Harper Adams University

A Thesis Submitted for the Degree of Doctor of Philosophy at

Harper Adams University

Aspects of the biology and ecology of saddle gall midge (*Haplodiplosis marginata*)

by
Charlotte H. C. Rowley

A thesis submitted for the degree of
Doctor of Philosophy

July 2017

Abstract

The sporadic nature of saddle gall midge (*Haplodiplosis marginata*) has meant that research into this pest has been, much like the outbreaks, patchy and intermittent. A lack of long term data has so far hampered attempts to develop a cohesive strategy towards the management of this pest. This thesis begins with a review of the existing literature on *H. marginata*, drawing together information from past studies done over many decades in a number of countries across Europe. Some of this information was previously unavailable in electronic format or required translation making the consolidation of these studies an important part of increasing the availability of current *H. marginata* knowledge and enabling the identification of research gaps. One example is the lack of information surrounding the developmental biology of *H. marginata* which is addressed in Chapter 2. Here, the effects of soil temperatures and rainfall events on *H. marginata* development are studied as a means of forecasting the adult emergence. This has a resulted in the development of degree day-based models to predict *H. marginata* phenology. Rainfall events followed by an accumulation of 512DD above 0°C can be used to predict peaks in *H. marginata* adult emergence and cumulative percentage emergence can be modelled using a probit-linked GLM or a bimodal function which can be used to predict the start of, or peaks in, *H. marginata* emergence as an early warning system for farmers. Chapters 3 and 4 further improve on current options for monitoring by investigating the chemical ecology of *H. marginata*. Electroantennography coupled with gas chromatography is used to confirm the male response to the female sex pheromone. Field experiments are used determine the optimum formulation for a pheromone lure as 0.5mg (*R*)-2 nonyl butyrate from a polyethylene vial dispenser This lure is then tested in further situations to define the optimal trap position as being placed at the height of the wheat ear, 20m into the crop with at least 20m between traps. Such experiments give further insight into behavioural aspects of this insect as well as providing practical monitoring solutions for farmers. The natural enemies of *H. marginata* are another understudied area of research which Chapter 5 attempts to improve upon through the development of a PCR-based assay for gut analysis of arthropod predators. Use of this assay in the field demonstrates the potential for further research in this area through the

identification of four carabid species that naturally predate on *H. marginata*: *Nebria brevicollis, Poecilus versicolor, Harpalus rufipes and Loricera pilicornis* Finally, a summary chapter suggests how this thesis can be used in further research towards integrated pest management solutions for this insect and places this work into the wider context of a dynamic agricultural environment.

Table of Contents

List of Figures

List of Tables

Chapter 1

A review of the biology, ecology and control of saddle gall midge, *Haplodiplosis marginata* (Diptera: Cecidomyiidae)

Abstract

Saddle gall midge *Haplodiplosis marginata* (Diptera: Cecidomyiidae) is a pest of cereals across Europe. The occasional nature of this pest has resulted in limited and sporadic research activity. There remain important gaps in knowledge due either to a genuine lack of research or to previous research being difficult to access. These knowledge gaps make the development of effective control options difficult. Here, we review the existing literature in an attempt to consolidate the information on *H. marginata* from research which spans several decades and encompasses many different countries. The current distribution and pest status of this insect are updated, along with the methods of cultural and chemical control available to growers. The biology and life history of the insect are described in detail and the ecological processes governing them are discussed. Finally, the areas in most need of further research are identified, along with suggestions of how this information can be used to help develop effective and sustainable management solutions for this pest

Published in: Rowley, C., Cherrill, A., Leather, S., Nicholls, C., Ellis, S. & Pope, T. (2016) A review of the biology, ecology and control of saddle gall midge, *Haplodiplosis marginata* (Diptera: Cecidomyiidae) with a focus on phenological forecasting. *Annals of Applied Biology*, **169**, 167–179.

1-1 Introduction

Saddle gall midge (*Haplodiplosis marginata* von Roser; *Haplodiplosis equestris* Wagner) is a polyphagous pest of cereal crops across Europe. The first reference to this species in the UK is by Omerod (1890), who received samples of injured barley stems from Lincolnshire exhibiting the characteristic galling of *H. marginata*. Identification was confirmed in 1909 when adults were reared from larvae found in infested wheat stems in Pembrokeshire (Enock, 1909). *Haplodiplosis marginata* has since been recorded as a sporadic pest of wheat, barley and rye crops in the UK, particularly in central and eastern areas of England. In the mid-1960s, a survey of the main cereal growing areas showed the species to be prevalent at low levels but of no economic interest (Empson, 1965). Between 1967 and 1972 however, severe outbreaks were reported in isolated areas across the country (Golightly & Woodville, 1974; Woodville, 1968, 1970, 1973). The pest was not considered to be a problem again until 2010, when localised outbreaks were reported in central England (Allison, 2010; Case, 2011). Reports of the midge being present at lower levels have continued since this time (HGCA, 2012).

The 40-year interval between economically damaging outbreaks of *H. marginata* in the UK and other European countries has resulted in a lack of continuity in research into this pest. For example, in the UK, prior to 2012 there had been no research published on *H. marginata* since 1974. A similar pattern can be observed in other European countries in which *H. marginata* has historically been economically damaging, with the last decade seeing an increase in research activity. The sporadic nature of this pest has frustrated research efforts as studies rarely coincide with serious outbreaks and long term information is sparse. Additionally, existing research on *H. marginata* is fragmented across several countries and several languages which, in combination with the age of the publications, can make accessing and translating them difficult, particularly where there is no digital copy available. The resulting knowledge gap has hampered attempts to respond to this re-emerging pest. This review aims to consolidate existing information available on this insect so that a consensus may be reached on key aspects of *H. marginata* biology and ecology, in particular the effect of environmental conditions on insect development. This will then provide a comprehensive source of information to inform and shape current and future research and management of *H. marginata*.

1-1.1 Geographical distribution

There is a long history of saddle gall midge attacking cereal crops in Europe. Reports of 'red vernicules' on wheat and barley in Bavaria in 1692 are thought to refer to *H. marginata* larvae (Weidner, 1985). The first reported economically important attack was in the former Yugoslavia in 1956 (Skuhravý *et al.*, 1983), with further serious outbreaks occurring across Europe over several decades in many countries including Belgium (Latteur, 1972), Romania (Popov *et al.*, 1998), Poland (Walczak, 1982), Hungary (Racz, 1973), the Netherlands (Nijveldt & Hulshoff, 1968), Sweden (Eklund *et al.*, 2005) and the Czech Republic (Skuhravý *et al.*, 1983) (Figure 1.1).

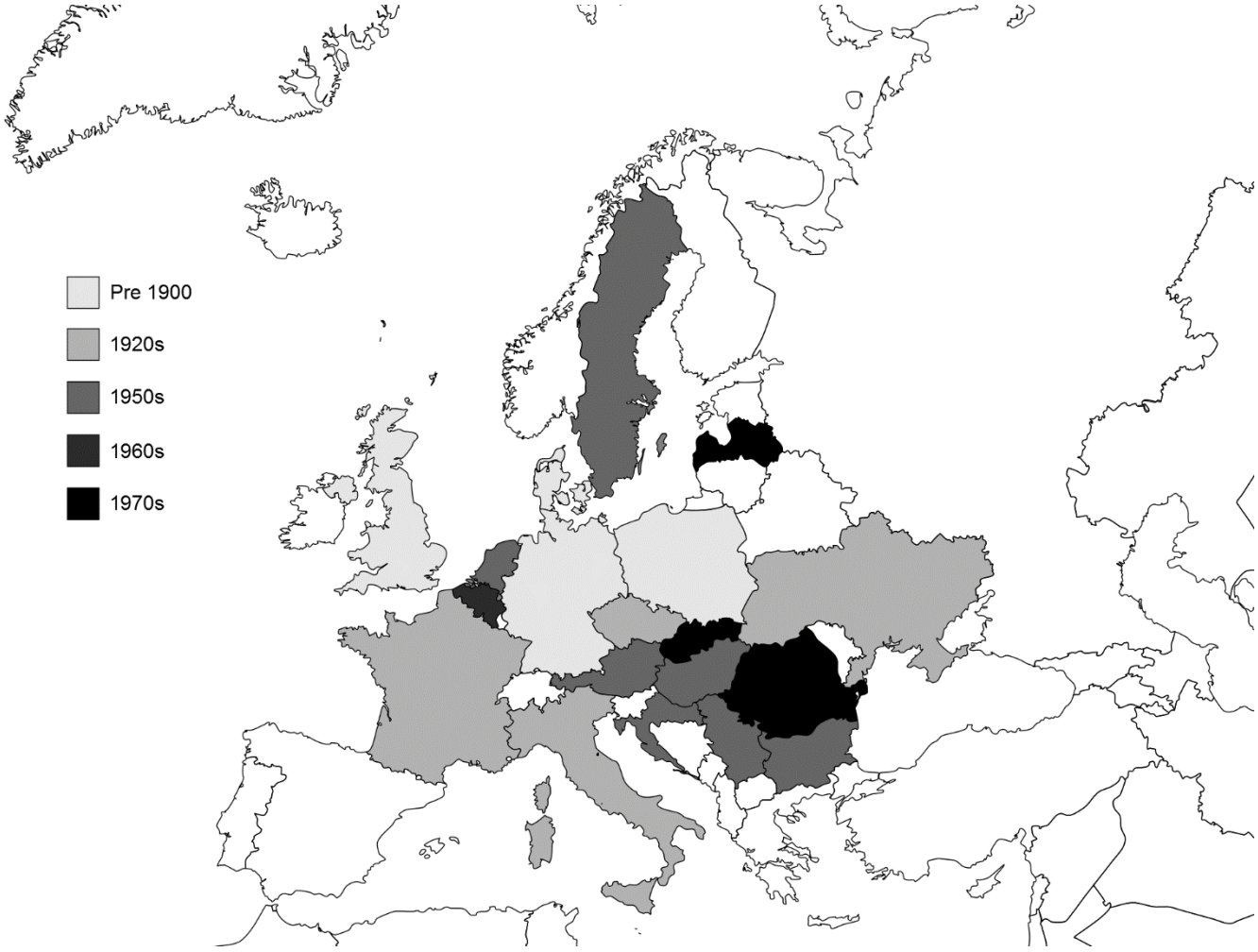


Figure 1.1. Map of European countries showing the decades in which *Haplodiplosis marginata* was first recorded, from 1692 until present

A survey of growers and agronomists in 2011 by HGCA (known as AHDB Cereals & Oilseeds from June 2015) in conjunction with ADAS, AICC, Dow AgroSciences, and NIAB TAG, demonstrated that the current UK distribution of *H. marginata* ranges from East Lothian to Cornwall (Caroline Nicholls, AHDB Cereals & Oilseeds, Pers. Comm., Figure 1.2). The known distribution of this pest is primarily based on reports following outbreaks and as such is likely to under-represent its range.

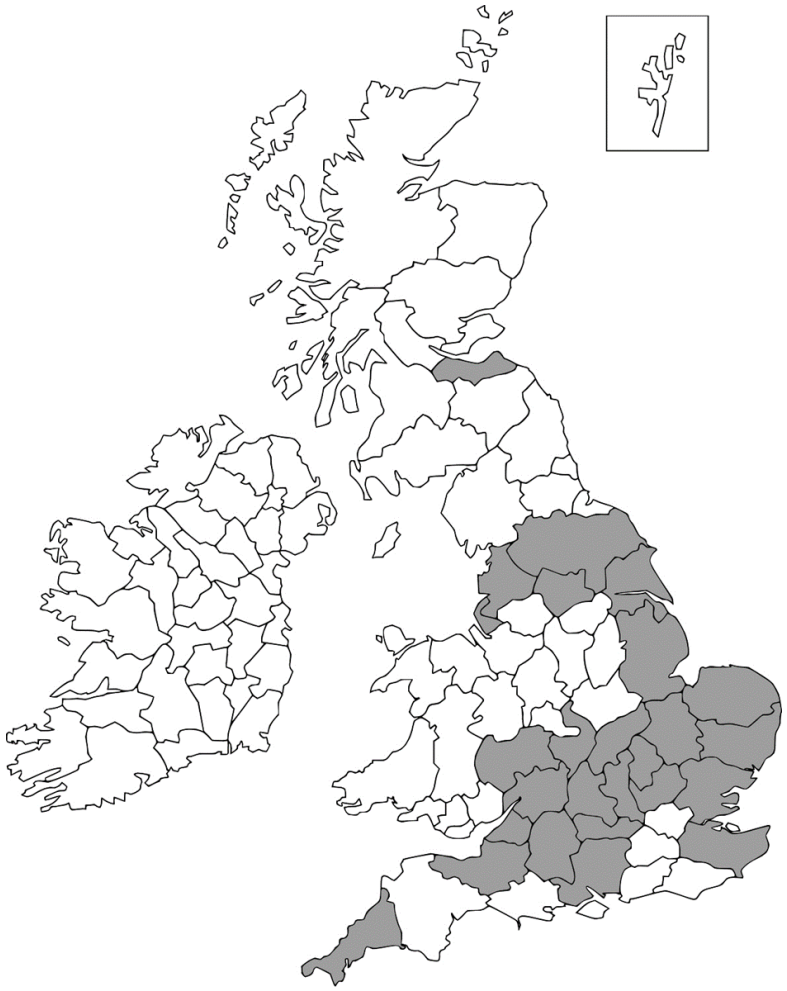


Figure 1.2. Map showing the 2011 areas with *Haplodiplosis marginata* infestation in the UK by county based on a survey of farmers and agronomists; shaded areas represent counties with at least one incidence of *H. marginata* infestation (Caroline Nicholls, AHDB Cereals & Oilseeds)

1-2 Taxonomy and morphology

1-2.1 Species history

The saddle gall midge belongs to the family Cecidomyiidae within the order Diptera and suborder Nematocera. This family is distinguished by the presence of a sclerotized sternal spatula in the larva and abdominal colouration that ranges from yellow to red. The subfamily Cecidomyiinae, to which *H. marginata* belongs, is characterised by reduced wing venation, antennae with fewer than 14 flagellomeres and larvae with only two dorsal papillae on the eighth abdominal segment (Harris, 1966; Gagné, 2004). Within the Cecidomyiinae, *H. marginata* belongs to the supertribe Cecidomyiidi, distinguished by the unique bi-nodal flagellomeres and many-looped circumfila on the male antennae (Gagné, 1994; Gagné, 2004).

Diplosis marginata was first described in 1840 by von Roser from specimens found on barley stems (Nijveldt & Hulshoff, 1968; Skuhravý *et al.,* 1993). In 1871, Wagner described *Diplosis equestris found* on wheat in Germany, giving it the common name "sattelmücke" (saddle midge) to make it easier for farmers to identify (Wagner, 1871). In 1900 *D. equestris* was moved into the genus Clinodiplosis by Kieffer and it was again moved in 1910 into the newly-created genus *Haplodiplosis* by Rübsaamen (Rübsaamen, 1910; Nijveldt, 1967). *Diplosis marginata* and *Haplodiplosis equestris* were later determined to be the same species by Nijveldt (1967), who concluded that *H. marginata* (von Roser) should be taken as the accepted name. *Diplosis equestris* (Wagner) is now listed as a synonym.

1-2.2 Descriptions of stages

1-2.2.1 Eggs and larval stages

Photographs of the different life stages of *H. marginata* are shown in Figure 1.3. The eggs of *H. marginata* are smooth and oval-shaped, normally 0.32 - 0.50 mm in length, with a slight red colouration that turns orange-red over time (Nijveldt & Hulshoff, 1968) (Figure 1.3 B.). Skuhravý *et al.* (1993) describe the morphology of the different developmental stages of *H. marginata*. As with other Cecidomyiidae, there are three larval instars. First instar larvae are 0.6 – 1.0 mm in length, ranging from white to pale red. The second instar larvae are up to 1.5 mm long and dark yellow in colour with a

visible gastrointestinal tract. Third instar larvae are between 2.5 mm and 4.0 mm long, 1.0 - 1.3 mm wide, pale red to red in colour, and have the characteristic sternal spatula on the ventral part of the third thoracic segment (Figure 1.3 D.). The pupae are 3.5 - 4.5 mm long and are initially red, however as they develop, the terminal section gradually turns black. Two pairs of horn-like protrusions are located at the anterior end, the largest of which are thought to serve as respiratory organs (Nijveldt & Hulshoff, 1968). The abdominal areas of the pupae are orange-red in males and bright red in females (Skuhravý *et al.,* 1993).

1-2.2.2 Adults

Adult midges range in size from 2 – 5 mm depending on the nutrient availability to the developing larvae. Females are generally larger than males. Egg numbers are correlated with female size and can be as high as 260 per individual (Skuhravý *et al.*, 1993). Adults of both sexes have a black head and thorax with a red-coloured abdomen, however, the abdomen of the females is wider and more brightly coloured due to the eggs inside (Figure 1.3 A.). The wing length of the female averages 3 - 4 mm. Adults are sexed on the basis of antennal and genital morphology, as well as the presence of eggs in the female. Both sexes have antennae with two basal segments and 12 flagellomeres (Nijveldt & Hulshoff, 1968). All but the terminal flagellomeres of the male antennae are binodose, having two swellings at either end, with rings of short circumfilar loops on each node as well as simple circumfila. The flagellar segments of the female are cylindrical and elongated with two rings of sensillary hairs on each (Figure 1.4) (Harris, 1966; Nijveldt & Hulshoff, 1968; Skuhravý *et al.*, 1993). The male copulatory organ is rod-shaped with a two-lobed upper lamella and shorter, lower lamella. The female ovipositor comprises three lamellae, the upper two larger than the lower, all with short sensory hairs (Figure 1.5) (Nijveldt & Hulshoff, 1968).

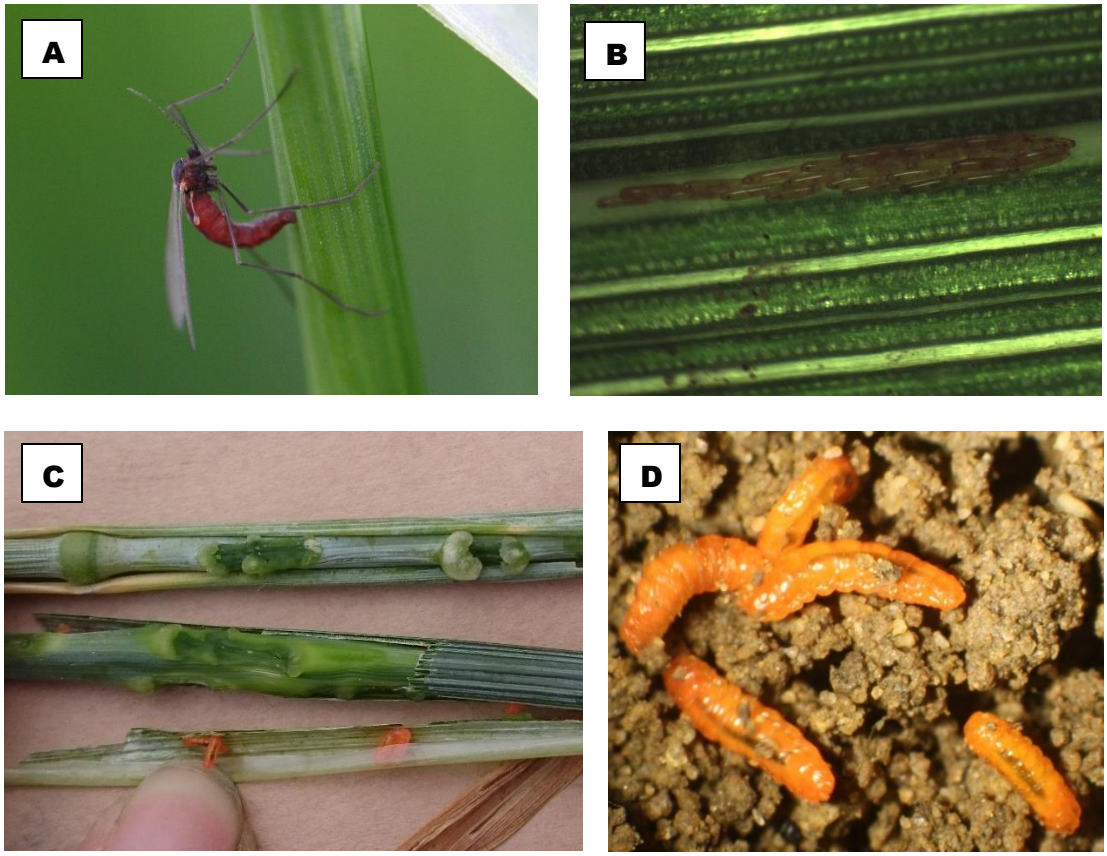


Figure 1.3. Photographs of different life stages of *Haplodiplosis marginata* **A.** adult female oviposition **B.** eggs laid along leaf veins **C.** immature larvae and gall formation on wheat stems **D.** mature larvae in soil

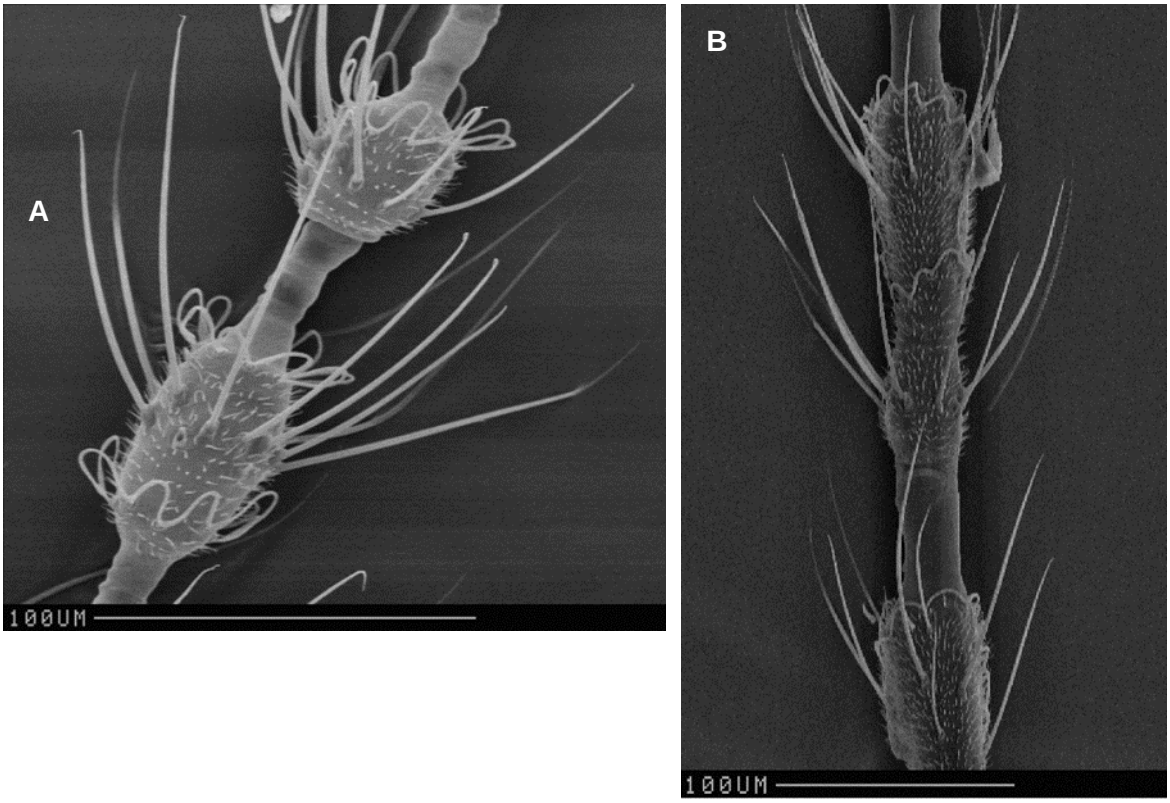


Figure 1.4. Scanning electron microscope images of antennal segments of *Haplodiplosis marginata* **A.** female **B.** male

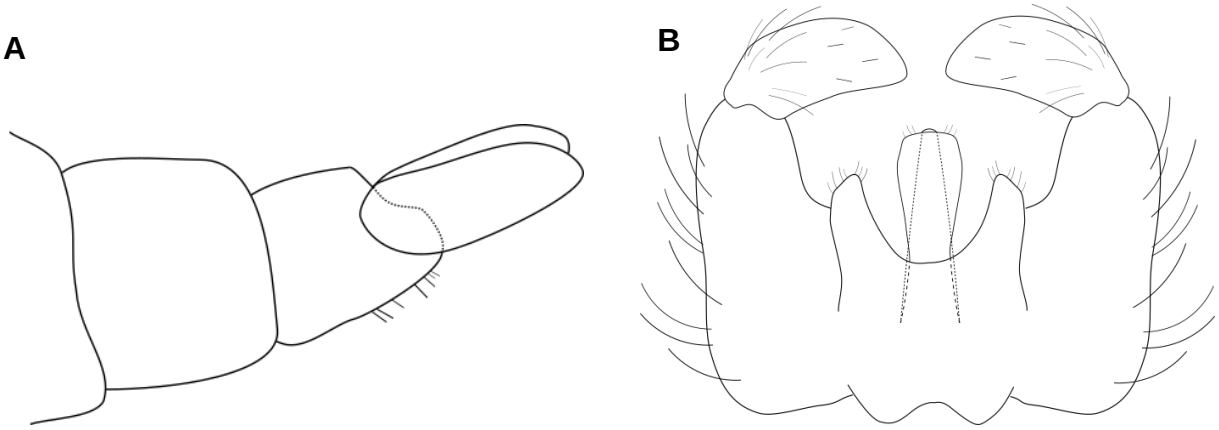


Figure 1.5. Depiction of *Haplodiplosis marginata* genitalia adapted from Harris (1966) **A.** female **B.** male

1-3 Life cycle and pest status

1-3.1 Life Cycle

The life cycle of *H. marginata* was recently described in detail by Censier *et al.* (2015). *Haplodiplosis marginata* is a univoltine species with the flight period beginning as early as mid-April and lasting until the beginning of July depending on environmental conditions (Censier *et al.*, 2012) (Figure 1.5). Adults are short-lived and have limited dispersal ability. Lifespan estimates vary between 1 and 7 days (Njveldt & Hulshoff, 1968, Popov *et al.*, 1998) and flight distances average 18 m (Schütte, 1964a), although male flight has been recorded as more than 120 m in some instances (Nijveldt & Hulshoff, 1968). Males generally emerge first and fly low to the ground in search of females (Skuhravý *et al.*, 1983). Females may undertake several short flights of 5 - 15 m when seeking a suitable oviposition site and appear to fly slightly higher than males (Skuhravý *et al.*, 1983; Skuhravý *et al.*, 1993). Adult *H. marginata* have been caught at heights of up to 6 m, meaning flight distances may be increased in high winds (Skuhravý *et al.*, 1993). Eggs are laid in a chain-like or raft-like formation along the leaf veins of cereals and grasses on either leaf surface (Dewar, 2012, Censier *et al.*, 2015). The location of oviposition is likely to depend on the position and angle of the leaf, apparently varying between crops (Nijveldt & Hulshoff, 1968). Barnes (1956) reports that females will preferentially lay on the topmost leaf, however this was only found to be true for spring barley in a later study by Nijveldt & Hulshoff, (1968), with the lowest leaf being more favourable for oviposition in wheat. Hatching occurs 1 - 2 weeks after oviposition depending on environmental conditions following which the larvae migrate down the leaf and begin to feed on the stem from beneath the leaf sheath (Golightly & Woodville, 1974). Larval feeding on the stem results in galls 2 - 5 mm in length which appear as the elongated 'saddle shaped' depressions characteristic of this species (Figure 1.3 C.). The larvae reach maturity between June and mid-July and drop from the stem to enter diapause in chambers in the soil where they overwinter (Golightly & Woodville, 1974; Skuhravý *et al.*, 1993). Pupation generally occurs the following spring, however larvae can remain in diapause in the soil for several years (Nijveldt & Hulshoff, 1968, Popov *et al.*, 1998; Dewar, 2012). Rarely, larvae can be found in cocoons in the soil stage (Censier *et al.*, 2014a).

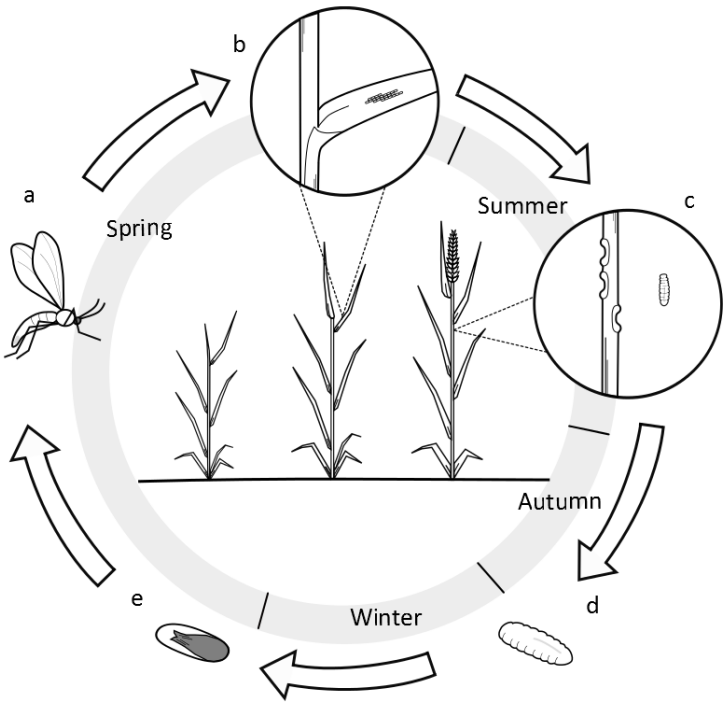


Figure 1.6. Life cycle of *Haplodiplosis marginata*; **A.** adult emergence, **B.** oviposition, **C.** gall formation and larval maturation, **D.** larval diapause, **E.** pupation.

1-3.2 Outbreaks

The reasons for the recent resurgence of *H. marginata* as a pest are as yet unknown. This species is thought to have benefitted in part from the intensification of farming methods, particularly the continuous sowing of wheat and barley crops (Skuhravý *et al.*, 1983). The HGCA survey, the results of which are summarised in Roberts *et al.,* 2014, showed that the majority of crops (48%) displaying symptoms consistent with *H. marginata* infestation were continuous cereal crops. A further 24% were however, first wheat crops, which can be explained by observations that larvae are able to persist in the soil for more than one year (Nijveldt & Hulshoff, 1968, Popov *et al.*, 1998; Dewar, 2012). Given the relatively low dispersal ability of this pest, localised outbreaks are more likely to occur where wheat is being grown

successively, or in close proximity to previously affected fields (Schütte, 1964b). Weed management through the use of crop rotations became less common as herbicide usage increased in the UK in the 1950s and 1960s allowing continuous cereal crops to be sown (Robinson & Sutherland, 2002). Low diversity monocultures can lead to increases in pest densities due to the availability of the host plant but also changes in the arthropod community which may include natural enemies (Matson *et al.*, 1997). These factors may have contributed to the severe outbreaks of *H. marginata* witnessed in the UK in the late 60s and early 70s (Woodville, 1968, 1973; Golightly & Woodville, 1974). Since 1980 however, the continuous cropping of wheat has steadily declined in the UK according to the Defra Winter Wheat Pest and Disease Survey (Judith Turner, Fera, Pers. Comm) suggesting that on a landscape scale at least, continuous wheat systems do not explain recent increases in *H. marginata* outbreaks.

Selective breeding of cereal varieties to increase productivity may have increased the availability of food resources for developing larvae and reduced levels of natural resistance to the pest (Skuhravý *et al.*, 1983), although the mechanism of any such resistance is unknown. Other possible factors include increased use of minimum tillage, which may allow better pupal survival as they are not buried as deeply by cultivations. For example in sorghum midge, *Stenodiplosis sorghicola*, the depth at which larvae diapause affects the post-diapausal adult emergence, presumably due to differences in exposure to environmental conditions (Franzmann *et al.*, 2006). The reduced disturbance from minimum tillage would however, also benefit natural enemies (Landis *et al.*, 2000), which could negate some of the benefit to pest populations. Rates of predation of *H. marginata* by natural enemies in the field however, are as yet unknown (see section 1-4.3). Pesticide usage is likely to have had an effect on *H. marginata* populations. This includes long term changes in the active substances being used and the rate of application. As mentioned previously, changes in arthropod communities as a result of agricultural practices may allow for the build-up of certain pest populations if the pressure from natural enemies is removed (Matson *et al.*, 1997). For example, the widespread use of broad spectrum insecticides in the UK during the 1960s and 1970s might have contributed to the outbreaks of *H. marginata* occurring at that time through the depletion of natural enemy populations. This was seen in cotton production in the USA during the same period, where a number of secondary pests emerged for this reason as a result

of broad-spectrum pesticide use (Begon *et al.*, 2006). More recently however, the total weight of insecticides applied to cereal crops in the UK has decreased by 54% since 1990 (Fera, 2016). This will in part have been due to improvements in the efficacy of the active substances in the products along with novel formulations and compounds. The total percentage area of cereal crops treated with an insecticide has also declined since 1990, with most crops only receiving a single treatment (Fera, 2016). Conversely, such reductions in pesticide use may have benefited *H. marginata* populations in the long term and allowed populations to build up to outbreak levels, although it is a complex area of study with no clear evidence at this stage. Changes in the abundance or diversity of grass species on arable land may also have influenced *H. marginata* populations. For example, a survey of arable weeds in 2000 showed the abundance of some common species such as black-grass (*Alopecurus myosuroides*) and couch grass (*Elymus repens*) had either increased or remained stable since the 1960s (Sutcliffe & Kay, 2000). Black-grass in particular is now a serious problem on arable land due to the increasing incidence of multi-herbicide resistance in this species (Hull *et al.*, 2014). Both species have the potential to act as wild hosts to *H. marginata* (Skuhravá & Skuhravý, 2014), allowing populations to persist in the absence of a cereal crop (see below).

1-3.3 Host range

In a study of host plant preferences comparing 48 different species of cereals and grasses in the field, *E. repens* was the most heavily attacked by *H. marginata* (Schütte, 1964b). *Haplodiplosis marginata* also had the lowest levels of larval mortality on *E. repens* when compared with other host plants such as wheat and barley (Skuhravý *et al.*, 1983). *Elymus repens* and other wild grasses have the potential to act as alternate hosts but it is as yet unclear whether increased availability of alternate hosts would facilitate pest populations or reduce pressure on the crop (Schütte, 1964b, Skuhravý *et al.*, 1983; Woodville, 1968). Variation in severity of attack, defined as the number of galls per stem, has been shown to occur between different varieties of wheat, barley and rye (Skuhravý *et al.*, 1993) and between countries in which the same variety is grown (Nijveldt & Hulshoff, 1968). Some degree of resistance to *H. marginata* was found in 28 out of 400 wheat varieties trialled in the Netherlands in 1966 as judged

by a lack of gall formation (Nijveldt & Hulshoff, 1968), and complete resistance has recently been found in an old Russian variety (Mike Taylor, Limagrain, Pers. Comm.), although the exact mechanism of this resistance is currently unknown. No modern variety has however, yet been identified that offers complete resistance, possibly as a result of outbreeding of any resistance traits (Skuhravý *et al.*, 1993, Censier *et al.*, 2015). Oats are a poor host with data suggesting that only 2 - 5% of the larvae survive following hatching (Skuhravý *et al.*, 1993). A more recent study found that oats were a less attractive host when sown next to spring wheat, and potential resistance was observed in one variety on which no galls were formed despite evidence of oviposition on the leaves (Censier *et al.*, 2013). Unlike with cereal leaf beetle (*Oulema melanopus*), a higher density of leaf pubescence does not reduce the rate of infestation of *H. marginata* on wheat (Schillinger & Gallun, 1968; Lange & Jochensen, 1987).

1-3.4 Crop Damage

1-3.4.1 Primary crop damage

Crops most at risk are spring crops, particularly wheat and barley (Skuhravý *et al.*, 1983, Skuhravý *et al.*, 1993) but damage has also occurred in late sown (after mid-November) winter wheat and barley (Pope & Ellis, 2012; HGCA, 2012). Golightly and Woodville (1974) observed that damage is most severe when egg-hatch coincides with stem extension, whilst losses are incurred on crops that are in or beyond the booting stage at the time of larval infestation are minimal. Cereal crops are therefore most vulnerable to attack between growth stages 31-39 (Tottman & Makepeace, 1979). Early sown spring crops appear to be less susceptible as the plant tissue is more mature at the time of egg hatch, potentially making it more difficult for the larvae to feed (Skuhravý *et al.*, 1993).

Where high population densities occur, there may be as many as 60 galls per stem (Skuhravá & Skuhravý, 2014). Galls are generally formed on the top three internodes where the plant tissue is least mature. A substance secreted by the larvae inhibits the development of epidermal cells in the immediate vicinity of the insect, while the surrounding tissues continue to develop, forming the gall (Nijveldt & Hulshoff, 1968). Development of vascular tissue is disrupted around the site of the gall, which can restrict the flow of nutrients to the ear. This can lead to shrivelled or underdeveloped grains

(Golightly, 1979) and reductions in stem length (De Clercq & D'Herde, 1972; Popov *et al.*, 1998), ear length (De Clercq & D'Herde, 1972), and thousand grain weight (Woodville, 1968). Galling has been shown to result in reductions in grain number and thousand grain weight in wheat by 63% and 64% respectively (Popov *et al.*, 1998).

1-3.4.2 Secondary crop damage

Destruction of the plant cuticle in the area of the gall leaves the plant vulnerable to secondary attack by bacteria or fungi, particularly in wet weather (Nijveldt & Hulshoff, 1968; Skuhravý *et al*., 1993; Eklund, 2005). Gall formation can also weaken the stem which increases the risk of lodging, where the stem breaks or bends so that the ear falls below the level of the combine and cannot be harvested (Woodville, 1970; Golightly & Woodville, 1974; Gratwick, 1992). This is of particular concern where attack coincides with a period of high winds and can be responsible for substantial yield losses.

1-3.5 Economic consequences

1-3.5.1 Potential yield loss

Estimates suggest that when the percentage of infested wheat stems reaches 70%, losses of 2.2 t/ha could occur (Skuhravá & Skuhravý, 2014). A recent study in Belgium showed a correlation between number of galls and yield loss in winter wheat, in the most severe case yields fell by 191 kg/ha (0.191 t/ha) for every increase of 100 galls per 100 stems (Censier *et al.*, 2016b). Past outbreaks of saddle gall midge in the UK have resulted in losses of 0.6 t/ha (Woodville, 1968). At the current average wheat yield in the UK of 7.9 t/ha, this would represent potential yield losses of 2.4 – 7.6% which could severely impact on revenue. For example, if only 1% of the current 5-year average total wheat yield of the UK (14.7 million tonnes/year) was affected by *H. marginata* damage, total yield could be reduced by around 11,000 tonnes a year representing a loss of £1.56 million to the industry at the current market value of £140/tonne. There are no published figures for yield losses or financial losses incurred in the recent UK outbreaks, however, the recent HGCA survey anecdotally reports that 52% of respondents who observed saddle gall midge infestation observed subsequent yield loss. In the most severe case, there

was an estimated 70% decrease in yield as reported by an agronomist in Buckinghamshire (Ellis *et al.*, 2014).

1-3.5.2 Economic thresholds

Estimates of thresholds of soil densities of larvae above which economic losses occur range from 12.4 million per hectare (Golightly & Woodville, 1974) to as little as 300,000 per hectare (Popov *et al.*, 1998). In terms of infestation, it has been estimated that more than three galls per stem leads toloss of yield (Skuhravý *et al.*, 1993). In Denmark, this threshold rises to five galls per stem (Woodville, 1973), in the UK it is between 4.5 and nine galls (Ellis *et al.*, 2014) and in Germany it is between five and ten (Schütte, 1983). The variation in these estimates demonstrates the current uncertainty surrounding the economic impact of this pest. In particular it is difficult to determine whether yield losses reported by farmers with *H. marginata* damage can be attributed entirely to the pest or if other factors are involved. As there are currently no estimates of the finiancial impact of saddle gall midge in the UK, economic thresholds cannot be determined. Aside from this, thresholds based on gall number are of limited use in pest management, as control measures are likely to be ineffective at this stage. Number of adult midges caught in traps might be a more appropriate measure on which to base pest management decisions. It is acknowledged that the actual damage caused depends on many factors such as crop type, growth stage and weather conditions (De Clercq & D'Herde, 1972; Censier *et al.* 2015).

1-4 Current control methods

1-4.1 Cultural control

Agricultural systems in which cereal crops are grown continuously are particularly susceptible to outbreaks of *H. marginata* as high densities of larvae accumulate in the soil. Break crops are generally accepted as an effective means of reducing infestation by depleting larval soil populations (Censier *et al.*, 2016b). Skuhravý *et al.* (1993) showed that infestations of wheat varieties were greatly reduced when sown after non-susceptible crops such as alfalfa or potato rather than susceptible cereals. Even so, with the potential for *H. marginata* larvae to enter extended diapause, breaks of one year may not

always be enough to reduce soil populations to below economically damaging levels. Field trials over six years in the Netherlands showed that a two-year break did not entirely eradicate *H. marginata* populations, and oats were often not particularly effective as a break crop despite being a relatively poor host plant (Nijveldt & Hulshoff, 1968). The introduction of the EU crop diversification requirement as part of the 2013 CAP reform aims to encourage farmers to grow a greater variety of crops by specifying a minimum number of crops and a maximum land cover amount for the two main crops (Regulation (EU) 1307/2013, 2013). This may result in fewer *H. marginata* outbreaks if continuous wheat systems are disrupted by widespread use of rotations and break crops.

1-4.2 Chemical control

Chemical controls applied directly to the soil are of limited efficacy, probably owing to insufficient penetration of the soil to the depths where overwintering larvae are found (Popov *et al.*, 1998). Foliar applications of organophosphates such as malathion and dimethoate applied to the crop have shown some efficacy against eggs and newly-hatched larvae of *H. marginata* on wheat in Romania (Popov *et al.*, 1998); and in the UK chlorpyrifos effectively reduced numbers of larvae and galls in wheat when applied at the visible flag leaf stage (GS 37) prior to its withdrawal (Roberts *et al.,* 2014). Control has also been achieved with pyrethroids such as alpha-cypermethrin (Popov *et al.*, 1998), and with deltamethrin, lambda-cyhalothrin and tau-fluvalinate on winter wheat (Censier *et al.*, 2012, 2015; Ellis *et al.*, 2014).

Early recommendations for chemical control advised using persistent insecticides and to time applications for three to five days after the first adults were recorded or when the eggs were found on 20% of leaves (Skuhravý *et al.*, 1993). There is a limited timeframe for application as once the larvae are beneath the leaf sheath they are protected from contact-acting insecticides (Gratwick, 1992). Repeated applications may be warranted as adult flight can persist for up to ten weeks (Censier *et al.*, 2012). Censier *et al.* (2012) found that treating the crop with pyrethroid insecticides twice with a two week interval resulted in 75 - 87% efficacy based on reductions in the percentage of attacked stems and mean gall number per stem. In a further study the authors recommended treating the crop to

coincide with peak adult flight (Censier *et al.*, 2016b). The authors, however, acknowledged that phenological monitoring was essential in order to synchronise applications with vulnerable life stages (Censier *et al.*, 2016b). Ellis *et al.* (2014) reported that chemical controls applied at the start of adult emergence resulted in the lowest yield loss, although treatments applied 7 – 10 days post emergence or when the first eggs were seen also reduced midge infestation. Ideally a forecasting model would be used to predict the onset of adult emergence and used to time in-field monitoring efforts on which chemical treatments may be based (see Chapter 2).

1-4.3 Natural enemies

Carabidae or Staphylinidae may contribute some degree of population control having been observed feeding on *H. marginata* larvae at the soil surface (Golightly & Woodville, 1974; Skuhravý *et al.*, 1993). Species from these families have similarly been shown to feed on orange wheat blossom midge larvae (*S. mosellana*); a species that shares many characteristics with saddle gall midge (Holland *et al.*, 1996). Larval stages may be parasitised by *Chrysocharis amyite* and *Platygaster taras* (Baier, 1963; Skuhravý, 1982), although research suggests that saddle gall midge mortality due to the latter is only 1 - 2% and the former only attacks larvae found on wild grasses as females are unable to penetrate the leaf sheaths of cereals with their short ovipositors (Nijveldt & Hulshoff, 1968; Woodville, 1968; De Clercq & D'Herde 1972). Parasitism of *H. marginata* eggs by a novel parasitic hymenopteran was found in Belgium in 1965. The species was described as *Platygaster equestris* in reference to the host's earlier name (*Haplodiplosis equestris*) and was found to parasitise up to 10% of *H. marginata* eggs (Spittler, 1969). An unidentified Chalcidid in Austria was found to parasitise up to 23% of *H. marginata* eggs according to Faber (1959 cited in Nijveldt & Hulshoff, 1968). Another *Platygaster* species was observed in 1966 attacking *H. marginata* larvae in the Netherlands, parasitising between 1 and 40% of larvae. Within a year, populations of the parasitoid overtook that of *H. marginata* although it is not clear whether declines in the latter were because of parasitism alone (Nijveldt & Hulshoff, 1968). *Holarcticesa clinius* is also recorded as a parasitoid of *H. marginata* in the Universal Chalcidoidea Database (Noyes, 2017).

Although populations of such parasitoids may help to keep *H. marginata* numbers in check, there is little evidence to suggest that any of these species would be appropriate for use as biological controls.

1-5 Influence of environmental conditions on *H. marginata*

Like many Cecidomyiidae, outbreaks of *H. marginata* are highly sporadic. Populations fluctuate from year to year and in the absence of a single correlating biotic or abiotic factor, predictions of future population size are difficult (Woodville, 1973; Basedow, 1986). Numbers of larvae in the soil can increase gradually over several years or rapidly within a generation (Basedow, 1986). High larval population densities in the soil can result in outbreaks (Skuhravý *et al.*, 1993), however, the level of damage further depends on elements such as reproductive success, crop susceptibility and weather conditions

1-5.1 Effects of temperature and moisture on *H. marginata* development

Skuhravý *et al.* (1983) have reported high larval mortality in the soil stage after recording emergence levels of just 5-12% in field experiments in Slovakia. It is not clear, however, what proportion of the population remained in diapause. Population declines have been observed following unfavourable weather conditions such as cold temperatures and extremes of soil moisture content, however this is not always consistent (Woodville, 1973; Popov *et al.*, 1998; Skuhravý *et al.*, 1983, 1993; Pope & Ellis, 2013). There is evidence of larval resilience in the soil stage. Cold tolerance was observed in a laboratory experiment by Nijveldt and Hulshoff (1968), where 49% of larvae survived being in frozen clay soil after 48 days, however survival was zero after two weeks at -10 ˚C in further experiments by De Clercq and D'Herde (1972). *Haplodiplosis marginata* larvae may also survive periods of flooding: over 50% of 100 larvae were able to survive immersion in water for 28 days. This supports field observations of larvae surviving in flooded soils (Nijveldt & Hulshoff, 1968) but disagrees with a recent UK study by Pope and Ellis (2013) who observed high levels of larval mortality following heavy rainfall. Additionally, very wet weather in summer may cause eggs to be washed off the leaves before hatching (Gratwick, 1992). On the other hand, very hot and dry summers may result in egg or larval desiccation

(Eklund, 2005). The prevalence of *H. marginata* in heavy soils that contain a high proportion of clay (Golightly & Woodville, 1974) is thought to be due to the higher moisture content of heavy soils protecting the larvae from desiccation (Andersson, 1969). Nonetheless, some drought tolerance has been recorded in experiments by Nijveldt and Hulshoff (1968): after 14 days of drought under controlled conditions, 52% of 600 larvae developed into adults while 15% remained in diapause. Larval survival dropped to 11% after 60 days of drought (Nijveldt & Hulshoff, 1968). It was thought that, as with the Cecidomyiid wheat blossom midges *Sitodiplosis mosellana* (orange wheat blossom midge) and *Contarinia tritici* (yellow wheat blossom midge) the larvae overwinter in cocoons, however cocoons have only ever been found in three field populations; one each in the UK (Barnes, 1956), the Netherlands (Nijveldt & Hulshoff, 1968) and Belgium (Censier *et al.*, 2014a). Cocoon formation is therefore considered to be rare in this species and is likely to be a response to drought, preventing desiccation (Nijveldt & Hulshoff, 1968; Censier *et al.*, 2014a).

Temperature and moisture are likely to be closely linked to the termination of diapause (Gratwick, 1992). *Sitodiplosis mosellana*, *Contarinia tritici* and *Contarinia sorghicola* (sorghum midge), also in the supertribe Cecidomyiidi, all require an interaction between temperature and moisture for diapause termination and adult emergence (Basedow, 1977; Baxendale & Teetes, 1983; Oakley & Ellis, 2009, Jacquemin *et al.*, 2014). Increased soil moisture may make it easier for larvae to move up through the soil profile to pupate whilst rising temperatures are likely to trigger the end of diapause for this species. This is supported in the literature, with numerous reports of warm, humid conditions prevailing shortly before an outbreak (Gratwick, 1992). It has been observed that under laboratory conditions, diapause in *H. marginata* is not terminated below 10°C (Baier, 1963) with larvae unable to survive prolonged temperatures of 5°C or 30°C (Nijveldt & Hulshoff, 1968). If conditions are too dry, about 75% of the larvae will remain in diapause for another year (Dewar, 2012) but even in suitable conditions, some 20% of larvae may extend diapause (Popov *et al.*, 1998). Extended larval diapause has been observed in other cecidomyiids (Harris & Foster, 1999). The orange wheat blossom midge, *S. mosellana* can remain in the soil within cocoons for ten years or more (Oakley & Ellis, 2009). The duration of diapause in *H.*

marginata has been shown to extend to at least six years, and is likely to vary according to both biotic and abiotic factors (Nijveldt & Hulshoff, 1968).

1-6 Summary

Integrated pest management programmes could offer a viable solution to reducing the risk and mitigating the consequences of future *H. marginata* outbreaks. In order for this to happen however, the current knowledge gaps concerning the biology and ecology of this insect outlined by this literature review need to be addressed. In particular, a breadth of research needs to be conducted over several years at a number of locations to understand how this species interacts with the wider environment and the implications for farmers. This information can be used as the foundation for management strategies and ecologically based IPM programmes. The ultimate aim for any IPM programme should be to consider the ecosystem as a whole, encompassing multiple pests within cropping system. This is not possible, however, without first conducting species-level research to understand each individual component of that ecosystem. This thesis therefore, aims to broaden existing knowledge of *H. marginata* and deliver a critical first step towards the development of IPM-compatible control options.

1-6.1 Thesis aims and objectives

Aims: To expand upon existing knowledge of the biology and ecology of *Haplodiplosis marginata* and improve current monitoring methods for this pest to aid the development of an effective integrated pest management programme

Objectives:

1. Study the effects of meteorological and soil conditions on the development of *H. marginata* in the soil stage through to adult emergence over three years. Use this information to develop a model based on thermal accumulations and other environmental factors in order to predict the timing of adult emergence.
2. Use electroantennography to assess the responses of male *Haplodiplosis marginata* to the major and minor components of the female sex pheromone. Test optimum

pheromone formulations, loadings and dispenser types in field experiments to develop an effective lure for trapping male *H. marginata.*

3. Identify the main factors affecting pheromone trap catch with the chosen lure in the field and determine a recommended best practice for users.
4. Identify the predatory natural enemies of *Haplodiplosis marginata* through the development a PCR-based assay to identify the presence of *H. marginata* in the guts of field-caught arthropod predators.

Chapter 2

Development of a degree-day based phenological forecasting model of saddle gall midge (*Haplodiplosis marginata*) (Diptera: Cecidomyiidae) emergence

Abstract

Outbreaks of saddle gall midge (*Haplodiplosis marginata*) affecting wheat and other cereals are difficult to anticipate and may not be identified until damage has occurred. The work presented here describes how simple degree day models can be used to predict *H. marginata* emergence based on soil temperatures. The importance of the availability of regular long-term trapping data is demonstrated by updating and improving upon initial models to predict the progression of emergence. Emergence of *H. marginata* adults at three sites in the UK was monitored over three years, showing that the date of emergence is closely associated with degree-day accumulations of soil temperatures. The presence of multiple peaks in emergence over several weeks was confirmed. Rainfall events followed by an accumulation of 512DD (±9.11DD) could be used to predict these peaks with greater precision than degree day accumulations alone. Cumulative percentage emergence as a function of degree day accumulations was best described by a probit model and a bimodal model. Probit model and bimodal models predicted *H. marginata* emergence at other sites and years to within 4 and 3 days respectively. Variation in trap catch is discussed in the context of soil texture and subsequent crop damage. Application of these models will enable growers to forecast peaks in emergence, make informed assessments of crop risk and time application of chemical controls appropriately where required.

Part-published in: Rowley, C., Cherrill, A., Leather, S., Nicholls, C., Ellis, S. & Pope, T. (2016) A review of the biology, ecology and control of saddle gall midge, *Haplodiplosis marginata* (Diptera: Cecidomyiidae) with a focus on phenological forecasting. *Annals of Applied Biology*, **169**, 167–179.

Remainder of chapter published as: Rowley., C., Cherrill, A., Leather, S. & Pope, T., (2017) Degree-day based phenological forecasting model of saddle gall midge (*Haplodiplosis marginata*) (Diptera: Cecidomyiidae) emergence, *Crop Protection,* **102**, 154-160.

2-1 Introduction

Saddle gall midge (*Haplodiplosis marginata*) is an occasional pest of cereals across Europe. The larval stage of this insect is phytophagous, causing the formation of saddle-shaped depressions (galls) on the stems of host plants (Censier *et al.*, 2015). Crops most at risk are spring wheat and spring barley (Skuhravý *et al.*, 1983; Skuhravý *et al.*, 1993), but the insect will also damage winter wheat and barley (Pope & Ellis, 2013). Damaged plants can exhibit a loss in yield due to shrunken grains as a consequence of galls disrupting the flow of nutrients to the ear (Woodville, 1968; Golightly, 1979). Stem breakage and secondary attack from pathogens at the site of the gall can also occur (Nijveldt & Hulshoff, 1968; Golightly & Woodville, 1974; Gratwick, 1992; Skuhravý *et al.*, 1993). Following a resurgence of *H. marginata* outbreaks in several European countries from 2010 onwards, attempts have been made to consolidate and extend current knowledge of this insect to better inform pest management options (Censier *et al.*, 2015; Rowley *et al.*, 2016). Such reviews have highlighted the lack of information concerning the factors affecting *H. marginata* development and life cycle events.

Haplodiplosis marginata populations can fluctuate wildly on a yearly basis, making outbreaks difficult to anticipate (Woodville, 1973; Basedow, 1986). Adult midges generally begin to emerge between the end of April and early May (Censier *et al.*, 2015; Rowley *et al.*, 2016), however early stages of infestation are seldom recognised due to the inconspicuous nature of the midge (Harris & Foster, 1999). Once damage is evident, chemical control applications are often unsuccessful as the larvae are protected by the leaf sheath (Gratwick, 1992). The use of reliable monitoring and forecasting tools are therefore critical in effective management of this pest (Censier *et al.*, 2015).

The sex pheromone of *H. marginata* has been identified as 2-nonyl butyrate (Censier *et al.*, 2014b) which has led to the development of species specific pheromone traps (Censier *et al.* 2016a, Rowley *et al.*, 2017). This advance has made it possible to reliably monitor the emergence and flight activity, providing opportunity to easily study populations in the field (Censier *et al.*, 2016a). In pest management, pheromone monitoring can be used to time chemical controls appropriately (Witzgall *et al.*, 2010). The traps, however only provide a limited amount of advanced warning of insect activity and cannot predict the peaks in emergence which have been observed previously in this species (Censier

et al., 2016b). In addition, traps can be difficult to maintain consistently over an entire flight season and give no indication as to the duration of emergence. Phenological forecasting is a tool used in pest management to predict insect emergence and activity by modelling the progression of a particular developmental stage in relation to environmental variables (Prasad & Prabhakar, 2012). Such models can be used to support pheromone monitoring, by predicting when to deploy traps and identifying periods of peak activity on a year-to-year basis. Successful forecasting models have so far been developed for other pest Cecidomyiidae such as orange wheat blossom midge (*Sitodiplosis mosellana*) (Elliot *et al.*, 2009; Jacquemin *et al.*, 2014); swede midge (*Contarinia nasturtii*) (Hallett *et al.*, 2007); sorghum midge (*Contarinia sorghicola*) (Baxendale *et al.*, 1984); blueberry gall midge (*Dasineura oxycoccana*) (Hahn & Isaacs, 2002); and pine needle gall midge (*Thecodiplosis japonensis*) (Son *et al.* 2007). When combined with meteorological data, models can provide assessments of crop risk over a wide geographical area and prompt farmers to inspect crops or deploy monitoring traps (Prasad & Prabhakar, 2012). Outputs from models may also feed into more complex decision support systems to guide farmers on when to employ pest management strategies (Strand, 2000).

Currently, there are no approved pesticides for the control of saddle gall midge in the UK, and there are few estimates of the relationship between crop damage and yield loss. In a study by Censier et al., (2016b) the authors estimated decline in yield of 0.191 t/ha for every increase of 100 galls per 100 stems. Ellis et al., (2014) reported that the minimum gall number which induced reductions in grain weight was between 4.5 and nine galls per stem, compared to unifested tillers. This will vary depending on variety, growth stage at the time of emergence and environmental conditions, however. The link between population size and galling intensity is yet to be established, but could provide the basis for economic thresholds to be determined. With this information, farmers could estimate the potential damage to the crop from numbers of midge caught in traps and decide if the expected reductions in yield would be greater than the cost of any control options before galling occurs.

Here, the feasibility of generating an early warning system for *H. marginata* based on environmental conditions is explored by using a simple degree-day based phenological forecasting model to predict insect emergence. Soil moisture has previously been identified as being important in

H. marginata emergence (Skuhravý *et al.*, 1983) and so an attempt is made to improve upon this initial model by identifying the role of rainfall in the phenology of this insect. Additionally, intensive sampling of *H. marginata* populations using pheromone traps has enabled the development of a model to describe the cumulative percentage emergence of the insect over the flight season. Soil textural analyses and assessments of crop damage are discussed in relation to trap catch in an initial attempt to study populations in the context of the wider environment. These models demonstrate the possibility of using phenological models to not only predict yearly emergence, but also to forecast periods of peak *H. marginata* emergence and provide a much more comprehensive understanding of the development of this insect in the soil stage.

2-2 Materials and Methods

2-2.1 Preliminary degree day emergence model

2-2.1.1 Field data 2014 and 2015

A study was completed to assess the feasibility of developing a model to reliably predict the adult emergence of *H. marginata* in the UK. Approximate dates of *H. marginata* emergence were established for sites across the UK in the years 2014 (four sites) and 2015 (seven sites) (see Table 2.1 for sites and counties). Emergence traps (2014) and pheromone traps (2015) were placed in fields in mid-April and monitored on a weekly basis. Emergence traps consisted of an upturned seedling tray which was coated on the underside with insect barrier glue (Agralan Ltd, Ashton Keynes, UK) and secured on the soil surface by wooden stakes. Pheromone traps consisted of a standard red delta trap with a removable sticky insert (Agralan Ltd, Ashton Keynes, UK) hung on a fibreglass cane. Pheromone lures comprised a polyethylene vial containing 0.5mg (*R*)-2-nonyl butyrate placed in the centre of the trap (Natural Resources Institute, University of Greenwich). The date midway between when adult midges were first found on the trap and when the trap was last checked was used as the emergence date. Hourly soil temperatures and daily rainfall data were obtained from the Met Office MIDAS network of weather stations (Met Office, 2012). Each station was within 20 km of each field site. The distance of the

meteorological stations to the emergence sites is likely to be a source of error, however the data are representative of that which would be available to farmers in order to use the model.

2-2.1.2 Preliminary model development

Two emergence models, *Preliminary model 1* and *Preliminary model 2,* were produced based on degree day models previously developed for *S. mosellana* in Canada (Elliott *et al.*, 2009) and Belgium (Jacquemin *et al.*, 2014). *Preliminary model 1* used hourly soil temperatures to calculate the accumulated degree days above a base temperature from 1st March until the date of emergence for each site. Degree hours were calculated by subtracting the base temperature from the mean hourly temperature and summing all positive values. The total was then divided by 24 to convert it to degree days (Cesaraccio *et al.*, 2001). The mean number of degree days was then used to predict emergence dates for all sites. Base temperatures ranging from 0 – 10°C were tested to determine the best model. The 1st March was chosen as a date at which any diapause requirements for this insect are likely to have been met, as is the case with *S. mosellana*, and there are no references to post-larval development occurring prior to this date in the field. *Preliminary model 2* incorporated rainfall data as current evidence suggests that moisture is important in the onset of *H. marginata* emergence (Nijveldt & Hulshoff, 1968; Gratwick, 1992; Popov *et al.*, 1998). The first date on which rainfall occurred after the mean daily soil temperature rose above a predetermined threshold was used as the date of biofix. Here we are using the term 'biofix' to represent the estimated date at which pupation begins. The date of biofix was then used to calculate accumulated degree days above a base temperature until emergence (as previously). Mean daily soil temperature thresholds of 5 - 12°C were tested, along with degree day base temperatures of 0 – 10°C to determine the best model.

For both models, the predicted dates of emergence were compared against the observed dates for the sites sampled in 2014 and 2015. The standard deviation of the differences were calculated to determine the precision of each model as described by Elliott *et al.* (2009). Previously recorded emergence dates were used for model validation. The models were used to predict emergence dates for *H. marginata* in North Bedfordshire for sites sampled in 1971 and 1972 (Woodville, 1973), although daily soil temperatures were used for the degree day calculations due to the unavailability of hourly data.

The models were further validated against emergence data for a site near Aylesbury from sampling done in 2012 and 2013 (Pope & Ellis, 2013; Ellis *et al.*, 2014).

2-2.2 Modelling peaks in *H. marginata* emergence

2-2.2.1 Field data 2015 and 2016

Based on the success of the two emergence models, detailed data were collected over two years to determine if peaks in emergence could be predicted. *Haplodiplosis marginata* activity was monitored over the entire flight season at three sites in the UK: Buckinghamshire (Bucks) and Oxfordshire (Oxon) in 2015, and additionally Wiltshire (Wilts) in 2016. Pheromone traps were placed in two fields at each site. All fields were in wheat with the exception of Field 1b at Bucks in 2015 which was in field beans and Field 2b at Oxon in 2016 which was in oilseed rape. The trapping period began approximately a week prior to the start of the flight season (mid-April to May) and sticky cards were changed every 3 - 4 days for 8 weeks, after which they were changed weekly until emergence ceased. The same pheromone lures were used throughout the field season. Numbers of *H. marginata* caught at each trapping interval were counted. Hourly soil temperatures and rainfall data were obtained as described in section 2-2.1.1.

2-2.2.2 Model development

Two degree day (DD) models were developed, *DD Model 1* and *DD Model 2* in an attempt to describe *H. marginata* emergence patterns. Peaks in *H. marginata* activity were identified from catch numbers and the start and end dates were approximated as occurring midway between counts. *DD Model 1* assumed a straightforward relationship between degree day accumulations from a single date of biofix to the start of each peak (Figure 2.1). Here, different DD accumulations do not represent exact physiological requirements but are used to approximate the time to emergence for groups of insects experiencing different temperatures lower down the soil profile. *DD Model 2* assumed equal DD accumulations between each rainfall event and the subsequent peak, as described by Jacquemin *et al.* (2014) from observations of *S. mosellana* emergence (Figure 2.1).

The same biofix was used as the start of DD accumulations for both models, defined as the date of first rainfall on or after 1st March. Here, biofix represents the time when conditions were suitable for pupation to occur post-diapause. The chosen biofix assumes the diapause requirements for *H. marginata* would have been met prior to 1st March as described in section 2-2.1.2. It also assumes moisture is necessary for pupation to occur, as with models of *S. mosellana* development (Oakley *et al.*, 1998; Elliot *et al.*, 2009). Degree day accumulations were calculated above 0°C as described in section 2-2.1.2, having been determined to be the most appropriate base temperature from the preliminary model (section 2-2.1.2). Rainfall events were classified as daily rainfall over 1 mm following 3 days without precipitation. The threshold of 1 mm was used to account for inaccuracies in monitoring equipment. For both models, the coefficient of variation of DD accumulations was calculated for all sites and years.

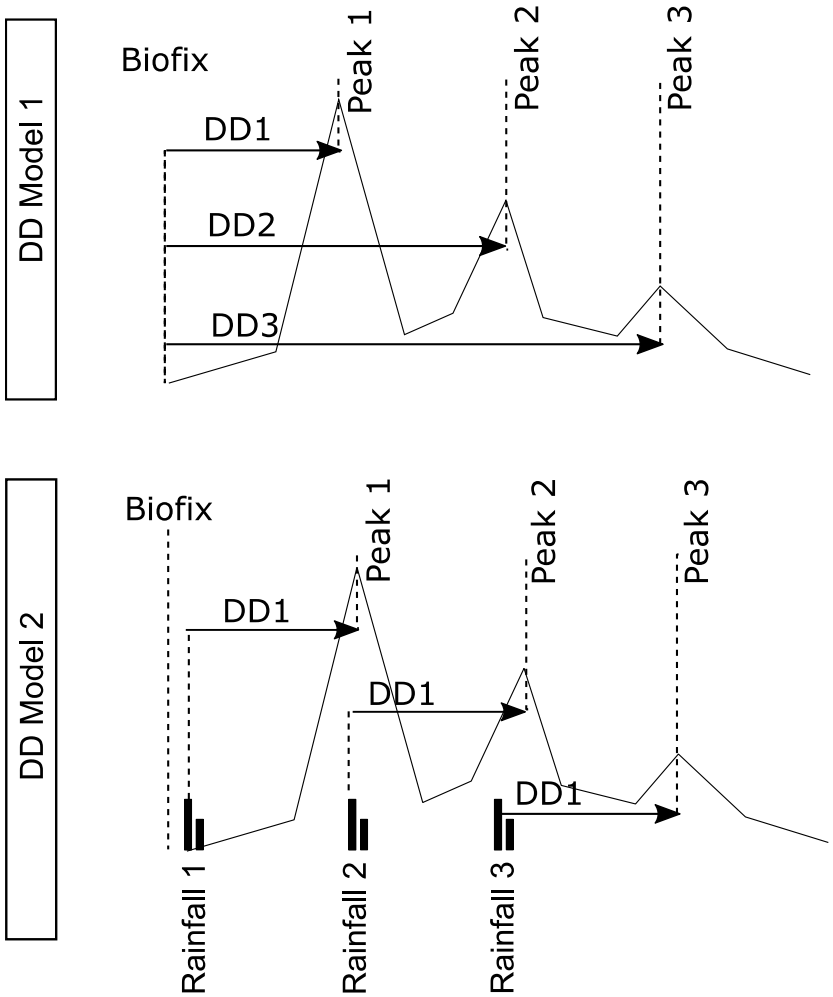


Figure 2.1. Representation of two different models to predict peaks in *Haplodiplosis marginata* emergence. DD refers to degree day accumulations with numbers indicating unique DD values.

2-2.2.3 Cumulative percentage emergence model

Pooled field data for each site and year were used to calculate the cumulative percentage emergence at each monitoring interval. Monitoring dates were converted to degree days calculated from the pre-determined date of biofix for each site and year, as described in section 2-2.2.2. Four models were tested to best describe the relationship between degree days and cumulative percentage emergence, all having previously been used successfully to describe insect development in response to time or temperature. For the *Weibull Model*, a two-parameter Weibull function was used:

(1) $y = 100(1 - \exp(-(x/\alpha)^{\beta})$

Where y is the cumulative percentage emergence, x is cumulative degree days and α and β are model parameters. A modified *Bimodal Model* developed by Kim *et al.* (2000) was used:

(2) $y = \alpha_1 \left\{ \left[\frac{1}{1+\exp\left[-\frac{x-\beta_1}{\gamma_1}\right]} \right] + \left(\frac{\alpha_2}{\alpha_1}\right) / \left[1 + \left(\frac{x}{\beta+\Delta\beta}\right)^{\gamma_2}\right] \right\}$

Where y is the cumulative percentage emergence, x is cumulative degree days and α to γ are model parameters as follows: α_1 and α_2 are the height of the first and second peaks respectively; β is the time in DD of the first peak; $\Delta\beta$ is the difference in DD between the first and second peak; γ_1 and γ_2 define the steepness of the first and second slopes respectively. Initial parameter estimates were made using the methods described in Kim *et al.* (2000). Both functions were fitted using nonlinear least squares regression. Two generalised linear models were also performed with binomial errors and logit or probit links (*GLM 1* and *GLM 2* respectively) (Forrest & Thomson, 2011). Model selection was done by comparing the adjusted r-squared and root mean square error (RMSE) values of models fitted to observed data (Damos & Savopoulou-Soultani, 2010; Parker *et al.*, 2011). The chosen model was validated against previous sites and years for which the date of *H. marginata* emergence is known and

compared with the previous emergence model from section 2-2.1. All statistical analyses were done in R-3.3.1 (R Core Team, 2016).

2-2.3 Soil textural analysis

Ten individual soil samples of approximately 200 g were taken from each field studied in 2016. Soil was removed using a trowel at a depth of 10 cm by walking a 'W' transect through the field and sampling at regular intervals. Samples were pooled for each field to create a composite soil sample which was air dried at 25°C for 7 days and then sieved through a 2.0 mm sieve to remove stones. Three 10 g samples were taken from the sieved soil for analysis. Soil texture was determined by mechanical analysis using the methodology described in Benton Jones (2001). Organic matter was removed prior to analysis by boiling the samples in hydrogen peroxide. Fractions based on particle size were then separated out according to the different settling velocities in a column of water. Soil organic matter content was determined by the loss on ignition method described by Ben-Dor and Banin (1989). Soil pH was measured using a Jenway 1305 pH meter with an epoxy bodied gel filled reference electrode.

2-2.4 Crop damage assessment

Tillers of wheat at growth stage at GS92 (Tottman & Makepeace, 1979) were sampled at random by walking along the tramlines of each field and sampling a stem at arm's length into the crop every 10 steps. In 2014, 25 galled and 25 non-galled stems were sampled from both fields (fields 1 & 2) at the Oxon site. In 2015, this was increased to 30 stems but samples were only taken from field 1 at Oxon due to the other being in field beans. In 2016, 30 stems were taken from each field, and additionally from field 2 at the Wilts site. Measurements were taken of ear length, stem height from the first node to the ear, grain number and grain weight per ear. In galled stems, the number of galls was also recorded. Evidence of damage by *Sitodiplosis mosellana* was recorded in 2016 when it became apparent that this pest was also infesting the crop at the Oxon and Wilts sites. Linear models on untransformed data were used to determine any differences in the measured parameters between galled and non-galled stems. Linear models were also use to identify any correlation between the number of galls and the crop measurements on damaged stems. All statistical analysis was done in R v.3.3.1 (R Core Team, 2016).

2-3 Results and Analysis

2-3.1 Preliminary degree day emergence model

Across all sites and years, the date of emergence varied from 30th April at the earliest, until 19th May at the latest. For *Preliminary Model 1*, the mean number of degree days accumulated above 0°C from 1st March until emergence was 588DD (± 9.7DD). A base temperature of 0°C was chosen as it gave the best results in terms of predicted emergence date compared with the observed emergence date. For *Preliminary Model 2*, a temperature of 6°C gave the best modelled results for the onset of pupation, followed by degree day accumulations above 0°C for the completion of adult development. The mean number of degree days calculated from the date of biofix until the date of emergence for each site was 548DD (± 8.4DD). *Preliminary Model 1* was able to predict emergence at the sampled sites to within 5 days (± 4 days) and *Preliminary Model 2* to within 4 days (± 2 days). The standard deviation of the differences between the observed dates and model predictions was also smaller for *Preliminary Model 2* suggesting a higher degree of precision (Table 2.1). From the historical data, predictions for the date of emergence from both models were within 5 days (± 3.5 days) for all sites (Table 2.2).

Table 2.1. Dates of observed and predicted emergence of adult *Haplodiplosis marginata* for years 2014 & 2015 from sampled sites, and difference in days for each model

		Preliminary Model 1		Preliminary Model 2	
Site	**Observed emergence date**	**Predicted emergence date**	**Days difference (Obs – Pred)**	**Predicted emergence date**	**Days difference (Obs – Pred)**
2014					
Royston (Herts)	30th April (± 0 days)	29th April	+1	27th April	+3
Bicester (Oxon)	3rd May (± 3.5 days)	2nd May	+1	2nd May	+1
H. Wycombe (Bucks)	3rd May (± 3.5 days)	3rd May	0	4th May	-1
Aylesbury (Bucks)	3rd May (± 3.5 days)	3rd May	0	6th May	-3
2015					
Royston (Herts)	2nd May (± 2 days)	4th May	-2	2nd May	0
Bicester (Oxon)	2nd May (± 2 days)	6rd May	+4	3rd May	-1
H. Wycombe (Bucks)	2nd May (± 2 days)	4th May	-2	5th May	-3
Aylesbury (Bucks)	9th May (± 4 days)	4th May	+5	8th May	+1
Glemsford (Suffolk)	3rd May (± 3 days)	5th May	+2	2nd May	+1
Thirsk (North Yorks)	9th May (± 2 days)	12th May	+3	13th May	-4
Devizes (Wiltshire)	3rd May (± 3 days)	4th May	1	1st May	+2
Max. difference			**+5 (± 4 days)**		**-4 (± 2days)**
SD (obs-pred)			**2.43**		**2.17**

Table 2.2. Dates of observed and predicted emergence for years 2012 & 2013 and 1971 & 1972, and difference in days for each model

		Preliminary Model 1		**Preliminary Model 2**	
Site	**Observed emergence date**	**Predicted emergence date**	**Days difference (Obs – Pred)**	**Predicted emergence date**	**Days difference (Obs – Pred)**
2013					
Aylesbury (Bucks)	17th May (± 3.5 days)	19th May	-2	19th May	-2
2012					
Aylesbury (Bucks)	10th May (± 3.5 days)	6th May	+4	5th May	+5
1972					
N. Bedfordshire	19th May	16th May	+3	21st May	-2
1971					
N. Bedfordshire	18th May	19th May	-1	16th May	+2

2-3.2 Modelling peaks in emergence

Emergence began no later than 2nd May (± 3 days) at all study sites and continued until as late as mid-July. Total site catches ranged from 1,755 to 20,384 individuals over the entire flight season. The catch data revealed apparent 'waves' of emergence of *H. marginata* over time, with a maximum catch rate of 200 individuals per trap per day. The first peak generally occurred soon after the initial emergence, with smaller subsequent peaks occurring at two- to three-week intervals (Figure 2.2).

At the two sites for which data were obtained in both years, mean soil temperatures in April and May 2015 differed by only 2.33°C whereas in 2016 the difference rose to 5.56 °C, reflecting a cooler April and warmer May than the previous year. Mean daily rainfall at the two sites was higher in 2016, averaging 2.28 mm compared with 1.31 mm in 2015. The maximum daily rainfall of 33.8 mm occurred in 2016 at the Oxon site.

DD Model 1 showed an average accumulation of 528.25DD (± 7.69DD) between the biofix and the onset of emergence. Accumulations between the biofix and subsequent peaks however, were more variable, averaging 796.82DD (± 39.14DD) and 1083.68 (± 54.81DD) for peaks 2 and 3 respectively (Table 2.3). Across all sites and years 46.7% of the identified rainfall events could be linked to peaks in

emergence (Figure 2.2). *DD Model 2* showed an average accumulation of 512.42DD (± 9.11DD) between a triggering rainfall event and a subsequent peak in emergence activity (Table 2.3, Figure 2.2).

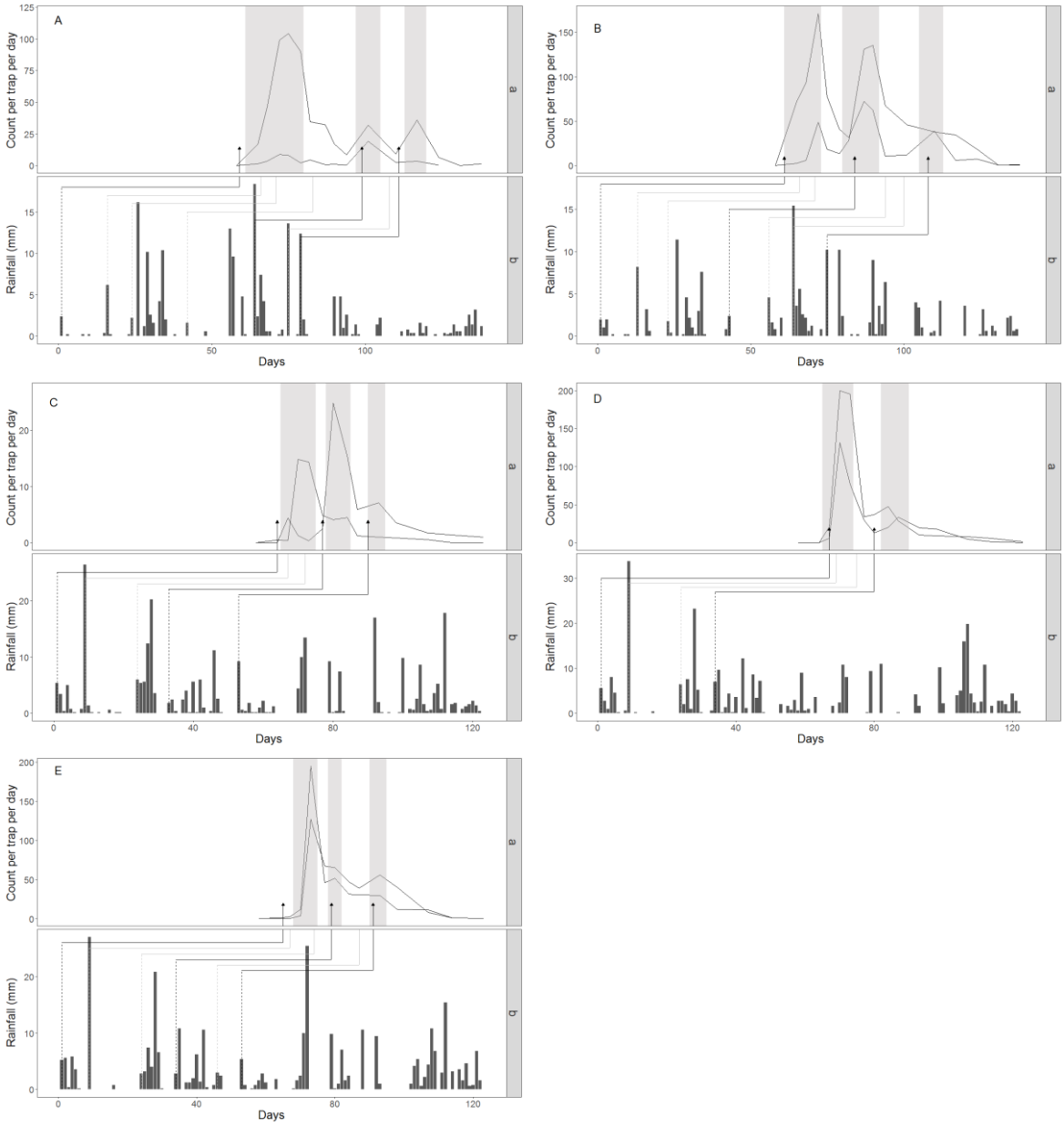


Figure 2.2 *Haplodiplosis marginata* catch per trap per day at two fields per site (panel a) and 24hr rainfall in mm (panel b) for each day of the trapping period. Black arrows represent inductive rainfall events, grey lines indicate non-inductive rainfall events. Horizontal lines represent degree day accumulations of 512DD. A) Bucks 2015, B) Oxon 2015, C) Bucks 2016, D) Oxon 2016, E) Wilts 2016

Table 2.3. Peaks in emergence: model development. Degree day accumulations for the periods between biofix and emergence peaks (*DD Model 1*) and the periods between inductive rainfall events and emergence peaks (*DD Model 2*), calculated for each site and year.

	DD Model 1: Biofix - Peak			**DD Model 2: Rain – Peak**		
	Peak 1 (DD1)	**Peak 2 (DD2)**	**Peak 3 (DD3)**	**Peak 1 (DD1)**	**Peak 2 (DD1)**	**Peak 3 (DD1)**
2015						
Bicester (Oxon)	537.24	764.91	1158.41	537.24	466.13	461.45
H. Wycombe (Bucks)	563.62	1054.46	1339.97	563.62	477.97	560.78
2016						
Bicester (Oxon)	484.68	746.86	-	484.68	551.44	-
H. Wycombe (Bucks)	534.13	694.30	926.43	534.13	486.59	516.16
Devizes (Wilts)	521.56	723.57	909.91	521.56	504.43	507.65
Mean	528.25	796.82	1083.68	528.25	497.31	511.51
SD	28.77	146.43	205.10	28.77	33.32	40.7
CV	**0.054**	**0.184**	**0.189**	**0.054**	**0.067**	**0.079**
				Mean (all peaks) (±SEM)		**512.42 (± 9.11)**

2-3.3 Cumulative percentage emergence model

The *Bimodal Model* was the best fitting model based on the adjusted r-squared value, accounting for 92% and of the variation in the data (Figure 2.3). Both the *Bimodal Model* and *GLM 2* had similar RSME values (Table 2.4). These models were therefore selected for validation. *GLM 2* predicted that 10% emergence of *H. marginata* would occur at 550DD post-biofix and the *Bimodal Model* at 576DD post-biofix. When validated against previous sites and years, *GLM 2* agreed with the observed date of emergence to within a maximum of 4 days (± 4 days) and the *Bimodal Model* to within 3 days (± 4 days), with the error reflecting uncertainty in the true emergence date as a result of the sampling interval. The *Bimodal Model* however had a lower standard deviation of differences between the observed and predicted dates indicating higher precision overall (Table 2.5).

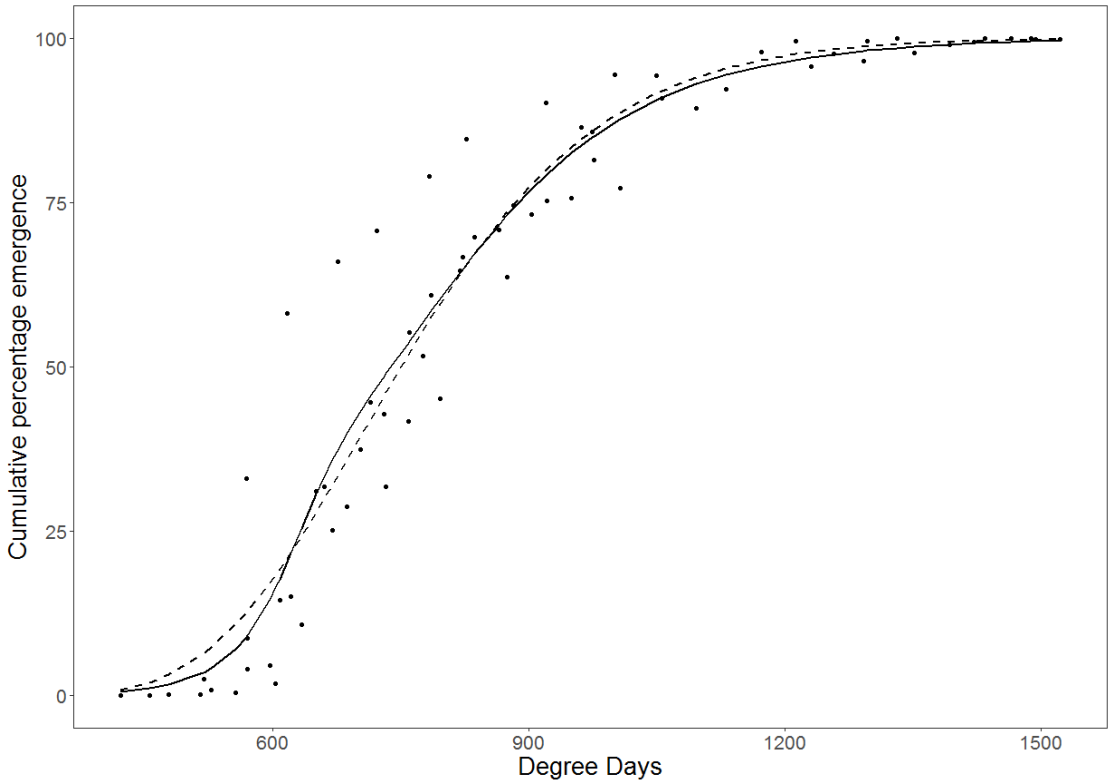


Figure 2.3. Percentage cumulative emergence of *Haplodiplosis marginata* as a function of accumulated degree days for all sites and years studied. Predicted emergence based on *GLM 2* (dashed line) and *Bimodal Model* (solid line) shown.

Table 2.4. Parameter estimates and standard error (SE) for all four cumulative percentage emergence models. RMSE and adjusted R^2 values shown. Predicted DD accumulations required for 10%, 50% and 90% based on the two selected models.

Model	Parameter	Est. value	SE	RMSE	Adj. R^2	Model predictions	
GLM 1	α	7.126	0.01616	10.03	0.89		
	β	-47.16	0.10725				
Weibull Model	α	822.061	10.6095	10.67	0.91		
	β	4.696	0.3915				
GLM 2	α	4.124	0.00845	9.98	0.89	10%	550.04
	β	-27.308	0.05616			50%	750.47
						90%	1023.93
Bimodal Model	α_1	25.409	48.365	9.79	0.92	10%	575.55
	β_1	623.233	29.473			50%	738.49
	γ_1	30.294	50.783			90%	1039.89
	α_2	75.063	50.114				
	$\Delta\beta$	187.541	136.260				
	γ_2	-7.322	3.286				

Table 2.5. Observed and predicted 10% emergence dates for *GLM 2* and the *Bimodal Model* for all sites and years. Differences in days between observed and predicted dates shown. Error in brackets represents uncertainty in emergence dates which are given as a midpoint between sampling dates.

		GLM 2		Bimodal Model	
Site	**Observed emergence date**	**Predicted emergence date**	**Days difference (Obs – Pred)**	**Predicted emergence date**	**Days difference (Obs – Pred)**
2014					
Royston (Herts)	30th April (± 0 days)	27th April	3	29h April	1
Bicester (Oxon)	3rd May (± 3.5 days)	30th April	3	2nd May	1
H. Wycombe (Bucks)	3rd May (± 3.5 days)	30th April	3	3rd May	0
Aylesbury (Bucks)	3rd May (± 3.5 days)	2nd May	1	3rd May	0
2015					
Royston (Herts)	2nd May (± 2 days)	1st May	1	3rd May	-1
Bicester (Oxon)	2nd May (± 2 days)	3rd May	-1	5th May	-3
H. Wycombe (Bucks)	2nd May (± 2 days)	1st May	1	3rd May	-1
Aylesbury (Bucks)	9th May (± 4 days)	5th May	4	6th May	3
Glemsford (Suffolk)	3rd May (± 3 days)	2nd May	1	4th May	-1
Thirsk (N. Yorks)	9th May (± 2 days)	9th May	0	10th May	-2
Devizes (Wiltshire)	3rd May (± 3 days)	30th April	3	3rd May	0
2016					
Bicester (Oxon)	7th May	8th May	-1	10th May	-3
H. Wycombe (Bucks)	7th May	5th May	2	7th May	0
Glemsford (Suffolk)	5th May (± 3 days)	6th May	-1	8th May	-3
Devizes (Wiltshire)	10th May	7th May	3	8th May	2
Max. difference			**4 (± 4days)**		**+3 (± 4days)**
SD (Obs - Pred)			**2.19**		**1.81**

2-3.4 Soil analysis

Soil textural analysis showed some variation between field sites, with all fields being clays or dominant in clay particles (Table 2.6). All soils had an organic matter content of over 6%. The pH did not vary significantly across sites and was either neutral or slightly alkaline in all fields.

Table 2.6. Soil textural classification, percentage organic matter and pH for all fields. Figures represent the mean (±SEM) of three 10g composite soil samples.

Site (Field)	% sand	% silt	% clay	Classification	% Organic matter	pH
Oxon (1)	22.35 (±0.33)	35.65 (±0.46)	42 (±0.71)	Clay	8.77 (±0.10)	7.37
Oxon (2)	32.51 (±2.12)	28.12 (±1.07)	39.36 (±1.05)	Clay	8.71 (±0.02)	7.27
Bucks (1)	36.9 (±0.54)	38.63 (±0.09)	24.47 (±0.49)	Clay loam	6.87 (±0.04)	6.97
Bucks (2)	34.31 (±1.05)	32.73 (±0.52)	32.95 (±0.59)	Clay loam	7.85 (±0.15)	7.20
Wilts (1)	16.26 (±0.51)	48.08 (±1.18)	35.66 (±0.66)	Silty clay	6.63 (±0.02)	7.47
Wilts (2)	15.68 (±0.43)	48.51 (±0.30)	35.81 (±0.33)	Silty clay	5.96 (±0.06)	7.40

2-3.5 Crop damage assessment

The mean number of galls found on damaged stems was 12.89, with little variation between sites and years despite total trap catch numbers ranging from 5,000 to 10,000 midges for each site. The highest number of galls found on one stem was 34 however 83% of galled stems had ten galls or fewer and more than half (55%) had five or fewer. In year 3, 40% of all sampled stems had evidence of *S. mosellana* larval infestation. Galled stems were shorter in length ($F_{1,333}$=46.44, P<0.001), had shorter ears ($F_{1,333}$=5.52, P<0.05), had fewer grains per ear ($F_{1,333}$=25.47, P<0.001) and lower grain weight per ear ($F_{1,333}$=6.75, P<0.01) when compared with non-galled stems (Figure 2.4). Removing data from year 3 in which *S. mosellana* damage was evident in some samples removed the effect of galling on grain weight ($F_{1,156}$=0.33, P=0.57) but had no impact on the other variables. There was no relationship between the number of galls and any of the measured parameters on galled stems. There was also no apparent relationship between mean grain weight, mean gall number or mean number of grain per ear and total trap catch for each site.

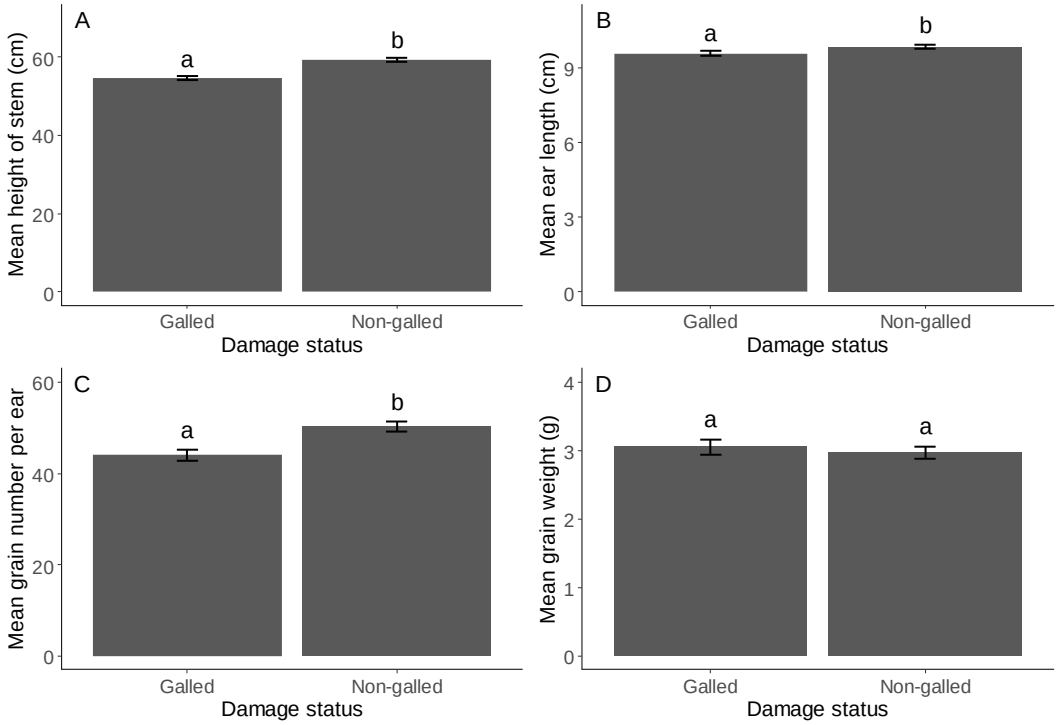


Figure 2.4. Crop damage assessment of stems sampled 2014 – 2016. Mean values (± SEM) show differences between galled and non-galled stems in **A.** height **B.** ear length **C.** grain number per ear **D.** grain weight per ear. N = 170 with the exception of **D**. where N = 134. Different lowercase letters represent where these differences are statistically significant at the P = 0.05 level.

2-4 Discussion

The work presented here clearly demonstrates how rainfall and soil temperature data can be used to develop forecasts of *H. marginata* emergence. The association between soil temperature and moisture and the onset of emergence in this species were initially demonstrated with a degree day model based on dates of emergence obtained in 2014 and 2015. With the availability of more detailed catch data, these preliminary models were then improved to develop a cumulative percentage emergence forecast for the entire flight season. Furthermore, the role of rainfall events in triggering *H. marginata* emergence was confirmed, enabling peaks in *H. marginata* emergence to be predicted. Waves of emergence in *H. marginata* have been observed before from data collected using non-specific traps (Censier *et al.*, 2016b). Such waves may arise because not all larvae encounter the conditions necessary for pupation all at once, for example due to their depth in the soil. From an ecological perspective, this strategy

increases the chance of coincidence between newly emerged adults and a suitable growth stage of the host plant. It is particularly relevant for *H. marginata*, which has a short adult lifespan of only 1 - 7 days (Nijveldt & Hulshoff, 1968; Popov *et al.*, 1998).

Moisture is an important but often underappreciated aspect of insect phenology and development (Tauber & Tauber, 1976; Tauber et al, 1998). The incorporation of soil moisture or rainfall into a degree day-based phenological model can lead to improved precision in some species (e.g. Baxendale & Teetes, 1983; Baxendale *et al.*, 1984; Tauber *et al.*, 1994). Early attempts to model the emergence pattern of orange wheat blossom midge, *S. mosellana,* recognised the importance of soil moisture in pupation and therefore emergence (Basedow, 1977; Basedow & Gillich, 1982; Oakley *et al.*, 1998; Elliot *et al.*, 2009). It was not until this insect was studied under controlled conditions and with more frequent field sampling that rainfall events could be linked to the inducement of this final stage of development (Jacquemin *et al.*, 2014). Here, there is apparently a similar effect of rainfall on *H. marginata* emergence based on field data. In the initial emergence models, *Preliminary Models 1 & 2*, the predictive ability was improved by incorporating rainfall into the selection of a date of biofix. In *DD Model 2*, the onset of a precipitation period followed by the accumulation of 512DD above 0°C predicted an increase in *H. marginata* emergence to within 3 days of the midpoint of the observed peaks. In both cases, models incorporating rainfall were more accurate than those using soil temperature alone. The models proposed here, and that proposed by Jacquemin *et al.*, (2014), agree with theories of insect development which state that post-diapause, insects can remain in a state of 'readiness' until an environmental cue triggers the onset of pupation (Tauber & Tauber, 1976; Hodek, 1996; Koštál, 2006). Such a mechanism ensures that development typically resumes when conditions are favourable regardless of when diapause is terminated. If no cue is received, the insect recommences diapause for another year (Tauber & Tauber, 1976). This would account for the proportion of *S. mosellana* and *H. marginata* larvae that undergo extended diapause (Nijveldt & Hulshoff, 1968; Basedow, 1977), which is thought to be up to twelve years in the case of *S. mosellana* (Barnes, 1952) and up to six years for *H. marginata* (Nijveldt & Hulshoff, 1968). The ability to predict waves of emergence are important in the management of this pest. Censier *et al.* (2016b) found that timing chemical controls to coincide with

peak emergence and good weather conditions eliminated the need for further applications. This was shown to be less effective when emergence was prolonged however, in which case repeated applications may be necessary. Careful crop monitoring remains essential if such chemical controls are to be timed appropriately (Ellis *et al.*, 2014; Censier *et al.*, 2016b).

Not all rainfall events were directly linked to increased catch rates: just under 50% of all rainfall events were found to be inductive. Smaller peaks in emergence may have been overlooked at the trapping interval used or due to weather conditions affecting the catch rate on particular days. Soil conditions in the preceding days might also determine the impact of a particular rainfall event. In their studies on sorghum midge, Baxendale *et al.* (1983, 1984) found that rainfall delayed emergence and drier years correlated with lower heat requirements. No such pattern was observed here, the site with the earliest emergence (Oxon 2016) also experienced 43% more rain than the next wettest site in the four weeks prior to emergence. This site also had a greater initial rate of emergence, resulting in only two clear emergence peaks rather than three (Figure 2.2). This may have been due to the 33.8mm of rainfall which the site received on the 9th March, resulting in a greater proportion of larvae encountering favourable pupation conditions at once. Factors such as landscape, soil type and structure will affect the permeability and moisture retention of soil. Soil type however, was not found to be of significance in the emergence of swede midge, when investigated alongside soil moisture (Chen & Shelton, 2007). *Haplodiplosis marginata* is known to favour heavy soils with a high clay content (Golightly & Woodville, 1974). Soil textural analysis of the sites used in this experiment shows that the fields studied are either clays or dominant in clay particles. Organic matter comprised at least 6% of the soil at the study sites. The moisture retention capabilities and open structure associated with these soils (Davies *et al.*, 2001) may result in greater pupation success for this species, however this would require more extensive analysis of mortality rates in the soil.

The models proposed here all calculate degree day accumulations above a base temperature of 0°C. This base temperature is unlikely to have any physiological relevance as the developmental threshold temperature for *H. marginata* has previously been reported as 10°C (Baier, 1963; Nijveldt & Hulshoff, 1968), although this is possibly an overestimation as temperatures between 5°C and 10°C

were apparently not tested in either study. In an analysis of the limitations of using degree day units, Bonhomme (2000) noted that, for plants at least, the threshold temperature used for degree day calculations is only of statistical relevance and is often unrelated to threshold temperature at which the rate of development is zero. Similarly, Snyder *et al.* (1999) reported good results when using a 0°C base temperature in degree day models compared with other developmental thresholds estimated from field observations.

The importance of the start date in calculating DD accumulations is widely recognised (Pruess, 1983) and model precision can be improved if there is a biological basis for the date selected (e.g. Riedl *et al.*, 1976). This date is commonly referred to as the 'biofix'. *Preliminary Model 2* was most accurate, with the proposed a point of biofix as the first rainfall event once mean daily soil temperatures rose above 6°C after the 1st March. With more comprehensive emergence data, the biofix was simplified to remove the temperature threshold in the model to predict peaks in emergence. The 1st March is often selected as a start date for DD accumulations where there is a no lower developmental threshold data to draw upon (Pruess, 1983), such as models of *S. mosellana* emergence (Wise & Lamb, 2004; Elliot, 2009). The same date was used here based on a lack of observed *H. marginata* development in the field prior to 1st March previously (Pope & Ellis, 2013), and the assumption that earlier soil temperatures had little effect on *H. marginata* development as they were at the lower end of the developmental threshold range. Photoperiodism can also play a major role in the termination of insect diapause, which further justifies removing the temperature threshold from the biofix estimate (Tauber & Tauber, 1976; Saunders, 2014).

The two DD-based cumulative emergence models for *H. marginata* proposed here, the *Bimodal Model* and *GLM 2*, are comparable in terms of their reliability as determined by the r-squared and RSME values. The *Bimodal Model* however has a slightly better predictive power as shown by the standard deviation of the observed and predicted 10% cumulative emergence from previous years. The value for 10% emergence was deemed to be an appropriate approximation for the start of emergence given the error involved in trapping insects at very low densities; it is unlikely that the earliest onset of emergence will have been recorded particularly in 2014 when pheromone traps were not available.

GLM 2 predicted that 10% emergence occurs at 550 DD post-biofix while the *Bimodal Model* estimated it to occur at 576DD. Both estimates fall well within the observed range of 538 – 621 DD. The *Bimodal Model* predicts a higher initial rate of emergence, which appears to fit the observed pattern of large initial peaks and smaller subsequent peaks of emergence. Over all sites and years, *GLM 2* predicted the onset of emergence to within 4 days (± 4 days) which is on a par with *Preliminary Model 2*. The *Bimodal Model* improved on this by predicting emergence to within 3 days (± 4 days). The advantage of the new models is the ability to predict cumulative percentage emergence over the entire flight season, rather than just the start date. This will enable the midpoint and conclusion of flight periods to be estimated and aid in the assessment of the need for chemical controls or the effectiveness of insecticides applied earlier in the emergence period. It may mean that pest management options can be used more judiciously, so that chemical controls are only applied if the crop is at a vulnerable growth stage prior to the mid-point of emergence.

The crop damage assessments presented here show that the effects of *H. marginata* damage are not always clear cut. Plant height, grain number and ear length were all compromised on damaged stems but total grain weight was not affected. The disparity between growth reduction and final grain weight may be due to a 'pruning effect' whereby the plant compensates for fewer grains through increased grain filling (Barnes, 1956). This has been noted previously in cases of *H. marginata* infestation, and originally led farmers to believe that the insect was beneficial to the crop (Barnes, 1956). As the attack becomes more severe however, losses may be incurred. In this study, gall number did not correlate to the observed reductions in growth, which may be due to relatively low levels of infestation recorded on many of the sampled stems. Similar numbers of galls have been correlated to losses in yield previously, however (Censier *et al.*, 2016b). The uncertainty surrounding the effect of galling on crops may also reflect the differences in wheat varieties grown at different sites and years. Such varietal variation in susceptibility indicates the potential of breeding for full or partial resistance to this pest. Antibiotic resistance to *S. mosellana* has been bred into some varieties of wheat, which prevents the larvae from developing past the first instar (Lamb *et al.*, 2000; Oakley, 2005). An equivalent development however, would require an understanding of the mechanism(s) of plant resistance to *H.*

marginata, which is currently unknown. Here, trap catches could not be linked to observed damage however variables such as growth stage, crop variety and local environmental conditions varied between sites making comparisons difficult. Meteorological conditions were thought to be largely responsible for crop damage observed in field trials in a two-year study by Censier *et al.* (2016b) which similarly did not correlate with trap catch. This demonstrates that even with high numbers of insects emerging over the flight period, the timing of emergence is likely to be crucial in the consideration of the potential threat to yield. Emergence models such as these are therefore extremely relevant to the determination of economic injury thresholds.

The models produced here rely on data collected by a national network of weather stations. This means that forecasts can be made for different parts of the country, providing estimates based on local weather conditions. Multiple pest forecast models could be used to identify different periods of activity from the same meteorological data, for example the VIPS automatic forecasting system developed in Norway (NIBIO, 2017) and the CIPRA model in Quebec (Bourgeois *et al.*, 2005). Ideally, such a forecasting system would be used alongside crop growth forecasts and economic injury thresholds to provide an assessment of crop risk throughout the flight period. Continued application and evaluation of predictive models for *H. marginata* and other pest species will further improve the reliability of such forecasts in the future.

Chapter 3

Development and optimisation of a sex pheromone lure for monitoring populations of saddle gall midge, *Haplodiplosis marginata*

Abstract

Saddle gall midge, *Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae), is a sporadic pest of cereals in Northern and Central Europe and is of increasing importance in the UK. Recently the major component of the sex pheromone produced by adult female *H. marginata* was reported to be 2-nonyl butyrate. The importance of absolute configuration on attractiveness, the effects on trap catches of the addition of minor pheromone components, dispenser type, and pheromone loading are described in the development of an optimised pheromone lure with which to trap *H. marginata* males. In analyses of volatiles collected from virgin female *H. marginata* by gas chromatography (GC) coupled to electroantennographic recording (EAG) from the antenna of a male *H. marginata*, two EAG responses were observed. Analyses by coupled GC-mass spectrometry (MS) indicated these were due to 2-nonyl butyrate and a trace amount (1%) of 2-heptyl butyrate. A similar trace amount of 2-nonanol was detected in GC-MS analyses but this compound did not elicit an EAG response when the synthetic compound was tested, whereas the other two compounds did. These three compounds were not observed in collections of volatiles made from male *H. marginata*. The 2-nonyl butyrate was shown to be the (*R*)-enantiomer. In field trapping tests (*R*)-2-nonyl butyrate was at least 10× more attractive to male *H. marginata* than the racemic compound, and the (*S*)-enantiomer was unattractive. Addition of the potential minor components individually or together at the naturally occurring ratios did not increase or reduce the attractiveness of the lure. Polyethylene vials and rubber septa were equally

Published as: Rowley, C., Pope, T.W., Cherrill, A., Leather, S.R., Fernández-Grandon, G.M. & Hall, D.R. (2017) Development and optimisation of a sex pheromone lure for monitoring populations of saddle gall midge, *Haplodiplosis marginata*. *Entomologia Experimentalis et Applicata*, **163**, 82–92.

effective as pheromone dispensers, lasting for at least five weeks in the field in the UK, although laboratory tests indicated release from the former was more uniform and more likely to last longer in the field. Increasing loading of pheromone in the dispenser increased attractiveness. Traps baited with polyethylene vials containing 0.5 mg of (*R*)-2-nonyl butyrate are recommended for monitoring *H. marginata* and these are far more sensitive than water or sticky traps currently used for monitoring this pest.

3-1 Introduction

Saddle gall midge, *Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae), is a sporadic pest of cereals of increasing importance in the UK and parts of continental Europe. Yield losses of up to 70% have been reported during recent UK outbreaks (Ellis *et al.*, 2014). It is a univoltine species with a short-lived adult stage and an overwintering phase in the larval stage. Adults begin emerging around the start of May (Skuhravý *et al.*, 1983; Gratwick, 1992) and mating occurs immediately (Golightly & Woodville, 1974). Females lay their eggs on the leaves of cereals and other grasses and, once hatched, the larvae begin feeding on the stem of the host plant resulting in the formation of saddle-shaped galls beneath the leaf sheath (Skuhravý *et al.*, 1983; Dewar, 2012). Gall formation can damage the plant by restricting nutrient flow to the ear leading to under-filled grains, and by leaving the plant vulnerable to attack from secondary pathogens (Nijveldt & Hulshoff, 1968; Dewar, 2012). Severe infestation results in multiple galls along the stems of a cereal plant, weakening the stems and increasing the risk of stem breakage which can cause substantial yield loss (Gratwick, 1992; Berry *et al.*, 1998). Crops most at risk from *H. marginata* are spring wheat, barley, and late-sown winter wheat, particularly in areas with heavy soils (Golightly & Woodville, 1974; Skuhravý *et al.*, 1983, 1993; Pope & Ellis, 2013).

The pest is very sporadic and the exact conditions which influence diapause termination, emergence, and the reproductive success of *H. marginata* are unknown, making outbreaks difficult to forecast (Woodville, 1973; Basedow, 1986). As in other Cecidomyiidae, monitoring populations of *H. marginata* is difficult due its cryptic nature (Harris & Foster, 1999). Populations can remain low for years

before increasing rapidly over a few generations making outbreaks difficult to forecast (Woodville, 1973; Basedow, 1986). Additionally the timing of pesticide application is critical as once the larvae begin feeding they are protected from contact insecticides by the leaf sheath. The best control is achieved when sprays coincide with the first appearance of adults or eggs in the crop, or 7-10 days after the start of adult emergence (Ellis *et al.*, 2014). Consequently, there is an urgent need for a simple and effective monitoring system for growers to use and on which pest management decisions can be based (Censier *et al.*, 2015, 2016b).

Monitoring of pest populations allows insecticides to be applied judiciously to target the temporal occurrence of the vulnerable life-stage of the organism (Jones, 1998). Use of pheromone-baited traps has been found to be an effective method of population monitoring in many pest species (Hardie & Minks, 1999; Witzgall *et al.*, 2010), including other cecidomyiid pests such as orange wheat blossom midge, *Sitodiplosis mosellana* (Gehin) (Bruce *et al.*, 2007; Bruce & Smart, 2009; Oakley & Ellis, 2009), apple leaf midge, *Dasineura mali* (Cross & Hall, 2009; Cross *et al.*, 2009) and Hessian fly, *Mayetiola destructor* (Anderson *et al.*, 2012).

The major component of the *H. marginata* sex pheromone was recently identified as 2-nonyl butyrate and the synthetic racemic compound was found to be attractive to male insects in field trials in Belgium (Censier *et al.*, 2014b, 2016a). Here, I confirmed the basic findings of Censier *et al.* (2014b) and extended them by using electroantennography (EAG) to detect potential minor pheromone components. The effects of these were evaluated in field trapping tests, as was the importance of the absolute configuration of the major pheromone component and the effect of pheromone loading on trap catches. Practical pheromone dispensers were evaluated to provide farmers and agronomists with an effective monitoring system on which to base pest management decisions.

3-2 Materials and methods

3-2.1 Insects

Larvae of *H. marginata* were collected from soil samples taken from affected fields between November 2013 and May 2014 and stored at 4°C for a minimum of 3 months. Each larva was transferred to an individual plastic container (1.5 cm diameter, 2.5 cm high) of moist sterilised compost covered with a

fine mesh and maintained at 20 °C, 60% r.h., and L16:D8 photoperiod, until adults emerged.

3-2.2 Pheromone collection

Volatiles were collected from individual virgin adult males and females separately, within 48 h of emergence. A single live midge was used per collection and was placed in a cylindrical glass vessel (5.3 cm diameter, 13 cm long; Hamilton Laboratory Glass, Margate, UK) with a glass frit and activated charcoal filter at one end (20 × 2 cm, 10-18 mesh; Fisher Chemicals, Loughborough, UK) and a collection filter at the other. The collection filter consisted of a Pasteur pipette (4 mm i.d.) containing Porapak Q (200 mg, 80-100 µm; Waters Associates, Milford, MA, USA) positioned between two glass wool plugs (Supelco, Gillingham, Dorset, UK). Air was drawn through the charcoal filter into the vessel containing the midge and out through the collection filter using a vacuum pump (M361C; Charles Austen Pump, Byfleet, UK) at a rate of 0.5 l per min. Collections were made continuously for a period of 48 h. Five collections were made from males and four from females. Volatiles were desorbed from the collection filters with dichloromethane (1.5 ml), concentrated under a stream of nitrogen, and refrigerated prior to analysis.

3-2.3 Coupled gas chromatography-mass spectrometry (GC-MS)

Aliquots of volatile collections were analysed using a Varian 3500 GC coupled to a Saturn 2200 MS (Agilent Technologies, Stockport, UK) operated in electron impact mode. A polar or non-polar GC column was used (30 m × 0.25 mm i.d., 0.25 µm film thickness) coated with DBWax (Supelco) or VF5 (Agilent), respectively, and the oven temperature was held at 40°C for 2 min and then programmed at 10°C per min to 240 °C. Compounds were identified by their mass spectra, their GC retention indices relative to the retention times of *n*-alkanes and comparison of retention indices and mass spectra with those of authentic synthetic standards.

3-2.4 Coupled gas chromatography-electroantennography (GC-EAG)

Antennal responses of male and female *H. marginata* to collections of volatiles from females were measured by EAG coupled with an Agilent 6890N GC with fused silica capillary columns (30 m × 0.32 mm i.d., 0.25 µm film thickness) coated with polar DB Wax (Agilent) and non-polar SPB1 (Supelco).

Injections were splitless (220 °C) for the polar column and with programmed temperature vaporising injector (held at 50°C for 0.2 min and then programmed at 60°C per min to 220 °C) for the non-polar column. The carrier gas was helium (2.4 ml per min) and the oven temperature was held at 50°C for 2 min and then programmed at 10°C per min to 250 °C. The ends of the GC columns went into a push-fit Y-connecter that lead through a second Y-connector fitted with two equal lengths of deactivated fused silica capillary going to the flame ionisation detector (FID) and a glass T-piece, splitting the GC effluent 50:50. The effluent was collected in the T-piece for 17 s before being blown over the antennal preparation for 3 s in a stream of air (200 ml per min) (Cork *et al.*, 1990).

The antennae were prepared by excising the head from a live specimen, then removing one of the antennae and the tip of the remaining antenna using a sharp microscalpel. Antennal responses were recorded using an INR-2 micromanipulator assembly (Syntech, Hilversum, The Netherlands). Two newly-pulled glass capillary electrodes were filled with an electrolyte solution of 0.1 M KCl with 1% polyvinylpyrrolidine (BDH Chemicals, Poole, UK) added to prevent evaporation. These were attached to silver wire electrodes mounted in micromanipulators. The insect preparation was mounted between the two glass electrodes with the head in the reference electrode and the distal end of the antenna in the recording electrode. The antennal responses were amplified 10× and converted to digital format through the second detector channel of the GC. Data from FID and EAG were captured and processed with EZChrom Elite v.3.3.1 software (Agilent).

3-2.5 Enantioselective gas chromatography

Enantoselective GC was carried out on a CP-Chirasil-Dex CB column (25 m × 0.32 mm i.d., 0.25 µm film thickness; Varian/Agilent) with He carrier gas (2.4 ml per min), split injection (220 °C, 20:1), and FID (220 °C). The oven temperature was held at 60°C for 2 min and then programmed at 5°C per min to 200 °C.

3-2.6 Chemicals

Unless otherwise stated, all chemicals were obtained from SigmaAldrich (Gillingham, UK) and were at least 98% pure. Racemic 2-nonyl butyrate was prepared by esterification of 2-nonanol with butyric acid

in the presence of N,N'-dicyclohexylcarbodiimide (DCCD) and 4-dimethylamino-pyridine (DMAP) in dichloromethane (Neises & Steglich, 1978). The product was obtained in 93% yield after purification by flash chromatography on silica gel eluted with 2% diethyl ether in hexane and kugelrohr distillation (at 70°C and 0.03 mm Hg). ^{1}H and ^{13}C nuclear magnetic resonance (NMR), infrared (IR) and mass spectral (MS) data were in agreement with those reported by Censier *et al.* (2014b).

Racemic 2-nonyl butyrate was resolved into the two enantiomers by stirring with a catalytic amount of lipase acrylic resin from *Candida antarctica* yeast in phosphate buffer (1 M K_2HPO_4) for 6 h with monitoring by enantioselective GC, which selectively hydrolysed the (*R*)-enantiomer (Hall *et al.*, 2012) The product was chromatographed on silica gel eluted successively with 2, 5, 10, 20, and 50% diethyl ether in hexane to give (*S*)-2-nonyl butyrate (98.7% enantiomeric excess by enantioselective GC) and (*R*)-2-nonanol. The latter was esterified as above to give (*R*)-2-nonyl butyrate (98.9% e.e.). Racemic 2-heptyl butyrate was prepared similarly from 2-heptanol. This was resolved into the enantiomers with lipase from *C. antarctica* to give the (*S*)- (97.8% e.e.) and (*R*)- (98.2% e.e.) enantiomers.

3-2.7 Pheromone dispensers

Two different dispenser types were tested: polyethylene vials (26 × 8 mm, 1.5 mm thick; Just Plastics, London, UK) and white rubber septa (20 × 10 mm; International Pheromone Systems, The Wirral, UK). These were loaded with the pheromone dissolved in hexane (100 µl) and the solvent was allowed to evaporate. Release rates were measured for dispensers loaded with 2-nonyl butyrate (1 mg) and maintained in a laboratory wind tunnel (27 °C, 2.2 m s^{-1} wind speed). Duplicate samples were removed at weekly intervals and the remaining pheromone was extracted individually in hexane (5 ml) containing dodecyl acetate (1 mg) as internal standard. Extracts were analysed by GC with FID on a capillary column (30 m × 0.32 mm i.d., 0.125 µm film thickness) coated with DB5 (Agilent) with splitless injection (220 °C) and the oven temperature held at 50°C for 2 min and then programmed at 10°C per min to 250 °C. The amount of pheromone remaining in lures returned from field trapping tests was measured similarly.

3-2.8 Field trapping experiments

Field trapping experiments were all carried out at sites with known soil populations of *H. marginata*. Five experiments were performed. Experiments 1, 2, 3, and 4 were carried out in Oxfordshire, UK (51°55"N, 1°10"W). Experiment 5 was carried out in Buckinghamshire, UK (51°37"N, 0°48"W). All fields were in winter wheat and the experiments were conducted during part of the flight season of *H. marginata*, coinciding with wheat growth stages 39-59 (Zadoks *et al.*, 1974).

For each experiment, pheromone dispensers were placed in standard red delta traps (Agralan, Wiltshire, UK) containing a removable sticky insert (15 × 15 cm). Polyethylene vials were used as dispensers for all experiments with the exception of experiment 1. Traps were hung from fibreglass canes and positioned at the height of the wheat ear. For experiments 1-4, traps were set out in a randomised complete block design. In each block, traps were laid out along a transect with 10 m between traps and 50 m between blocks. Adult *H. marginata* were identified based on antennal and genital morphology (Harris, 1966) and counted using a bifocal microscope.

3-2.8.1 Experiment 1 – pheromone dispensers

Catches of male *H. marginata* in traps baited with racemic 2-nonyl butyrate (1 mg) formulated in the two types of pheromone dispenser, rubber septa, and polyethylene vials, were compared with catches in an unbaited trap. Traps were laid out in four replicated blocks and were in place between 15 May and 19 June 2014 and the sticky inserts of the traps were changed after 6 days, at which time the treatments were re-randomised within the blocks.

3-2.8.2 Experiment 2 – pheromone chirality

Catches in traps baited with lures containing (*R*)-2-nonyl butyrate (0.5 mg), (*S*)-2-nonyl butyrate (0.5 mg), the racemic mixture (1 mg), and an unbaited trap as control were compared. Traps were laid out in four replicated blocks and were in place between 5 and 19 June 2014.

3-2.8.3 Experiment 3 – effect of minor components

The effects of addition to (*R*)-2-nonyl butyrate (0.5 mg) of two minor components were tested: (*R*)-2-nonanol and (*R*)-2-heptyl butyrate, each at 2% of the major component, separately and in combination.

These treatments were compared with lures containing (*R*)-2-nonyl butyrate (0.5 mg), lures containing the racemic mixture (1 mg), and with an unbaited trap as control. Traps were laid out in 10 replicated blocks and were in place between 18 and 29 May 2015. The sticky inserts of the traps were changed on days 4 and 9 of the experiment, with the treatments re-randomised within the blocks after each change.

3-2.8.4 Experiment 4 – pheromone loading

Trap catches with lures containing loadings of 2.5, 0.5, 0.05, and 0.005 mg of the major pheromone component, (*R*)-2-nonyl butyrate, were compared. Traps were laid out in 10 replicated blocks and were in place between 2 and 11 June 2015. The sticky inserts of the traps were changed on days 4 and 8 of the experiment, with the treatments re-randomised within the blocks after each change.

3-2.8.5 Experiment 5 – comparison with other traps

Numbers of midges caught in delta traps baited with lures containing (*R*)-2-nonyl butyrate (0.5 mg) were compared with existing trapping methods, i.e., unbaited sticky traps and water traps. Standard yellow insect sticky traps (25 × 10 cm) were mounted on fibreglass canes at crop height. Water traps (Nickerson Brothers, Lincoln, UK) comprised a yellow bowl (25 cm diameter, 10 cm deep), partly filled with water to which several drops of Fairy™ dishwashing liquid were added, and mounted on a cane at crop height. All three traps were compared in two 3 × 3 Latin squares. All traps were checked at weekly intervals between 11 and 29 May 2015.

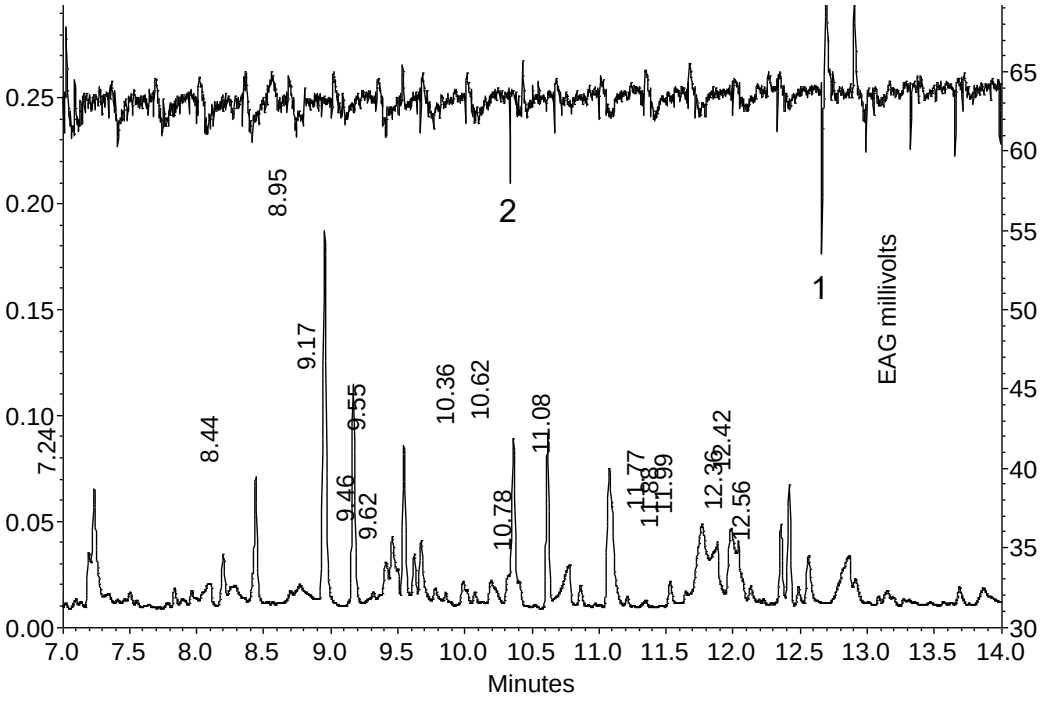
3-2.9 Statistical analysis

Numbers of *H. marginata* caught per day for each trap were log(x+1) transformed to improve the homoscedasticity of the data and were analysed with a two-way ANOVA with treatment and block as factors. The least significant difference (LSD) test was used to test for significant differences between means ($\alpha = 0.05$). All analyses were done in R v.3.2.2 (R Core Team, 2015). Results in experiment 5 were not analysed statistically due to the extreme heteroscedasticity of the data.

3-3 Results

3-3.1 Pheromone identification

Analyses of collections of volatiles from female *H. marginata* on the non-polar GC column with a male antenna EAG preparation indicated one strong EAG response and a weaker response to a compound eluting earlier (Figure 3.1). Analyses on the polar column showed a strong EAG response but the minor response was not so clear (data not shown). Retention data for the EAG responses and synthetic compounds are shown in Table 1.



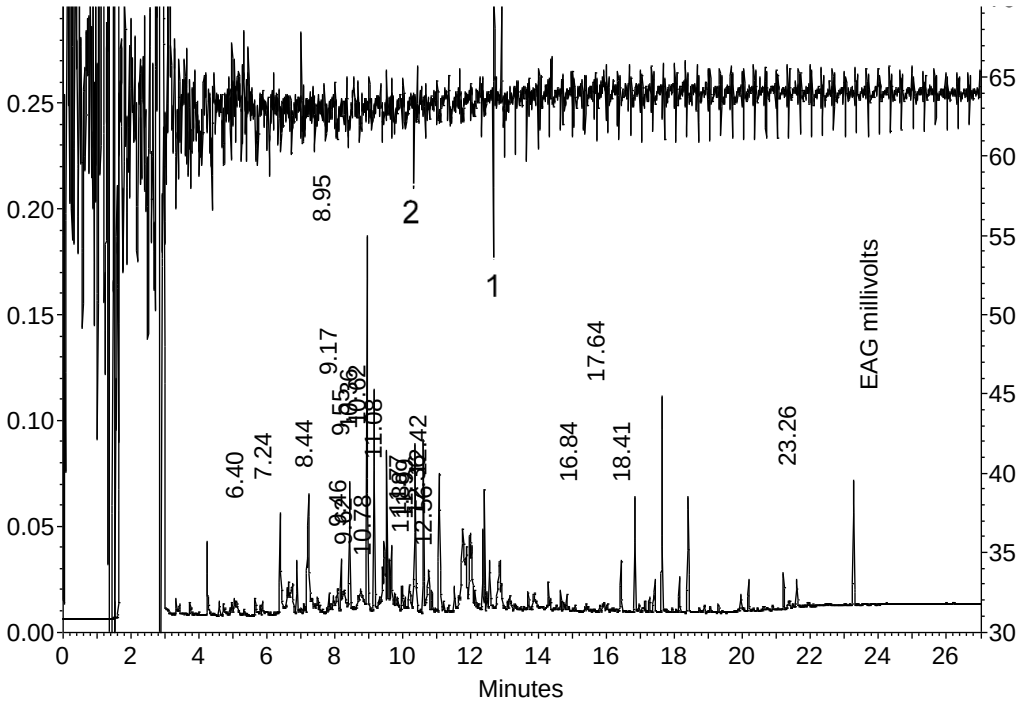


Figure 3.1. Coupled gas chromatography-electroantennography (GC-EAG) analysis of collection of volatiles from female *Haplodiplosis marginata* on non-polar column. Note that the lower panel is an expansion of the upper panel and FID signal is lower trace and EAG upper trace in each; major response (1) to 2-nonyl butyrate at 12.42 min, minor response (2) at 10.2 min; 2-heptyl butyrate at 10.00 min, 2-nonanol at 8.25 min.

Analyses of collections of volatiles from female and male *H. marginata* by GC-MS on both non-polar and polar GC columns (Figure 3.2) indicated a female-specific compound that was identified as 2-nonyl butyrate by comparison of retention times (Table 3.1) and mass spectrum with those of the authentic synthetic compound, and the identification was confirmed by co-chromatography on both GC columns. Up to 50 ng per female of 2-nonyl butyrate was collected during 48 h. This compound had retention data consistent with that of the major response in the GC-EAG analyses (Table 3.1).

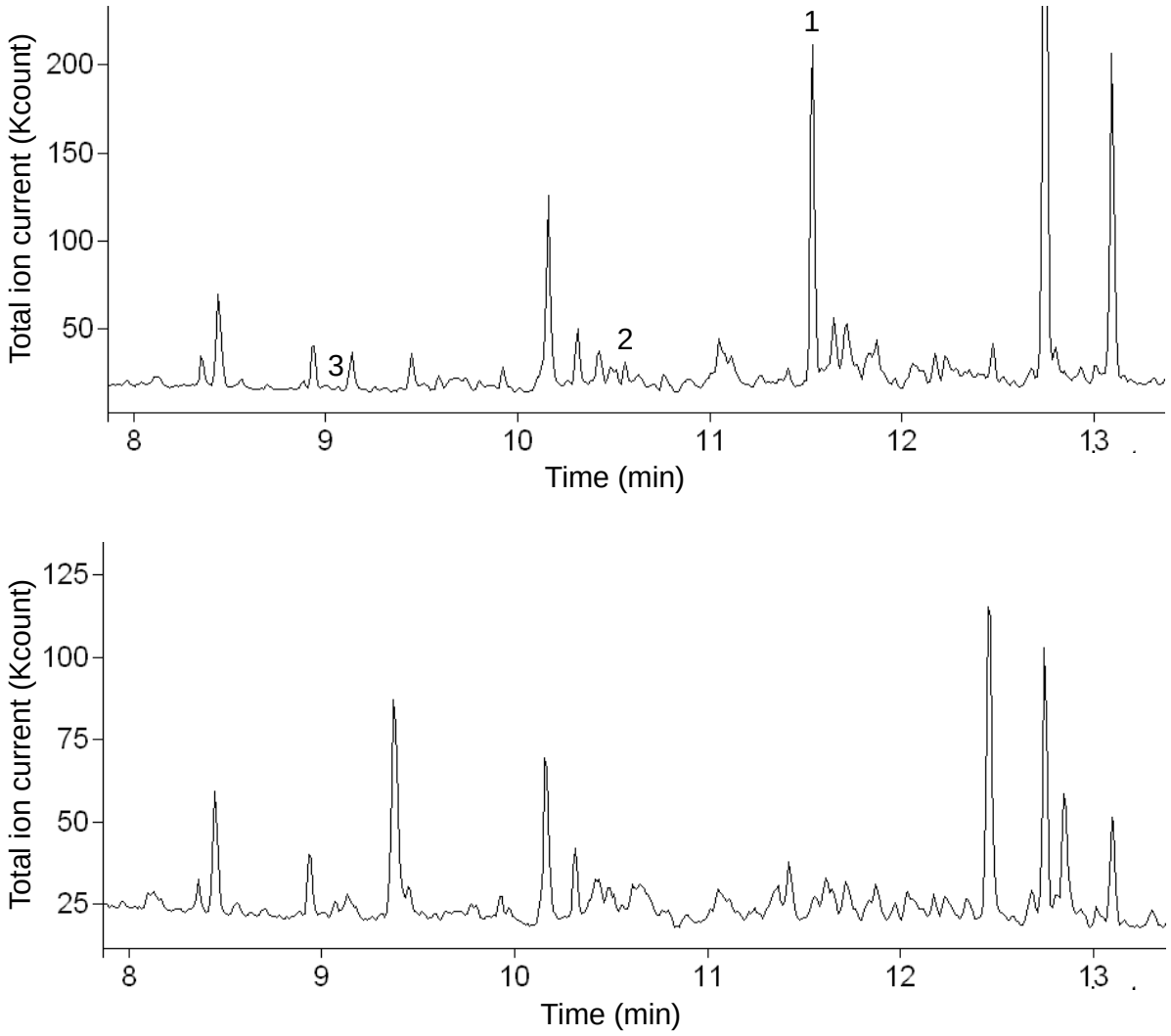


Figure 3.2. Coupled gas chromatography-mass spectrometry (GC-MS) analyses on polar GC column of volatiles from female *Haplodiplosis marginata* (upper panel) and volatiles from male *H. marginata* (lower panel). (1) 2-Nonylbutyrate, (2) 2-nonanol, (3) 2-heptyl butyrate.

2-Nonanol was detected in GC-MS analyses at approximately 2% of the 2-nonyl butyrate. Single ion scanning of the GC-MS analyses of volatiles from female *H. marginata* at *m/z* 71 and 89, characteristic of butyrate esters, showed the presence of 2-heptyl butyrate at approximately 1% of the 2-nonyl butyrate. 2-Undecyl butyrate, an analogue reported to be present by Censier *et al.* (2014b), could not be detected (<0.1% of major component). Similarly, 2,7-dibutyroxynonane, the female sex pheromone of the closely related orange wheat blossom midge, *S. mosellana* (Gries *et al.*, 2000), could not be detected by comparison with the authentic synthetic compound. Other potential minor pheromone

components related to 2-nonyl butyrate, such as 2-nonanone and 2-nonyl acetate, could not be detected (Table 3.1). In GC-EAG analyses of the synthetic compounds (10 ng injected), strong EAG responses were observed to 2-nonyl butyrate and 2-heptyl butyrate, but there was no detectable response to 2-nonanol (data not shown). The retention indices of 2-heptyl butyrate were consistent with those of the component responsible for the minor EAG responses in analyses of volatiles from female midges on both non-polar and GC columns in the GC-EAG system used (Table 3.1). Analysis of the volatiles from female *H. marginata* on the enantioselective cyclodextrin GC column indicated a peak at the retention time of (*R*)-2-nonyl butyrate (15.69 min), but no peak (<5%) at the retention time of the (*S*)-enantiomer (15.30 min).

Table 3.1. Retention indices relative to retention times of *n*-alkanes of electroantennography (EAG) responses in gas chromatography (GC)-EAG analyses of volatiles from virgin female *Haplodiplosis marginata* with male *H. marginata* EAG preparation, and of synthetic compounds

	Non-polar		Polar	
	GC-EAG (SPB1)[1]	GC-MS (VF5)[1]	GC-EAG (DBWax)[1]	GC-MS (DBWax)[1]
EAG major response	1389		1601	
EAG minor response	1235		1415	
2-Nonyl butyrate	1389	1403	1601	1591
2-Nonanol	1082	1104	1528	1513
2-Nonyl acetate	1218	1234	1460	1456
2-Heptyl butyrate	1201	1215	1400	1392
2,7-Dibutyroxy-nonane	1846	1861	2282	2245
2-Nonanone	1075	1092	1376	1378

[1] GC column phase

3-3.2 Pheromone dispensers

Polyethylene vials were found to release 2-nonyl butyrate more uniformly than the rubber septa under laboratory conditions (Figure 3.3). The rubber septa released over 90% of the pheromone within the 1st week at 27°C and 2.2 m s^{-1} wind speed. In contrast, 30% of the compound remained after 28 days in the polyethylene vials. Polyethylene vials containing an initial loading of 1 mg racemic 2-nonyl butyrate and returned from field tests after 2 weeks contained (mean ± SEM =) 0.72 ± 0.02 mg (n = 3). Polyethylene vials and rubber septa returned from the field after 6 weeks contained 0.41 ± 0.02 and 0.31 ± 0.02 mg, respectively.

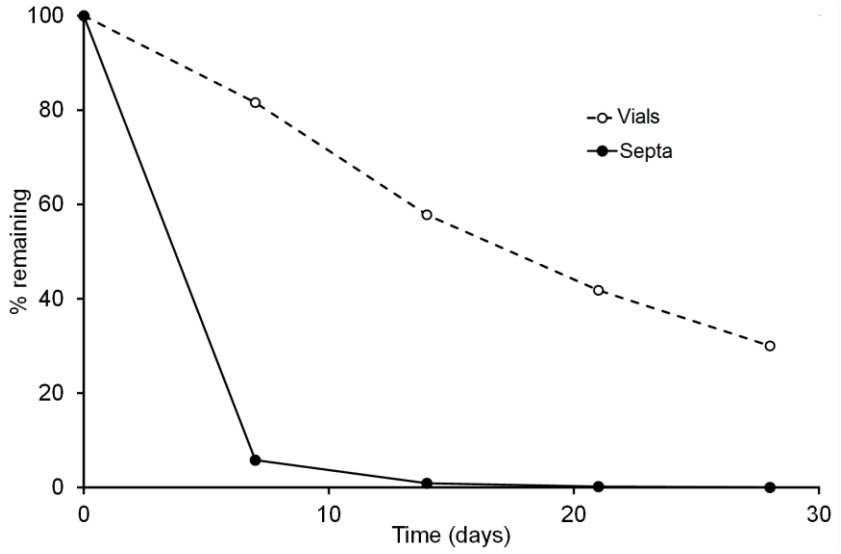


Figure 3.3. Release of 2-nonyl butyrate (1 mg) from rubber septa and polyethylene vials in laboratory wind tunnel at 27°C and 2.2 m s^{-1} wind speed as measured by gas chromatography analyses of the amount remaining at intervals.

3-3.3 Field trapping experiments

3-3.3.1 Experiment 1 – pheromone dispensers

Traps baited with 1 mg racemic 2-nonyl butyrate dispensed from either rubber septa or polyethylene vials caught more male *H. marginata* than the unbaited traps ($F_{2,9}$ = 21.33, P<0.001) during the 1st week of trapping. There was no difference however, in catches with the two dispenser types (Figure 3.4A). Catches during the next 2 weeks were too low for analysis but showed the same trend with mean catches per trap over the period of 4.3 ± 1.9 with vials, 5.3 ± 1.4 with septa, and no catches in unbaited

traps.

3-3.3.2 Experiment 2 – pheromone chirality

Traps baited with (*R*)-2-nonyl butyrate caught significantly more male *H. marginata* compared with the other treatments ($F_{3,9}$ = 22.56, P<0.001). During the 14-day trapping period no adults were caught on the unbaited traps or the traps baited with (*S*)-2-nonyl butyrate, and the catch with racemic 2-nonyl butyrate was less than 5% of that with (*R*)-2-nonyl butyrate (Figure 3.4B).

3-3.3.3 Experiment 3 – effect of minor components

A total of 26 658 male *H. marginata* was caught during the 11-day trapping period. Traps baited with racemic 2-nonyl butyrate caught significantly more than unbaited traps but less than 10% of the number caught in traps baited with (*R*)-2-nonyl butyrate ($F_{5,45}$ =253.66, P<0.001; Figure 3.4C). Addition of the minor components, (*R*)-2-nonanol and/or (*R*)-2-heptyl butyrate, did not increase or decrease trap catches compared with catches with the major component, (*R*)-2-nonyl butyrate, alone. There was no interaction between treatment and block but the effect of block was significant ($F_{9,45}$ = 6.799, P<0.01).

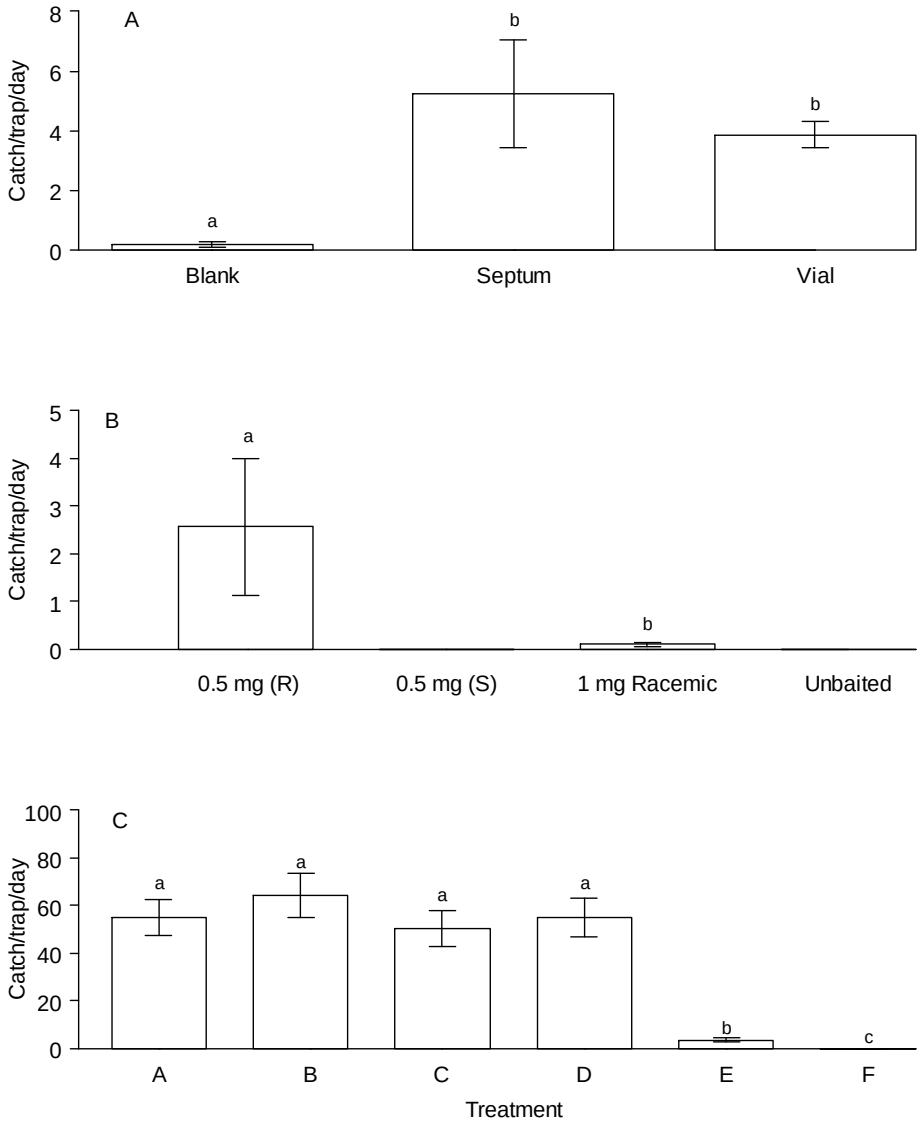


Figure 3.4. Mean (± SEM) daily catches of male *Haplodiplosis marginata* in traps baited with (A) racemic 2-nonyl butyrate (1 mg) dispensed from polyethylene vials or rubber septa (experiment 1, 15-21 May 2014); (B) racemic 2-nonyl butyrate (1 mg), (*R*)-2-nonyl butyrate (0.5 mg), (*S*)-2-nonyl butyrate (0.5 mg), and unbaited (experiment 2, 5-19 June 2014); and (C) a range of treatments (experiment 3, 18-29 May 2015): A, 0.5 mg (*R*)-2-nonyl butyrate; B, 0.5 mg (*R*)-2-nonyl butyrate + 2% (*R*)-2-nonanol; C, 0.5 mg (*R*)-2 nonyl butyrate + 2% (*R*)-2 heptyl-butyrate; D, 0.5 mg (*R*)-2-nonyl butyrate + 2% (*R*)-2-nonanol + 2% (*R*)-2 heptyl-butyrate; E, 1 mg racemic 2-nonyl butyrate; F, unbaited control. Bars show back-transformed means. Means within a panel capped with different letters are significantly different [LSD tests: $P<0.001$ (panels A and B), $P<0.05$ (panel C)].

3-3.3.4 Experiment 4 – pheromone loading

A total of 13 775 male *H. marginata* was caught during the 9-day trapping period. Significant differences in numbers caught were observed between all treatments ($F_{4,36}$ = 187.42, P<0.001) and trap catches were dose-dependent with more male *H. marginata* caught when higher pheromone loadings were used (Figure 3.5A). Log mean catch plotted against log pheromone loading indicated a linear association (Figure 3.5B).

3-3.3.5 Experiment 5 – comparison with other traps

Substantially more male *H. marginata* were caught in the pheromone trap compared with both the unbaited sticky and water traps. During the trapping period of 18 days with six replicates, over 6 500 *H. marginata* were caught using the pheromone traps compared with 26 and 27 in the sticky and water traps, respectively.

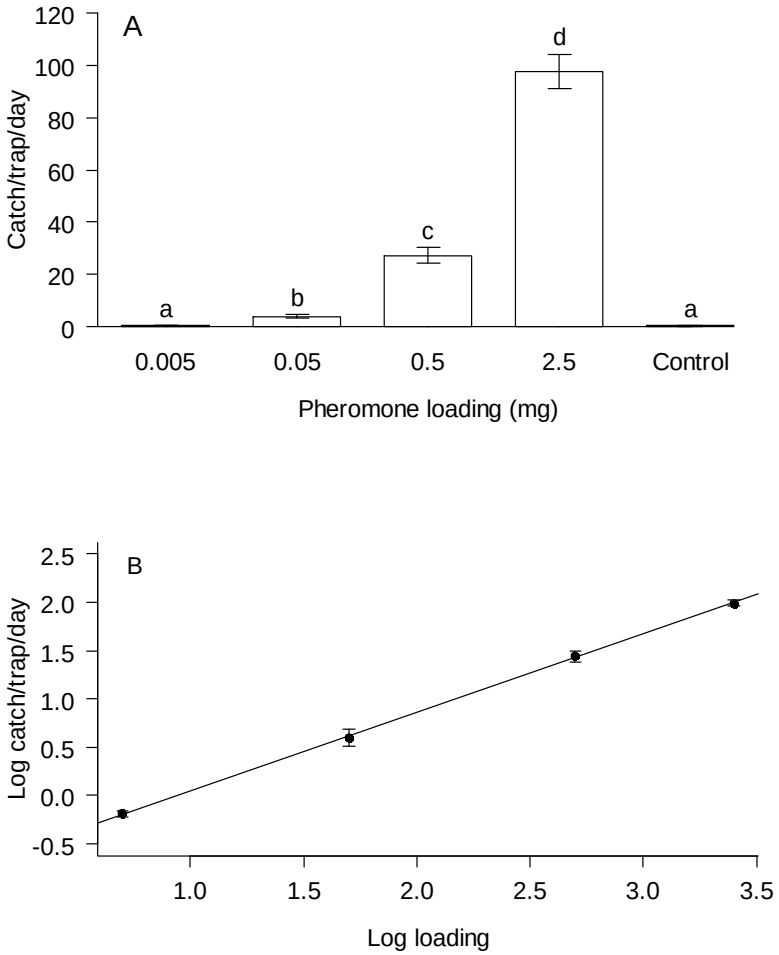


Figure 3.5. (A) Mean (± SEM) catches of male *Haplodiplosis marginata* in experiment 4 with different lure loadings of (*R*)-2-nonyl butyrate and unbaited control (2–11 June 2015; bars show back-transformed means). Means capped with different letters are significantly different (LSD test: P<0.05). (B) Log mean daily catch per trap of *H. marginata* against log pheromone loading in experiment 4.

3-4 Discussion

Although Censier *et al.* (2014b, 2016a) identified the major component of the sex pheromone produced by female *H. marginata* as (*R*)-2-nonyl butyrate, they only tested the racemic compound in field trapping tests. The racemic compound was reported to be attractive to male *H. marginata*, but the effects of potential minor pheromone components detected were not investigated. Initial studies (Censier *et al.*, 2014b) used 20 mg of the racemic compound in polyethylene sachet dispensers with a short field life of

several days, and subsequently a rubber septum loaded with 5 mg of the racemic compound was recommended for monitoring (Censier *et al.*, 2016a). Here, I confirmed that virgin female *H. marginata* produce (*R*)-2-nonyl butyrate with trace amounts (approximately 2% relative to the major component) of 2-heptyl butyrate and 2-nonanol. The former two compounds elicited EAG responses from male *H. marginata*, but the latter did not. Censier *et al.* (2014b) reported relatively large amounts of 2-nonanol were detected in volatiles from crushed pheromone glands, presumably due to enzymatic hydrolysis of the butyrate ester (cf. Ho & Millar, 2002). In field tests, traps baited with (*R*)-2-nonyl butyrate caught more than 10× the numbers of male *H. marginata* caught in those baited with an equivalent amount of the racemic compound. The (*S*)-enantiomer was unattractive, but clearly has an antagonistic effect on the attractiveness of the (*R*)-enantiomer.

Absolute configuration is often important in the bioactivity of pheromones (Mori, 2007). The sex pheromone components of cecidomyiid midges identified so far all have one or two chiral centres and the females have been shown or are deduced to produce one stereoisomer (Hall *et al.*, 2012). In components with two chiral centres the correct chirality is invariably critical for attraction. However, in the majority of those with one chiral centre the females produce one enantiomer that is attractive to males but the other enantiomer is neither attractive nor interferes with attraction of the active enantiomer (Hall *et al.*, 2012). *Haplodiplosis marginata* is an exception to this trend with the (*S*)-enantiomer not only being unattractive but also reducing the attractiveness of the naturally-produced (*R*)-enantiomer. Enantiomeric inhibition has only been observed in one other Cecidomyiid to date, the pea midge (*Contarinia pisi*) (Hillbur et al., 2001). It is thought to increase reproductive isolation in related species or to reduce the signal-to noise ratio in insect chemical communications (Hillbur et al., 2001; Leal, 1996). This is demonstrated in the Japanese and Osaka beetles (*Popillia japonica* and *Anomala osakana*), which both naturally produce different enantiomers of the same pheromone compound, the activity of which is inhibited by the presence of the antipode (Tumlinson et al., 1977; Leal, 1996). (*R*)-2-Nonyl butyrate is the simplest midge pheromone reported to date, having an unbranched carbon chain with an odd number of carbon atoms and the characteristic oxygenated functionality at the 2-position (Hall *et al.*, 2012). 2-Nonanol and its esters are relatively easily resolved into the two enantiomers with high

enantiomeric excess by kinetic hydrolytic resolution with a lipase enzyme (Hall *et al.*, 2012). The extra cost involved in using (*R*)-2-nonyl butyrate in lures rather than the racemate would probably be outweighed by the more than 10-fold increase in catches, given that the cost of the active ingredient would be a small part of the overall cost of a commercially produced lure. The absence of any effect of the potential minor pheromone components on attractiveness of the major component was somewhat surprising, although the sex pheromones of many of the cecidomyiid midge species identified to date consist of a single component (Hall *et al.*, 2012). In the chrysanthemum midge, *Rhopalomyia longicauda* the sex pheromone is (2*S*,8*Z*)-2-butyroxy-8-heptadecene, and addition of even 2% of the corresponding alcohol reduces attractiveness significantly (Liu *et al.*, 2009).

Polyethylene vials and rubber septa were compared as commercially available, practical pheromone dispensers. These were equally effective in field trapping tests and were still attractive after 5 weeks in the field in the UK. Laboratory tests however, indicated release from the vials was more uniform than from the septa and the former were likely to last longer in the field. The flight period of *H. marginata* can extend to up to 10 weeks (Censier *et al.*, 2015), and the emergence pattern appears to show small peaks of activity throughout the season. Lure longevity must therefore allow for monitoring to extend over the duration of this period. After 6 weeks in the field polyethylene vials still contained over 40% of the pheromone originally loaded, but the longevity of the lures beyond this remains to be tested in order to determine the need for lure renewal over entire the *H. marginata* flight period.

Pheromone loadings of (*R*)-2-nonyl butyrate ranging from 0.005 mg to 2.5 mg were tested to determine the optimum for a pheromone lure. The quantity of pheromone used can affect lure longevity and the aerial concentration of the pheromone in the field. If the pheromone concentration is too low, the sensitivity of the trap may be compromised. If the concentration is too high, receptor neurons in the antennae of the target insect may lose sensitivity resulting in reduced trap catches (Mayer, 1993). Increasing the loading of (*R*)-2-nonyl butyrate in polyethylene vial dispensers increased catches of male *H. marginata* with a positive linear association between log mean catch and log pheromone dose over the range tested from 0.05 to 2.5 mg. This is useful in estimating the amount of pheromone required for monitoring purposes. A catch rate that is too high can make counts difficult and quickly saturate the

sticky trap, necessitating more frequent changes. Cross and Hall (2009) determined that a mean daily catch of 25 midges per trap was suitable for monitoring apple leaf midge, *Dasineura mali* (Kieffer). The catch rate will inevitably vary greatly depending on the background population and time of trapping, but during the period of this experiment a loading of 0.5 mg of (*R*)-2-nonyl butyrate gave a mean daily catch rate of 27.3 midge per trap. These data were obtained mid-season and therefore did not represent the peak catch rate which appears to occur soon after the start of emergence (Censier *et al.*, 2016b). The higher loading of 2.5 mg would therefore be unsuitable for monitoring in many field situations. Meanwhile the lower loading of 0.05 mg, although still effective during this experiment, may not be sensitive enough to detect early emergence if there are fewer males around. Higher pheromone loadings may be appropriate if the lures are to be used for control of the pest by mass trapping or lure-and-kill approaches when the maximum catch is required. Use of (*R*)-2-nonyl butyrate rather than the racemic compound might also be advantageous in these situations. For example, a lure containing 2.5 mg of the (*R*)-enantiomer would be equivalent in attractiveness to one containing 50 mg of the racemic compound, and this latter loading would be above the capacity of the dispensers used here.

The pheromone has a limitation in that it only monitors male activity and females are rarely caught. This is the same with other successful pheromone trapping systems currently in use for monitoring cecidomyiid pests such as *S. mosellana* (Bruce *et al.*, 2007). Unlike *S. mosellana* however, where females disperse after mating, there is little evidence for female dispersal in *H. marginata*. Skuhravý *et al.* (1983) observed that females made several short flights until a suitable host plant was found. The same study also recorded a sex ratio of females to males of 59:41 and 54:46 based on emergence trap and Möricke trap catches, respectively. Female numbers are therefore likely to be slightly higher than, or comparable with, the number of males being caught. In practice, the enhanced performance of these pheromone traps in comparison to existing methods for trapping cecidomyiids far outweighs this limitation. The differences in trap catches shown between blocks in the field experiments is an indication that trap position in the field is important. Trap position has been shown to have a significant impact on catch rate in other pheromone trap systems due to interactions with the habitat (McNally & Barnes, 1981; Edde *et al.*, 2005; Mori & Evenden, 2013). The block in which the lowest

number of insects caught was located at the field boundary adjacent to farm buildings. It is likely these structures were having an effect on catch rates and traps used for monitoring should ideally be placed away from field boundaries to mitigate such effects.

The present study indicates that a polyethylene vial loaded with 0.5 mg of (*R*)-2-nonyl butyrate is a suitable lure for trapping adult *H. marginata* in the field, and would be equivalent in attractiveness to a lure containing 10 mg of the racemic compound. This system will greatly improve detection in areas of low *H. marginata* populations, and will provide a greater degree of precision when monitoring for the start of adult activity. There is still more work to be done to enable growers to manage the threat of *H. marginata* effectively. Ideally, the relationship between trap catches and crop damage should be established in order to provide a threshold above which treatments should be applied. This information will help to prevent ineffective and prophylactic insecticide applications. The longevity of the lure under field conditions will also need to be determined to define the frequency at which it should be renewed. The pheromone trap itself could be further optimised through trials to investigate trap design, trap positioning, and the range over which *H. marginata* males respond.

Chapter 4

Factors affecting trap catch in pheromone-based monitoring of saddle gall midge *Haplodiplosis marginata* (Diptera: Cecidomyiidae)

Abstract

Saddle gall midge *Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae), is a pest of cereal crops in Europe. Outbreaks are difficult to predict and effective monitoring tools are required to ensure the effectiveness of pest management options. The female sex pheromone, (*R*)-2-nonyl butyrate provides the basis of a highly effective lure for this insect. This chapter demonstrates how the success of this lure can be influenced by parameters such as trap location, lure age, and interference between traps fitted with these lures. A pheromone lure containing (*R*)-2-nonyl butyrate has the capacity to attract male midges after 9 weeks under field conditions. Traps performed best when situated away from field margins and below the height of the crop. Interference between nearby traps was evident at distances less than 20 m. The results here offer new insights into the behavioural responses of male *H. marginata* to the female sex pheromone and provide practical recommendations for the use of *H. marginata* pheromone traps in the field.

4-1 Introduction

Saddle gall midge, *Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae), is a pest of cereal crops in Europe that has exhibited a sporadic pattern of outbreaks over several decades. This is a univoltine species, with adults emerging in May following a larval overwintering stage. After mating, *H. marginata* females oviposit on the leaves of cereal plants and wild grasses. Upon hatching, larvae

Published as: Rowley, C., Cherrill, A., Leather, S., Hall, D. & Pope, T. (2017) Factors affecting trap catch in pheromone-based monitoring of saddle gall midge *Haplodiplosis marginata* (Diptera: Cecidomyiidae). *Pest Management Science* (In press)

begin to feed on the stem of the host plant from beneath the leaf sheath. Larval feeding causes the formation of saddle-shaped galls on the stem which can affect plant development and cause yield loss (Woodville, 1968; Golightly, 1979; Popov *et al.*, 1998). Spring crops of wheat and barley are most at risk from this pest (Skuhravý *et al.*, 1983; Skuhravý *et al.*, 1993), particularly where damage coincides with stem extension (Golightly & Woodville, 1974). Regular crop rotations can reduce *H. marginata* numbers through removal of the host crop (Skuhravý *et al.*, 1993), but as the overwintering larvae can survive in the soil for several years the population may still persist (Nijveldt & Hulshoff, 1968). The biology and ecology of this insect have been reviewed in detail in recent attempts to consolidate the existing information on this insect (Censier *et al.*, 2014b, Rowley *et al.*, 2015). Such reviews have highlighted the need for more effective detection and monitoring tools given the sporadic and often inconspicuous nature of the pest. This is also of importance for application of chemical controls which need to be timed to coincide with the vulnerable egg-laying stage of the insect to be effective (Censier *et al.*, 2012; Ellis *et al.*, 2014). Currently, farmers and agronomists must regularly check the crop for adults and eggs which is time-consuming and may miss the early stages of outbreaks of this pest.

Pheromone traps are regularly used for detection of pest species and their sensitivity means that insects can be detected even when population density is low, such as at the start of emergence (Witzgall *et al.*, 2010). The sex pheromones of pest species of gall midges are relatively well-studied (Hall *et al.*, 2012) and have been successfully applied to in-field monitoring and detection in a range of species such as Hessian fly, *Mayetiola destructor* (Anderson *et al.*, 2012); orange wheat blossom midge, *Sitodiplosis mosellana* (Bruce *et al.*, 2007, Bruce & Smart, 2009); and apple leaf midge, *Dasineura mali* (Cross & Hall, 2009; Cross *et al.*, 2009). Pheromone monitoring of swede midge (*Contarinia nasturtii*) has been recommended for use in combination with a predictive model to determine the time of emergence (Hallett et al, 2009). Censier *et al.* (2014b) identified the major component of the female sex pheromone of *H. marginata* as 2-nonyl butyrate. More recently, an effective lure for this pest has been developed, based on the optimised blend and loading of pheromone and dispenser type (Rowley *et al.*, 2017). For this information to be of practical benefit however, more information is needed on how best to deploy traps baited with these pheromone lures.

The longevity of a pheromone lure is dependent on the initial loading and the subsequent rates of release and degradation of the compound, which are in turn influenced by environmental conditions such as temperature and UV light (Howse *et al.*, 1998). An ideal lure should exhibit a constant rate of release and last for the duration of the flight season. In previous work, polyethylene vials loaded with 0.5 mg (*R*)-2-nonyl butyrate were identified as effective dispensers for the *H. marginata* pheromone, lasting for at least four weeks under laboratory conditions (Rowley *et al.*, 2017). Here I determine the effectiveness of the lure over time under typical field conditions. This information has implications for catch interpretation and the need to refresh lures if they are in use over the entire flight period of *H. marginata*.

In the development of many pheromone trap systems, trap position has been found to have a considerable influence on the catch rate of the insect (e.g. Bartelt *et al.*, 1994; Kong *et al.*, 2013; Rhainds *et al.* 2016). Pheromones disperse in the form of plumes, which insects detect and follow upwind to the source of the odour. Pheromone plume structure and the ability of the insect to navigate to the source are both influenced by external factors such as wind speed and direction, landscape features, pheromone concentration, and signal interference from other sources. The positioning of a trap in relation to the surrounding environment and the insect itself is therefore of importance. Pheromone plumes of the same compound have been shown to interact causing disruption of the catch rate in a particular trap (e.g. Wall & Perry, 1978). Given that several traps are often deployed within an area to increase confidence in the numbers caught, it is essential to know the minimum inter-trap distance at which interference occurs to ensure optimum catch rates in all traps (Jones, 1998). This information would also help evaluate the suitability of this lure for use in mass trapping strategies if traps have a considerable range of attraction. *Haplodiplosis marginata,* like many Cecidomyiidae, are not thought to be strong flyers and may be particularly influenced by factors affecting the pheromone plume. We therefore aim to determine the optimal positioning of *H. marginata* pheromone traps in relation to height, distance from the field margin and proximity to other traps.

4-2 Experimental Methods

4-2.1 Field sites

Three sites with existing populations of *H. marginata* located in Oxfordshire (51°55"N, 1°10"W); Buckinghamshire (51°37"N, 0°48"W) and Wiltshire (51°2"N, 1°57"W) were used. Pheromone dispensers were placed in standard red delta traps (Agralan, Wiltshire, UK) containing a removable sticky insert (15 cm x 15 cm). Polyethylene vials (26 mm x 8 mm x 1.5 mm thick, Just Plastics Ltd., London, UK) containing (*R*)-2-nonyl butyrate (0.5mg; 98% enantiomeric excess) synthesised as described previously (Rowley, 2016) were used as lures for all experiments. Traps were hung from fibreglass canes and positioned at the height of the ear of the wheat crop unless otherwise stated. Mean wind speed and prevailing wind direction for the duration of the trapping periods were obtained by pooling data for the three nearest weather stations to each field site from the Met Office MIDAS dataset (Met Office, 2012). Adult *H. marginata* were identified based on antennal and genital morphology (Harris, 1966) and counted using a bifocal microscope. All statistical analyses were done in R 3.3.1 (R Core Team, 2016). Linear mixed effects models were fitted with the lme function from the nlme package (Pinheiro *et al.*, 2016) and post-hoc multiple comparisons (Tukey's Contrasts) were performed using the glht function from the multcomp package.

4-2.2 Field experiments

4-2.2.1 Lure longevity

Traps were positioned in two fields of winter wheat: one each at the site in Oxfordshire and Buckinghamshire, between 3rd May – 1st July 2016. Winter wheat growth stages were approximately 37 at the start of the experiment and 69 by the end (Zadoks, *et al.*, 1974). Traps were positioned at the height of the ear along two parallel transects 20 m apart. Four traps were placed at intervals of 40 m along each transect. Traps placed at the same distance along the two transects represented a pair, each trap baited with either a pheromone lure that remained in the trap throughout the season or a lure that was replaced weekly. New lures were replaced on days 6, 13, 20, 29, 34, 43, 50 and 59 of the experiment at which time the sticky inserts of all traps were renewed and the positions of traps within a pair were switched to reduce positional effects. Trap height was adjusted each week to match the

growth of the crop. At the end of the experiment aged lures were retained. The remaining pheromone was extracted from each lure individually in hexane (5 ml) containing dodecyl acetate (1 mg) as the internal standard. Extracts were analysed by GC with FID on a capillary column (30 m x 0.32 mm i.d. x 0.125 µ film thickness) coated with DB5 (Agilent) with splitless injection (220°C) and the oven temperature held at 50°C for 2 min and then programmed at 10°C/min to 250°C. Data for the first two weeks of the experiment at the Bucks site and the first week at the Oxon site were removed due to low catches in all traps unduly influencing the model fit.. Numbers of *H. marginata* caught per day for each trap were log transformed to improve the homoscedasticity of the data. The effect of field, days elapsed and lure type (old or new) on catch were analysed using a linear mixed model with pair as a random effect. The total catch of traps with old lures was calculated as a percentage of the total catch of traps with new lures for each time period. A linear regression of this data against days elapsed was used to analyse the effect of time on lure performance.

4-2.2.2 Trap height

Traps were positioned at the site in Oxfordshire between 13th – 19th May 2016 in two adjacent fields. One field was in winter wheat and the other in spring wheat, which were at growth stages 45-47 and 29-31 respectively over the experimental period. Traps were laid out in two 4 x 4 Latin squares, one in each field with at least 200 m between the two squares. Four height treatments were used, measured from the ground to the base of the trap: 0 cm, 40 cm, 80 cm and 120 cm. Treatment 0 cm was below the height of the crop in both fields. Treatment 40 cm was at the height of the ear in the field of winter wheat, and above crop height in the field of spring wheat. Treatments 80 cm and 120 cm were above crop height in both fields. Sticky cards were removed and counted on day three and at the end of the experiment. Treatments within each Latin square were re-randomised on day three. Both sets of counts were used in the analysis. Numbers of *H. marginata* caught in each trap were log(x+1) transformed to improve the homoscedasticity of the data and were analysed using a two-way analysis of variance (ANOVA). Tukey's honestly significant difference (HSD) test was used to compare means of different height treatments overall and between fields.

4-2.2.3 Distance from field margins

Traps were positioned at all three sites in fields of winter wheat between 19th May – 1st June 2016. The crop was at growth stage 47 at the start of trapping and 59 at the end. Three traps were positioned at 20 m intervals on a transect perpendicular to the field margin, with the first trap placed in the margin itself. Transects were placed on field margins of each aspect (north, south, east and west facing) in each field giving 12 transects in total. Each transect was later classified as upwind, downwind or crosswind according to the prevailing wind direction for the trapping period. Sticky inserts were changed weekly. Count data were pooled over the entire trapping period and the effects of trap position in relation to wind direction and distance from the field margin on catch were analysed using a linear mixed model with transect as a random effect. Diagnostic plots of residuals were used to check that the assumptions of the model were met. Multiple comparisons of means were used to test for significant differences in catch between traps at different distances from the field margin.

4-2.2.4 Range of interference

Traps were positioned in a field of winter wheat at each of the three sites between 1st – 22nd June 2016. The crop was at growth stage 59 at the start of trapping and 65 at the end. In each field were positioned four hexagonal arrays of traps with an additional central trap, so that all traps were equidistance apart with at least 80 m between arrays (Elkinton & Cardé, 1988; Wedding *et al.*, 1995). Each array had a different inter-trap distance (treatment): 5 m, 10 m, 20 m and 40 m, with each treatment occurring once per field. The sticky inserts of all traps were changed three times at an interval of one week. On each occasion the treatments were re-randomised within each field. The design gave nine replicates of each inter-trap distance. The central trap remained in the same location regardless of the inter-trap distance. Following log transformation, the relationship between inter-trap distance and mean catch of the outer and central traps was investigated using a linear mixed effects model with array as a random effect. Significant outliers were found in both downwind traps of one of the 20 m arrays over one particular trapping period. As these traps were determined to be unduly influencing the fit of the models, it was decided that these should be removed prior to analysis. Diagnostic plots of residuals were used to check that the assumptions of the models were met.

4-3 Results

4-3.1 Field experiments

4-3.1.1 Lure Longevity

Over the entire experimental period, traps baited with the same lure caught fewer insects than traps baited with new lures ($F_{1,94}$=50.65, P<0.001) but this effect did not change significantly over time (Figure 4.1). There were clear differences between the numbers of insects caught at each field site ($F_{1,6}$=43.95, P<0.001) and fewer insects were caught as the experiment progressed ($F_{1,94}$=466.54, P<0.001) (Figure 4.1).

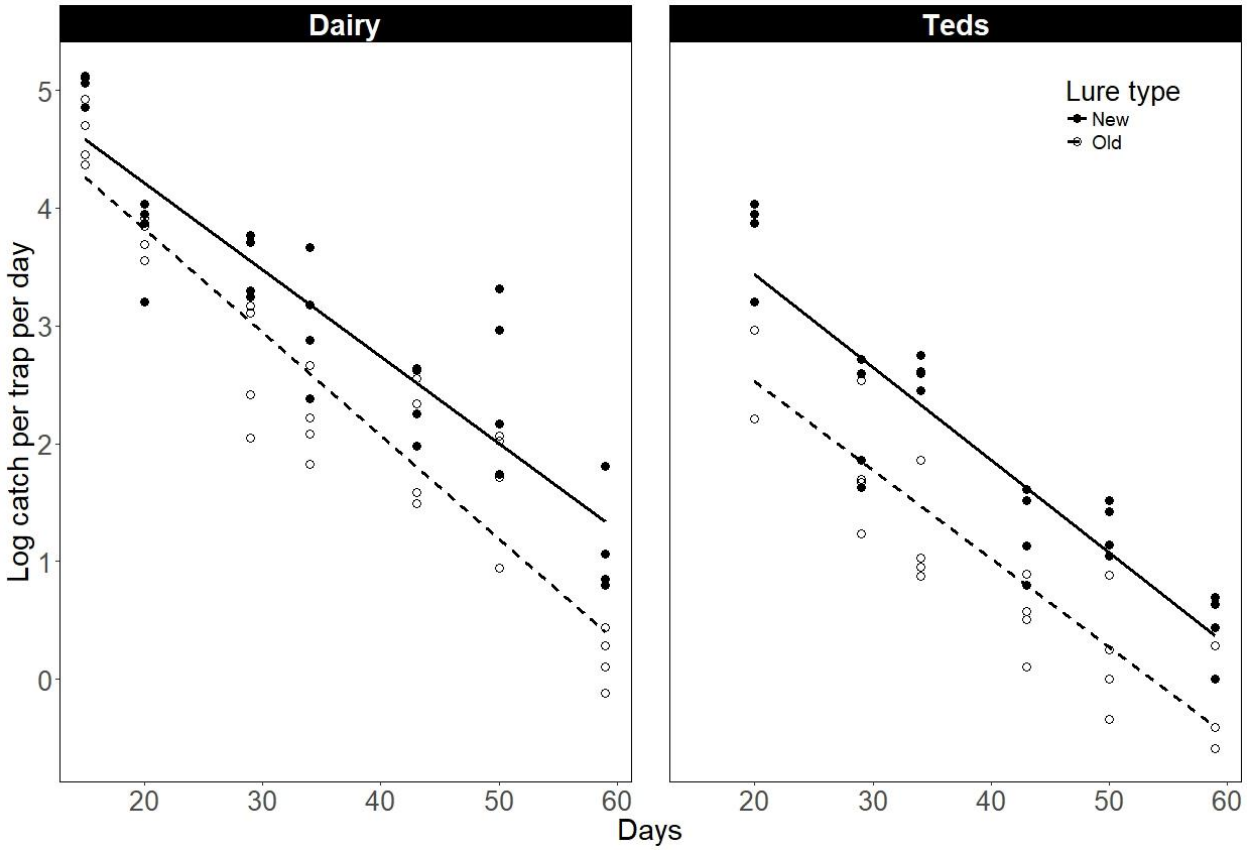


Figure 4.1. Catches of *Haplodiplosis marginata* males in traps baited with lures maintained continuously (old) or renewed at approximately weekly intervals (new) at two sites (3 May – 16 July 2016; *N* = 4 at each site; dots show log counts, lines show regressions)

The number of insects caught in traps with old lures expressed as a percentage of the catch in traps baited with new lures did not decrease significantly over time ($F_{1,5}$=4.536, P = 0.086) although a negative trend was evident (Figure 4.2). Analysis of the old lures (*N* = 4) revealed that 39.4% ± 0.7 of the pheromone from site 2 (Bucks) and 36.1% ± 1.4 of the pheromone at site 3 (Oxon) remained in the lures after the 59-day trapping period. Mean air temperatures during this time were 13.43 ± 0.10°C and 13.36 ± 0.11°C at sites 2 and 3 respectively; with the maximum air temperature not exceeding 25°C at either site.

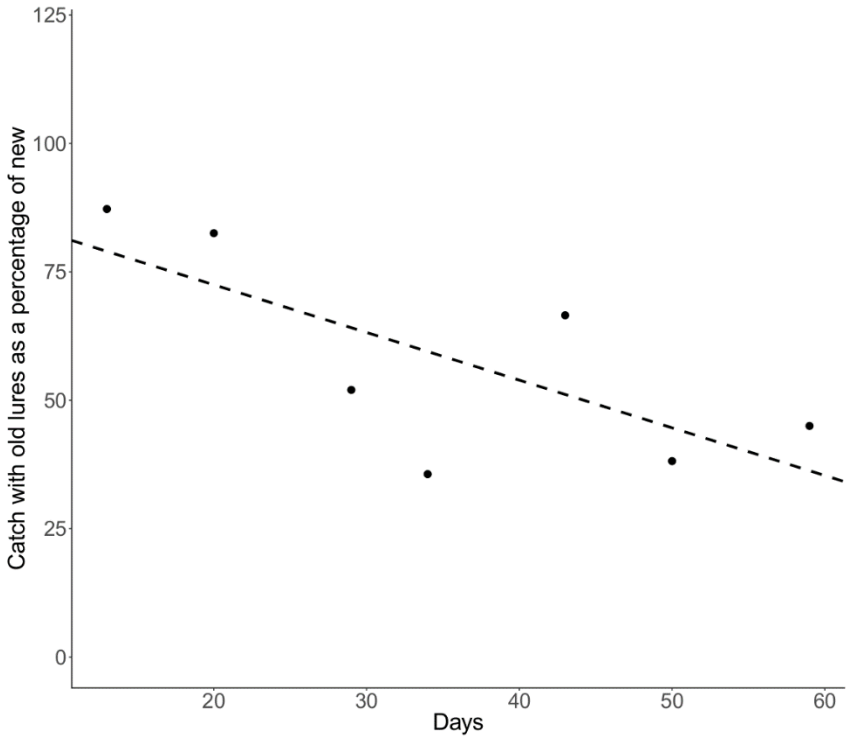


Figure 4.2. Total catches of *Haplodiplosis marginata* males in traps baited with lures maintained continuously expressed as a percentage of catches in traps baited with lures renewed for each trapping period at two sites (3 May – 16 July 2016; *N* = 4 at both sites).

4-3.1.2 Trap Height

Numbers of insects caught in each field were nearly identical: 49% of the total trapped were caught in field 1 and 51% in field 2. The fewest insects were caught at 80 cm and 120 cm; catches at 0 cm and

40 cm accounted for 98.3% of the total 3,100 trapped over the period of the experiment. Catch numbers differed between heights ($F_{3,30}$ =110.33, P<0.001), and catch rates at 0 cm and 40 cm heights differed between fields ($F_{9,24}$ =5.78, P<0.001) (Figure 4.3). This difference was accounted for by trap height in relation to crop height. *Post hoc* tests revealed that field 1 in spring wheat (crop height of approximately 10 cm) had far higher numbers of insects trapped at 0 cm than 40 cm (P<0.001). Field 2 in spring wheat (crop height of approximately 40 cm) had no difference in catches at these two heights and had a higher number of insects caught at 40 cm compared with field 1 (P<0.001). Both fields therefore showed high catches in traps positioned below crop height and low catches in traps positioned above crop height (Figure 4.3).

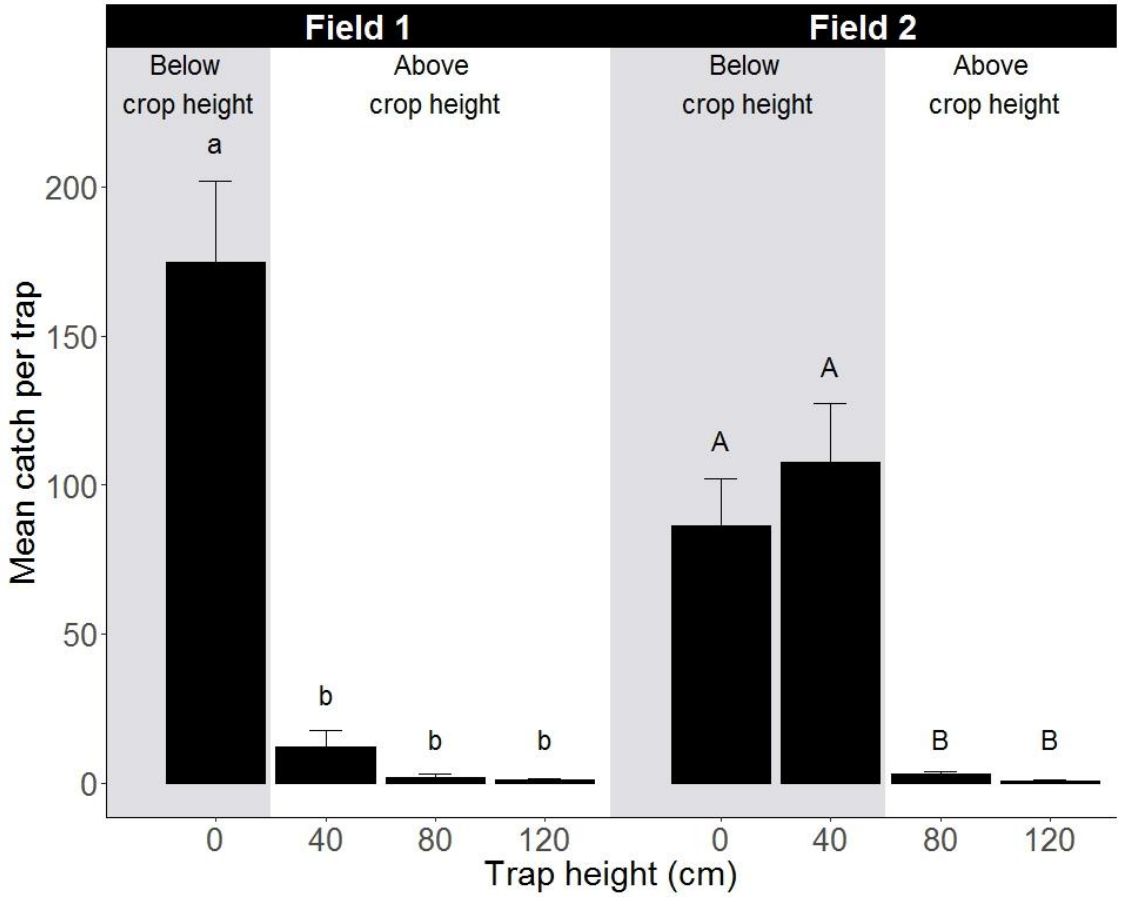


Figure 4.3. Mean catches (+SEM) of *Haplodiplosis marginata* males in traps positioned at different heights in fields of spring wheat (Field 1) and winter wheat (Field 2) at the Oxfordshire field site (13-19 May 2016; *N* = 4 at each site and height; shaded areas represent traps at or below the height of the crop). Different letters indicate significant differences between heights.

4-3.1.3 Distance from field margins

Transect direction in relation to prevailing wind direction had no effect on catch rate ($F_{2,9}$ = 0.29, P=0.75). The number of *H. marginata* males caught was affected by the distance of the trap from the field margin ($F_{2,22}$ =8.19, P<0.01) (Figure 4.4). *Post hoc* testing revealed lower catches in traps positioned in the field margin compared with those positioned 20 m (P<0.05) and 40 m into the crop (P<0.001). There was no difference in catch between the traps placed 20 and 40 m into the crop (P=0.54).

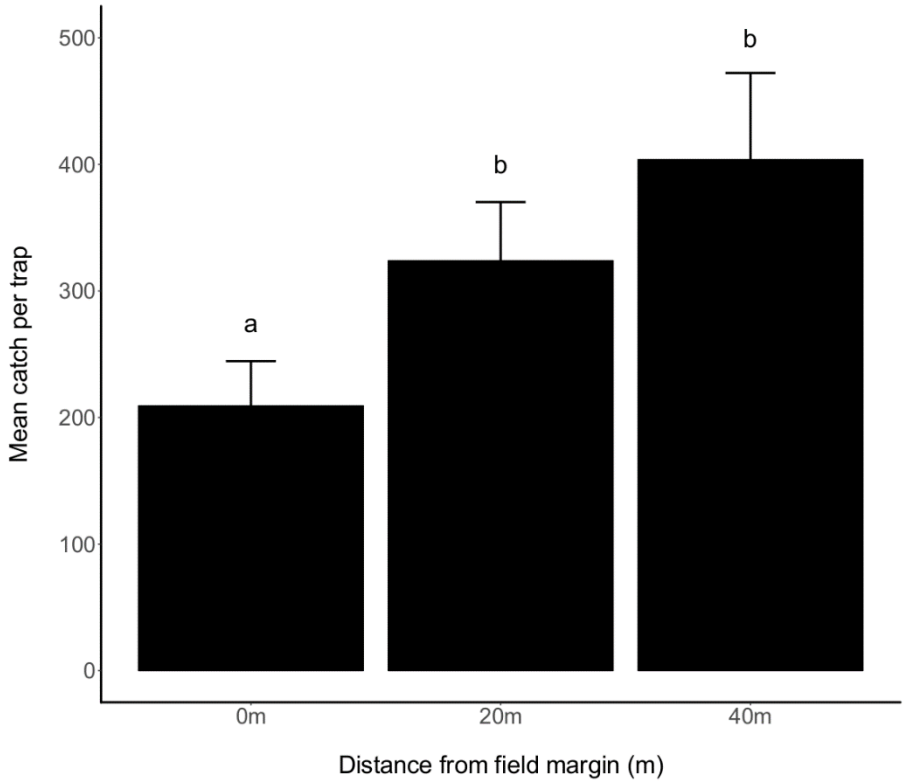


Figure 4.4. Mean catches (+SEM) of *Haplodiplosis marginata* males in traps positioned at increasing distance from the field margin (19 May – 1 June 2016; three sites, N = 4 at each site). Lowercase letters indicate significant differences between distances.

4-3.1.4 Range of interference

The number of male *H. marginata* caught per day in outer traps of the hexagonal array was higher compared to central traps ($F_{1,49}$=22.58, P<0.001) and was higher overall (all traps combined) in arrays with a greater inter-trap distance ($F_{1,6}$=49.21, P<0.001). Differences between the catch rate of outer and inner traps reduced with increasing inter-trap distance ($F_{1,49}$=12.93, P<0.001) (Figure 4.5).

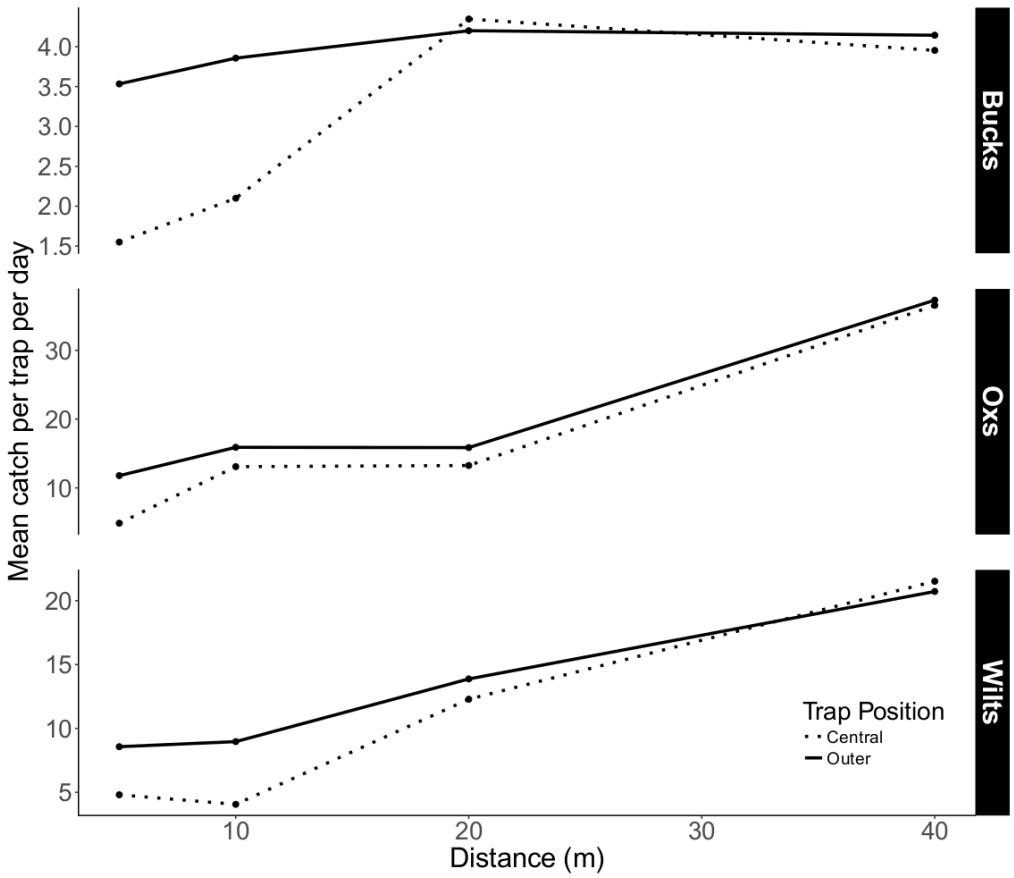


Figure 4.5. Mean numbers of *Haplodiplosis marginata* males caught in central and outer traps in hexagonal arrays of different inter-trap distances at three sites (1-22 June 2016; *N* = 9).

4-4 Discussion

The results presented here provide new insights into factors affecting the performance of pheromone-baited traps for *H. marginata* that will contribute to design of protocols for their use in monitoring and potentially control of this pest.

Pheromone lures were still attracting male *H. marginata* adults after nearly nine weeks in the field which is comparable to data for commercially available lures for other pest species (Mcnally & Barnes, 1980; Vanaclocha et al, 2016) and longer than the recommended usage time of six weeks for *S. mosellana* lures (Bruce *et al.*, 2007; Bruce & Smart, 2009). Lures replaced each week consistently caught more midges than lures maintained continuously, even at the beginning of the experiment. Release of pheromone from the polyethylene vials is first order, i.e. proportional to the amount remaining, and it seems unlikely that the small decrease in release rate from the old lures during the

first two weeks would have resulted in a significant decrease in catches. Lures for the experiment were stored in sealed aluminium foil bags, and it is possible that, after removal from the bags and installation in the traps, there was an initial "burst" of pheromone from the surface of the lures that may have given consistently higher catches during the first day (Hodges *et al.*, 2004).

The number of insects caught with old lures as a percentage of new lures did not decrease significantly over time in these experiments, but there was a clear negative trend to the relationship which would probably become statistically significant with more data points. In the final week of trapping, old lures trapped 45% of the number of insects caught by new lures which is to be expected from the finding that 35 – 40% of the pheromone remained in the old lures at the end of the experiment. This concurs with earlier experiments where 6-week old field aged lures containing 1 mg of racemic 2-nonyl butyrate had 0.41 ± 0.02 mg of the compound remaining (Rowley *et al.*, 2016) (see Chapter 3, section 3-3.2). Thus these lures are likely to remain attractive over the entire flight period of *H. marginata*, which is typically 8 – 10 weeks (Censier *et al.*, 2015). This will reduce the cost and time required to operate this system in the field. There is some decrease in attractiveness during this period and further work is required to relate catches directly to population levels, but it is anticipated that population peaks may be reliably identified mid-season relative to catches in the previous weeks, alerting the farmer to a potential increase in oviposition activity.

The height of a pheromone trap can strongly affect catch rate (e.g. Mori & Evenden, 2013). Cecidomyiidae are typically not known to be strong fliers (Gagné, 1994) and *H. marginata* appears to be no exception: the furthest flight distance recorded for males is just 120 m (Skuhravý *et al.*, 1983). Cecidomyiidae males also tend to exhibit a lack of vertical movement during flight (Harris & Foster, 1999). An earlier trapping study of *H. marginata* using passive traps at the same heights used in the present experiment found that 9.8 – 17% of males were caught in traps at 80 cm or above, compared with 25 – 33% of females (Skuhravý *et al.*, 1983). Here, I found traps at heights of 80 cm and above accounted for just 1.7% of the total insects caught. This may be a consequence of using active rather than passive traps, wind conditions during the experiments, and the absence of females caught as they generally fly at greater heights (Skuhravý *et al.*, 1983, 1993). The effect of the lower trap heights varied

between fields as a consequence of crop type. In the field of spring wheat, 92% of insects were caught at ground level which was the only trap below crop height. In the field of winter wheat only 43.7% of insects were caught at ground level while 54.4% were caught at 40 cm which was at the height of the ear of crop. As with most midge species, adult *H. marginata* are often found close to the soil surface as this is the location of emergence and mating (Skuhravý *et al.*, 1993). The results indicate that the adults are relatively evenly dispersed within the crop, although not above, even though the pheromone plume from traps above the crop level would have extended into the crop. This is in contrast to results with several other midge species (Hall *et al.*, 2012). For apple leaf midge, catches in traps at 0.5 m above ground were only 30% of those at ground level, even though the canopy was much higher (Cross & Hall, 2009). A similar effect of the interaction between habitat and trap height was observed in lesser grain borers (*Rhyzopertha dominica*) responding to an aggregation pheromone (Edde *et al.*, 2005). The presence of volatiles from the crop may also enhance mate seeking behaviour in this insect, as is the case with males of the brassica pod midge *Dasineura brassicae* (Murchie *et al.*, 1997). A study of codling moth in orchards recommended that trap height be considered relative to the tree height rather than in absolute terms (Riedl *et al.*, 1979). Although the height of wheat crops may not vary to the same extent, this study supports the idea that the crop is important in standardising catch rates in monitoring traps between fields. Based on these findings, it would be most practical for farmers to position pheromone traps at the height of the ear, as is recommended for pheromone traps of *S. mosellana* (AHDB, 2016). This height gives a good level of performance and also makes the traps easier to find than those placed at ground level.

Catch rates of *H. marginata* declined when pheromone traps were situated in field margins. Of the total number of insects caught, 22% were in the field margin traps compared with 35% and 43% caught in traps 20 m and 40 m into the field respectively. This result may be a function of the reduced area from which *H. marginata* could be attracted to the traps, given that most margins were not adjacent to areas with *H. marginata* populations. There were no differences in catch rates in traps positioned 20 m and 40 m into the crop, yet there was a trend towards increased catch rates with increasing distance from margins at two out of three study sites. The third site had the lowest catch rates with just 14% of

the total insects trapped, which may account for the lack of a similar trend. Couch grass (*Elymus repens*) and other wild grasses have been shown to be excellent host plants for *H. marginata* (Schütte, 1964; Skuhravý *et al.*, 1983) and weeds can increase pest populations by acting as alternate host plants (Norris & Kogan, 2005). The presence of grass weed species in field margins here did not appear to increase numbers of *H. marginata* in these areas, possibly because the in-field populations were substantial or because the particular grass species were not favoured by *H. marginata*. Obstacles such as hedgerows and trees adjacent to the margins may have impeded the flight of insects (Lewis, 1969), but the direction of the transect in relation to wind direction had no effect on the catch rate which suggests this was not the case. There were signs of predation on traps and although not surveyed here, it is possible that natural enemy populations associated with the field margins could have affected *H. marginata* counts in these areas. Field margins can augment natural enemy populations in arable fields (Bianchi *et al.*, 2006), but any suppressive effect may be reduced with increasing distance into the crop (Dennis & Fry 1992). In a study on European corn borer (*Ostrinia nubilalis*) trap location, the authors suggest that in addition to increased catch rates, within-field trap placement is advantageous in that the uniform habitat of the crop results in a more reliable trapping system (Derrick *et al.*, 1992). It is therefore sensible to propose that *H. marginata* pheromone traps should be placed in an open space in an area of the field with known populations to maximise capture rates. In practice, given that traps placed 40 m into the crop would increase maintenance time with no appreciable gain in catch rate, a position 20 m into the crop should be sufficient in most cases.

Female Cecidomyiidae have been shown to produce sex pheromones that act as attractants over long distances rather than eliciting short-range behavioural effects (Harris & Foster, 1999). The high numbers of *H. marginata* caught in traps baited with (R)-2-nonyl butyrate support this however it raises the possibility of interference occurring between lures of nearby traps. The flight behaviour of *H. marginata* is not well studied, but *M. destructor* males exhibit plume following behaviour very similar to that of male moths when responding to female sex pheromones (Harris & Foster, 1991). The range of interference within moth pheromone trap systems has been studied based on the idea that pheromone traps in the centre of an array of traps will catch fewer individuals than traps on the outer edges if plumes

are interacting (Wall & Perry, 1978; Houseweart, 1981; Elkinton & Cardé, 1988; Bacca *et al.*, 2006). In the case of *H. marginata*, central traps caught fewer insects than the outer traps and this difference declined with increasing inter-trap distance. This indicates the occurrence of plume interactions, where the overlapping plumes from upwind lures divert the insect away from the central trap (Wall & Perry, 1978, 1980, 1987). On this basis, trap interference occurs at inter-trap distances below 20 m and that this should be considered the minimum trap spacing to avoid plumes from overlapping. There was also an overall reduction in catches in the traps with decreasing inter-trap spacing and it is conceivable that this resulted from a trapping out of insects in the area. In a detection or monitoring trap it would be advantageous to use larger inter-trap distances where possible to avoid the possibility of interactions occurring at higher wind speeds. For mass trapping or pheromone disruption strategies, at least 25 traps would need to be deployed per hectare to ensure coverage of the area at the current pheromone concentration. However, far higher catches can be obtained by increasing the pheromone loading to 2.5 mg or more (Rowley *et al.*, 2016). Further research would be required to determine the minimum distance between traps at a higher pheromone loading but it is likely to be large enough to offset the increased pheromone production costs in order to get complete coverage over an area.

The recommendations for use describe not only aspects of practical consideration for consumers which are important in achieving reliable results from the product (Wall, 1990); but also provide insight into the flight of male *H. marginata* following emergence and their responses to pheromone lures.

Chapter 5

PCR-based gut content analysis to identify arthropod predators of *Haplodiplosis marginata*

Abstract

Saddle gall midge (*Haplodiplosis marginata*) is a cereal pest exhibiting sporadic outbreaks for which chemical control options are limited. Integrated Pest Management programmes may offer a means of suppressing *H. marginata* outbreaks, reducing pesticide input. Many IPM programmes benefit from the natural population suppression inflicted through predation and parasitism. The larval stage of *H. marginata* overwinters in the soil and may be predated by ground-dwelling arthropods, however the natural enemies of *H. marginata* are poorly studied. A PCR-based assay for detecting *H. marginata* in the guts of predators was designed using novel species-specific primers. Feeding trials involving *H. marginata* larvae showed a detectability half-life of 31.07 hours post-feeding in *Nebria brevicollis.* The guts of field-caught Carabidae were screened for *H. marginata* DNA. Four species: *Poecilus versicolor, Nebria brevicollis, Harpalus rufipes* and *Loricera pilicornis* were identified as natural enemies of *H. marginata* for the first time. A higher proportion of positive results were obtained at the end of *H. marginata* emergence (July) compared with the beginning (May). The importance of understanding trophic interactions in the management of *H. marginata* is discussed in addition to the potential uses for the newly designed assay and primers.

Rowley, C., Cherrill, A., Leather, S., McCormack, A., Skarp, J. & Pope, T., (*Submitted*) PCR-based gut content analysis to identify arthropod predators of *Haplodiplosis marginata***.** *Biological Control*

5-1 Introduction

Saddle gall midge *Haplodiplosis marginata* (von Roser) is a pest of cereals that has been the focus of relatively little research in Europe due to the sporadic nature of outbreaks, often with decades in between. Outbreaks in the United Kingdom and elsewhere from 2010 onwards have highlighted gaps in knowledge regarding the best options for the control and long-term management of this pest. Recent reviews have consolidated existing literature on the biology and ecology of this insect (Censier *et al.*, 2015; Rowley *et al.*, 2016) to further address this issue.

Briefly, *H. marginata* is a univoltine insect that overwinters in the larval stage. Adults emerge in late April through May and oviposit on the leaves of cereals and grasses (Censier *et al.*, 2015; Rowley *et al.* 2016). Newly hatched larvae then feed on the stems of the plant until maturity, forming saddle-shaped galls in the process (Golightly & Woodville, 1974). The larvae then drop from the plant in late July and burrow down into the soil to enter diapause, which can extend to more than one year when environmental conditions are not conducive for pupation to occur in spring (Nijveldt & Hulshoff, 1968). Gall formation on the stems of cereal plants can lead to inhibition of growth and yield loss, as well as increasing the risk of pathogen attack and stem breakage (Nijveldt & Hulshoff, 1968; Woodville, 1970; Golightly, 1979; Popov *et al.*, 1998).

Application of pyrethroid insecticides, timed to coincide with adult emergence or the egg laying stage, can be effective against this pest (Ellis *et al.*, 2014). Later applications may be ineffective as larvae are protected from the insecticide by the leaf sheath whilst feeding (Gratwick, 1992). It is widely accepted, however, that an over-reliance on chemical pesticides is undesirable due to detrimental effects on human health and the environment (Aktar *et al.*, 2009; Geiger *et al.*, 2010). In particular, pesticides such as pyrethroids can have a negative impact on non-target organisms such as carabids (Holland & Luff, 2000; van Toor, 2006). Integrated Pest Management (IPM) programmes aim to employ control measures that minimise the impact on the wider environment (Kogan, 1998) and are promoted by the EU Sustainable Use of Pesticides Directive as a means of minimising chemical inputs in pest management (Directive 2009/128/EC). Such programmes are based on decision support systems that rely on knowledge of the biology and ecology of the target organism, including interactions with other

organisms in the crop environment (Kogan, 1998). One strategy that may be adopted in IPM programmes is to increase pest mortality from natural enemies through conservation or augmentative biological control (Naranjo, 2001; Östman *et al.*, 2003). Currently, the impact of predation on *H. marginata* population dynamics is poorly understood and there is a clear lack of information on the natural enemies of this insect (see below). Such knowledge would greatly benefit decision making in IPM programmes concerning this pest.

Predatory interactions involving invertebrates in the field can be difficult to study, often being short-lived, inconspicuous, and unobservable without intervention (Stuart & Greenstone, 1990; Symondson, 2002). The problems are exacerbated with belowground interactions (Juen & Traugott, 2004) which has led to a distinct lack of information on the arthropod species that predate on primarily soil dwelling species such as *H. marginata*. An important component of IPM programmes is an understanding of the impact of natural enemies on pest populations. In many cases, effective maintenance of natural enemy assemblages can help to suppress pest populations (Symondson *et al.*, 2002; Wilby & Thomas, 2002; Cardinale *et al.*, 2003). This is generally achieved by increasing numbers of existing predator populations either artificially through introductions (augmentative biological control) or naturally through beneficial environmental practices (conservation biological control). Generalist predators are potentially less effective against dipteran pests due to a large proportion of the pests' life cycles being belowground or within the host plant (Symondson *et al.*, 2002). Nonetheless, the presence of predatory natural enemies has been shown to have an effect on populations of dipteran pests such as brassica pod midge (Büchs & Nuss, 2000), onion maggot (Grafius & Warner, 1989) and cabbage root fly (Mowat & Martin, 1981).

Larvae of *Haplodiplosis marginata* are most vulnerable to predation in April and early May, when they move towards the soil surface to pupate, and in July and August, when mature larvae drop from the plant to the soil. Predation of the larvae of another Cecidomyiid, orange wheat blossom midge *Sitodiplosis mosellana* (Géhin), by Carabidae and Staphylinidae is thought to occur in the soil stage (Speyer & Waede, 1956), during pupation (Floate *et al.*, 1990) and on return to the soil to overwinter (Basedow, 1973; Holland & Thomas, 2000). Arthropod predators active during these periods could

therefore be exploited to enhance the suppressive effects of regular crop rotations as a means of reducing the frequency and severity of *H. marginata* outbreaks.

Current information on natural enemies of *H. marginata* or the associated mortality rates at any life stage is limited. The parasitoids *Chrysocharis amyite* (Walker) and various *Platygaster spp*. are known to attack *H. marginata* larvae, but they have little impact on overall population size (Nijveldt & Hulshoff, 1968; Baier, 1963; Skuhravý, 1982; Rowley *et al.*, 2016). As with *S. mosellana*, Carabidae and Staphylinidae have been reported to predate on larvae of *H. marginata,* however, field observations are scarce and the exact species remain unidentified (Golightly & Woodville, 1974; Skuhravý *et al.*, 1993). Nothing is known about the species that predate on adults. A study in Canada, identified 14 species of carabid predating on *S. mosellana* in the field (Floate *et al.*, 1990). This study utilised immunological markers to identify evidence of predation from gut content analysis. Over the past few decades, PCR-based molecular gut analysis has been developed as an alternative to immunological assays to identify predation through the detection of the of target organism DNA in the guts of predators (Chen *et al*., 2000; Symondson, 2002; Gariepy *et al.*, 2007). The relatively quick, cheap and easily reproducible nature of this technology means it has become a widespread and reliable means of detecting trophic interactions in the field. PCR-based gut assays have been used extensively in agroecosystems to identify the natural enemies of pest species such as cereal aphids (Chen *et al.*, 2000), western corn rootworm (Lundgren *et al.*, 2009), cotton whitefly (Zhang *et al.*, 2007), slugs (Hatteland *et al.*, 2011), and pollen beetle (Öberg *et al.* 2011), including multiplex reactions with multiple target pest species (Harper *et al.*, 2005; King *et al.*, 2010). The method is highly suited to predator surveys such as this where prey spend a large proportion of the time belowground, making observational studies impossible. Despite the potential for increased false negatives from soil contamination (Juen & Traugott, 2006) this technique has been used successfully to identify trophic interactions of belowground species in the field (Eitzinger *et al.*, 2013).

Here, the development of species-specific primers for *H. marginata* for use in a PCR-based gut assay is described. Results from a field survey of natural enemies of *H. marginata* in the UK using the assay are reported and predators of this insect are identified to species level for the first time.

Knowledge of the key species that predate on the larval stage of this insect will help to inform decisions aimed at encouraging populations of beneficial insects as a means of aiding pest population suppression. This work may also lead to future applications of molecular techniques in further research efforts on this relatively understudied cereal pest insect.

5-2 Methods

5-2.1 Insects

Haplodiplosis marginata larvae were collected in soil samples from fields in Oxfordshire and Buckinghamshire, UK, between April and June 2015. Larvae were maintained in plastic containers of moist, sterilised compost at 4°C until use. Adult *Nebria brevicollis* (Fabricius) beetles were collected in pitfall traps at Harper Adams University, UK, in June 2015. Beetles were maintained in clear plastic containers at 20°C, 16:8 L:D, 60% RH and fed on *Tenebrio molitor* (Linnaeus) larvae prior to the feeding assay. Insect specimens used in cross-reactivity tests were collected by hand (Harper Adams University), pitfall traps and pan traps (Oxfordshire) and stored at -80°C prior to DNA extraction.

5-2.2 DNA Extraction

DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's supplementary protocol for insect DNA extraction. Whole insect specimens were washed in Tris-EDTA (TE) buffer prior to extraction, followed by grinding with a sterile micro-pestle. Single whole *H. marginata* larvae and undissected invertebrates were used for sequencing and assay cross-reactivity testing. For gut analyses, the elytra of the beetles were removed and entire guts were dissected out, before being used for DNA extraction. Following extraction, DNA was pelleted by centrifugation and resuspended in 100 µL TE buffer before being stored at -20°C until use. One negative control (no insect material) was included for every 20 extractions.

5-2.3 PCR amplification and sequencing of *H. marginata* COI region

A 521bp fragment of *H. marginata* DNA from the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the universal insect primers C1-J-1718 and C1-N-2191 (Simon *et al.*, 1994; King *et al.*, 2010). Individual PCR reactions (25 µL) comprised of; 1X PCR master mix (Invitrogen, Carlsbad,

CA, USA), 0.625 U *Taq* polymerase (Invitrogen), 4 mM $MgCl_2$ (Invitrogen), 2.5 µg bovine serum albumin (Sigma-Aldrich, Dorset, UK), 0.05 mM dNTPs (Invitrogen), 0.1 µM of each primer and 2.5 µL of target DNA. PCR conditions consisted of an initial denaturation at 94°C for 2 min 30 s, then 35 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 45 s, followed by a final extension period at 72°C for 10 min. PCR products were separated on a 1.5% agarose gel stained with GelRed™ Nucleic Acid Gel Stain (Biotium, Fremont, USA) and photographed under UV light (Sint *et al.*, 2011). Unpurified PCR products were sequenced by Eurofins Genomics (Ebersberg, Germany) on a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were deposited in the European Nucleotide Archive (accession number LT852755).

5-2.4 Primer design and PCR assay development

Primers specific to *H. marginata* were designed from the sequencing products using the program Primer-BLAST (Geer *et al.*, 2010). Individual primer pairs were synthesised by Eurogentec Ltd. (Liège, Belgium) and validated for use using a T100 Thermal Cycler (Bio-rad, Watford, UK). Validation of the primer pairs consisted of specificity testing against *H. marginata* and 40 non-target organisms from orders Diptera, Coleoptera, Hymenoptera, Hemiptera, and Araneae. Following this the primer pairs showing no cross-reactivity were selected and the optimum PCR conditions examined by altering the annealing temperature across individual reactions (55°C to 77 °C) observing for a strong single band. The primer pair with the highest optimum annealing temperature was selected for use in the assay. Assay sensitivity was determined using a serial dilution of *H. marginata* DNA at concentrations from 10 ng μL^{-1} to 0.0001 ng μL^{-1}, with 10 replicates of each dilution.

5-2.5 Rate of digestion of *H. marginata* DNA in predator guts

The digestion half-life of *H. marginata* DNA in the guts of a predator was determined under controlled conditions using the carabid *N. brevicollis*. The half-life is the time at which *H. marginata* DNA can only be detected in 50% of the predators following feeding (Greenstone & Hunt, 1993). Prior to feeding, individual *N. brevicollis* specimens were separated into clear plastic containers (10 cm diameter x 6 cm height) with moist cotton wool and starved for 5 days. A single live larva of *H. marginata* was placed

into each container at time 0h and beetles were observed feeding. Beetles that did not consume the larva within 15 minutes were excluded from the experiment. Beetles were maintained at 20°C, 16:8 L:D, 60% RH for the duration of the trial. Groups of beetles were killed by freezing at 0h, 2h, 4h, 8h, 12h, 24h and 36h post-feeding. All groups comprised 10 beetles with the exception of the 24h group which had 9 beetles. Five beetles were left unfed and killed at 0h. All specimens were stored at -80°C and entire guts were dissected from each beetle prior to DNA extraction (see section 5-2.2). PCR reactions proceeded as described in section 5-2.3. Positive results were expressed as a percentage of the total insects screened at each time point and a probit model was fitted to the data to determine the time post-feeding at which the detection half-life occurred. Statistical analysis was performed in R v.3.3.1 (R Core Team, 2016).

5-2.6 Field survey

Carabidae were collected using live pitfall traps from the field in Oxfordshire which was planted with spring wheat. Five pitfall traps were positioned in a cross-shaped array connected with barriers (10 cm height x 30 cm length) made from galvanised lawn edging to improve the catch rate (Hansen & New, 2005). Each trap was comprised of a plastic beaker (8 cm diameter x 10.6 cm height) with small rocks placed in the bottom as refugia (Sunderland *et al.*, 2005). A corrugated plastic cover (12 cm x 12 cm) on wire supports was positioned 5 cm above the trap. On each sampling date, six arrays were set up making 30 traps in total, positioned in various field locations with at least 30 m between arrays. Traps were set in the late afternoon or early evening and collected before noon on the following day. Live specimens were immediately placed on ice in an insulated container at the point of collection, prior to storage at -80 °C. Trapping took place in early May 2016 on 2 occasions, 10 days apart, with an additional collection made in late July using just 20 traps (4 arrays).

5-3 Results

5-3.1 Primer design and PCR assay development

The selected primer pair amplified a fragment of 348bp and had an optimum annealing temperature of 65°C which was used for all subsequent reactions. The sequences of the selected primers were as

follows: F-COI-12 5'-GAGCACCAGATATAGCATTTCC and R-COI-360 5'-CCAGCCAATACTGGTAAAGAAAG. No cross-reactivity of the primers was observed with any of the non-target species tested, which included representative individuals from 8 different orders including the Cecidomyiid *S. mosellana*. Using the newly designed primers, it was possible to detect pure *H. marginata* DNA at concentrations as low as 0.001 ng μL^{-1}.

5-3.2 Rate of digestion of *H. marginata* DNA in predator guts

Digestion time had a significant effect on the probability of detecting *H. marginata* DNA from the guts of *N. brevicollis* ($F_{1,5}$=16.297, P<0.01). The detectability half-life of *H. marginata* DNA under these conditions was determined to be 31.07h (Figure 5.1). The assay was successful in 100% of individuals killed immediately after feeding, while the unfed beetles did not produce any positive results. The greatest decline in probability of detection in the time points tested occurred between 12h and 24h post-feeding.

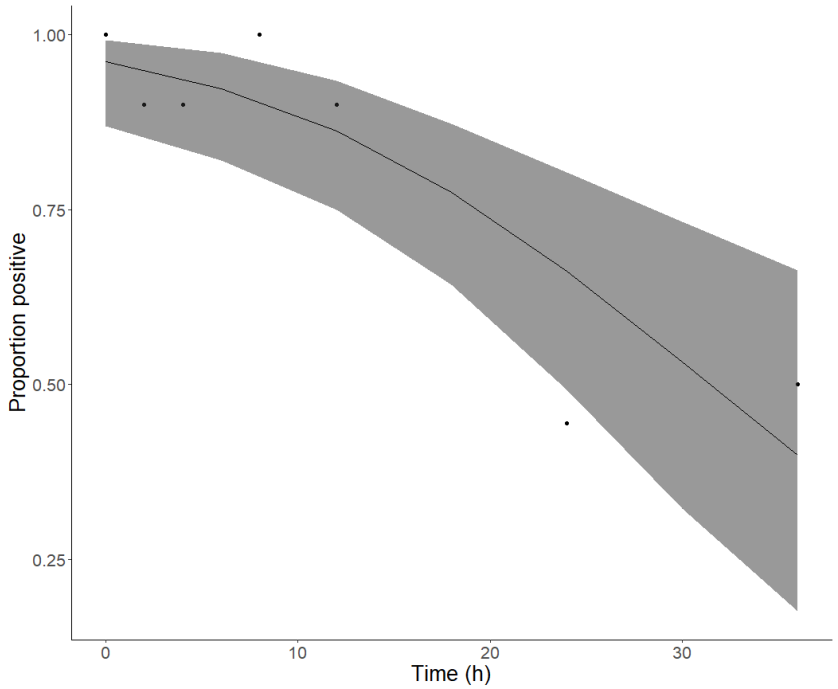


Figure 5.1. Proportion of positive assays for *Haplodiplosis marginata* DNA in the guts of *Nebria brevicollis* at time post-consumption of a single prey larvae. Fitted line represents probit model with 95% CI.

5-3.3 Field survey

From all trapping occasions, 110 individual carabid specimens of 11 different species were trapped. The majority of beetles (47%) were caught in the central traps of the arrays. Positive results for the presence of *H. marginata* DNA were found in 7.2% of specimens and were obtained from 4 different species (Table 5.1). Beetles trapped late in the season (July) represented only 15% of all specimens tested, but had a much higher rate of positive results (23.5%) compared with beetles trapped in May (4.3%). This is despite the activity density of the beetles being almost identical in May and July (0.84 and 0.85 beetles per trap per day respectively).

Table 5.1. Number of individuals of each carabid species tested for the presence of *H. marginata* DNA during the field survey in Buckinghamshire, UK, and expressed as a percentage of the total carabids tested (in brackets). Number of individual assays testing positive for the presence of *H. marginata* for each carabid species tested and the percentage positive for that species (in brackets).

Species	**Number tested (*% of total carabids*)**	**Number positive (*% for species*)**
Poecilus cupreus	9 (*8.18*)	0 (*0*)
Nebria brevicollis	15 (*13.64*)	3 (*20*)
Abax parallelepipedus	1 (*0.91*)	0 (*0*)
Anchomenus dorsalis	1 (*0.91*)	0 (*0*)
Bembidion deletum	2 (*1.82*)	0 (*0*)
Bembidion tetracolum	1 (*0.91*)	0 (*0*)
Harpalus affinis	9 (*8.18*)	0 (*0*)
Poecilus versicolor	45 (*40.9*)	2 (*4.44*)
Harpalus rufipes	19 (*17.27*)	2 (*10.53*)
Loricera pilicornis	2 (*1.82*)	1 (*50*)
Pterostichus melanarius	6 (*5.45*)	0 (*0*)
Total	110 (*100*)	8 (*7.27*)

5-4 Discussion

The development of species-specific primers and PCR protocol for *H. marginata*, described here, increases the potential for research on this cryptic insect at a molecular level. Here, we have applied this to the development of a viable gut analysis assay, enabling highly specific and reliable detection of *H. marginata* in DNA the guts of predatory natural enemies. A field survey using this assay has, for the first time, identified 4 carabid species feeding on this pest in the wild which has implications for the effective management of this insect.

The COI region of the genome is commonly used for species-specific primer design as it is less highly conserved than other regions (King, 2008). It is particularly appropriate for gut analysis studies as it is located in the mitochondria, therefore each cell will have multiple copies making the probability of detection greater than for nucleic DNA (Hoy, 1994). The target amplicon is 348bp, which slightly exceeds the recommended maximum length of 300bp (King *et al.*, 2008) based on the theory that shorter fragments will be subject to less digestion in the gut. The work done by Sint *et al.* (2011) however, suggests that this recommendation might be too conservative. For example, Juen and Traugott (2006) found no difference in the amplification success of 463bp and 127bp amplicons of *Amphimallon solstitiale* (Linnaeus) DNA in the guts of *Poecilus versicolour* (Sturm) larvae. Furthermore, no significant relationship was found between fragment length and the detectability half-life taken from a range of studies (Greenstone *et al.*, 2014). These primers performed well at a high annealing temperature of 65°C which reduces the chance of erroneous base matching at the primer sites (King, 2008), but was not the highest temperature at which an amplicon was obtained to ensure the sensitivity of the assay (Sint *et al.*, 2011). The specificity of the assay was supported by the lack of cross reactivity with DNA from non-target species commonly found on agricultural land including the Cecidomyiid *S. mosellana*.

The assay was able to reliably detect *H. marginata* DNA at concentrations of 0.001 ng μL^{-1} which is comparable with other insect primers used in gut analysis (e.g. Ekbom *et al.*, 2014). The effects of digestion or inhibitors present in the guts of the predator may further reduce assay sensitivity in some instances. Nonetheless, the ability of the assay to detect the DNA from a single *H. marginata* larva in

starved predator guts was repeatedly demonstrated in the feeding assay giving confidence in the reliability of the test. The feeding assay further demonstrated that the half-life of detection for this assay was 31h post-consumption, which is comparable to assays for other predator-prey interactions (e.g. Juen & Traugott, 2004, Waldner *et al.*, 2013) and is well within the range so far reported for other carabids of 18 – 88.5h (Monzó *et al.*, 2011). A long detectability half-life is vital if the assay is to be used on field-caught specimens particularly when predators are mainly nocturnal, as with many carabids (Kromp, 1999). The results suggest the assay was more than adequate for the field survey described here where traps were in place for no more than 18h. Additionally, the feeding trial was conducted at 20°C which is higher than typical field temperatures, and may reflect an underestimation of detection half-life in the field (Hoogendoorn & Heimpel, 2001). The carabid species used in this trial, *N. brevicollis*, is a common predator in arable environments (Luff, 2007) however detection half-life will vary depending on the predator species (Greenstone *et al.*, 2007). For example, the detectability of aphid DNA was higher in *N. brevicollis* compared with another common carabid, *Pterostichus melanarius* (Illiger), independent of the effects of ambient temperature or target amplicon size (von Berg *et al.*, 2008). Detectability appears to vary less between species of the same taxa than between taxa however (Waldner *et al.*, 2013), which suggests that the data shown here represent a reasonable benchmark for carabids of a similar size and activity level.

This field survey shows for the first time the species of carabid beetle that are feeding on *H. marginata*. Of the 12 species caught on the surveyed site, 4 tested positive for the presence of *H. marginata* DNA. All of the species which tested positive are relatively common, highly generalist feeders of medium to large size (above 5 mm long). A number of these species are known to predate on dipteran adults and larvae (Penney, 1966; Allen & Hagley, 1990; Lys, 1995; Sunderland *et al.*, 1995; Luff, 2002; King *et al.* 2010). Although many species display burrowing behaviours, belowground predation by adult carabids has not been well studied. Many carabid larvae are active belowground predators (Lövei & Sunderland, 1996) and have been shown to feed on *S. mosellana* in the field (Floate *et al.*, 1990). While not surveyed here, carabid larvae are potentially a significant source of predation for *H. marginata* larvae. The proportion of positive assays was higher in July, despite the activity density being

comparable between early and late season sampling. Drier soil in the late season may have prevented *H. marginata* from burrowing into the soil, or enabled carabids easier access to larvae belowground via the formation of fissures. Basedow (1973) reported from field observations of the Cecidomyiids *Dasineura brassicae* (Winnertz), *Contarinia tritici* (Kirby) and *S. mosellana* mortalities of up to 65%, 58% and 43% respectively from predation of larvae returning to the soil to overwinter. This was supported by the findings of Floate *et al.*, (1990) and Holland & Thomas (2000) who found that larvae were more likely to be predated upon when returning to the soil to overwinter rather than during pupation. The results presented here suggest that the same is true of *H. marginata* larvae.

As with other predator surveys using PCR-based gut analysis, there is the chance that a positive result could have resulted from scavenging or secondary predation of adult or larval *H. marginata* (Juen & Traugott, 2004; Foltan *et al.*, 2005; Sheppard *et al.*, 2005). Carabid beetles frequently exhibit intraguild predation (Snyder & Wise, 1999; Lang, 2002) and will feed on carrion, sometimes in preference to fresh prey (Mair & Port, 2001; Foltan *et al.*, 2005). In this scenario, the surveyed predators will not be affecting *H. marginata* populations directly, and may indirectly benefit them by consuming pest predators. Partially decayed organisms are harder to detect in the gut however (Foltan *et al.*, 2005), therefore it seems reasonable to assume the positive results obtained here are as a result of predation. This has implications for pest management, as these predatory arthropods could be contributing to suppression of *H. marginata* populations. As pitfall traps are only effective at sampling surface active arthropods, of which only carabids were surveyed here, the actual range of organisms predating on *H. marginata* could be much larger. Dipteran larvae are a primary food source of Staphylinidae (Good & Giller, 1991) and dipteran species are thought to be an important dietary component for spiders (Harwood *et al.*, 2007; Schmidt *et al.*, 2012). Although there are no label recommendations for chemical control of *H. marginata* in the UK, pyrethroid pesticides have been shown to be effective in field trials (Ellis *et al.*, 2014) and are currently used to reduce *S. mosellana* damage in wheat (AHDB, 2016). There is clear evidence however that pyrethroid pesticides can have a negative effect on carabid beetles (Van Toor, 2006; Navntoft *et al.*, 2006) and other natural enemies (Theiling & Croft, 1988; Desneux *et al.*, 2007).

The EU Sustainable Use of Pesticides Directive (Directive 2009/128/EC) and the potential for active ingredients to be withdrawn in the future under EU regulations for plant protection products (Regulation EC 1107/2009) further highlights the need to move towards low pesticide-input farming (Hillocks, 2012). One strategy will be to tailor pest management methods to support existing populations of natural enemies (e.g. Landis *et al.*, 2000; Bianchi *et al.*, 2006) and limit the application of insecticides through IPM compatible measures (Hillocks, 2012). In the case of *H. marginata*, such practices could include regular crop rotations involving non-cereal crops to supress pest populations (Skuhravý *et al.*, 1993) and regular monitoring of pest numbers through the use of pheromone traps (Censier *et al.*, 2016a; Rowley *et al.*, 2017).

The primers developed for this study provide a useful resource for further molecular research on this insect. Such molecular tools could be used in the identification of this species in traps, which is particularly useful when specimens are partial or degraded (Frey *et al.*, 2004). This could be of value not only in monitoring tools, but also in expanding current knowledge on the distribution of *H. marginata* in the UK which at present is based on limited data (Rowley *et al.*, 2016). The assay described here could also be used as a tool in field-based predation experiments (Furlong, 2015) or included in multiplex PCRs to simultaneously screen for many pest species at once (King *et al.*, 2010). The detectability half-life of DNA in the guts of fluid feeders such as centipedes, heteropterans and spiders is generally much longer than that described in carabids (Harwood et al 2007; Greenstone et al 2007; Waldner *et al.*, 2013), which gives confidence that this assay would be suitable for use in other predator taxa. Such surveys could reveal further trophic links involving *H. marginata* in agroecosystems which are at present unknown. Information on the interactions between pest species and other organisms in the environment is key to the development of more sophisticated IPM programmes (Kogan, 1998). Additionally, these primers could be used to investigate parasitoid enemies of *H. marginata* (Rougerie *et al.*, 2011), providing further information on pest interactions in the field. The field survey identifies for the first time, species which consume *H. marginata* in the field. Different rates of digestion and therefore prey DNA degradation between species means that further data are required to quantify rates of predation of *H. marginata*. The next step would be to obtain species-specific digestibility data under controlled

conditions and conduct further field surveys to identify the most important predators of this pest. Nonetheless, the information presented here is vital in the management of this pest as it demonstrates that these and other species of arthropod predators are likely to be having an impact on *H. marginata* populations. This represents an important first step in understanding the predation pressures exerted on *H. marginata* populations, which may be a key aspect in the development of an effective IPM programme for this insect.

Chapter 6

General Discussion

In 2009, the EU sustainable use of pesticides directive (Directive 2009/128/EC) came into force which aims to reduce the impact of pesticides on human health and the environment. A major component of the directive was the requirement for Member States to promote the use of Integrated Pest Management and ensure that the principles of IPM were adopted by all industry professionals by 2014. The principles of IPM aim to encourage more robust, flexible and sustainable crop production methods using a suite of measures that are ecologically and economically justified. These principles begin with the prevention and suppression of pests, move on to monitoring and decision making, and finally move on to control starting with the lowest impact option available.

The importance of IPM Programmes in ensuring a sustainable agricultural industry in the future can be demonstrated using the example of saddle gall midge, *Haplodiplosis marginata*. This is a highly sporadic pest and the exact cause of outbreaks is unknown (Skuhravý *et al.*, 1983; Rowley *et al.*, 2016). Insect numbers can vary greatly from year to year and population size is difficult to predict (Woodville, 1973; Basedow, 1986). There is little opportunity for continued monitoring given that the majority of the life cycle is spent belowground (Censier *et al.*, 2015; Rowley *et al.*, 2016) and extraction of larvae from soil is laborious, requiring specialist equipment (Salt and Hollick, 1944). In the event of a severe infestation, chemical controls must be applied prior to gall formation to be effective (Gratwick, 1992; Ellis *et al.*, 2014). There is however, currently no approval for the use of any pesticide against *H. marginata* in the UK (HGCA, 2012). The uncertainty surrounding this insect and lack of reliable IPM-based strategies mean that in the event of a pesticide gaining approval, such as those shown to be effective in trials (Ellis *et al.*, 2014; Roberts *et al.*, 2014), prophylactic applications will be relied upon for the

protection of crops. This would be, however, highly detrimental to non-target organisms and increases the likelihood of resistance occurring. The use of chemical controls might be reduced if farmers could reliably monitor the presence of this pest and estimate potential damage. Such monitoring would also enable any chemical controls to be timed appropriately improving the effectiveness of the application, or allow for the use of less persistent formulations of biopesticides. Out of the 13 main invertebrate pests of UK cereal crops, *H. marginata* is one of only two for which no economic threshold has yet been established, the other being autumn aphids for which the advice is to spray if present (Ramsden *et al.*, 2017). The development of action thresholds based on the economic injury level would allow for the option of 'informed inaction' in non-outbreak years resulting in no pesticide input (Kogan, 1998).

Haplodiplosis marginata is a prime example of a pest for which IPM-compatible options are badly needed. *Sitodiplosis mosellana* is a Cecidomyiid pest which presents many of the same challenges as *H. marginata* and has been the subject of extensive IPM research involving crop rotations, plant resistance, monitoring traps, degree day models, economic thresholds and biological control in several countries including the UK (Knodel & Ganehiarachchi, 2008; Bruce & Smart, 2009; Elliot *et al.*, 2009; Chavalle *et al.*, 2017). Farmers are encouraged to adopt a range of complementary measures rather than relying on one tactic as this has been shown to be more successful and reduces the probability of resistance emerging in the insect (Barzman et al, 2015). The development of such IPM strategies relies on a thorough understanding of the biology and ecology of the target insect (Kogan, 1998). Unfortunately, in the case of *H. marginata,* the lifecycle and the sporadic nature of outbreaks, combined with periods of low economic impact in many countries has led to a severe lack of research into this pest (Rowley *et al.*, 2016). This meant that few IPM-compatible options were available when outbreaks occurred in several countries in 2010 and subsequent years (HGCA, 2012). The research presented here therefore, represents a significant contribution to the existing knowledge base from which effective prevention and control measures can be developed, in addition to providing practical recommendations on monitoring tools. The following describes how the present research could be effectively applied using the principles of IPM.

6-1 Prevention and suppression

Prevention of *H. marginata* damage aims to reduce the probability of crops being infested, while suppression aims to mitigate the economic consequences of that damage (Barzman *et al.*, 2015). Cleaning of machinery and equipment between infested and non-infested fields may help to prevent the spread of larvae and prevent populations from building up (Bajwa & Kogan, 2004). Pheromone trap experiments described in Chapter 4 show the adults have a tendency to fly at crop height and therefore tall hedges may act as a barrier to dispersal. Crop rotations with non-cereal crops or oats are an effective way to reduce populations by interrupting the life cycle (Skuhravý et a., 1993; Censier *et al.*, 2015). Monitoring of larval numbers in the present study between 2014 and 2015 showed a decrease of over 70% at a site with a break crop of oilseed rape compared with two other sites in continuous wheat where numbers remained steady or increased. Crop diversity, both spatially and temporally, has the added benefit of preventing the accumulation of other pests and encouraging diversity in natural enemy communities (Barzman *et al.*, 2015; Bianchi *et al.*, 2006).

6-1.1 Identification of natural enemies

The suppressive effect of natural enemies on pest populations through predation and parasitism is an important aspect of many IPM programmes. Previously, there was very little information on the natural enemies of *H. marginata* and no predators had been identified to species level (Rowley *et al.*, 2016). The difficulties of observing predator-prey interactions in the field are exacerbated in species such as *H. marginata*. The larval and pupal stages are the least mobile and therefore the most vulnerable to predation however being primarily belowground they are also the most difficult to observe. Advances in molecular biology have allowed the gut contents of predators to be screened for the DNA of target pest species. The work presented here describes the design of species specific primers which were successfully applied to a PCR-based assay to detect *H. marginata* DNA in gut contents. From this, four Carabid species were identified as predators of *H. marginata* in the field for the first time. This information is important in shaping IPM programmes for *H. marginata*. Several studies have shown the negative effects of insecticides, including the pyrethroids which are used in *H. marginata* control, on carabids (van Toor, 2006). This is an example of how current practices could be disrupting predator-

induced suppression and contributing to the accumulation of pest populations (Matson *et al.*, 1997).

There are an estimated 50-250 natural enemy species for each agricultural pest (van Lenteren, 1995), although many of these will be polyphagous. Almost certainly there are more predators of *H. marginata* yet to be identified, for example spiders and carabid larvae which were not surveyed in this project. Additionally, faeces from vertebrates could be screened in a PCR-based assay as the bright orange larvae of *H. marginata* might well be a target for larger organisms when they move to the surface to pupate. There is also the opportunity to extend screening beyond predators. It may be possible to identify parasitoids of *H. marginata* using these primers (Rougerie *et al.*, 2011). Molecular assays specific to the identified parasitoid species could then be used to give a clearer picture of the extent of parasitism in the field (Greenstone, 2006). Similarly, molecular techniques could be used to identify entomopathogenic nematodes and fungi that might be inducing lethal or sublethal effects in populations (Shapiro-Ilan, 2003). Even if the individual contribution to pest mortality of individual species is small, a diverse natural enemy complex could be sufficient to regulate *H. marginata* populations. Research suggests that natural enemy diversity has more of an impact on the pest status of concealed, endopterygote insects such as *H. marginata* (Wilby & Thomas, 2002).

The primers described here could be incorporated into a multiplex PCR reaction to screen for multiple pest species simultaneously which would give a clearer picture of the most important predators in a particular agri-ecosystem (King *et al.* 2010). This is essential if agricultural practices are to be modified to encourage certain species, as techniques that may be of benefit to one species may be detrimental to another. Additionally, augmentation of species may lead to increased levels of intraguild predation which may disrupt predation on the target species (Rosenheim *et al.*, 1995; Finke & Denno, 2005) although this effect may be lessened in more complex habitats (Finke & Denno, 2003).

6-2 Monitoring of *Haplodiplosis marginata*

Insect populations in agricultural systems are often unstable due to the rapidly changing environment and management interventions (Prasad & Prabhakar, 2012). Pest monitoring is therefore a key principle of IPM as it alleviates some of the uncertainty and provides information on which programmes and

management decisions can then be based. Monitoring programmes may be aimed at identifying pest species or giving early warnings of infestation or outbreaks; estimating population size or providing information on the timing of critical life stages.

6-2.1 Phenological forecasting

Data on insect species development can be used to establish degree day models from which phenological events such as yearly adult emergence can be predicted. The life cycle of *Haplodiplosis marginata* has been relatively well described (Skuhravý *et al.*, 1983; Censier *et al.*, 2015), however there is little known about the developmental biology of this insect. Existing research into developmental thresholds only tested a limited number of temperatures meaning the posited lower developmental threshold of 10°C is potentially inaccurate (Baier, 1963; Nijveldt & Hulshoff, 1968). This is demonstrated by observations of *H. marginata* pupation in soil temperatures below 10°C in the UK (Pope & Ellis, 2013). Furthermore, laboratory studies so far completed did not fully recreate field conditions for *H. marginata* due to the variation that exists within soils, and potential differences in developmental time at constant and fluctuating temperatures (Hagstrum & Milliken, 1991). In addition to thermal thresholds for development, other environmental factors are important in the seasonal ecology of insects which are relevant when attempting to predict emergence (Tauber & Tauber 1976; Tauber *et al.*, 1998, Leather *et al.*, 1993; Koštál, 2006). The research presented here confirms that in *H. marginata*, rainfall has an effect on pupation, potentially by acting as an environmental cue. The role of moisture in insect emergence has been studied in other insect species including *S. mosellana* (Oakley *et al.*, 1998; Elliot *et al.*, 2009; Jacquemin *et al.*, 2014). It has been suggested that increased soil moisture might improve mobility (Menu, 1993; Ellis *et al.*, 2004) or act a behavioural stimulus (Tauber et al, 1994). More research needs to be done to understand the role moisture plays specifically in the development of *H. marginata* such as whether it is important for diapause termination or post-diapause development (Leather *et al.*, 1993). Nonetheless, the information shown here can be used to successfully predict the initiation of a degree day accumulation period, following which adult *H. marginata* will emerge. This is significant as for the first time, farmers could be provided with a window of time during which to inspect crops or deploy traps. Similar degree-day based models of varying complexity have long been used to monitor other

insect pests (e.g. Riedl *et al.*, 1976) and a database of over 500 insect developmental requirements has been created to support the creation of further phenology models (Nietschke *et al.*, 2007). Advances in automated data collection and software programming have made it possible to set up national networks that incorporate models for multiple pests and provide monitoring alerts based on local conditions. Linking phenology models to geographical information systems (GIS) can aid the generation of regionally based risk assessments (Fand *et al.*, 2013). Multi-species networks are already in place such as the SOPRA system in Swiss orchards (Samietz, 2011), VIPS pest notifications in Norway (NIBIO, 2017); and NAPPFAST in North Carolina (Margarey *et al.*, 2007). Increased sophistication of basic degree-day phenology models could be achieved by simulating the effects of other relevant ecological or biological variables. For example, degree day accumulations also provide the basis for crop growth models (Miller *et al.*, 2001). This means that pest forecast models could be combined with growth models of the host crop to give a more detailed estimate of crop risk. For example, the CIPRA software based in Quebec combines models of insect pests, disease and crop phenology to provide real-time forecasting based on meteorological data (Bourgeois, 2008). In the case of *H. marginata*, cereal crops are most vulnerable during stem extension (Golightly & Woodville, 1974) therefore a forecast of adult emergence during this period would pose a greater risk to the crop than emergence occurring after the crop has booted. Combining forecasting models with decision support systems means that suitable control options can be presented to users based on their specific circumstances.

The observed waves of emergence of *H. marginata* mean that predictive models such as this are more important given that activity will need to be monitored throughout the flight season. Peaks in emergence could be an indication to survey the crop for eggs. No data has yet been collected on the extent of egg laying throughout the flight season, and even if the crop is no longer at a vulnerable growth stage continued checking of eggs could give forewarning of future outbreak populations. Over a longer period, forecasts could also be used to reveal trends in the phenology of *H. marginata* relating to changes in climate which may not be evident from year to year. For example, milder overwintering conditions reduces fecundity and survival in the goldenrod gall fly (Irwin & Lee, 2000). Changes in climate are predicted to impact upon the phenology of insect pests in the future which may have

implications for the severity of outbreaks (Cannon, 1998).

6-2.2 *Haplodiplosis marginata* pheromone traps

The use of pheromone traps in IPM has greatly improved the ease and reliability of insect monitoring (Witzgall *et al.*, 2010). In 2014, Censier *et al.* identified (*R*)-2-nonyl butyrate as the female sex pheromone of *H. marginata*. The work presented here confirms response of male *H. marginata* to this compound through EAG. Field experiments, were used to determine the optimum lure formulation for this insect so that trap catch could be maximised with a minimal amount of compound required. This is an important consideration in the development of a commercial lure. An interesting finding in this research was the inhibitory effect of the (*S*)-enatiomer of 2-nonyl buryrate, which is a chiral molecule. This is not a common finding in gall midges and has only been observed once before (Hillbur, 1999). It has been observed in other organisms however, and is thought to have evolved to further increase the specificity of pheromone communication (Mori, 1998). It highlights the need to test for antagonism in racemic mixtures of chiral compounds and may help to inform further research into the chemical ecology of other gall midge species. The position of the trap in relation to the crop and other traps was also important in optimising the catch rate, as has been shown with other pheromone research (Kong *et al.*, 2014; Rhainds *et al.*, 2016). The research presented here provides a basis for an effective pheromone lure and guidelines for best practice in the use of the trap.

Further innovations could concentrate on trap design to further increase catch rate (Edde *et al.*, 2005; Diaz-Gomez, 2010) or provide automated solutions for checking the traps. For example, 'Z-Traps' have been developed which count insects as they enter and upload the data instantly (Spensa Technologies, 2017). Another automated system has been developed known as Trapview™ which uploads pictures of trap captures at regular intervals. The software then uses auto-recognition to identify certain insect pests from the pictures, provide threshold-based alerts and has the capability to incorporate weather data into a database of insect activity (EFOS, 2017). The large amounts of data collected from automated pest monitoring systems could then be used to further improve integrated pest management programmes (Okuyama *et al.*, 2011).

The development of this simple monitoring tool means that the presence of *H. marginata* can be

recognised with greater ease and precision than previously. The phenological forecasting system described previously could be used to predict the date of adult emergence and estimates of when to begin inspecting the crop for egg-laying. Pheromone traps could be deployed to monitor emergence throughout the flight season or confirm pest presence, and in the future may be used for estimating population size. If found to be present in high numbers, farmers could implement crop rotations in subsequent years to prevent numbers building up to outbreak levels. This may lead to a much clearer understanding of the distribution of this insect and may lead to reports of its presence in location where it was previously unknown. The newly designed primers can also be used for detection purposes as is currently done for crop pathogens (Henson & French, 1993; Fang & Ramasamy, 2015). Real-time PCR has several advantages over classical PCR in that it is less laborious and can be done on relatively inexpensive, portable thermocyclers which offers the possibility of in-field testing of specimens (Schaad & Frederick, 2002). This would make it easier for farmers to identify new populations of *H. marginata* from individuals caught in traps which might be useful if the insect is degraded. Again, this could be combined with other pest species in a multiplex reaction to screen the contents of a non-specific traps such as pan traps or suction traps as part of a widespread pest monitoring system. For example, the Rothamsted Insect Survey provides information on aphid migration to farmers across the UK based on collections from a network of traps (Storkey *et al.*, 2016).

6-3 Practical applications of this research

The work described here is of direct relevance to farmers who are concerned about the risk of crop damage due to saddle gall midge. The evidence for arthropod predation of *H. marginata* has clear implications for the need to encourage a diversity of natural enemies in the field to contribute to the suppression of this pest. Wide, diverse field margins or uncultivated land provide essential habitats in which there are minimal inputs of pesticide that may otherwise negatively affect these species. Regular crop rotations will also encourage arthropod diversity as well as suppressing populations of *H. marginata* by disrupting the life cycle. Populations of *H. marginata* can then be monitored to provide advance warning of increasing numbers or potential outbreaks. The phenological forecasting models can be

used to estimate the timing of adult emergence based on rainfall and degree day accumulations derived from either Met Office temperature data or equipment on site.

Once the date of adult emergence has been predicted, pheromone traps can be deployed in the field. Polyethylene vials containing 0.5mg (*R*)-2 nonyl butyrate should be used as the pheromone lure and placed within a delta trap. Traps should be positioned in a site of previous *H. marginata* infestation, at least 20 m away from field margins and other structures and with at least 20 m between adjacent traps. The base of the trap is should be level with the top of the crop to maximise trap catch. A minimum of two traps should be used per field, ideally in areas with varying microclimates so as to increase the chances of trapping midges at the very start of emergence. Traps should be checked every 1 – 2 days for the presence of adults and once observed the crop should be checked for eggs 3 – 5 days later.

In the event of pesticide approval for this species, control measures are likely to be most effective if applications are timed to coincide with the emergence of adults or 7 – 10 days after to coincide with the egg-laying period. Pheromone traps can then be used to monitor for additional peaks in emergence for the duration of the flight period without the need to refresh the lure. The cumulative emergence model is also based on degree day accumulations and can allow farmers to estimate when 50% and 90% of emergence is complete. This can be used alongside crop growth assessments so that where peak emergence occurs after growth stage 39 in wheat, crop damage is likely to be minimal.

6-4 Future Work

6-4.1 Improved monitoring and risk assessment

The pheromone trap described here is an important step in the development of standardised way of assessing populations. Further research to relate trap catch to the severity of galling and ultimately the effect on yield would greatly improve the accuracy of risk assessments for this pest. Action thresholds could then be developed based on factors such as trap catch, crop type and growth stage to enable farmers to determine whether to use pest management measures based on the risk to the crop. Thresholds might also be developed to predict imminent outbreaks of *H. marginata* based on trap catches (Shepherd *et al.*, 1985). Pheromone traps could also be utilised in research to improve the

phenological models presented here. Many forecasting models use degree day accumulations calculated from the earliest emergence date, which cannot be reliably monitored in this insect without the use of pheromone traps (Knutson & Muegge, 2010). Trap data documenting the start of the emergence period for several years combined with crop monitoring of insect life stages could allow estimates of the timing of important events such as egg laying or egg hatch. Using the start of emergence as the point of biofix will be more precise than estimating the start of pupation based on soil conditions and could lead to greater precision in the phenological models. Predicting the timing of key life stages will be necessary if chemical controls are to be applied more judiciously or if biopesticides are to be applied effectively.

6-4.2 Mass trapping

The sex pheromones of other pests such as codling moth, *Cydia pomonella*, have successfully been exploited for the purposes of mass trapping in IPM programmes (El-Sayed *et al.*, 2006; Witzgall *et al.*, 2010). The pheromone traps for *H. marginata* described here proved to be extremely effective at catching high numbers of insects in the field. Trap catch could be further increased by increasing the number or size of traps deployed per field and by increasing the quantity of compound used in the lures. With the current formulation, approximately 25 traps would need to be deployed per hectare of crop to ensure full coverage. It was found however, that the catch rate did not decrease at the highest quantity of compound tested, 2.5mg (*R*)-nonyl-butyrate, therefore the upper threshold for lure loading is still undetermined. Further cost-benefit analysis would be required to determine the optimal quantity of compound that remains attractive, reduces operating costs by requiring fewer traps and is economical to manufacture. Male *H. marginata* reportedly emerge several days before females (Skuhravý *et al.*, 1983) which would mean mass trapping could be employed to reduce the initial number of males available to mate with females upon emergence. The attractiveness of the pheromone lure would ideally need to be compared with the attractiveness of calling virgin females in the field to ensure competition from these sources did not reduce effectivity (El-Sayed, 2006).

6-4.3 Biopesticides

Biopesticides are broadly defined as biologically based crop protection products such as those containing a microbial or insect- or plant-derived active ingredient (Bailey *et al.* 2010). These products are an increasingly important component of IPM programmes where they offer an alternative to synthetic pesticides, particularly in crops where the control options are limited.

One widely used biopesticide is the entomopathogenic fungus *Beauveria bassiana* which can provide some level of protection from the cecidomyiid swede midge, *Contarinia nasturtii*, in crucifers. The authors made clear that although synthetic pesticides were more effective overall, used in conjunction with other IPM-based strategies, *B. bassiana* could prevent yield losses to this pest (Evans & Hallett, 2016). Beneficial microorganisms such as *B. bassiana* are widespread in nature. An entomopathogenic fungus of the genus *Lecanicillium* has been observed inducing mortality in field sampled *H. marginata* larvae (AHDB, 2016). Not all microorganisms are suitable for biological control purposes however, and extensive research is needed to determine the likelihood of success of a particular pest-pathogen combination (Bale *et al.*, 2008). For example, a different entomopathogenic fungus *Metarhizium brunneum* was shown to induce high levels of mortality in *C. nasturtii* larvae under controlled conditions however there were mixed result when tested under field conditions (Evans *et al.*, 2015). Rather than identifying a pathogen specific to *H. marginata* which may not be desirable commercially, it may be that an existing biopesticide could provide the level of control needed as part of an effective IPM programme. The sensitivity of microorganisms to environmental conditions can play a large role in their success or failure as biospesticides (Rodgers, 1993) although this is being addressed as new biopesticides are developed (Glare *et al.*, 2012). For example, many entomopathogenic nematodes may only be effective within a certain temperature range (Griffin, 1993). Automated environmental monitoring networks described in section 6-2.1 for predicting *H. marginata* emergence could also be used to determine the ideal timing of biopesticide applications. Similarly, entomopathogenic organisms may only be effective against a certain life stage and will need to be targeted appropriately, for example ensuring nematode products are applied before the larvae pupate into adults (Rodgers, 1993).

6-5 Summary

The agricultural industry is constantly responding to new challenges that arise as a result of being dependent on the environment and consumer markets. One of the biggest issues that the industry currently faces is how to ensure that current farming practices are sustainable in order to withstand the challenges of population growth and increased demand in the context of fewer natural resources (FAO, 2017). Losses in crop yield due to pests can be substantial despite increases in pesticide use worldwide (Oerke, 2006). Predicted changes in climate and agroecosystems in an increasingly connected world may lead to changes in pest distribution, outbreaks becoming more unpredictable and a greater risk of invasive species emerging (Cannon, 1998; Ziska et al., 2010; Rosenzweig, 2001). Intensive use of synthetic pesticides has led to resistance among pest populations, the risk of which is set to increase as products are withdrawn under tightening legislation in the EU and options become more limited (Brattsten *et al.*, 1986; Hillocks *et al.*, 2012). The move towards low pesticide-input farming in the EU reflects the consensus that overreliance on these products is unsustainable both in terms of crop protection and the wider environment (Pretty, 2008).

In order to effectively protect crops from ever-changing threats, farmers need a flexible approach to pest management based on scientific research that is revisited regularly. Integrated pest management programmes comprise a suite of control tactics that build resilience into the cropping system (Barzman *et al.*, 2015). In order to achieve this however, research needs to be done into the basic aspects of pest biology and ecology that shape the pest management solutions. The example of *Haplodiplosis marginata* shows how a lack of consistent research activity can severely limit control options when they are most needed. This is not a unique example, and even widely accepted practices such as economic thresholds suffer from a lack of academic rigour on which to base decisions (Ramsden *et al*., 2017). Furthermore, pests do not exist in isolation and the ideal integrated pest management programme is one that is based on an understanding of the agri-ecosystem in which it is based (Kogan, 1998).

As the landscape, climate, crops and farming practices and agricultural organisms are constantly

shifting, research into sustainable pest management must work to keep ahead of new challenges so that famers have the tools to respond when they arise. Genetic screening of resistance genes and computer modelling could provide early warnings of emerging resistance (Denholm & Rowland, 1992; Renton et al., 2014). Biopesticides offer a safer, more environmentally benign alternative to their synthetic counterparts and offer a high degree of specificity, reducing the negative impact on non-target organisms (Rodgers, 1993; Glare *et al.*, 2012). Chemical attractants and repellents could aid monitoring programmes and provide species-specific crop protection through the use of push-pull strategies or mating disruption (Cook *et al.*, 2007, Witzgall *et al*., 2010). Such technologies cannot be developed if the fundamental aspects of a species' biology and ecology, including specific crop-pest interactions, are not understood. Even where the knowledge exists, the application of that research into something that can be easily used by farmers is another challenge: a lack of training and technical support was highlighted as one of the main reasons why IPM programmes were not more widely adopted, particularly in developing countries (Parsa *et al.*, 2014).

In the case of *H. marginata*, this thesis lays the groundwork for new advances to be made in the development of an effective IPM programme for this species. Knowledge gained on insect development and chemical ecology has been applied to providing a practical solution for the problem of monitoring for this pest. This information can be further drawn upon to inform the management of other pests with similar life history strategies or ecological interactions. The accumulation of high quality scientific research into different pest species can be thought of as another component of the IPM toolkit and one that may help us to anticipate and sustainably mitigate risks to crops in the future.

Acknowledgements

I would firstly like to thank my funders, AHDB Cereals & Oilseeds and Harper Adams University for providing the finances and facilities necessary for me to undertake this research.

I am indebted to my supervisory team for their advice and guidance over the years. As my Director of Studies, Dr Tom Pope has been a constant source of support and an excellent mentor. Dr Andrew Cherrill and Professor Simon Leather have contributed a great deal of their knowledge and experience to all aspects of the project.

I have been fortunate to benefit from the assitance of a number of collaborators on this research, to whom I would like to express my thanks. Professor David Hall for his valuable expertise in chemical ecology and insightful comments on manuscripts. Dr Mandela Fernández-Grandon for giving up his time to instruct me in the use of GC-EAG. Dr Steve Ellis for assisting me with data collection and offering helpful comments on manuscripts. Caroline Nicholls for her support as research manager at AHDB. Janetta Skarp for the excellent work she did designing PCR primers and Alex McCormack for taking time out of his own PhD to advise on the process.

I am extremely grateful to the many farmers and agronomists who gave up their time to help me locate field sites, permitted me to set up experiments on their land and took and interest in my work. I would like to thank Joseph Roberts for his assistance in fieldwork and setting up experiments, not to mention putting up with excessively long journeys on the A14. I am fortunate to have made some amazing friends in the past four years, in particular my fellow PhD students and postdocs in G.26 without whom I might have gone slightly mad.

And finally, I would especially like to thank my fiancé Lee Boot for sharing in the many highs and lows of this PhD, and for supporting me every step of the way.

References

AHDB (2016) Orange wheat blossom midge, Information Sheet 53. AHDB, Warwickshire, UK.

Aktar, M.W., Sengupta, D. & Chowdhury, A. (2009) Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary Toxicology*, **2**, 1–12.

Allen, W.R. & Hagley, E.A.C. (1990) Epigeal arthropods as predators of mature larvae and pupae of the apple maggot (Diptera: Tephritidae). *Environmental Entomology*, **19**, 309–312.

Allison, R. (2010) Gall midge outbreak may force rethink on 2010 cropping plans. *Farmers Weekly*. URL http://www.fwi.co.uk/arable/gall-midge-outbreak-may-force-rethink-on-2010-cropping-plans.htm [accessed June 2017]

Anderson, K.M., Hillbur, Y., Reber, J., Hanson, B., Ashley, R.O. & Harris, M.O. (2012) Using sex pheromone trapping to explore threats to wheat from Hessian fly (Diptera: Cecidomyiidae) in the Upper Great Plains. *Journal of Economic Entomology*, **105**, 1988–1997.

Andersson, K. (1969) Sadelgallmyggan förekommer allmänt i Skåne och Halland / Saddel gall midge commonly found in Skåne and Halland. *Växtskyddsnotiser*, **2**–**3**.

Bacca, T., Lima, E.R., Picanço, M.C., Guedes, R.N.C. & Viana, J.H.M. (2006) Optimum spacing of pheromone traps for monitoring the coffee leaf miner *Leucoptera coffeella*. *Entomologia Experimentalis et Applicata*, **119**, 39–45.

Baier, M. (1963) Zur biologie und gradologie der sattelmücke *Haplodiplosis equestris* Wagner (Diptera, Cecidomyiidae). *Zeitschrift für Angewandte Entomologie*, **53**, 217–273.

Bailey, A., Chandler, D., Grant, W., Grant, W.P., Greaves, J., Prince, G., *et al.* (2013) *Biopesticides: Pest Management and Regulation*. CABI, Wallingford, Oxfordshire, UK.

Bajwa, W.I. & Kogan, M. (2004) Cultural Practices: Springboard to IPM. In *Integrated Pest Management: Potential, Constraints and Challenges*. CABI Publishing, Wallingford, UK.

Bale, J., Lenteren, J. van & Bigler, F. (2008) Biological control and sustainable food production. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**, 761–776.

Barnes, H.F. (1952) Studies of Fluctuations in Insect Populations Xii. Further evidence of prolonged larval life in the wheat-blossom midges. *Annals of Applied Biology*, **39**, 370–373.

Barnes, H.F. (1956) Gall Midges of Economic Importance. Vol. VII. Gall Midges of Cereal Crops. *Proceedings of the Royal Entomological Society of London. Series A, General Entomology*, **31**, 86–86.

Bartelt, R.J., Vetter, R.S., Carlson, D.G. & Baker, T.C. (1994) Influence of pheromone dose, trap height, and septum age on effectiveness of pheromones for *Carpophilus mutilatus* and *C. hemipterus* (Coleoptera: Nitidulidae) in a California date garden. *Journal of Economic Entomology*, **87**, 667–675.

Basedow, T. (1973) The influence of predatory arthropods of the soil surface on the abundance of phytophagous insects in agriculture. / Der Einfluss epigaischer Raubarthropoden auf die Abundanz phytophager Insekten in der Agrarlandschaft. *Pedobiologia*, **13**, 410–422.

Basedow, T. (1977) The effect of temperature and precipitation on diapause and phenology in the wheat blossom midges *Contarinia tritici* (Kirby) and *Sitodiplosis mosellana* (Gehin) (Dipt., Cecidomyidae). *Zoologische Jahrbucher Abteilung fur Systematik Okologie und Geographie der Tiere*, **104**, 302–326.

Basedow, T. (1986) The abundance dynamics of the saddle gall midge, *Haplodiplosis marginata* (von Roser) (Dipt., Cecidomyiidae), in wheat grown for one year, two successive years or continuously. / Die Abundanzdynamik der Sattelmücke, *Haplodiplosis marginata* (von Roser) (Dipt., Cecidomyiidae), bei Fruchtwechsel, bei wiederholtem und bei permanentem Anbau von Weizen. *Journal of Applied Entomology*, **102**, 11–19.

Basedow, T. & Gillich, H. (1982) Untersuchungen zur Prognose des Auftretens der Weizengallmücken *Contarinia tritici* (Kirby) und *Sitodiplosis mosellana* (Géhin) (Dipt., Cecidomyidae). *Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz*, **55**, 84–89.

Baxendale, F.P. & Teetes, G.L. (1983) Factors influencing adult emergence from diapausing sorghum midge, *Contarinia sorghicola* (Diptera: Cecidomyiidae). *Environmental Entomology*, **12**, 1064–1067.

Baxendale, F.P., Teetes, G. & Sharpe, P. (1984) Temperature-dependent model for sorghum midge (Diptera: Cecidomyiidae) spring emergence. *Faculty Publications: Department of Entomology*.

Begon, M., Townsend, C.R. & Harper, J.L. (2006) *Ecology: From Individuals to Ecosystems*. John Wiley & Sons, Malden, MA.

Ben-Dor, E. & Banin, A. (1989) Determination of organic matter content in arid-zone soils using a simple “loss-on-ignition” method. *Communications in Soil Science and Plant Analysis*, **20**, 1675–1695.

Benton Jones Jr, J. (2001) *Laboratory Guide for Conducting Soil Tests and Plant Analysis*. CRC Press, Boca Raton, USA.

Berg, K. von, Traugott, M., Symondson, W.O.C. & Scheu, S. (2008) The effects of temperature on detection of prey DNA in two species of carabid beetle. *Bulletin of Entomological Research*, **98**, 263–269.

Berry, P.M., Spink, J.H., Griffin, J.M., Sylvester-Bradley, R., Baker, C.J., Clare, R.W., *et al.* (1998) *Research to understand, predict and control factors affecting lodging in wheat*. HGCA Project Report No. 169. HGCA, Warwickshire, UK.

Bianchi, F.J.J.A., Booij, C.J.H. & Tscharntke, T. (2006) Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society of London B: Biological Sciences*, **273**, 1715–1727.

Bonhomme, R. (2000) Bases and limits to using “degree day” units. *European Journal of Agronomy*, **13**, 1–10.

Bourgeois, G., Beaudry, N., Plouffe, D., Chouinard, G., Audet, R. & Deaudelin, G. (2005) Forecasting pests in field crops using real-time weather information: the CIPRA network in Quebec. *Acta horticulturae*, **674**, 303-304

Bruce, T.J., Hooper, A.M., Ireland, L., Jones, O.T., Martin, J.L., Smart, L.E., *et al.* (2007) Development of a pheromone trap monitoring system for orange wheat blossom midge, *Sitodiplosis mosellana*, in the UK. *Pest Management Science*, **63**, 49–56.

Bruce, T.J. & Smart, L.E. (2009) Orange wheat blossom midge, *Sitodiplosis mosellana*, management. *Outlooks on Pest Management*, **20**, 89–92.

Büchs, W. & Nuss, H. (2000) First steps to assess the importance of epigaeic active polyphagous predators on oilseed rape insect pests with soil pupating larvae. *IOBC-WPRS Bulletin*, **23**, 151–163.

Cannon, R.J.C. (1998) The implications of predicted climate change for insect pests in the UK, with emphasis on non-indigenous species. *Global Change Biology*, **4**, 785–796.

Cardinale, B.J., Harvey, C.T., Gross, K. & Ives, A.R. (2003) Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. *Ecology Letters*, **6**, 857–865.

Case, P. (2011) Midge found in continuous wheat. *Farmers Weekly*, **155**, 63.

Censier, F., Chavalle, S., Wittouck, D., De Proft, M. & Bodson, B. (2012) Chemical control of *Haplodiplosis marginata* von Roser (Diptera: Cecidomyiidae). *Communications in Agricultural and Applied Biological Sciences*, **77**, 667–675.

Censier, F., Chavalle, S., San Martin Y Gomez, G., De Proft, M. & Bodson, B. (2013) Study on the sensitivity of three oat varieties to the saddle gall midge, *Haplodiplosis marginata* (von Roser) (Diptera: cecidomyiidae). *Communications in Agricultural and Applied Biological Sciences*, **78**, 287–292.

Censier, F., Chavalle, S., Knor, S., Proft, M.D., Bodson, B. & Skuhravá, M. (2014a) Unusual occurrence of cocoons in population of *Haplodiplosis marginata* (Diptera: Cecidomyiidae) in Belgium. *Journal of Insect Science*, **14**, 239.

Censier, F., Fischer, C.Y., Chavalle, S., Heuskin, S., Fauconnier, M.-L., Bodson, B., *et al.* (2014b) Identification of 1-methyloctyl butanoate as the major sex pheromone component from females of the saddle gall midge, *Haplodiplosis marginata* (Diptera: Cecidomyiidae). *Chemoecology*, **24**, 243–251.

Censier, F., De Proft, M. & Bodson, B. (2015) The saddle gall midge, *Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae): Population dynamics and integrated management. *Crop Protection*, **78**, 137–145.

Censier, F., Heuskin, S., San Martin Y Gomez, G., Michels, F., Fauconnier, M.-L., De Proft, M., *et al.* (2016a) A pheromone trap monitoring system for the saddle gall midge, *Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae). *Crop Protection*, **80**, 1–6.

Censier, F., Chavalle, S., San Martin Y Gomez, G., De Proft, M. & Bodson, B. (2016b) Targeted control of the saddle gall midge, *Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae), and the benefits of good control of this pest to winter wheat yield. *Pest Management Science*, **72**, 731-737

Cesaraccio, C., Spano, D., Duce, P. & Snyder, R.L. (2001) An improved model for determining degree-day values from daily temperature data. *International Journal of Biometeorology*, **45**, 161–169.

Chavalle, S., Jacquemin, G. & De Proft, M. (2017) Assessing cultivar resistance to *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) using a phenotyping method under semi-field conditions. *Journal of Applied Entomology*, [*in press*]

Chen, M. & Shelton, A.M. (2007) Impact of soil type, moisture, and depth on swede midge (Diptera: Cecidomyiidae) pupation and emergence. *Environmental Entomology*, **36**, 1349–1355.

Chen, Y., Giles, K.L., Payton, M.E. & Greenstone, M.H. (2000) Identifying key cereal aphid predators by molecular gut analysis. *Molecular Ecology*, **9**, 1887–1898.

Cork, A., Beevor, P.S., Gough, A.J.E & Hall, D.R. (1990) Gas chromatography linked to electroantennography: a versatile technique for identifying insect semiochemicals. In *Chromatography and Isolation of Insect Hormones and Pheromones*. Plenum Press, New York , NY, USA. p. 271-279

Cross, J.V. & Hall, D.R. (2009) Exploitation of the sex pheromone of apple leaf midge *Dasineura mali* Kieffer (Diptera: Cecidomyiidae) for pest monitoring: Part 1. Development of lure and trap. *Crop Protection*, **28**, 139–144.

Cross, J.V., Hall, D.R., Shaw, P. & Anfora, G. (2009) Exploitation of the sex pheromone of apple leaf midge *Dasineura mali* Kieffer (Diptera: Cecidomyiidae): Part 2. Use of sex pheromone traps for pest monitoring. *Crop Protection*, **28**, 128–133.

Damos, P.T. & Savopoulou-Soultani, M. (2010) Development and statistical evaluation of models in forecasting moth phenology of major lepidopterous peach pest complex for Integrated Pest Management programs. *Crop Protection*, **29**, 1190–1199.

Davies, D.B., Eagle, D. & Finney, B. (2001) *Soil*. Farming Press, Tonbridge, UK.

De Clercq, R. & D'Herde, C.J. (1972) *Bijdrage tot de studie van de biologie, de verspreiding, de pathogeniteit en de bestrijding van de tarwestengelgalmug* Haplodiplosis marginata *(von Roser 1840) Rübsaamen 1911.* (PhD) Rijksstation voor nematologie en entomologie, Merelbeke, Belgium.

Dennis, P. & Fry, G.L.A. (1992) Field margins: can they enhance natural enemy population densities and general arthropod diversity on farmland? *Agriculture, Ecosystems & Environment*, Biotic Diversity in Agroecosystems, **40**, 95–115.

Derrick, M.E., Duyn, J.W.V., Sorenson, C.E. & Kennedy, G.G. (1992) Effect of pheromone trap placement on capture of male european corn borer (Lepidoptera: Pyralidae) in three North Carolina crops. *Environmental Entomology*, **21**, 240–246.

Desneux, N., Decourtye, A. & Delpuech, J.-M. (2007) The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*, **52**, 81–106.

Dewar, A. (2012) *Ecology and control of saddle gall midge,* Haplodiplosis marginata *von Roser (Diptera; Cecidomyiidae)*. HGCA Research Review No. 76. HGCA, Warwickshire, UK .

Diaz-Gomez, O., Malo, E.A., Patiño-Arrellano, S.A. & Rojas, J.C. (2012) Pheromone trap for monitoring *Copitarsia decolora* (Lepidoptera: Noctuidae) activity in cruciferous crops in Mexico. *Florida Entomologist*, **95**, 602–609.

Edde, P.A., Phillips, T.W. & Toews, M.D. (2005) Responses of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) to its aggregation pheromones as influenced by trap design, trap height, and habitat. *Environmental Entomology*, **34**, 1549–1557.

EFOS (2017) *Automated Trap Monitoring System* URL http://www.trapview.com/en/#monitor [accessed June 2017]

Eitzinger, B., Micic, A., Körner, M., Traugott, M. & Scheu, S. (2013) Unveiling soil food web links: New PCR assays for detection of prey DNA in the gut of soil arthropod predators. *Soil Biology and Biochemistry*, **57**, 943–945.

Ekbom, B., Kuusk, A.-K., Malsher, G., Åström, S. & Cassel-Lundhagen, A. (2014) Consumption of flea beetles (Phyllotreta, Coleoptera: Chrysomelidae) by spiders in field habitats detected by molecular analysis. *The Canadian Entomologist*, **146**, 639–651.

Eklund, E. (2005) Sadelgallmygga som skadegörare på stråsäd Försök och erfarenheter från Västra Götalands län. (Master's) *Sveriges Lantbruksuniversitet,* Uppsala, Sweden*.*

Elkinton, J.S. & Cardé, R.T. (1988) Effects of intertrap distance and wind direction on the interaction of gypsy moth (Lepidoptera: Lymantriidae) pheromone- baited traps. *Environmental Entomology*, **17**, 764–769.

Elliott, R.H., Mann, L. & Olfert, O. (2009) Calendar and degree-day requirements for emergence of adult wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) in Saskatchewan, Canada. *Crop Protection*, **28**, 588–594.

Ellis, J.D., Hepburn, R., Luckman, B. & Elzen, P.J. (2004) Effects of soil type, moisture, and density on pupation success of *Aethina tumida* (Coleoptera: Nitidulidae). *Environmental Entomology*, **33**, 794–798.

Ellis, S., Ashlee, N.J. & Maulden, K.A. (2014) Improving risk assessment and control of saddle gall midge (*Haplodiplosis marginata*). *Aspects of Applied Biology*, **127**, 29–34.

El-Sayed, A.M., Suckling, D.M., Wearing, C.H. & Byers, J.A. (2006) Potential of mass trapping for long-term pest management and eradication of invasive species. *Journal of Economic Entomology*, **99**, 1550–1564.

Empson, D.W. (1965) *Cereal Pests*. MAFF Reference Book. HMSO, London, UK.

Enock, F. (1909) *Clinodiplosis equestris* (Wagner); an insect new to Great Britain. *The Entomologist,* **42**, 217.

Evans, B.G., Jordan, K.S., Brownbridge, M. & Hallett, R.H. (2015) Effect of temperature and host life stage on efficacy of soil entomopathogens against the swede midge (Diptera: Cecidomyiidae). *Journal of Economic Entomology*, **108**, 473–483.

Evans, B.G. & Hallett, R.H. (2016) Efficacy of biopesticides for management of the swede midge (Diptera: Cecidomyiidae). *Journal of Economic Entomology*, **109**, 2159–2167.

Faber, W. (1959) Untersuchungen über ein katastrophales Auftreten der Sattelmücke (*Haplodiplosis equestris* Wagner) in Ostirol. *Pflanzenschutzber*, **23**, 65–90.

Fand, B.B., Choudhary, J.S., Kumar, M. & Bal, S.K. (2014) phenology modelling and GIS applications in pest management: a tool for studying and understanding insect-pest dynamics in the context of global climate change. In *Approaches to Plant Stress and their Management*, Springer India, pp. 107–124.

Fang, Y. & Ramasamy, R.P. (2015) Current and prospective methods for plant disease detection. *Biosensors*, **5**, 537–561.

FAO (2017) *The future of food and agriculture – Trends and challenges*. Food and Agriculture Organization of the United Nations, Rome, Italy.

Fera (2016) *Pesticide Usage Survey*. Fera Science Ltd., UK. URL https://secure.fera.defra.gov.uk/pusstats/index.cfm

Finke, D.L. & Denno, R.F. (2003) Intra-guild predation relaxes natural enemy impacts on herbivore populations. *Ecological Entomology*, **28**, 67–73.

Finke, D.L. & Denno, R.F. (2005) Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecology Letters*, **8**, 1299–1306.

Floate, K.D., Doane, J.F. & Gillott, C. (1990) Carabid predators of the wheat midge (Diptera: Cecidomyiidae) in Saskatchewan. *Environmental Entomology*, **19**, 1503–1511.

Foltan, P., Sheppard, S., Konvicka, M. & Symondson, W.O.C. (2005) The significance of facultative scavenging in generalist predator nutrition: detecting decayed prey in the guts of predators usinPCR. *Molecular Ecology*, **14**, 4147–4158.

Forrest, J.R.K. & Thomson, J.D. (2011) An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. *Ecological Monographs*, **81**, 469–491.

Franzmann, B.A., Lloyd, R.J. & Zalucki, M.P. (2006) Effect of soil burial depth and wetting on mortality of diapausing larvae and patterns of post-diapause adult emergence of sorghum midge, *Stenodiplosis sorghicola* (Coquillett) (Diptera: Cecidomyiidae). *Australian Journal of Entomology*, **45**, 192–197.

Frey, J.E., Frey, B. & Baur, R. (2004) Molecular identification of the swede midge (Diptera: Cecidomyiidae). *The Canadian Entomologist*, **136**, 771–780.

Furlong, M.J. (2015) Knowing your enemies: Integrating molecular and ecological methods to assess the impact of arthropod predators on crop pests. *Insect Science*, **22**, 6–19.

Gagné, R.J. (1994) *The Gall Midges of the Neotropical Region*. Cornell University Press, New York, USA.

Gagné, R.J. (2004) *A catalog of the Cecidomyiidae (Diptera) of the world.* Entomological Society of Washington, Washington D.C., USA.

Ganehiarachchi, M. & Knodel, J. (2008) Integrated pest management of the wheat midge in North Dakota. NDSU Extension Service, USA.

Gariepy, T.D., Kuhlmann, U., Gillott, C. & Erlandson, M. (2007) Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of Arthropods. *Journal of Applied Entomology*, **131**, 225–240.

Geer, L.Y., Marchler-Bauer, A., Geer, R.C., Han, L., He, J., He, S., *et al.* (2010) The NCBI BioSystems database. *Nucleic Acids Research*, **38**, 492–496.

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W.W., Emmerson, M., Morales, M.B., *et al.* (2010) Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology*, **11**, 97–105.

Glare, T., Caradus, J., Gelernter, W., Jackson, T., Keyhani, N., Köhl, J., *et al.* (2012) Have biopesticides come of age? *Trends in Biotechnology*, **30**, 250–258.

Golightly, W.H. (1979) *Saddle gall midge*. Ministry of Agriculture, Fisheries and Food, London, UK.

Golightly, W.H. & Woodville, H.C. (1974) Studies of recent outbreaks of saddle gall midge. *Annals of Applied Biology*, **77**, 97.

Good, J.A. & Giller, P.S. (1991) The diet of predatory staphylinid beetles–a review of records. *Entomologist's Monthly Magazine*, 77–89.

Grafius, E. & Warner, F.W. (1989) Predation by *Bembidion quadrimaculatum* (Coleoptera: Carabidae) on *Delia antiqua* (Diptera: Anthomyiidae). *Environmental Entomology*, **18**, 1056–1059.

Gratwick, M. (1992) Saddle gall midge. In *Crop Pests in the UK.* Chapman & Hall, London, UK, p. 306–309

Greenstone, M.H. & Hunt, J.H. (1993) Determination of prey antigen half-life in *Polistes metricus* using a monoclonal antibody-based immunodot assay. *Entomologia Experimentalis et Applicata*, **68**, 1–7.

Greenstone, M.H. (2006) Molecular methods for assessing insect parasitism. *Bulletin of Entomological Research*, **96**, 1–13.

Greenstone, M.H., Rowley, D.L., Weber, D.C., Payton, M.E. & Hawthorne, D.J. (2007) Feeding mode and prey detectability half-lives in molecular gut-content analysis: an example with two predators of the Colorado potato beetle. *Bulletin of Entomological Research*, **97**, 201–209.

Greenstone, M.H., Payton, M.E., Weber, D.C. & Simmons, A.M. (2014) The detectability half-life in arthropod predator–prey research: what it is, why we need it, how to measure it, and how to use it. *Molecular Ecology*, **23**, 3799–3813.

Gries, R., Gries, G., Khaskin, G., King, S., Olfert, O. *et al.* (2000) Sex pheromone of orange wheat blossom midge, *Sitodiplosis mosellana*. *Naturwissenschaften*, **87**, 450–454.

Griffin, C.T. (1993) Temperature responses of entomopathogenic nematodes: implications for the success of biological control programmes. In *Nematodes and the Biological Control of Insect Pests*. Csiro Publishing, Clayton, Australia.

Hagstrum, D.W. & Milliken, G.A. (1991) Modeling differences in insect developmental times between constant and fluctuating temperatures. *Annals of the Entomological Society of America*, **84**, 369–379.

Hahn, N.G. & Isaacs, R. (2012) Distribution and phenology of *Dasineura oxycoccana* (Diptera: Cecidomyiidae) in Michigan blueberries. *Environmental Entomology*, **41**, 455–462.

Hall, D.R., Amarawardana, L., Cross, J.V., Francke, W., Boddum, T. & Hillbur, Y. (2012) The chemical ecology of cecidomyiid midges (Diptera: Cecidomyiidae). *Journal of Chemical Ecology*, **38**, 2–22.

Hallett, R.H., Goodfellow, S.A. & Heal, J.D. (2007) Monitoring and detection of the swede midge (Diptera: Cecidomyiidae). *The Canadian Entomologist*, **139**, 700–712.

Hansen, J.E. & New, T.R. (2005) Use of barrier pitfall traps to enhance inventory surveys of epigaeic Coleoptera. *Journal of Insect Conservation*, **9**, 131–136.

Hardie, J. & Minks, A.K. (Eds.). (1999) *Pheromones of Non-lepidopteran Insects Associated with Agricultural Plants*. CABI Publishing, Wallingford, Oxon, UK.

Harper, G.L., King, R.A., Dodd, C.S., Harwood, J.D., Glen, D.M., Bruford, M.W., *et al.* (2005) Rapid screening of invertebrate predators for multiple prey DNA targets. *Molecular Ecology*, **14**, 819–827.

Harris, K.M. (1966) Gall midge genera of economic importance (Diptera: Cecidomyiidae) Part 1: Introduction and subfamily Cecidomyiinae; supertribe Cecidomyiidi. *Transactions of the Royal Entomological Society of London*, **118**, 313–358.

Harris, M.O. & Foster, S.P. (1991) Wind tunnel studies of sex pheromone-mediated behavior of the Hessian fly (Diptera: Cecidomyiidae). *Journal of Chemical Ecology*, **17**, 2421–2435.

Harris, K.M. & Foster, S. (1999) Gall Midges. In *Pheromones of Non-Lepidopteran Insects Associated With Agricultural Plants*. CABI Publishing, Wallingford, Oxon, UK.

Harwood, J.D., Bostrom, M.R., Hladilek, E.E., Wise, D.H. & Obrycki, J.J. (2007) An order-specific monoclonal antibody to Diptera reveals the impact of alternative prey on spider feeding behavior in a complex food web. *Biological Control*, **41**, 397–407.

Hatteland, B.A., Symondson, W.O.C., King, R.A., Skage, M., Schander, C. & Solhøy, T. (2011) Molecular analysis of predation by carabid beetles (Carabidae) on the invasive Iberian slug *Arion lusitanicus*. *Bulletin of Entomological Research*, **101**, 675–686.

HGCA (2012) *Biology and control of saddle gall midge*. Information sheet 15. HGCA Publications, Warwickshire, UK.

Hillbur, Y., Anderson, P., Arn, H., Bengtsson, M., Lofqvist, J., Biddle, A.J., *et al.* (1999) Identification of sex pheromone components of the pea midge, *Contarinia pisi* (Diptera : Cecidomyiidae). *Naturwissenschaften*, **86**, 292–294.

Hillocks, R.J. (2012) Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Protection*, **31**, 85–93.

Hodek, I. (1996) Diapause development, diapause termination and the end of diapause. *European Journal of Entomology*, **93**, 475–487.

Ho, H.Y. & Millar, J.G. (2002) Identification, electroantennogram screening, and field bioassays of volatile chemicals from *Lygus hesperus* Knight (Heteroptera: Miridae). *Zoological Studies,* **41**, 311–320.

Hodges, R.J., Addo, S., Farman, D.I. & Hall, D.R. (2004) Optimising pheromone lures and trapping methodology for *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). *Journal of Stored Products Research*, **40**, 439–449.

Holland, J.M. & Luff, M.L. (2000) The effects of agricultural practices on carabidae in temperate agroecosystems. *Integrated Pest Management Reviews*, **5**, 109–129.

Holland, J.M. & Thomas, S.R. (2000) Do polyphagous predators help control orange wheat blossom midge, *Sitodiplosis mosellana* Gehin (Dipt.,Cecidomyiidae) in winter wheat? *Journal of Applied Entomology*, **124**, 325–330.

Holland, J.M., Thomas, S.R. & Hewitt, A. (1996) Some effects of polyphagous predators on an outbreak of cereal aphid (*Sitobion avenae* F.) and orange wheat blossom midge (*Sitodoplosis mosellana* Géhin). *Agriculture, Ecosystems & Environment*, **59**, 181–190.

Hoogendoorn, M. & Heimpel, G.E. (2001) PCR-based gut content analysis of insect predators: Using ribosomal ITS-1 fragments from prey to estimate predation frequency. *Molecular Ecology*, **10**, 2059–2067.

Houseweart, M.W., Jennings, D.T. & Sanders, C.J. (1981) Variable associated with pheromone traps for monitoring spruce budworm populations (Lepidoptera: Tortricidae). *The Canadian Entomologist,* **113**, 527–537.

Howse, P., Stevens, J.M. & Jones, G.A.D. (1997) *Insect Pheromones and their Use in Pest Management*. Springer, London, UK.

Hoy, M.A. (1994) *Insect molecular genetics: an introduction to principles and applications*. Academic Press, San Diego, USA.

Hull R., Tatnell L.V., Cook S.K., Beffa R., Moss S.R. (2014) Current status of herbicide-resistant weeds in the UK. *Aspects of Applied Biology*, **127**, 261–272.

Irwin, J.T. & Lee, R.E. (2000) Mild winter temperatures reduce survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis* (Diptera : Tephritidae). *Journal of Insect Physiology*, **46**, 655–661.

Jacquemin, G., Chavalle, S. & De Proft, M. (2014) Forecasting the emergence of the adult orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) in Belgium. *Crop Protection*, **58**, 6–13.

Jones, O.T. (1998) Practical applications of pheromones and other semiochemicals. In *Insect Pheromones and their Use in Pest Management*. Springer, London, UK, p.261-355

Juen, A. & Traugott, M. (2004) Detecting predation and scavenging by DNA gut-content analysis: a case study using a soil insect predator-prey system. *Oecologia*, **142**, 344–352.

Juen, A. & Traugott, M. (2006) Amplification facilitators and multiplex PCR: Tools to overcome PCR-inhibition in DNA-gut-content analysis of soil-living invertebrates. *Soil Biology and Biochemistry*, **38**, 1872–1879.

Kim, D.-S., Lee, J.-H. & Yiem, M.-S. (2000) Spring emergence pattern of *Carposina sasakii* (Lepidoptera: Carposinidae) in apple orchards in Korea and its forecasting models based on degree-days. *Environmental Entomology*, **29**, 1188–1198.

King, R.A., Read, D.S., Traugott, M. & Symondson, W.O.C. (2008) Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular Ecology*, **17**, 947–963.

King, R.A., Moreno-Ripoll, R., Agustí, N., Shayler, S.P., Bell, J.R., Bohan, D.A., *et al.* (2010) Multiplex reactions for the molecular detection of predation on pest and nonpest invertebrates in agroecosystems. *Molecular Ecology Resources*, **11**, 370–373.

Knutson, A.E. & Muegge, M.A. (2010) A degree-day model initiated by pheromone trap captures for managing pecan nut casebearer (Lepidoptera: Pyralidae) in pecans. *Journal of Economic Entomology*, **103**, 735–743.

Kogan, M. (1998) Integrated pest management: historical perspectives and contemporary developments. *Annual Review of Entomology*, **43**, 243–270.

Kong, W.N., Hu, R.S., Zhao, Z.G., Li, J., Zhang, Z.W., Li, S.C., *et al.* (2014) Effects of trap height, location, and spacing on pheromone-baited trap catch efficacy for oriental fruit moths (Lepidoptera: Tortricidae) in a peach orchard. *Canadian Entomologist*, **146**, 684–692.

Koštál, V. (2006) Eco-physiological phases of insect diapause. *Journal of Insect Physiology*, **52**, 113–127.

Kromp, B. (1999) Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. *Agriculture, Ecosystems & Environment*, **74**, 187–228.

Lamb, R., McKenzie, R., Wise, I., Barker, P., Smith, M. & Olfert, O. (2000) Resistance to *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) in spring wheat (Gramineae). *The Canadian Entomologist*, **132**, 591–605.

Landis, D.A., Wratten, S.D. & Gurr, G.M. (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology*, **45**, 175–201.

Lang, A. (2003) Intraguild interference and biocontrol effects of generalist predators in a winter wheat field. *Oecologia*, **134**, 144–153.

Lange, W. & Jochensen, G. (1987) Leaf hairs in common wheat are no deterrent to attack by the saddle gall midge. *Cereal Research Communications*, **15**, 255–258.

Latteur, G. (1972) The size of larval populations of the saddle gall midge (*Haplodiplosis marginata* von Roser = *H. equestris* Wagner, Diptera, Cecidomyiidae) in wheat fields in the main cereal-growing areas of southern Belgium in 1970 and 1971. / Importance des populations larvaires de la Cecidomyie equestre (*Haplodiplosis marginata* von Roser = *H. equestris* Wagner, Diptera, Cecidomyiidae) dans les froments des principales zones cerealieres du Sud de la Belgique en 1970 et 1971. *Parasitica*, **28**, 16–24.

Leal, W.S. (1996) Chemical communication in scarab beetles: reciprocal behavioral agonist-antagonist activities of chiral pheromones. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 12112–12115.

Leather, S.R., Walters, K.F.A. & Bale, J.S. (1993) *The Ecology of Insect Overwintering*. Cambridge University Press, Cambridge, UK.

Lenteren, J.C. van. (1995) Success in biological control of arthropods through augmentation of natural enemies. In *Biological Control: Measures of Success*. Kluwer Academic Publishers, Dordrecht, The Netherlands, p.77-103

Lewis, T. (1969) The Distribution of Flying Insects Near a Low Hedgerow. *Journal of Applied Ecology*, **6**, 443–452.

Liu, Y., He, X-K., Hall, D., Farman, D., Amarawardana, L. *et al.* (2009) (2*S*,8*Z*)-2-Butyroxy-8-heptadecene: major component of the sex pheromone of chrysanthemum gall midge, *Rhopalomyia longicauda*. *Journal of Chemical Ecology,* **35**, 715–723.

Lövei, G.L. & Sunderland, K.D. (1996) Ecology and behavior of ground beetles (Coleoptera: Carabidae). *Annual Review of Entomology*, **41**, 231–256.

Luff, M.L. (2002) Carabid assemblage organization and species composition. In *The Agroecology of Carabid Beetles*. Intercept, Andover, UK.

Luff, M.L. (2007) *The Carabidae*. Royal Entomological Society, St. Albans, UK.

Lundgren, J.G., Ellsbury, M.E. & Prischmann, D.A. (2009) Analysis of the predator community of a subterranean herbivorous insect based on polymerase chain reaction. *Ecological Applications*, **19**, 2157–2166.

Lys, J.-A. (1995) Observation of epigeic predators and predation on artificial prey in a cereal field. *Entomologia Experimentalis et Applicata*, **75**, 265–272.

Magarey, R.D., Fowler, G.A., Borchert, D.M., Sutton, T.B., Colunga-Garcia, M. & Simpson, J.A. (2007) NAPPFAST: An internet system for the weather-based mapping of plant pathogens. *Plant Disease*, **91**, 336–345.

Mair, J. & Port, G.R. (2001) Predation by the carabid beetles *Pterostichus madidus* and *Nebria brevicollis* is affected by size and condition of the prey slug *Deroceras reticulatum*. *Agricultural and Forest Entomology*, **3**, 99–106.

Matson, P.A., Parton, W.J., Power, A.G. & Swift, M.J. (1997) Agricultural intensification and ecosystem properties. *Science*, **277**, 504–509.

Mayer, M.S. (1993) Responses of three antennal specialist neurons of male *Trichoplusia ni* (Hübner) to sex pheromone components at and above naturally emitted levels. *Journal of Insect Physiology*, **39**, 401–412.

McNally, P.S. & Barnes, M.M. (1980) Inherent characteristics of codling moth pheromone traps. *Environmental Entomology*, **9**, 538–541.

McNally, P.S. & Barnes, M.M. (1981) Effects of codling moth pheromone trap placement, orientation and density on trap catches. *Environmental Entomology*, **10**, 22–26.

Menu, F. (1993) Strategies of emergence in the chestnut weevil *Curculio elephas* (Coleoptera: Curculionidae). *Oecologia*, **96**, 383–390.

Met Office (2012) Met Office Integrated Data Archive System (MIDAS) Land and Marine Surface Stations Data (1853-current). NCAS British Atmospheric Data Centre, URL http://catalogue.ceda.ac.uk/uuid/220a65615218d5c9cc9e4785a3234bd0 [accessed June 2017]

Miller, P., Lanier, W. & Brandt, S. (2001) Using growing degree days to predict plant stages. MSU Extension Service, USA.

Monzó, C., Sabater-Muñoz, B., Urbaneja, A. & Castañera, P. (2011) The ground beetle *Pseudophonus rufipes* revealed as predator of *Ceratitis capitata* in citrus orchards. *Biological Control*, **56**, 17–21.

Mori, B.A. & Evenden, M.L. (2013) Factors affecting pheromone-baited trap capture of male *Coleophora deauratella*, an invasive pest of clover in Canada. *Journal of Economic Entomology*, **106**, 844–854.

Mori, K. (2007) Significance of chirality in pheromone science. *Bioorganic & Medicinal Chemistry*, **15**, 7505–7523.

Mowat, D.J. (Department of A.Z. & Martin, S.J. (1981) The contribution of predatory beetles (Coleoptera: Carabidae and Staphylinidae) and seed-bed-applied insecticide to the control of cabbage root fly, *Delia brassicae* (Wied.), in transplanted cauliflowers. *Horticultural Research,* **21**, 127-136.

Murchie, A.K., Smart, L.E. & Williams, I.H. (1997) Responses of *Dasineura brassicae* and its parasitoids *Platygaster subuliformis* and *Omphale clypealis* to field traps baited with organic isothiocyanates. *Journal of Chemical Ecology*, **23**, 917–926.

NIBIO. (2017) Varsling Innen Plante Skadegjørere. URL https://www.vips-landbruk.no/ [accessed June 2017]

Naranjo, S.E. (2001) Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. *Crop Protection*, **20**, 835–852.

Navntoft, S., Esbjerg, P. & Riedel, W. (2006) Effects of reduced pesticide dosages on carabids (Coleoptera: Carabidae) in winter wheat. *Agricultural and Forest Entomology*, **8**, 57–62.

Neises, B. & Steglich, W., (1978) Simple method for the esterification of carboxylic acids. *Angewandte Chemie International Edition,* **17**, 523-525.

Nietschke, B.S., Magarey, R.D., Borchert, D.M., Calvin, D.D. & Jones, E. (2007) A developmental database to support insect phenology models. *Crop Protection*, **26**, 1444–1448.

Nijveldt, W. (1967) Notes on Cecidomyidae, I. *Entomologische Berichten*, **27**, 8–14.

Nijveldt, W.C. & Hulshoff, A.J.A. (1968) *Waarnemingen inzake de tarwestengelgalmug (*Haplodiplosis equestris *Wagner) in Nederland*. Centrum voor Landbouwpublikaties en Landbouwdocumentatie, Wageningen.

Norris, R.F. & Kogan, M. (2005) Ecology of interactions between weeds and arthropods. *Annual Review of Entomology*, **50**, 479–503.

Noyes, J.S. (2017) *Universal Chalcidoidea Database*. URL http://www.nhm.ac.uk/chalcidoids

Oakley, J.N., Cumbleton, P.C., Corbett, S.J., Saunders, P., Green, D.I., Young, J.E.B., *et al.* (1998) Prediction of orange wheat blossom midge activity and risk of damage. *Crop Protection*, **17**, 145–149.

Oakley, J.N. (2005) *Integrated control of wheat blossom midge: Variety choice, use of pheromone traps and treatment thresholds*. HGCA Project Report No. 363. HGCA, Warwickshire, UK.

Oakley, J.N. & Ellis, S. (2009) Orange wheat blossom midge - guidelines for assessment and control. HGCA, Warwickshire, UK

Öberg, S., Cassel-Lundhagen, A. & Ekbom, B. (2011) Pollen beetles are consumed by ground- and foliage-dwelling spiders in winter oilseed rape. *Entomologia Experimentalis et Applicata*, **138**, 256–262.

Okuyama, T., Yang, E.-C., Chen, C.-P., Lin, T.-S., Chuang, C.-L. & Jiang, J.-A. (2011) Using automated monitoring systems to uncover pest population dynamics in agricultural fields. *Agricultural Systems*, **104**, 666–670.

Ormerod, E.A. (1890) *Report of observations of injurious insects and common farm pests during the year 1889, with methods of prevention and remedy: 13th report*. Simpkin, Marshall, Hamilton, Kent & Co., London, UK.

Östman, Ö., Ekbom, B. & Bengtsson, J. (2003) Yield increase attributable to aphid predation by ground-living polyphagous natural enemies in spring barley in Sweden. *Ecological Economics*, **45**, 149–158.

Parker, A. K., De Cortázar-Atauri, I. G., Van Leeuwen, C. & Chuine, I. (2011) General phenological model to characterise the timing of flowering and veraison of *Vitis vinifera* L. *Australian Journal of Grape and Wine Research*, **17**, 206–216.

Penney, M.M. (1966) Studies on certain aspects of the ecology of *Nebria brevicollis* (F.) (Coleoptera, Carabidae). *Journal of Animal Ecology*, **35**, 505–512.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, (2015) nlme: linear and nonlinear mixed effects models. R Package Version 3. 1–131. URL http://CRAN.R-project.org/package=nlme

Pope, T. & Ellis, S. (2013) *Monitoring saddle gall midge (*Haplodiplosis marginata*) larvae and adult emergence*. HGCA Project Report No. 516. HGCA, Warwickshire, UK.

Popov, C., Petcu, L. & Barbulescu, A. (1998) Researches on biology, ecology and control of saddle gall midge (*Haplodiplosis marginata* von Roser) in Romania. *Romanian Agricultural Research*, **9**, 67–73.

Prasad, Y. & Prabhakar, M. (2012) Pest Monitoring and Forecasting. In *Integrated Pest Management: Principles and Practice*. CABI Publishing, Wallingford, UK, pp. 41–57.

Pruess, K.P. (1983) Day-degree methods for pest management. *Environmental Entomology,* **12**, 613-619.

Racz, U.V. (1973) Damage caused by *Haplodiplosis equestris* Wagn. in Hungary and the analysis of its extent. *Acta Phytopathologica Academiae Scientiarum Hungaricae*, **8**, 221-225.

Ramsden, M.W., Kendall, S.L., Ellis, S.A. & Berry, P.M. (2017) A review of economic thresholds for invertebrate pests in UK arable crops. *Crop Protection*, **96**, 30–43.

R Core Team (2015, 2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/

Regulation (EU) No 1307/2013 (2013) of the European Parliament and of the Council of 17 December 2013 establishing rules for direct payments to farmers under support schemes within the framework of

the common agricultural policy and repealing Council Regulation (EC) No 637/2008 and Council Regulation (EC) No 73/2009. *Official Journal of the European Union* L347

Rhainds, M., Therrien, P. & Morneau, L. (2016) Pheromone-based monitoring of spruce budworm (Lepidoptera: Tortricidae) larvae in relation to trap position. *Journal of Economic Entomology*, **109**, 717–723.

Riedl, H., Hoying, S.A., Barnett, W.W. & Detar, J.E. (1979) Relationship of within-tree placement of the pheromone trap to codling moth catches. *Environmental Entomology*, **8**, 765–769.

Roberts, D.A., Harris, D., Corr, I. & Corrigan, W. (2014) Saddle gall midge (*Haplodiplosis marginata*) in winter wheat. *Crop Protection in Northern Britain*. The Association for Crop Protection in Northern Britain, pp. 125–130.

Robinson, R.A. & Sutherland, W.J. (2002) Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology*, **39**, 157–176.

Rodgers, P.B. (1993) Potential of biopesticides in agriculture. *Pesticide Science*, **39**, 117–129.

Rosenheim, J.A., Kaya, H.K., Ehler, L.E., Marois, J.J. & Jaffee, B.A. (1995) Intraguild predation among biological-control agents: theory and evidence. *Biological Control*, **5**, 303–335.

Rougerie, R., Smith, M.A., Fernandez-Triana, J., Lopez-Vaamonde, C., Ratnasingham, S. & Hebert, P.D.N. (2011) Molecular analysis of parasitoid linkages (MAPL): gut contents of adult parasitoid wasps reveal larval host. *Molecular Ecology*, **20**, 179–186.

Rowley, C., Cherrill, A., Leather, S., Nicholls, C., Ellis, S. & Pope, T. (2016) A review of the biology, ecology and control of saddle gall midge, *Haplodiplosis marginata* (Diptera: Cecidomyiidae) with a focus on phenological forecasting. *Annals of Applied Biology*, **169**, 167–179.

Rowley, C., Pope, T.W., Cherrill, A., Leather, S.R., Fernández-Grandon, G.M. & Hall, D.R. (2017) Development and optimisation of a sex pheromone lure for monitoring populations of saddle gall midge, *Haplodiplosis marginata*. *Entomologia Experimentalis et Applicata*, **163**, 82–92.

Rübsaamen, E.W. & Rübsaamen, E.W. (1910) Über deutsche Gallmücken und Gallen. *Zoologischen Wiss Insekten Biologie*, **6**, 125–133.

Salt, G. & Hollick, F.S.J. (1944) Studies of wireworm populations. *Annals of Applied Biology*, **31**, 52–64.

Samietz, J., Graf, B., Höhn, H., Schaub, L., Höpli, H.U. & Razavi, E. (2011) Web-based decision support for sustainable pest management in fruit orchards: development of the Swiss system SOPRA.

Saunders, D.S. (2014) Insect photoperiodism: effects of temperature on the induction of insect diapause and diverse roles for the circadian system in the photoperiodic response. *Entomological Science*, **17**, 25-40.

Schaad, N.W. & Frederick, R.D. (2002) Real-time PCR and its application for rapid plant disease diagnostics. *Canadian Journal of Plant Pathology*, **24**, 250–258.

Shepherd, R.F., Gray, T.G., Chorney, R.J. & Daterman, G.E. (1985) Pest management of Douglas-fir tussock moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae): monitoring endemic populations with pheromone traps to detect incipient outbreaks. *Canadian entomologist*, **117**, 839–848.

Schillinger, J.A. & Gallun, R.L. (1968) Leaf pubescence of wheat as a deterrent to the cereal leaf beetle, *Oulema melanopus*. *Annals of the Entomological Society of America*, **61**, 900–903.

Schmidt, J.M., Harwood, J.D. & Rypstra, A.L. (2012) Foraging activity of a dominant epigeal predator: molecular evidence for the effect of prey density on consumption. *Oikos*, **121**, 1715–1724.

Schütte, F. (1964a) Zur Anfälligkeit einiger Getreide- und Gräserarten gegen *Haplodiplosis equestris* (Wagner). *Anzeiger für Schädlingskunde*, **37**, 129–132.

Schütte, F. (1964b) Zum Wirtspflanzenkreis und zur Vagilität der Sattelmücke (*Haplodiplosis equestris* Wagner). *Zeitschrift für Angewandte Entomologie*, **54**, 196–201.

Schütte, F. (1983) Saddle gall midge. Information on integrated control. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes*, **35**, 109.

Shapiro-ilan, D.I., Gardner, W.A., Fuxa, J.R., Wood, B.W., Nguyen, K.B., Adams, B.J., *et al.* (2003) Survey of entomopathogenic nematodes and fungi endemic to pecan orchards of the southeastern

United States and their virulence to the pecan weevil (Coleoptera: Curculionidae). *Environmental Entomology*, **32**, 187–195.

Sheppard, S.K., Bell, J., Sunderland, K.D., Fenlon, J., Skervin, D. & Symondson, W.O.C. (2005) Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. *Molecular Ecology*, **14**, 4461–4468.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, **87**, 651–701.

Sint, D., Raso, L., Kaufmann, R. & Traugott, M. (2011) Optimizing methods for PCR-based analysis of predation. *Molecular Ecology Resources*, **11**, 795–801.

Skuhravá M., Skuhravý V. (2014) *Haplodiplosis marginata*, In CABI: *Crop Protection Compendium*. CABI Publishing, Wallingford, UK, p. 25.

Skuhravý, V. (1982) The saddle gall midge *Haplodiplosis marginata* (von Roser) (Diptera, Cecidomyiidae), an important pest of wheat and barley in Czechoslovakia. *Folia Facultatis Scientiarum Naturalium Universitatis Purkynianae Brunensis, Biologia*, **23**, 133–135.

Skuhravý, V., Skuhravá, M. & Brewer, W.J. (1983) Ecology of the saddle gall midge *Haplodiplosis marginata* (von Roser) (Diptera, Cecidomyiidae). *Zeitschrift für Angewandte Entomologie*, **96**, 476–490.

Skuhravý, V., Skuhravá, M. & Brewer, T.W. (1993) The saddle gall midge *Haplodiplosis marginata* (Diptera: Cecidomyiidae) in Czech Republic and Slovak Republic from 1971-1989. *Acta Societatis Zoologicae Bohemoslovacae*, **57**, 117–137.

Snyder, R.L., Spano, D., Cesaraccio, C. & Duce, P. (1999) Determining degree-day thresholds from field observations. *International Journal of Biometeorology*, **42**, 177–182.

Snyder, W.E. & Wise, D.H. (1999) Predator interference and the establishment of generalist predator populations for biocontrol. *Biological Control*, **15**, 283–292.

Son, Y., Lee, J.-H. & Chung, Y.-J. (2007) Temperature-dependent post-diapause development and prediction of spring emergence of the pine needle gall midge (Dipt., Cecidomyiidae). *Journal of Applied Entomology*, **131**, 674–683.

Spenser Technologies (2017) URL http://www.spensatech.com/z-trap/ [accessed June 2017]

Speyer, W. & Waede, M. (1956) Feinde und Parasiten der Weizengallmücken. *Anzeiger für Schädlingskunde*, **29**, 185–191.

Spittler, H. (1969) Beiträge zur Morphologie, Biologie und Ökologie des Sattelmückenparasiten *Platygaster equestris* nov. spec. (Hymenoptera, Proctotrupoidea, Scelionidae) unter besonderer Berücksichtigung seines abundanzdynamischen Einflusses auf *Haplodiplosis equestris* Wagner (Diptera, Cecidomyiidae). *Zeitschrift für Angewandte Entomologie*, **64**, 1–34.

Storkey, J., Macdonald, A.J., Bell, J.R., Clark, I.M., Gregory, A.S., Hawkins, N.J., *et al.* (2016) The Unique Contribution of Rothamsted to Ecological Research at Large Temporal Scales. *Advances in Ecological Research*, Large-Scale Ecology: Model Systems to Global Perspectives, **55**, 3–42.

Strand, J.F. (2000) Some agrometeorological aspects of pest and disease management for the 21st century. *Agricultural and Forest Meteorology*, **103**, 73–82.

Stuart, M.K. & Greenstone, M.H. (1990) Beyond ELISA: a rapid, sensitive, specific immunodot assay for identification of predator stomach contents. *Annals of the Entomological Society of America*, **83**, 1101–1107.

Sunderland, K., Powell, W. & Symondson, W. (2005) Populations and Communites. In *Insects as Natural Enemies: A Practical Perspective*. Springer, Berlin, Germany, pp. 299–434.

Sunderland, K.D., Lovei, G.L. & Fenlon, J. (1995) Diets and reproductive phenologies of the introduced ground beetles *Harpalus affinis* and *Clivina australasiae* (Coleoptera, Carabidae) in New-Zealand.

Sutcliffe, O.L. & Kay, Q.O.N. (2000) Changes in the arable flora of central southern England since the 1960s. *Biological Conservation*, **93**, 1–8.

Symondson, W.O.C. (2002) Molecular identification of prey in predator diets. *Molecular Ecology*, **11**, 627–641.

Symondson, W.O.C., Sunderland, K.D. & Greenstone, M.H. (2002) Can generalist predators be effective biocontrol agents? *Annual Review of Entomology*, **47**, 561–594.

Tauber, M. & Tauber, and C.A. (1976) Insect Seasonality: Diapause maintenance, termination, and postdiapause development. *Annual Review of Entomology*, **21**, 81–107.

Tauber, M.J., Tauber, C.A. & Nyrop, J.P. (1994) Soil moisture and postdormancy emergence of Colorado potato beetles (Coleoptera: Chrysomelidae): Descriptive model and field emergence patterns. *Environmental Entomology*, **23**, 1485–1496.

Tauber, M.J., Tauber, C.A., Nyrop, J.P. & Villani, M.G. (1998) Moisture, a vital but neglected factor in the seasonal ecology of insects: Hypotheses and tests of mechanisms. *Environmental Entomology*, **27**, 523–530.

Theiling, K.M. & Croft, B.A. (1988) Pesticide side-effects on arthropod natural enemies: A database summary. *Agriculture, Ecosystems & Environment*, **21**, 191–218.

Toor, R.F. van. (2006) The effects of pesticides on Carabidae (Insecta: Coleoptera), predators of slugs (Mollusca: Gastropoda): literature review. *New Zealand Plant Protection*, **59**, 208–216.

Tottman, D.R., Makepeace, R.J. & Broad, H. (1979) An explanation of the decimal code for the growth stages of cereals, with illustrations. *Annals of Applied Biology*, **93**, 221–234.

Tumlinson, J.H., Klein, M.G., Doolittle, R.E., Ladd, T.L. & Proveaux, A.T. (1977) Identification of the female japanese beetle sex pheromone: inhibition of male response by an enantiomer. *Science*, **197**, 789–792.

Vanaclocha, P., Jones, M.M., Monzó, C. & Stansly, P.A. (2016) Placement density and longevity of pheromone traps for monitoring of the citrus leafminer (Lepidoptera: Gracillariidae). *Florida Entomologist*, **99**, 196–202.

Wagner, B. (1871) *Diplosis equestris* n. sp., Sattelmücke. *Entomologische Zeitung Stettin*, **32**, 414–423.

Walczak, F. (1982) Incidence of *Haplodiplosis equestris* Wagner on cereals in Poland during 1979-1981. *Ochrona Roslin*, **26**.

Waldner, T., Sint, D., Juen, A. & Traugott, M. (2013) The effect of predator identity on post-feeding prey DNA detection success in soil-dwelling macro-invertebrates. *Soil Biology and Biochemistry*, **63**, 116–123.

Wall, C. & Perry, J.N. (1978) Interactions between pheromone traps for the pea moth*, Cydia Nigricana* (f.). *Entomologia Experimentalis et Applicata*, **24**, 155–162.

Wall, C. & Perry, J.N. (1980) Effects of spacing and trap number on interactions between pea moth pheromone traps. *Entomologia Experimentalis et Applicata*, **28**, 313–321.

Wall, C. & Perry, J.N. (1987) Range of action of moth sex-attractant sources. *Entomologia Experimentalis et Applicata*, **44**, 5–14.

Wall, C. (1990) Principles of Monitoring. In *Behaviour Modifying Chemicals for Insect Management*. CRC Press, Florida, USA.

Wedding, R., Anderbrant, O. & Jönsson, P. (1995) Influence of wind conditions and intertrap spacing on pheromone trap catches of male European pine sawfly, *Neodiprion sertifer*. *Entomologia Experimentalis et Applicata*, **77**, 223–232.

Weidner, H. (1985) The saddle-gall midge *Haplodiplosis equestris* (Wagner, 1871), a barley and wheat pest in north-eastern Bavaria in the years 1692 and 1693 (Diptera, Cecidomyiidae). / Die Sattelmücke *Haplodiplosis equestris* (Wagner, 1871), ein Gersten- und Weizenschädling in Nordost-Bayern in den Jahren 1692 und 1693 (Diptera, Cecidomyiidae). *Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz*, **58**, 65–68.

Wilby, A. & Thomas, M.B. (2002) Natural enemy diversity and pest control: patterns of pest emergence with agricultural intensification. *Ecology Letters*, **5**, 353–360.

Wise, I.L. & Lamb, R.J. (2004) Diapause and emergence of *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) and its parasitoid *Macroglenes penetrans* (Hymenoptera: Pteromalidae). *The Canadian Entomologist*, **136**, 77–90.

Witzgall, P., Kirsch, P. & Cork, A. (2010) Sex pheromones and their impact on pest management. *Journal of Chemical Ecology*, **36**, 80–100.

Woodville, H.C. (1968) Saddle gall midge survey on barley 1967. *Plant Pathology*, **17**, 64–66.

Woodville, H.C. (1970) Results of a three year survey of saddle gall midge (*Haplodiplosis equestris* (Wagn.) on Spring Barley. *Plant Pathology*, **19**, 141–145.

Woodville, H.C. (1973) Observations on saddle gall midge (*Haplodiplosis equestris* (Wagn.)) in eastern England. *Plant Pathology*, **22**, 177–181.

Zadoks, J.C., Chang, T.T. & Konzak, C.F. (1974) A decimal code for the growth stages of cereals. *Weed Research*, **14**, 415–421.

Zhang, G.-F., Lü, Z.-C., Wan, F.-H. & Lövei, G.L. (2007) Real-time PCR quantification of *Bemisia tabaci* (Homoptera: Aleyrodidae) B-biotype remains in predator guts. *Molecular Ecology Notes*, **7**, 947–954.