26 August 2009 when *A. ocularis* appeared (●).

Although all the recorded plants were not damaged (100% Infection) in 2009, the number of plants damaged increased at later plant growth stages. Therefore the intensity of damage per plant was categorized in order to determine how individual plants are damaged and have to compensate for damage.

3.2 Number of tillers damaged per plant

In both 2008 and 2009 the percentage tillers per plant that were damaged were less on the barley than on the wheat (Fig. 6). This could probably be due to macro and micro environmental differences between the sites and the different cultivation practices. In 2008 there were no significant differences between growth stages of wheat in terms of the percentage damaged tillers per plant (Chi-Square=2.412; P=0.299). In barley significant differences occurred between growth stages (Chi-Square=36.41; P<0.001). During 2009 there was significant differences in the percentage damaged tillers between growth stages for both wheat (Chi-Square=9.642; P<0.001) and barley (Chi-Square=35.32; P< 0.001).

In 2009 there was a gradual increase in mine activity in the wheat as expected because of the late arrival of the flies. In barley during both 2008 and 2009, the leaf mining activity was low at the beginning of the season with a sudden peak towards the end of the season. This can probably be a result of environmental differences and cultivation practices. Barley is sown with very little spacing between plants and therefore the soil might be screened from the sun during the early growth stages by the plant canopy. Wheat in this field was planted in rows with ± 30 cm inter row spacing, which allowed sunlight to reach the larva and flies. The heat of the sun can therefore penetrate through the plant canopy to the soil. It is therefore argued that soil temperature could be a factor that triggers leaf miner emergence and need further investigation.

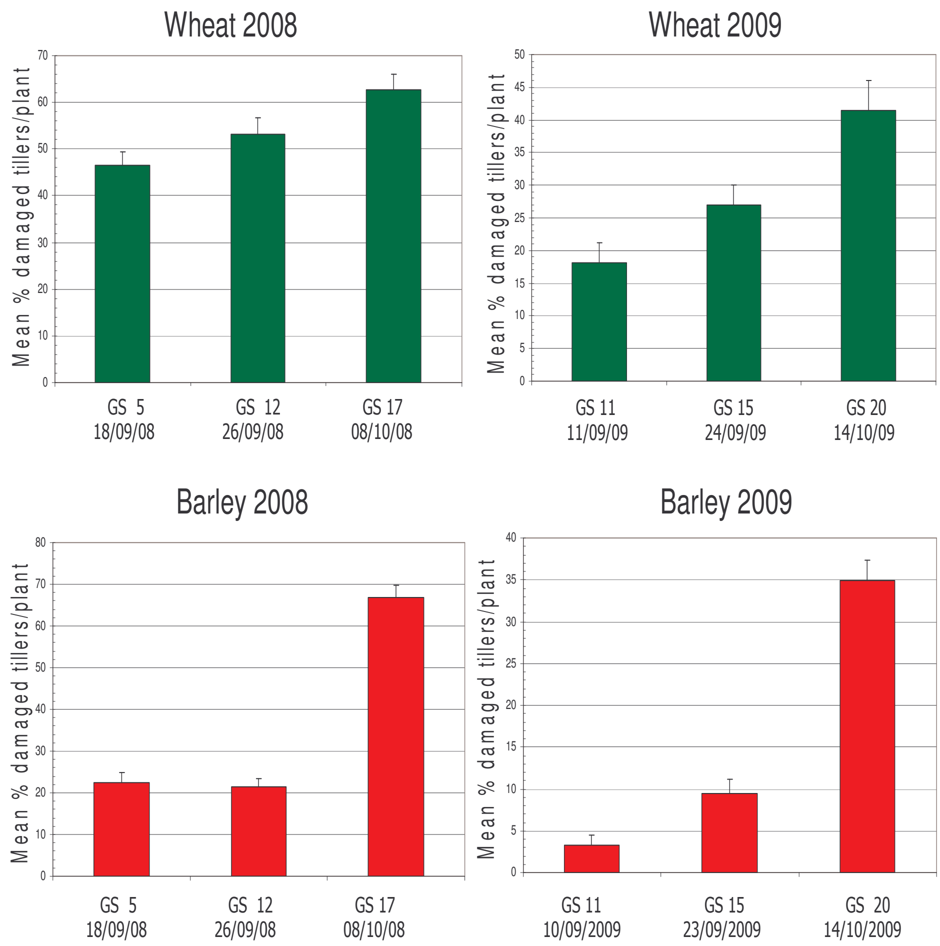

Figure 6. **Mean percentage of infested tillers (± se) per plant for wheat and barley in the Douglas area during 2008 and 2009.**

In 2008, the damage categories with the highest number of tillers attacked per plant were 11% – 20%, 31% - 40%, or 41% - 50% (Table 2). Calculations based on this data showed that the average percentage of tillers damaged per plant was 45%. This implies that whilst practically half of a plant's tillers were attacked throughout the season, the remaining tillers had to compensate for the loss of photosynthesis and the translocation of nutrients and water.

In 2009 only 23% of the tillers per plant were infested by larvae, the lowest damage category was recorded for the most of the tillers, indicating that damage was very severe in 2008. This could be an indication that the fly population in this area is stabilizing and mining injury is becoming less intense or that the late in the season arrival of the flies had an influence.

Table 2. Total number of plants, with tillers attacked per plant according to a damage sliding scale, in the Douglas area in 2008 and 2009.

	Number of plants per season	
Damage category	**2008**	**2009**
0 – 10	26	122
11 - 20	50	39
21 - 30	27	45
31 - 40	40	39
41 - 50	45	29
51 - 60	25	9
61 - 70	25	3
71 - 80	32	5
81 - 90	10	0
91 - 100	20	9
Mean % leaves attacked	45%	23%

As mentioned the number of tillers attacked was much less in 2009 and the plants were attacked later in the season. The question is, if the flies arrive later in the season do they still attack the older leaves at the bottom of the plant or does leaf age have no significance in adult plants?

3.3 Leaf damage increase on wheat and barley

Leaf mining began on the lower leaves of the young plant and, as new leaves are formed, the new leaves were attacked upwards towards the growth apex (Table 3). This was also found for other leaf miner species (Dove 1985, Godfray 1985, Videla & Valladares 2007). The reason could be that nutrient flux and water content of young leaves will be more favourable for insect nutrition (Godfray 1985). Whilst this is an important factor, feeding directly relates to reproduction and therefore, according to Videla & Valladares

(2007), the ultimate reason that lower leaves are attacked is because the female prefers the older, sturdier leaves for oviposition.

Table 3. Leaf damage progression on wheat and barley, in the Douglas area in 2008 and2009, based on the leaf numbering system in Fig. 4.

				Wheat				
% of	**7**	-	-	0.20	**7**	-	-	
leaf	**5 & 6**	-	-	4	**5 & 6**	-	-	1
number	**3 & 4**	12	13	31	**3 & 4**	0	8	25
attacked	**1 & 2**	88	87	65	**1 & 2**	100	92	75
	2008	**GS 5**	**GS 12**	**GS 17**	**2009**	**GS 11**	**GS 15**	**GS 20**
				Barley				
% of	**7**	**-**	**-**	0.30	**7**	-	-	0.60
leaf	**5 & 6**	**-**	**-**	5	**5 & 6**	-	-	20
number	**3 & 4**			27	**3 & 4**	0	8	57
attacked	**1 & 2**	100	100	68	**1 & 2**	100	92	23
	2008	**GS 5**	**GS 12**	**GS 17**	**2009**	**GS 11**	**GS 15**	**GS 20**

In 2008 the leaf miner was detected two weeks earlier than during 2009 and therefore the plant was attacked at a younger growth stage. Grasses can usually withstand greater defoliation during the early, rapid growth stages, but ultimately limits the plant later in the season when reproduction structures are more important and therefore yield can be affected (Trlica 2006). In 2009 the damage increased faster towards the upper leaves on the plant, but still commenced on the lower leaves and spread upwards. This increase of damage may be an indication that the adult flies stays inside the plant canopy and attack younger growth after the leaf emergence.

The flag leaf was affected in GS 17 and GS 20. Since flag leaves are important in the translocation of food to the developing grain, damage to these leaves could depress the yield (Duthoit 1968). Although all the leaves (as numbered in Fig.4) can be attacked on a tiller, the question was how many leaves per tiller in the 2008 and 2009 seasons were attacked?

3.4. Number of leaves damaged per plant

Wheat leaf damage in 2008 was again more than on barley (Figs 7 & 8), as expected from the results in 3.2. The damage to wheat leaves was similar in GS 5 and GS 12 (Fig. 7). Only in GS 17 did the damage increase and there was a significant difference (Chi-square=9.505; P<0.01) between this growth stages and the rest (Fig. 7).

The leaf damage to barley (Fig. 7) was significantly lower at GS 12 than at GS 5 and GS 17 (Chi-square=5.557; P<0.01). This could indicate that a second generation of flies invaded the fields after GS12, more eggs were laid and damage increased on the leaves

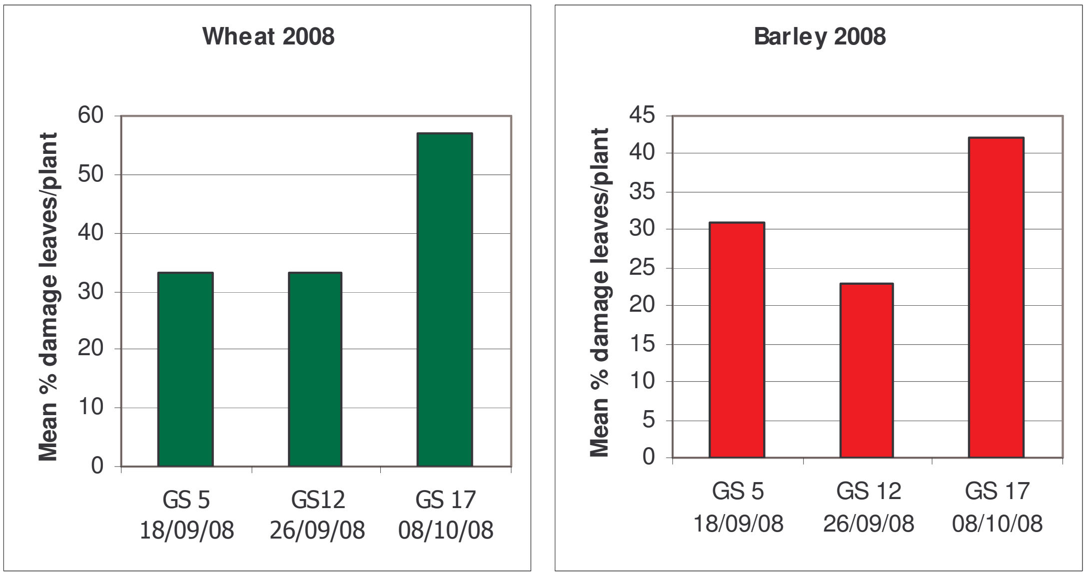

Figure 7. Mean percentage infested leaves per plant for wheat and barley in the Douglas area during 2008.

In 2009 there was a significant increase in the number of mines per leaf from GS 11 - 20 in both wheat (Chi-square=7.282; P<0.01) and barley (Chi-square=8.918; P<0.01) (Fig. 8). As mentioned the flies arrived later in the season in 2009.

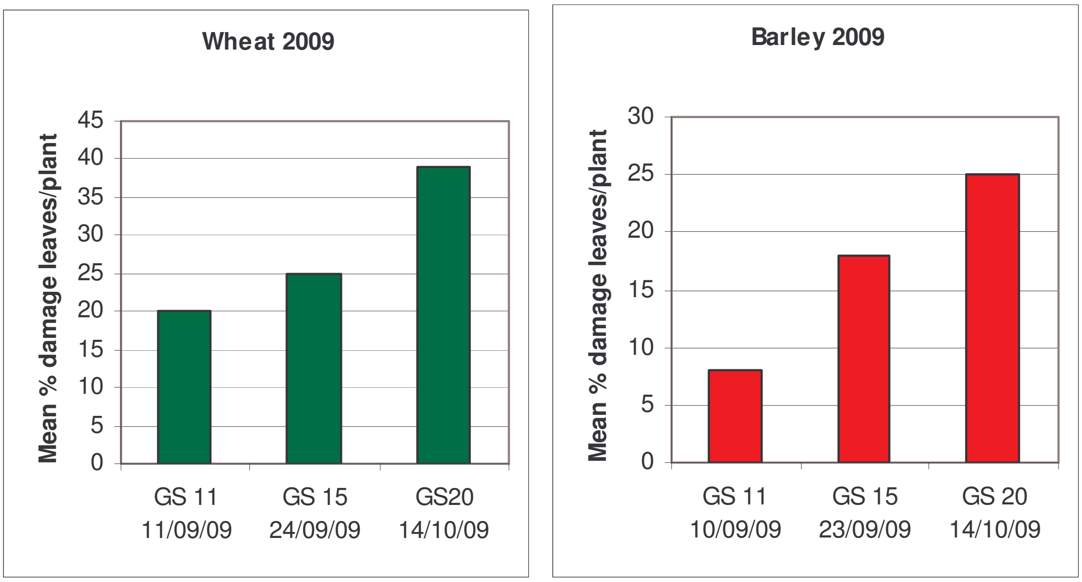

Figure 8. Mean percentage infested leaves per plant for wheat and barley in the Douglas area during 2009.

In 2008 the pooled leaves of wheat and barley had 37% damage per tiller (Table 4), whilst 25% of leaves were infested in 2009 (Table 4). According to the damage categories most of the tillers had between 10% and 50% damaged leaves in 2008. In 2009 most of the tillers had between 0% and 40% damaged leaves, again showing that the number of leaves damaged were less in 2009 than in 2008. Photosynthesis is important for the production of carbohydrates for plant growth and maintenance (Trlica 2006). Since the chlorophyll in part of the leaf is destroyed during larval feeding, part of the plants' photosynthesis production is destroyed.

Table 4. Percentage leaves attacked (of both plants pooled), according to, damage categories, in the Douglas area in 2008 and 2009.

	The total number of tillers per 300 plants (barley & wheat combined).	
% leaves damaged per tiller	**2008**	**2009**
0 to 10	3	107
11 to 20	176	136
21 to 30	329	134
31 to 40	131	91
41 to 50	262	74
51 to 60	58	14
61 to 70	53	6
71 to 80	39	4
81 to 90	3	2
91 to 100	5	1
Mean % leaves damaged	37%	25%

If a plant can no longer produce enough carbohydrates for its own needs, growth and reproduction and eventually yield can be affected (Trlica 2006). In order to proactively calculate this, leaf area damaged has to be determined.

3.5. Percentage leaf area damaged

In 2008, 100% of the leaves in wheat and barley were damage with the number of leaves per damage category spread over the range from 10% – 100% (Table 5).

At GS 5, 51% of the mean wheat leaf area was damaged and this increased to 59% at GS 17 (Fig 9). However the number of leaves per damage category shows a sharp increase in the 100% category during GS 17 (Table 5). It is thus clear that a definite increase in the degree of leaf area damage was recorded in wheat (Table 5). At GS 5 the percentage damage is relatively low, but as the plant is using its energy during this growth stage to establish the number of spikelets per spike (Fig 3), damage to the leaves can affect the size of the heads and number of spikelets per spike later in the season (Miller 1992). With plants experiencing stress, such as the destruction of foliage during GS 5, potential seed set can be reduced (Miller 1992).

There was a sharp increase in the percentage leaf area damaged in barley from GS 5 to GS 17 (Table 5) (Fig 9). At GS 5 the barley leaf area damage was mostly only 10%. There is a peak in GS 17 again, with mostly 100% leaf area damage. Although the damage of barley leaves is more severe at GS 17 than that of wheat at GS 17, the total barley yield loss is expected to be lower because the damage was not as severe at GS 5.

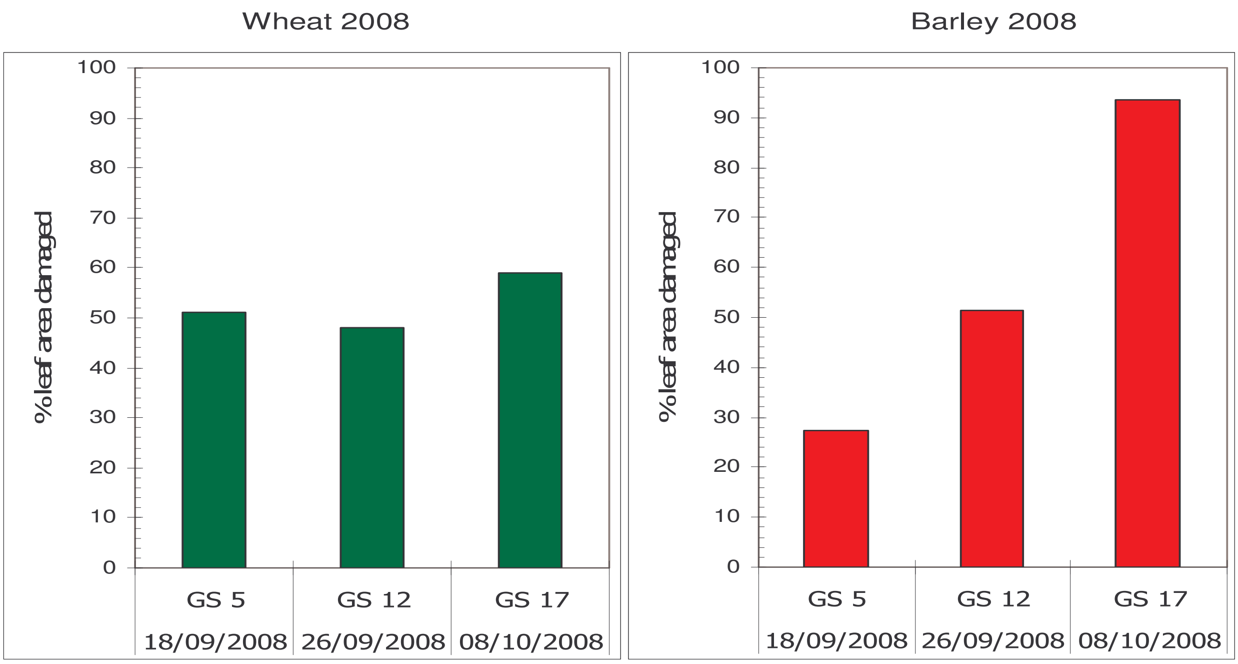

Figure 9. Mean percentage area damaged per leaf in wheat and barley in the Douglas area in 2008.

Table 5. Total number of leaves damaged, categorised according to percentage leaf area damage at GS 5, 12 and 17 for wheat and barley in the Douglas area in 2008.

% area damaged	2008					
	Wheat			Barley		
	GS 5	GS12	GS 17	GS 5	GS12	GS 17
0	0	0	0	0	0	0
10%	25	47	53	50	13	25
20%	27	39	49	25	20	9
30%	26	77	80	26	39	22
40%	33	28	33	18	27	4
50%	12	18	18	11	10	0
60%	21	28	26	5	11	3
70%	19	14	11	2	7	2
80%	15	12	4	5	9	0
90%	10	10	2	0	4	0
100%	29	57	179	0	35	693

In 2009 some leaves stayed undamaged in both wheat and barley (Table 6). At GS 11 only 23.5% of the mean leaf area of wheat was damaged but from GS 15 damage increased to 64% at GS 20 (Fig 10). Most of the damaged leaves in GS 11 fell in the 20 % and 30% categories but as the damage increased there was a shift towards the 100 % category in GS 20.

Very little (2.3%) leaf area was damaged in barley at GS 11 but increased to 45.5% at GS 20 (Fig 10). At GS 15 most damaged leaves was present in the 20% – 40% categories showing only minimal damage at this stage. The number of leaves in the 100% category was minimal.

Between GS 11 and GS 15 all meaningful tiller development of leaves has ceased and no effect on the number of heads per square meter is expected (Miller 1992). Most damage occurred at GS 20, but at this stage the head is fully developed and the damage impact on yield is minimal (Miller 1992).

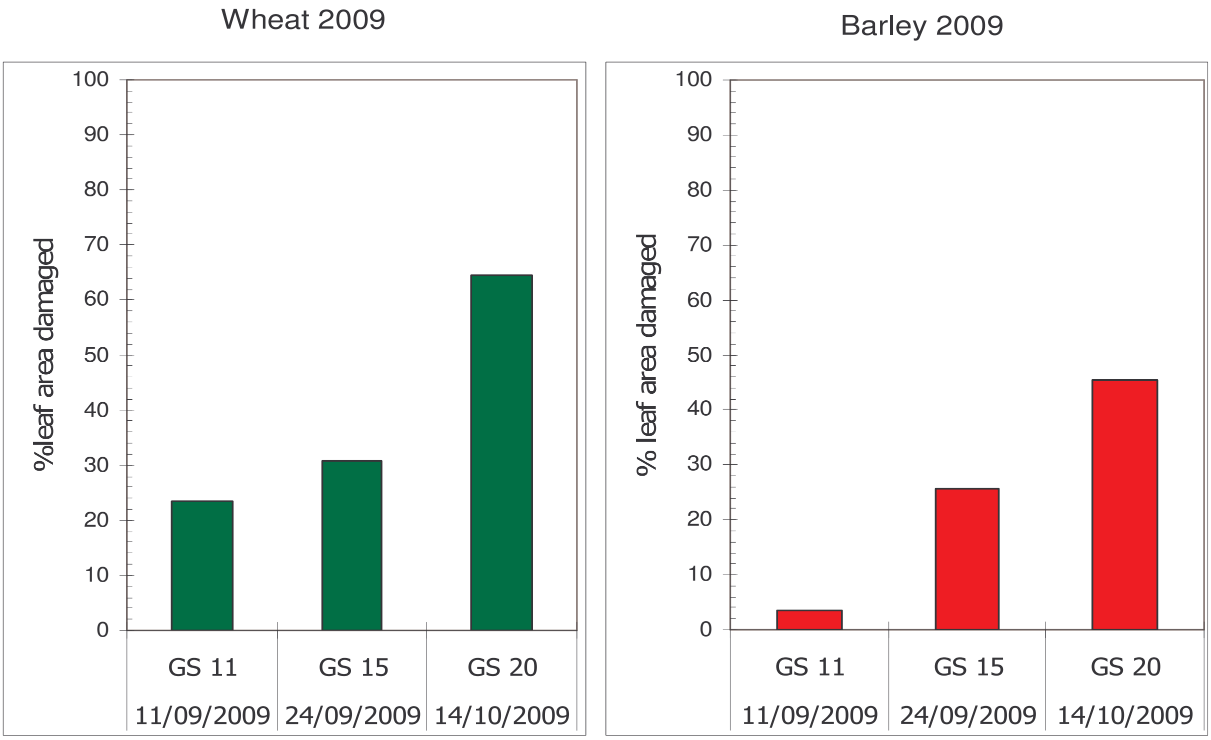

Figure 10. Mean percentage area damaged per leaf in wheat and barley in the Douglas area in 2009.

Table 6. Total number of leaves damaged, categorised according to percentage leaf area damage at GS 5, 12 and 17 for wheat and barley in the Douglas area in 2009.

% area damaged	2009					
	Wheat			Barley		
	GS 11	GS 15	GS 20	GS 11	GS 15	GS 20
0	24	12	8	39	23	2
10	3	16	27	11	5	32
20	11	28	30	0	13	60
30	13	32	12	1	16	44
40	7	20	8	1	10	28
50	2	6	27	0	1	38
60	3	2	0	0	2	16
70	6	11	4	0	0	23
80	0	1	13	0	2	54
90	0	1	13	0	3	12
100	0	2	107	0	2	4

If 50% of a leaf area is damaged, at least 12 cm of the leaf area (and photosynthesis area) is lost. If too much leaf area is destroyed, additional reserves for plant regrowth may be required (Trlica 2006). Since photosynthesis area loss may also affect root growth (Trlica 2006), the plant become less successful regarding nutrient retrieval, resulting in plants as a whole becoming weakened. Weak plants could lodge and result in yield loss. Leaf miner infestations can be very destructive if the leaf miner reaches pest status at a very early growth stage affecting the ability of the plant to compensate for the leaf area loss. Therefore monitoring for leaf miners early in the crop growth season is very important.

4. Conclusion

In both the 2008 and 2009 season the flies only emerged after GS 5. Damage can become severe when foliage experience high damage percentages and the plant has to compensate for approximately half of the tillers that has been attacked. The loss of leaf area can also cause intense stress on the plant. Therefore, the question arises: If the plant must compensate for this loss of foliage, will it be able to compensate if any other pest or environmental stress is placed on it?

No obvious relationship could be found between aerial temperature and growth stages of the crop on the one side and emergence of the flies and infestation on the other side. Further research is needed to determine the stimulus for fly emergence during a specific time frame during the wheat and barley growth season.

5. References

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CHAPTER 5

EFFICIENCY AND SELECTIVITY OF INSECTICIDES AGAINST *AGROMYZA OCULARIS* IN IRRIGATED WHEAT AND BARLEY CROPS

1. Introduction

Leaf miners attacking grasses or cereal crops are not well studied (Scheirs *et al*. 2001). Grasses have a low nutrient content with rare active feeding deterrents and structural defences (Scheirs *et al.* 2001). Agromyzidae feeding on cultivated cereals are found throughout the world and belong to five genera *i.e*. *Agromyza*, *Liriomyza*, *Pseudonapomyza*, *Phytomyza* and *Cerodontha*. A small degree of overlapping can occur, but different parts of the world usually harbour their own unique species (Spencer 1973).

Fluctuating populations and sporadic outbreaks can occur in Agromyzidae feeding on cereals (Spencer 1973). According to Spencer (1973) plant damage by grass leaf miners undeniably elevate the species to pest status level, especially when large population groups attack young plants.

Grass leaf miner species attacking cultivated crops is a world wide phenomenon. For instance, during 1986/87 insect pests of spring wheat were documented in central Saudi Arabia (El-Hag & El-Meleig 1991a). The two insect species ranked as primary pests of wheat was the greenbug, *Schizaphis graminum* (Rondani) and a wheat leaf miner, *Agromyza* sp. (El-Hag & El-Meleig 1991a). El-Hag & El-Meleig (1991b) also reported *Agromyza megalopsis* (Hering), *A. intermittens* (Becker) and *A. prespana* (Spencer) on wheat and barley in Hungary and *A. megalopsis* in Iraq. In Poland, Agromyzidae has been observed in cereal crops causing a reduction in the number of grains in a head (Walckzak 1995). According to Iwasaki (1999), the economic injury level for a barley leaf miner based on yield loss combined with spray cost, was estimated at 12% and 16% respectively for spring and winter wheat in North Japan. In Bulgaria, *A. megalopsis* caused yield decreases in barley of 26.8% (Khristov 2000).

No control measures were available for the recent outbreak of *Agromyza ocularis* on wheat and barley in the Northern Cape Provence (Prinsloo 2001). According to Prinsloo (2001) the following should be taken in consideration when implementing chemical management for an outbreak as mentioned above:

1.1 Contact pesticide will not be effective since the pesticide has to be sprayed directly onto the pest in order to attain physical contact. Adult flies will be killed, but no impact will be made on the larvae causing the leaf damage (Lochner 2000; Norris *et al*. 2003). The most important leaf miner stages to target, through chemical control, are the eggs and larvae and to date no pesticide has been registered that is effective against the eggs (Weintraub & Horowitz 1995). Chemical control should be systemic, since the larva feeds inside the leaf on the tissue. Systemic insecticides are taken up by the plant and transported throughout the plant towards the growth tips (Cloyd 2002) and as such the chemical is most effective against insects with piercing-sucking mouthparts (Cloyd 2002). Translaminar insecticides penetrate leaf tissues, forming a reservoir of active ingredients inside the leaf (Cloyd 2002). This pesticide treatment is effective against spider mites and leaf miners because of the active ingredient’s ability to distribute through the leaves (Cloyd 2002).

1.2 It is essential that the chemicals should be applied correctly as a management strategy in order to guard against chemical resistance (Prinsloo 2001). If chemicals are not applied properly resistance can build up within 6 years (Lochner 2000).

1.3 Chemicals should also be used sensibly to allow the least effect on the natural enemy population (Prinsloo 2001). Insecticides can be disruptive to natural enemies of pests and often after such an event, there is, more often than not, a breakout of the pest itself after treatment with the insecticide (Capinera 2005; Cranshaw *et al*. 2009).

1.4 Ideally chemical control should be integrated with cultural practices, but none exist for implementation against *A. ocularis* (Prinsloo 2001).

According to the guidelines for Good Plant Protection (GPP) practices (Anon 1997) on wheat, *Agromyza* spp. are important economic pests in northern Europe. The basic strategy to use is the application of insecticides in the pyrethroid, organophosphate and synthetic pyrethroid groups. The particular insecticide is applied during growth stage (GS) 12 in the case of heavy attacks, or when a threshold of more than 20% mining is reached on the lower leaves, accompanied with oviposition punctures on the upper leaves at GS 17. It was also recorded that aphid control also affects *Agromyza* spp. occurrence (Anon 1997).

In Israel, Weintraub and Horowitz (1995) determined that the most effective insecticides for the control of leaf miner larva were: Oxamyl®, Abamectin®, Cyromazine® and Thiocyclam hydrogen oxalate®. The main pesticides used in Turkey are Abamectin® and Cyromazine®, both of which are translaminar (Civelek & Weintraub 2003) and effective for reducing the larval leaf miner population size of both the *L. trifolii* and *L. huidobrensis* (Weintraub 1999).

In South Africa Unimectin 18EC® was registered as an emergency measure for leaf miner control on barley (Kotzé 2009). Avermectin, the active agent in Unimectin® and Abamectin®, has a translaminar systemic action (Pereira 2009).

According to Lochner (2000) the control measure for the potato leaf miner in South Africa is the application of Abamectin® at the first visible sign of the puncture wounds induced by the female adult. Abamectin® is sensitive to ultraviolet light and must therefore not be sprayed in the afternoon (Lochner 2000). A second application must be sprayed 7-10 days after the first application which effectively breaks the cycle and controls the pest for at least 4 weeks or longer (Lochner 2000). After the second application of Abamectin®, the insecticide must be retracted and not sprayed in the same season again (Lochner 2000).

During the 2007 and 2008 wheat and barley seasons in the Northern Cape, experiments were conducted with insecticides already in use in chemical programs against *L. trifolii* and *L. huidobrensis* in order to determine the efficacy of the chemicals against *A. ocularis*. The following questions were posed:

- Do the insecticides reduce the larval population?
- Is there a difference in the yield between untreated and treated wheat and barley plants?

2. Material and methods

Insecticide studies were carried out in 2007 and 2008 during the wheat and barley season at sites with leaf miner infestations in the Douglas district.

2.1 Year 2007

On 8 October 2007 four trials were sprayed with Unimectin® to determine its efficacy against *A. ocularis* in irrigated wheat and barely crops. One barley trail was planted at each of the chosen Blinkklip and Eldorine sites; whilst Vaalboskop and Amar were chosen as wheat trial sites.

At all four localities maize was planted between 1 December and 20 December. After harvesting the maize in June, wheat and barley were planted. All fields were irrigated by centre pivot systems. When the wheat and barley were at flag leaf stage the leaf miner larval stage was targeted for chemical application. Weather conditions at the time were 29°C, 90% relative humidity, 100% cloud cover and no wind at the time of application. Application of insecticide was conducted between 9:30 and 14:00 during the day. Soil was sandy with 0% - 10% clay texture.

The equipment used was a multispray gas sprayer with Agritop® blue nozzles 80-03 (flat fan). A pre-spray count of larvae was done by sampling a 100 leaves randomly per plot for each treatment. One post treatment count on 30 infested leaves per plot was conducted five days after application. Yield and grain quality for these plots were determined at harvest.

The trial plan was a randomized block design with three treatments and an untreated control (Table 1). The plots were 20m^2 in size and the trial was replicated 5X. Aqua Rite® (100ml/ 100L water) used to correct the pH of water, was added prior to the mixing.

Table 1. Trial plan for chemical application against grass leaf miner at sites in the Douglas area in 2007.

Treatment	Active ingredient	Concentrations used	Formulation	Adjuvant
Untreated	-	-	-	-
Unimectin® (1)	avermectin	300ml/ha	18EC	Citrex® 0.5L/ha
Unimectin® (2)	avermectin	600ml/ha	18EC	Citrex® 0.5L/ha
Unimectin® (3)	avermectin	1200ml/ha	18EC	Citrex® 1.0L/ha

2.2 Year 2008

Four trials were conducted at the following sites; Barley at Blinkklip and Higgs Hope; wheat at Wouterspan and Higgs Hope.

At all localities maize was planted between 1 December and 20 December. After harvesting the maize in June, wheat and barley were planted. All fields were irrigated by centre pivot systems. On 24 September 2008, 6 October 2008 and 17 October 2008 chemical applications were conducted to determine the efficacy of different insecticides against *A. ocularis*. The wheat and barley were at growth stage (GS) 12 with the first application, at GS 14 with the second and GS 16 with the third application. Weather conditions at the time were 20°C, 90% relative humidity, 0% cloud cover and no wind. Application of insecticide was conducted at 8:00 and 16:00 during the day. Soil was sandy with 0% - 10% clay texture. The same spray equipment as the previous year was used for application. The trial plan was a randomized block design containing eight treatments and an untreated control (Table 2). The plots were 20m^2 in size and the trial was replicated 5X. Aqua Rite® (100ml/ 100L water) used to correct the pH of water, was added prior to the mixing.

A pre-spray count of larvae was conducted by sampling 100 leaves randomly per plot for each treatment. One post-treatment count on 30 infested leaves per plot was conducted five days after each application. Yield and grain quality for the plots were again determined at harvest.

Table 2 Trial plan for chemical application against grass leaf miner at sites in the Douglas area in 2008.

Pesticides	**Active ingredient**	**Concentrations used**	**Formulation**	**Adjuvant**
Untreated	-	-	-	-
(1) Cartap hydrochloride®	Cartap hydrochlorid	400g/100L H_2O	500g/kg SP	Citrex® 0.5L/ha
(2) Cartap hydrochloride®	Cartap hydrochlorid	800g/100L H_2O	500g/kg SP	Citrex® 1.0L/ha
Cyromazine® (1)	cyromazine	40g/100L H_2O	750g/kg WP	Citrex® 0.5L/ha
Cyromazine® (2)	cyromazine	80g/100L H_2O	18EC	Citrex® 1.0L/ha
Unimectin® (1)	abamectin (avermectin)	600ml/ha	18EC	Citrex® 0.5L/ha
Unimectin® (2)	abamectin (avermectin)	1200ml/ha	18EC	Citrex® 1.0L/ha
Abamectin® (1)	avermectin	600ml/ha	18EC	Booster oil® 30ml/10L H_2O
Abamectin® (2)	avermectin	1200ml/ha	18EC	Booster oil® 30ml/10L H_2O

2.4 Data analysis

Data were analyzed by using GraphPad software (GraphPad Software 1998). The comparisons were done by applying One-way Analysis of Variance (ANOVA) and the Turkey-Kramer Multiple comparisons at 5% significance. Data from each year was analyzed separately.

3. Results and discussion

3.1 Year 2007

Data for both barley and both wheat trials were analysed and compared with each other. There was no significant difference between the results of the wheat trials and also not between the barley trials and therefore the data from both the wheat and barley trials were pooled before analysis.

3.1.1 Barley

In barley (Fig. 1) there was no significant differences (F = 3.990, P>0.05) between the number of live larvae present in the control vs. Unimectin® 1 and Unimectin® 2. In the Unimectin® 3 treatment (twice the recommended dosage for *L. huidobrensis* and *L. trifolii)* the larval survival rate was significantly less than the control (F = 3.990, P<0.001).There was no significant difference (F=0.8886, P>0.05) in yield between the control and any of the treatments (Fig. 2).

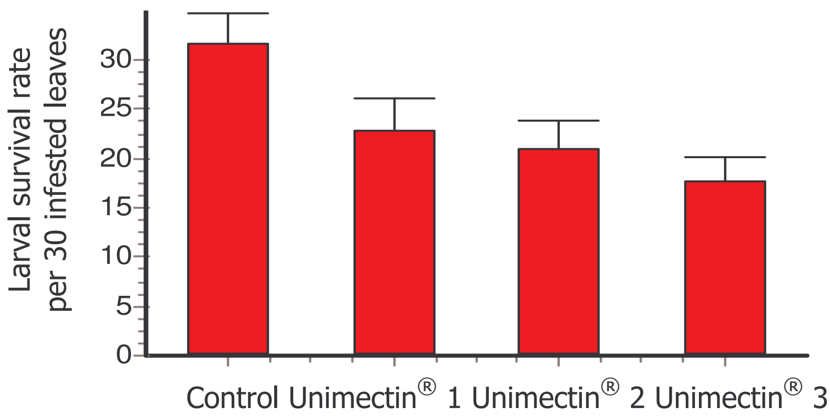

Figure 1. Mean of number live larvae (± se) in barley after treatment with Unimectin® 1, (300ml), Unimectin® 2 (600ml) and Unimectin® 3 (1220ml) in the Douglas area in 2007.

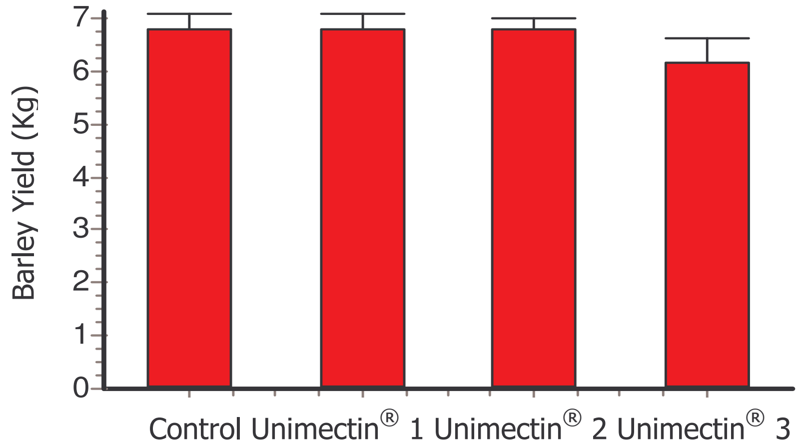

Figure 2. Mean barley yield (±se) treated with Unimectin® 1 (300ml), Unimectin® 2 (600ml) and Unimectin® 3 (1220ml) in the Douglas area in 2007.

3.1.2. Wheat

In wheat (Fig. 3), the larval survival rate was significantly lower (F=19.973, P<0.001) with Unimectin® 1, Unimectin® 2 and Unimectin® 3 treatments than on the control. There was no significant difference (F=0.07687, P>0.05) between the yield of the control and any of the treatment yields of wheat (Fig. 4).

The low larval survival rate in wheat could be due to a number of factors that should be taken into consideration. During application the canopy of barley was denser than that of the wheat, which could have influenced the dispersal efficacy of the insecticide. Treatment application was only done at flag leaf stage and some of the spraying occurred during midday, which could have influenced the effectiveness of the application.

The residual action of Unimectin® is ±40 days. The time period between flag leaf stage (GS 15) and harvest of both crops could be between 40 and 50 days depending on the climate. Although the double dosage of Unimectin® showed effective control of larvae, it is not recommended, since it is possible that residues on the plants may still be present at the time of harvest. Despite the dosage, it is necessary that the presence of residues should always be tested after harvest.

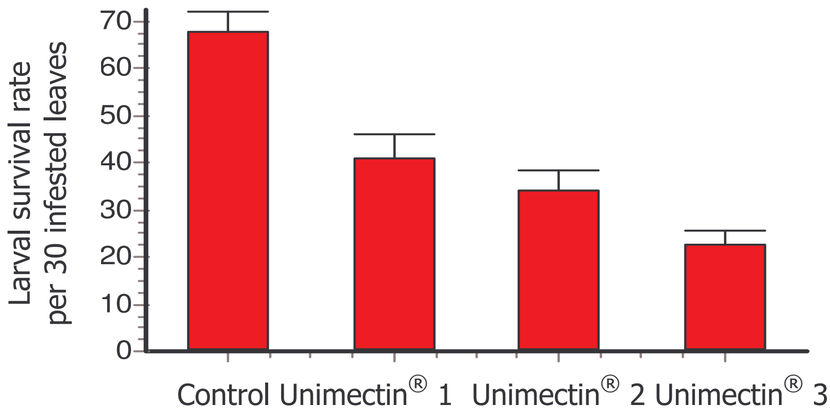

Figure 3. Mean number of live grass leaf miner larvae (±se) in wheat after treatment with Unimectin® 1 (300ml), Unimectin® 2 (600ml) and Unimectin® 3 (1220ml) in the Douglas area in 2007.

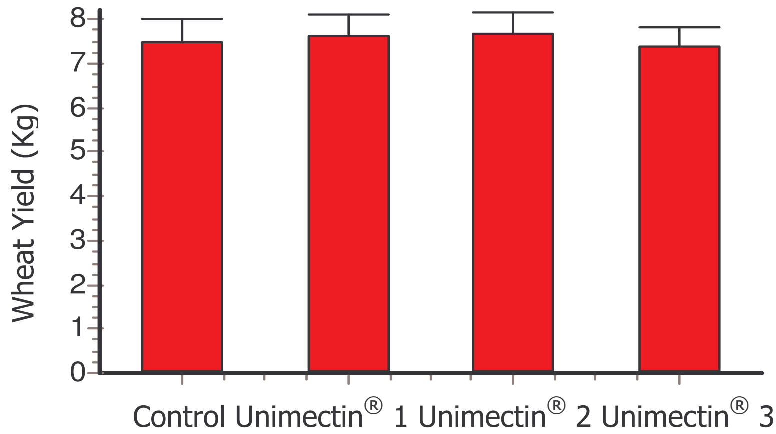

Figure 4. Mean wheat yield (± se) treated with Unimectin® 1 (300ml), Unimectin® 2 (600ml) and Unimectin® 3 (1220ml) in the Douglas area in 2007.

There was in the yield of both wheat and barley had no significant difference between the control and treated plots. Although larvae were still present on the treated plots, albeit less, the yield was not damaged significantly (Figs 2 & 4). However bear in mind that the insecticide could only have been applied when the damage was already inflicted in both the control and treated plots. Other influences could also have determined the results such as infestations occurring earlier in the season or encountering higher infestation levels.

3.2 Year 2008

The data for the barley and wheat trials were again pooled before analysis.

3.2.1. Barley

All insecticide treatments (Fig. 5) reduced the larval survival rate significantly compare to the control ($F=5.51$, $P<0.01$). The Unimectin® 2 and Abamectin® 2 treatments were, however, the most effective on barley and both of them show significantly fewer larvae than all the other chemical treatments although they did not differ from each other (Fig.5).

Although these double dosage treatments were the most effective it cannot be recommended because of the probability of residue occurrence on the crop. Therefore any of the single dosage treatment would probably be a better control option (Fig 5). There was no significant difference ($F=2.821$, $P>0.05$) between the yield of the control and treated plots of barley (Fig. 6).

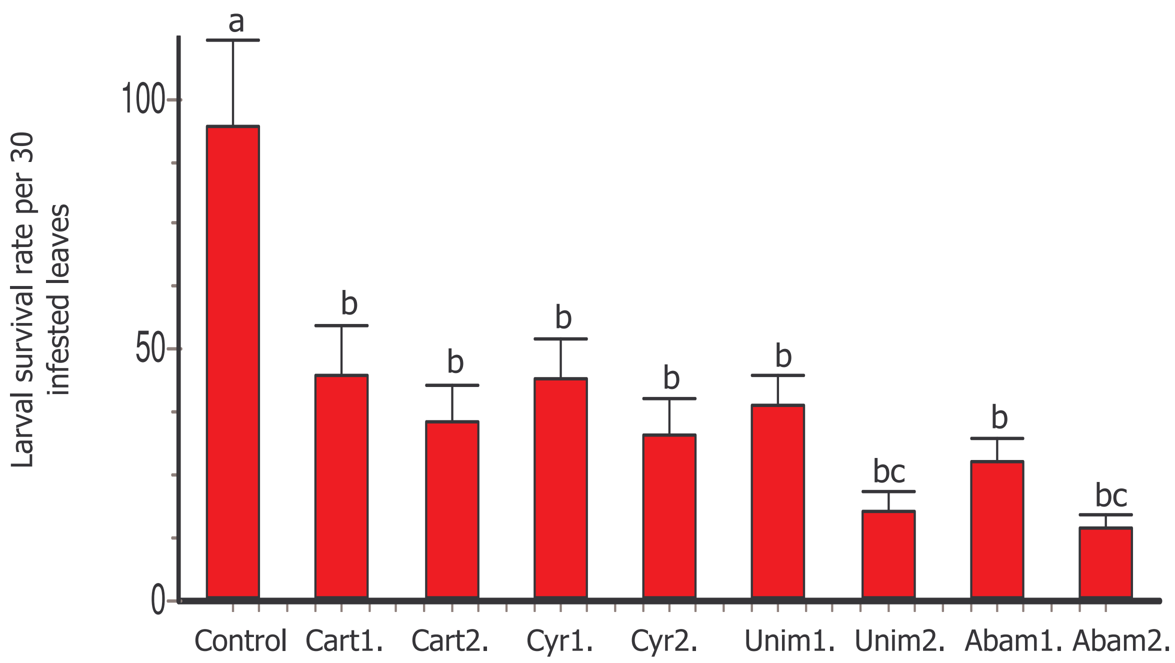

Figure 5. Mean number of live grass larvae in barley (±se) after treatment with the folowing variety of insecticides: Cartap hydrochloride® 400g (Cart1.), Cartap hydrochloride® 800g (Cart2.), Cyromazine® 40g (Cyr1.), Cyromazine® 80g (Cyr2.), Unimectin® 600ml (Unim1.), Unimectin® 1200ml (Unim2.), Abamectin® 600ml (Abam1.) and Abamectin® 1200ml (Abam2.) in the Douglas area in 2008. (Letters above bars indicates significant differences between treatments.)

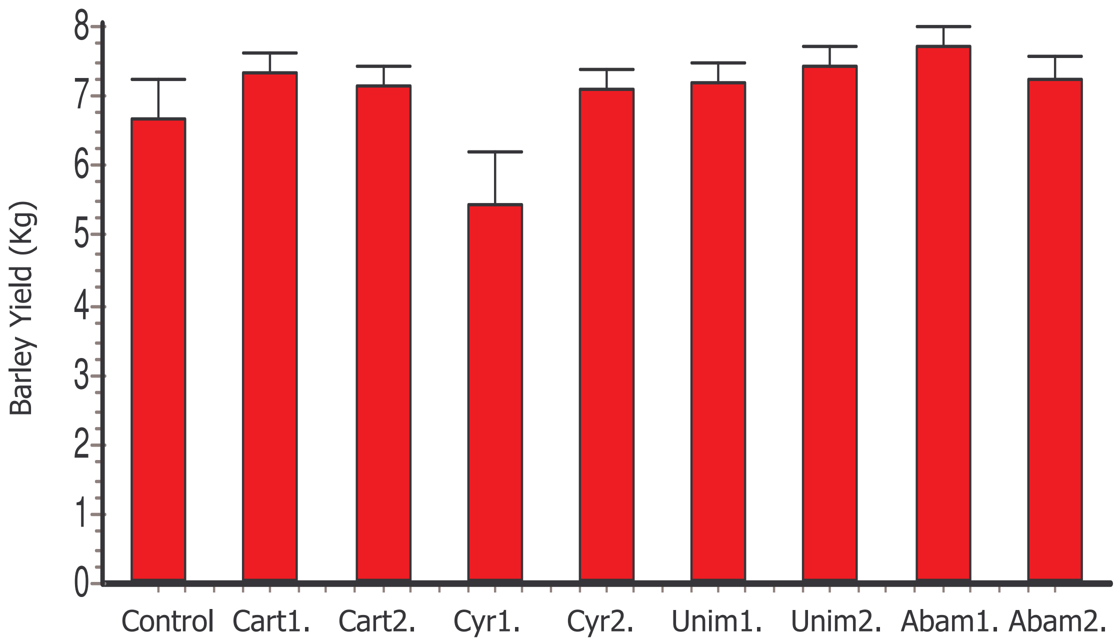

Figure 6. Mean of barley yield (± se) after treatment with the following variety of insecticides: Cartap hydrochloride® 400g (Cart1.), Cartap hydrochloride® 800g (Cart2.), Cyromazine® 40g (Cyr1.), Cyromazine® 80g (Cyr2.), Unimectin® 600ml (Unim1.), Unimectin® 1200ml (Unim2.), Abamectin® 600ml (Abam1.) and Abamectin® 1200ml (Abam2.) in the Douglas area in 2008.

3.2.2. Wheat

In wheat all chemical treatments also reduced the larval survival rate significantly compared to the control (F=10.61, P<0.001) (Fig. 7). The Cartap 1 treatment, however, shows weakest control and has significantly more larvae than the rest of the chemical treatments. The rest of the treatments did not differ significantly in the number of life larvae present. Although chemical treatments have a significant effect on the larval survival rate the difference was not expressed in the yield of the different plots (F=0.5824, P>0.05) (Fig. 8).

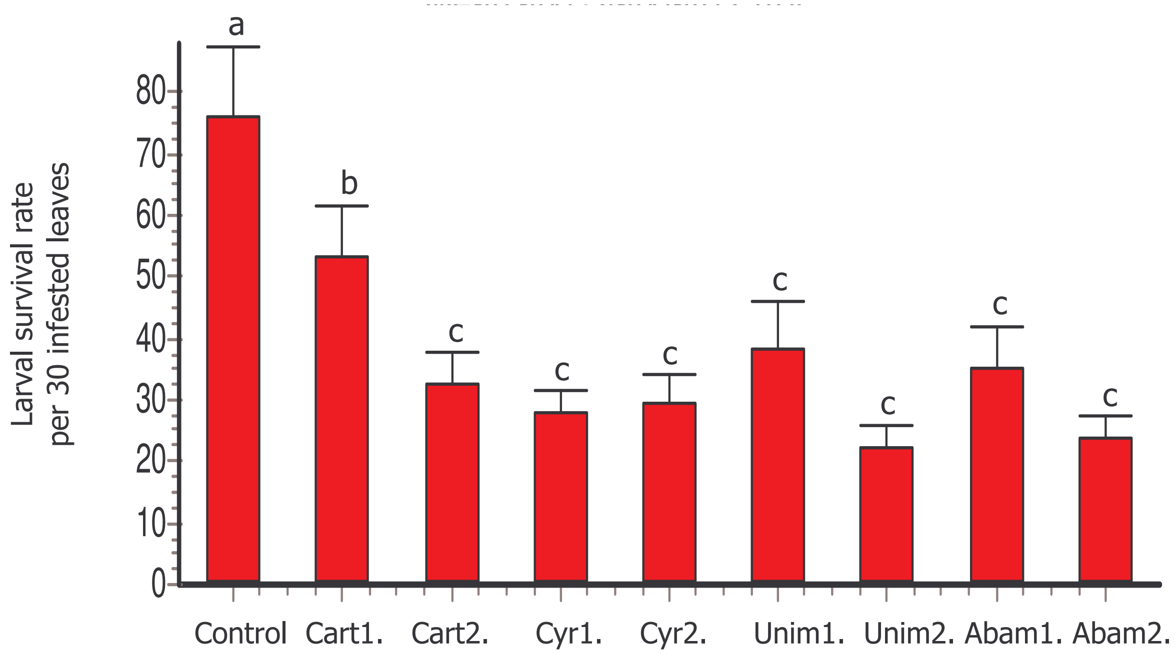

Figure 7. Mean number of live grass larvae in wheat (±se) after treatment with the folowing variety of insecticides: Cartap hydrochloride® 400g (Cart1.), Cartap hydrochloride® 800g (Cart2.), Cyromazine® 40g (Cyr1.), Cyromazine® 80g (Cyr2.), Unimectin® 600ml (Unim1.), Unimectin® 1200ml (Unim2.), Abamectin® 600ml (Abam1.) and Abamectin® 1200ml (Abam2.) in the Douglas area in 2008. (Letters above bars indicates significant differences between treatments.)

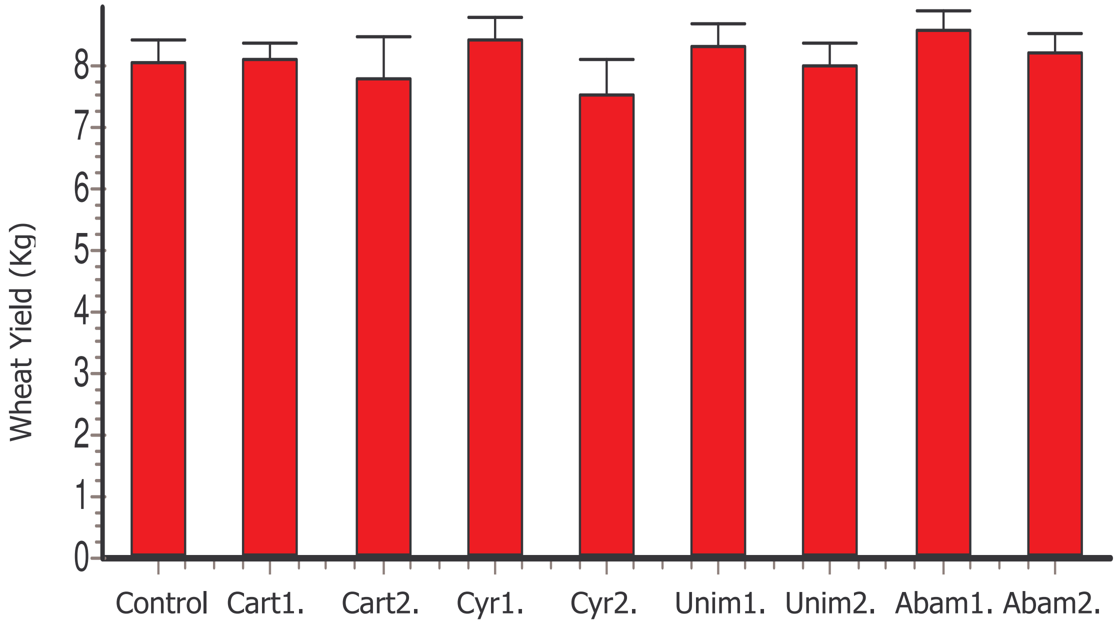

Figure 8. Mean of barley yield (± se) after treatment with the following variety of insecticides: Cartap hydrochloride® 400g (Cart1.), Cartap hydrochloride® 800g (Cart2.), Cyromazine® 40g (Cyr1.), Cyromazine® 80g (Cyr2.), Unimectin® 600ml (Unim1.), Unimectin® 1200ml (Unim2.), Abamectin® 600ml (Abam1.) and Abamectin® 1200ml (Abam2.) in the Douglas area in 2008.

The treatments reduced the larval survival rate effectively on both crops. Albeit that a number of larvae survived each of the treatments the yield of both wheat and barley was not significantly effected, as can be observed when comparing the treatment and control plots.

4. Registration of insecticide

According to registration regulations (Act No. 36 of 1947), the level of control must be stipulated in the registration application. According to the application the suitable and sufficient control is at least 80% control before it is registered as a pesticide for this particular pest and crop ([7]André Coetzee, personal communication). The pesticides tested were systemic and translaminar insecticides which penetrate the leaves and are known to be effective against leaf miners (Cloyd 2002). The mentioned required level of control was only achieved on barley in 2008 with the application of Unimectin® and Abamectin® double dosages (Tables 4 & 5).

Table 4. Treatment efficacy percentage for *Agromyza ocularis* larvae on barley and wheat in the Douglas area in 2007, using Unimectin® 1 (300ml), Unimectin® 2 (600ml) and Unimectin® 3 (1220ml).

	Barley		
Chemical	Unimectin 1	Unimectin 2	Unimectin 3
% Treatment Efficacy	28%	34%	44%
	Wheat		
Chemical	Unimectin 1	Unimectin 2	Unimectin 3
% Treatment Efficacy	40%	50%	66%

[7] André Coetzee, GWK (Pty) Ltd. in Douglas, Northern Cape, South Africa.

Table 5. Treatment efficacy percentage for *Agromyza ocularis* larvae on barley and wheat in the Douglas area in 2008, using Cartap hydrochloride® 400g (Cart1.), Cartap hydrochloride® 800g (Cart2.), Cyromazine® 40g (Cyr1.), Cyromazine® 80g (Cyr2.), Unimectin® 600ml (Unim1.), Unimectin® 1200ml (Unim2.), Abamectin® 600ml (Abam1.) and Abamectin® 1200ml (Abam2.). Highlighted is the percentage fulfil registration requirements.

	Barley							
Chemical	Cart (1)	Cart (2)	Cyr (1)	Cyr (2)	Unim (1)	Unim (2)	Abam (1)	Abam (2)
% Treatment Efficacy	53%	62%	53%	66%	59%	80%	71%	85%
	Wheat							
Chemical	Cart (1)	Cart (2)	Cyr (1)	Cyr (2)	Unim (1)	Unim (2)	Abam (1)	Abam (2)
% Treatment Efficacy	30%	57%	62%	63%	50%	71%	53%	68%

The reasons for poor control could be the following:

1. The crop canopy was dense and the chemicals might not have penetrated effectively to the lower leaves where the larvae are most abundant (as discussed in Chapter 4). Therefore, care should be taken during chemical application that the penetration levels into the crop canopy are optimal.

2. Compared to 2007 (Table 4) and 2008 (Table 5), the treatment of 2008 was more effective in the control of the larvae than is the case in 2007. The reason for this could be that the 2007 treatments were applied during the late morning and afternoon, while in 2008 applications were conducted during the early mornings and late in the afternoon. Pesticide efficiency is always compromised on account of

ultraviolet light (Lochner 2000) and there should be compensation for this.

3. In 2007 the treatment was applied only once during the outbreak season, while the treatments during 2008 were applied three times throughout the season. As mentioned, early seasonal application is more successful by applying a repeat second treatment to break the life cycle (Lochner 2000). The method of repeated application could therefore be the explanation for the more successful 2008 (Table 5) treatments.

5. Conclusion

From both trials it is clear that the survival rate of larvae could be reduced by the application of the tested chemicals. The double dosage treatments of both Unimectin® and Abamectin® in 2008 was the most effective and fit the recommendations stipulated in the registration application. However because both chemicals were applied at a double dosage which enhances the probability of high residues on the grain after harvest these chemicals could not be registered as a pesticide treatment against *A. ocularis* on barley and wheat in the Northern Cape. Based on the yield analyses of 2007 and 2008 the reduction in numbers of leaf miner larvae do not have a noticeable effect on crop yield even for the most effective treatments, which implies that the plant could compensate for the damage under the optimal cultivation conditions that exist in irrigated fields.

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CHAPTER 6

GENERAL DISCUSSION, SUGGESTIONS AND FUTURE FOCUS FOR RESEARCH ON *AGROMYZA OCULARIS*

1. Summary

1.1 Leaf mining, which refers to an insect larvae feeding or "mining" inside the leaves and stems of plants, occurs in nine families of the Diptera order, of which Agromyzidae is the most diverse.

1.2 Several species of the Agromyzidae are important insect pests which can cause yield loss on different crops. Fluctuating populations and sporadic outbreaks of the genus *Agromyza,* or the grass leaf miners, can occur on cereals and must be considered as actual and potential pests especially when large populations attack young plants.

1.3 *Agromyza ocularis* Spencer is an indigenous grass leaf miner of South Africa. Although described in 1961, it was first recorded attacking wheat under irrigation in the Bergville area of KwaZulu Natal, during 1992. This outbreak was sporadic but since 2000 annual outbreaks started occurring in the Northern Cape and are spreading towards other irrigation areas along the Gariep (Orange) River and Vaal River where wheat and barley are produced.

1.4 *A. ocularis* is a small Diptera species and the adult is mostly black in colour with clear wings, with larvae which are yellow in colour and cylindrical in form. They are oligophagous with a preference for plants in the grass family *e.g.* wheat and barley.

1.5 Two distinct types of injury are caused by *A. ocularis* namely feeding punctures and leaf mining. The feeding punctures are inflicted by the adult female ovipositor and are either used for oviposition or to feed on plant sap and as such cause a stippling symptom on the leaf. Mines are caused by the larvae feeding on tissue inside the leaf. A mine originates where the larvae hatch. The mines are usually linear towards the stem or leaf apex and the area mined is totally necrotic and cannot be revived by the plant. Photosynthesis by the leaves is severely diminished in this way. Characteristic black frass granules can be seen throughout the mine.

1.6 Under laboratory conditions the larvae can mature within 4 days at 25°C, feeding continuously in the leaf whilst tunnelling moving forward. Several larvae can feed in a single leaf simultaneously.

1.7 The mature larva cuts a slit drops from the mine, drops from the plant and burrows into the damp soil next to the plant where it pupates. The pupal stage can last 23 days at 25°C. A pupal diapause period can be present which and can last for approximately 10 months. The specific importance of this diapause period is not clear but could be a mechanism to survive through periods of low food reserves and extreme temperature.

1.8 The lifespan of the adult fly is 30 days at 25°C. This could lead to overlapping generations of flies in crop fields, which obviously increases feeding damage on the crop. The flies were recorded to be active in the field when mean daily temperatures ranged between 10°C and 30°C, during periods when relative humidity is high due to irrigation practices. This could be regarded as the optimal conditions the flies need to survive and thrive.

1.9 The following alternate host plants were identified: *Phalaris minor* (small canary grass), *Bromus catharticus* (rescue grass), *Lolium perenne* (perennial rye grass) and *Avena fatua* (common wild oats). These grasses were recorded growing on the outside edge of the wheat fields during the wheat growing season but not during off-season or in the natural vegetation surrounding the fields.

1.10 The presence of flies in wheat fields can be detected by three different methods. Firstly by walking through the field at least twice a week searching for feeding punctures or mines on the leaves. A second method is to sweep the foliage with a sweep net. This method could be ineffective in dense stand of plants since the flies would be able to take shelter in the lower parts of the plants where they will escape detection. Thirdly, a suction trap can be used which has to be on the same level as mature plants in order to function optimally.

1.11 The emergence of the flies differed by 14 days between the 2008 and 2009 growing season. In spite of this difference, the crops in both years were older than growth stage (GS) 5 (Joubert scale) and plants were in a stem elongation phase when this happened. It thus seems as if the appearance of flies is not linked to plant growth stage, but rather to climatic conditions.

1.12 At plant growth stage 5 the number of tillers per plant is already set and the plants' energy reserves are used to initiate the number of heads per plant, as well as, the number of florets per head. If the plant is heavily damaged at this time the number of heads and florets per plant will be influenced. However the leaf miner behaviour showed that oviposition commences on the lower, older leaves and as a result these are mined first. The rest of the leaves on the plant are attacked as they appear.

1.13 In 2008, 100% of both barley and wheat plants was damaged by leaf miners when investigated from GS 5 and onwards. The number of damaged tillers per wheat plant increased from 48% - 63% between GS 5 and GS 17, prior to flowering. The number of damaged wheat leaves per tiller increased from 33% - 58% between GS 5 and 17 and between 10% and 100% of the leaf surface was damaged during this time. This demonstrates the severity of damage caused by the leaf miner. The damage varies between fields and between years and impact levels could be influenced by climatic conditions. Economic losses in crop yield are thus expected. However, insecticide trials conducted under similar conditions during 2008 showed no significant difference in yield between the treatment and the control plots even though not all larvae were controlled. The plant seems therefore to compensate for the damage caused by the leaf miner larvae. If the optimal cultivation conditions under which the crop is produced *e.g*. adequate water and fertiliser, is considered, this is understandable.

1.14 The testing of different insecticides in field trials between 2007 and 2008 provide varying results, with only double dosage of Unimectin® showing 80% reduction in larval numbers on barley, which is according to pesticide registration application. Abamectin® double dosage was the most successful in 2008 showing 53% - 85% reduction in larval numbers on barley. In spite of all this variation, no significant increase in yield could be measured on any of the treatments in any of the years, suggesting that the plants have the ability to compensate for the damage. However, this could also be an indication that the damage was inflicted by the larvae before spraying and could not be rectified by chemical treatment.

2. Suggestions

2.1. For early detection:

- In the beginning of the season monitoring must be repeated regularly by walking throughout the field searching for early signs of infestation.
- Especially the lower leaves near the ground must be monitored.

2.2. For further spread:

- Contamination must be avoided by cleaning machinery regularly
- Soil movement between fields must be contained, since leaf miner pupa can be distributed in this matter.

2.3. Chemical application:

- Applications early in the season, followed by a second treatment to break the life cycle are more successful.
- Application of the chemicals Abamectin® and Unimectin® must be applied properly to penetrate through the dense foliage in order to reach the leaf miner larvae on the lower leaves.
- For pesticide efficiency early morning or late afternoon application must be conducted, since, the active ingredient avamectin of Unimectin® and Abamectin® breaks down in ultraviolet light.

3. Future focus for research on *Agromyza ocularis*

Based on the current study, various other aspects came to light which need to be investigated.

- The lifecycle must be studied under controlled laboratory conditions at different temperatures regimes.

- Yellow sticky traps within the crop fields can be applied for early detection of leaf miner flies, since; *A. ocularis* does not fly far from the crop and usually takes refuge between the leaves of the crop.
- Natural enemies of *A. ocularis* have to be investigated as a control tactic. None could be recorded from the Northern Cape region in spite of concerted efforts.