“Joy in looking and understanding is nature’s most beautiful gift.”

Albert Einstein (1879–1955)

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Development of IPM tools for the management of *Oulema* beetles in winter wheat (*Triticum aestivum* L.)

Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD) of Applied Biological Sciences

Dutch translation of the title:

Ontwikkeling van IPM-tools voor een geïntegreerd management van het graan- en grashaantje (*Oulema* spp.) in wintertarwe (*Triticum aestivum* L.)

Cover illustration:

Cage trials during the spring of 2016. The photo of this trial was taken in Bottelare, Belgium. Ghent University – Photo by Hilde Christiaens

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List of abbreviations

BBCH	A growth stage scale, aimed to provide a uniform standard of the visible growth stages of plants, using a two-digit decimal code. Zadoks et al. (1974) described this scale for wheat plants. The abbreviation originally comes from the German Biologische Bundesansalt Bundessortenamt and Chemistry industry.
CLB	Cereal leaf beetle, a complex of species belonging to the genus *Oulema*
Cox1	Subunit 1 of cytochrome c oxidase
CV	Cross-validation
CYCL	Cross-year cross-location validation (Landschoot et al., 2012)
DDT	Dichlorodiphenyltrichloroethane, an organochlorine insecticide
DIMBOA	2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, a hydroxamic acid
DSS	Decision support system
EC	Electrical conductivity (Sm^{-1})
EPPO	European and Mediterranean Plant Protection Organisation. Published reports on several pests as on how to study the organism of the interaction with its host.
FAB	Functional agro-biodiversity
GDD	Growing degree days
GLV	Green leaf volatile
HDPE	High-density polyethylene
HIPV	Herbivore induced plant volatile
IPM	Integrated pest management
LCG	Landbouwcentrum granen
MAE	Mean absolute error
NPK	Nitrogen – phosphorus – potassium composed fertiliser
SNE	Semi natural element
UAN	Urea-ammonium nitrate
VOC	Volatile organic compound
WUE	Water-use efficiency

Chapter 1: Problem statement, objectives and research outline

General introduction

Pest insects have been a problem since the start of agriculture, around 10.000 years ago. The Neolithic revolution proved a huge jump forward in feeding billions of people. Ever since then, scientists and growers have been searching for ways to increase the yield per area unit. By selecting and breeding some species out of a wild, natural ecosystem, an artificial ecosystem, i.e. a natural ecosystem altered by men, was created: the agricultural ecosystem. The past has shown that this 'new' ecosystem often lacks heterogeneity, combined with a low biodiversity. To date, this system enables us to produce food for around seven billion people. Nonetheless, this system proves prone to numerous biotic stress inputs, mainly a variety of weeds, pests and diseases, accounting for yield losses up to 50% or even 82% in the absence of management practices (Oerke, 2006). The green revolution, post-World War II, enabled the grower to control many of these stresses by using external inputs such as pesticides and chemical fertilisers. Although these inputs were cheap and readily available and often considered as the '*silver bullet'* for pest control, many problems arose with using them on a large scale (Ameen and Raza, 2017). Repeated and excessive use during the last 70 years gave rise to resistance of target organisms, strongly reducing the efficiency of many pesticides (Hajek, 2004). While some gave problems to the applier due to a high acute toxicity (e.g. the carbamate aldicarb), others led to problems further on in the food chain, due to a high stability in the environment (e.g. DDT) (Lee and Barr, 1976). Both the term 'Integrated control', as well as the idea of economic thresholds were first introduced by Stern et al. (1959). In 1962, the public awareness towards an aversion to pesticide use was triggered by Carson with her book "Silent Spring" (Carson and Darling, 1962). Politics followed in 1987 with the commission Brundtland, publishing the rapport "Our common future", introducing the term '*sustainability*' for the first time. This led to the introduction of an integrated pest management (IPM) practice (Stern et al., 1959). An IPM-organised agricultural system integrates multiple pest management practices, making it more sustainable. Identification of pest, disease or weed is essential, implicating that the IPM practice is a knowledge-based management. IPM combines many management practices such as biological, cultural, mechanical, genetics such as breeding and chemical practices. Chemical pesticides are thus used as a final intervention only, when other methods fail in keeping the yield loss under an economical threshold. Unfortunately, despite more than 50 years of research, development and political support, IPM has not yet become the common practice in crop protection. Even today, pest management often relies on single tactics such as pesticide use. To change this, Europe introduced directive 2009/128/EC in 2014, obligating professional users of pesticides to implement the principles of IPM in order to limit the use and misuse of pesticides.

Cereal leaf beetles (CLBs; Chrysomelidae: Coleoptera) are recognised as pest insects in many countries of the world, resulting in yield losses up to 30% (Van Duyn et al., 1997; Ihrig et al., 2001; Pike et al., 2002; Kher et al., 2011). Although, in many countries, cereals encounter major yield losses due to this pest, knowledge about CLBs, e.g. their life cycle or distribution, is still fragmentary in Belgium. In Flanders, yield losses vary from year to year and are occasionally significantly noticeable (LCG, 2011). As the presence of CLBs and their larvae in cereals can result in significant yield losses in only a few days, they are often controlled in combination with an aphid control product. As both pest insects share a population of natural enemies, a wrong choice and timing of this treatment can result in an uncontrolled explosion of the aphid population due to the absence of natural enemies in the field, often ensuring the need for a second application.

Indeed, timing is crucial to control this occasional pest insect. Thresholds can help determining this timing and support the grower to manage this pest insect in the field. These thresholds vary depending on region and crop (Philips et al., 2011). Literature mentions thresholds varying from 0.4 larvae per tiller (Buntin et al., 2004) to 25 eggs or larvae per 100 tillers (Herbert and Van Duyn, 1999; Herbert and Van Duyn, 2009). Even within Western Europe, these thresholds vary from 0.5 larvae per tiller (Tschumi, 2015) to 2.5 larvae per tiller (Chambon et al., 1983). This variability makes timing an insecticide treatment in the field difficult. In addition, this pest insect lives in an ecosystem where natural enemies are present as well. The effect of these natural enemies is yet to be included in economic thresholds, as they will reduce the potential yield loss accompanied with any number of adults or eggs found on the plants. In conclusion, more knowledge is needed to manage this pest, using an IPM-guided approach.

The aim of this PhD study is to drive growers to an IPM-oriented management for CLBs in the region of Flanders, Belgium. To achieve this goal, we try to obtain an answer to several research questions concerning the management of CLBs, leading up to a combination of practices that can help to decrease the potential economic loss caused by CLBs. Crop husbandry, management of surroundings and weather variables are potential parameters steering the phenology of CLBs. Crop husbandry practices such as choice of variety, sowing date and density, fertiliser applications, dose and timing of pesticide applications and many more can have a significant influence on the presence of CLBs in the field. Surroundings influence not only the presence (survival) of natural enemies, but will have an impact on the mortality rates of CLBs as well and therefore impact the presence in the field during following years. Prediction models that simulate pest densities within the field will help translating our knowledge in a concrete, readily available tool for the grower to use. A combination of these tools will help growers to achieve a more sustainable pest management in their wheat fields. However, several questions need to be addressed first.

Hypotheses and research questions

In the course of this PhD thesis, the following **hypotheses (1, 2 and 3)** and **research questions (RS 1.1 and 1.2; 2.1, 2.2, 2.3 and 2.4; 3.1 and 3.2)** will be tested and answered:

1. The CLB population in Flemish wheat fields is dominated by two species from the genus *Oulema* (*O. melanopus* and *O. obscura*), as in other Western European countries.

   RS 1.1 Which species of the genus *Oulema* occur in Flanders?

   RS 1.2 What is their distribution throughout Flanders, Belgium?

2. The larvae of CLBs significantly reduce yield in wheat in Flanders.

   RS 2.1 Which parameters influence the phenology and distribution of CLBs in and around the field?

   RS 2.2 Which preventive or curative management practices can be used to control CLBs?

   RS 2.3 What is the population density of CLB larvae in Flemish winter wheat?

   RS 2.4 How many CLB larvae can be tolerated compared to wheat prices (i.e. what are correct economic threshold levels in Flanders, Belgium)?

3. A decision support system (DSS) is an effective tool for the wheat grower to control CLBs.

   RS 3.1 Can insecticide treatments be integrated in a CLB management?

   RS 3.2 Which model is suitable to predict the presence and population density of the CLBs in winter wheat fields?

Thesis outline

A literature overview introduces the topic of this research. In **Chapter 2**, a run-through the current knowledge concerning the CLB problem is presented. **Chapter 3** contains the experimental framework in which several trials were executed. Every subsequent chapter in this thesis will review different parts of the CLB problem in Flanders, leading up to **Chapter 7**, in which the potential of some IPM tools for the management of CLBs is introduced.

Firstly, the distribution of CLBs was examined throughout Flanders. Within the genus *Oulema*, six species are described on the species list in Belgium (Belgium Species List, 2018) and although it is commonly accepted that only a few species are responsible for the loss of yield in cereals, actual research determining species in the North-Western region of Europe is rare. Furthermore, research has shown that there is still a lot of confusion in determining CLB species, which often results in misclassification of beetles. To eliminate any remaining doubts concerning the species distribution and the influence on yield, the species distribution in Flanders was investigated. With this intention, CLBs were collected at approximately 30 fields each season, spread over the wheat-growing regions in Flanders. **Chapter 4** gives an overview of the within-field and between-fields distribution of the occurring CLB species in Flanders. Besides the species-distribution, this chapter also analyses the contribution of the different species to the loss of yield in winter wheat fields.

In relation to the ecology of CLBs, questions still remain unanswered. Thus, secondly, an extensive field monitoring trial was executed. During four growth seasons, each year, 30 commercial wheat fields were selected and monitored on a weekly basis during spring and summer. Monitoring from BBCH 32 (second node) until BBCH 90 (the end of ripening) enabled us to examine the effect of agronomical practices and influencing environmental parameters such as temperature, humidity and precipitation on CLB development. Based on the results of **Chapter 4, 5 and 7**, an IPM-oriented approach for the management of CLBs was constructed in **Chapter 8.**

Thirdly, **Chapter 6** includes a review of current prediction models, as well as an attempt to create a holistic model for predicting CLB presence in the field, specified for Flemish wheat fields. This predictive model relies on a combination of parameters, which are mainly environmental and agronomical parameters. As a result, this model aims to predict the following variables:

- First appearance of CLB eggs and larvae. This can guide the grower to monitor his field properly;
- Peak date of CLB eggs and larvae. This will help the grower estimating damage and making proper decisions concerning curative treatment.

We started with building a growing degree day (GDD) model, as this model type is often used for predicting insect presence in the field and timing of insecticide applications. As these types of models do not always have big predictive value, using machine learning techniques, other models were constructed as well.

Fourthly, we combined data of the monitoring, insecticide, manual defoliation and cage trials. These trials contribute to determine economic thresholds on a semi-field/greenhouse experimental scale and are presented in **Chapter 7**. To start, we examined the effect of several larval densities on the yield of winter wheat. In these cage trials, CLBs were introduced in field planted cages at various densities, in order to mimic field circumstances. Secondly, in the insecticide trial, several insecticides were tested on a field scale for the effect on CLBs. Flag leaf damage was monitored as well. Finally, manual defoliation trials were executed on a field and greenhouse level. These help to support the underlying correlation, gathered from the cage trials. Here, leaves were cut manually, in an attempt to mimic leaf damage due to CLB feeding as close as possible. Results coming out of these monitoring trials can be used to determine solid economic thresholds on the one hand and to support growers to take the right control decisions at the optimal time in order to control the pest properly and to minimise economic losses on the other hand.

Finally, in **Chapter 8** we conclude with a discussion on the results gathered during a period of four years, as well as perspectives on possibilities for future research.

Chapter 2: Literature overview

Several organisms have a decreasing effect on the productivity (yield and quality) of wheat. Oerke (2006) stated that in Western Europe the average yield loss due to pest insects is eight percent (actual losses), even after using crop protection products. As potential losses (without the use of management practices) are estimated at nine percent, it is clear that current management practices lack efficiency (Oerke, 2006). Papers concerning CLBs, conclude that this pest can cause even more damage: yield loss of infested fields reach 10 to 20%, regionally up to 30% without crop protection products (Van Duyn et al*,* 1997; Ihrig et al., 2001; Pike et al., 2002; Kher et al*.,* 2011).

Taxonomy

Several beetles are named as CLBs. In Western Europe, apart from the most common *Oulema melanopus* (Linnaeus) and *O. obscura* (Heyden), *O. duftschmidi* (Redtenbacher), *O. rufocyanea* (Suffrian), *O. erichsoni* (Suffrian) and *O. septentrionis* (Weise) occur. Figure 1 gives an overview of the beetles within the genus *Oulema* that are described as pest insects in Belgium. CLBs are often described as part of a 'CLB-complex', which belongs to the family of the Chrysomelidae. This complex, characterised by their red pronotum ('*graanhaantjes',* referred to in this work as complex 1), often described in literature as the CLB-complex (*O. melanopus; O. duftschmidi; O. rufocyanea*), clearly differs in morphology compared to the other complex within the genus of *Oulema*. This second complex is characterised by a blue pronotum. Species belonging to the latter complex are *Oulema erichsonii, O. septentrionis,* and *O. obscura*. Figure 1 lists these two complexes: the upper part represents a complex of three beetles ('*grashaantjes*', referred to in this work as complex 2), while the bottom part represents the complex 1 species. Literature concerning complex 2 is limited as these species are not common in the USA, where the majority of the research concerning the *Oulema* genus has been done.

While observations have shown that species from complex 1 are most common in Belgium and other parts of Europe, some species from complex 2 are observed in Belgian wheat fields as well (Stilmant, 1995). The distribution of the following species covers Europe: *Oulema erichsonii, O. septentrionis,* and *O. obscura*. Especially *O. obscura* is believed to have an active contribution to yield losses in Northwestern European winter wheat fields (Walczak, 2005).

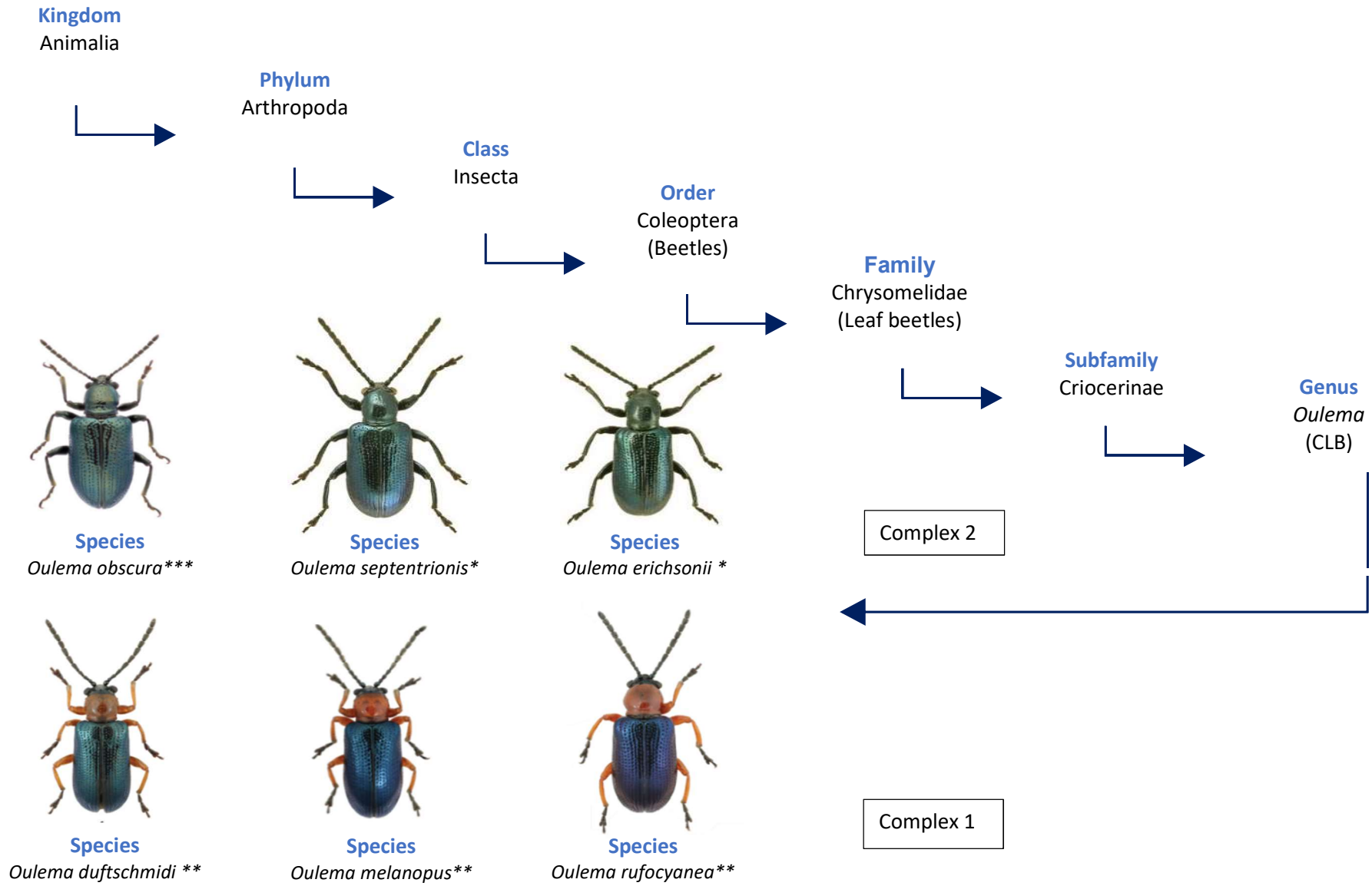


*Figure 1: Classification tree of CLBs listed on the official Belgian species list; * (Borowiec, 2007); ** (Bezděk and Baselga, 2015); *** (Noordijk et al., 2016). Based on Meutermans et al., 2018.*

Oulema species classification

Within each complex, morphology often differs very little, making it hard to classify up to species level without dissection. Leaf beetles or Chrysomelids are classified as such by a particular structure of the tarsi, antennae, their short and round body shapes and their phytophagous habits. Although many of these beetles prefer wild plant species, many leaf beetles are known pest insects of agricultural and forest crops (Jolivet et al., 1988). Examples of these pest insects are: the Colorado potato beetle (*Leptinotarsa decemlineata* (Say); Alyokhin, 2009), the CLB (genus *Oulema*) and the corn rootworm (*Diabrotica virgifera* (LeConte)*;* Schellenberger et al., 2016). Even though some species can cause serious crop damage, others can be beneficial, being herbivorous and host specific, some of these leaf beetles are also used for biological control of noxious weeds (Jolivet et al., 1988). *Lema cyanella* (Linnaeus) is an example of such a leaf beetle and is often used for the control of plume thistles (*Cirsium* spp.) in Canada (Peschken and Johnson, 1979; Peschken 1984). CLBs are often described as a complex due to the fact that determination up to species level is difficult and time-consuming. The genus *Oulema* consists of seventeen different species (NCBI, 2017). However, the Belgian official species list only mentions six species: *O. duftschmidi, O. melanopus, O. rufocyanea, O. erichsonii, O. septentrionis* and *O. obscura* (Schmitt and Rönn, 2011; Belgian Species List, 2018). Next to these species, the following species are described in Europe: *O. hoffmannseggii* (Lacordaire)*, O. magistrettiorum* (Ruffo) and *O. tristis* (Herbst) (Fauna Europaeae, 2017; PESI, 2017). It is known that the spatial distribution of these species is not homogeneous and only a few of them can cause economic losses. Possible explanations for this heterogeneous distribution can be found in differential influence of climate on the development of these insect species. For example, Lesage et al. (2007) mentioned *O. duftschmidi* to be distributed more southern, in a more Mediterranean climate.

Like many other organisms, classifications led to a series of discussions between scientists in the past. An example for this is the genus of CLBs. Some researchers suggest that a specialised adaptation of *Lema* spp. on cultivated grasses such as wheat, led to a separation into a new genus *Oulema* (Schmitt, 1988). Small morphological differences confirmed the need for more accurate classification methods to correctly identify the genus of a beetle. Therefore, Ninan et al. (1968) studied the genetic background of *O. melanopus*. They concluded that the genome of *Lema* spp. evolved from a $9XY_p$ karyotype to a smaller genome due to failed meisosis or fusions. As *Lema* spp. have more genetic material than *Oulema* spp., this study showed clear differences in chromosome number and genetic foundation between both genera. *Oulema* spp. are characterised by a $7Xy_p$ karyotype, presented in Figure 2. This corresponds to 7 pairs of autosomes with Xy_p representing the heteromorphic chromosomal pair with a small male chromosome (y) and a big female chromosome (Y). The small '*p*' refers to the shape of both sex chromosomes, i.e. the typical parachute shape (Kher, 2014).

Nonetheless, other researchers (Wellso and Hoxie, 1988; Kher, 2014) state that there is not enough evidence to support this claim and suggested to separate species based on host plants, as *Lema* spp. survive on a wider range of plant species, i.e. species that have broad leaves such as S*olanacea*, *Cirsium* and *Basellacea* spp., while *Oulema* spp. survive on *Poacea* spp. only (Wellso and Hoxie, 1988).

Figure 2: 7Xy$_p$ karyotype of Oulema melanopus during the mitosis (Ninan et al., 1968).

Classification within both complexes is based on differences in morphology (length or dissections of the genitalia) or DNA (*Cox1* gene). Although sequence differences within the *Cox1* gene are often used to classify insects (Liu and Beckenbach, 1992; Lunt et al., 1996; Hybert et al., 2003), research (Bertini et al., 2006; Bezděk and Baselga, 2015) has demonstrated that identifying CLB species using the *Cox1* is not adequate. *Cox1* cytochrome (cycochrome c oxidase subunit 1) is a mitochondrially encoded protein. Since *Cox1* is present in almost every living organism, studying sequence variation is especially useful. Closely related species differ in just a few nucleotides in the sequence of the gene. Nevertheless, for classifying CLB species this technique shows little success, as the haplotype within complex 1 showed 90.5–99.5% similarities, while similarities with complex 2 species ranged from 91.6–100%, making classification based on this gene difficult (Bezděk and Baselga, 2015).

Studying external morphology and measuring lengths of different parts of the insect body is an approved protocol to classify species as well. Within each complex, these species have a very similar external morphology (Figure 4): the black coxa, trochanter and tarsus are even the foundation of the species name. The species name '*melanopus*' is indeed a contraction of the Greek words '*melas*' and '*pus*', which mean black and paw, respectively. Other morphological similarities are the reddish femur, tibia and pronotum as well as the dark, metallic blue cephalon, thorax, scutellum and elytra. Bezděk and Baselga (2015) investigated the possibilities to classify CLBs, studying differences in these externally measured body parts (Figure 3; Figure 4). They noticed differences in (a) body length (BL), varying from 3.7 to 6.2 mm; (b) width of the elytra (EW), varying from 1.4 to 2.2 mm and (c) length of the antennae (AL), varying from 1.8 to 3.1 mm. They noticed that ratios can be used to classify some species: the ratio of the length to the width of the elytra led to a first indication of species: *O. duftschmidi* and *O. melanopus* show a greater ratio than *O. rufocyanea* and *O. mauroi*. Ratios vary respectively from 1.87 to 2.05 for the first and 1.69 to 1.87 for the latter species.

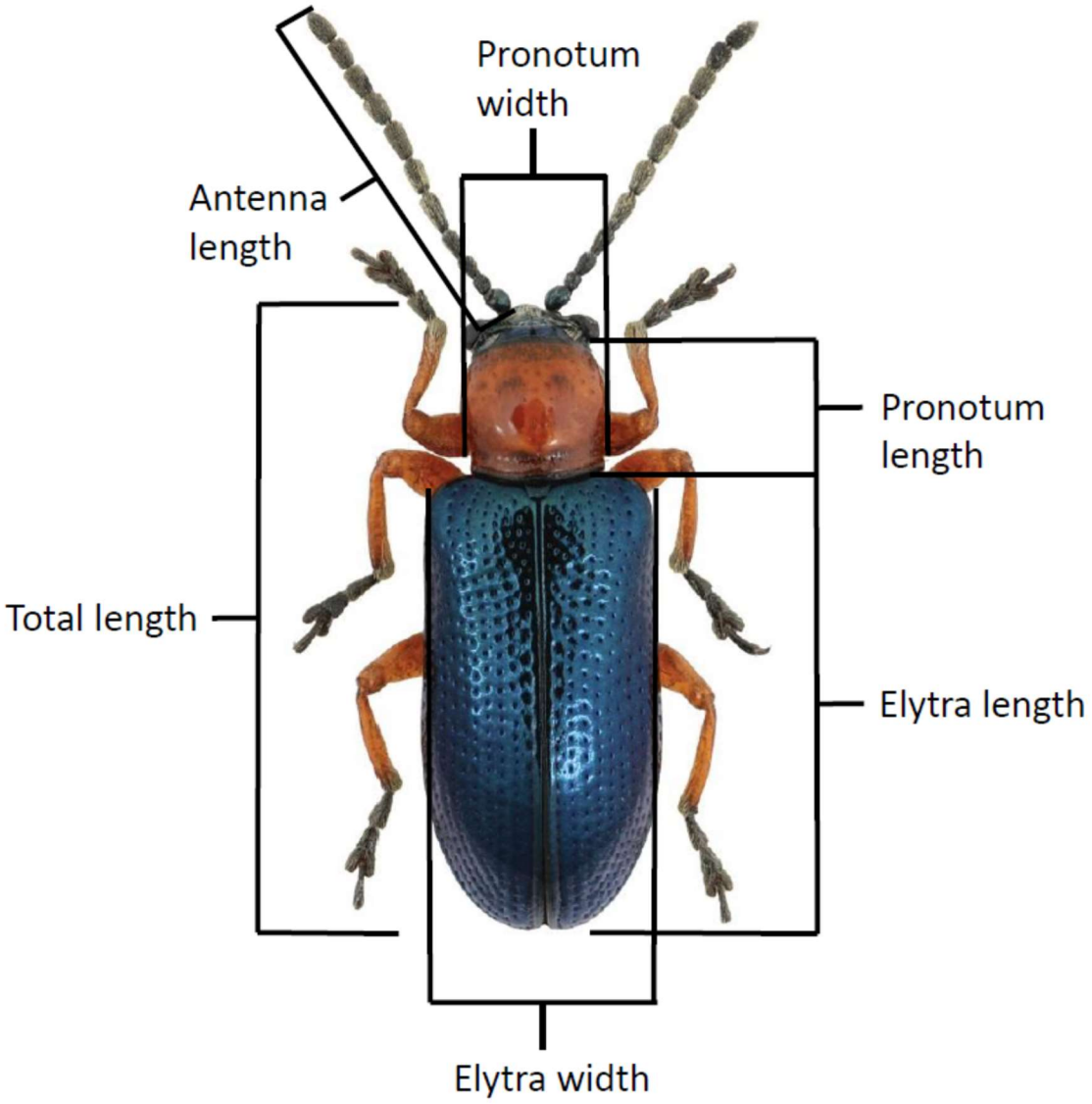


Figure 3: Body measurements of O. melanopus (Bezděk and Baselga, 2015).

	O. duftschmidi	*O. melanopus*	*O. rufocyanea*	*O. mauroi*	*O. verae*
					
BL	4.2–5.7 mm	4.5–6.2 mm	4.0–4.6 mm	3.7–4.6 mm	3.8–4.3 mm
EL	3.2–3.9 mm	3.2–4.1 mm	2.8–3.1 mm	2.6–3.1 mm	2.6–3.0 mm
EW	1.6–2.1 mm	1.7–2.2 mm	2.0–2.4 mm	1.5–1.7 mm	1.4–1.6 mm

*Figure 4: Species from complex 1 with their body measurements (BL= body length; EL= elytra length; EW= elytra width) (*Bezděk and Baselga, 2015).

Recalling the overlapping ratios of the externally measured body parts of the different species, other differences in morphology are more frequently used to classify species within both complexes of the genus *Oulema*. More accurate to classify CLBs is analysing the genitals of the male and female beetles. An accurate classification up to species level starts by determining the beetle's gender. Sexing CLBs is not an easy task and a (destructive) dissection is required (Myser and Schultz, 1967). Figure 5 gives an overview of the spermatheca and ductus spermathecae of *O. melanopus, O. rufocyanea, O. mauroi* and *O. verae* (female genitalia).

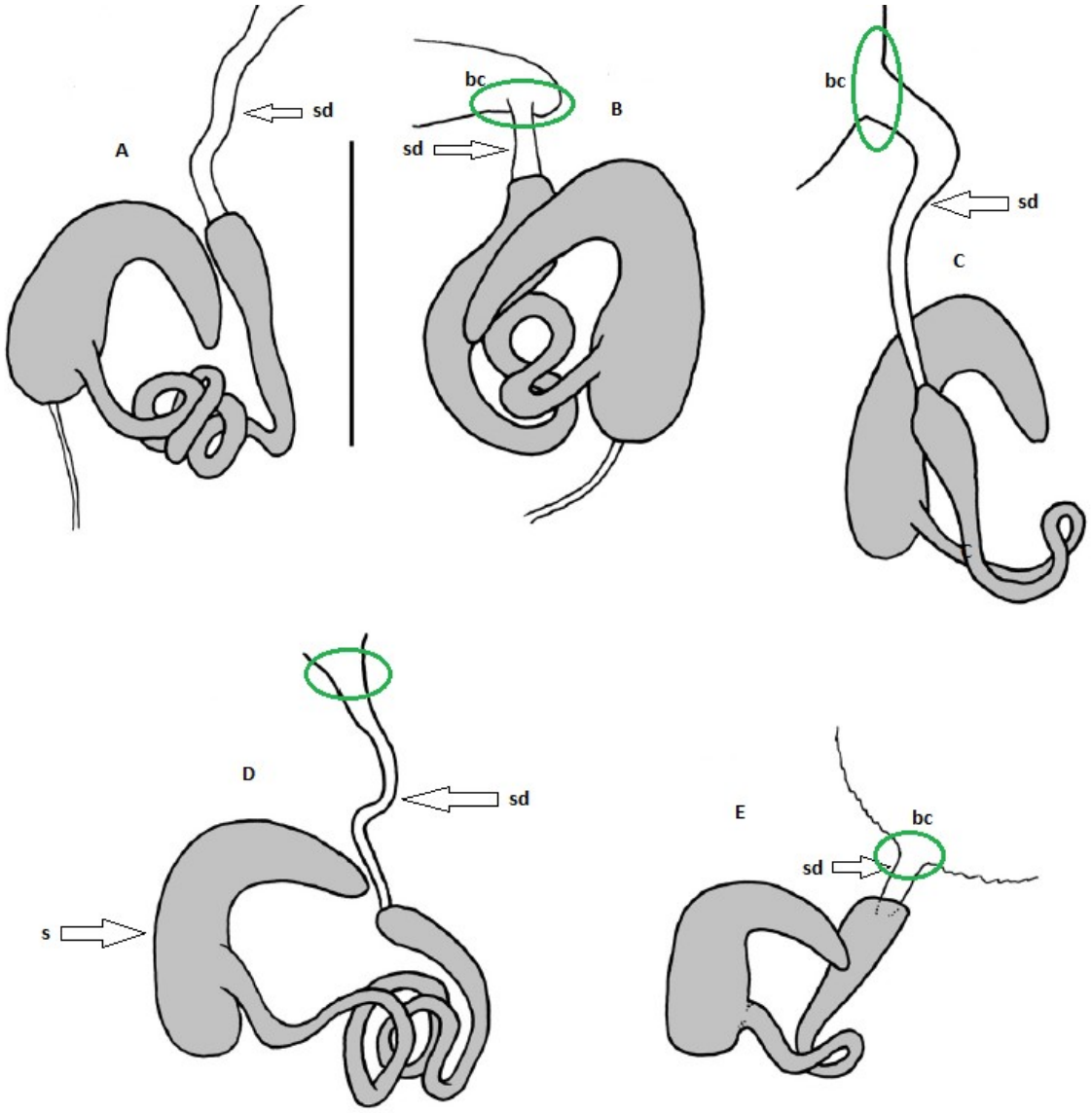


Figure 5: Spermatheca and ductus spermathecae: A - Oulema duftschmidi; B - O. melanopus; C - O. rufocyanea; D - O. mauroi; E - O. verae. Abbreviations: bc = bursa copulatrix; s = spermatheca; sd = spermathecal duct. The terminal portion of the bursa copulatrix is encircled in green (Bezděk and Baselga, 2015).

Generally, females of complex 1 are classified by comparing the length of the spermathecal duct as well as the width of the terminal portion of the bursa copulatrix between the several species. Categorising the males within this complex up to species level is done by measuring the elytra and antennae, but more importantly, by dissecting the male genitalia as there are clear differences in the shape of the flagellum of the aedeagus (Figure 6). To separate *O. rufocyanea* from the other species, measuring the elytra and antennae is useful (Bezděk and Baselga, 2015).

Figure 6: Lateral (top) and dorsal (bottom) view of the flagellum of O. duftschmidi (left), O. melanopus (middle) and O. rufocyanea (right) (Bezděk and Baselga, 2015).

Overlapping external body measurements, as well as lacking differences in the genome, led to incorrect classifications. Especially *O. melanopus* and *O. duftschmidi* are often confused (Berti, 1989). Overlapping geographical spread as well as a shared within-field distribution are both a reason for these incorrect classifications (Bechini et al., 2013).

Although body measurements are not useful for classifying species belonging to complex 1 due to too much overlap, this technique is still used to distinguish complex 2 species. More specifically, *O. erichsoni*, *O. obscura* and *O. septentrionis* are classified based on morphological ratios such as elytra length (EL) to elytra width (EW). *Oulema obscura* can easily be separated from other species: its pronotum is relatively small (3.0–4.2 mm), while the other two species have a longer pronotum of 4.0–4.5 mm. The elytra of *O. obscura* are also relatively short (EL = 1.25 × EW), while the length of the elytra of the other two species measures 1.3–1.45 times the width (Hubble, 2012). According to Allen (1976) and Cox (2000), the other species within complex 2 can be differentiated based on: (1) differences in the angle at the widest point of the pronotum; (2) presence or absence of a metallic reflection in the front thoracic segment and (3) differences in the lamella of the aedeagus.

Nonetheless, some confusion within the classification of species belonging to this complex continues to exist. Indeed, species within this complex are often confused with *Lema cyanella*, a beetle that can occur in wheat fields, but feeds on thistle species instead of on *Poaceae* species, thus not being of economic importance. Moreover, this leaf beetle has been used as biocontrol organism of Canada thistle (*Cirsium arvense* (L.) Scop.) (Peschken, 1984).

Distribution in the Northern Hemisphere

Relative abundance of species within both complexes differs between years and regions. Some species from complex 1 (especially *O. melanopus*) are described all over the world as a major pest insect in cultivated *Poaceae* species, while others are rarer and are only occasionally found to be of economic importance. Originating from Eurasia, *O. melanopus* migrated over the years to several parts of the world. For example, this species was first described in 1962 in Michigan, causing major damage to oats (Haynes and Gage, 1981). This species is probably the most widespread species within complex 1, causing damage to wheat crops in the USA and Europe (Schmitt and Rönn, 2011). It is believed that an increase in monoculture cropping systems gave rise to its widespread distribution (Wenda-Piesik and Piesik, 1998; Ulrich et al., 2004). In addition, transportation of wheat straw with Christmas trees is often pointed to as another reason for its global spread (LeSage et al., 2007). The distribution of CLBs is dependent on weather conditions as they influence development, fertility and mortality. Generally, CLBs are found in almost every country with a humid and subhumid climate, although they are considered to cause most damage in countries with a continental climate (Tanasković et al., 2012). These climates are characterised by cold winters and warm, dry summers (Balachowsky and Mesnil, 1936). Regions subjected to such a climate often experience high annual temperature variability (warm summers and cold winters). Especially the Balkan region seems to have an excellent climate for the distribution and spread of CLBs (Kostov, 2001). Wheat fields in countries as Switzerland and Italy are often infested with CLBs due to a lower mortality and higher oviposition rate (Bezděk, 2001).

The economic importance of some other species within each complex is unknown due to misidentifications in the past. *Oulema duftschmidi* is believed to occur evenly as *O. melanopus* (Schmitt and Rönn, 2011), though the economic importance is presumed to be unequal (Beenen and Winkelman, 1992; Chrobok and Borowiec, 1993; Bezděk, 2001). The third species within complex 1, *O. rufocyanea*, is believed to be rare and only to appear in Central and Southern Europe. Recently, Bezděk and Baselga (2015) described two new species belonging to this species complex, which are morphologically very close to *O. rufocyanea*: *O. verea* and *O. mauroi*, respectively. Based on differences in the length of the elytra and antennae, *O. verae* was distinguished from CLBs that were

originally described as *O. rufocyanea* (Baselga and Novoa, 2006). Though, to date, these two species are only described in the southern parts of Europe (resp. Spain and Italy).

Generally, *Oulema* spp. are recognised as pest insects in many countries (Canada (Kher et al., 2011); Balkan region (Kostov, 2001); most regions of the U.S.A (Ihrig et al., 2001; Buntin et al., 2004); Hungary (Kadocsa, 1916; Papp and Masterhazy, 1996; Pozgai and Saringer, 2006); Poland (Miczulski, 1973; Ulrich et al., 2004); Moldova (Livia, 2006); Russia (Sphanev and Golubev, 2008); Slovakia (Jeloková and Gallo, 2008); Bulgaria (Kostov, 2001); Pakistan (Khan et al., 2008); Iran (Nikbakhtzedh and Tirgari, 2002); Austria (Fuss et al., 2005); Serbia (Dimitrijevic et al., 2001); Yugoslavia (Hadzistevic, 1975); Romania (Popov et al., 2005); Kazakhstan (Bedin, 1971); Greece (Pelecassis, 1951); Spain (Olfert et al., 2004); The Netherlands (Daamen and Stol, 1993); Belgium (Stilmant, 1995); France (Chambon et al., 1983); Germany (Schmitt, 1988); Great Britain (Hodson, 1929, Allen, 1976); Switzerland (Tschumi, 2015) and Italy (Morlacchi et al., 2007)). However, crop losses and accompanying pest status depend on factors such as weather-related mortality and mortality caused by parasitoids (Buntin et al., 2004). Stilmant (1995) concludes that CLBs obtain a pest status in those areas where a short spring is followed by dry summers. Despite the variety of CLBs found and determined in Europe, Jossi and Bigler (1996) stated that in Switzerland, more than 90% of the population consists of the *O. melanopus*. Other studies confirmed this trend as well for Northern Europe (LeSage et al., 2007). Figure 7 shows the distribution of CLBs in the world. Countries in which CLBs have been described as pest insects are coloured in red.

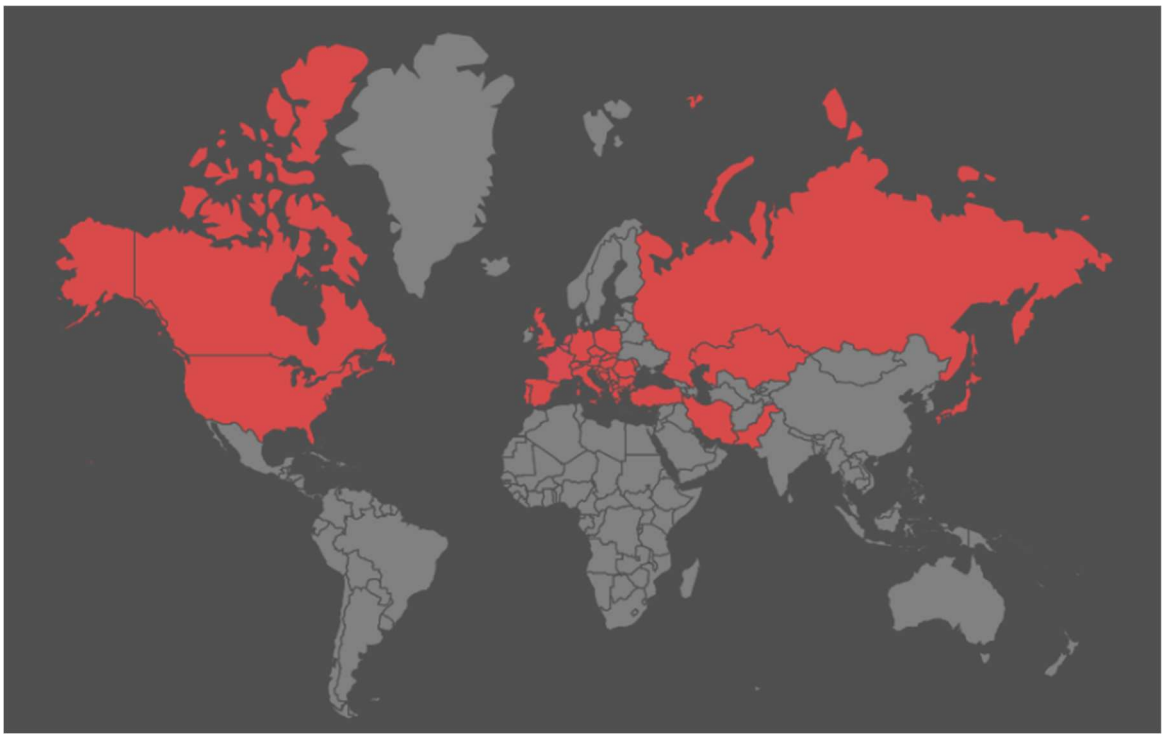


Figure 7: Countries in which CLBs have been described as pest insects are coloured in red. By: amCharts.

Ecology

The ecology of *O. melanopus* has been studied and reported extensively (Gallun et al., 1967; Yun, 1967; Helgesen, 1969; Shade et al., 1970; Gage, 1972; Helgesen and Haynes, 1972; Ruesink, 1972; Ruppel, 1972; Wellso et al., 1972; Haynes, 1973; Casagrande et al., 1977; Lecigne and Roehrich, 1977; Logan, 1980; Haynes and Gage, 1981; Battenfield et al., 1982; Hatchett et al., 1987; Grant and Patrick, 1993; Buntin et al., 2004). Although most of the phenology of the different *Oulema* spp. is comparable, some small differences exist. For example, the place of pupation varies between complex 1 (pupates under the ground) and complex 2 (pupates on the plant) species. In addition, the influence of temperature and other environmental variables differs between species.

Life cycle

Oulema species have one generation per year (univoltine) (Wellso et al., 1973; Casagrande et al., 1977), granting a short second generation of *O. melanopus* that was monitored in spring cereals in the past (McPherson, 1983). Being a poikilothermic organism, the life cycle of CLBs is strongly controlled by temperature (Fulton and Haynes, 1975; Evans et al., 2006; Philips et al., 2012; Evans et al., 2014). Figure 8 presents the phenology of *O. melanopus* adults, eggs, larvae, pupae and post-diapause adults with their respective host plants at each moment.

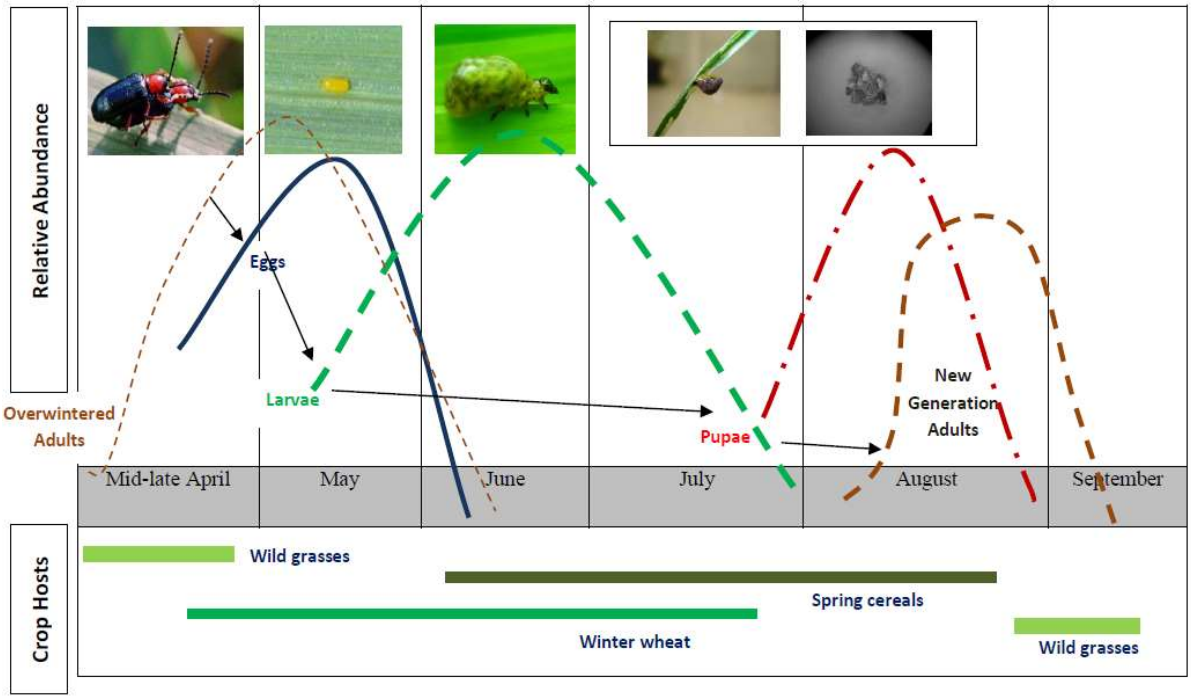


Figure 8: Phenology of O. melanopus: adults, eggs, larvae, pupae and post-diapause adults and their host plants at each moment during their life cycle (Kher, 2014).

Oulema species overwinter as an adult in habitats that protect the insect from temperature extremes. Forest litter, fence rows, dense woods, croplands (grass crowns, grain stubble), sparse woods (Castro et al., 1965; Yun, 1967; Casagrande et al., 1977) have been shown as favourable habitats. Mortality of *O. melanopus* seems lowest at the edge of woodlots (40%) and highest in croplands (99%; Casagrande et al., 1977). Within these sites, most CLBs were found in crop residues, cracks of bark and rolled leaves, which could suggest these as favoured overwintering place for the beetles (Piesik and Piesik, 1998; Ulrich et al., 2004). Even though mortality is high due to the absence of crevices in grain stubble, it remains an overwintering site in high density CLB areas (Casagrande et al., 1977).

In spring, when temperatures rise above 10–15 °C, CLBs become active again and start looking for a suitable host plant (Chambon et al., 1983). In literature, disagreement exists over the spring temperature at which the CLBs become active. While some authors (Tanasković et al., 2012) note 10 °C, others mention 14.4 °C (Guitierrez et al., 1974), or even 15 °C as threshold (Piesik and Piesik, 1998).

Before starting oviposition, female *Oulema* beetles test their host plant by a short feeding period. Host selection is determined by a couple of factors such as plant volatiles, plant architecture, morphology and anatomy (Rausher, 1981; Renwick, 1989; Coley and Kursar, 1996). When a suitable host crop is found, the CLBs mate and lay eggs.

Oviposition starts seven to ten days post-diapause and continues for six to eight weeks. Eggs are 1 mm long, orange coloured and cylindrical. Eggs are laid singly or in clusters of two or three, usually on the adaxial leaf surface (Wilson and Shade, 1964; Helgesen and Haynes, 1972; Piesik and Piesik, 1998; Hoffman and Rao, 2011). Eggs are generally laid on the highest placed leaf of the plant at moment of oviposition (Wellso and Cress, 1973). Aging eggs colourise brown and, depending on temperature, hatch in four to six days (Barton and Stehr, 1970). During her entire life, each female *O. melanopus* beetle lays about 40 eggs (Hodson, 1929), although some authors monitored 100–150 (Venturi, 1942), 275 (Schmitt, 1988) or even 400 eggs per female (Ruppel, 1964, 1972). The number of eggs laid per female varies depending on nutrition and temperature (Schmitt, 1988).

After hatching, *Oulema* beetles go through four larval stages, whereas temperatures for development range from 8 to 32 °C. At optimal temperatures for survival (for *O. melanopus*: < 30% mortality at 22–32 °C; Guppy and Harcourt, 1978; Herbert et al., 2007), this stage takes ten to fourteen days to complete. Mortality of *O. melanopus* larvae increases strongly from 34 °C onwards (Guppy and Harcourt, 1978). The larvae feed on the upper epidermis and parenchyma down to the lower epidermis between the veins, causing elongated windowpanes in the leaves (Gallun et al., 1967; Grant and Patrick, 1993; Buntin et al., 2004). Larvae have a greyish yellow corpus and cover themselves with a

black globule of mucus and faecal matters (Piesik and Piesik, 1998; Philips, 2013). Next to a difference in oviposition rate between host crops, Helsegen and Haynes (1972) also discovered a difference in larval mortality based on host crop. Variations in phenology and morphology of the wheat plants could contribute to a 10% higher mortality in crops compared to oats.

Before pupation, the larvae of the fourth stage enter the prepupal stage. Leading up to this stage, the larvae drop the faecal coat and start secreting an adhesive material to form a cocoon (Wellso et al., 1973). The prepupae of complex 1 species then drop on the ground at the base of the plant and form the exarate, yellow coloured pupae under the ground at a depth of approximately 5 cm (Dysart et al., 1973; Philips et al., 2011). Prepupae of CLBs from complex 2 (e.g. *O. obscura*) migrate to the leaf sheath or tiller and pupate on the plant. Post-diapause adults emerge three weeks later and start feeding on various grasses before looking for a suitable overwintering habitat (Grant and Patrick, 1993).

Diapause

CLBs have an '*obligate*' diapause, which is described as being "*the most highly evolved system of dormancy for surviving cyclic, long term and extreme environmental conditions*" (Jolivet et al., 1988). Apart from this diapause, *Oulema* species use an aestivation to help survive dry, warm summers (Jolivet et al., 1988). Diapause is initiated by photoperiod and temperature. The corpora allata is responsible for the initiation and duration of the diapause. A decrease of daylength or average temperature initiates a pre-diapausal phase, prior to the diapause itself. During this pre-diapausal phase, CLBs reduce feeding, become photonegative and start the search for a dark, covered overwintering site. Pre- or nondiapausal male beetles are unable to fertilise females (Jolivet et al., 1988). During diapause, CLBs rely on a reserve of triglycerides that was built up during the pre-diapausal phase (Watanabe and Tanaka, 2000). During this phase, spermatogenesis is initiated, causing the male beetles' testes to reach their maximum size. Female beetles' ovarioles only start developing after diapause (Jolivet et al., 1988).

Abiotic conditions (temperature, humidity, wind)

Being poikilothermic organisms, research has already illustrated that the life cycle of CLBs is largely driven by temperature, in contrast to day length or other cues (Figure 9; Fulton and Haynes, 1975; Evans et al., 2006; Philips et al., 2012). Temperature steers CLB development, but also causes the biological need for a diapause or aestivation.

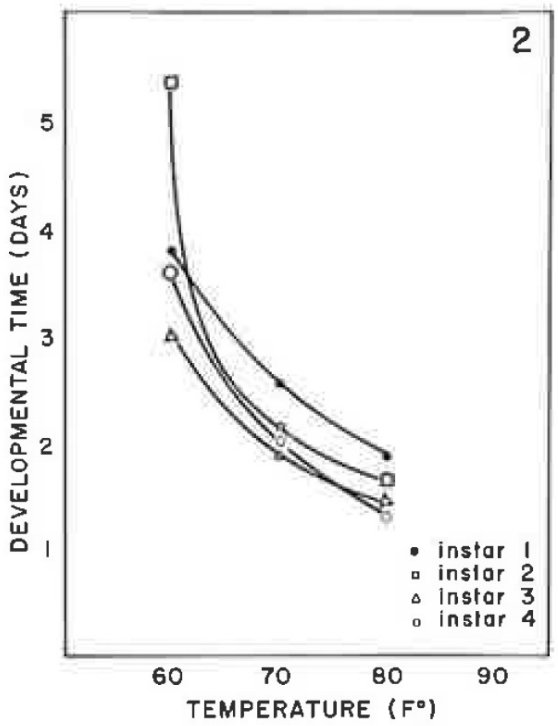


Figure 9: Interaction between temperature and developmental time of the larvae of O. melanopus (Helgesen and Haynes, 1972).

Many organisms have a lower and upper temperature threshold under and above which the development stops (Figure 9). For *O. melanopus*, the interaction of development and temperature was studied by Guppy and Harcourt in 1978. In agreement with Yun's (1967) work, they found an upper thermal limit between 32 and 34 °C. In contrast, discussion remains about the lower temperature threshold for CLB development. Guppy and Harcourt (1978) found that 7 °C was the minimum development temperature, lying closely to developmental zero. Based on laboratorial trials, Yun (1967) used a higher threshold of 8.9 °C, while others rounded this to 9 °C (Barr et al., 1973; Tummala et al., 1975). Comparing lower thresholds in a trial did not result in significant differences in mortality (Guppy and Harcourt, 1978).

Between the lower and upper threshold, developmental rate and temperature are positively correlated. Table 1 shows the result of the work by Guppy and Harcourt in 1978. In this table, the mean duration (days) of every life stage of *O. melanopus* is presented for every tested temperature. Percentage mortality is given between parentheses. It is clear that the higher the temperature, until a certain upper threshold, the shorter the total duration of the egg, larval and pupal stage, maintaining a low mortality up to 30 °C. It is also clear that the pupal stage is the most critical, leading to a mortality from 60% (18–22 °C) to even 97% (32 °C).

Table 1: Mean duration in days of the immature stages of O. melanopus at various temperatures. Percentage mortality for each stage is given in parentheses (Guppy and Harcourt, 1978)

	Temperature (°C)										
Stage	**8**	**10**	**12**	**14**	**18**	**22**	**25**	**28**	**30**	**32**	**34**
Egg	41.3 (64)	38.5 (71)	25.3 (23)	17.9 (24)	10.1 (37)	6.1 (28)	4.5 (17)	3.8 (11)	3.7 (8)	3.9 (22)	- (100)
Larvae	47.4 (41)	46.2 (42)	32.5 (17)	25.2 (28)	15.7 (20)	10.8 (17)	8.5 (20)	7.0 (27)	6.4 (60)	6.6 (43)	- (100)
Pupae	61.3 (93)	- (100)	61.9 (90)	45.5 (63)	27.1 (60)	17.1 (60)	13.2 (67)	11.3 (63)	10.2 (73)	10.0 (97)	- (100)

These results were confirmed by the work of Ali et al. (1979). For other species within complex 1, these data can differ, mostly based on what region they appear in, e.g. *O. duftschmidi* shows a higher lower threshold for development: 11.2 °C (Severini et al., 2003), as they are more commonly found in warmer regions in Europe.

In contrast to spring, negative correlations with temperature exist during winter, when adult CLBs are in diapause. Temperature fluctuations, cold periods during winter followed by warmer periods can accelerate the termination of the diapause resulting in an earlier CLB development. Furthermore, negative correlations between winter temperatures and CLBs have been found in literature. In the Netherlands, Daamen and Stol (1993) found that the phenology and crop injury due to CLBs to be negatively correlated with mean temperatures during the winter months December, January and February. Correspondingly, Ali et al. (1979) proved that mortality of overwintering CLBs severely increases above 10 °C.

Comparing complex 1 species to complex 2 species, differences in thermal influence on the phenology can be found. In 2005, Walczak studied the differences between *O. melanopus* and *O. obscura* and noticed that while in the lab differences between both species appeared to be small, in the field these differences enlarged (Figure 10). This study showed that under natural field conditions, *O. melanopus* beetles develop slower than *O. obscura* beetles. Walczak (2005) suggested that this could be explained by the fact that complex 2 species (*O. obscura*) pupate on the plant and that therefore air temperature controls the length of this period, while species from complex 1 (*O. melanopus*) pupate under the ground, subject to colder temperatures.

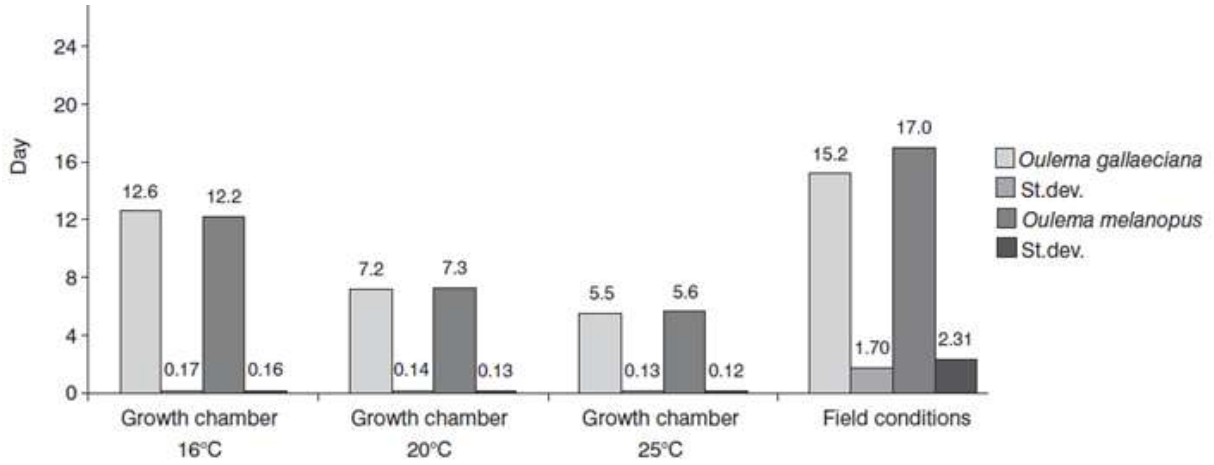


Figure 10: Comparison between O. obscura and O. melanopus adult beetle development under controlled (16, 20 and 25 °C) and field conditions (Walczak, 2005).

Ali et al. (1979) compared both species as well. An evaluation of both species showed that *O. obscura* generally experiences lower mortality rates compared to *O. melanopus*, at the same temperatures.

Differences in development of the same species between different regions were also studied. Especially interesting here is the migration of *O. melanopus* to the USA. Being introduced in Michigan in 1962, the beetle spread over many states, leading to major crop losses (Haynes and Gage, 1981). A comparison of populations of North America and Europe revealed that European larvae develop faster at colder temperatures and slower at higher temperatures compared to the American population (Table 2; Guppy, 1979). These researchers believed that this difference can be attributed to region adaption, where species adapt to the local climate.

*Table 2: Mean number of days (± SE) of the egg and larval stages of a European (EUR.) and American (N.A.) population of O. melanopus (Guppy, 1979) at constant temperatures. The results of a t-test are presented under t-value, where ** points to a significant difference at the 1% level*

Stage and population		Temperature (°C)				
		17	22	25	27	31
Egg	N.A.	11.3 ± 0.06	6.0 ± 0.07	4.9 ± 0.04	4.2 ± 0.03	3.8 ± 0.06
	EUR.	11.5 ± 0.10	6.0 ± 0.07	4.9 ± 0.03	4.2 ± 0.03	3.8 ± 0.07
Larvae	N.A.	17.3 ± 0.27	10.3 ± 0.09	8.3 ± 0.12	7.2 ± 0.06	6.3 ± 0.12
	EUR.	16.5 ± 0.15	10.6 ± 0.15	8.1 ± 0.06	7.3 ± 0.13	6.9 ± 0.14
t-value		2.86**	1.41	1.42	0.39	3.15**

Temperature not solely influences the growth of the pest insect, but also affects the growth of its host crop and natural enemies and thus the tritrophic interaction. In cooler seasons, lower temperatures will help the host crop to outgrow the damaged leaves, while the growth of the pest insect is slowed down. Studies show a lower temperature threshold for wheat leaf initiation at 3 °C (Porter and Gawith, 1999), for oats this lower thresholds varies between 3.3 and 4.4 °C (Webster et al., 1978), while CLBs' lower temperature thresholds sits between 7–10 °C, depending on the literature source.

Not only temperature, but also humidity is known to play a role in the development of CLBs. Ali et al. (1979) found a negative Pearson correlation between relative humidity and mortality in the egg stage, showing that a higher relative humidity decreases egg mortality. Even though for both *O. melanopus* and *O. obscura* this correlation was valid, *O. obscura* seems more sensitive to this abiotic factor, leading to a difference of more than 20% in mortality at 85% relative humidity.

Physical damage can also be detrimental to the larvae: i.e. wind and rain can be lethal as these factors can wash and brush a larva from the host plant. Shade et al. (1970) showed that mortality during a single rainstorm ranged from 3 to 28% in various larval instars. Knocked to the ground, the larvae are exposed to ground predators, dehydration or drowning, pathogens and starvation (Helsegen and Haynes, 1972).

Dispersal

At the start of the season, when average day temperatures increase, the adults become active and start migrating to adjacent fields. Sawyer and Haynes (1985) found that the type and number of useful overwintering habitats near a field influences the initial CLB density within a crop. Nevertheless, they also state that the between-year effect is influenced by a number of factors such as weather, sowing date, wind patterns and spatial arrangements of the fields. This makes it hard to predict the adult and larval density within one field.

Within fields, population dynamics of the different species can be highly variable, depending on the tritrophic interaction between plant, pest and natural enemies, which is in turn influenced by environmental conditions. Based on comparisons of absolute numbers of beetles at different distances within a field, simulation models suggested more beetle activity near the edge of the field, nearby possible overwintering sites (Sawyer and Haynes, 1986). Other empirical studies (Lecigne and Roehrich, 1977; Reay-Jones, 2010) showed similar results. CLBs also show a clear preference for younger plants. When adults move through a wheat field, studies show that these insects tend to remain at plants with young growth (Haynes, 1973).

At the end of the season, when post-diapause adults leave the pupae, they start looking for a suitable host and overwintering site. Host plants for these post-diapause adults include various adjacent wild or cultivated *Poaceae* species (Grant and Patrick, 1993).

Host plants

Host searching, oviposition, but also feeding are influenced by visual plant characteristics, e.g. colour, size, structure and plant architecture (Médiène et al., 2010). Other factors e.g. nitrogen content, secondary components and plant volatiles, are also known to influence the interaction between host plant and insect pest (McNeill and Southwood, 1978; Rosenthal and Janzen, 1979; Scriber and Slansky, 1981; Scriber, 1984; Renwick, 1989). It is a set of these parameters that make one host more preferable over another.

The interaction between CLBs and the host crop (cereals) is well documented (Everson et al., 1966; Gallun et al., 1966; Ringlund and Everson, 1968; Smith et al., 1971; Wallace et al., 1974; Papp and Masterhazy, 1996; Konyspaevna, 2012). Studying this interaction can be useful in light of resistance breeding (Haynes and Gage, 1981; Kher et al., 2011; Philips et al., 2011). Empirical studies show a difference in preference of CLBs between small grain cereals: oats were preferred above other cereals as barley, wheat, spelt and rye (Gallun et al., 1966; Wilson and Shade, 1966; Piesik and Piesik, 1998).

Against *O. melanopus* infestation, two mechanisms of resistance are described: antixenosis and antibiosis (Gallun et al., 1966; Schillinger, 1966; Wellso, 1973; Hoxie et al., 1975; Wellso, 1979). The first, antixenosis, is described as non-preference by the insect for the potential host crop (Price et al., 1980; Kher, 2014) and is a well-studied major mechanism for resistance to CLBs (Gallun et al., 1966; Wellso, 1973; Hoxie et al., 1975). Antibiosis can be explained as the negative effect of resistant host plants on the physiology of the pest insect (Painter, 1958; Renwick, 1983; Smith, 2005). Leaf pubescence promotes resistance against CLBs and can be catalogued under both mechanisms, causing the deterability of oviposition (antixenosis), affect hatchability (antibiosis), larval mortality (antibiosis) and adult feeding (antixenosis) on the resistant genotype (Gallun et al., 1966; Wellso, 1973; Hoxie et al., 1975; Papp et al., 1992). The number and length of trichomes on the leaves affect oviposition and larval feeding, giving the plant a potential benefit. The number of trichomes strongly influences oviposition and survival rates of first-instar larvae. A higher density of trichomes on the leaves causes a decline of 93% in the number of eggs laid. Mortality of eggs and first-instar larvae seems similar, going from 90% to 80% for the eggs and first-instar larvae respectively (Gallun et al., 1972). First-instar larvae that survive and feed on the resistant varieties, show a lower body weight (Schillinger, 1969). The presence of silica in the trichomes of resistant varieties can cause indigestibility (Wellso et al., 1973). Granting leaf pubescence delivers a potential successful defence strategy of the plant against herbivores, breeding towards higher yield has caused the current small grain cereal varieties to have little and short trichomes (Hoffman and Rao, 2011).

The difference in preference between oats, barley, wheat, spelt, rye can partially be explained by differences in silica content of the leaves. Silica (mainly opaline phytoliths) reduce digestibility and

increase leaf rigidity and roughness (Lucas et al., 2000; Laing et al., 2006; Reynolds et al., 2009). Silica content of the oat leaves is lowest compared to other small grains (Handreck and Jones, 1968).

Other morphological characteristics of the leaves will also play a role in resistance towards CLBs. Narrow-leaved varieties are undesirable because they limit the space for feeding and larval activity. A minimal width of 75 µm between the veins is necessary for easy feeding by the first-instar larvae (Shade and Wilson, 1967).

Finally, the production of green leaf volatiles (GLVs) also plays a role in antibiotic effects of the host plant to CLBs. GLVs have a double effect: Delaney et al. (2013) have demonstrated that these volatiles are used by CLBs for locating a suitable host crop, although they can also induce resistance. Studies show the effect of DIMBOA (2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one) on the growth of post-diapause adults (Wellso, 1978), inhibiting larval development. Herbivore induced plant volatiles (HIPVs) can influence the feeding behaviour of insects on resistant hosts (Piesik et al., 2009, 2010). Cis-jasmone derivates, such as indoles (shikimic acid derivate) and terpenes (mono- and sesquiterpenes), will deter continued feeding on resistant hosts and attract natural enemies, negatively influencing the feeding of CLB larvae (Piesik et al., 2011; Delaney et al., 2013; Piesik et al., 2013).

The effect of plant volatiles is not limited to inhibiting growth of adults or influencing feeding behaviour of larvae, but acts as a biocontrol mechanism for pest organisms. Volatiles produced by plants after feeding of herbivore insects can attract parasitoids (Cortesero et al., 2000). Antibiotic effects will also chronically or acutely lower general fitness of CLB larvae, limiting economic yield losses (Smith et al., 1971).

Even if the general importance of host plant resistance is well known (Everson et al. 1966; Gallun et al. 1966; Papp et al. 1992), further research on the interaction between host plant and CLBs is necessary (Kher, 2014). Especially as it is known that indirect effects of antibiosis can enlarge the exposure and susceptibility of the pest organism to natural enemies, supporting biocontrol (Singh, 1986). Since CLB is a global pest insect, genetic diversity is large, causing a different response to certain antibiotic characteristics (Kher, 2014).

Although resistance breeding in crops is often a challenge, literature shows that wheat has the greatest genetic potential compared to the other preferred hosts of *O. melanopus* (Hahn, 1968; Steidl et al., 1979), therefore making it a possible cornerstone for the preventive control of CLBs.

Crop damage

Oulema spp. cause direct or indirect damage to *Poaceae* in both larvae and adult life stages (Kher et al., 2011). Direct damage is caused by the feeding of the larvae and adults on leaf tissue, causing the

'frosted' windowpaned view of the wheat plants (Figure 11; Grant and Patrick, 1993). Although both larvae and adults feed on the leaves, reducing the photosynthetic capacity of their host plant, research has shown that only the larvae cause significant economic damage (Haynes and Gage, 1981; Grant and Patrick, 1993; Kostov, 2001).

Figure 11: Flag leaf damage by CLB larvae causes a 'frosted' windowpaned view of the leaves of winter wheat plants. Photo by Elias Van de Vijver.

Larval damage to leaves, e.g. defoliation of leaves, causes many physiological responses within the plant. Many studies have investigated these effects by manually defoliating cereal plants. Direct effects on photosynthesis are clear: defoliation of leaves not only decreases the photosynthetic area of the plant (leading to a smaller total photosynthetic capacity), it also decreases stomatal conductivity and transpiration (Macedo et al., 2007). Reasons for these effects are the interruption of the capillarity in the leaves, causing a disruption of the source-sink relationship, an altered water balance within the plant and even an accumulation of sugars at the source (Shao et al., 2010). All these effects impede photosynthesis. Defoliation has a direct effect on photosynthesis, although the effect on yield appears to differ significantly depending on crop growth stage (Ahmadi and Joudi, 2007). This can be caused by many factors. For example, many studies show an increased speed of photosynthesis when plants are

defoliated (Trumble et al., 1993; Rosenthal and Kotanen, 1994; Macedo et al., 2006). This is caused by a decrease in water, nutrient and hormonal competition among the remaining leaves (McNaughton, 1983; Meyer, 1998; Collin et al., 2000; Shao et al., 2010).

Final yields in cereals are determined by three components: number of ears per area unit, number of kernels per ear and kernel weight (measured by thousand grain weight) (Buntin et al., 2004). Each of these factors is determined on different crop growth stages, implying that the time of defoliation plays a crucial role in determining final yield loss (Olson et al., 1989). Buntin et al. (2004) investigated the effect of defoliation by *O. melanopus* on these three factors. Their study showed that defoliation did not affect tillering, thus the number of ears per area unit. On the contrary, several other studies (Zhu, 2001; Zhu et al., 2004) described a paradoxal increase in grain yield and water-use efficiency (WUE). In these cases, an early and minor defoliation caused an increase in grain yield.

Generally, empirical studies show that defoliation, in a vegetative stage, does not affect final yield, implying that the plant compensates for the lost leaf surface: an increased photosynthesis enables the plant to buffer the final grain yield (Shao et al., 2010). This can be an explanation for the fact that CLB adults and early larvae instars do not cause significant economic damage. When plants become generative, yields are significantly influenced by defoliation (Najad, 1981). From flowering stage on, most studies report a decrease in thousand grain weight by defoliating the flag leaf and the other two top leaves (Najad, 1981; Sairam and Dube, 1984; Rao et al., 1989; Asghar and Ingram, 1993).

Severe larval and adult leaf feeding cause loss of kernel weight and number of kernels per ear (Gallun et al., 1967; Merritt and Apple, 1969). As the larvae go through different stages, their feeding activity increases. Most crop losses are induced by fourth-instar larvae, leading up to 70% of all damage by the CLBs (Kher et al., 2011). Although larvae of the last stage show the highest feeding activity, their high activity is believed not to be the only contributing factor for high crop losses at that stage (Wilson et al., 1969). Like many other herbivorous arthropods, the larvae are attracted to the highest placed leaf since it is a rich source of nitrogen and hydrogen compounds. However, the flag leaf is an important site for photosynthesis that determines grain filling and affects the plant's ability to cope with stress (Evans et al., 1975; Maydup et al, 2010). For these reasons, it is critical to minimise damage when plants reach this stage during development.

Defoliation not only affects photosynthesis. The evapotranspiration of the plants is affected as well, as root length can be affected by defoliating leaves. Indeed, the allocation of resources to root growth is decreased in order to increase shoot growth (De Roover et al., 1999; Todorovic et al., 1999). This all causes a lower WUE, which, during dry periods, affects yields. Undeniably, it is known that water stress shortens seed filling and therefore thousand grain weight (Frederik and Camberato, 1995).

Grain yield losses, due to leaf feeding by CLBs, ranged, depending on the region and cereal species from 0.5–4% (Poland, Ulrich et al., 2004), 25.6% (USA, Webster et al., 1981), 70% (Central Europe, Knechtel and Manolache, in Wilson et al., 1969 and Dimitrijevic et al., 2001) or even 80% (Teofilovic, 1969) in winter wheat; 20% (USA, Wilson et al., 1969) or 48% (Merritt and Apple, 1969) in winter oats and 3–8% (Poland, Ulrich et al., 2004) in winter barley. Comparing winter wheat with spring wheat, losses tend to be higher in spring wheat, going up to 28–55% (resp. Kolarov, 1988; Webster et al., 1972).

Grain quality (e.g. parameters which affect the baking characteristics) is not altered by CLBs' leaf feeding (Gallun et al., 1967; Ahmadi et al., 2009; Zhang et al., 2012). General defoliating studies revealed that defoliating did not affect final protein content and water content. On the other hand, the concentration of micro-elements appeared to differ with a decrease of manganese and an increase of iron, zinc and copper.

Control of CLBs

To minimise crop losses caused by CLBs, various practices can be implemented. An integrated management of a pest organism states that preventive strategies should be considered before leading to curative control plans (bottom-up approach, Figure 12). The integrated management of CLBs is a bottom-up approach, meaning that tactics suggested on the bottom of the IPM pyramid should receive most attention, compared to the tactics more to the top that can help to correct and curatively treat the pest insect in the field. On the right, a summary is given of measurements which are available within each tactic for CLB management.

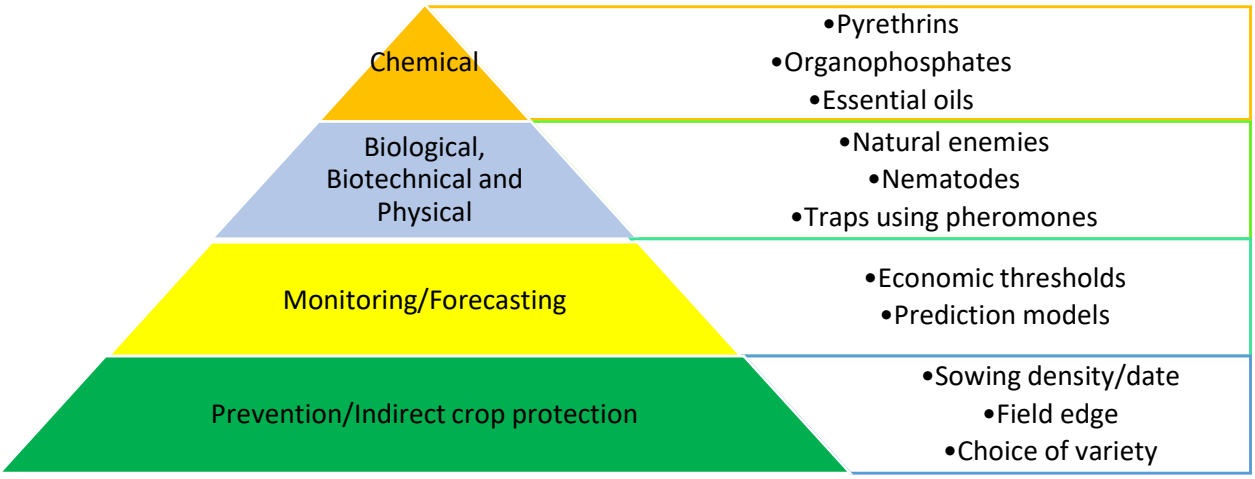


Figure 12: IPM pyramid applied to CLB management in Belgium, based on Meissle et al., 2011 and adapted from Laridon, 2018.

Preventive control tactics

Preventive tactics are an essential part of IPM. Knowledge of the pest organism is key to adjust the management to minimise yield losses. Figure 13 visualises the interactions between crop and fauna in the field and how management can be used to change this interaction. The idea of most preventive tactics is to cause a spatial or temporal (or both) desynchronisation between pest population and the crop grown as these tactics reduce the biotic interactions, promoting crop production (Aubertot et al., 2004).

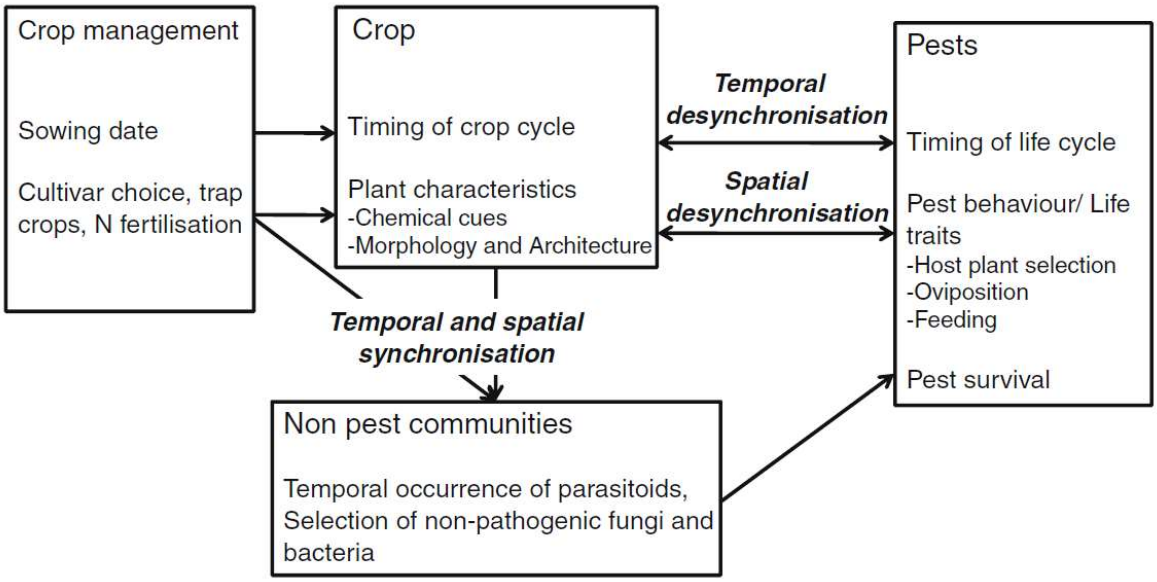


Figure 13: Preventive tactics can be used to cause a spatial and temporal desynchronisation between plant and pest insect (Mediéne et al., 2010).

At the same time, it seems essential to keep in mind that natural enemies can help in controlling pest organisms. Therefore, we want to aim for a temporal and/or spatial synchronisation of the crop with non-pest organisms (functional agro-biodiversity, FAB). It is clear that this balance is often difficult to make, keeping in mind that managing a crop combines an IPM for multiple organisms. However, for insect pest organisms, sowing date seems critical for shifting the phenology and to cause a temporal shift. In case of CLBs, delaying sowing, can synchronise the population of eggs and larvae with natural enemies (parasitoids and predators). It is known that these natural enemies can manage the population densities of CLBs well under economic thresholds (Tschumi, 2015).

Sowing date

A lot of discussion remains in literature about the effect of sowing date on the presence of CLBs in winter wheat fields. Generally, the idea of shifting sowing date is to cause an offset between both plants and the pest insect's life cycle (Figure 13).

In North America, the general guideline to manage CLBs recommends an early sowing date (Philips et al., 2011). Grant and Patrick (1993) found more CLBs in thinner stands and later planted fields. Others

have monitored that on fields that are planted late, lack nitrogen fertilisation or have poor soil quality, a lower number of CLBs were active (Casagrande et al., 1977; McPherson, 1983). Hoffman and Rao (2010, 2011) described that *O. melanopus* has a preference for late planted spring cereals for oviposition. Older leaves tend to have a higher thickness and more silica and are therefore less desirable for oviposition, compared to younger leaves. Haynes (1973) also believed that CLBs prefer younger leaves for feeding and oviposition. Nevertheless, Gage (1972) found that the younger the plant at initial attack of the larvae, the higher the crop damage will be. Older cereal plants tend to have a more developed root system and are therefore less prone to stress caused by the loss of photosynthetic material. Although Gage (1972) initially studied the effect on oats, later he confirmed this for winter wheat as well. In this trial, he found more eggs and larvae of CLBs in late planted winter wheat fields. Comparing winter to spring cereals, spring cereals were most prone to CLB damage (Lee et al., 1976). As spring cereals are in an earlier growth stage at moment of infestation, the synchrony between plant and insect is optimal for supporting the development of the larvae.

Plant density

Plant density has, next to sowing date, also an important effect on the presence of insect pests in the crop. Similar to the effect of sowing date, discussion remains on the influence of plant density on the presence, oviposition and leaf damage by *O. melanopus.* Webster et al. (1978) found that lower seeding rates in oats can be successful to minimise yield losses attributed to CLB activity. A thinner stand enables the plant to compensate the lost leaf material on the upper leaves. Webster et al. (1978) concluded that a sparse stand resulted in fewer eggs and larvae per area unit. Honek (1991) found similar results in the Czech Republic. Dense stands of large plants were preferred by CLBs for oviposition. Although the number of eggs found on the plants per unit of field area increased with crop density, the number of eggs per unit of leaf area appeared independent of crop density. Despite this, leaf acceptability for the larvae increased with crop density, assuring favourable trophic conditions for the beetle. On the other hand, Herbert (1990) and Grant and Patrick (1993) found that *O. melanopus* adults were attracted to a thinner stand.

Although plant density and sowing date do have an effect on the presence of CLBs, attaining a lower plant density or advance the sowing date for the preventive management of CLBs, is not recommended (Casagrande et al., 1977; Webster et al., 1978). Yield loss accompanied with a lower stand or later sowing date cannot be compensated by the yield loss due to CLBs, making it economically uninteresting.

Host plant: intercropping and trap cropping

Due to the fact that CLBs show clear differences between several hosts for oviposition and beetle fitness, design strategies such as trap cropping or intercropping can be used within an IPM outline.

Intercropping, the coexistence of two or more crops in the same field at the same time (Wezel et al., 2014), has much potential in productivity, ecological sustainability, yield stability, resilience to (a)biotic stress. Literature shows that combining multiple crops proves to have a positive effect on insect species richness in and around the field (Mason and Macdonald, 2000; Weibull et al., 2000; Atauri and de Lucio, 2001; Steffan-Dewenter et al., 2002, 2003; Purtauf et al., 2005; Vanbergen et al., 2005). As natural enemies are more present, this could influence the control of CLBs. For CLBs, a mixed cropping system of oats with barley, triticale or other oat varieties, resulted in a considerable reduction of oat injuries (Piesik and Piesik, 1998).

Trap cropping is another effective approach to control pests. Because most insects are attracted to some allelopathic compounds and show a certain preference for a crop, variety or crop stage, these differences can be used to attract potential pest insects to the trap crop (Hokkanen, 1991; Shelton and Badenes-Perez, 2006). Specifically, trap crops appear to have an effect on Coleoptera, Heteroptera, Lepidoptera and Homoptera (Shelton and Badenes-Perez, 2006). Kher et al. (2011) suggested the use of Waldern oat as trap crop as it appeared to be an attractive host for oviposition, while showing a negative effect on the feeding behaviour of the larvae. Waldern oats are spring oat varieties from Canada with high yield potentials (Kibite, 1991).

Crop rotation

Research concerning the effect of crop rotation on CLB presence is rare. Nevertheless, the general effect of crop rotation is known: an increased rate of monocultural cropping, reduced crop rotation or reduced tillage, all increase the presence of pests in the crop (Oerke, 2006). Also, it is believed that large-scale monoculture cropping systems enabled CLBs to spread globally (Wenda-Piesik and Piesik, 1998; Ulrich et al., 2004).

Fertilisation

It is known that chemical leaf composition affects a number of factors in the insect development. As chemical leaf composition is determined by fertilisation, this can be managed. Especially nitrogen content seems detrimental for larval feeding and general larval viability. However, Hoffman and Rao (2011) found that nitrogen content in the leaves did not affect oviposition. The effect of nitrogen on larval feeding seems indirect. Studies indicate that an increasing nitrogen fertilisation causes a reduction in tissue toughness, making the leaves more attractive for feeding (Wait et al., 1998; Kerpel et al., 2006).

Although nitrogen seems most important, phosphorous and potassium are important as well. A study of Dimitrijevic et al. (1999) mentioned an increase of leaf damage due to a combined fertilisation scheme with nitrogen, phosphorous and potassium. This scheme increased the plant's growth and

development. Also, it is possible that an intensive fertilisation scheme reduces the plant's resistance. Potassium increases the water content in the leaf cells and therefore the turgor pressure of the leaves, which could make the leaves more tasteful, therefore increasing CLB damage.

Landscape architecture

Complex and diversified landscapes favour natural enemy population due to a combination of reasons. Plant-provided resources can be crucial for many generalist and specialist arthropod natural enemies. To survive through some life stages, insects require shelter, overwintering sites and food sources as floral, extra-floral nectar and pollen. Although woody and herbaceous vegetation can provide these parameters, a well-adapted habitat management is often necessary in order to enhance natural enemies, without benefitting the pest insect (Lundgren, 2009). This population of natural predators or parasitoids could offer a feasible alternative to a pesticide-based control of pest insects (Andow, 1991; Barbosa, 1998; Bianchi et al., 2006; Rusch et al., 2010). Tschumi et al. (2015) showed that perennial, species-rich wildflower strips (e.g. a seed mixture of indigenous forbs, legumes and grasses) can reduce CLBs population density and therefore increase winter wheat yield (Figure 14). These strips ensure an early and continuing intervention of natural enemies and cause a negative impact on the spread and establishment of CLBs (Tschumi et al., 2015).

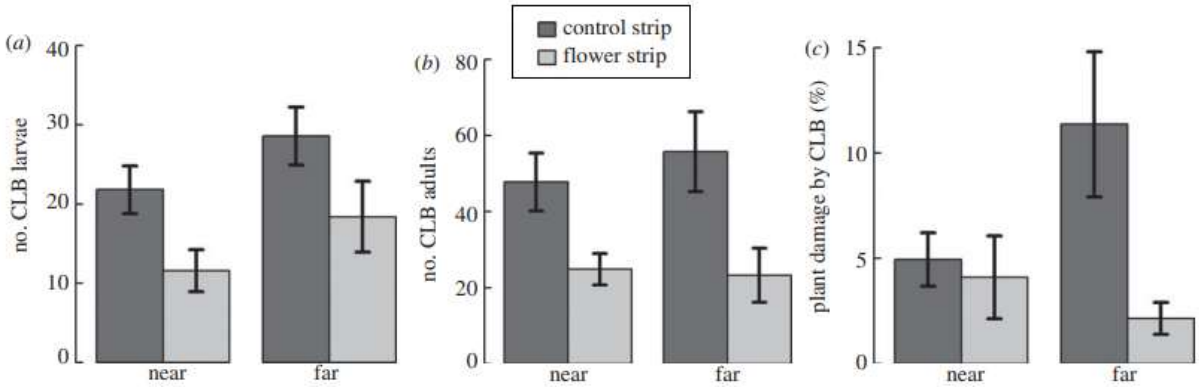


Figure 14: Influence of flower strips on the number of CLB larvae (a), number of CLB adults (b) and flag leaf damage caused by CLBs (%; c), monitored on two distances: near the flower strip = 0.5–10.4 m and far from the flower strip (a mixture of indigenous forbs, legumes and grasses, width of the strip = 3 m) = 10.5–20.4 m . In the control strip, another crop field (winter wheat, maize, sunflowers, grassland) is adjacent (Tschumi et al., 2015).

In this study, egg numbers were reduced with 44% at a distance of 5 m from the strip, lowering the level under the economic threshold of 0.5 eggs per tiller. CLB larvae density and flag leaf damage were reduced by 66% and 40%, respectively. Flower strips also had a considerable effect on CLB larvae and adult presence. Next to affecting CLBs, these flower strips can also enhance natural enemy population. Tschumi et al. (2015) showed a significant increase of adult natural enemies such as ground beetles, lacewings and ladybirds, although the effect was clearly strongest near the strip.

Curative control tactics

Economic thresholds for CLBs

Worldwide, many economic thresholds are set for CLBs. While it is clear that different regions need different economic thresholds, the variation in the tolerable level of pest insects within the crop often makes deciding whether or not to apply a curative treatment hard for growers. For CLBs, older thresholds mentioned in literature range from 0.25 eggs or larvae per tiller (Van Duyn et al., 1997) to 2.5 larvae per tiller (Chambon et al., 1983; Stilmant, 1995). While most of this research was done in the USA, pre-1970, more recent studies mention lower thresholds, e.g. 0.5 larvae per tiller (Tschumi, 2015). Table 3 lists the thresholds used for CLB control that are mentioned in literature. Because crop damage also depends on the crop stage, some authors also mention a certain crop stage at which their threshold level applies.

Thresholds are quite useful to determine timing for a curative control tactic. Nonetheless, the pest insect is only a part of the cropping system in which the plant and natural enemies evolve as well. This tritrophic interaction will clearly influence this threshold as well. Some studies on CLBs therefore set multiple thresholds, depending on the crop stage. For example, Webster and Smith (1983) mentioned a threshold of three eggs or larvae per tiller. As the plant is most vulnerable after the emergence of the flag leaf, this threshold was updated by Roberts et al. (2014): from flag leaf appearance, growers are advised to spray earlier, when counting 1 egg or larvae per tiller in their fields.

To date, no thresholds described in literature take the effect of natural enemies on CLB population density into account.

Table 3: Economic thresholds for CLBs mentioned in literature

Threshold	Crop stage	Crop	Region	Source
Eurasia				
2.5 larvae per tiller	-	wheat	France	Anglade et al., 1976
0.5–1 larvae per tiller	-	wheat	Germany	Heyer and Wetzel, 1990
2.5 larvae per tiller	-	wheat	Belgium	Stilmant, 1995
0.5–1.5 larvae per tiller	-	wheat	Russia	Sokolov, 1999
0.5–1 larvae per tiller	-	cereals	Croatia	Barcic and Culjak, 2001
1 larvae per tiller	-	wheat	Servia	Tanaskovic et al., 2012
0.5 larvae per tiller	-	wheat	Switzerland	Tschumi, 2015
USA				
1 larvae per tiller	-	wheat		Haynes and Gage, 1981
3 eggs or larvae per plant	< BBCH 39[1]	cereals	Washington state	Webster and Smith, 1983
1 larvae per flag leaf	> BBCH 39			updated by Roberts et al., 2014
0.25 eggs or larvae per tiller	-	wheat	N. Carolina	Herbert and Van Duyn, 1997
0.63 eggs or 0.43 fourth-instar larvae per tiller	-	wheat	N. Carolina and Virginia	Ihrig et al., 2001
0.4 larvae per tiller	BBCH 51[2]–69[3]	wheat	Georgia	Buntin et al., 2004
> 3 eggs or larvae per tiller	< BBCH 39	cereals	Montana	Blodgett et al., 2004
> 1 larvae per flag leaf	> BBCH 39			

[1] Flag leaf stage: flag leaf fully enrolled, ligule visible
[2] Beginning of heading: tip of inflorescence emerged from leaf sheath
[3] End of flowering: all spikelets have completed flowering

Prediction models

There is a more than 40-years tradition of modelling wheat, CLBs and natural enemy interactions (Coulman et al., 1972; Casagrande et al., 1976; Guppy and Harcourt, 1978; Philips et al., 2012). However, the use of such prediction models in plant protection is scarce, mainly because of their insufficient forecast accuracy. The most important problems in modelling CLBs and antagonist population dynamics have to do with describing the inter-field dynamics, estimation of mortality rates and survival, which are influenced by several variables (Helgesen and Haynes, 1972). In order to develop more accurate prediction models, a good understanding of the population dynamics, both spatially and temporally, is critically important. Weather conditions, crop husbandry practices and landscape management (e.g. surrounding vegetation) are important factors affecting the tritrophic interactions between plants, CLBs and their natural enemies (Haynes and Gage, 1981).

A wide range of modelling approaches has been used to predict insect development, ranging from simple GDD models (Evans et al., 2006; Philips et al., 2012) to more complex mechanistic models (Helgesen and Haynes, 1972; Tummala et al., 1975; Lee and Barr, 1976). Mechanistic models adopt a bottom-up approach to understanding system dynamics, and in case the life cycle of the various organisms is not completely described, these models will often not fit data as well as empirical models that use statistically derived functions. Detailed population models tend to be very complex, because it is often believed that higher complexity leads to higher accuracy. However, it is overlooked that such multiple input models are also very sensitive to measurement errors rising with each parameter and can hence result in highly variable predictions (Klüken, 2008). For this reason, we will first focus on the relatively simple GDD models, while making an attempt for a more holistic model in the following part of this chapter. Although GDD models have a long history of use in predicting plant and insect phenology, this modelling approach is still amended in recent warning systems (Cayton et al., 2015; Akyuz et al., 2017; Calero et al., 2017) and certainly offers, with appropriate thresholds, opportunities. Since insects are poikilothermic, temperature is the major driver of phenology. Indeed, there exists a profound correlation between temperature and the phenology of insects. Therefore, insect phenology is often predicted via the calculation of heat units, or GDD. GDD accrual is typically initiated after a discrete biological event, referred to as a 'biofix', or a calendar date (e.g. January, 1). A GDD model can be represented according to (1).

$$\mathrm{GDD} = \left[\frac{T_{max} + T_{min}}{2}\right] - T_{base} \qquad (1)$$

where T_{max} is the maximum daily temperature, T_{min} is the minimum daily temperature and T_{base} is the lower threshold for development. In case the average temperature is lower than T_{base}, no degree days are accumulated and 0 is recorded for that day, since no insect growth or development occurs. Since each developmental stage has its own total heat requirement, insect development can be estimated

by accumulating GDD throughout the season until a certain maximum GDD threshold is reached (Wilson and Barnett, 1983; Zalom et al., 1983). The warmer the weather, the faster GDD accumulate and the faster the maximum GDD threshold is reached. To predict the date of the various life cycle stages of CLBs, numerous GDD models have been developed, each with its specific maximum GDD threshold and base temperature.

In Table 4, an overview of the growing degree days necessary to reach a certain life cycle stage of CLBs is given. All models start to accumulate GDD on the biofix date of January, 1. These data were based on field trials and in some cases supported by data retrieved from laboratory trials. It can be seen that the observations of the various researchers at different locations led to a wide range of base temperatures and maximum GDD thresholds. Therefore, based on the thresholds set by Gage and Haynes (1975), Guppy and Harcourt (1978), Kidd et al. (2002), Blodget et al. (2004), Evans et al. (2006) and Hoffman and Rao (2010), a combined GDD model was developed and implemented on http://uspest.org/wea, a webserver of the Oregon State University on which GDD models for several organisms can be accessed. The suggested combined CLB model uses a base temperature of 9 °C and the first egg, egg peak, first larvae and larvae peak can be expected at 80 GDD, 150 GGD, 180 GDD and 360 GDD, respectively. Furthermore, note that Philips et al. (2012) did not use a fixed base temperature and GDD threshold to predict the larvae peak. According to these authors, the larvae peak is expected to occur in average 17.5 days (within a range of 7–35 days) after the egg peak. Additionally, it can be seen that Evans et al. (2014) considered a variable threshold to predict the date of CLB egg peaks in Utah. Based on data from 2001 until 2011, these authors concluded that there was a considerable variation among years in the number of GDD associated with the occurrence of the CLB egg peak (ranging from 145 to 325 GDD). Much of this variability could be accounted for by considering early spring warmth. Indeed, there was a positive linear relationship between the GDD for egg peaks and the accumulated heat during spring, the higher the temperatures in spring the higher the GDD threshold. Thus, instead of using a fixed GDD threshold, these authors suggest that the GDD threshold should take into account early spring warmth. For instance, the threshold for egg peak (y) can be calculated according to

$$y = 67.45 + 1.41\,x \qquad (2)$$

with x the accumulated degree days from January, 1 until April, 21.

Table 4: Summary of GDD necessary to reach a certain life cycle stage of CLBs, between brackets the minimum development threshold is mentioned

Source	Location	First egg	Egg peak	First larvae	Larvae peak
Gage and Haynes (1975)	N. America			220 (8.9)	
Ali et al. (1977)	Germany	85–90 (10.5)			
Guppy and Harcourt (1978)	Ottawa	105 (7)	166 (7)		
Guppy and Harcourt (1978)	Ottawa	87 (9)	137 (9)		
Kidd et al. (2002)	N. Carolina			165 (8.9)	349 (8.9)
Blodgett et al. (2004)	Montana	253 (7.17)			
Evans et al. (2006)	Utah	90 (8.9)	150 (8.9)	105 (8.9)	240 (8.9)
Hoffman and Rao (2010)	Canada	88 (9)			
Philips et al. (2012)	Virginia		182 (8)		Egg peak+17.5
Evans et al. (2014)	Utah		Variable		
Combined (http://uspest.org/wea)		80 (9)		180 (9)	360 (9)

All the approaches mentioned in Table 4 studied the relationship between GDD and a certain life cycle stage. However, for practical insight into the phenology of the pest insect, predicting pest incidence on field level is important as well. Therefore, Ihrig et al. (2001) studied the relationship between CLB egg counts and the number of insects in the fourth-instar stage and the impact of fourth-instar population on winter wheat. A significant linear relationship was found between the 50th percentile of the number of eggs (*x*) and the density of the fourth-instar population (*y*) ($y = 0.36 x - 0.01$ (3); $R^2 = 0.79$). Potentially detrimental larval infestations were forecast from egg populations present during the tiller elongation to flag leaf emergence developmental stages. A significant positive linear relationship between total fourth-instar larvae population estimates (x) and percent flag leaf defoliation (y) was detected ($y = 20.29 x + 1.34$ (4); $R^2 = 0.60$). Furthermore, a weaker, but still significant linear relationship between the total fourth-instar population estimates (x) and percent yield loss (y) was found ($y = 11.74 x + 6.51$ (5); $R^2 = 0.26$), indicating that factors in addition to flag leaf injury, primarily by fourth instars, also contributed to reduced yields.

Biological control

The past has proven that biocontrol, using parasitoids, has been an effective management strategy for CLBs (Wellso, 1982). Though, not only parasitoids are present in the field and contributing to predating or parasitising CLBs. Syrphidae (larvae), Chrysopidae and Hemerobiidae, Coccinellidae, Phytoseiidae and Anthocoridae species are all described being a predator for CLB eggs or larvae. Especially ladybird beetles (Coccinellidae species) are a proven effective predator. Roberts (2016) mentioned a consumption rate of 40% of CLB eggs or small larvae due to this insect. Table 5 lists all possible predators of CLB eggs or larvae.

Table 5: Insect species that are described as being an active or passive predator for CLBs

Family	Species name	Source
Coccinellidae	*Hippodamia parenthesis* (Say)	Shade et al., 1970; Bragg, 2009
	Hippodamia tredecimpunctata (Linnaeus)	
	Coccinella novemnotata (Herbst)	
	Coleomegilla maculata (De Greer)	
	Hippodamia convergens (Guerin)	
Chrysopidae	*Chrysoperla carnea* (Stephens)	Speyer, 1954
Carabidae	Several species	Holland and Thomas, 1997; Holland, 1998; Meindl et al., 2001
Staphylinidae	*Philonthus cognatus* (Stephens)	Meindl et al., 2001
Erythraeidae	*Balaustium* spp.	Shade et al., 1970
Nabidae	*Nabis ferus* (Linnaeus), other *nabis* spp.	Bjegovic, 1968; Schärer, 1994
Several fungi species	*Alternaria alternata* (Keissler); *Isaria farinose* (Holmsk); *Verticillium lecanii* (Zimmerman)	Machowicz-Stefaniak and Miczulski, 1985
Nematodes	*Steinernema carpocapsae* (Weiser)	Laznik et al., 2010

Nonetheless, parasitoids have a higher rate of success and are easily introduced in heavily CLB-infested fields (Roberts, 2016). Only a few of these species are specialised on CLB eggs or larvae; examples are *Diaparsis* spp. and *Lemophagus* spp. Table 6 mentions all described parasitoids active on the different CLB life stages.

Table 6: Insect species that are described as CLB parasitoids for different life stages

Family	Species name	Parasitised life stage CLBs	Source
Ichneumonidae	*Gelis instabilis* (Förster)	Larvae, pupae	Stehr and Haynes, 1972; Pavlov, 1981; Haeselbarth, 1989; Cox, 1994; Gallo and Jeloková, 2006
	Diaparsis carinifer (Thomson)	Larvae	Dysart et al., 1973; Haynes and Gage, 1981; Haeselbarth, 1989;
	Diaparsis temporalis (Horstmann)	Larvae	Haynes and Gage, 1981; Lampert and Haynes, 1985; Haeselbarth, 1989
	Itoplectis maculator (Fabricus)	Pupae	Pavlov, 1981; Haeselbarth, 1989; Gallo and Jeloková, 2006
	Itoplectis alternans (Gravenhorst)	Pupae	Pavlov, 1981
	Scambus annulatus (Kiss)	Pupae	Pavlov, 1981; Haeselbarth, 1989

	Bathythrix maculmatus (Hellin)	Pupae	Pavlov, 1981; Haeselbarth, 1989; Gallo and Jeloková, 2006
	Lemophagus curtus (Townes)	Larvae	Dysart et al., 1973; Haynes and Gage, 1981; Pavlov, 1981; Lampert and Haynes, 1985; Haeselbarth, 1989; Gallo and Jeloková, 2006;
Chalcidoidae	*Habrocytus* spp.		Pavlov, 1981
	Eupteromalus spp.		Pavlov, 1981
	Trichomalopsis microptera (Lindeman)		Pavlov, 1981
	Necremnus leucarthos (Nees)	Pupae	Pavlov, 1981; Haeselbarth, 1989; Horvath and Szabolcs, 1992; Miczulski, 1994; Šedivý, 1995; Gallo and Jeloková, 2006
	Tetrastichus julis (Walker)	Eggs	Dysart et al., 1973; Haynes and Gage, 1981; Lampert and Haynes, 1985; Haeselbarth, 1989; Gallo and Jeloková, 2006
	Anaphes flavipes (Förster)	Eggs	Haynes and Gage, 1981; Lampert and Haynes, 1985; Haeselbarth, 1989; Horvath and Szabolcs, 1992; Jeloková and Gallo, 2008
	Camptoptera papaveris (Förster)	Eggs	Natural History Museum, 2014
	Pteromalus chrusos (Walker)	Eggs	Natural History Museum, 2014
	Pteromalus semotus (Walker)	Eggs	Natural History Museum, 2014
	Pteromalus vibulenus (Walker)	Eggs	Pavlov, 1981; Gallo and Jeloková, 2006
	Trichomalopsis microptera (Lindeman)	Eggs	Pavlov, 1981; Haeselbarth, 1989
	Trichogramma spp.	Eggs	Maltby et al., 1969
Diptera: Tachinidae	*Hyalomyodes triangulifer* (Loew)	Adult	Bjegovic, 1967; Bjegovic, 1968
	Meigenia mutabilis (Fallén)	Larvae, pupae	Natural History Museum, 2014
Diptera: Phoridae	*Phalacrotophora fasciata* (Fallén)		Šedivý, 1995
Diptera	*Duophoria nigrata*		Šedivý, 1995

Although a variety of species parasitise CLBs at various life stages, only a few of them are known to be effective to keep CLBs under economic threshold levels. A selection of four species of parasitoids were introduced from Italy to the USA in the early seventies: *Tetrastichus julis* (Walker), *Diaparsis carinifer* (Thomson), *Lemophagus curtus (*Townes) and the egg parasitoid *Anaphes flavipes* (Förster). In '*field nurseries*', these parasitoids were reared in a protected field area and then relocated to heavy infested fields (Dysart et al., 1973). Compared with an earlier chemical eradication attempt, this biocontrol strategy has proven to be successful in the management of CLBs. Especially *T. julis* and *A. flavipes* proved effective (Roberts, 2016), with parasitism rates of 95% (*T. julis*) and 12.3% (*A. flavipes*; Anderson and Paschke, 1968).

While *A. flavipes* is the most effective parasitoid in some parts of Europe (Horvath and Szabolcs, 1992), researchers question its potential to keep CLB eggs under economic thresholds levels (Jeloková and Gallo, 2008). An answer for this could be found in the asynchronic phenology between the egg population of CLBs and the egg parasitoid (Figure 15). It is clear that this egg parasitoid lags behind its host (Dysart, 1971).

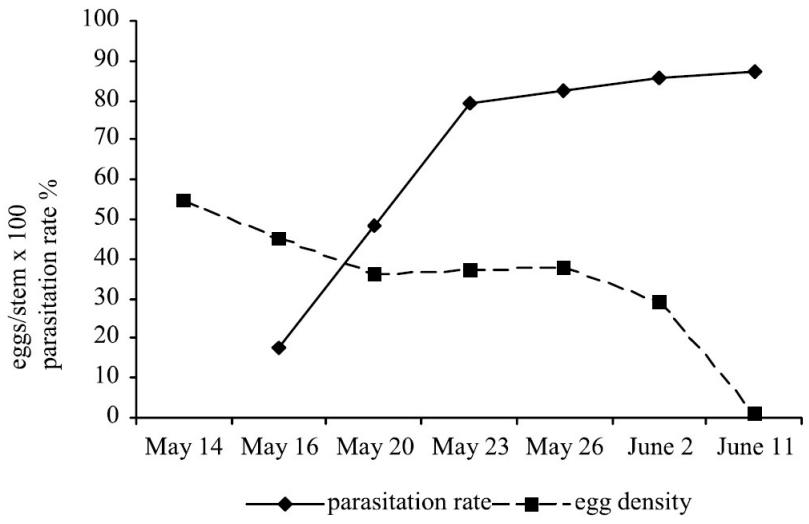


Figure 15: CLB egg density (eggs per tiller x 100) and parasitation rate (%) of A. flavipes on CLB eggs in time (Meindl et al., 2001).

It appears globally that especially *T. julis* wasps are effective in controlling CLB larvae. This insect's first adult peak period (bivoltine) is well synchronised with early CLB larvae, while the second generation parasitises later host larvae. This almost perfect synchronisation (Gage and Haynes, 1975), in combination with a high host specificity (only described to be active on CLBs; Cárcamo et al., 2012) and its capacity to find its host well beyond its geographic range (Philips et al., 2011) make *T. julis* a very successful biocontrol organism.

Chemical control in Europe

After its introduction in the USA, eradication attempts were executed in an effort to minimise the economic loss due to the presence of CLBs. Initial lack of natural enemies in these fields ensured the beetle population growth and caused major crop losses during the 1960s (Haynes and Gage, 1981). Nonetheless, most of the chemical control agents used in the USA are not allowed in the European Union (EU). Products allowed in the EU for the control of CLBs in small grains are zeta-cypermethrin, deltamethrin (synthetic pyrethroids) or dimethoate (organophosphate) (Fytoweb, 2017). While pyrethroids have advantages compared to other older insecticides, as they are effective at a low dosage, biodegraded and among the least toxic compounds for mammals, they are contact-based and lethal to beneficial organisms such as *T. julis* (Coats et al., 1979). In spring barley, seed treatments could reduce the CLB population with 40%, without affecting the natural enemies. However, foliar sprays have much higher effectiveness (90% mortality; Tharp et al., 2000) compared to seed treatments. Neonicotinoids are controversial due to their possible detrimental effect on bees. The EU issued a prohibition for neonics for agricultural use in 2018. Comparing the effectiveness towards CLBs of dimethoate to other insecticides, Speese (1995) showed a significant lower effectiveness compared to other insecticides such as λ-cyhalothrin. In fact, Speese (1995) discovered that, by killing the beneficial insect species in the field, the tested organophosphates induced outbreaks of summer aphids (*Sitobion avenae* (Fabricius)). Philips et al. (2011) concluded that chemical treatments of CLBs should be executed very careful, taking into account a very strict timing.

Chapter 3: Experimental framework

All trials ran in light of a project to bring IPM closer to growers. While some trials were executed on Bottelare UGent research farm (independently), most of the observations were done on growers' fields, as this could bridge the gap between research and practice. In this chapter, the observation network that formed the backbone of this PhD thesis is described. During four years, several growers' fields were selected and monitored. In an attempt to link management practices and surroundings to the biology of the beetles, the CLBs' phenology (adult, egg and larva) was studied in these fields. Sweep net samples were also collected on these fields to discover which *Oulema* species are generally active in Belgium.

Monitoring of CLB eggs and larvae in Flanders

To unravel inscrutabilities concerning CLBs phenology and distribution or their interactions with different insect species in natural conditions, several monitoring fields throughout Flanders were surveyed during four subsequent growth seasons.

Starting from April, when plants reached BBCH 32 (second node), until hard dough stage (BBCH 87), the CLBs present were counted weekly on every field. Depending from year to year, approximately 30 fields scattered over the most important wheat growing regions of Flanders were monitored (Polders, Schelde-Polders, Zuid-Vlaanderen, Vlaams-Brabant and Haspengouw) (Figure 16).

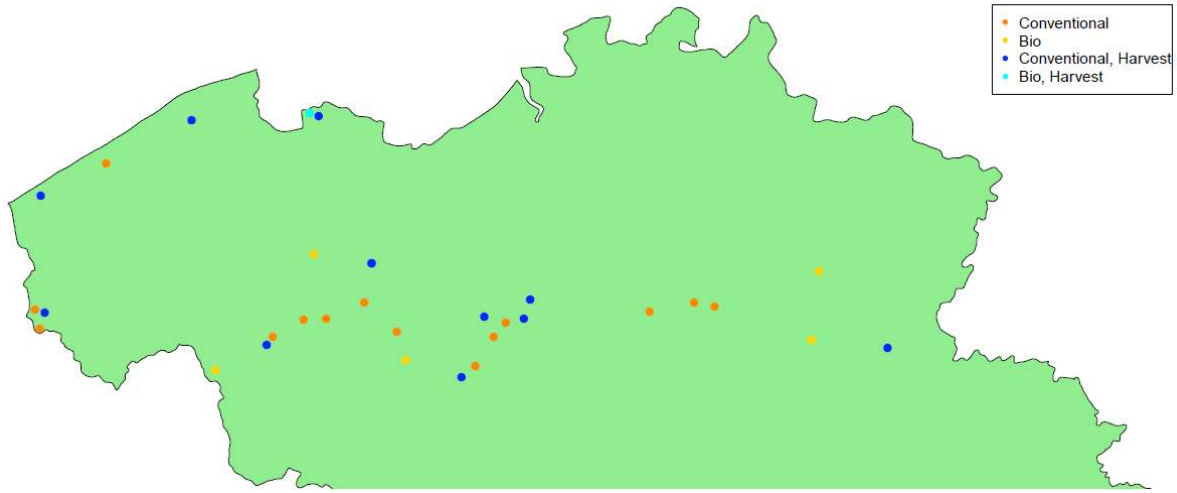


Figure 16: Overview of the locations that were observed during growth season 2015–2016. Red dots indicate fields with a conventional cultivation, yellow: organic cultivation; (dark and light) blue dots: on these fields, grain yield losses due to CLBs presence were determined.

During four years, 122 fields were monitored in total. Although most of the fields were managed using conventional practices (105 fields), some organic fields were monitored as well (17). These fields were selected based on differences in management practices (although these differences are small in Belgian wheat cultivation) and the presence of a semi-natural element (SNE) next to the field. Examples of these SNEs are (catalogued according to their effect on CLB development; Casagrande et al., 1977): hedges (33 fields), low density forests (12), high density forests (6), arable land (18), perennial grassland (14), grassy field edge (14) and flower borders (4). Most of the monitored fields consisted of winter wheat, although, some spring wheat was monitored as well.

Conventional management followed good agricultural practices, which generally included a broad crop rotation scheme. Crop rotation in Belgium is often limited to a 3 or 4-year rotation scheme. Rotation crops on the monitored fields included mostly potatoes (21 fields), maize (20) and winter wheat (4). Other, less common rotation crops were included as well, i.e. chicory (3), sugar beets (2), flax (3) and different types of cabbages (4). Soil tillage techniques before sown included plowing at 28 cm and harrowing. Most fields were sown at normal sowing time (80% of the monitored fields were sown between October, 15 and November, 10), while some fields were sown rather early (13% before October, 15). General practices dictate a sowing density of 350 grains/m², which was followed by most growers (67%). Fertiliser was mostly applied in three different fractions, with a total application of approximately 200 kg/ha of nitrogen (62% of the growers added between 160 and 220 kg/ha), following the N-index advice system (Soil Service of Belgium). A herbicide application was applied in April and fungicide applications were applied in May and June. No insecticides were applied on any of the wheat fields before and during the period in which the field was monitored.

Organic fields were managed following a broad rotation scheme. Most of the organic wheat fields monitored planted spring wheat, using manure as fertilisation (one application before sowing). Sowing densities applied were higher than regular sowing densities (400–450 grains/m²) and weeds were controlled mechanically. Harvested grains were sifted and dried to 15% humidity.

During wheat growth season, CLBs were weekly monitored in eight 16 m² plots, placed inside an insecticide-free zone. To investigate the effect of distance from the field edge on the phenology of *Oulema* species, four of these plots were placed at the field edge, the other four at the field centre (Figure 17). CLBs were counted on 30 randomly selected plants per plot. In total, 240 plants per field were monitored (8 plots × 30 plants) on a weekly basis. The corners of the insecticide-free zone were marked with flags for the grower.

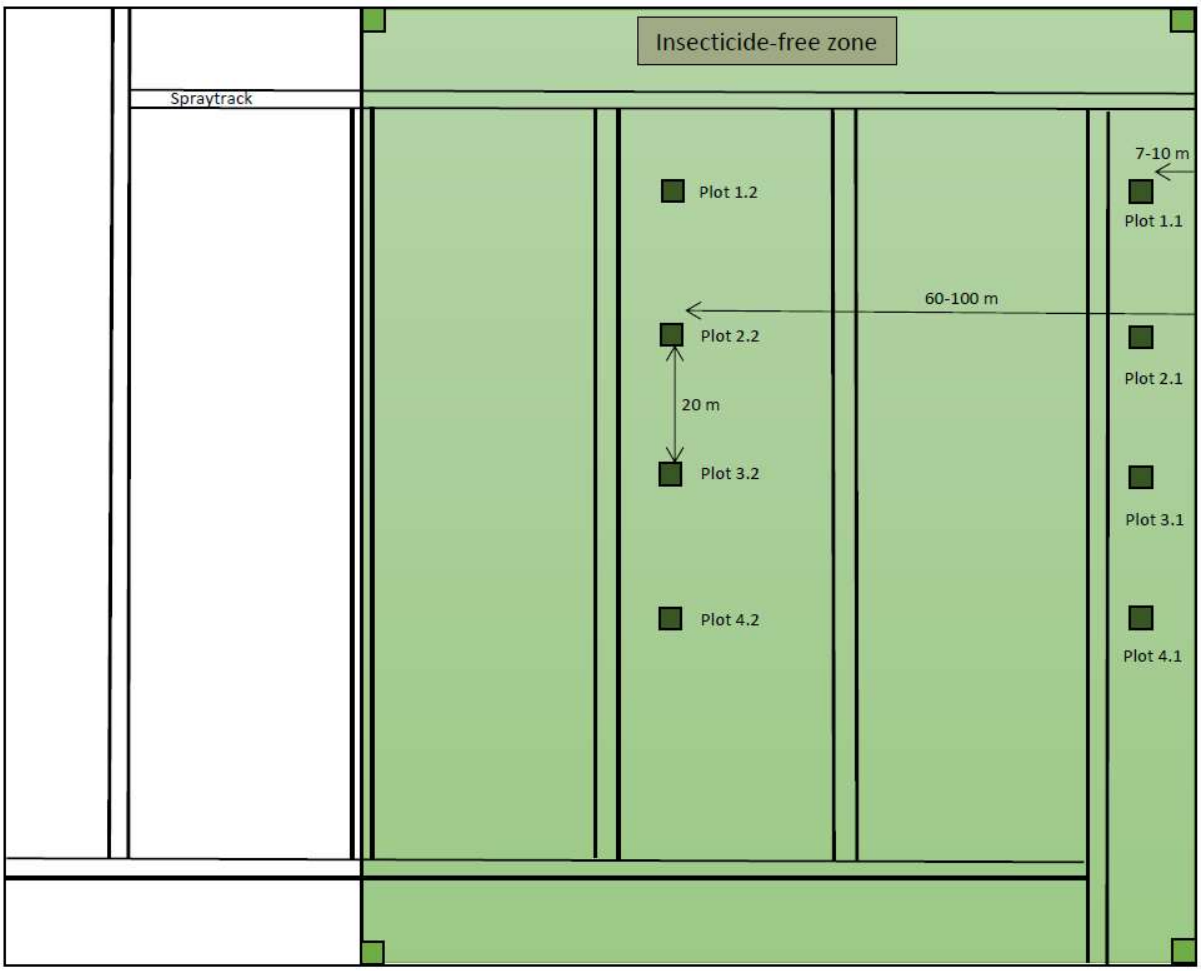


Figure 17: Example of the plot layout on a monitored field: eight monitoring plots (4 × 4 m) were placed inside an insecticide-free zone, which was marked by flags (green cubics) for the grower. Within each field, four monitoring plots (plots 1.1, 2.1, 3.1 and 4.1) were positioned close (7–10 m) to the field edge, while four plots were placed further away (60–100 m) from the field edge (plots 1.2, 2.2, 3.2, 4.2).

On each plant, the number of eggs and larvae of CLBs was monitored. Collected monitoring data were saved in a spreadsheet straight away, using a specialised field computer (Psion Teklogix). Hourly weather data were collected using the automated weather stations across Flanders (distance < 5 km from each field), directed from the Agricultural Centre for Potato Research (Kruishoutem).

Collection of CLB adults in Flanders

To assess the abundance of each species, CLBs were collected from several fields that were also monitored for the eggs and larvae of CLBs. For this trial, beetles were collected from 81 fields in total: 2016, 28 fields; 2017, 30 fields and 2018, 23 fields. Each field was catalogued to the different Flemish agricultural regions (SL, sandy loam; S, sand; P, polder; L, loam).

For the investigation of CLB between-field distribution, beetles were collected following the protocol described by Reay-Jones (2010). Using a sweep net with a diameter of 30 cm, sampling was done at two distances from a selected field edge, next to the observation plots in which the eggs and larvae of

Oulema spp. were monitored. At each distance, two samples were collected by sweeping through the top canopy of eight rows per sweep. One sample consisted of 30 sweeps over a width of ca. 1 m (indicating that one sweep covers one metre length, this covers 30 m²). Using an aspirator (pooter), the insects were collected from the sweep net and then transferred to collection pots. The collected beetles were stored in a solution of 70% (v/v%) ethanol.

Collection events took place at three time points during the first growth season (2016, April_P2 (16-04, 19-04, 20-04, 21-04), May_P1 (03-05, 09-05, 11-05), May_P2 (18-05, 19-05)). Due to later arrival of the CLBs in the monitored fields in 2017, CLBs were collected at only two time points (May_P1 (03-05, 04-05), May_P2 (30-05, 31-05)). In 2018, only one time point was selected for collecting (May_P1 (03-05, 04-05, 08-05)). Time points were determined according to the expected peak densities based on a growing degree day model developed in Belgium during the same time period of the experiments described here (**Chapter 6**).

Another sampling scheme investigated the within-field distribution and possible shifts during the growing season at five similar fields with a grassy edge in 2018. These fields were managed uniformly and laid nearby the research station for practical reasons. During the period starting from April 18 until May 5, beetles were collected twice a week at four distances in the field from a chosen field edge (10, 20, 30 and 40 m). The protocol for sweeping, collecting, storing and storage of the beetles was similar to that of the between-field distribution sampling.

Belgian climate and weather conditions

According to the Köppen climate classification, the Belgian climate has an Oceanic (maritime) climate (type Cfb). This climate zone is characterised by relatively mild winters, summer and temperate rainfall throughout the year. Temperature extremes are rather rare. Definition of a maritime climate states that monthly maximum temperatures stay below 22 °C in the warmest month, while minima stay above 0 °C in the coldest month. Precipitation should reach 40 mm per month. Regions that also house an oceanic climate and known for CLBs infestations are: large parts of Western Europe, as well as western parts of Oregon and Washington State (USA).

Nonetheless, the weather conditions fluctuate within and between growth seasons, which could influence parts of the tritrophic interaction such as the phenology of CLBs, natural enemies or the growth of their host plants. Therefore, a review per growth season follows below: Figure 18–Figure 21.

Generally speaking, it is clear that the global climate is changing. In the past decade, the overall global temperature has risen with 0.7 °C (Rosenzweig et al., 2000) and some researchers predict a total increase of 3.5–7.5 °C in worst case scenarios (Cohen and Miller, 2001). This not only influences the

insect population dynamics by altering development rates, generation times, overwintering mortality, but also causes possible shifts in species distribution. Plant dynamics are influenced as well: e.g. a shift in the growth season, changing mortality or emergence rates (Porter et al., 1991). Even in the course of this research, possible effects of climate warming were present as during two years, Belgian heat records were broken (July, 2 (2015) and matched again on July, 27 (2018)).

2015

Figure 18 represents the main environmental variables (2015, Bottelare) compared to the climatic normals for Belgium during 1984–2010: mean monthly temperature (°C) and precipitation (mm). While it is clear that most months follow climatic normals for temperature, November and December stand out to be significantly warmer than average. Generally, 2015 proved to be significantly warmer and sunnier compared to the climatic normals from Ukkel, Belgium (1984–2010).

Comparing precipitation with the climatic normals for Belgium, more deviations are visible. A wetter January and November are visible, while April, June, July and October are drier than average. Generally, 2015 was slightly drier compared to the climatic normals.

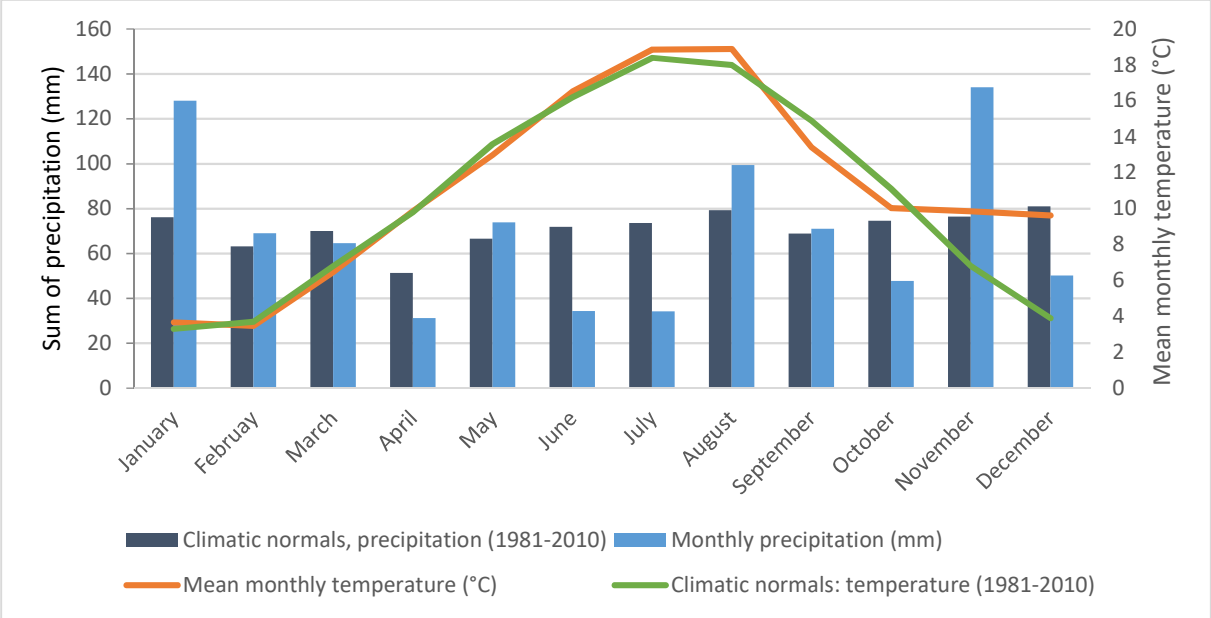


Figure 18: Mean monthly temperature (°C) and precipitation (mm) in Bottelare (2015) compared to climatic normals for Belgium (1984–2010).

2016

Although temperatures were slightly higher than the climatic normals, 2016 proved to be a 'normal year'. Spring ended wet, while later on in the season, a drier period followed (July and September).

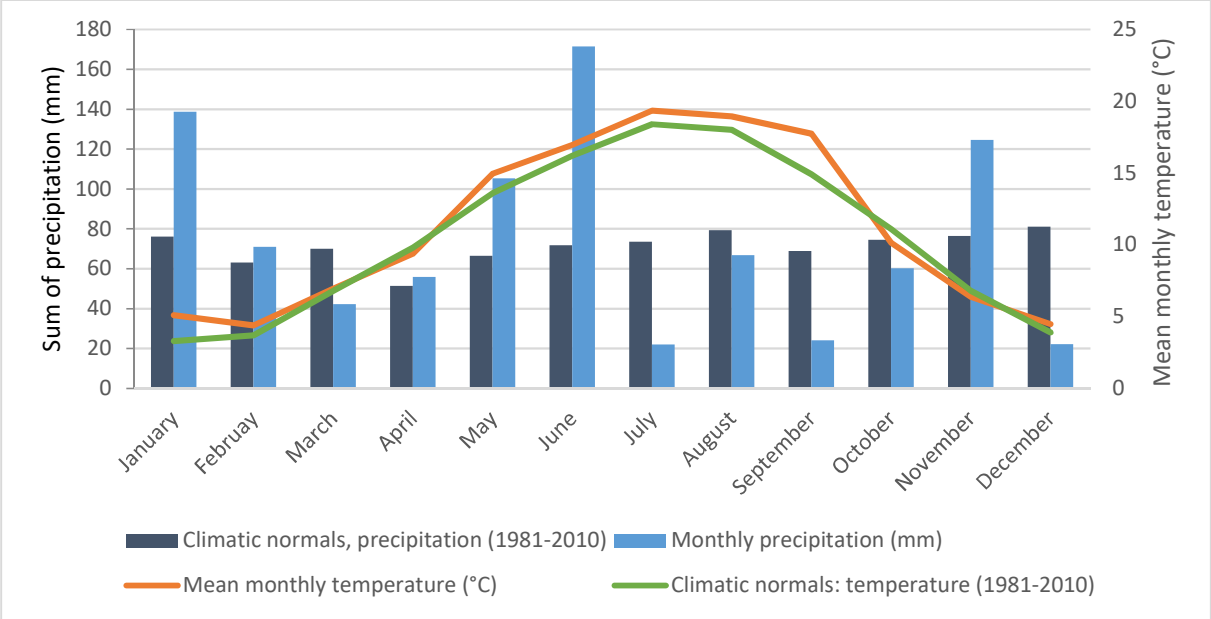


Figure 19: Mean monthly temperature (°C) and precipitation (mm) in Bottelare (2016) compared to climatic normals for Belgium (1984–2010).

2017

While 2017 started rather cold (January), generally this year proved to be unusually warm (February, March, June, October, November and December), with a low overall precipitation. December proved to be a somber month with lots of precipitation and even snowfall.

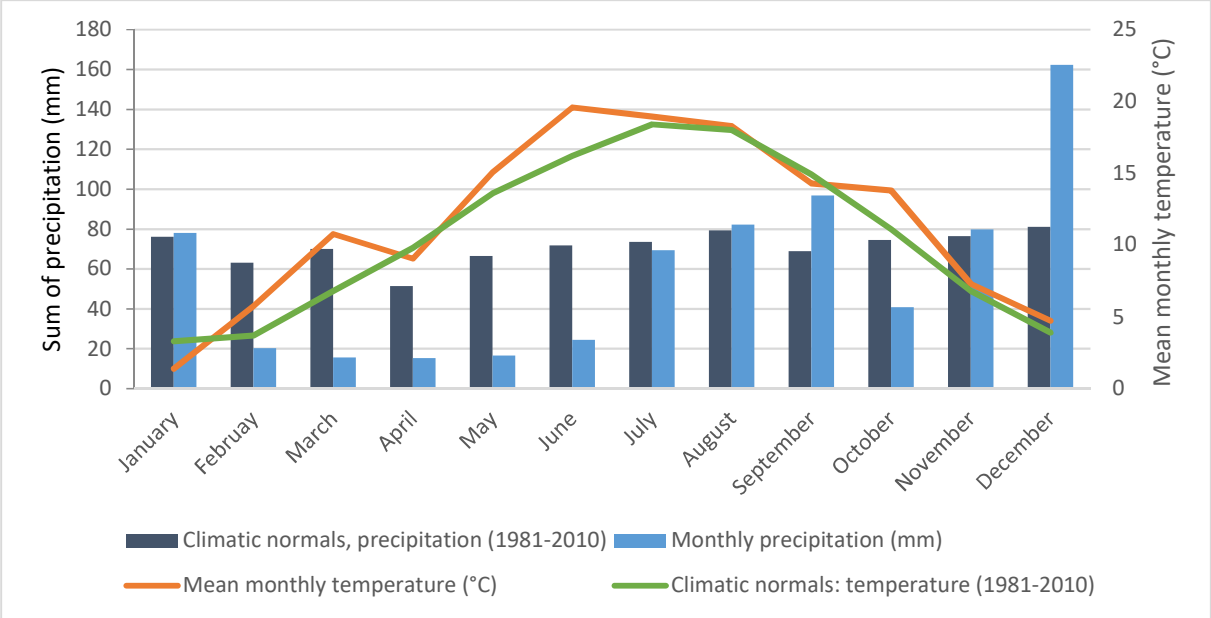


Figure 20: Mean monthly temperature (°C) and precipitation (mm) in Bottelare (2017) compared to climatic normals for Belgium (1984–2010).

2018

While February and March were unusually cold for the time of the year, the general spring temperature was remarkably warm compared to the climatic normals, with warm months such as January, April, May, June and July. Later on in the season, the summer of 2018 started exceptionally dry. In May (34.4 mm/m² precipitation), June (5 mm) and July (12.4 mm), only a fraction of the climatic normal precipitation has fallen during these months.

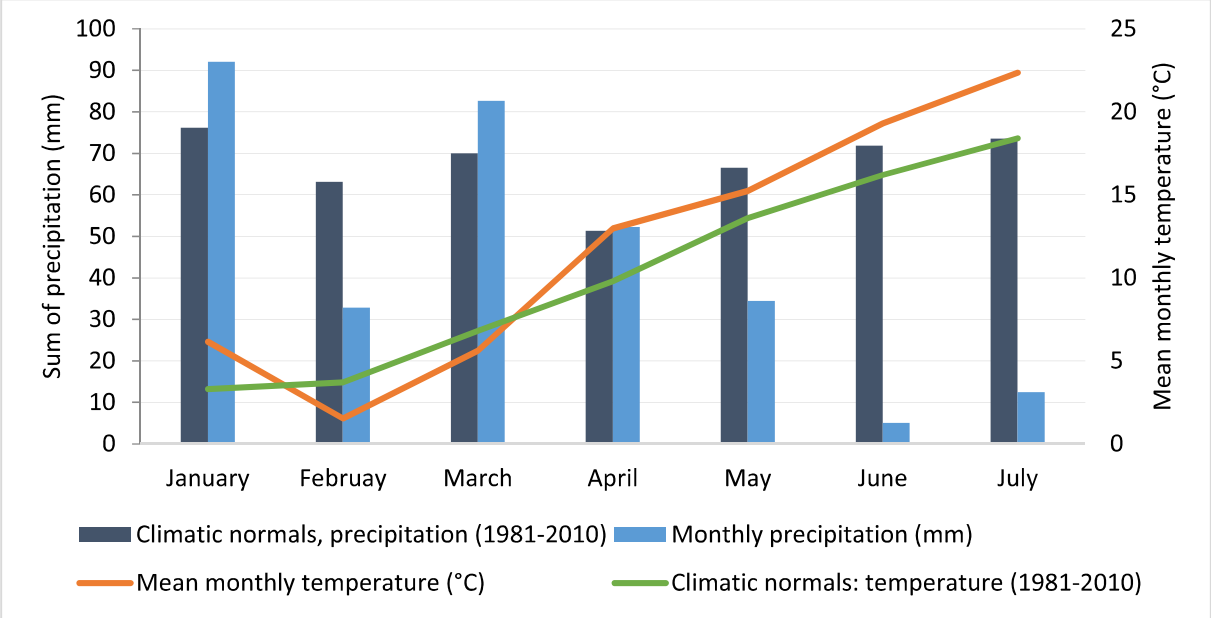


Figure 21: Mean monthly temperature (°C) and precipitation (mm) in Bottelare (2018) compared to climatic normals for Belgium (1984–2010).

Chapter 4: Between and within-field distribution of CLBs in Flemish winter wheat[4]

Introduction

Oulema species (Coleoptera: Chrysomelidae) are widespread all over the Western Palearctic area, causing damage to major crops within the *Poaceae* family (Balachowsky and Mesnil, 1936). Several species within the genus *Oulema* are known to cause damage in winter wheat (*Triticum aestivum* L.): *O. melanopus* (Linnaeus, 1758), *O. rufocyanea* (Suffrian, 1847), *O. duftschmidi* (Redtenbacher, 1874), *O. obscura* (Stephens, 1831), *O. septentrionis* (Weise, 1880) and *O. erichsonii* (Suffrian, 1841) (Walczak, 2005; Schmitt and Rönn, 2011; Chapelin-Viscari and Maillet-Mezeray, 2015). A more detailed description of the taxonomy, classification and distribution of CLBs can be found in **Chapter 2** of this thesis.

Due to the increasing problems with CLBs on small-grain cereals in recent years, a thorough understanding of the problem is a prerequisite of a knowledge-guided IPM-oriented strategy. As CLB incidence is caused by a complex of different species, insight into the species composition and dynamics between and within growth seasons is of paramount importance to develop accurate control strategies. In this light, the aim of this study was to gain insight into the between- and within-field species distribution in Flemish wheat (*Triticum aestivum* L.) fields. It is known that the various CLB species react differently to temperature and humidity and therefore show a different phenology (Ali et al., 1977, 1979). Current Flemish CLB management consists mostly of a single insecticide treatment with a broad-spectrum pyrethroid, which sometimes interferes with growing aphid and natural enemy populations. Therefore, insight into the species composition and their phenology is important for accurate timing of insecticide treatments, which has proven to be essential but very difficult with CLBs.

[4] Adapted from an accepted publication: Van De Vijver E., S. Landschoot, M. Van Roie, F. Temmerman, J. Dillen, K. De Ceuleners, G. Smagghe, B. De Baets, and G. Haesaert. 2018. Inter- and intra-field distribution of cereal leaf beetle species (Coleoptera: Chrysomelidae) in Belgian winter wheat. Environ. Entomol. 2018, doi: 10.1093/ee/nvz002.

Materials and methods

Collection of CLBs

A more detailed description of how the beetles were collected and how the grower's fields were managed can be found in **Chapter 3**.

Identification of the collected CLBs

In order to identify *O. melanopus*, *O. duftschmidi* and *O. rufocyanea*, we first determined beetle gender. Gender was determined by a dissection of the genitalia. Dissections were executed by means of a Zeiss Stemi 2000 0.8*10 (80X maximum magnification) and a standard dissection set. Only males were selected for further dissection and determination, as the female genitalia are a lot smaller and more fragile to handle. The female specimens were classified as either *O. mel/duf/ruf* (the species belonging to complex 1) or *O. obscura* (species belonging to complex 2). Identifications of the males were achieved by comparison of the flagella, following a protocol described in Bezděk and Baselga (2015) (Figure 22). To distinguish *O. erichsonii*, *O. obscura* and *O. septentrionis*, differences in morphology of pronotum and the ratio of the elytra length (EL) to elytra width (EW) were used (Hubble, 2012). Finally, body length (BL), elytra length (EL), elytra width (EW), antennae length (AL), pronotum length (PL) and pronotum width (PW) of a random subset of 10 beetles per species were measured.

Figure 22: Aedeagus of the main Oulema species (from left to right): O. duftschmidi, O. melanopus and O. obscura. Scale: 1 cm represents 0.5 mm.

Statistical analyses

All analyses were conducted in R (version 3.5, R Development Core Team, 2017). To test whether or not the species distribution was influenced by the sampling period and the sampling position in the field, a Pearson's Chi-squared Test for Count Data (`chisq.test`) was used. In case it turned out that the species distribution was dependent on the sampling period/position (p-value < 0.05), pairwise Chi-squared tests (at a significance level of $\alpha = 0.05/n$, with n the number of tests) were performed. A letter code above the bar plots was used to denote significant differences between populations. Since the normality and homoscedasticity assumptions of parametric tests were not fulfilled, a Kruskal-Wallis test (significance level $\alpha = 0.05$) was performed to test whether the CLB population size (complex 1

and complex 2) differed according to the sampling period. In case there were significant differences, Dunn's Multiple Comparison Test was run to detect which groups significantly differed.

To gain insight into the potential relationship between the CLB population size and weather conditions, Pearson correlation coefficients were calculated and tested for their significance at a significance level of $\alpha = 0.05$.

Finally, a linear discriminant analysis (LDA) was applied to determine whether based on the body measurements of the different CLB species an identification up to species level is possible. To test the accuracy of the LDA, leave-one-out cross-validation (LOCV) was performed. LOCV uses all but one of the data points to determine the decision boundaries and then uses these boundaries to predict the omitted data point's group membership. The procedure was repeated for each observation.

Results

Oulema species composition

To gain insight in the species composition, in a first step, collected beetles were dissected and identified to species. On most observation moments male and female beetles had a similar share in the population. Except for April 2016, in this period the frequency of the male *O. obscura* individuals was $^1/_3$ of the frequency of the females and during the second part of May 2017, complex 1 consisted of $^3/_4$ males and $^1/_4$ females (Table 7). Furthermore, in 2016 and 2018, beetles belonging to complex 1 were more abundant compared to beetles belonging to complex 2, whereas in 2017 both complexes were equally present.

Table 7: Relative frequency (%) of the males (m) and females (f) from the species belonging to complex 1 (O. melanopus, O. rufocyanea, O. duftschmidi) and the species belonging to complex 2 (O. obscura), based on averages across fields and across distances from the field edge during seasons 2016 (28 fields), 2017 (30 fields) and 2018 (23 fields)

	2016			**2017**		**2018**
	April_P2	**May_P1**	**May_P2**	**May_P1**	**May_P2**	**May_P1**
Complex 1 (m)	56.06	42.96	38.89	21.13	36.96	43.18
Complex 1 (f)	37.88	44.63	39.58	22.54	13.04	32.82
Complex 2 (m)	1.52	6.21	9.72	33.80	21.74	12.92
Complex 2 (f)	4.55	6.21	11.81	22.54	28.26	11.08

A Chi-squared test on the gender of the beetles indicated that only in 2018, a significant higher number of male beetles from complex 1 were collected (p-value = 0.009). The results of the Chi-squared test are added in **Appendix 1**.

Results of a Chi-squared test also indicated that the distribution of CLB species was dependent on the growing season and that shifts in population composition occurred during single growth seasons (Figure 23).

In the second part of April 2016, *O. melanopus* (55.3%) was the most abundant species, whereas *O. rufocyanea* (2.6%) and *O. obscura* (2.6%) were only marginally present. Both in the first and second part of May 2016, *O. duftschmidi* was the predominant species, with relative abundances of 51.0% and 58.6%, respectively *O. obscura*, which was a minor species in 2016, was the main species in the first part of May 2017 (61.5%). In the second part of May 2017, *O. obscura* and *O. duftschmidi* had an almost equal share in the population (37.0% and 40.7%). In 2018, *O. duftschmidi* was, with a relative frequency of 58.3%, the main species on the collection dates in the first half of May.

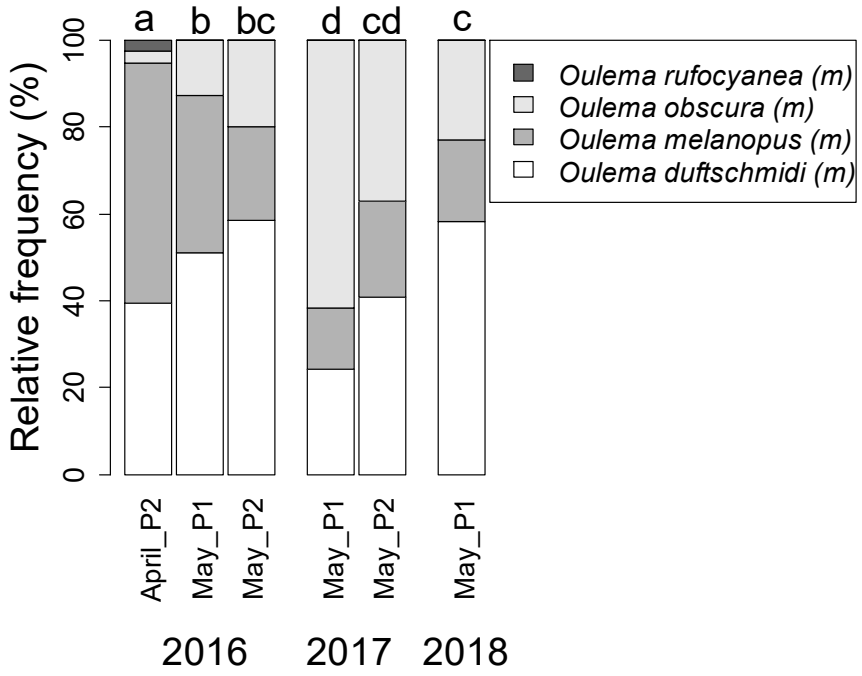


Figure 23: Relative frequency of the different Oulema species (males), on the sampling dates during the second part of April (April_P2), the first part of May (May_P1) and the second part of May (May_P2) during the seasons 2016 (28 fields), 2017 (30 fields) and 2018 (23 fields). Different letters above the bars point to significant differences between population distributions (Chi-squared test).

Studying the species composition at different sampling locations (near the field edge or in the centre of the field), revealed that in two of the sampled growth seasons, significant differences in the species composition were found (Figure 24). In 2016, there were no significant differences between the population composition near the edge of the field and the composition in the centre of the field. In 2017 and 2018, the population composition was dependent on the position of sweeping. In May 2017, no *O. melanopus* adults were sampled near field edge, whereas in the centre of the field the frequency of this species in the collected samples was 19.0%. In May 2018, an increased abundance of both *O. duftschmidi* and *O. melanopus* was noted when going from the edge to the centre of the field.

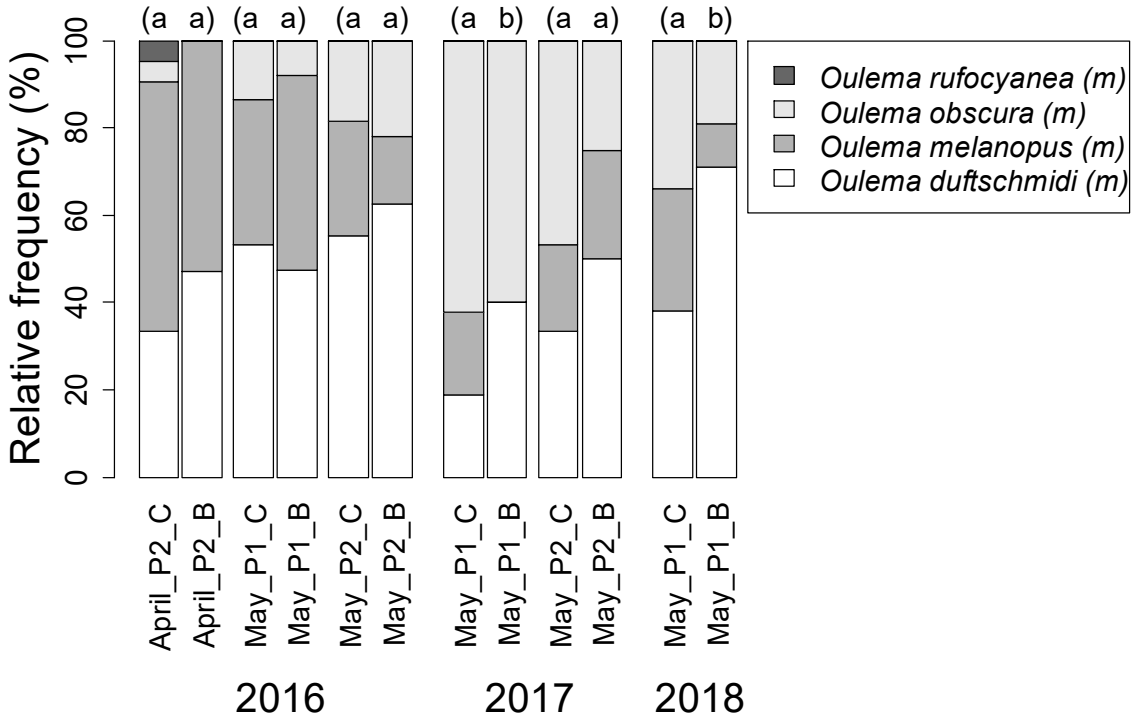


Figure 24: Relative frequency of the different Oulema species (males), during the second part of April (April_P2), the first part of May (May_P1) and the second part of May (May_P2) near to the edge of the field (B) and in the centre of the field (C) during the seasons 2016 (28 fields), 2017 (30 fields) and 2018 (23 fields). Different letters above the bars point to significant differences between population distributions within one sampled period (Chi-squared test). Sampling close by the field edge was done at 4–8 m, while sampling in the centre was executed at 30–50 m from the field edge.

Geographical distribution of *Oulema* species

The geographical distribution of the different CLB species in Flemish wheat fields was determined as well (Figure 25 and Figure 26), according to the different agricultural regions. Pie charts present the population distribution in the different regions in 2018 (Figure 26). In 2016 and 2018, the CLBs population composition in the loamy region significantly differed from the composition in the other regions (p-value < 0.001 (Chi-squared test)), the fact that in 2017 no significant differences can be found is partially due to the fact that the total number of CLBs collected in 2017 was lower compared to the other years. Within this region, the relative abundance of *O. obscura* was highest (36% in 2016; 80% in 2017, 65% in 2018) in the collected samples. The relative abundance of *O. duftschmidi* was lower in the loamy region compared to the other regions. In the polders, *O. obscura* was the least important species, with frequencies of 0%, 16.7%, and 2.1% in 2016, 2017, and 2018, respectively. In comparison, *O. duftschmidi* was the primary species, with frequencies of 71%, 67%, and 91% in 2016, 2017, and 2018, respectively.

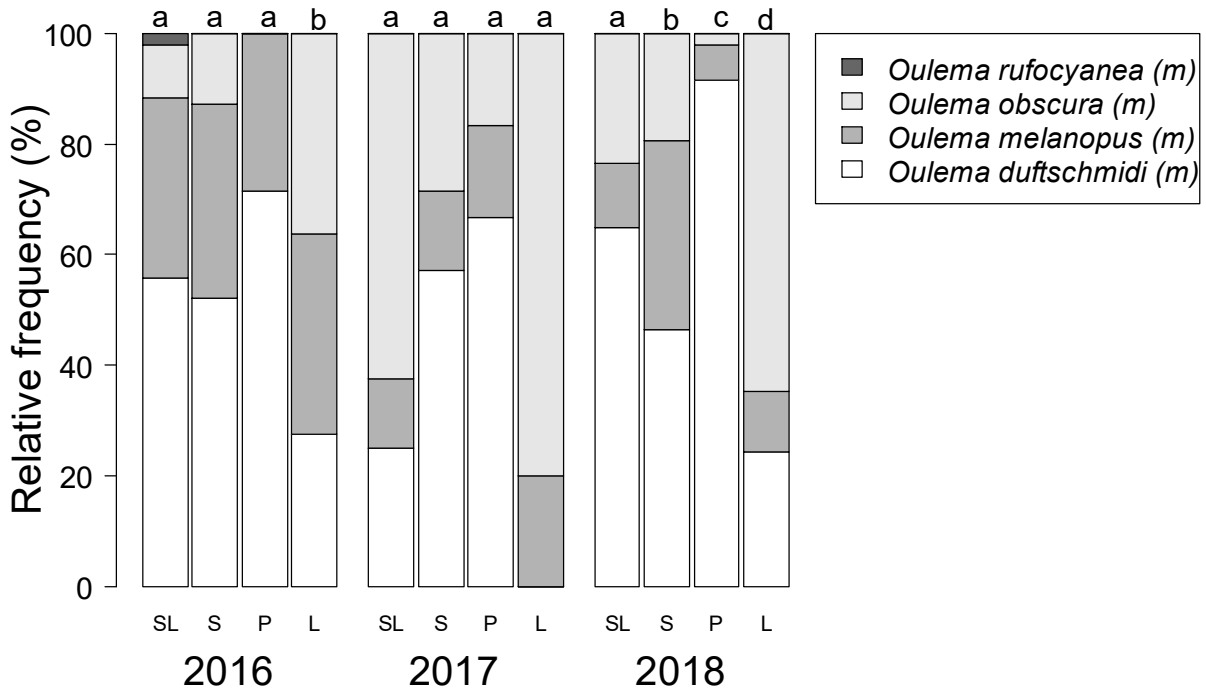


Figure 25: Relative frequency of the different Oulema species (males) for each agricultural region in Flanders during the seasons 2016 (28 fields), 2017 (30 fields) and 2018 (23 fields). Different letters above the bars point to significant differences between population distributions (Chi-squared test). SL = Sandy Loam; S = Sand; P = Polders; L = Loam.

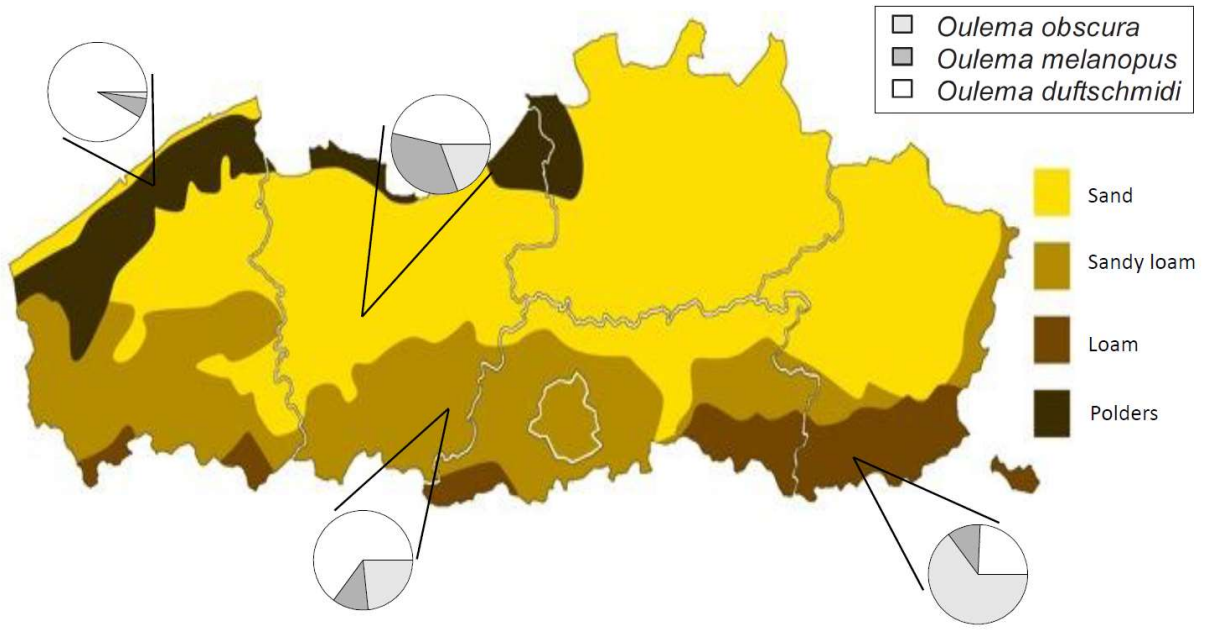


Figure 26: Geographical relative distribution of Oulema species (%, males) across agricultural regions in Flanders, 2017–2018.

Within-field distribution of *Oulema* species

Presenting the average number of CLBs per sweep and the species composition at different moments at various distances from the field edge (10, 20, 30 and 40 m) revealed significant differences in species composition depending on the sampling position (Figure 27). The species distribution at each position in the field differed significantly at April, 28 and May, 5, while no significant differences were found at the other sampling moments.

Concerning the number of CLBs, it can be seen that on average more beetles were found at 30 and 40 m (except at May, 2) within the field compared to closer to the field edge (10 or 20 m), however, these differences were never significant (p-values 0.836, 0.349, 0.073, 0.092, 0.957 and 0.072 for each date). Concerning the evolution of the number of *Oulema* adults during the growth season, following normal phenology, a gradual increase followed by a decrease was expected. However, it can be seen that the sampled population size increases until April, 21, then decreases until May, 2, and at May, 5, a steep increase in the number of *Oulema* adults was recorded. These fluctuations in number of male *Oulema* adults present in the sweep net can partially be attributed to the weather conditions during sweeping. For example, the number of male *Oulema* adults per sweep was positively correlated with the temperature (0.38, p-value = 0.044).

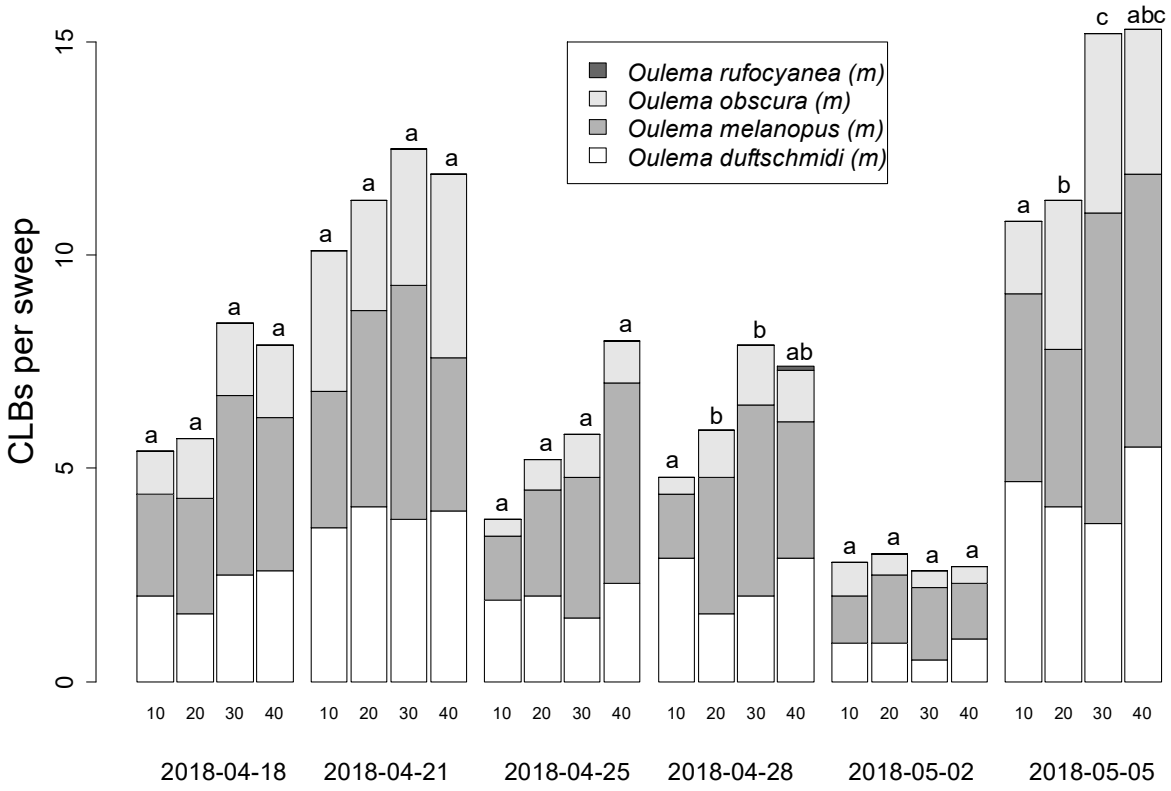


Figure 27: Average number of CLBs (males) per sweep at 10, 20, 30 or 40 m from the edge of the field at different time points during the growth season (2017–2018). Different letters per date point to significant differences in species composition between distances (Chi-squared test; comparison within dates only).

Classification based on morphometric analysis

As mentioned above, *Oulema* species determination up to species level is a challenging task due to their similar morphology. In Table 8 the average measurements (minimum and maximum) of the body length, elytra length, elytra width, antennae length, pronotum length, pronotum width and the ratios between these body parts, measured on ten individuals per species, are given.

Table 8: Average measurements (minimum–maximum) of the various body parts of different Oulema species (m = male, f = female)

	***O. duftschmidi* (m)**	***O. mel/duf/ruf* (f)**	***O. melanopus* (m)**	***O. obscura* (m)**	***O. obscura* (f)**
Body length (mm) (BL)	4.3–4.8	4.5–5.6	4.5–5.3	3.8–4.2	4.0–4.6
Elytra length (mm) (EL)	3.2–3.5	3.3–4.0	3.4–3.8	2.8–3.1	3.0–3.4
Elytra width (mm) (EW)	1.6–2.0	1.8–2.3	1.8–2.2	1.7–2.0	1.1–2.2
Antennae length (mm) (AL)	2.5–3.0	2.4–3.0	2.5–3.0	2.2–2.5	2.2–2.5
Pronotum length (mm) (PL)	0.9–1.1	1.0–1.3	1.0–1.1	0.8–1.0	0.9–1.1
Pronotum width (mm) (PW)	1.0–1.1	1.1–1.3	1.1–1.2	0.9–1.0	1.0–1.1
EL/BL	0.70–0.76	0.71–0.78	0.7–0.79	0.71–0.78	0.70–0.81
EL/EW	1.60–2.12	1.59–1.83	1.64–2.00	1.46–1.67	1.45–3.09
AL/BL	0.54–0.68	0.50–0.58	0.51–0.62	0.55–0.62	0.52–0.61
PL/PW	0.90–1.10	0.83–1.00	0.91–1.00	0.89–1.11	0.90–1.00

Using linear combinations of these measurements taken on our samples, a possible identification up to species level and a separation between genders (*O. obscura*) was verified. The first two LDA functions accounted for respectively 90.39% and 0.08% of the variance. It was clear that based on the morphological measurements, the species belonging to complex 1 (*O. melanopus, O. duftschmidi* and O*. rufocyanea*) can be separated from the species belonging to complex 2 (*O. obscura*) (Figure 28). Within both complexes, LDA was also able to capture the differences between species. Based on LOOCV, an accuracy of 78% was obtained. The first LDA function explains the biggest part of the variation (90.39%) and relies on a combination of coefficients, of which EL/BL and the length of the elytra are the most important. The contingency table (Table 9) shows that all *O. duftschmidi* beetles

were correctly predicted. Seven out of the ten *O. melanopus* individuals were correctly assigned, while the remaining three were classified as *O. duftschmidi*. Gender was classified as well. Here, two out of the ten male *O. obscura* were classified as females, whereas three female *O. obscura* beetles were classified as males and one was assigned to *O. duftschmidi*.

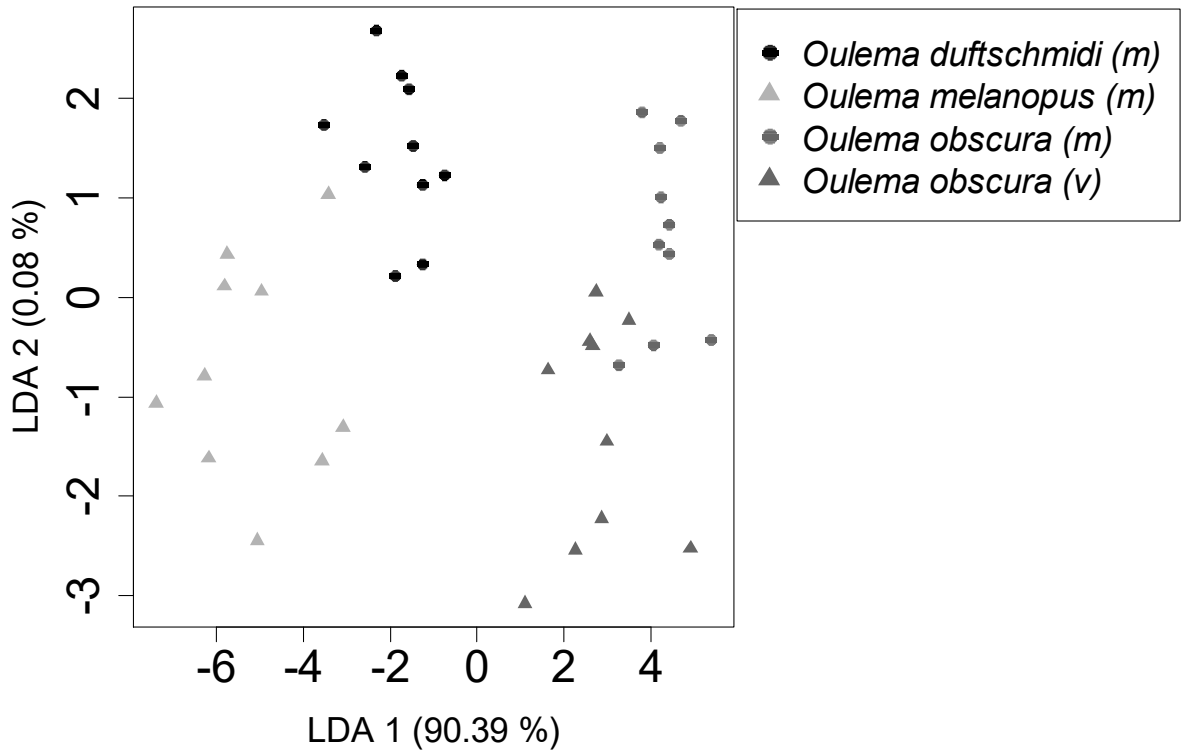


Figure 28: Linear discriminant analysis (LDA) plot of the first two discriminant functions showing the separation of the various Oulema species and gender.

Table 9: Contingency table obtained for linear discriminant analysis (LDA) with LOOCV

	O. duftschmidi (m)	***O. melanopus*** (m)	***O. obscura*** (m)	***O. obscura*** (f)
O. duftschmidi (m)	10	3	0	1
O. melanopus (m)	0	7	0	0
O. obscura (m)	0	0	8	3
O. obscura (f)	0	0	2	6

Discussion

Knowledge of the species composition is quite useful in a knowledge-based IPM context. As the impact of temperature and other variables differs between species, a different species composition could also cause a difference in phenology and therefore change the timing of a possible insecticide treatment. Concerning the distribution of the different species, we found that in Flanders both *O. melanopus* and *O. duftschmidi* appeared sympatric, which is in accordance with the hypothesis by Berti (1989) and in agreement with later empirical research from France (Chapelin-Viscardi and Maillet-Mezeray, 2015). *Oulema duftschmidi* was, during most periods, the main species in Flanders. While some authors suggest that the distribution of both *O. melanopus* and *O. duftschmidi* is similar (Bechini et al., 2013), others have found different results, supporting our findings that *O. duftschmidi* is a more abundant species in Western European countries such as Belgium (Chapelin-Viscardi and Maillet-Mezeray, 2015). The relative abundance of *O. obscura* adults in Flemish winter wheat fields was variable during the sampled period, depending on the growth season and the period within the season. The frequency of this species in the sweep net samples varied between 2.6% (April 2016) and 61.5% (first part of May 2017). Although no definite explanation can be found for this variable distribution, literature suggests a differential influence of temperature on the phenology of both complexes (Walczak, 2005). Temperature is indeed the main variable influencing insect development. Ali et al. (1977) showed that developmental threshold temperature of adult *O. obscura* is lower than that of *O. melanopus*, suggesting that development starts earlier in the growth season. Moreover, Ali et al. (1979) found differences between mortality rates for both species. From their work, it was clear that *O. obscura* showed a lower mortality compared to *O. melanopus*. Walczak (2005) also concluded that *O. obscura* was receptive to variations in temperature. This could also explain why more *O. obscura* individuals were found on 'lighter' soil types (sandy, sandy loam and loamy soils), as these soil types are more prone to variations in temperature. While *O. obscura* was frequently found in our Flemish wheat fields, no other species (*O. septentrionis* and *O. erichsonii*) from complex 2 were observed. Although these species were not found in our trial fields (winter wheat), this does not necessarily mean that these species do not occur in Flanders. It is known that these species have other preferred host plants such as oats (*Avena* spp.) or bulrushes (*Typha* spp.) for *O. septentrionis* and floating sweet-grass (*Glyceria fluitans* (L.) Brown) for *O. erichsonii*, possibly causing them to be absent in cultivated wheat fields. *Oulema duftschmidi* was found to be more abundant in the polders (heavier soil type). These soils are also closer to the North Sea, possibly subject to a microclimate. It is known that generally, this region receives less precipitation, more insolation, more wind, has cooler summers and warmer winters. These factors could influence the species composition. For example, Lesage et al. (2007) observed this species to appear more frequently in southern, Mediterranean climates. Insect development is also

influenced by other variables (besides temperature) such as radiation and humidity, which should in turn coincide with the presence of an appropriate host crop. However, more (long-term) research is needed to link species abundance with environmental factors. Species composition also shifts within the season. While growth seasons 2016 and 2018 showed clear significant differences in the species composition between the different agricultural regions, no significant differences were found in 2017, the year with the lowest CLB activity. The total number of CLBs collected in this year was lower compared to the other years, which could influence the species composition. In 2018, sampling was executed at only one time point. Therefore, caution is required when comparing these species compositions between seasons. As for the between-field distribution, on some fields, sampling took place only once within the season. Variable CLB densities between the growth seasons made adequate sampling difficult. This makes comparing relative species abundances between different years difficult.

Generally, no significant differences in sex ratio were found. While most authors found an equal distribution of both sexes (Chambon et al., 1983), others noticed clear differences (Chapelin-Viscardi and Maillet-Mezeray, 2015). Chapelin-Viscardi and Maillet-Mezeray (2015) suggests differences between both sexes could be searched in differences in food preferences, flight activity or climatic factors. As female genitalia are smaller and more fragile to handle, female individuals were not classified up to species level. Chapelin-Viscardi (2015) mentions that there should be no reason to suggest a difference in species composition between different sexes. It is known that during mating season, the males are actively searching for a partner, while the females are more passive (Chapelin-Viscardi, 2015). Therefore, more male beetles can be retrieved from sweep catches. This was also observed in their work. However, as no research has yet been executed to investigate the exact species composition between genders, to date, it cannot be excluded that other species compositions could be found for the females.

During the within-field trial in 2018, it was seen that even over a short time period, the weather conditions during sweeping influenced the number of CLBs caught per sweep, e.g. during periods with a higher temperature, beetles are more active and therefore easier to catch. This was also observed by Guttierez et al. (1974) and Stilmant (1995). When studying the within-field distribution of both complexes, it was seen that the *Oulema* distribution and composition in the centre and at the edge of the field was similar on most of the dates. This confirms the work by Ruesink and Haynes (1973) and the results from Chambon et al. (1983). Nevertheless, more research investigating this in detail on different fields with different field edges over multiple years could provide more revealing results. However, based on the results from 2018, it was concluded that the species density was higher in the centre of the field, which was also found by Reay-Jones (2010).

Finally, the dissections revealed a high variability in body measurements. This was also observed by other authors (Chapelin-Viscardi and Maillet-Mezeray, 2015; Bezděk and Baselga, 2015). Nonetheless, body measurements taken from our dissected CLBs are similar to those taken by Bezděk and Baselga (2015). A LDA analysis showed that by combining these body measurements, the complex 1 species were clearly separable from the complex 2 species. However, for obvious reasons, separation to species level within the complexes is more relevant. An accuracy of 78% was obtained for separating to species level, indicating that for an exact determination a dissection of the genitalia is still necessary.

In conclusion, it is clear that while *O. melanopus* is spread globally, in Flanders, it is not always the most abundant species. Although differences in *Oulema* species composition were found between different regions, soil types or climates, we found that *O. duftschmidi* and *O. obscura* are also commonly found in our wheat fields.

Chapter 5: CLB phenology and population dynamics in Flanders

Introduction

The phenology of *O. melanopus* has been studied and reported extensively (Gallun et al., 1967; Yun, 1967; Helgesen, 1969; Shade et al., 1970; Gage, 1972; Helgesen and Haynes, 1972; Ruppel, 1972; Wellso et al., 1972; Haynes, 1973; Casagrande et al., 1977; Lecigne and Roehrich, 1977; Logan, 1980; Haynes and Gage, 1981; Battenfield et al., 1982; Hatchett et al., 1987; Grant and Patrick, 1993; Buntin et al., 2004). Although most of the phenology of the different *Oulema* spp. is comparable, small differences do exist. An example of this is a differential influence of temperature on the life cycle of the different species. A more detailed description of the CLB ecology can be found in **Chapter 2** of this thesis. A detailed knowledge of an insect's phenology is crucial in an IPM-oriented approach. Therefore, in this chapter an overview is made of the general phenology of CLBs in Flanders and the effect of several abiotic parameters such as temperature, humidity, precipitation and crop husbandry practices on the development of CLBs was studied.

Material and methods

As phenology could differ between regions, management, soil type and surrounding, a number of fields were monitored for CLB presence during four subsequent growth seasons (2014–2015; 2015–2016; 2016–2017 and 2017–2018). A detailed description of the monitoring protocol and the location of the monitored fields can be found in **Chapter 3**.

Categorising the data

As the monitored fields laid across the main wheat growing regions in Flanders, the various soil types (loam, sandy loam, clay (in this work catalogued as 'Polder') and sand) were represented. Apart from a geographical spread, fields were selected based on differences in surroundings or management practices. Some organic fields were selected for weekly monitoring as well, to unravel the link between management and the CLBs' phenology or to estimate the effect of semi-natural elements (SNE) on the CLBs mortality/phenology. To statistically analyse the effect of the crop management on CLBs' phenology, the different parameters of the observed growers' fields were catalogued.

The variable SNE (the element that was next to the monitored field) was included in the analysis, where label '1' represents fields that had a heterogeneous (species-rich) field edge, stimulating the development of natural enemies. Examples of these habitats are flower borders and meadows. Label '2' represents biotopes such as tree rows and woody field edges. Habitats that have little effect on the presence of natural enemies or species-poor habitats such as grassland, croplands that are known to lower biodiversity are given a label '3'.

Crop rotation was included during analysis as well. Label ‘1’ is given to fields that have a previous crop that could enlarge the risk on CLBs presence in the field (monoculture wheat). Label ‘2’ is given to fields that hosted another *Poaceae* species in the previous year (maize, grassland). Fields with a value ‘3’ hosted any other crop during the previous season.

For including information about sowing date and densities, fields are categorised using variables 'sowing date' and 'sowing density'. Winter wheat fields that are sown before October, 14 are considered to be sown early (label ‘1’). From October, 15 until November, 10 is considered a normal sowing date (label ‘2’), while fields sown later than November, 11 are considered to be sown late (label ‘3’). For spring wheat, fields that are sown before March, 14 are considered to be sown early (label ‘1’). From March, 15 until April, 10 is considered a normal sowing date (label ‘2’), while fields sown later than April, 11 are considered to be sown late (label ‘3’).

High density sown fields (> 400 grains/m²) are categorised with label ‘1’, while fields sown with a density of 300–399 grains/m² are considered to follow standard sowing guidelines (label ‘2’). Densities below 299 grains/m² are considered low (label ‘3’).

Soil tillage was included as well and categorised between the influence of the choosen soil tillage technique applied on the population CLBs: label ‘1’ are fields that are managed with a no-tillage technique, while fields with a label ‘2’ are managed conventionally, i.e. ploughed and harrowed before sowing.

The last variable that was included in the analysis was 'fertilisation' (total nitrogen fertilisation in kg per hectare during the growth season). Fields that received less than 175 kg of nitrogen are categorised as receiving little fertilisation (label ‘1’). A normal fertilisation regime (label ‘2’) is considered to receive between 175 and 225 kg per year per hectare. Fields that received more than 240 kg of nitrogen fertilisation (label ‘3’) are considered to be highly manured (based on guidelines set by the Soil service of Belgium). Table 10 lists the number of fields that were included in the analysis for each parameter.

Table 10: Number of fields catagorised under the different crop husbandry practices or surroundings. Semi-natural elements (SNE): label ‘1’: flower border, meadows, ‘2’: tree rows or woody edges, ‘3’: grassland or cropland; Crop rotation: label ‘1’: monoculture wheat, ‘2’: other Poaceae species, ‘3’: other crop; Sowing date: label ‘1’: sown before October, 14, ‘2’: sown between October, 15 and November, 10, ‘3’: sown after November, 11; Sowing density: label ‘1’: sown at > 400 grains/m², ‘2’: sown at 300–399 grains/m², ‘3’: sown at > 300 grains/m²; Soil tillage: label ‘1’: no-tillage, ‘2’: regular soil tillage, i.e. ploughing and harrowing; Fertilisation: label ‘1’: < 175 kg nitrogen per ha, ‘2’: between 175 and 225 kg nitrogen per ha and ‘3’: > 240 kg nitrogen per ha

Label	SNE	Crop rotation	Sowing date	Sowing density	Soil tillage	Fertilisation
‘1’	12	4	11	23	5	13
‘2’	71	29	76	57	75	45
‘3’	38	52	10	5	-	20

More detailed information about materials and methods of the observation fields are given in **Chapter 3**. The results are presented based on week numbers, starting from January, 1. Table 11 presents the corresponding dates for each growth season.

Table 11: Corresponding dates and week number for growth seasons 2014–2015, 2015–2016, 2016–2017 and 2017–2018

Week number	2014–2015	2015–2016	2016–2017	2017–2018
15	April, 6–12	April, 11–17	April, 10–16	April, 9–15
16	April, 13–19	April, 18–24	April, 17–23	April, 16–22
17	April, 20–26	April, 25–May, 1	April, 24–30	April, 23–29
18	April, 27–May, 3	May, 2–8	May, 1–7	April, 30–May, 6
19	May, 4–10	May, 9–15	May, 8–14	May, 7–13
20	May, 11–17	May, 16–22	May, 15–21	May, 14–20
21	May, 18–24	May, 23–29	May, 22–28	May, 21–27
22	May, 25–31	May, 30–June, 5	May, 29–June, 4	May, 28–June, 3
23	June, 1–7	June, 6–12	June, 5–11	June, 4–10
24	June, 8–14	June, 13–19	June, 12–18	June, 11–17
25	June, 15–21	June, 20–26	June, 19–25	June, 18–24
26	June, 22–28	June, 27–July, 3	June, 26–July, 2	June, 25–July, 1
27	June, 29–July, 5	July, 4–10	July, 3–9	July, 2–8
28	July, 6–12	July, 11–17	July, 10–16	July, 9–15

Statistical analyses

All analyses were conducted in R (version 3.5, R Development Core Team, 2017). Since the normality and homoscedasticity assumptions of parametric tests were fulfilled, a one-way ANOVA analysis (significance level $\alpha = 0.05$) was performed to test whether the number of eggs or larvae differed according to the specific management practice analysed. In case there were significant differences, Tukey's HSD range test was executed to detect which groups significantly differed. To gain insight into the potential relationship between several key CLB life events, Pearson correlation coefficients were calculated and tested for their significance at a significance level of $\alpha = 0.05$.

Results

CLB egg and larval development in Flanders

Figure 29 presents the average number of eggs (left) and larvae (right) monitored during growth seasons 2014–2015, 2015–2016, 2016–2017 and 2017–2018. Egg peak densities varied depending on the growth season. The observed egg peak densities in Flemish wheat fields varied from an average of 0.06 (2016–2017) to 0.25 (2017–2018) eggs per tiller and can be considered low. Observations started from crop growth stage BBCH 32 (second node). The first oviposition was only observed in growth season 2015–2016, during week 15.

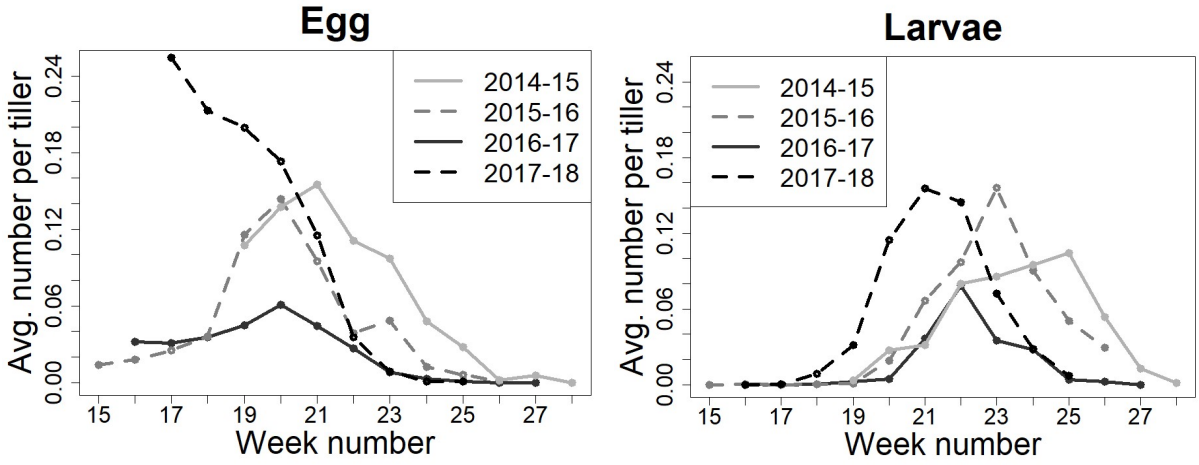


Figure 29: Weekly changes in egg (left) and larval (right) densities (represented by the average number per tiller) of Oulema species in winter wheat during growth seasons 2014–2015, 2015–2016, 2016–2017 and 2017–2018.

Egg densities reached a peak density in week 21 (2015), 20 (2015–2016 and 2016–2017) and earlier for 2018. Peak heights differed depending on the growth season. Densities reached in 2016–2017 were very low at 0.06 eggs per tiller. Monitored eggs in 2014–2015 and 2015–2016 reached a similar level at 0.15 eggs per tiller, while in 2017–2018, eggs reached a peak density of more than 0.25 eggs per tiller. Last eggs were found in week 24 (2016–2017 and 2017–2018) or 26 (2014–2015 and 2015–2016). The total duration of the egg laying period could only be estimated in 2015–2016 to 10 weeks. On average, CLB egg densities of 0.07, 0.05, 0.03 and 0.10 eggs per tiller were reached in growth seasons 2014–2015, 2015–2016, 2016–2017 and 2017–2018 respectively.

Larval activity was observed from week 17 (2017–2018) or week 19 (2014–2015, 2015–2016 and 2016–2017). Peak dates varied from year to year. Peak larval densities were reached in week 25 (2014–2015), 23 (2015–2016), 22 (2016–2017) and week 21 in 2017–2018. Absolute peak larval densities (average per tiller) varied from 0.08 larvae per tiller to 0.16 larvae per tiller (2015–2016 and 2017–2018). Larvae were active on the plants until week 25 (2016–2017 and 2017–2018) or 28 (2014–2015). Larval end date was not observed in growth season 2015–2016. Total duration of the larval activity on the plants varied from 7 weeks (2016–2017), to 10 weeks (2014–2015). In 2017–2018, larvae were 9 weeks active

on the monitored fields. On average, CLB larval densities of 0.05, 0.04, 0.02 and 0.06 larvae per tiller were reached in growth seasons 2014–2015, 2015–2016, 2016–2017 and 2017–2018, respectively.

Observed peak dates of the eggs (day number from January, 1) were positively correlated (Pearson correlation) with the observed peak date of the larvae, later on in the season (r = 0.414, n = 110, p-value < 0.001). Figure 30 shows this relationship. In this graph, a linear regression line was added to the graph (R^2 = 0.16). This indicates that when the egg peak date is observed on day 121 (May, 01), the larval peak date is estimated on day 144 (May, 24).

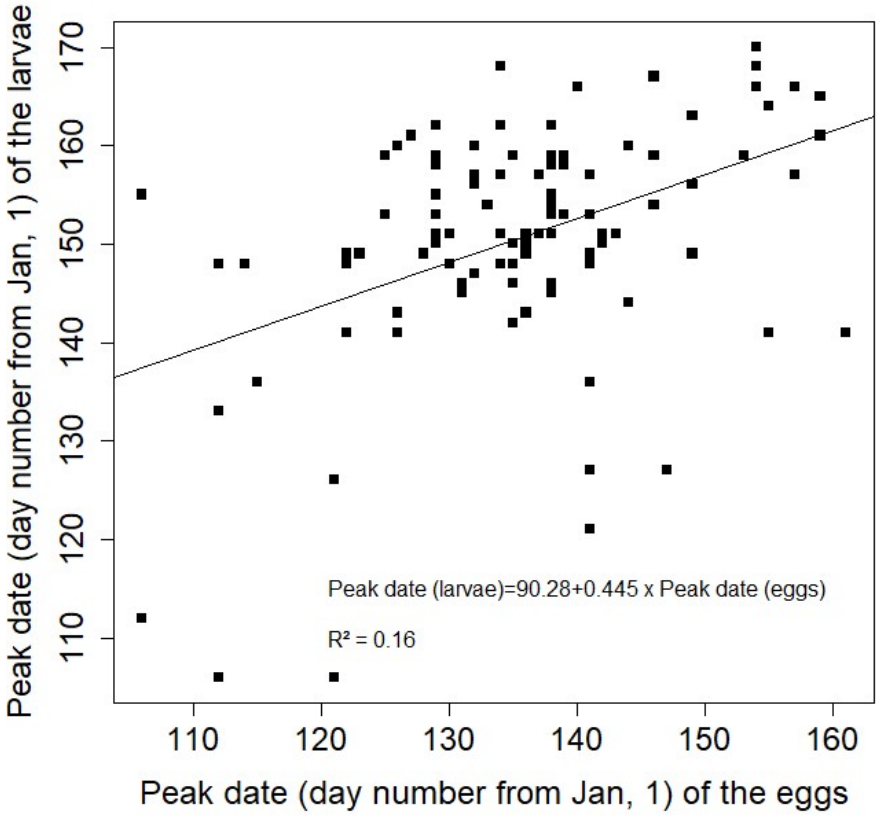


Figure 30: Relationship between the observed peak dates of the eggs (day numbers from January, 1) and the observed peak dates of the larvae.

Observed peak heights of the eggs (number per tiller) were also positively correlated (Pearson correlation) with the observed peak height of the larvae, later on in the season (r = 0.60, n = 113, p-value < 0.001). In Figure 30, a linear regression line was added to the graph (R^2 = 0.35). This linear regression line indicate that when an egg height of 0.5 eggs per tiller is observed, a larval peak height of 0.28 larvae per tiller can be expected.

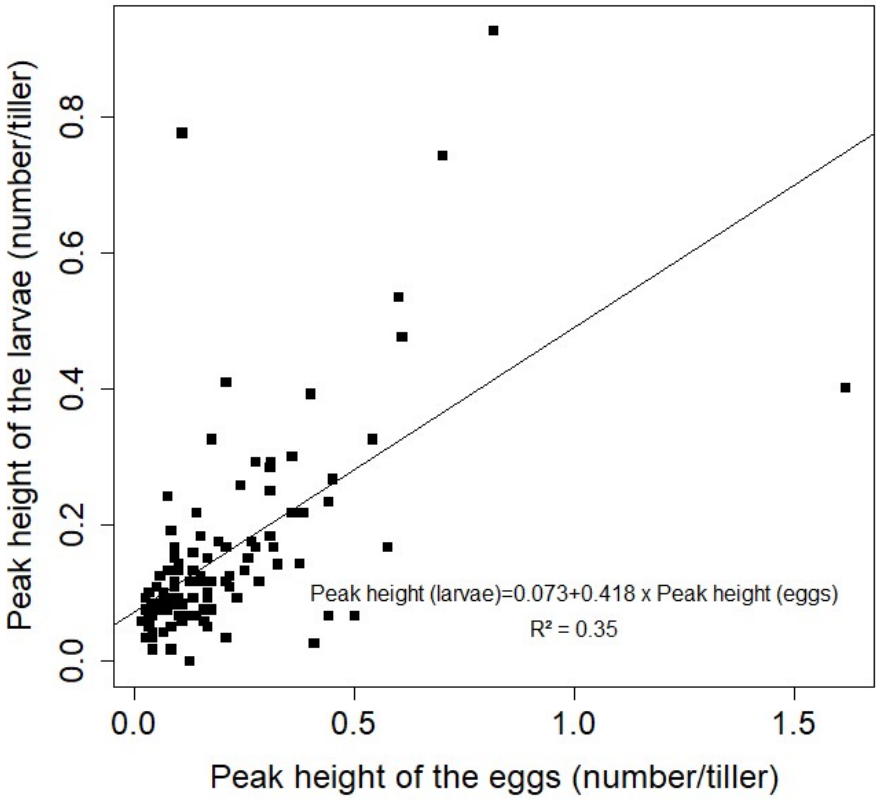


Figure 31: Relationship between the observed peak heights of the eggs (number per tiller) and the observed peak heights of the larvae.

Figure 32 presents the relationship between the observed oviposition starting dates (day numbers from January, 1) and the total observed duration of the larval presence in the field (days; r = 0.39, n = 114, p-value < 0.001; Figure 30). In this graph, a linear regression line was added to the graph (R^2 = 0.15). This regression indicates that when the first eggs are observed on day 121 (May, 01 (in 2019)), the larvae can be expected to be active in the field for 36 days (days from start oviposition to the pupation of the last larvae).

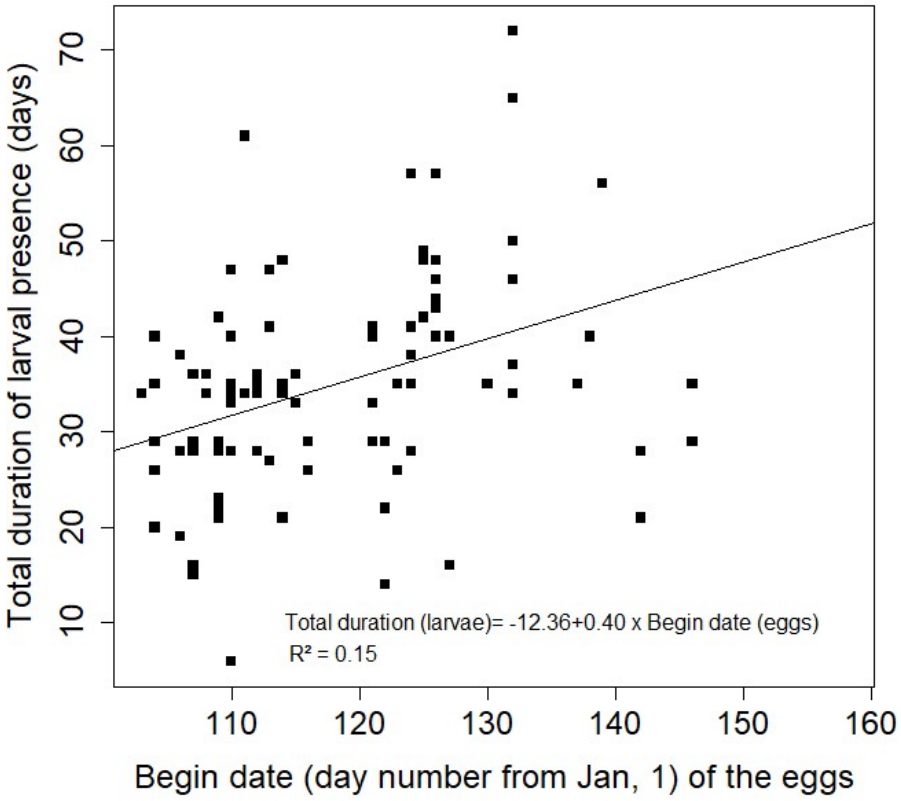


Figure 32: Relationship between the observed oviposition starting dates (day numbers from January, 1) and the total duration of the observed larval presences in the field.

Influence of temperature on the phenology of CLBs

Influence of temperature during the observed growth seasons showed to be a crucial parameter determining the phenology of CLBs. Temperatures varied depending on the season (Chapter 3). Therefore, correlations between dates at which key events in the egg and larvae phenology took place with average monthly temperatures during the monitored growth season were determined (Table 12). Some key events that were included in this analysis are: start date (eGH.begin and lGH.begin for respectively the oviposition starting dates and larvae begin dates), peak dates (eGH.peak and lGH.peak), end date (eGH.end and lGH.end), total observed duration of population development (eGH.duration and lGH.duration) and observed peak heights (eGH.PH and lGH.PH).

Table 12: Correlations between average monthly temperatures (°C) and (1) CLB oviposition starting dates (eGH.start), peak dates (eGH.peak), end dates (eGH.end), total duration of population development (eGH.duration) and peak heights (eGH.PH); (2) CLB larvae start dates (lGH.start), peak dates (lGH.peak), end dates (lGH.end), total duration of population development (lGH.duration) and peak heights (lGH.PH) with p-values < 0.05 (*); < 0.01 (**) and < 0.001 (***). Values in red indicate a significant negative correlation, while correlations in green indicate significant positive correlations. Green cells indicate the developmental 'gap' of four to five weeks between the eggs and the larvae

	Feb_Temp	Mar_Temp	Apr_Temp	May_Temp	Jun_Temp
eGH.start	-0.252**	-0.202*	0.021	-0.418***	-0.301
eGH.peak	0.099	-0.007	-0.247*	-0.236*	-0.118
eGH.end	0.161	0.002	-0.356***	-0.502***	-0.340***
eGH.duration	0.390***	0.129	-0.343***	0.000	-0.023***
eGH.PH	-0.314***	-0.198*	0.341***	0.132	0.026***
lGH.start	0.395***	0.177	-0.526***	-0.314***	-0.157***
lGH.peak	0.192	-0.140	-0.403***	-0.478***	-0.444***
lGH.end	0.103	-0.103	-0.405***	-0.737***	-0.558***
lGH.duration	-0.099	-0.190*	-0.135	-0.601***	-0.495***
lGH.PH	-0.267**	-0.243**	0.230*	0.040	-0.088

In general, correlations between observed starts of the CLB egg and larval development and average monthly temperatures are negative. This means that the warmer these months are, the earlier the eggs or larvae were observed in the fields (based on weather data presented in **Chapter 3**). As adults generally start laying eggs from week 15 (second week of April), correlations with average temperature during later months (May and June) can be considered as biologically irrelevant.

As the adults start oviposition from week 17–19 (last week of April–second week of May), temperatures in June can be considered to be irrelevant for influencing the start of the larvae. Significant negative correlations of -0.526 (average temperature in April, p-value < 0.001) and -0.314 (average temperature in May, p-value < 0.001) show that the warmer these months were, the earlier the larvae were observed in the fields. The developmental 'gap' of four to five weeks between the egg and larval population can be extracted from these correlations as well (cells highlighted in green).

Peak dates and densities were generally negatively correlated with average monthly temperatures. This is especially visible for the observed larval peak date, where the warmer April, May or June was, the earlier the peak was observed. For example (Figure 18–Figure 21), April and May were warmer than average in 2018, while in 2016 and 2017 only May was warmer than average. In 2015, temperatures were normal in this period of the year. Correspondingly, larval peak height (Figure 29) was observed earliest in 2018, followed by 2017, 2016 and 2015.

Egg peak height can be influenced by temperatures in February. A significant negative correlation (-0.314, p-value < 0.001) suggests that the warmer this month is, the lower the observed egg peak will be. From the moment the beetles start laying eggs, these correlations shift to a significant positive correlation (0.341, p-value < 0.001), suggesting that a warm April can cause a higher egg peak density.

The end of the CLB egg and larval population seemed influenced by temperature as well, with clear significant negative correlations with the egg and larval population. The warmer April, May or June was, the earlier the last eggs or larvae were found in the observed fields. This is also found in the correlation between temperature and population development duration where significant negative correlations with the larvae population (-0.601 in May, p-value < 0.001 and -0.495 in June, p-value < 0.001) suggest a faster development in warmer months.

Effect of crop husbandry practices or surrounding on CLB development

No significant links were found between the sowing date and the different variables that described the population development of the eggs or the larvae. Also, no significant links were found between the different tillage techniques of the monitored fields and the egg or larvae development (a detail of the statistics can be found in **Appendix 2**).

At the same time, a one-way ANOVA analysis pointed out that significant differences could be found between different close-by SNEs, surrounding the field and the egg peak height ($F(2, 107) = 3.948$, p-value = 0.022). A post-hoc analysis pointed out that a flower border or meadow significantly increased the observed peak height (compared to monitored fields with woody edges or tree lines).

The previous crop type influenced the observed peak height of the eggs significantly as well ($F(2, 74) = 9.767$, p-value < 0.001). Especially monoculture wheat caused a significant increase in egg peak height, whereas other crops within the family of the *Poaceae* or other previous crop types gave no significant increase in egg peak height.

The observed egg peak date was significantly affected by sowing density ($F(2,62) = 7.246$, p-value = 0.001). A post-hoc analysis indicated that over the years, the egg peak date is observed significantly later on fields with a higher sowing density (sown at > 400 grains/m²), compared to a normal sowing density (sown at 300–399 grains/m²). An example of this significant link is graphed in Figure 33.

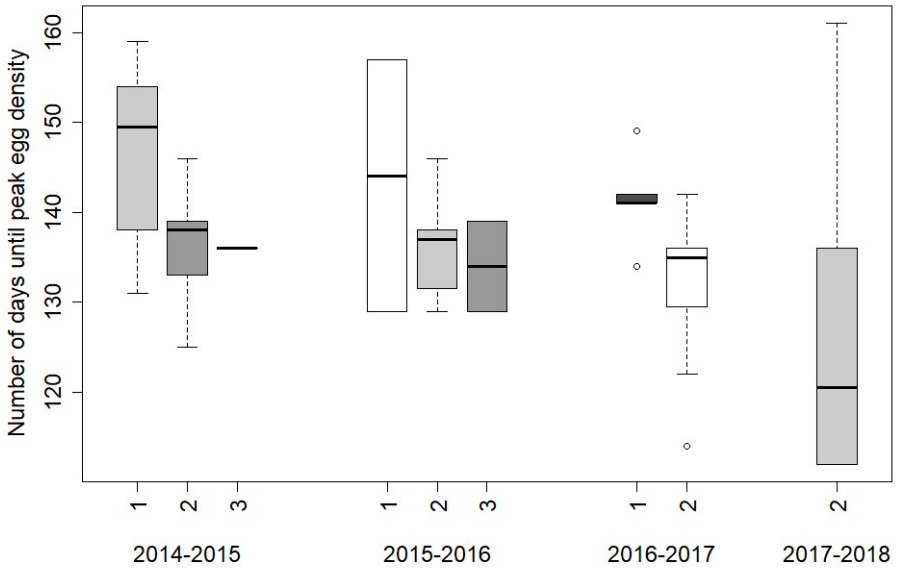


Figure 33: Effect of different sowing densities (value '1': sown at > 400 grains/m² (23 fields), '2': sown at 300–399 grains/m² (57 fields), '3': sown at > 300 grains/m² (5 fields)) on the observed egg peak dates for all monitored growth seasons (2014–2015, 2015–2016, 2016–2017 and 2017–2018).

Investigating the effect of fertilisation with the observed egg and larval development, we found that over all the monitored years, the egg peak date was observed later in fields that received the least nitrogen fertilisation (< 175 kg nitrogen per ha, compared to a regime in which plants received between 175 and 225 kg nitrogen per ha) ($F(2,54) = 3.437$, p-value = 0.039).

No clear links were found with fertilisation and observed larval development. The results of the one-way ANOVA analysis of all crop husbandry practices in relation to the egg and larval development can be found in **Appendix 2**.

Discussion

CLB egg and larval development differed between all monitored growth seasons. Nonetheless, some findings are in agreement with older Belgian research (Stilmant, 1995). In his study, Stilmant found that larval activity starts from the last week of April (week 17), while the egg peak took place in week 19 (middle of May). The larval peak on the two fields he monitored in his work, took place during the last week of May (week 21). Nevertheless, the number of observations done by Stilmant was limited. We observed some variation on this peak date. Nonetheless, in 2014–2015, the observed peak date closely matched the peak date mentioned for Belgium by Stilmant in his work. The first oviposition was not observed in all seasons due to (extreme) varying temperatures (**Chapter 3**), which shifted the population development.

Other years though, the larval peak date was observed later, up to week 25 (third week of June). This seems to be significantly influenced by a leading year-effect. A correlation analysis pointed out that this year-effect is mainly a temperature effect. Many authors have already proven the link between CLB as poikilothermic insect organism and temperature (Ali et al., 1977, 1979; Philips et al., 2012; Evans et al., 2014). It is clear that average monthly temperatures could be of significance to model development. However, as not all months have an equal influence on key moments such as start of population build up (egg or larval), peak date, peak height and date of last activity (end of population in the field), GDD do not always perform best. Indeed, these models rely on a sum of temperatures from a biofix date (January, 1 in most cases) until the date at which a certain life stage occurs. It is clear that these models can be improved by selecting only these months that have a significant influence.

Peak densities found during the four growth seasons observed, are considered low. Comparing our observed peak densities of the CLB eggs with common thresholds of 0.5 eggs per tiller (Tschumi, 2015), it is clear that with an average observed peak density of 0.25 eggs per tiller, a curative chemical treatment was not required. Larval densities remained low as well (thresholds vary from 0.4 (Buntin et al., 2004) to 2.5 larvae per tiller (Chapelin-Viscardi and Maillet-Mezeray, 2015)), and never reached economical threshold levels. Nonetheless, on field level, caution still is required as peak densities vary and a possible break through the economic threshold is surely possible at an individual field level.

The presence of a flower border or meadow increased the observed egg peak height significantly. It is known that CLBs overwinter at the edge of the field. Therefore, it is possible that these SNEs also decrease the mortality of CLBs by providing alternative sheltering. Although the effect of crop rotation has not yet been investigated for CLBs specifically, it is already known that rotating with different crops has many benefits on the long term. Our study pointed out that monoculture cropping of wheat increased the observed egg peak height. Beetles that overwinter nearby fields with wheat in

monoculture, surely have a potential benefit as they do not have to migrate to other fields. Finally, the effect of fertilisation on CLB presence was also investigated. Our study was only able to link fertilisation with the egg peak date, where a higher nitrogen fertilisation caused a delay of the egg population. Nitrogen should increase the leaves' attractiveness towards pest insects, as it reduces tissue toughness (Wait et al., 1998; Kerpel et al., 2006).

Although promising results were retrieved from the large-scale monitoring trials, more research studying the impact of crop husbandry practices on CLB development is necessary. The monitored fields in our study were not directed in their crop management, which caused a multitude of differences in applied crop management practices. While our study was able to study the phenology of CLBs and correlate these data with temperature and relative humidity, the effect of each crop husbandry practice individually still needs further investigation.

The influence of other abiotic parameters was not significant in this study. While literature suggests an effect of rainfall, wind and relative humidity on CLB presence, variability on these parameters appeared to be too high during our trials to make statements about the effect of these individual parameters on CLB development.

In conclusion, our study shows that CLBs in Flanders are an occasional pest insect and that monitoring is key for making economical decisions. Comparing observed pest densities with economic thresholds will enable the growers to spray only when necessary. To observe the larval phenology in the field and determine the need and optimal timing of an insecticide application, monitoring should start from week 20 (peak eggs) until week 25 (peak larvae). However, as temperature influences this peak date significantly, monitoring during these weeks can help to estimate the pest pressure in the monitored field and make guided decisions concerning a treatment. For example, temperatures in April, 2018 were abnormally high (**Chapter 3**), which resulted in a shift in the phenology. This would explain why monitoring started too late to observe the peak egg density.

Chapter 6: Development of predictive models for Oulema species in winter wheat[5]

Introduction: existing GDD models to predict CLB life stages

There is a more than 40-year tradition of modelling wheat, CLBs and natural enemy interactions (Coulman et al., 1972; Casagrande et al., 1976; Guppy and Harcourt, 1978; Philips et al., 2012). However, the use of such prediction models in plant protection is scarce, mainly because of their insufficient forecast accuracy. The most important problems in modelling CLBs and antagonist population dynamics have to do with describing the inter-field dynamics, estimation of mortality rates and survival, which are influenced by several variables (Helgesen and Haynes, 1972). In order to develop more accurate prediction models, a good understanding of the population dynamics, both spatially and temporally, is critically important. Weather conditions, crop husbandry practices and landscape management (e.g. surrounding vegetation) are important factors affecting the tritrophic interactions between plants, CLBs and their natural enemies (Haynes and Gage, 1981). A more detailed description of prediction models for CLBs can be found in **Chapter 2** of this thesis.

Material and methods

Calculations in this chapter were executed on a dataset including the monitoring data (gathered with a protocol described in **Chapter 3**), weather data and management data from each monitored field. Daily weather data included maximum, minimum and mean temperature, precipitation and/or relative humidity for the regression models. All analyses were conducted in R (version 3.5, R Development Core Team, 2017).

To determine the length and starting time of the periods in which environmental variables were associated with annual fluctuations of CLB eggs and larvae, the window-pane methodology was adopted. The concept underlying window-pane analysis is the specification of a time window of predefined length or duration and the construction of summary environmental variables (e.g., means) for the specified window. This time window is moved along the total time frame of interest (e.g., a year or a growth season) in daily increments, so that the environmental data from the entire time frame are ultimately considered in the data analysis (Kriss et al., 2010). For this study a window length of 25 days was chosen. The first window began on January, 1 (time 0) and ended on January, 25, the second window began on January, 2 and ended on January, 26, etc. until the end of June. So, two successive windows share all but one day of data. For each 25-day window, summary variables of

[5] Adapted from an accepted publication: Van De Vijver E., S. Landschoot, G. Smagghe, B. De Baets, F. Temmerman, J. Dillen, and G. Haesaert. 2018. Potentials and limitations of a growing degree day approach to predict the phenology of cereal leaf beetles. Environ. Entomol. 47(4): 1039–1046.

temperature (sum), rainfall (sum) and relative humidity (mean) were calculated and the correlation with the date of the first CLB egg or larvae and the peak dates were calculated. Based on this window-pane methodology, the variables with the highest correlation with a certain life stage and with a low mutual correlation (i.e. a low correlation between the predictor variables) were chosen. So, the variable selection was based on the correlation analysis.

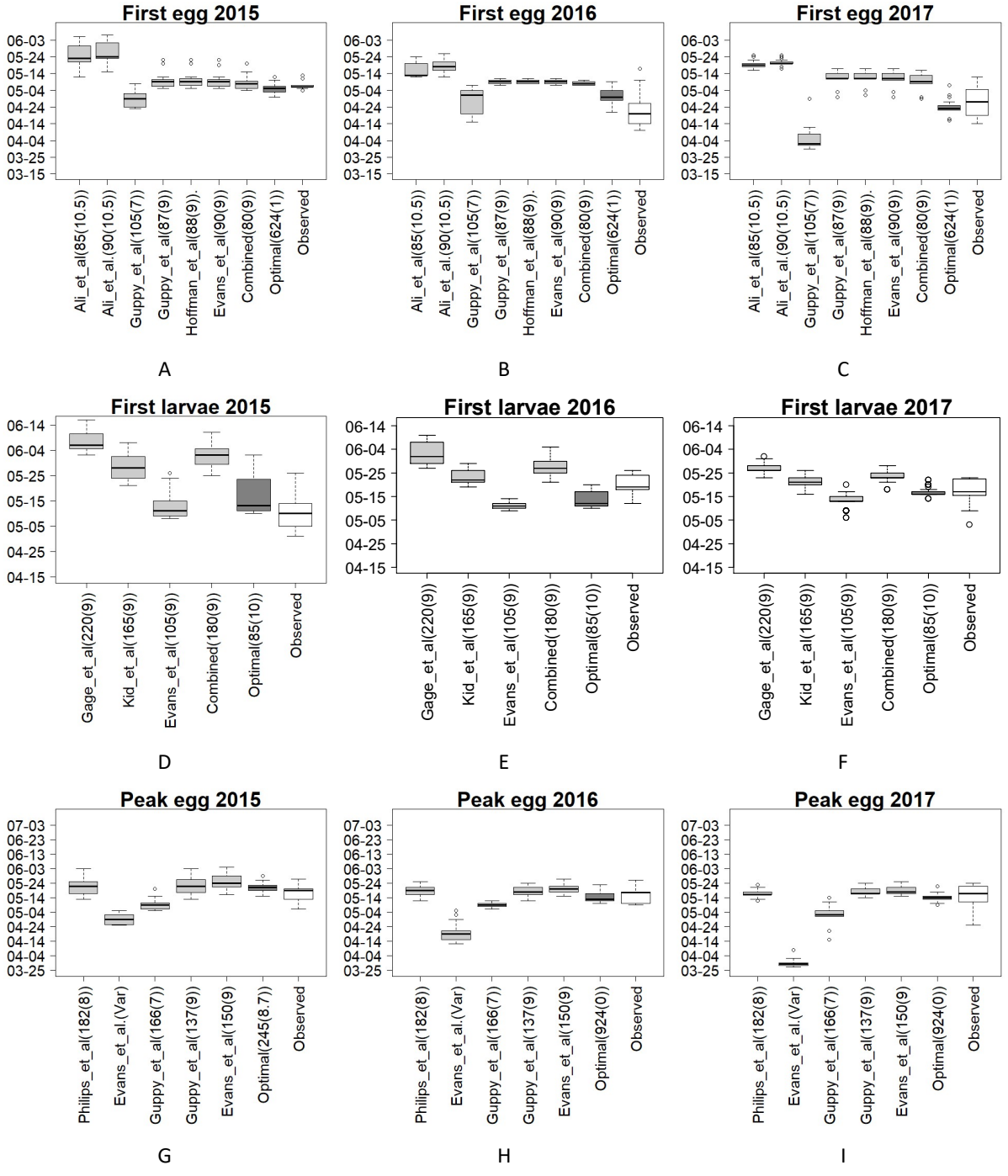
To construct prediction models for the occurrence of CLB eggs and larvae, we used an ordinary multiple linear regression approach and a ridge regression approach. Ordinary multiple linear regression is the simplest and best-known metric regression model. This modelling approach involves a minimisation of the residual sum of squares. Yet, it is known that least-squares minimisation in multiple linear regression may not always provide accurate predictions for a number of reasons, such as a lack of robustness to outliers and missing mechanisms for handling multicollinearity and controlling the complexity in the presence of many predictor variables. Ridge regression offers a solution to the last two problems by shrinking the regression coefficients towards zero. As such, a penalised residual sum of squares is minimised (Hastie et al., 2008). Regression trees were used as a third type of prediction model, which allows for the construction of non-linear functions, unlike the two former regression techniques. Trees are built using a process known as binary recursive partitioning. The algorithm recursively splits the data into two groups based on a splitting rule. The split that maximises the reduction in impurity is chosen, the data set split and the process repeated. Splitting continues until the terminal nodes are too small or too few to be split. The partitioning intends to increase the homogeneity of the two resulting subsets or nodes, based on the response variable. The partitioning stops when no splitting rule can improve the homogeneity of the nodes significantly (Hastie et al., 2008).

To validate the models, two versions of cross-validation (CV) were considered. The first version involved a standard random 10-fold cross-validation for which the data were randomly subdivided into ten parts. Subsequently, ten iterations of model calibration and validation were performed, also known as training and testing, leaving out one particular fold for testing in each iteration, while using the remaining nine folds for training. The second evaluation strategy was a more specific cross-year cross-location (CYCL) validation strategy proposed in Landschoot et al. (2012). This strategy allows to obtain an unbiased estimate of the model performance for future years and new locations.

Results

Validation of the GDD models

In Figure 34 the variability of predicted dates for the first CLB egg, the first CLB larvae, the egg and larvae peak are shown together with the dates on which the first eggs, first larvae or larvae peak were observed. These boxplots give a first indication of the model performance. It is clear that some models from literature do not succeed in predicting the various life stages within an acceptable margin of error (clearly different boxes), necessary for being suitable as a tool for growers. However, even if two boxes are similar this does not guarantee that the observations are correctly predicted.



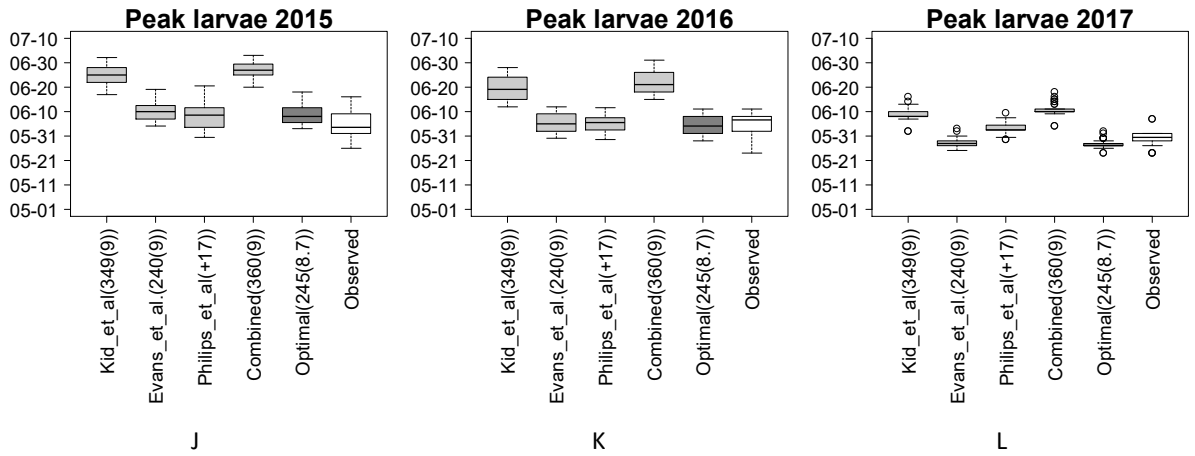


Figure 34: Variation in predicted (grey) and observed (white) dates of the first CLB eggs (A–C), first larvae (D–F), egg peak (G–I) and peak larvae (J–L) in the monitored fields. The dark grey box represents the variation of the predicted dates using a GDD threshold of 624 and a T_{base} of 1 °C for the first egg, a GDD threshold of 85 and a T_{base} of 10 °C for the first larvae, a GDD threshold of 924 and a T_{base} of 0 °C for the egg peak, and a GDD threshold of 245 and a T_{base} of 8.7 °C for the peak larvae. The specific threshold and base temperature are mentioned for each author between parentheses.

To gain insight in the predictive performance, the mean absolute error (MAE) was calculated. Furthermore, based on these observations and temperature data, similar to literature, the base temperatures and GDD thresholds resulting in the best fit for our data were determined. So, for a set of base temperatures, ranging from 0 °C until 10 °C (with jumps of 1 °C), the average GDD threshold was determined. In a next step, the predictive performance of the thresholds associated with each base temperature was calculated. The GDD thresholds and base temperatures listed in Table 13–Table 16 are the values resulting in the lowest MAE. On average, the first eggs in Belgium were observed at 624 GDD (with a base temperature of 1 °C) and the egg peak was observed at 924 GDD (with a base temperature of 0 °C). However, the MAEs were still eight and six days, respectively (Table 13 and Table 15). The best prediction for the first and peak larvae were obtained for a 85 GGD threshold and a base temperature of 10 °C) and a 245 GDD threshold and a base temperature of 8.7 °C, respectively. The MAEs were six days and five days, respectively (Table 14 and 16). The predicted dates vs. the observed dates of the first egg, peak egg, first larvae and peak larvae are represented in Figure 35. The dots are scattered randomly around the line $x = y$ and do not follow an increasing trend illustrating the poor predictive power of the models.

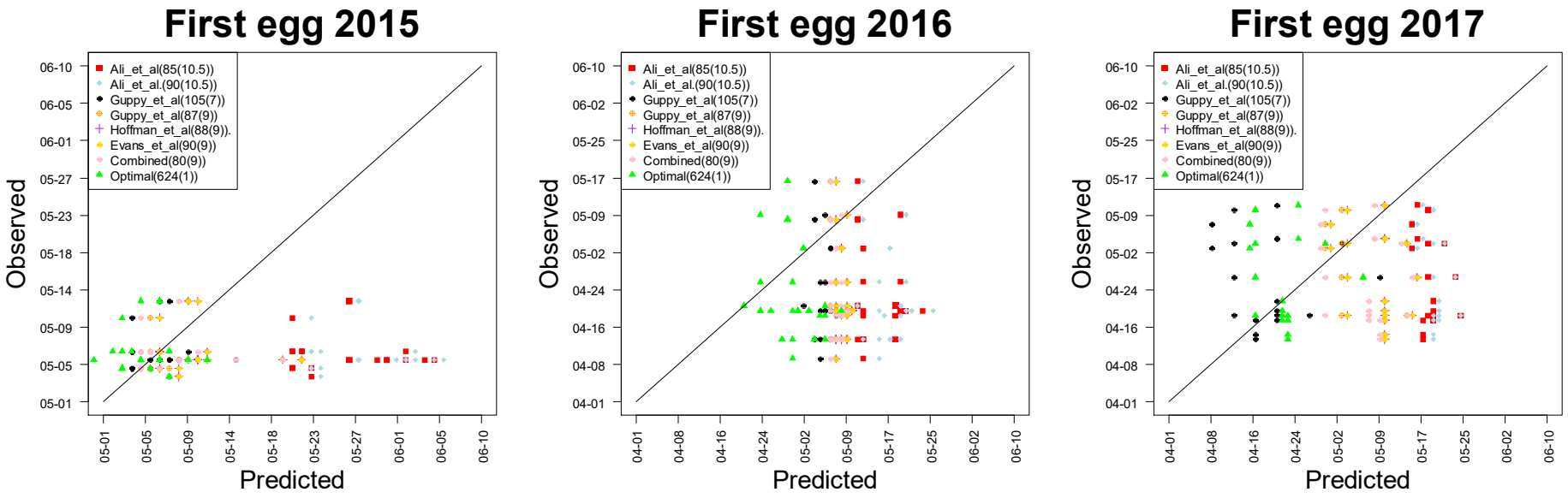
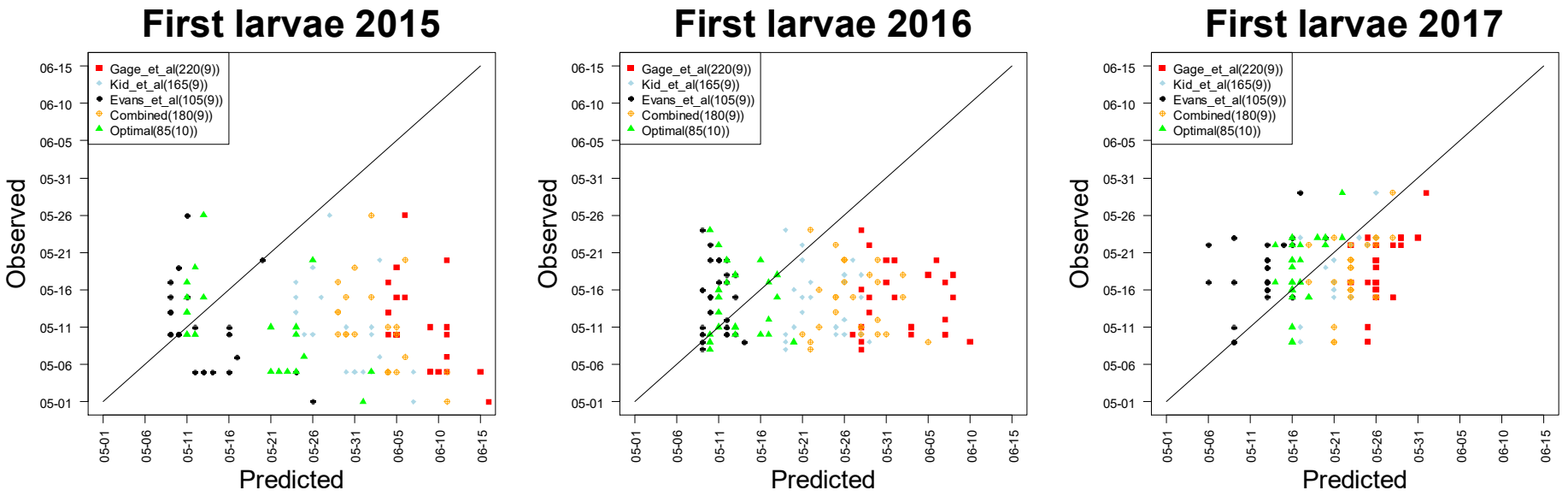


Figure 35: Observed vs. Predicted dates of the first egg (A, B, C), first larvae (D, E, F), egg peak (G, H, I) and larvae peak (J, K, L) during 2015, 2016 and 2017 with the different GDD models from literature and the optimal GDD temperature and threshold based on our data. Points on the line y=x are perfectly predicted, above this line are predicted too early and below this line are predicted too late. The specific threshold and base temperature are mentioned for each author between parentheses.



D E F

Figure 35: (continued) Observed vs. Predicted dates of the first egg (A, B, C), first larvae (D, E, F), egg peak (G, H, I) and larvae peak (J, K, L) during 2015, 2016 and 2017 with the different GDD models from literature and the optimal GDD temperature and threshold based on our data. Points on the line y=x are perfectly predicted, above this line are predicted too early and below this line are predicted too late. The specific threshold and base temperature are mentioned for each author between parentheses.

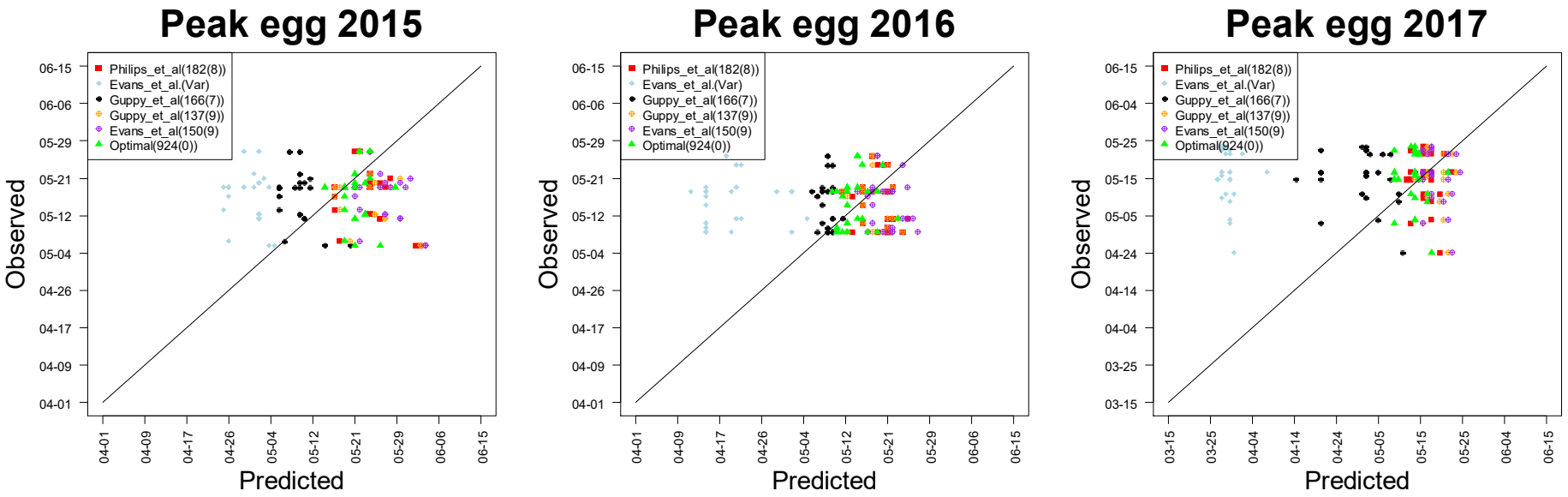
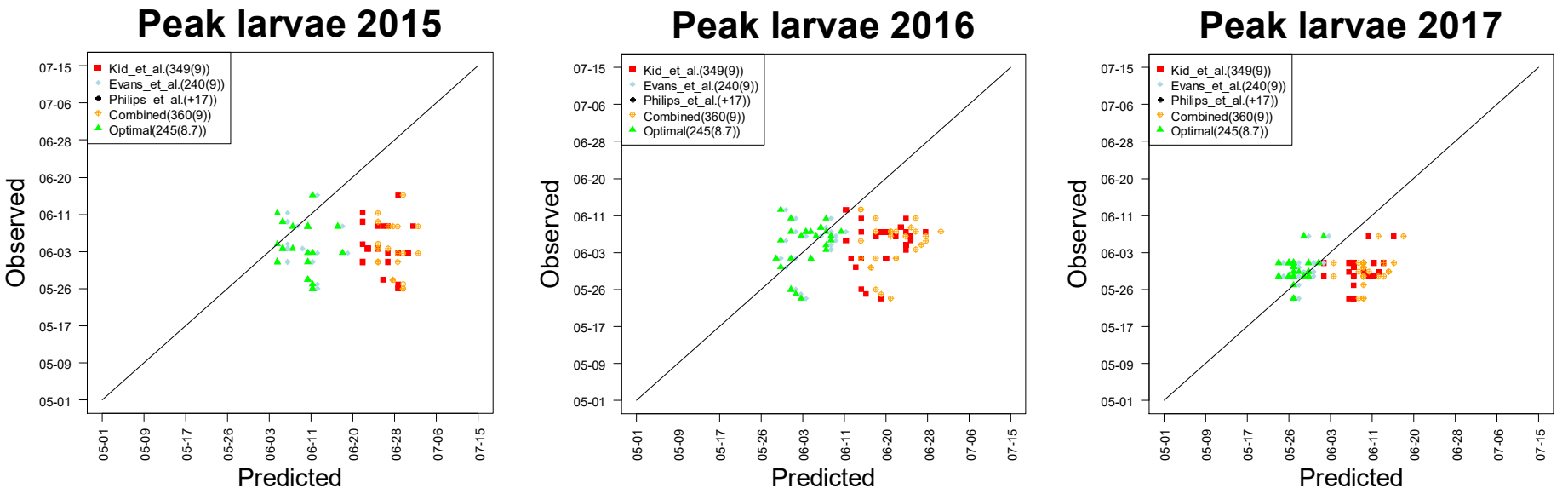


Figure 35: (continued) Observed vs. Predicted dates of the first egg (A, B, C), first larvae (D, E, F), egg peak (G, H, I) and larvae peak (J, K, L) during 2015, 2016 and 2017 with the different GDD models from literature and the optimal GDD temperature and threshold based on our data. Points on the line y=x are perfectly predicted, above this line are predicted too early and below this line are predicted too late. The specific threshold and base temperature are mentioned for each author between parentheses.



J K L

Figure 35: (continued) Observed vs. Predicted dates of the first egg (A, B, C), first larvae (D, E, F), egg peak (G, H, I) and larvae peak (J, K, L) during 2015, 2016 and 2017 with the different GDD models from literature and the optimal GDD temperature and threshold based on our data. Points on the line y=x are perfectly predicted, above this line are predicted too early and below this line are predicted too late. The specific threshold and base temperature are mentioned for each author between parentheses.

Table 17 lists the correlations between the predicted and observed values. None of these correlations appears to be significant. Furthermore, note that the standard deviations of the date of the first egg (Table 13), egg peak (Table 15), first larvae (Table 14) and peak larvae (Table 16) are 9.21 days, 6.18 days, 5.74 days and 5.58 days, respectively. These values are in line with the MAEs, and for a good model we expected MAEs to be considerably lower than the standard deviation. So, it turned out that a GDD approach alone is not sufficient to predict CLB populations in Flanders. A more detailed study is necessary to map the driving factors determining the CLB populations.

Table 13: MAEs of the different GDD models predicting the date of the first eggs. The specific threshold and base temperature are mentioned between parentheses. 'C' represents a combined model, based on the three modelled years

	Ali et al. (85(10.5))	Ali et al. (90(10.5))	Guppy et al. (105(7))	Guppy et al. (87(9))	Hoffman et al. (88(9))	Evans et al. (90(9))	Combined (80(9))	Optimal (624(1))
2015	19.58	21.08	8.29	3.96	4.13	3.92	3.46	3.25
2016	24.14	26.00	12.41	17.66	17.59	9.03	16.86	12.03
2017	22.32	23.32	23.21	14.26	14.21	14.05	13.37	9.37
C	22.14	23.65	10.24	12.19	12.21	12.13	11.47	8.40

Table 14: MAEs of the different GDD models predicting the date of the first larvae. The specific threshold and base temperature are mentioned between parentheses. 'C' represents a combined model, based on the three modelled years

	Gage et al. (220(9))	Kid et al. (165(9))	Evans et al. (105(9))	Combined (180(9))	Optimal (85(10))
2015	28.26	18.48	7.00	22.91	10.83
2016	18.19	8.96	4.81	12.52	4.59
2017	8.23	3.70	5.60	5.43	3.03
C	17.35	9.73	5.74	12.85	5.80

Table 15: MAEs of the different GDD models predicting the date of the egg peak. The specific threshold and base temperature are mentioned between parentheses. 'C' represents a combined model, based on the three modelled years

	Philips et al. (182(8))	Evans et al. (Var)	Guppy et al. (166(7))	Guppy et al. (137(9))	Evans et al. (150(9)	Optimal (924(0))
2015	7.95	16.82	9.27	8.55	10.18	5.64
2016	6.46	25.38	6.77	6.27	6.92	4.81
2017	5.88	46.52	15.28	5.96	6.32	6.80
C	6.71	30.04	10.44	6.85	7.70	5.74

Table 16: MAEs of the different GDD models predicting the date of the larvae peak. The specific threshold and base temperature are mentioned between parentheses. 'C' represents a combined model, based on the three modelled years

	Kid et al. (349(9))	Evans et al. (240(9))	Philips et al. (+17))	Combined (360(9))	Optimal (245(8.7))
2015	22.27	8.00	7.45	24.23	7.45
2016	13.85	4.41	4.65	16.26	4.62
2017	9.57	3.11	4.11	11.54	3.42
C	14.70	4.96	5.25	16.82	4.92

Table 17: Pearson correlation between the observed and predicted dates of the first egg (A), first larvae (C), egg peak (B) and larvae peak (D) during 2015, 2016, 2017 for the different GDD models from literature and the optimal GDD threshold based on our data. The specific threshold and base temperature are mentioned between parentheses.

A

	Ali et al. (85(10.5))	Ali et al. (90(10.5))	Guppy et al. (105(7))	Guppy et al. (87(9))	Hoffman et al. (88(9))	Evans et al. (90(9))	Combined (80(9))	Optimal (624(1))
2015	-0,0341	-0,0489	0,0055	-0,0676	-0,0596	-0,0656	-0,0473	-0,0399
2016	-0,1685	-0,2245	-0,3257	-0,3107	-0,1726	0,9540	-0,1726	-0,2690
2017	-0,3317	-0,4810	0,0109	-0,3493	-0,3472	-0,3210	-0,3343	-0,0682

B

	Philips et al. (182(8))	Evans et al. (Var)	Guppy et al. (166(7))	Guppy et al. (137(9))	Evans et al. (150(9)	Optimal (924(0))
2015	-0,3684	-0,1654	-0,4148	-0,3829	-0,3908	0,0058
2016	-0,2093	0,0313	-0,0555	-0,1820	-0,2341	0,1910
2017	-0,1402	-0,0785	-0,1459	-0,2150	-0,2089	-0,1520

C

	Gage et al. (220(9))	Kid et al. (165(9))	Evans et al. (105(9))	Combined (180(9))	Optimal (85(10))
2015	-0,5502	-0,4863	-0,4300	-0,4842	-0,5625
2016	-0,0954	-0,1439	-0,1088	-0,0684	-0,1229
2017	0,5417	0,5266	0,4375	0,4504	0,5064

Table 17: (continued) Pearson correlation between the observed and predicted dates of the first egg (A), first larvae (C), egg peak (B) and larvae peak (D) during 2015, 2016, 2017 for the different GDD models from literature and the optimal GDD threshold based on our data. The specific threshold and base temperature are mentioned between parentheses.

D

	Kid et al. (349(9))	Evans et al. (240(9))	Philips et al. (+17))	Combined (360(9))	Optimal (1214(0))
2015	-0,1565	-0,1272	-0,0826	-0,1321	-0,1350
2016	0,1529	0,2123	0,0659	0,1561	0,2049
2017	0,3368	0,3582	0,2248	0,3389	0,3406

Development of a predictive model

As mentioned above, a window-pane analysis was carried out to find the periods during which the weather conditions have the highest influence on the development of CLBs. For each independent variable (day number of the first egg, egg peak, first larvae and larvae peak) six variables were selected as predictor variables (Table 18). As temperature is the main factor influencing the growth and development, most predictor variables are temperature based. Furthermore, it can be seen that for the date of first oviposition and egg peak, the correlations with the temperature for the selected periods were negative, indicating that in case temperatures are higher, the first egg and egg peak come earlier. Furthermore, the correlations with rainfall during spring were positive indicating that the first egg and egg peak occur later in case of a lot of rainfall during that period. For the larvae the correlations with temperature were sometimes positive and sometimes negative, meaning that during some periods higher temperatures fasten development, while during other ones they delay development. These variables were used as predictors to construct multiple linear regression models, ridge regression models and regression trees.

Table 18: Pearson correlations of the six variables (period + specific weather variables which were correlated with the first egg, egg peak, first larvae and peak larvae of observed CLBs) that were used to construct the prediction model. Based on the data from 2014 until 2017. P-values < 0.05 (*); < 0.01 (**) and < 0.001 (***)

	First egg		Egg peak		First larvae		Peak larvae	
No.	**Variable + period**	**Cor.**	**Variable + period**	**Cor.**	**Variable + period**	**Cor.**	**Variable + period**	**Cor.**
1	Avg. temp. 16-I– 9-II	-0.50***	Avg. temp. 3-I– 27-I	-0.11	Avg. temp. 3-I– 27-I	-0.36***	Avg. temp. 3-I– 27-I	0.54***
2	Avg. temp. 10-III– 3-IV	-0.23**	Avg. temp. 15-II– 11-III	-0.11	Avg. temp. 28-I– 21-II	0.41***	Avg. temp. 11-I– 4-II	0.48***
3	Avg. temp. 29-III– 22-IV	-0.13	Avg. temp. 3-III– 27-III	-0.10	Avg. temp. 22-II– 18-III	0.19*	Avg. temp. 22-II– 18-III	-0.48***
4	Avg. temp. 1-V– 25-V	-0.39***	Rel. humidity 2-V– 26-V	-0.29**	Avg. temp. 23-III– 16-IV	0.46***	Avg. temp. 23-III– 16-IV	-0.28**
5	Rainfall 22-II– 18-III	-0.56***	Rainfall 14-III– 7-IV	0.15	Avg. temp. 17-IV– 11-V	-0.46***	Avg. temp. 17-IV– 11-V	0.36***
6	Rainfall 25-IV– 19-V	0.49***	Rainfall 31-III– 24-IV	0.14	Rainfall 19-II– 15-III	0.47***	Rainfall 10-V– 3-VI	0.20*

The coefficients of the resulting models are listed in Table 19; it can be seen that the absolute values of the coefficients of the ridge regression model are smaller compared to the coefficients of the linear regression model since ridge regression puts constraints on the coefficients to avoid over-fitting. The MAE of each model, validated without CV, with random CV and with CYCL validation are represented in Table 20. Without validation, the regression tree-model performed best (lowest MAE), followed by the ridge regression model and the linear regression model. In case random CV was applied, the ridge regression model performed better than the linear regression model. The CYCL validation led to the highest MAE for each modelling approach. Furthermore, the regression trees outperformed the other models.

In the previous section, the optimal GDD thresholds and optimal base temperatures for our data were based on the entire data set, so the data were not split into a test and training set. In a next step, the applicability of these thresholds and base temperatures to predict the first egg, first larvae, larvae peak and peak was determined. Since no test-train splitting was done to determine the optimal thresholds and base temperatures, the MAE of the GDD models should be compared with the MAE of the regression models without cross-validation. It can be seen in Table 20 that the MAE of the GDD models is always higher compared to the MAE of the models without CV. So, our proposed models perform better compared to the GDD approach. However, it can be argued that an MAE of 3 to 4.88 days is still too high for an accurate prediction model to be used in practice.

Table 19: Coefficients associated with the different variables included in the multiple linear regression model and the ridge regression model. Coefficients < 0.005 are indicated with '-'

No.	First egg		Egg peak		First larvae		Peak larvae	
	Linear	**Ridge**	**Linear**	**Ridge**	**Linear**	**Ridge**	**Linear**	**Ridge**
intercept	76.11	92.17	167.72	166.96	117.89	128.76	162.15	157.00
1	-0.08	-0.06	-0.05	-0.04	-0.18	-0.07	0.05	0.03
2	-0.05	-0.03	-0.07	-0.02	0.15	0.03	0.03	0.05
3	0.05	0.05	0.10	0.05	-0.08	-0.03	-0.09	-0.04
4	0.17	0.10	-0.48	-0.45	-0.06	0.03	0.03	-0.01
5	-0.22	-0.19	0.03	0.02	0.14	-	-0.01	-
6	0.04	0.06	-0.04	-	0.04	0.04	-0.11	-0.06

Table 20: Performance (MAE) of the multiple linear regression models, ridge regression models and regression trees predicting the day number of the first egg, egg peak, first larvae and larvae peak, without cross-validation, with random 10-fpm CV and with CYCL validation

	Without CV			Random 10-fold CV			CYCL V		
	Linear	**Rigde**	**Tree**	**Linear**	**Rigde**	**Tree**	**Linear**	**Rigde**	**Tree**
First egg	5.38	6.96	3.51	6.11	6.01	3.51	14.12	10.30	3.51
Egg peak	4.57	4.80	3.81	5.02	5.03	3.81	8.33	5.24	3.81
First larvae	3.75	4.70	2.81	5.24	4.35	2.14	5.95	5.57	4.88
Larvae peak	3.58	4.08	3.00	4.02	4.01	3.00	19.23	19.03	3.00

Implementation of the prediction model into a webtool for growers

Finally, the constructed model was implemented into a webtool, available for growers (Figure 36). This webtool is run on a platform at the Soil Service of Belgium and Landbouwcentrum Granen (LCG). In a first part of this webtool, information is provided for the grower concerning the phenology of CLBs in Flanders, crop damage and possible preventive crop husbandry practices for CLBs. In this tool, a grower can include the location of each field. Based on weather data from the nearest automated weather station as input, predictions are made available for the growers during the growth season. The output of the model is presented graphically, together with the modelled presence of aphids. For CLBs, this model predicts the date of first oviposition, first occurrence of the larvae and peak dates of the eggs and larvae.

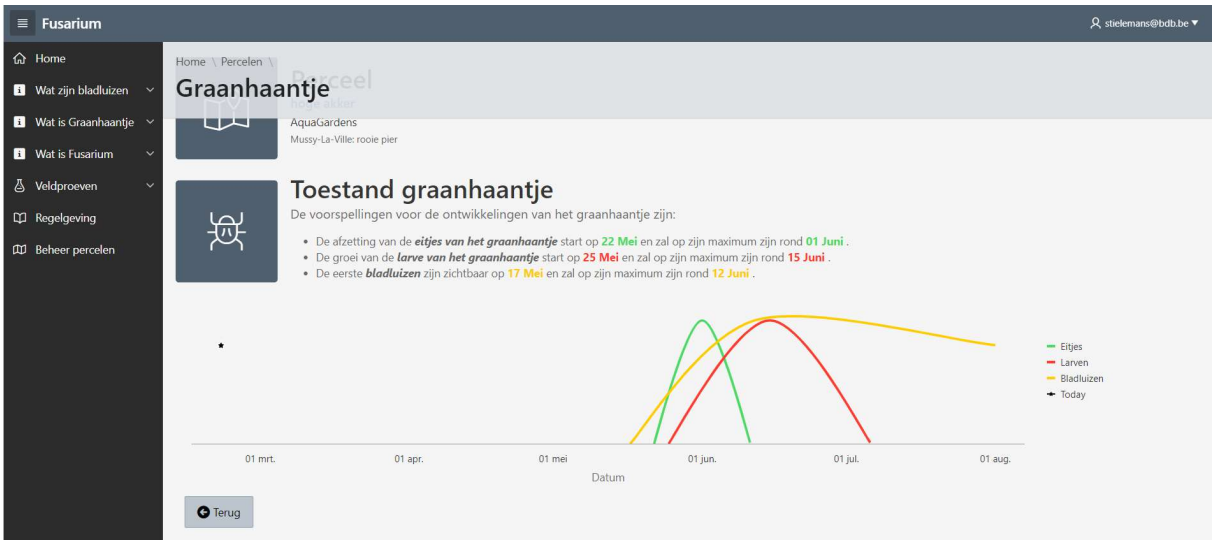


Figure 36: The constructed model was implemented into a webtool, available for growers. Information about aphids (yellow), CLB (eggs in green, larvae in red) and Fusarium are included in this webtool, as well as a prediction model for each of these pest insects.

Discussion

During seasons with a high insect pressure, CLBs can cause significant grain yield losses in winter wheat. To avoid potential economic damages, unwarranted calendar-based insecticide sprays are applied. Since the population size of these organisms fluctuates greatly from year to year and from location to location, the need and optimal timing of an insecticide application varies from year to year and from location to location. To avoid unnecessary insecticide treatments in view of IPM, models predicting the timing and magnitude of CLBs infestation are critically important.

The different aspects of the CLBs' life cycle are mainly estimated using simple GDD models. The main differences between the various models are the accumulated GDD threshold and base temperature. The available GDD models from literature were subjected to an evaluation on our data, however, it turned out that none of the previously established thresholds was appropriate for our data. Literature shows that with decreasing latitude, the base temperature will be higher and the sum of effective temperatures will be lower (Honek, 1996). This suggests that development will be slower at lower latitudes, which is in agreement that the American models predict specific life stages too late for our regional CLB population. However, many researchers (Tauber et al., 1987; Lamb and Mackay, 1988; Mogi, 1992; Groeters, 1992) agree that the base temperature remains constant within species level, although others mention that an adaptation of this base temperature to latitude is possible (Umeya and Yamada, 1973; Rae and Death, 1991). Different species within the same genus can show differences in base temperature. Trudgill and Perry (1994) showed that species that occur in warmer regions have higher base temperatures, which implies a lower sum of effective temperatures to reach a certain life stage. Even in case we reparametrised the models with the optimal GDD threshold and

base temperature for our data, the mean absolute errors were too high, in general as high as the standard deviation of our data. So, these GDD models have little predictive value. It can be concluded that models based on accumulated temperature (from January, 1) are not appropriate to predict CLB populations under the prevailing Belgian weather conditions. The low predictive power can be explained by several factors. Firstly, in contrast to regions for which the GDD models were developed, the CLB incidence in the monitored fields was very low. Secondly, the CLB population in Belgium is a composite of several species, all with a similar, though slightly different temperature-based phenology (Ali et al., 1977, 1979; Morlacchi et al., 2007), which makes it more difficult to set a general temperature-based threshold. Most of the models developed for CLB management are based on research from the USA, where only one species is actively found, i.e. *O. melanopus*. In addition, Guppy (1979) proved that there exist slight differences in optimum temperatures between European species and CLBs found in North America. For GDD models it is assumed that a temperature increase influences the insect in exactly the same way whether it comes one day before the start of the season or a couple of months before the season starts. However, that might not be a correct assumption. Keeping this in mind, we performed a window-pane correlation analysis to reveal the periods during which weather conditions are most influential. Based on this information various modelling approaches were explored to predict the date of the first egg, first larvae, egg peak and larvae peak. These models performed considerably better compared to the common GDD models. Based on our correlation analysis it was seen that for the larvae the correlations with temperature are sometimes positive and sometimes negative, meaning that during some periods higher temperatures fasten development, while during others they delay development. Based on the results gathered during three years, we found that CLB larvae were observed earlier in the years with e.g. a 'colder' winter. This seems at first sight biologically irrelevant, however, it has to be added that Flanders has a mild climate, e.g. the average temperatures in February 2015, 2016 and 2017 were respectively 6.3 °C, 4.5 °C and 6.1 °C. It seems that temperatures during winter were not really a limiting factor in delaying development. In addition, temperature fluctuations, cold periods during winter followed by warmer periods can accelerate the termination of the diapause resulting in an earlier CLB development. Furthermore, negative correlations between winter temperatures and CLBs have also been found in literature. In the Netherlands, Daamen and Stol (1993) found that the phenology and crop injury due to CLBs to be negatively correlated with mean temperatures of winter months December, January and February. Correspondingly, Ali et al. (1979) proved that mortality of overwintering CLBs severely increases above 10 °C.

Concerning the different modelling approaches, multiple linear regression, ridge regression and regression trees, it was concluded that regression trees outperformed linear regression and ridge regression. In case cross-validation was applied, the multiple linear regression performed worst, since linear regression is prone to overfitting, whereas ridge regression and regression trees are more robust towards overfitting. Ridge regression puts constraints on the model coefficients by adding a penalty term to the loss function. Minimising loss with this penalty term means we tend to avoid very large coefficient values, which ensures that the coefficients are not skewed due to outliers. Furthermore, the performance based on CYCL validation is the worst, but also the most realistic, as this is the performance that can be expected for new years and new locations.

In conclusion, this analysis shows that GDD models, even with optimised thresholds for our data, have little predictive value to predict CLBs. Linear regression, ridge regression and regression trees performed considerably better. However, in case cross-validation was applied, to gain insight in the future predictive value, the MAEs were too high.

As crop husbandry practices also influence CLB occurrence these can be included as additional variables. After four years of monitoring, correlation analysis pointed out that for a good prediction of *Oulema* species, more data is required. As this remains an occasional pest insect in Flanders and collection of CLBs was executed on growers' fields, four years appeared to be a too short period for including parameters such as fertilisation, sowing practices, choice of variety, as these parameters also influence each other, introducing extra variance. Nevertheless, to support growers, a webtool was developed. While the model implemented does not predict absolute densities, making monitoring still a requirement, it does provide a time window during which monitoring can be executed. This can limit time spent on observations and help the grower to manage his crop.

Chapter 7: Development of potential IPM tools for the management of CLBs in Flemish winter wheat

Introduction

CLBs cause direct and indirect damage to several *Poaceae* species. Research has shown a correlation between the presence of the larvae of CLB and leaf damage (Ihrig et al., 2001; Buntin et al., 2004). Nonetheless, it seems that extending this correlation to grain yield loss is not always that easy. Therefore, the need for trials that independently examine this correlation rises. Studies correlating leaf damage to yield losses have been done by researchers all over the world. To simulate the damage caused by herbivores, manually defoliating plants is a popular technique (Buntin et al., 2004; Macedo et al., 2006; Cerkal et al., 2009). Buntin et al. (2004) tested what effect cutting leaves could have on the grain yield of winter wheat plants. To estimate the effect of a reduced leaf area on crop yield loss, we adopted their protocol for a manual defoliation trial in the greenhouse and on the field.

Research has shown that these direct crop losses mainly occur when CLBs develop through the fourth larval stage, being the most active instar (Kher et al., 2011). However, especially the interaction with their host crop seems important (Kher, 2014). Since the flag leaf plays an important role in grain filling, damage at the flag leaf is in most cases associated with grain yield loss. In Belgium, depending on temperature, this plant growth stage is often accompanied by the fourth-instar larvae, potentially causing major crop losses. It is known that fourth-instar larvae are responsible for 70% of all the damage caused by the CLBs (Kher et al., 2011). As the larvae go through the different stages, their feeding activity increases. Severe larval and adult leaf feeding cause loss of kernel weight and kernel numbers per head (Gallun et al., 1967).

Even though the phenology of CLBs has been well studied, still, timing a pesticide application is often very difficult. Combining the fact that defoliating leaves causes many morphological and physiological changes within the plant and the fact that (a)biotic factors differ between regions, it is clear that economic thresholds can differ between regions (Haynes and Gage, 1981; Buntin et al., 2004). Additionally, as economic thresholds remain a cost-benefit analysis, it is clear that different grain prices or different costs of treatments between different regions have their effect on these thresholds as well.

Much research has already been done to set thresholds for CLBs. In the USA, Webster et al. (1972) calculated a threshold of three or more eggs and larvae per tiller or more than one larvae per flag leaf. Webster et al. (1972) noted one larvae per tiller. Although fields were being treated earlier, this threshold still let too much room for economic losses. Therefore, Ihrig et al. (2001) introduced a

threshold for aerial applications at which a grain yield loss of 185 kg/ha was tolerated in winter wheat. Based on this level, Buntin et al. (2004) calculated a threshold of 0.4 larvae per tiller between spike emergence and anthesis stage of growth. Ihrig et al. (2001) also found a profound correlation between eggs laid and the number of fourth-instar larvae per tiller, leading to a threshold of 0.5 eggs per tiller. Even though a long oviposition period could make an early treatment ineffective, Buntin et al. (2004) ratified this, favouring a tank-mix application of λ-cyhalothrin with a foliar fungicide. In Europe, thresholds mentioned in literature vary significantly as well. In France, Chambon et al. (1983) mentioned a threshold of 2.5 larvae per tiller, while in Switzerland the threshold is based on Buntin's research and set on 0.5 eggs per tiller or 0.4 larvae per tiller (Tschumi, 2015).

In this study, we tried to investigate the effect of CLB larvae on the grain yield of winter and spring wheat. Cage trials were set up to explore the link between the larvae, loss of leaf material and grain yield. To support findings extracted from the cage trials, plants were manually defoliated in the greenhouse as well as on the field. Results from these greenhouse and field trials will help set adequate thresholds for Flanders.

Even though CLB is an occasional pest insect in Belgium (**Chapter 5**), insecticide treatments are almost a general practice every season. As crop losses can increase at a fast rate, this concern often leads to superfluous insecticide treatments, indicating that often it is more an emotional than an economical decision. Going for an integrated approach, the setting of a threshold could substantiate the output of monitoring, hopefully leading to a reduction of insecticide use.

Next to optimising pesticide management, other practices could be optimised for CLB management as well. Sowing date could desynchronise the life cycles of plant and pest, enabling the plant to compensate better for the lost leaf material, or inhibiting the pest's reproduction (or increasing the pest's general mortality). Current Flemish CLB management mostly consists of a singly insecticide treatment, which is, in addition, not always timed correctly. Clearly, there is need for optimisation and a more durable approach that can be implemented by growers.

Material and methods

Several trials were conducted to investigate potential IPM tools. An overview of these trials is presented in Table 21.

Table 21: Overview of conducted trials during growth seasons 2014–2015, 2015–2016, 2016–2017 and 2017–2018 on several test levels

Trial level	Trial name	Growth season	Location
Greenhouse	Manual defoliation	2017–2018	Greenhouse ILVO
Semi-field	Cage trials	2014–2015, 2015–2016, 2016–2017, 2017–2018	Bottelare and Rumbeke-Beitem
Field	Insecticide trials	2015–2016, 2016–2017	Bottelare
Field	Sowing density trials	2016–2017, 2017–2018	Bottelare

Cage trials

To assess the damaging effect and potential IPM tools for the management of *O. melanopus*, *O. duftschmidi* and *O. obscura* on the leaves of winter wheat, cage field trials were executed at two locations in Belgium, Flanders during a period of three years: Bottelare and Rumbeke-Beitem. Cages were placed in different varieties of winter wheat and spring wheat. Table 22 lists the varieties and treatments executed at both trial locations during the three growth seasons. These field cages were used to isolate wheat plants, in which different densities of CLBs were introduced to assess the interaction between plant and larvae (Figure 37; Table 23). To limit all interference with other insects, all cages were treated with a broad-spectrum insecticide (λ-cyhalothrin, 5 g/ha), two weeks before introduction of the beetles in the cages. Because of a low *Oulema* pressure during growth season 2016–2017, it was difficult to collect enough adult CLB individuals for the trials. As a result, in growth season 2016–2017 the number of replicates was reduced to three instead of four. Within 32 cages, four separate degrees of infestation were simulated: In 2016, 0, 6, 10 and 20 CLBs were introduced. As a density of 20 CLBs did not lead to significant grain yield losses in 2015–2016, higher densities were introduced during the trials in 2016–2017: 0, 10, 20 and 50 CLBs. In 2017–2018, an even higher number of adult beetles per cage was introduced: 0, 20, 50 and 100 CLBs per cage. In the control cages, no beetles were introduced.

Table 22: Varieties and treatments included in the cage trials at both locations

Location	Growth season	Variety	Treatments (beetles per cage)
Bottelare	**2015–2016**	Intro (winter wheat)	0, 6, 10 and 20
		Cellule (winter wheat)	0, 6, 10 and 20
	2016–2017	Intro (winter wheat)	0, 10, 20 and 50
		Cellule (winter wheat)	0, 10, 20 and 50
	2017–2018	Intro (winter wheat)	0, 20, 50 and 100
		Tybalt (spring wheat)	0, 20, 50 and 100
Rumbeke-Beitem	**2015–2016**	Skerzzo (winter wheat)	0, 6, 10 and 20
		Feeling (spring wheat)	0, 6, 10 and 20
	2016–2017	Activus (winter wheat)	0, 10, 20 and 50
		Feeling (spring wheat)	0, 10, 20 and 50

To ensure the damage potential is similar as in a grower's field, *Oulema* beetles were collected randomly on fields with a high CLB infestation and then introduced into the cages. The species composition of the collected *Oulema* beetle population was not determined. The introduced adults are therefore a species composition, as found in the field. Beetles were introduced when plants reached BBCH 32 (second node) (2015–2016, 2016–2017) or BBCH 31 (start of tiller elongation) (2017–2018).

Table 23: Cage trial dates of events at both locations and during all three years

Location	Growth season	Variety	Placing cages	Introducing CLBs	Start monitoring	End monitoring	Harvest
Bottelare	**2015–2016**	Intro	13-IV-16	13-V-16	31-V-16	16-VI-16	08-VIII-16
		Cellule	13-IV-16	13-V-16	31-V-16	24-VI-16	08-VIII-16
	2016–2017	Intro	27-IV-17	22-V-17	12-VI-17	26-VI-17	31-VII-17
		Cellule	27-IV-17	23-V-17	12-VI-17	26-VI-17	31-VII-17
	2017–2018	Intro	25-IV-18	2-V-18	14-V-18	19-VI-18	08-VII-18
Rumbeke-Beitem	**2015–2016**	Skerzzo	18-IV-16	20-V-16	03-VI-16	23-VI-16	20-VII-16
		Feeling	13-V-16	20-V-16	03-VI-16	23-VI-16	08-VIII-16
	2016–2017	Activus	11-IV-17	22-V-17	08-VI-17	21-VI-17	28-VII-17
		Feeling	08-V-17	22-V-17	08-VI-17	21-VI-17	28-VII-17

During three years, flag leaf damage was scored using qualified scales from the European and Mediterranean Plant Protection Organisation (EPPO). Collection of the CLBs was done using sweep nets with a diameter of 30 cm, after which the CLBs were transferred to the cages, installed at each of the trial locations. Cages measured 1 m² and were covered with a special insect net (95 g/m² UV-stabilised high-density polyethylene (HDPE), mesh of 0.95 × 1.35 mm, wire thickness of 0.28 mm), which are equipped to isolate CLB adults and larvae, minimising interactions with other insects. During one month after infestations, 30 randomly selected plants were monitored within the cages on a

weekly basis for flag leaf damage and presence of larvae. Flag leaf damage was estimated using a pre-set scale (Figure 38; EPPO PP1/236(1), 2004).

Figure 37: Cages equipped with specialised insect netting were used to isolate CLB adults and larvae on the wheat plants inside the cages. Photo by Hilde Christiaens.

At the end of each season, when grains reached approximately 15% humidity, 100 ears (main and secondary tillers) per cage were collected from the middle of the plot and harvested in order to determine the grain yield. Ears were cut for collection with standard scissors at the base of the spikelet. Afterwards, using a spike thresher (Wintersteiger), grains were separated from the chaff. The grains were collected, weighed and analysed with a Dickey John GAC 2100 GI analyser for dry matter content and test weight. The number of grains per 100 tillers was determined as well. Afterwards, the grain yield was corrected to 15% humidity. To assess the thousand grain weight, the number of grains per plot was counted using a Contador Pfeuffer, a mechanical seed counter.

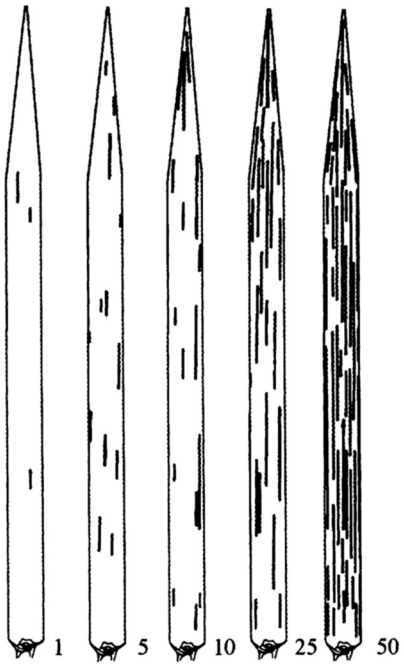


Figure 38: EPPO PP1/236(1) (2004) scale to evaluate the flag leaf damage caused by CLBs. Numbers represent the percentage leaf damage.

Manual defoliation trials

During the growth season 2017–2018, a pot trial was set up with spring wheat (Var.: Tybalt) plants. These plants were sown in pots on November, 10 (2017). In the manual defoliation trial, three crop stages were selected for defoliation at which different amounts of leaf damage were inflicted (Figure 39), following a protocol similar to Buntin et al. (2004). To determine the amount of leaf mass that had to be cut away, 20 randomly selected flag leaves were measured in length. Afterwards, an average proportion was calculated ($1/4^{th}$, $1/2^{th}$ and $3/4^{th}$).

All different treatments can be found in Table 24. Different crop stages included: (1) BBCH 39 (flag leaf stage, flag leaf fully enrolled, ligule just visible); (2) BBCH 59 (end of heading stage, when inflorescence was fully emerged) and (3) BBCH 77 (late milk stage). This factor was combined with cutting different leaf densities from the plants. Five defoliation levels were managed: (1) flag leaf fully defoliated (Tr. 1, 5, 9); (2) flag leaf half defoliated (Tr. 2, 6, 10); (3) upper two leaf layers fully defoliated (Tr. 3, 7, 11); (4) all leaves fully defoliated (Tr. 4, 8, 12), ending with a control treatment (5), where no leaves were cut (Tr. 13, control). This trial ran in six replicates.

Table 24: Treatments of the manual defoliation trial executed in the greenhouse

Treatment	Crop growth stage	Removed leaf biomass
1	BBCH 39[6]	Entire flag leaf
2	BBCH 39	1/2th of flag leaf
3	BBCH 39	Upper two leaf layers
4	BBCH 39	All leaves
5	BBCH 59[7]	Entire flag leaf
6	BBCH 59	1/2th of flag leaf
7	BBCH 59	Upper two leaf layers
8	BBCH 59	All leaves
9	BBCH 77[8]	Entire flag leaf
10	BBCH 77	1/2th of flag leaf
11	BBCH 77	Upper two leaf layers
12	BBCH 77	All leaves
13	Control	No leaves were cut

For this trial, wheat was sown in standard sterile and sifted potting substrate (brand: 'Jardino basic'; pH_{H2O} 5.0–6.0; electric conductivity (EC) 1.2 mS/cm; with added fertiliser: NPK 14-16-18). In each pot, ten grains were sown. After germination, pots were corrected to a fixed eight plants per pot. Plants were watered automatically, using an ebb and flow system. The indoor growing conditions were changed according to the growth of the plant: (1) until booting stage (BBCH 40): 15/10 °C (day/night regime) with no additional artificial light; (2) BBCH 41 (early boot stage) until harvest: 20/16 °C (day/night regime) with additional artificial light until 150 W/m² (from 5 am until 9 pm). Relative humidity was not altered during the trial period.

[6] Flag leaf stage
[7] End of heading stage
[8] Late milk stage

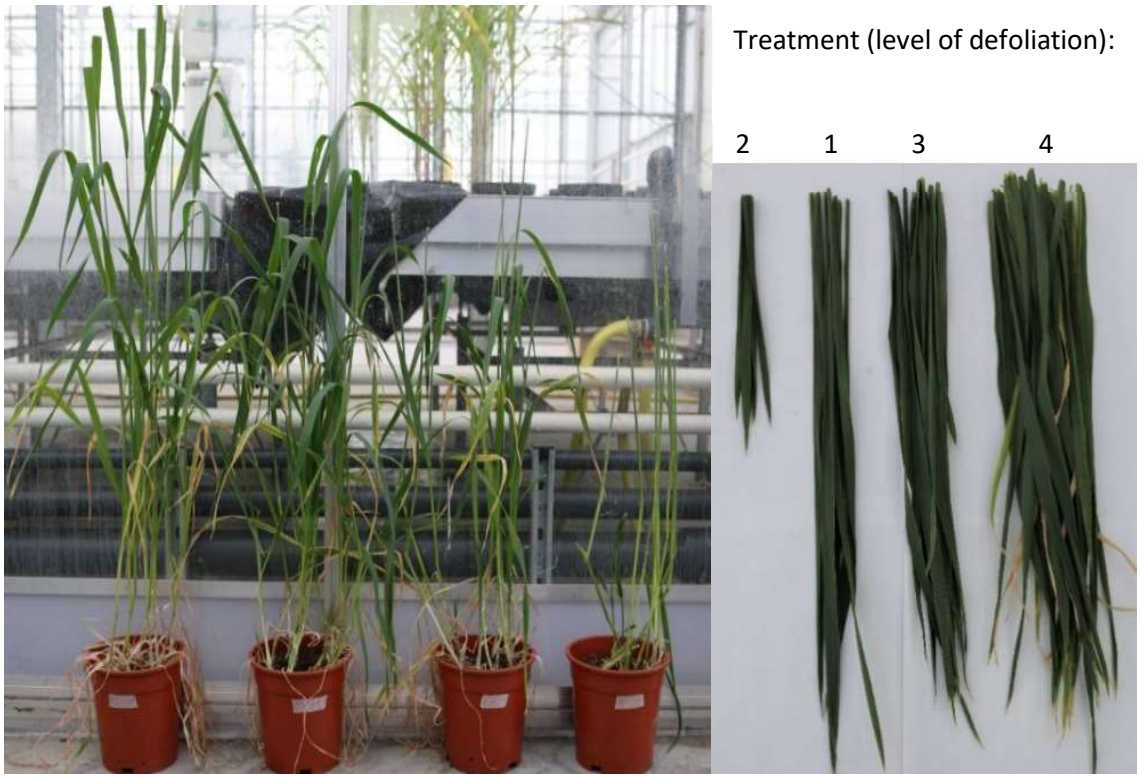


Figure 39: Different degrees of biomass removal at flag leaf stage (BBCH 39 = flag leaf stage). Total removed leaf biomass is presented on the right. From left to right (both figures): Tr. 2 (flag leaf half removed), Tr. 1 (entire flag leaf removed), Tr. 3 (upper two leaf layers removed) and Tr. 4 (all leaves removed).

In order to keep the yield loss due to other (a)biotic factors minimal, plants were monitored regularly and sprayed accordingly (Table 25). The spraying solution was prepared with 300 litre water per hectare. Spraying was executed with a hand pump sprayer.

Table 25: Plants in the manual defoliation trial were treated regularly during their growth. The following formulations were used: Okapi, Adexar (Jan., 2); Evora XPRO (Feb., 1 and 8); Mesurol SC 500, Ceriax (Feb., 15); Granovo and Bravo (Apr., 30)

Date (2018)	**Growth stage**	**Dose of active ingredients (g/ha)**	**Pest or disease treated**
January, 2	BBCH 16 (six leaves unfolded)	Λ-cyhalothrin (3.75) + Pirimicarb (75); Epoxyconazol (125) + Fluxapyroxad (125)	Aphids, mildew and rust
February, 1	BBCH 17 (seven leaves unfolded)	Bixafen (94) + Prothioconazol (125) + Tebuconazol (125)	Mildew
February, 8	BBCH 17 (seven leaves unfolded)	Bixafen (94) + Prothioconazol (125) + Tebuconazol (125)	Mildew
February, 15	BBCH 18 (eight leaves unfolded)	Methiocarb (750); Epoxiconazol (125) + Fluxapyroxad (125) + Pyraclostrobin (200)	Thrips, leaf spot disease and mildew
April, 30	BBCH 59 (end of heading, inflorescence fully emerged)	Boscalid (350)+ Epoxiconazol (125); Chloortalonil (1000)	Leaf spot disease, mildew and rust

When plants reached tillering stage (BBCH 15), fertiliser was added (30 units nitrogen (urea-ammonium nitrate (UAN), 39%); 60 units potassium sulphate, K_2SO_4) to the plants. To avoid lodging, support gauze was added when tiller elongation started (BBCH 31). The various treatments applied in the greenhouse are described below. The length and maximal width of each cut leaf was measured. Based on a formula described by Chanda and Singh (2002), the leaf area was then calculated.

Trials were harvested by cutting the main tillers above the substrate. Harvest occurred at two separate moments. The plants were harvested early, at BBCH 85 (soft dough stage; June, 18, 2018). Per pot, the main tillers were selected and five ears were harvested and labelled individually. The length and weight of each of the harvested main tillers were measured. Afterwards, ears were cut from the tiller. As dry matter content of the harvested grains showed a big variability (due to the early harvest), all ears were dried for three days at 60 °C in a Vötsch Industrietechnik GmBH drying oven. After drying the ears, using a spike thresher (Wintersteiger), grains were separated from the chaff. Both the grains and chaff were collected, weighed and analysed using a Dickey John GAC 2100 GI analyser for dry matter content and test weight. Afterwards, the grain yield was corrected to 15% humidity.

Insecticide trials

To test frequently used insecticides for their effectiveness towards CLBs, insecticide trials were executed. A protocol was assembled based on the Manual for Field Trials in Plant Protection and the EPPO protocol for CLBs (CIBA-GEIGY AG, 1992, EPPO PP1/236(1), 2004). Trials ran during two following seasons in Bottelare, Belgium on winter wheat (var.: Intro) (2015–2016 and 2016–2017). Seven treatments were included in four replicates in a randomised complete block design. Chosen insecticides are frequently used in small grain cereals in Flanders or do have an effect on Chrysomelidae beetles. Biscaya 240-OD (thiacloprid, 96 g/ha) is frequently used in potato against the Colorado potato beetle, while Decis EC 2,5 (deltamethrin, 5 g/ha) and Karate Zeon (λ-cyhalothrin, 5 g/ha) are frequently used against aphids in wheat. Teppeki (flonicamid, 80g/ha) is a selective translaminar product used against aphids in wheat. Fury 100 EW (zetacypermethrin, 10 g/ha) is an approved insecticide against CLB in winter wheat. NeemAzal-T/S (azadirachtin, 25 g/ha) is a biopesticide with an active ingredient that is often used against many Chrysomelid pest insects (Table 26). Not all these insecticides are approved in wheat in Flanders. For those insecticides that were not approved at the moment of application, an admission was requested at FOD (federal government, health department) Volksgezondheid. To prevent neighbour effects due to drift, two untreated buffer plots around each treated plot were included. The control plots were kept insecticide-free.

Table 26: Overview of treatments during growth seasons 2015–2016 and 2016–2017, Bottelare research farm

Treatment	Chemical family	Systemic/contact mode of action	Sensitive target	Active ingredient	Dosage per ha	Registered in winter wheat?
Control	/	/	/	No application	/	/
Teppeki	Pyridine-carboxamides	Systemic	Aphids	Flonicamid 50%	160 g	Yes
Decis EC 2,5	Pyrethroids	Contact and stomach action	Broad-spectrum	Deltamethrin 2.5 g/l	200 ml	Yes
Karate Zeon	Pyrethroids	Contact and stomach action	Broad-spectrum	λ-cyhalothrin 100 g/l	50 ml	Yes
Fury 100 EW	Pyrethroids	Contact and stomach action	CLBs, aphids	Zetacypermethri 100 g/l	100 ml	Yes
NeemAzal-T/S	Azadirachtins	Stomach action	Multiple insects	Azadirachtin 10 g/l	2500 ml	No
Biscaya 240 OD	Neonicotinoids	Contact and stomach action + some systemic properties	Aphids, Chrysomelidae	Thiacloprid 240 g/l	400 ml	No

These field trials were executed in Bottelare research farm (Oost-Vlaanderen; N50°57'45.8", E3°45'43.1") and Rumbeke-Beitem (West-Vlaanderen; N50°54'17.7"; E3°07'45.3"). Soil type in Bottelare is a moderately dry sandy loam with a strongly spotted, crumbled texture B horizont (soil type Ldc, Lcc), while in Rumbeke-Beitem, trials were executed on a moderately dry, light sandy soil with a crumbled texture B horizont (Pcc, Ldc type) (Geopunt Vlaanderen, 2011).

At both locations, before sowing, soil preparation was done using conventional tillage techniques. Sowing was executed with a row spacing of 12.5 cm. Trials were sown at 350 grains/m² in good circumstances following good agricultural practices. Fertilisation application followed recommendations set by Soil Service of Belgium and was based on soil samples (nitrogen was applied in three fractions: (1) 2015–2016: 60, 70 and 58 kg/ha; (2) 2016–2017: 72, 58 and 58 kg /ha; phosphor was applied once together with a potassium fertiliser: (1) 2015–2016: 41 kg/ha P_2O_5 + 96 kg/ha K_2O; (2) 2016–2017: 41 kg/ha P_2O_5 + 84 kg/ha K_2O). To limit biotic stresses to the plant that would influence grain yield, the trials were treated intensively during the growth season including one herbicide treatment with florasulam, tritosulfuron, mesosulfuron and esterified rapeseed oil in spring (March), two fungicide treatments with (1) boscalid, epoxyconazole and chloortalonil in May or (2) epoxyconazool, fluxapyroxad and pyraclostrobin in June.

When plants reached BBCH 32 (April, second node), a growth regulator was also applied on the winter wheat plants consisting of mepiquatchloride and prohexadion.

All treatments consisted of a single insecticide application at the start of the booting stage, when the flag leaf was fully enrolled (BBCH 40). Applications were applied using specialised trial spraying

equipment (two stroke back sprayer with a spray boom of 1.5 metre, equipped with 50% drift reducing Teejet AVI 110.02) spraying nozzles. Spraying was executed with a pressure of 3 bar. Nozzle flow rate was tested to ensure a dispense of 800 ml/min. Fields were treated at 3.4 km/h. The presence of the eggs and larvae of CLBs was counted at five moments (before application, 3, 7, 14 and 21 days after application) on 30 plants, selected randomly in each plot of 10 m². Flag leaf damage was estimated at two moments (before application and 14 days after application), using qualified scales from the European and Mediterranean Plant Protection Organisation (EPPO) (Figure 38).

At the end of the season, treated plots were selected for harvest. These plots of 1.5 × 10 m were harvested using specialised harvesting equipment (Wintersteiger Delta plot combine). The harvested grains were collected and dried to determine dry matter content and test weight using a Dickey John GAC 2100 GI analyser. Afterwards, grain yields were corrected to a fixed 15% humidity.

Sowing density trials

To evaluate the effect of altering the sowing date of winter wheat towards the presence of CLBs, field trials were executed at the Bottelare research farm during two growth seasons: 2016–2017 and 2017–2018. Soil type in Bottelare is a moderately dry sandy loam with a strongly spotted, crumbled texture B horizont (soil type Ldc, Lcc). In the first year, winter wheat (var.: Intro) was sown on October, 27 (2016) at four different densities: 250, 300, 350 and 400 grains/m². In 2017–2018, spring wheat (var.: Tybalt) was sown on April, 21 (2018) at four densities: 100, 300, 400 and 600 grains/m². The experiments ran in a randomised complete block design with four replicates. Soil preparation was done using conventional tillage techniques. Sowing was executed with a row spacing of 12.5 cm. Trials were sown in good circumstances following good agricultural practices. Fertilisation application followed recommendations set by Soil Service of Belgium and was based on soil samples (nitrogen was applied in three fractions in winter wheat (1) and two fractions in spring wheat (2): (1) 2016–2017: 72, 58 and 58 kg /ha; (2) 2017–2018: 90 and 30 kg/ha ; phosphor was applied once together with a potassium fertiliser: (1) 2016–2017: 41 kg/ha P_2O_5 + 84 kg/ha K_2O; (2) 2017–2018: 40 kg/ha P_2O_5 + 210 kg/ha K_2O). To limit biotic stresses to the plant that would influence grain yield, the trials were treated intensively during the growth season including one herbicide treatment with florasulam, tritosulfuron, mesosulfuron and esterified rapeseed oil in spring (March), two fungicide treatments with (1) boscalid, epoxyconazole and chloortalonil in May or (2) epoxyconazool, fluxapyroxad and pyraclostrobin in June.

When plants reached BBCH 32 (April, second node), a growth regulator was also applied on the winter wheat plants consisting of mepiquatchloride and prohexadion. Every plot consisted of 10 m² in which 30 plants were randomly selected for monitoring insects. From BBCH 32 (second node), each week 120 (30 x 4 replicates) plants were monitored. On each plant, the number of eggs and larvae of CLBs was monitored. Countings were recorded using a specialised pocket computer (Psion Teklogix).

Plants were harvested on July, 31 (2017) and August, 6 (2018) using specialised harvesting equipment (Wintersteiger Delta plot combine). The harvested grains were collected and dried to determine dry matter content and test weight Dickey John GAC 2100 GI analyser. Afterwards, grain yields were corrected to a fixed 15% humidity.

Statistical analyses

All analyses were conducted in R (version 3.5, R Development Core Team, 2017). As the data of the greenhouse trials were normally distributed and the assumptions concerning homoscedasticity were fulfilled, a two-way ANOVA (significance level $\alpha = 0.05$) was performed to test whether the grain yield differed significantly, using the time of defoliation and the amount of removed leaf area as factors. In case there were significant differences, Tukey's HSD range test was executed to detect which groups significantly differed. The field data were analysed using a Kruskal-Wallis test (significance level $\alpha = 0.05$), since the normality and homoscedasticity assumptions of parametric tests were not fulfilled. This test was used in the (1) insecticide trial to analyse the effect of different insecticides on the number of eggs and larvae, (2) sowing density trial to analyse the effect of different sowing densities on the number of eggs and larvae and (3) cage trials to study the effect of the number of beetles introduced in each cage on the flag leaf damage and grain yield. In case there were significant differences, Dunn's Multiple Comparison Test was run to detect which groups significantly differed. Since the normality and homoscedasticity assumptions of parametric tests were fulfilled on the yield data of the insecticide trial, a one-way ANOVA analysis (significance level $\alpha = 0.05$) was performed to test the effect of insecticide treatment on grain yield. In case there were significant differences, Tukey's HSD range test was executed to detect which groups significantly differed. To gain insight into the potential relationship between the observed larvae, flag leaf damage and grain yield in the cage trials, Pearson correlation coefficients were calculated and tested for their significance at a significance level of $\alpha = 0.05$.

Results

Cage trials

Table 27 lists the results of the trials executed at two locations (Bottelare, Rumbeke-Beitem) during three subsequent growth seasons (2015–2016, 2016–2017 and 2017–2018). During these three years, we found that the average number of observed larvae and flag leaf damage were highly correlated (2015–2016, Beitem, r = 0.868, n= 32, p-value < 0.001; 2016–2017, Beitem, r = 0.959, n = 16, p-value < 0.001; 2015–2016, Bottelare, r = 0.771, n = 32, p-value < 0.001; 2016–2017, Bottelare, r = 0.874, n = 24, p-value < 0.001; 2017–2018, Bottelare, r = 0.923, n = 16, p-value < 0.001).

Table 27: Grain yield (g grains per 100 tillers), number of grains per 100 tillers and thousand grain weight (g, ±SE) in relation to the number of larvae per tiller observed and total flag leaf damage (%) for each location (Bottelare and Rumbeke-Beitem) and growth seasons (2015–2016, 2016–2017 and 2017–2018)

Location	Growth season	Treatment (number of *Oulema* adults per cage)	Grain yield (g/100 tillers)	Thousand grain weight (g)	Number of grains per 100 tillers	Av. Number of larvae per tiller	Peak larval density per tiller	Total flag leaf damage (%)
Bottelare	**2015–2016**	**0**	168.8±51			0.120±0.08	0.235±0.15	3.64±2.9
		6	177.6±52			0.158±0.05	0.320±0.09	4.36±2.0
		10	155.6±41			0.247±0.15	0.425±0.22	6.16±3.9
		20	163.7±49			0.369±0.14	0.520±0.13	9.76±5.2
	2016–2017	**0**	266.2±52	50.89±7.3	5200.2±299	0.008±0.02	0.027±0.07	0.25±0.4
		10	297.0±48	54.01±5.2	5476.7±486	0.088±0.07	0.233±0.18	4.45±2.8
		20	279.9±35	51.41±4.5	5432.0±246	0.108±0.05	0.193±0.09	4.34±2.8
		50	290.8±46	53.20±4.5	5438.2±416	0.178±0.08	0.347±0.14	9.36±2.4
	2017–2018	**0**	287.8±14	53.82±0.7	5345.5±208	0.078±0.07	0.215±0.16	1.58±1.3
		20	275.9±7	53.38±1.0	5167.8±130	0.378±0.21	0.960±0.65	6.44±3.8
		50	287.3±9	54.08±0.8	5312.0±116	0.572±0.26	1.400±0.73	16.23±9.2
		100	273.3±11	52.31±1.0	5223.8±129	0.990±0.43	2.420±1.09	22.19±8.8
Rumbeke-Beitem	**2015–2016**	**0**	161.9±28	48.81±5.1	3298.3±252	0.068±0.07	0.143±0.13	4.34±6.4
		6	166.1±25	48.20±4.7	3439.8±359	0.135±0.09	0.274±0.18	8.19±5.8
		10	161.7±32	47.69±5.9	3369.3±354	0.181±0.10	0.368±0.22	11.45±6.3
		20	159.4±35	47.10±7.6	3363.8±368	0.231±0.10	0.495±0.22	16.98±9.5
	2016–2017	**0**	227.9±18	48.96±2.8	4649.8±113	0.003±0.01	0.010±0.01	0.11±0.1
		10	228.7±16	49.04±3.0	4661.0±86	0.052±0.04	0.053±0.04	0.98±0.3
		20	222.4±19	47.91±3.0	4637.8±141	0.143±0.08	0.233±0.14	4.29±2.9
		50	208.2±11	47.69±1.6	4370.3±280	0.258±0.13	0.570±0.26	12.44±4.6

Regardless of the observed flag leaf damage caused by the larvae, we were not able to find a direct significant link between the total flag leaf damage and total grain yield (r = -0.042, n = 120, p-value = 0.646) or the number of grains per 100 harvested tillers (r = -0.151, n = 88, p-value = 0.160). A significant link was found with the thousand grain weight (r = 0.210, n = 88, p-value = 0.050), indicating that grains harvested from plants with flag leaf damage were heavier than grains from undamaged plants. Also, the correlations between larval presence on the plants and final grain yields (r = 0.104, n = 120, p-value = 0.258) and between larval presence and the number of grains per 100 tillers (r = 0.176, n = 88, p-value = 0.176; Figure 43) appeared not to be significant. In contrast, thousand grain weight was significantly positively correlated with larval presence (r = 0.235, n = 88, p-value = 0.027).

Introducing more CLBs led to a higher number of larvae per tiller ($\chi^2(5) = 47.414$, p-value < 0.001; Figure 40) and more total flag leaf damage ($\chi^2(5) = 45.924$, p-value < 0.001; Figure 41). Introducing 10 CLBs in a cage in Bottelare during growth season 2015–2016 led to an average of 0.25 larvae per tiller and 6% damage on the flag leaf, while introducing 100 CLBs during growth season 2017–2018 resulted in 0.99 larvae per tiller and 22.19% damage on the flag leaf. 10 CLBs per cage resulted in a grain yield loss of 7.82% in growth season 2015–2016 (relative difference to the control), while introducing 100 beetles led to a grain yield loss of 5.04% in growth seasons 2017–2018 (relative difference to the control).

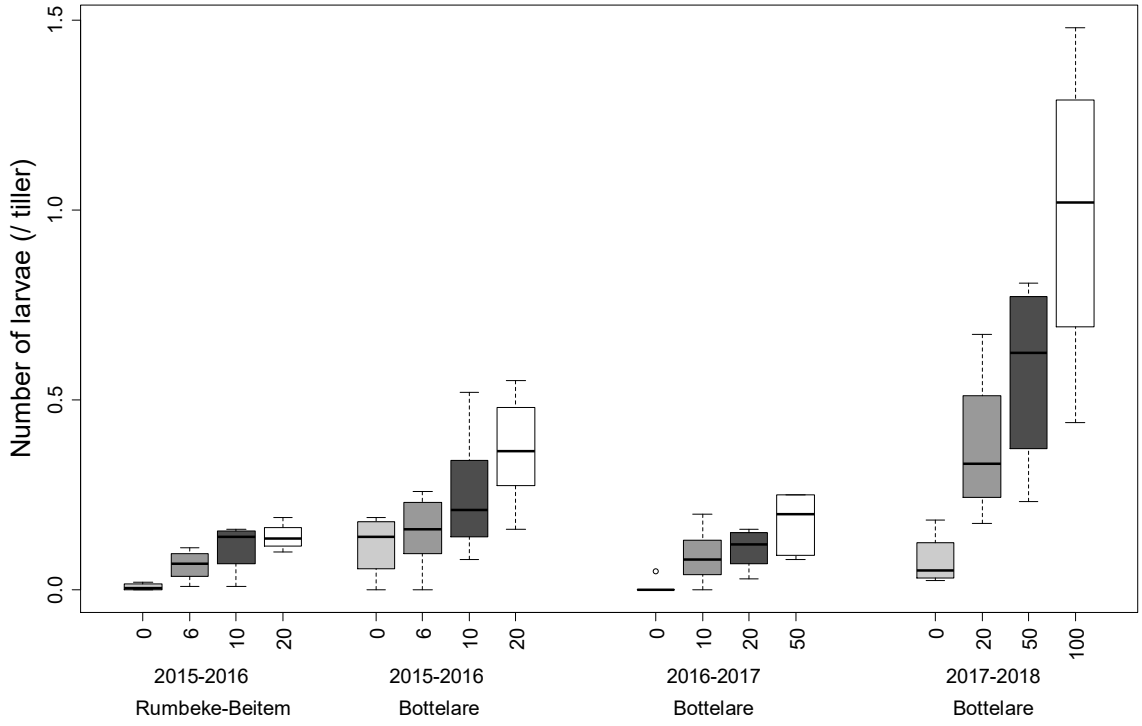


Figure 40: Number of Oulema larvae per tiller in relation to the introduced number of Oulema adults for two locations and three growth seasons (winter wheat cage trials): 0 = control treatment, no beetles introduced; 6 = 6 beetles introduced per cage; 10 = 10 beetles introduced per cage; 20 = 20 beetles introduced per cage; 50 = 50 beetles introduced per cage; 100 = 100 beetles introduced per cage.

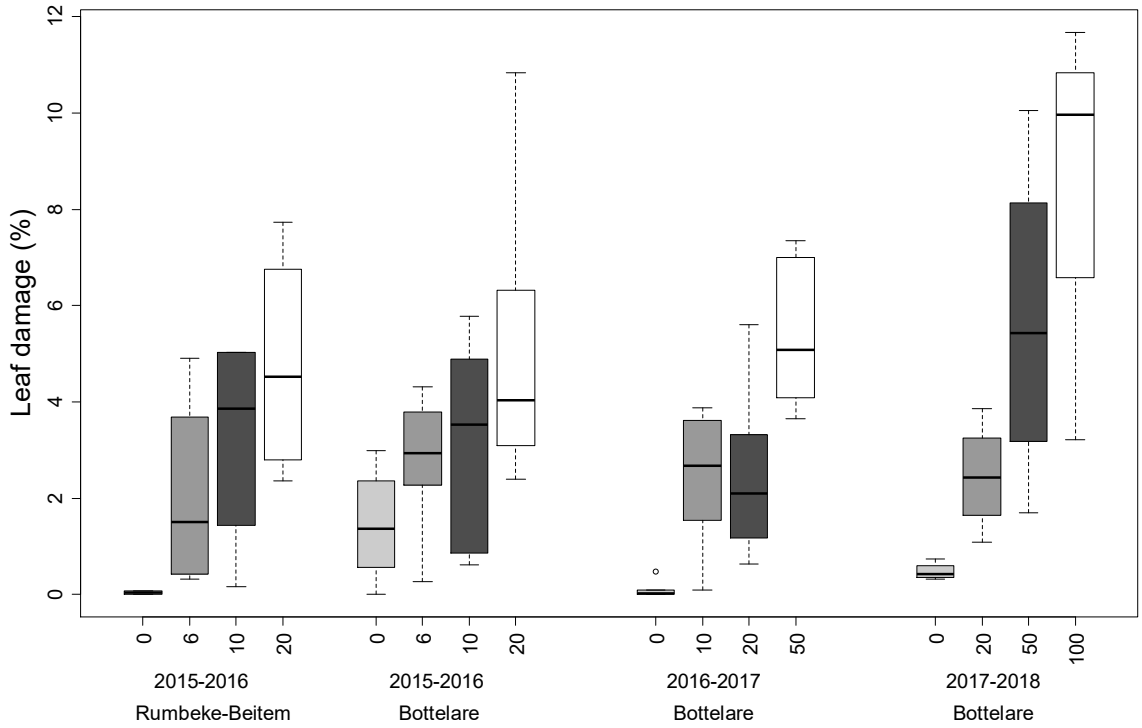


Figure 41: Average flag leaf damage (%) in relation to the introduced number of Oulema adults for two locations and three growth seasons (winter wheat cage trials): 0 = control treatment, no beetles introduced; 6 = 6 beetles introduced per cage; 10 = 10 beetles introduced per cage; 20 = 20 beetles introduced per cage; 50 = 50 beetles introduced per cage; 100 = 100 beetles introduced per cage.

Although the introduction of CLBs led to leaf damage, this was not accompanied with an equivalent grain yield loss, no significant differences between the control plots and the plots with CLBs were observed (Table 28 and Figure 42). Correspondingly, the number of grains harvested from 100 tillers (Figure 43) and thousand grain weight (**Appendix 3**) did not differ significantly between different treatments in all monitored growth seasons (2015–2016, 2016–2017 and 2017–2018) or crops (spring or winter wheat).

Table 28: Kruskal-Wallis test statistics of the hypothesis that grain yield (g/100 tillers) significantly differs between treatments

Growth season	Crop	χ^2	Df	n	p
2015–2016	spring wheat	0.507	3	16	0.917
	winter wheat	0.891	3	48	0.828
2016–2017	spring wheat	4.478	3	16	0.214
	winter wheat	2.800	3	24	0.423
2017–2018	winter wheat	5.382	3	16	0.146

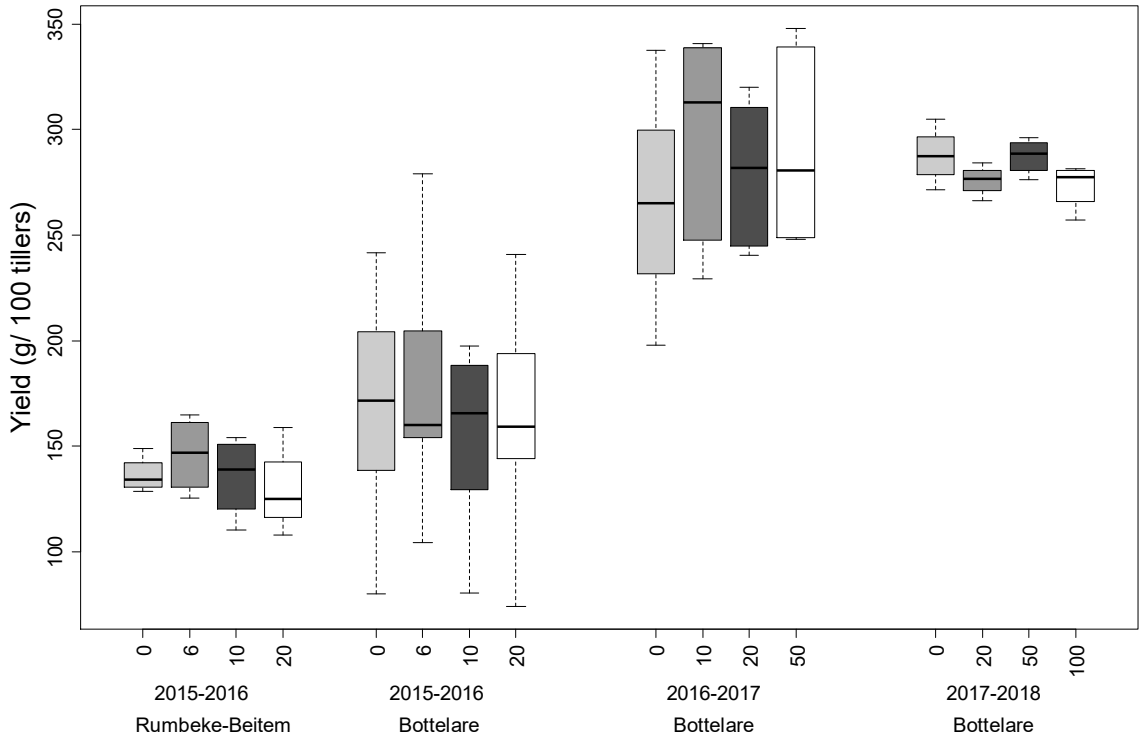


Figure 42: Grain yield (g per 100 tillers) in relation to the introduced number of Oulema adults for two locations and three growth seasons (winter wheat cage trials): 0 = control treatment, no beetles introduced; 6 = 6 beetles introduced per cage; 10 = 10 beetles introduced per cage; 20 = 20 beetles introduced per cage; 50 = 50 beetles introduced per cage; 100 = 100 beetles introduced per cage.

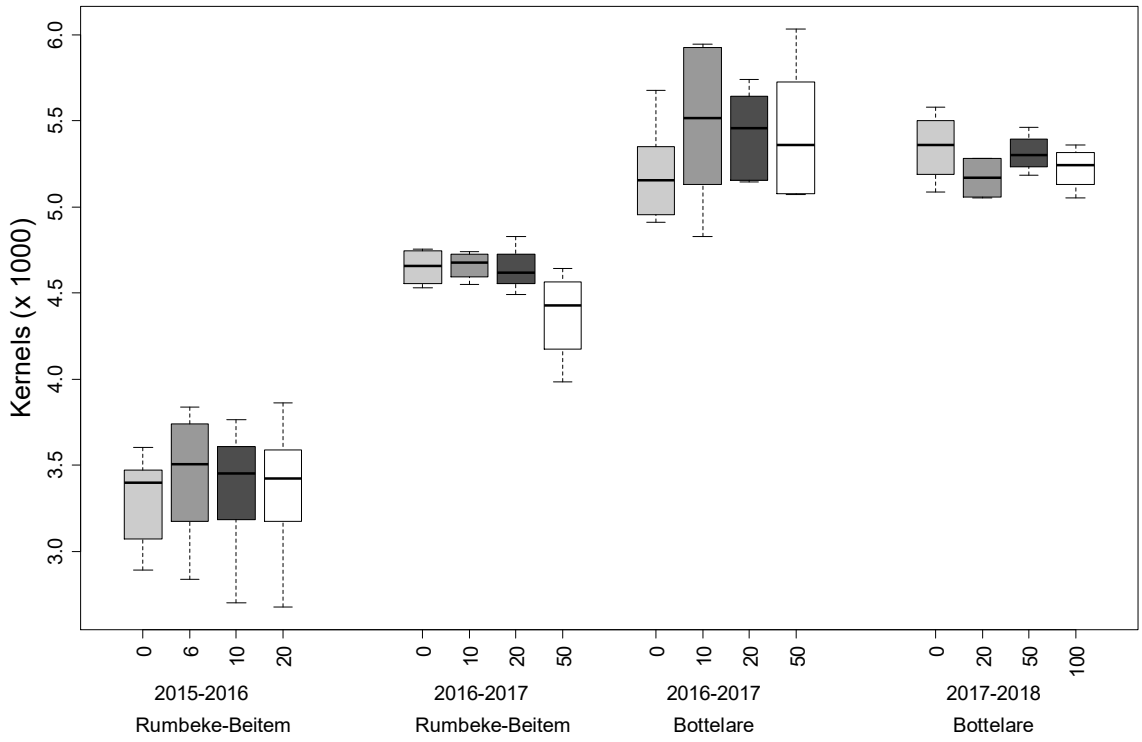


Figure 43: Number of kernels (x 1000) per treatment in relation to the introduced number of Oulema adults for two locations and three growth seasons (winter wheat cage trials): 0 = control treatment, no beetles introduced; 6 = 6 beetles introduced per cage; 10 = 10 beetles introduced per cage; 20 = 20 beetles introduced per cage; 50 = 50 beetles introduced per cage; 100 = 100 beetles introduced per cage.

Figure 44 shows the relationship between the observed number of larvae on the plants and the total flag leaf damage estimated using the EPPO-scale (EPPO PP1/236(1), 2004). A linear regression line was added to the graph ($R^2 = 0.64$, p-value < 0.001), which indicates that an observed presence of 0.4 larvae per tiller leads to more than 10% leaf damage on the flag leaf.

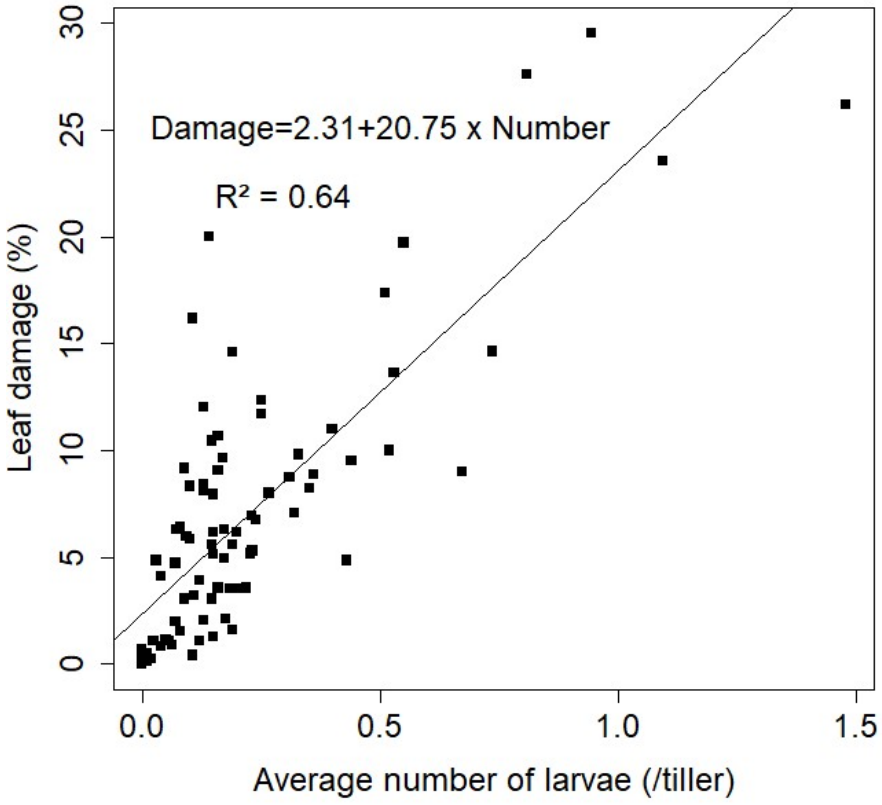


Figure 44: Relationship between average number of larvae per tiller and total flag leaf damage estimated using EPPO scales in the field cages.

Total grain yield was significantly positively correlated with other yield parameters such as thousand grain weight (r = 0.76, n = 86, p-value < 0.001) and number of grains per 100 tillers (r = 0.95, n = 86, p-value < 0.001).

Manual defoliation trials – greenhouse

Table 29 lists the results of the manual defoliation trial executed in the greenhouse. A two-way ANOVA analysis (Table 30) pointed out that during these trials, dry matter content of the grains significantly differed between crop growth stages at which the plants were defoliated ($F(2,61) = 4.113$, p-value = 0.021). The weight (g) and length (cm) of the harvested tillers, measured at harvest and grain yield (g per tiller) did not significantly differ between crop growth stages at which the plants were defoliated (resp. $F(2,61) = 2.120$, p-value = 0.129 ; $F(2,61) = 1.739$, p-value = 0.184 and $F(2,61) = 2.905$, p-value = 0.062). The degree of defoliation seemed to influence grain yield ($F(3,61) = 6.948$, p-value < 0.001) and the length of the tiller ($F(3,61) = 2.757$, p-value = 0.05) significantly, while tiller weight and dry matter content were not significantly influenced by the removed amount of leaf mass (resp. $F(3,61) = 0.312$, p-value = 0.817 and $F(3,61) = 1.770$, p-value = 0.162). Analysis of the removed leaf area, a significant interaction effect was observed ($F(6,61) = 11.352$, p-value < 0.001), indicating that removed leaf area decreased significantly when plants aged. Looking at each treatment (removed leaf mass) individually, it was clear that while the first two and the fourth degree of defoliation differed significantly between growth stages (a significant decrease in leaf area was observed), the third degree of defoliation did not significantly differ between crop growth stages ($F(2,15) = 0.876$, p-value = 0.437).

Table 29: Grain yield (g per tiller), dry matter content (%), tiller weight (g per tiller) and length of defoliated tiller (±SE) in relation to the amount (1 = flag leaf half removed; 2 = flag leaf fully removed; 3 = upper two leaves fully removed and 4 = all leaves of the plant removed; control = no leaves removed) and moment (BBCH 39 = flag leaf stage; BBCH 59 = end of heading stage and BBCH 77 = soft dough stage) of defoliation

Crop growth stage	Degree of defoliation	Removed leaf area (cm^2)	Tiller length (cm)	Tiller weight (g/tiller)	Grain yield (g/tiller)	Dry matter content (%)
BBCH 39	**1**	19.82±2.06	75.8±5.78	6.09±0.89	1.97±0.32	58.9±4.64
	2	41.83±3.64	70.8±4.64	5.45±1.40	1.75±0.30	55.9±2.09
	3	76.29±5.09	65.8±5.94	5.64±1.04	1.30±0.25	70.1±10.80
	4	167.36±21.14	64.5±2.27	5.07±1.46	0.75±0.09	55.4±12.66
BBCH 59	**1**	20.68±4.06	72.2±7.67	5.80±1.48	1.77±0.50	55.8±3.76
	2	39.15±6.41	75.8±3.63	5.08±1.44	1.76±0.25	56.5±4.31
	3	78.26±16.58	75.1±6.51	5.08±0.75	1.44±0.30	52.6±3.57
	4	125.81±35.65	73.8±3.45	4.89±0.84	1.41±0.37	54.2±5.45
BBCH 77	**1**	15.50±3.73	73.9±2.82	5.36±1.20	1.85±0.47	54.1±3.62
	2	33.33±3.59	76.6±2.78	5.46±1.65	1.88±0.44	56.3±7.52
	3	69.89±9.64	74.5±2.44	5.23±1.53	1.86±0.42	57.0±2.67
	4	87.90±10.15	73.7±1.73	5.71±2.33	1.63±0.25	55.3±4.66
Control	**No defoliation**	0	77.5±2.77	6.03±0.83	2.14±0.43	57.0±3.83

Table 30: Results (F-value, degrees of freedom and the p-value) of a two-way ANOVA (factors: crop growth stage and degree of defoliation; α = 0.05) analysis on the various variables (dry matter content, tiller weight, tiller length, grain yield and removed leaf area) in the manual defoliation trial

	Variable	F	Df	p-value
Main effect: crop growth stage	Dry matter content (%)	4.113	2, 61	0.021
	Tiller weight (g)	2.120	2, 61	0.129
	Tiller length (cm)	1.739	2, 61	0.184
	Grain yield (g per tiller)	2.905	2, 61	0.062
Main effect: degree of defoliation	Dry matter content (%)	1.770	3, 61	0.162
	Tiller weight (g)	0.312	3, 61	0.817
	Tiller length (cm)	2.757	3, 61	0.05
	Grain yield (g per tiller)	6.948	3, 61	< 0.001
Interaction effect	Removed leaf area (cm^2)	11.352	6, 61	< 0.001

Figure 45 presents the removed leaf area for each treatment at each crop growth stage. Figure 46–Figure 49 present the effect of both factors (crop growth stage and degree of defoliation) on the monitored variables (grain yield in g per tiller, tiller length (cm), dry matter content (%) and tiller weight (g per tiller). A clear significant interaction effect was observed ($F(6,65) = 4.483$, p-value = 0.001), indicating that grain yield loss decreases when defoliating plants at a later crop growth stage. While grain yield did not significantly differ between the first two degrees of defoliations (removing the flag leaf (1/2th or fully)), removing the upper two leaf layers or all leaves of the plant resulted in significant yield differences. It seems that especially at BBCH 39 (flag leaf stage), defoliation resulted in a significant decrease of grain yield (removing upper two leaf layers: $F(2,15) = 7.883$, p-value = 0.005; removing all leaves from the plant: $F(2,15) = 21.268$, p-value < 0.001). Comparing the grain yield of all treatments with the control treatment (no leaves cut), it was clear that only these predescribed treatments resulted in a significant different grain yield (compared to the control treatment). An average grain yield reduction of 1.39 g was observed when removing all the leaves of the plant compared to the control treatment. Tiller length also showed a clear interaction effect between crop growth stage and degree of defoliation ($F(6,65) = 3.113$, p-value = 0.010), indicating that the effect of defoliation on tiller length decreased when defoliating an older plant. Removing half the flag leaf on the different crop growth stages resulted in no significant differences in tiller length, while removing the flag leaf fully, the upper two leaf layers or all leaves of the plant on the different crop growth stages resulted in significant differences of tiller length (resp. $F(2,15) = 4.161$, p-value = 0.037; $F(2,15) = 4.628$, p-value = 0.027 and $F(2,15) = 5.021$, p-value = 0.021). A post-hoc analysis revealed that removing leaf mass at an early, vegetative crop growth stage results in a significant shorter tiller. Removing leaf mass at the flag leaf stage (BBCH 39) reduced the length of the tiller with 6.7 cm, while defoliation in a later crop growth stage (BBCH 77 (late milk stage)) only caused a shortening of 0.9 cm (compared with the control treatment).

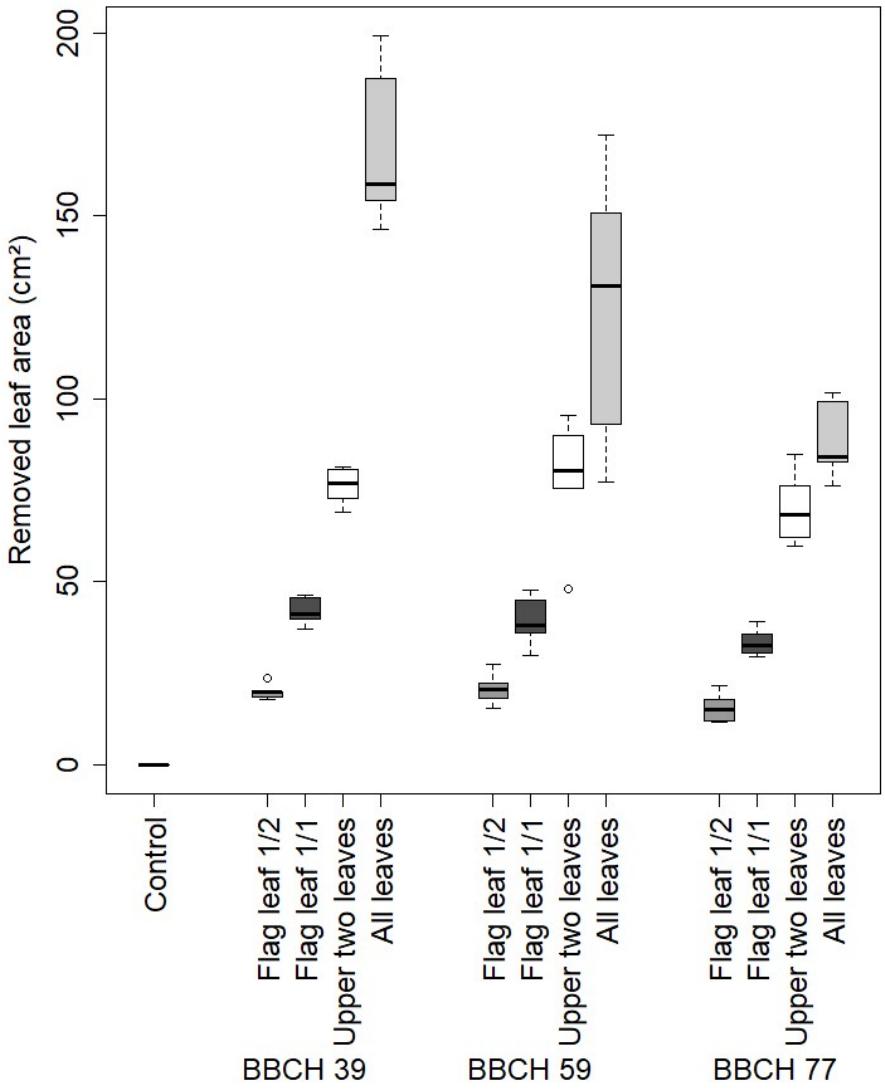


Figure 45: Removed leaf area (cm²) for each treatment in the manual defoliation (greenhouse) trial. BBCH 39 = flag leaf stage; BBCH 59 = end of heading stage; BBCH 77 = late milk stage.

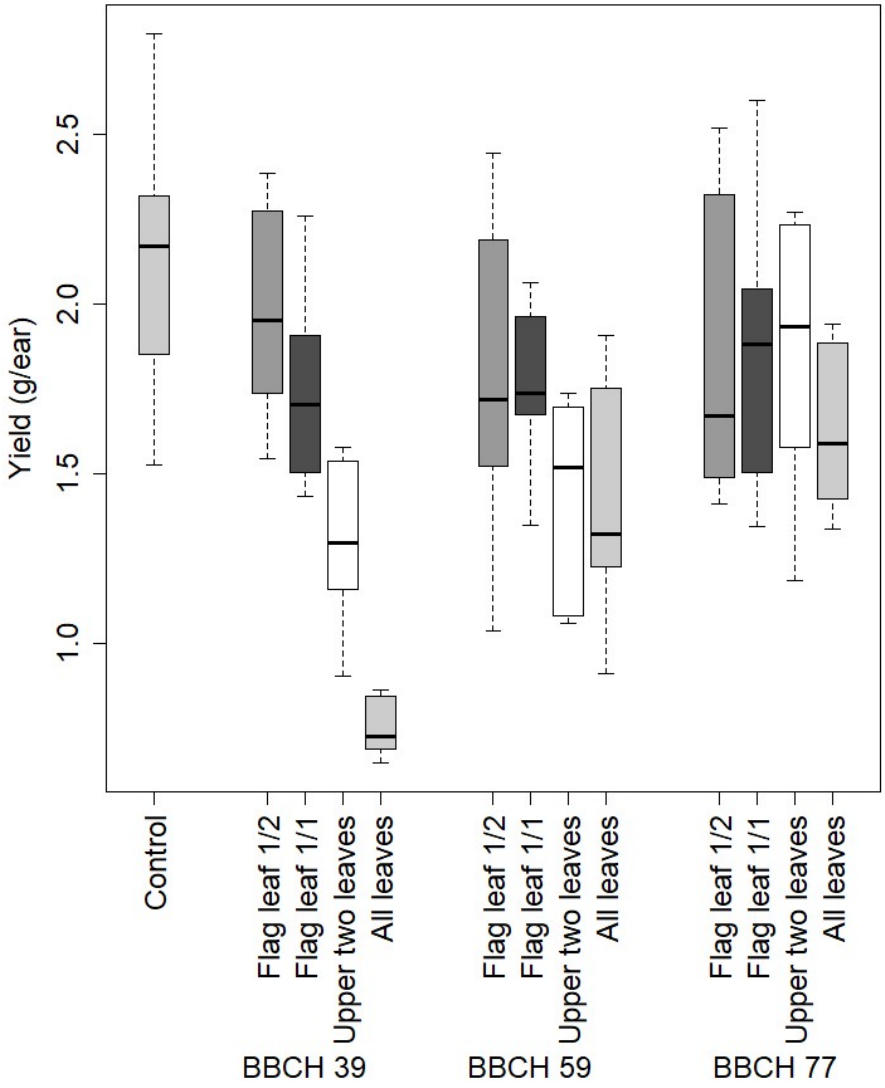


Figure 46: Grain yield (g per tiller) for each treatment in the manual defoliation (greenhouse) trial. BBCH 39 = flag leaf stage; BBCH 59 = end of heading stage; BBCH 77 = late milk stage.

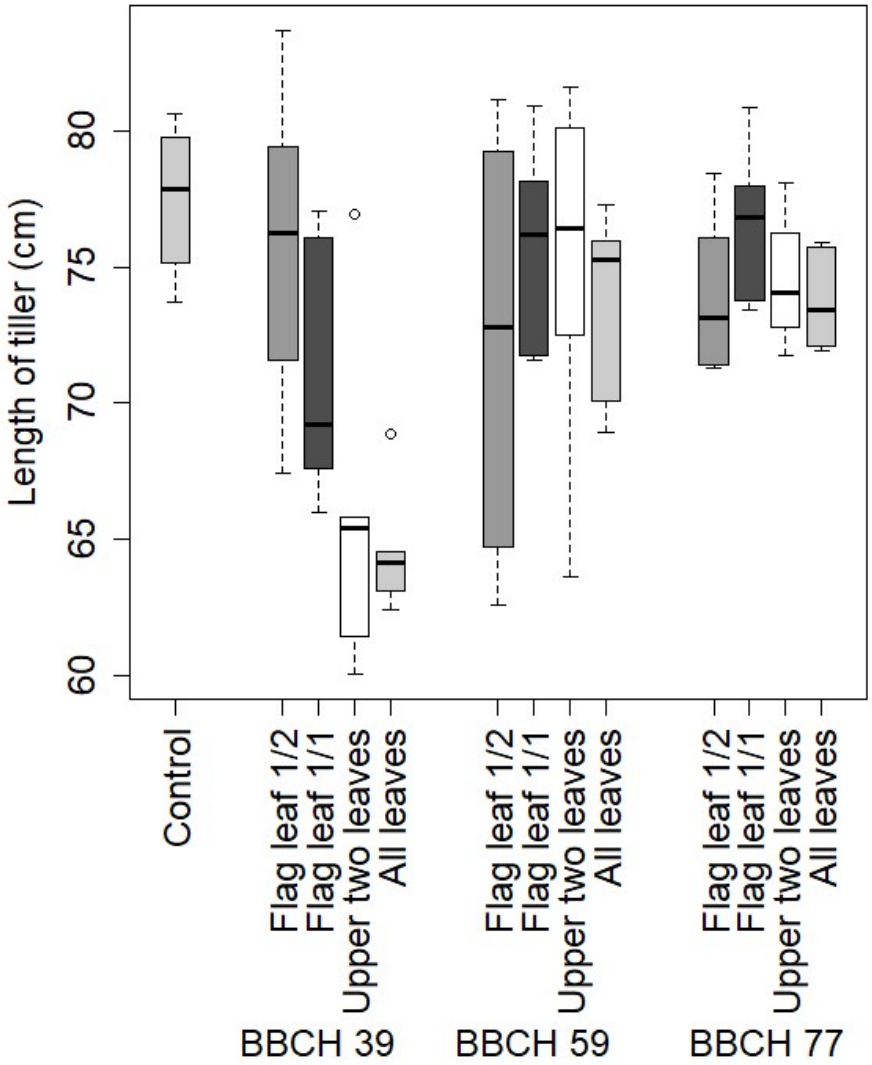


Figure 47: Tiller length (cm) for each treatment in the manual defoliation (greenhouse) trial. BBCH 39 = flag leaf stage; BBCH 59 = end of heading stage; BBCH 77 = late milk stage.

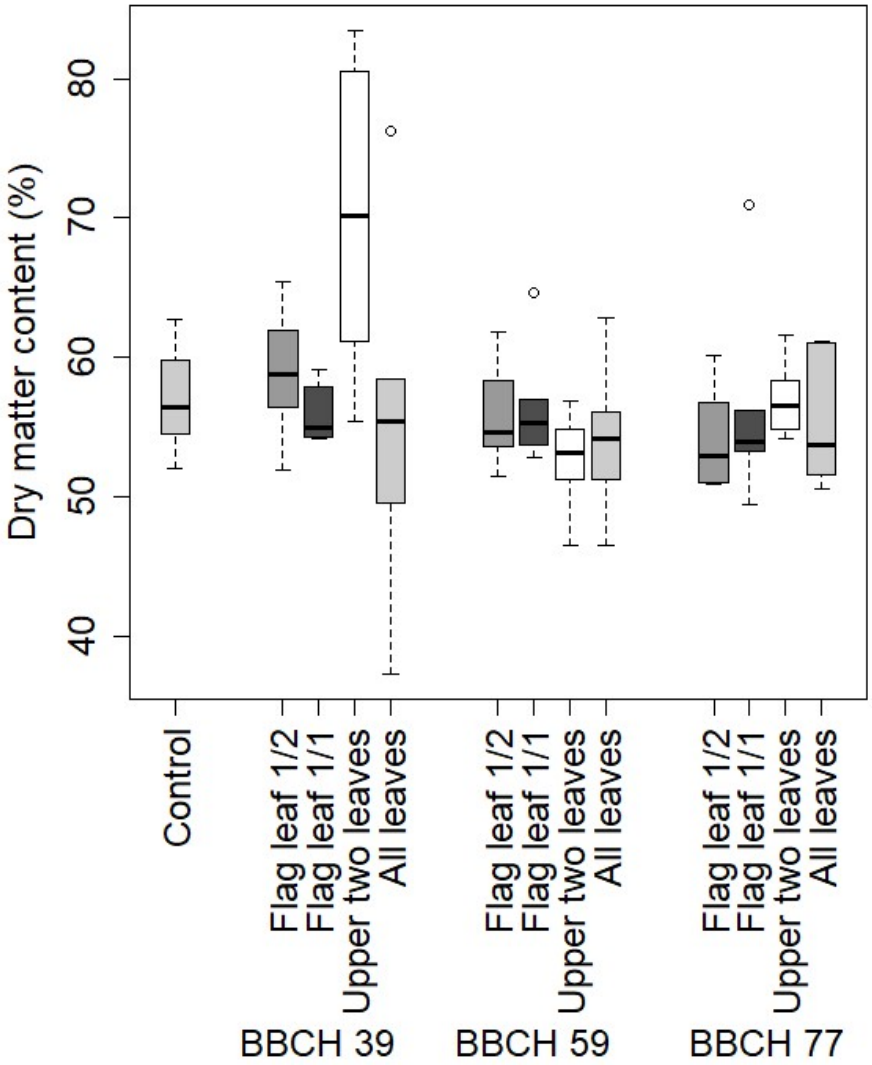


Figure 48: Dry matter content (%) per pot for each treatment in the manual defoliation (greenhouse) trial. BBCH 39 = flag leaf stage; BBCH 59 = end of heading stage; BBCH 77 = late milk stage.

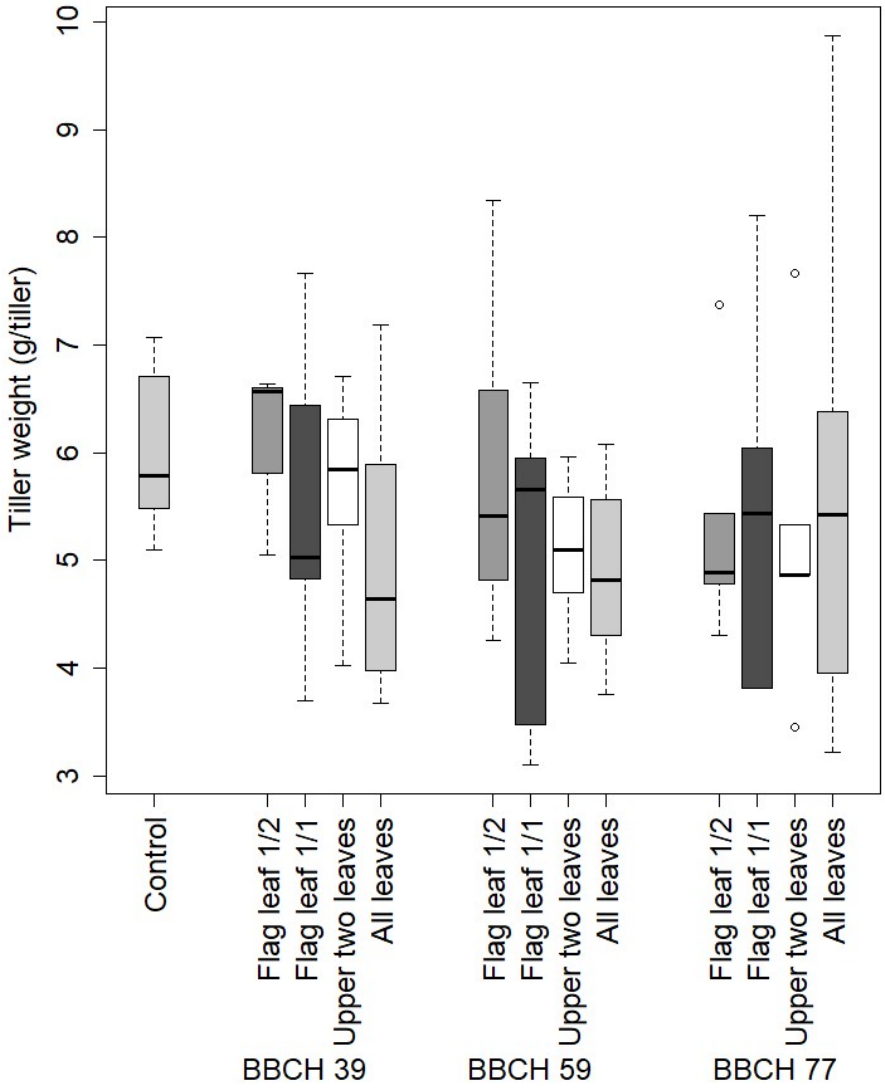


Figure 49: Tiller weight (g per tiller) for each treatment in the manual defoliation (greenhouse) trial. BBCH 39 = flag leaf stage; BBCH 59 = end of heading stage; BBCH 77 = late milk stage.

This study revealed a significant correlation between the removed leaf area and the final grain yield (g per tiller) ($r = -0.633$, $n = 76$, p-value < 0.001). A linear regression line was added to the graph (Figure 50; $R^2 = 0.37$), which indicates that removing 25 cm^2 of leaf material results in an estimated grain yield of 3.29 g (per ear), while removing 200 cm^2 reduces this grain yield to 1.04 g.

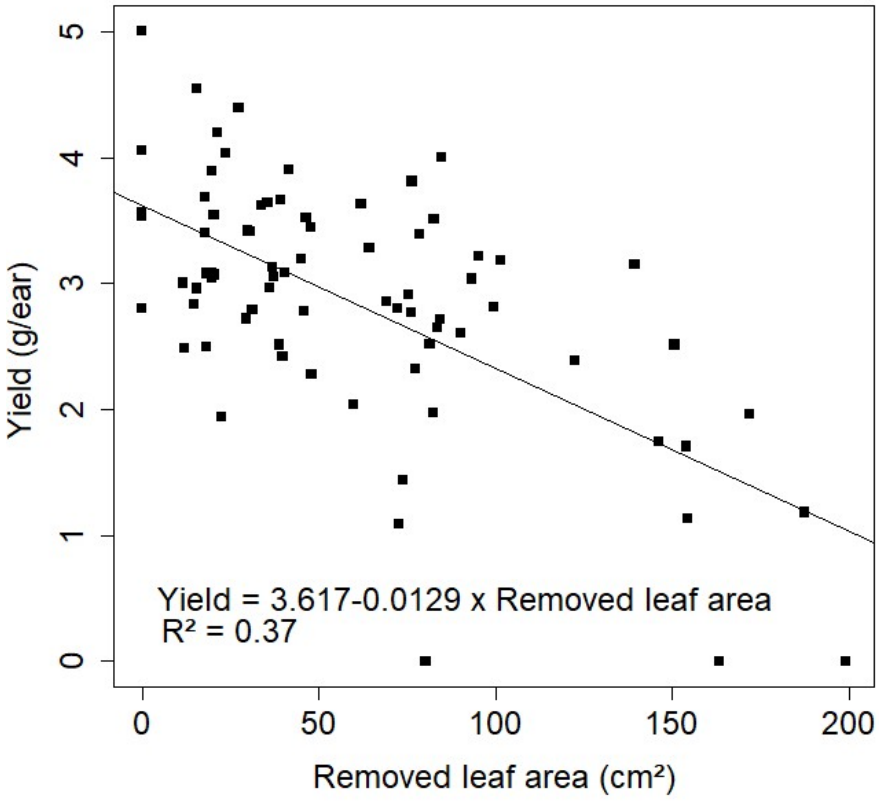


Figure 50: Relationship between the removed leaf area (cm²) and the grain yield (g per tiller) in the manual defoliation trial.

The manual defoliation study also revealed a significant correlation between the length of the tiller (cm) and the final grain yield (g per tiller) ($r = 0.638$, $n = 76$, p-value < 0.001). A linear regression line was added to the graph (Figure 51; $R^2 = 0.40$), which indicates that a observed length of 65 cm leads to a grain yield of 1.21 g, while a tiller length of 80 cm leads to a grain yield of a approximately 2 g.

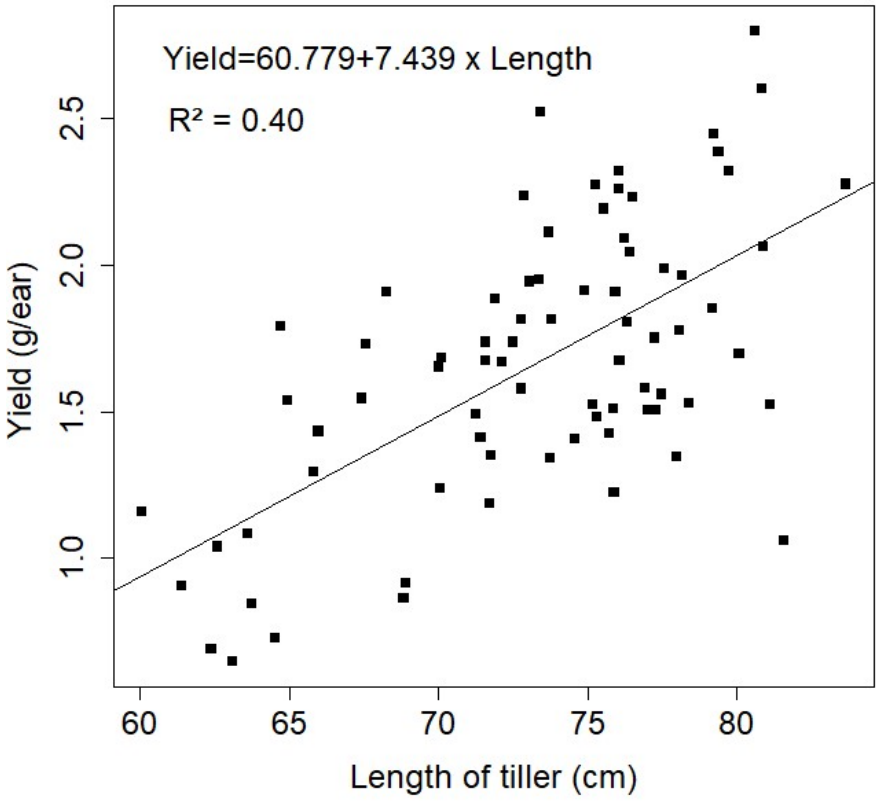


Figure 51: Relationship between the length of the tiller (cm) and the grain yield (g per tiller) in the manual defoliation trial.

Insecticide trials

Table 31 enlists the results of the trials during two subsequent growth seasons (2015–2016 and 2016–2017). A Kruskal-Wallis test on the monitoring data revealed that mean number of eggs or peak egg density (amount per tiller) did not significantly differ (2015–2016: $\chi^2(6) = 5.385$, p-value = 0.496; 2016–2017: $\chi^2(6) = 10.182$, p-value = 0.117) between tested insecticides, for both growth seasons. In addition, based on the data from growth season 2015–2016, no significant differences between the tested insecticides were found for the mean number of larvae ($\chi^2(6) = 10.000$, p-value = 0.125), peak density of larvae ($\chi^2(6) = 12.278$, p-value = 0.056; Figure 52) and total flag leaf damage ($\chi^2(6) = 8.000$, p-value = 0.238; Figure 53).

Table 31: Grain yield (kg/ha), average number of eggs and larvae (per 100 tillers), egg and larval peak numbers (per 100 tillers) and total flag leaf damage (%, ±SE) in relation to the different treatments for each growth seasons (2015–2016 and 2016–2017): Decis EC 2,5 (deltamethrin, 5 g/ha); Karate zeon (λ-cyhalothrin, 5 g/ha); Fury 100 EW (zetacypermethrin, 10 g/ha); Neemazal-T/S (azadirachtin, 25 g/ha); Biscaya 240 OD (thiacloprid, 96 g/ha) and Teppeki (flonicamid, 80 g/ha)

Growth season	Treatment	Grain yield (kg/ha)	Average number of eggs per 100 tillers	Peak number of eggs per 100 tillers	Average number of larvae per 100 tillers	Peak number of larvae per 100 tillers	Total flag leaf damage (%)
2015–2016	Control	8910.7±814	0.21±0.42	0.83±1.67	4.38±3.75	10.83±8.33	6.32±2.35
	Decis EC2,5	9176.7±992	0	0	0.63±0.80	1.67±1.92	4.10±0.98
	Karate Zeon	9220.8±791	0	0	0.21±0.42	0.83±1.67	4.52±1.69
	Fury 100 EW	9418.0±844	0	0	1.46±1.42	5.00±4.30	3.67±0.75
	NeemAzal-T/S	9205.1±1010	0	0	1.67±1.80	5.00±5.77	4.63±1.00
	Biscaya 240 OD	9041.3±827	0.21±0.4	0.83±1.67	1.88±1.04	5.83±3.19	5.32±1.92
	Teppeki	8653.3±1138	0	0	1.04±1.25	3.33±3.84	8.26±2.18
2016–2017	Control	11068.1±714	1.46±1.85	4.17±4.19	3.33±2.64	8.33±6.38	6.62±3.65
	Decis EC2,5	11047.2±518	0.63±0.80	2.50±3.19	1.04±0.42	3.33±0.00	2.45±0.97
	Karate Zeon	10978.7±755	0.21±0.42	0.83±1.67	0.63±1.25	2.50±5.00	4.40±2.89
	Fury 100 EW	11130.0±501	0	0	0.21±0.42	0.83±1.67	3.83±2.67
	NeemAzal-T/S	11408.1±471	0	0	0.83±0.68	2.50±1.67	4.02±2.40
	Biscaya 240 OD	11054.1±499	0.63±0.80	1.67±1.92	1.46±1.72	2.50±3.19	1.63±1.10
	Teppeki	10844.7±281	1.04±0.80	3.33±2.72	3.13±1.25	7.50±1.67	8.51±7.08

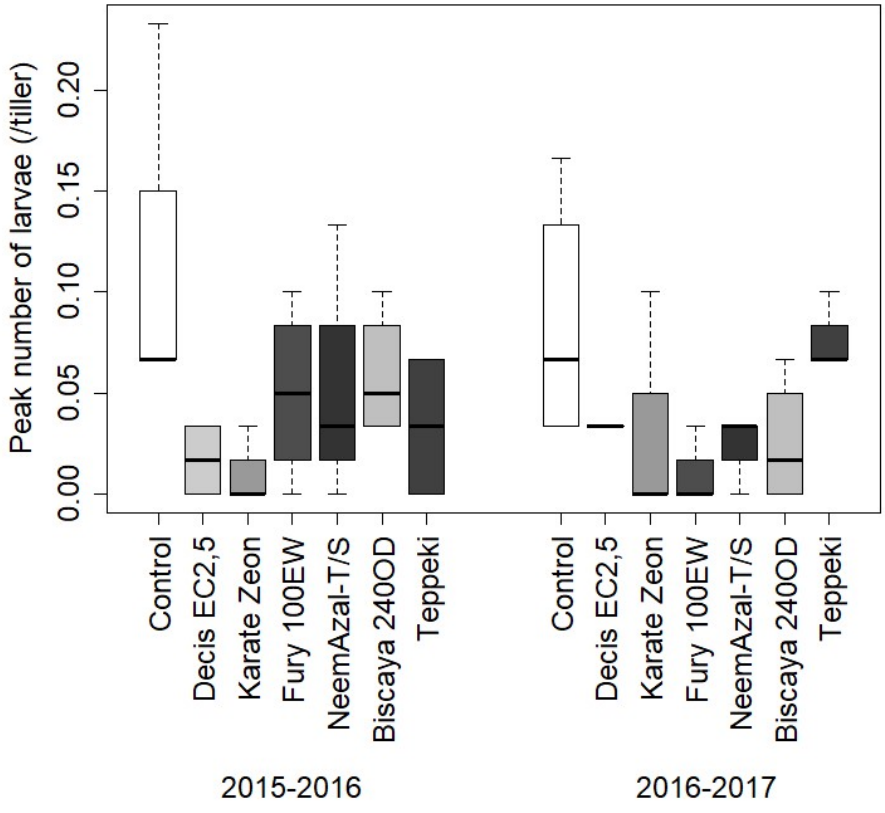


Figure 52: Peak number of larvae (mean number per tiller) for both growth seasons (2015–2016 and 2016–2017) and each treatment: Decis EC 2,5 (deltamethrin, 5 g/ha); Karate zeon (λ-cyhalothrin, 5 g/ha); Fury 100 EW (zetacypermethrin, 10 g/ha); Neemazal-T/S (azadirachtin, 25 g/ha); Biscaya 240 OD (thiacloprid, 96 g/ha) and Teppeki (flonicamid, 80 g/ha)

In contrast, during the second growth season (2016–2017), the various treatments gave rise to significant differences in the observed peak larval density ($\chi^2(6) = 15.750$, p-value = 0.015; Figure 52) and in the total flag leaf damage ($\chi^2(6) = 14.000$, p-value = 0.030; Figure 53). During this growth season, peak densities were highest in the control treatment (8.33 larvae per 100 tillers), followed by Teppeki (flonicamid, 80 g/ha, 7.5 larvae per 100 tillers). During this growth season, flag leaf damage was highest in the plots treated with Teppeki (flonicamid, 80 g/ha, 8.51% of damage to the flag leaf), followed by the control treatment (6.62% of leaf damage).

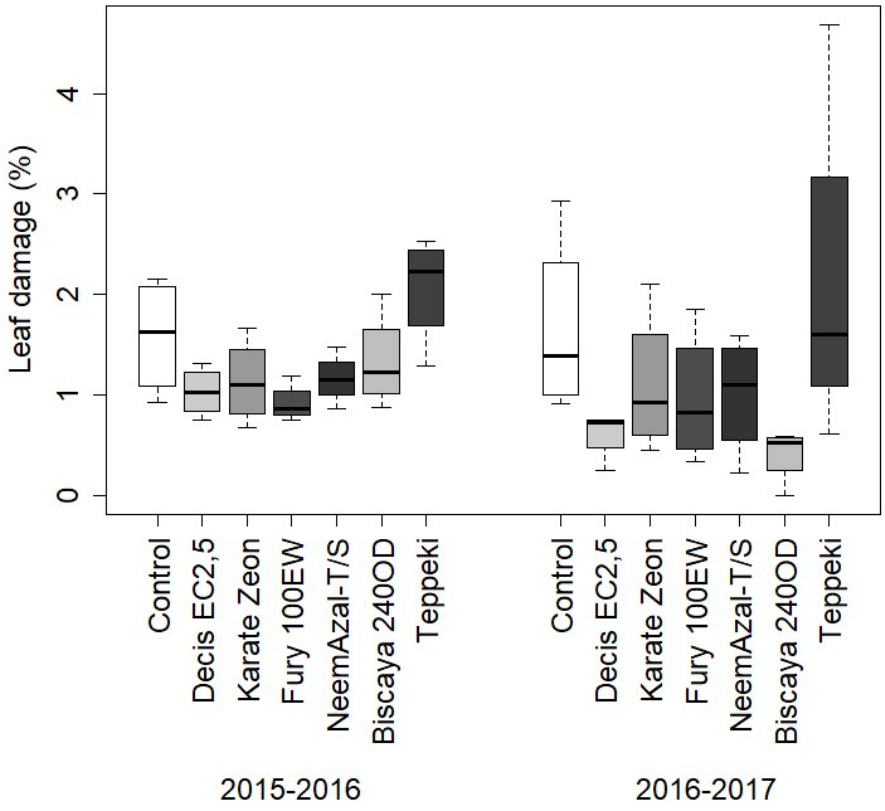


Figure 53: Total flag leaf damage (%) for both growth seasons (2015–2016 and 2016–2017) and each treatment, observed 14 days after treatment application. Treatments include Decis EC 2,5 (deltamethrin, 5 g/ha); Karate zeon (λ-cyhalothrin, 5 g/ha); Fury 100 EW (zetacypermethrin, 10 g/ha); Neemazal-T/S (azadirachtin, 25 g/ha); Biscaya 240 OD (thiacloprid, 96 g/ha) and Teppeki (flonicamid, 80 g/ha).

A one-way ANOVA revealed no significant differences between grain yield (ton per ha; Figure 54) for different treatments during both growth seasons (2015–2016, (F(6,21) = 0.289, p-value = 0.936); 2016–2017, (F(6,21) = 0.385, p-value = 0.880)). Also, test weight (**Appendix 4**) did not significantly differ between treatments during both growth seasons (2015–2016, F(6,21) = 1.192, p-value = 0.348; 2016–2017, F(6,21) = 0.863, p-value = 0.58).

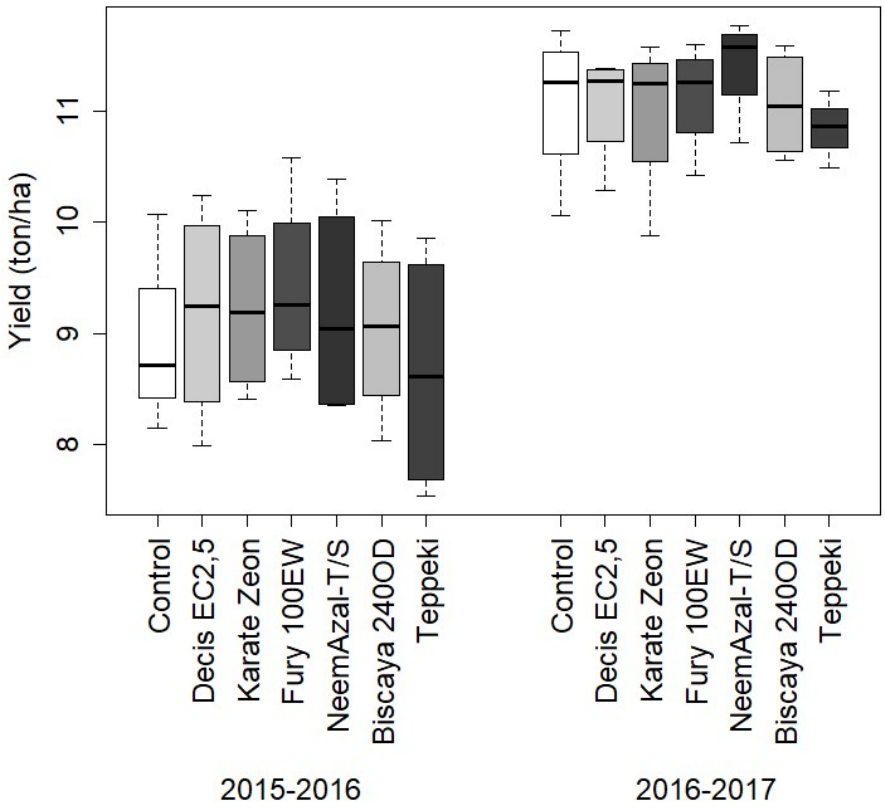


Figure 54: Grain yield (ton per ha) for each treatment and both growth seasons (2015–2016 and 2016–2017) in the field insecticide trial. Treatments include Decis EC 2,5 (deltamethrin, 5 g/ha); Karate zeon (λ-cyhalothrin, 5 g/ha); Fury 100 EW (zetacypermethrin, 10 g/ha); Neemazal-T/S (azadirachtin, 25 g/ha); Biscaya 240 OD (thiacloprid, 96 g/ha) and Teppeki (flonicamid, 80 g/ha)

Based on the results of both insecticide trials, it seemed that Karate Zeon (λ-cyhalothrin, 5 g/ha) is able to keep the population of CLB larvae lowest during the monitored period (3 weeks). Both the peak number of larvae (an average peak density of 0.017 larvae per tiller) as well as the mean number of larvae (0.0042 larvae per tiller) were lowest in this treatment and highest in the control treatment (resp. 0.096 larvae per tiller and 0.039 larvae per tiller). However, flag leaf damage was lowest for Decis EC2,5 (deltamethrin, 5 g/ha) with a total observed flag leaf damage of 3.28%. Plots that were treated with Teppeki (flonicamid, 80 g/ha) showed the highest flag leaf damage (8.38%). Grain yields were highest for NeemAzal-T/S (azadirachtin, 25 g/ha) and Fury 100EW (zetacypermethrin, 10 g/ha) and lowest for Teppeki (flonicamid, 80 g/ha) and the control treatment. The grains harvested from the plots that were treated with Decis EC2,5 (deltamethrin, 5 g/ha) showed the highest test weight (76.16 g). The control treatment showed lowest test weight (75.05 g).

Sowing density trials

Table 32 lists the results of the trials during two subsequent growth seasons (2016–2017 and 2017–2018). The first growth season (2016–2017) revealed no significant differences in the observation data of the eggs (mean number of eggs per tiller ($F(3,12) = 1.715$, p-value = 0.217) and peak number of eggs per tiller ($\chi^2(3) = 5.333$, p-value = 0.149)) and the larvae (mean number of larvae per tiller ($\chi^2(3) = 6.000$, p-value = 0.112) and peak number of larvae per tiller ($\chi^2(3) = 3.200$, p-value = 0.362)). During the second growth season (2017–2018), the various sowing densities resulted in significant differences for egg peak density ($\chi^2(3) = 8.889$, p-value = 0.031). The highest sowing density (600 grains/m²) gave rise to the lowest peak egg density (0.28 eggs per tiller), while the highest peak egg density (0.87 eggs per tiller) was observed in the plots with the lowest sowing density (100 grains/m²). The effect of sowing density was also visible in the observed peak number of larvae per tiller (Figure 55). Nonetheless, these differences were not significant ($\chi^2(3) = 4.000$, p-value = 0.261). Likewise, no significant differences were found during this growth season for the mean number of observed larvae per tiller ($\chi^2(3) = 2.794$, p-value = 0.425).

Table 32: Grain yield (kg/ha), average number of eggs and larvae (per tillers), egg and larval peak numbers (per tillers, ±SE) in relation to the different sowing densities for each growth seasons (2016–2017 and 2017–2018)

Growth season	Sowing density (grains/m²)	Grain yield (kg/ha)	Average number of eggs per tiller	Peak number of eggs per tiller	Average number of larvae per tiller	Peak number of larvae per tiller
2016–2017	250	8602.2±1506	0.02±0.014	0.06±0.03	0.01±0.005	0.07±0.03
	300	8567.8±1514	0.03±0.009	0.08±0.03	0.01±0.006	0.06±0.03
	350	9105.0±423	0.04±0.015	0.13±0.06	0.02±0.012	0.13±0.07
	400	9200.9±642	0.03±0.015	0.15±0.08	0.01±0.004	0.08±0.02
2017–2018	100	6129.7±289	0.58±0.133	0.87±0.26	0.13±0.053	0.24±0.11
	300	8421.2±656	0.24±0.077	0.38±0.09	0.08±0.024	0.10±0.04
	400	8349.0±645	0.26±0.058	0.39±0.13	0.06±0.025	0.08±0.04
	600	8050.8±528	0.18±0.046	0.28±0.06	0.07±0.044	0.10±0.09

A Kruskal-Wallis analysis revealed no significant differences between grain yield (kg per ha; Figure 56) for the different treatments for growth season 2016–2017 ($\chi^2(3) = 2.000$, p-value = 0.572). In 2017–2018, the lowest sowing density yielded significanthly less than the other treatments ($\chi^2(3) = 8.757$, p-value = 0.033). Grain yield also revealed significant correlations with the observed mean density of the eggs ($r = -0.682$, p-value < 0.001) and the larvae ($r = -0.606$, p-value < 0.001), as well as with the observed peak densities of the eggs ($r = -0.648$, p-value < 0.001) or the larvae ($r = -0.491$, p-value = 0.004).

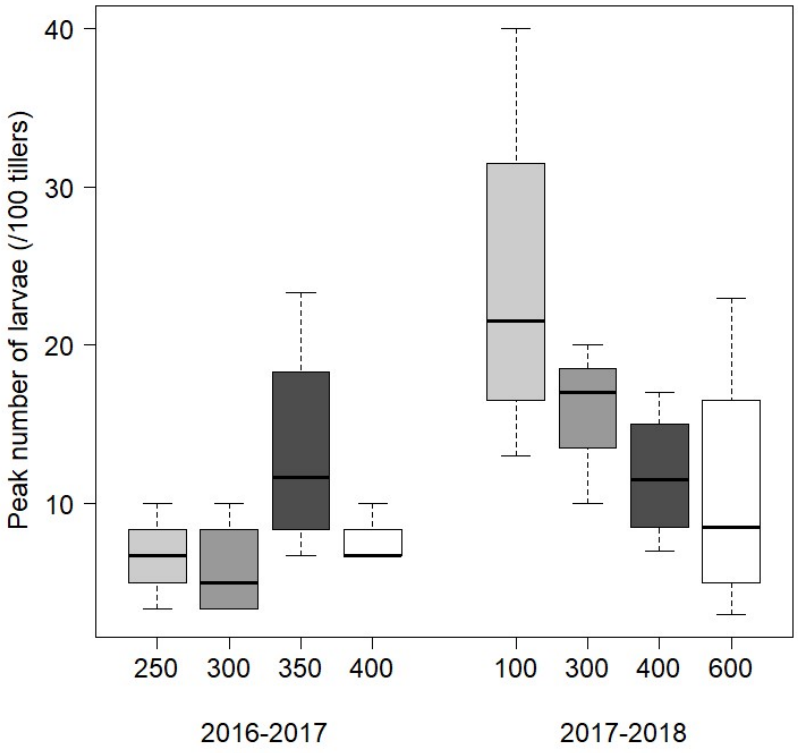


Figure 55: Peak number of larvae (number per 100 tillers) in relation to the sowing density (grains per m²) for two growth seasons: 2016–2017 and 2017–2018.

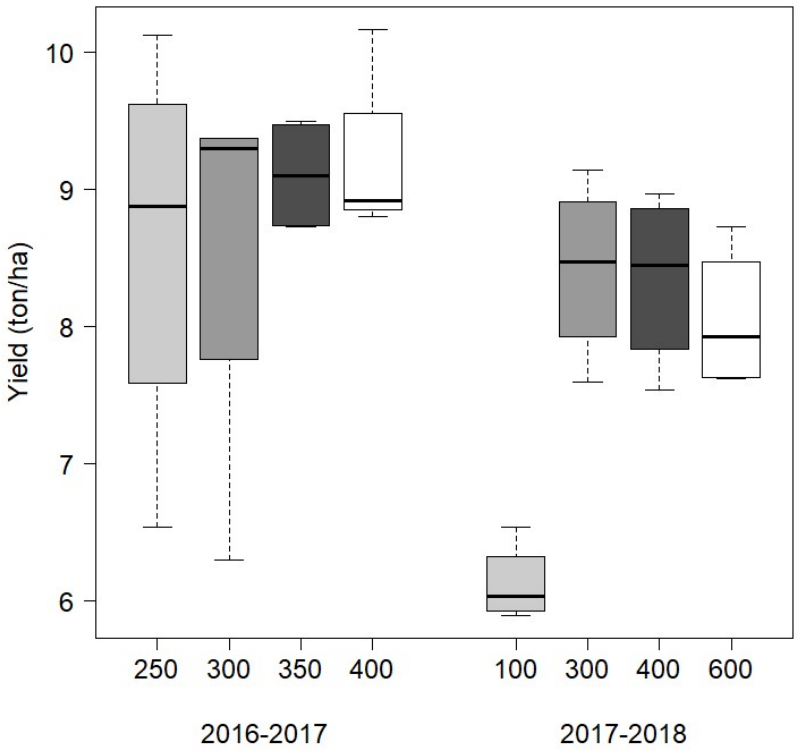


Figure 56: Grain yield (ton per ha) in relation to the sowing density (grains per m²) for two growth seasons: 2016–2017 and 2017–2018.

Discussion

Cage trials

Total flag leaf damage was significantly (positively) correlated with the observed larval presence. Nevertheless, this leaf damage did not correlate with grain yield loss. These findings are in agreement with findings in literature (Ihrig et al., 2001, Buntin et al., 2004) showing that although larvae are present in the fields, they do not always lead to significant grain yield losses. Our study also showed an effect of flag leaf damage towards the thousand grain weight of the plants, making them heavier. The effect of defoliation on the thousand grain weight of the kernels is not unambiguous and depends on other (abiotic) stress inputs at the moment of defoliation (Frederik and Camberato, 1995). Also, the effect of larval defoliation differs with crop growth stage, with a critical period three weeks before anthesis until flowering (Frederik and Bauer, 1999). Introducing adult CLBs (and not larvae) in our cages could cause a desynchronisation between plant and pest insect life cycle. When CLBs reached the fourth-instar larval stage, plants were long past anthesis. Studies show that grain yield loss after anthesis, due to biotic stress factors, is minimal. To minimise this desynchronisation, adults were introduced as early as possible in the cages in growth season 2017–2018, when plants reached BBCH 32 (second node). Even during this growth season, with larval densities peaking at 2.5 larvae per tiller, no significant grain yield loss was observed. Similar results were observed in previous growth seasons and trial locations. While we can assume that at these densities, plants were stressed, it remains possible that other factors were more limiting: e.g. drought stress or other pest insects such as aphids. Indeed, it is known that abiotic stress factors such as drought can determine the final grain yield. Especially during flowering, the plants are in need of water for completing the anthesis stage (Frederik and Bauer, 1999). As plants were isolated inside a cage, this can create a microclimate that could influence the plants but also the mortality and activity of the adult beetles and larvae. An increase in temperature (until 32 °C) and humidity decreases the larval mortality (Ali et al., 1979). Nevertheless, an increase in temperature also shortens the life cycle of insects and therefore the time the larvae can defoliate leaf tissue. An increased temperature also causes the plants to ripen faster, decreasing the time that the larvae can defoliate leaf tissue. Especially during late spring 2017 and 2018, plants experienced a dry period that lasted until anthesis, provoking drought stress to the plants. Subject to a microclimate within the cages, it is possible that this stress was even higher on the tested plants, lowering grain yields. Next to these abiotic interactions within the cages, isolating the adult beetles in high numbers per area unit can introduce an extra stress factor, potentially increasing the mortality of eggs and larvae and therefore minimising the impact on final grain yield.

While it is clear that an infestation of CLB larvae during critical moments in the plant's life will reduce grain yields, due to a number of reasons (mentioned in **Chapter 2,** Crop damage), it is not possible to predict in situ grain yield losses caused by larval feeding based on our cage trial data. Nevertheless, a clear link with flag leaf damage was found.

Manual defoliation trials

This trial succeeded in bridging the existing gap between the defoliating effect of the larvae and grain yield. Similar to the work of Buntin et al. (2004), a clear grain yield loss was observed by defoliating in early crop growth stages (BBCH 39, flag leaf stage). In the treatments that involved fully defoliating the flag leaves, upper two leaves or all the leaves of the plant, some tillers did not develop an ear. This effect was especially visible at the plants that were defoliated at an early crop growth stage (BBCH 39). At this stage, the plant was still actively growing. It is possible that the plant reacted to the inflicted damage and relocated its resources to other tillers. It is already known that the plant, when damaged, is able to shift its resources to other shoots (Rubia et al., 1996), or even change its architecture (Tiffin, 2000). Possibly the tillers without developed ear are secondary tillers.

Defoliation after late milk stage did not seem to affect the plant significantly, when the plants are already at the start of scenescening. Therefore, we can assume that the assimilation rate is already lower at this stage. The removed leaf area (cm^2) was a lot smaller compared to the removed area in earlier crop growth stages. Therefore, we can assume that the differences in removed leaf area between different defoliation levels were smaller. In addition, a significant effect of defoliation was observed in case leaf tissue was removed during the first two growth stages, whereas defoliation in the last growth stage did not affect grain yield. Tiller length was only significantly influenced in the early crop growth stage (BBCH 39), with a significant difference between only removing flag leaf material or also defoliation the other leaves.

At boot stage (BBCH 59), grain yield differed significantly between the treatments that included the removal of flag leaf material and the treatments that removed more leaf material (all leaves removed and upper two leaves removed).

Although extrapolating these results to a field scale is not possible, based on these results, it can be concluded that leaf damage at an early crop growth stage causes the most stress to the plant and therefore has the most adverse effect on growth and consequently grain yield components. Caution is also required when extrapolating data based on artificial leaf defoliation (used as a surrogate for herbivory caused by *Oulema* larvae) experiments to natural field circumstances. CLBs' feeding pattern is different, leaving the lower epidermis tissue unharmed. This damage is not easily mimicked by manually defoliating leaves. A leaf scar due to CLB feeding increases water loss and forms a possible

infection site for diseases (Wellso, 1978). It is clear that, while it remains a useful practice to mimic herbivory damage on plants, serious caution is needed when using this data to apply on a field-basis (Baldwin, 1990).

Insecticide trials

During these two trial seasons, synthethic pyrethroids seemed most effective in controlling CLBs and maximising grain yields. Products such as Karate Zeon (λ-cyhalothrin, 5 g/ha), Decis EC2.5 (deltamethrin, 5 g/ha) and Fury 100EW (zeta-cypermethrin, 10 g/ha) were almost identical in effectivity, keeping the number of larvae and observed flag leaf damage low during the monitoring period. The highest flag leaf damage was seen in plots treated with a systemic aphid-product such as Teppeki (flonicamid, 80 g/ha). These plots also yielded the least, comparable with the control treatment. It is clear that this product does not improve the management of CLBs. Its use should therefore be limited to moments when aphid levels reach economic thresholds and not be used for CLB control.

While most of the control strategies used in the past for CLBs relied heavily on insecticides (Ruppel, 1972), quickly, it became clear that a curative treatment alone was not sufficient in areas where CLBs were very active. While many insecticides were tested for their effectivity towards CLBs, only a handful of these tested products are still allowed in the EU. Comparing organic carbamates (methomyl) and organic phosphosinsecticides (malathion) with synthetic pyrethroids, Buntin et al. (2004) proved that the synthetic pyrethroids were not only the cheapest, but the most effective as well. Darab et al. (2017) observed similar results in Romania, comparing pyrethroids with neonicotinoids.

Despite the negative impact of these broad-spectrum pyrethroids on the natural enemy population, the lack of (approved) alternatives, their high effectivity and their low price makes this class of insecticides currently the best choice for growers to control CLBs.

Sowing density trials

While the results of the sowing density trials during the first growth season (2016–2017) did not highlight major effects of the sowing density on the phenology of CLBs, altering the sowing densities to extremes led to better insights in the reaction of the pest insect to the plant density. In 2017–2018, it became clear that in the high plant density treatments significantly less larvae (number per tiller) were present. This is in agreement with research by Herbert (1990) and Grand and Patrick (1993). On the other hand, these authors also highlighted the fact that while CLB larval presence is lower per plant unit, it remains equal when recalculated on area unit basis. Therefore, most researchers (Casagrande et al., 1977; Webster et al., 1978) do not recommend to alter sowing densities as a preventive measurement, but advise to follow general management practices (350 grains/m² for winter wheat,

sown in good circumstances in a sandy loam soil). During growth season 2017–2018, we also found that the lower sowing density treatment yielded significantly less than the other treatments. While we found significant correlations with observed egg and larval presence in the field, this correlation can be assumed to be non-causal. It is known that increasing the sowing density is the basis for increasing grain yield (Hiltbrunner et al., 2007) and decreasing nitrogen accumulation (Arduini et al., 2006; Dai et al., 2014). Indeed, when wheat is grown without abiotic stress inputs, the grain yield increases with sowing density (Wilson and Swanson 1962; Blue et al., 1990; Tompkins et al., 1991). Therefore we can assume that this could be the main reason for the significant difference in grain yield. On the other hand, when the crop is subjected to drought stress, a higher density is known to reduce grain yield per area unit (Wilson and Swanson 1962; Blue et al., 1990; Tompkins et al., 1991). This was observed in 2018, as this year had a very dry spring and summer (**Chapter 3**), resulting in a grain yield drop in the higher sowing density.

As we were not able to find conclusive evidence of the effectiveness of altering sowing density for CLB management, we recommend to follow guidelines and sow at a sowing density of 300 to 350 grains/m², as these densities resulted in the highest grain yield, with tolerable levels of CLB larvae, well below economic thresholds.

Chapter 8: Conclusions and future perspectives

Recalling the research objectives

In this chapter, an attempt is made to answer the predetermined research questions. Managing CLB occurrence in wheat is not an easy task to accomplish, as this pest only occasionally surpasses the economic thresholds levels currently used in many regions (0.4 larvae per tiller), making the timing of an insecticide treatment difficult. Also, currently, no economic thresholds for CLB management were set specifically for our region. Furthermore, CLBs share this population of natural enemies with aphids that are present later on in the growth season. As most insecticides approved in winter wheat for CLB management could reduce the number of natural enemies active in the field, application timing is crucial. It is known that these natural enemies are crucially important to keep aphid numbers from increasing exponentially. In the first part of this thesis, we aimed to investigate what species of the genus *Oulema* are active in Flemish wheat fields (**Chapter 4**). A second part summarised the vast monitoring trials that ran for four subsequent growth seasons, covering the main wheat growing regions in Flanders (**Chapter 5**). The knowledge gathered in this chapter was then used to construct a prediction model (**Chapter 6**). This model can support growers in their decision concerning a curative treatment. In **Chapter 7**, a known effective preventive tactic for insect management was investigated for its effectiveness towards CLBs. In addition, trials were executed to support the grower with a curative treatment (timing, choice of product). In the present chapter (**Chapter 8**), these research goals are addressed. In a conclusion, a potential IPM-system is described for Flanders. Furthermore, this chapter will end with addressing potential areas for future research.

Hypothesis 1: The CLB population in Flemish wheat fields is dominated by two species from the genus *Oulema* (*O. melanopus* and *O. obscura*), as in other Western European countries.

- **RS 1.1 Which species of the genus *Oulema* occur in Flanders?**

Sweep net catches during three subsequent years on the observed wheat fields proved that next to the worldwide main species *O. melanopus*, *O. duftschmidi* and *O. obscura* occur in Flemish wheat fields as well. *Oulema rufocyanea*'s occurrence can be described as being rare.

- **RS 2.1 What is their distribution throughout Flanders?**

The relative distribution of each species was highly influenced by a year-effect. The within-field distribution did not significantly differ between species. The species distribution of *O. melanopus* and *O. duftschmidi* showed to be sympatric in Flanders. The least abundant species, *O. obscura,* was observed most in the Polders, the heavier soil type.

⇨ As a conclusion, based on both research questions, this hypothesis can only partially be supported.

Hypothesis 2: The larvae of CLB significantly reduce grain yield in wheat in Flanders.

- **RS 2.1 Which parameters influence the phenology and distribution of CLBs in and around the field?**

The monitoring trials pointed out that growth and development of these poikilothermic organisms are mainly influenced by temperature. We were able to link the yearly fluctuations in CLB development with temperature data gathered from each location. It is clear that temperatures in February and March influence the starting date of the egg population, while temperatures in March and May influence the start, peak and end date of the larval population. Peak heights were negatively correlated with mean temperatures in February and March, indicating that warm months decrease the peak density of the eggs and the larvae. On the contrary, temperatures in April were positively correlated with peak heights of the eggs and the larvae, indicating that a warm April will increase the peak heights of the eggs and the larvae.

- **RS 2.2 Which preventive or curative management practices can be used to control CLBs?**

Concerning preventive tactics, we observed that increasing sowing densities decreases the pest density per plant unit. Current literature investigated the effect of sowing density on the presence of *Oulema melanopus*. In Belgium though, our research showed multiple species to be active in winter wheat. The effect on sowing density on the other species was yet to be investigated. Nonetheless, as increasing sowing density could increase other problems and therefore decrease the financial benefit of introducing this tactic, we follow the recommendation made by Webster et al. (1979) not to alter sowing densities for CLB management.

- **RS 2.3 What is the population density of CLB larvae in Flemish winter wheat?**

During the four monitored growth seasons, CLB larvae reached an average of 0.04 larvae per tiller, while average peak levels of 0.14 larvae per tiller were observed. Comparing the observed larval densities with current available economic thresholds (0.4 larvae per tiller or 1 larvae per tiller), these densities are considered low to very low. We can conclude that currently, CLBs remain an occasional pest insect in Belgium.

- **RS 2.4 How many CLB larvae can be tolerated compared to wheat prices (i.e. what are correct economic thresholds levels in Flanders, Belgium)?**

Based on a combination of the manual defoliation and cage trials, we conclude that growers should monitor their crop and execute a treatment when plants are still in the vegetative stage (before flowering) and larvae reach densities of **1.8 larvae per tiller**. Although this threshold is much higher than thresholds mentioned in more recent literature: 0.4 larvae per tiller (Buntin et al., 2004) or 1 larvae per tiller (Tanaskovic et al., 2012), older (regional) studies mention a threshold of 2.5 larvae per tiller (Stilmant, 1995; Anglade et al., 1976) for Belgium, indicating that relevant grain yield losses only occur at (very) high larval densities. Nevertheless, we stress that caution is required when applying this threshold as it is not based on in situ field-based research. Therefore, we can conclude that more research is necessary to confirm the validity of this threshold under field circumstances.

⇨ As a conclusion, based on these research questions, this hypothesis can only partially be supported, as in Flanders, the presence of the larvae is only occasionally of significant importance.

Hypothesis 3: A decision support system (DSS) is an effective tool for the wheat grower to control CLBs.

- **RS 3.1 Can insecticide treatments be integrated in a CLB management?**

Concerning a curative (insecticide) treatment, when necessary, an insecticide treatment with an approved synthetic pyrethroid is recommended. We do not recommend the use of older organophosphates as these products are not only toxic for the user, but literature also proves their lack of effectiveness. As currently, a curative insecticide treatment with a synthetic pyrethroid is broadly used by growers to control CLBs, only observing and timing the treatment needs optimisation.

- **RS 3.2 Which model is suitable to predict the presence and population density of the CLBs in winter wheat fields?**

The monitoring data were used to construct a predictive model. Based on correlations with temperature data from each location, we were able to predict the dates of the first eggs and larvae (mean absolute error of 4 days). This model was included into a webtool, available for growers. Although monitoring will still be a requirement before executing a curative treatment, this tool will help the grower to minimise both product input as well as time spent on monitoring.

⇨ As a conclusion, based on both research questions, this hypothesis can be supported. An error of 4 days can be considered acceptable and will definitely support the grower.

Conclusion

To start with, we can conclude that the timing at which the larvae defoliate the plants seems to critically influence the final grain yield. While the cage trials did not conclusively prove the effect of the larvae on grain yield loss, a significant link was found between grain yield and the amount of removed leaf mass. The defoliation trial proved that if the plants are still developing (before boot stage) when being defoliated by the larvae, the grain yield loss can be extensive. In the defoliation trial, it became clear that when the plants lost even only half of the flag leaf at this stage, this resulted in a grain yield drop of 10%. At boot stage (BBCH 59), grain yield differed significantly between the treatments that included the removal of flag leaf material and the treatments that removed more leaf material (all leaves removed and upper two leaves removed). Therefore, based on the gathered knowledge from both trials, we can conclude that if larval presence is extensive (> 1.8 larvae per tiller) and leaf damage is visible at early crop growth stages or at lower leaf levels, caution is required and a treatment could be necessary. At field level, this was observed in spring wheat fields, as development starts later in spring wheat than in winter wheat.

Based on the knowledge gathered in this thesis, the following advice concerning an IPM for CLBs can be formulated. Preventive tactics do not seem to be economically interesting for this pest insect.

While crop rotation has a proven effect on pest insects, it is still unclear how a certain rotation will influence the density of CLBs in a certain field. Our trials indicated that monoculture cropping of wheat can cause a higher egg peak in the field, therefore a grower should try to maintain a proper rotation scheme with three or more crops included. Choosing a variety, a grower could ask for varieties with pubescence on the leaves. Nevertheless, as the genetic diversity between the varieties in Flanders is small, there is little benefit here. A known effective preventive tool that could be implemented is the use of a ‘trap crop’ (i.e. a field edge with young oats around a winter wheat field), where only the edge of the field is being treated for CLBs. As CLB infestation remains an occasional pest in Flanders (**Chapter**

5), we recommend to follow general management practices concerning sowing dates and densities. We found a significant effect of nitrogen fertilisation on the egg peak date, which was observed later in fields that received little fertilisation. Nevertheless, as we found no effect on the larvae, we suggest to apply fertiliser following the recommendations based on soil samples. For preventive tactics, as a conclusion, a grower should aim to cultivate a healthy crop, with no special focus on altering practices for CLB management.

Monitoring remains an essential part of IPM. To stimulate monitoring while keeping time spent on monitoring small, a webtool (**Chapter 6**) was implemented for growers. Here, the dates of first oviposition and first larvae and peak dates of the eggs and larvae are predicted based on weather data nearby the grower's field. The predicted data of the CLB density in one field can then be compared with current thresholds.

The regression analysis from Figure 44 and Figure 50 (**Chapter 7**) pointed out that **1.8 larvae per tiller** result in a grain yield loss of 5% (grain weight), at this threshold an insecticide treatment is economically justified, based on a grain price of 130 euro/ton and a cost of 50 euro per treatment per hectare. Comparing this threshold with other aforementioned economic thresholds in literature (0.4 larvae per tiller (Buntin et al., 2004) or 2.5 larvae per tiller (Stilmant, 1995)), it is clear that this threshold is higher and therefore delays the moment of a curative treatment, or even make it unnecessary. A possible explanation for this high threshold can be found in the fact that no extreme densities were reached during any of the trial years and that the data supporting this threshold were obtained from greenhouse or semi-field trials. Therefore, more in situ field-based research will be required to test and confirm this economic threshold. Thresholds also vary depending on numerous factors such as grain prices, local yields, treatment costs, crop growth stage, efficacy level, choice of insecticide and many more. It is clear that this threshold should be used with due care.

To conclude, when the larval population reaches levels of 1.8 larvae per tiller, a curative insecticide treatment is recommended. In addition, field monitoring is crucially important, as the development of the CLB population depends on variables such as temperature and precipitation. While **Chapter 5** showed that the peak date and density of the eggs can be a valid predictor for the peak date and density of the larvae, going in the field remains a cornerstone practice in IPM. Nevertheless, egg peak heights can deliver valid information about whether or not a curative treatment will be necessary. This curative treatment is only effective against the larvae as most of the approved products rely on contact between the active ingredient and the targeted pest insect. Treatment is recommended with a synthetic pyrethroid, following recommended spraying guidelines concerning dosage, as these products seemed to deliver the most 'bang for the buck'.

Future perspectives

Intensive cropping management ensures the need for intensive inputs (fertilisers, pesticides). As IPM combines several practices, including biological, physical, mechanical, chemical and preventive management, only parts of the IPM puzzle were investigated and highlighted in this thesis. For the purpose of making our cropping system more stable, sustainable and profitable, more research concerning the potential of other IPM pilars such as biological control of CLBs is required. How can we increase a population of parasitoids in the field, while remaining economically competitive? It is known that especially these natural enemies are capable of keeping the population of CLBs under the economic threshold. What other preventive tactics could be used for CLB management, e.g. trap cropping? Are there physical or mechanical management practices that significantly influence the phenology of CLBs and are these profitable for growers to invest in?

Agriculture is developing at a fast rate with the introduction of new technologies (i.e. use of GIS, multispectral imaging, drones and specialised spraying equipment capable of spraying specific areas of the field), going towards a '*precision agriculture*' in the third wave of agricultural revolutions. By using these new technologies and sensors, 'big data' is collected. Analysing these data could enable the grower to limit his inputs while maintaining his outcome. It is already possible to treat individual plants, instead of treating whole areas. This can minimise the wastage of pesticides and fertilisers, making agriculture more efficient and sustainable (Blackmore et al., 2003). While at the moment monitoring happens by going to the field and monitoring a certain number of plants across the field, new technologies (sensors) could make this unnecessary (Shinghal et al., 2010). It is clear that agriculture is evolving at a fast rate and new research could enhance current IPM strategies, even for CLB management in Flanders.

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Summary

Globally, CLB (*Oulema* spp.) can cause serious economic losses to winter wheat cropping. Although damage due to the cereal leaf beetle is highly variable in this crop, has the past already shown that these organisms can cause major grain yield losses (2011, 2013). Currently an IPM for CLB is mostly absent or consisting of a correctional insecticide treatment. Even for this, knowledge of optimal timing is absent in Europe, combined with a lack of selective pesticides approved in Flanders for the control of CLB (Cereal Leaf Beetle). As thresholds specifically for our region are still absent, guided control is mainly based on older economic thresholds from the United States. It is clear that more research is necessary to hand out tools towards growers and therefore enable the possibility to integrate the management of CLB in winter wheat cropping.

During four subsequent growth seasons, several aspects of the CLB management were studied intensively. Large-scale field experiments included monitoring trials. During these trials, approximately 30 years were monitored for CLB presence annually. To examine whether or not correlations with the phenology of the beetle exist, other data such as weather data and crop husbandry practices were logged for each field as well. These correlations were also used to model the phenology and construct a predictive model that guides the grower in his management. These models were afterwards implemented into a webtool, available for growers. On the monitored fields, sweep catches were performed during the three last growth seasons. This enabled us to study the species from the genus *Oulema* that are active in Belgium.

Apart from these large-scale field trials on other locations (growers' fields), other field trials were executed on our specialised institutions (Bottelare and Rumbeke-Beitem). This includes sowing density trials (to test the effect of sowing density on the presence of CLB and grain yield), insecticide trials (to test currently approved and used insecticides) and cage trials. A manual defoliation trial was executed in the greenhouse to link leaf damage to grain yield loss.

The monitoring trials revealed that CLB in Flanders is only occasionally of economic importance (occasional pest insect). Clear correlations with temperature were found, confirming the effect this variable has on the development of this insect. Warm years will fasten development, which can cause the population to develop earlier in the field and cause more damage to the plants. Especially in combination when the plant is stressed by other a(biotic) factors such as drought or the presence of aphids, it is clear that CLB damage can be determining. The sweep catches revealed that in Flanders *O. melanopus*, *O. duftschmidi* and *O. obscura* are the main species found in our wheat fields.

The cage trials revealed that CLB presence causes serious flag leaf damage, which can cause grain yield loss. In the greenhouse (manual defoliation trial), we were able to link this leaf damage to grain yield loss and therefore construct an economic threshold of 1.8 larvae per tiller. The insecticide trials pointed out that when a curative treatment is necessary, a synthetic pyrethroid (deltamethrin, 5 g/ha and zetacypermethrin, 10 g/ha are both approved for CLB management in Belgium) has the highest efficiency for CLB management. The sowing density trials showed that altering the sowing density as a preventive measure for CLB management will not result in a grain yield gain, although higher densities are accompanied by lower CLB densities (per plant unit). Therefore, we recommend to follow general management practices concerning the sowing of winter wheat.

Samenvatting

Wereldwijd zorgen het graan- en grashaantje (*Oulema* spp.) jaarlijks voor grote verliezen in de wintertarweteelt. Hoewel schade door het graan- en grashaantje vaak erg variabel is in onze contreien, heeft het verleden reeds meermaals aangetoond dat dit insect wel van economisch belang kan zijn (LCG, 2011). Tot op heden was een IPM voor het graan- of grashaantje afwezig of bestond het slechts uit een curatieve chemische behandeling. Zelfs voor dit laatste bleek dat een correcte timing erg moeilijk was door het gebrek aan kennis in Europa. Daarnaast maakt het gebrek aan selectieve middelen voor dit insect de behandeling moeilijker. Doordat economische schadedrempels specifiek voor onze regio afwezig zijn, werd een timing tot op heden vooral gebaseerd op (oude) schadedrempels uit de Verenigde Staten. Alles samengenomen is het duidelijk dat meer onderzoek nodig is om IPM voor het graan-en grashaantje in de wintertarweteelt over te brengen aan de landbouwer.

Verscheidene deelaspecten van het management van gras- en graanhaantje werden gedurende vier opvolgende groeiseizoenen intensief bestudeerd. In grootschalig veldonderzoek werden jaarlijks 30 praktijkvelden wekelijks opgevolgd. Hierbij werd de fenologie van het gras- en graanhaantje bestudeerd. Daarnaast werden ook de weersgegevens en het teeltmanagement bijgehouden per perceel. Correlatiestudies tussen al deze variabelen lieten ons toe een webtool met een geïmplementeerd predictiemodel te ontwikkelen dat landbouwers kan helpen bij het beheersen van deze kevers. Naast deze waarnemingen werden op dezelfde locaties ook sleepnetvangsten uitgevoerd. Deze gevangen kevers werden vervolgens op soortnaam gebracht, wat ons informatie gaf over de soortenverdeling binnen het genus *Oulema* in Vlaanderen.

Naast deze grootschalige veldproeven op praktijkniveau, werden op onze gespecialiseerde proeflocaties (Bottelare en Rumbeke-Beitem) ook nog andere veldproeven uitgevoerd. Proeven waarbij het effect van de zaaidichtheid op de fenologie en dichtheid van het graan- en grashaantje bestudeerd werd; insecticideproeven waarin de toegelaten en vaak gebruikte graaninsecticiden getoetst werden naar hun effectiviteit t.o.v. het graan- en grashaantje. Als laatste lieten de kooiproeven in combinatie met manuele ontbladeringsproeven ons toe de aanwezigheid van het graanhaantje te koppelen aan opbrengstverlies. Dit was nodig voor het opstellen van actuele schadedrempels.

De waarnemingen op praktijkniveau toonden aan dat het graan- en grashaantje slechts gedurende sommige jaren van economisch belang is (occasioneel plaaginsect). Daarnaast werd een duidelijk verband tussen de ontwikkeling en temperatuur gevonden. Warme jaren versnellen de ontwikkeling,

wat ervoor kan zorgen dat het insect vroeger in het perceel actief wordt en schade aanricht. In combinatie met andere (a)biotische stressfactoren zoals droogte of andere plaaginsecten (bladluizen), kan de bladschade door het graan-of grashaantje bepalend zijn. De sleepvangsten toonden aan dat in Vlaanderen de populatie graan- en grashaantje voornamelijk bestaan uit *O. melanopus*, *O. duftschmidi* (beide graanhaantjes) en *O. obscura* (een voorbeeld van een grashaantje).

De kooiproeven toonden aan dat de aanwezigheid van de larven resulteert in bladschade, welke vervolgens opbrengstverlies kunnen geven. Via de manuele ontbladeringsproef (serreproef), konden we vervolgens de bladschade doortrekken naar effectief opbrengstverlies. De combinatie van beide proeven leidde ons naar een economische schadedrempel van 1.8 larven per halm. De insecticideproef toonde aan dat, wanneer een curatieve behandeling economisch verantwoord is, een synthetisch pyrethroide (deltamethrin en zetacypermethrin zijn beide erkend voor *Oulema* spp. in België) het efficiëntst zal werken. De proef met de zaaidichtheden toonde aan dat de larvale dichtheid in het gewas afnam bij hogere zaaidichtheden. Desondanks zagen we geen verschil in opbrengst. Ondanks dat het aantal per plant afnam, bleef de dichtheid per oppervlakte-eenheid gelijk, waardoor de opbrengst per oppervlakte ook niet significant beïnvloed werd.

Appendices

Appendix 1 – results of the Chi-squared test on the number of beetles for each gender of complex 1 and complex 2

Table 33: Results of the Chi-squared test on the absolute frequencies of the males (m) and females (f) from the species belonging to complex 1 (O. melanopus, O. rufocyanea, O. duftschmidi) and the species belonging to complex 2 (O. obscura), based on averages across fields and across distances from the field edge during seasons 2016 (28 fields), 2017 (30 fields) and 2018 (23 fields)

	Growth season	Period	X^2	p-value
Complex 1	2016	April_P2	1,17	0,28
		May_P1	0,07	0,80
		May_P2	0,00	0,95
	2017	May_P1	0,03	0,86
		May_P2	2,23	0,13
	2018	May_P1	6,78	0,009
Complex 2	2016	April P2	0,53	0,47
		May_P1	0,00	1,00
		May_P2	0,26	0,61
	2017	May_P1	1,62	0,20
		May_P2	0,09	0,77
	2018	May_P1	0,69	0,41

Appendix 2 – The effect of different sowing densities, sowing dates, fertilisation schemes, crop rotations and semi-natural elements on the development of CLBs

Effect of SNE

Table 34: Results of a one-way ANOVA analysis comparing the effect of different SNEs over all monitored growth seasons (2014–2015, 2015–2016, 2016–2017 and 2017–2018). α = 0.05

	Df	F	p-value
eGH.start	2, 107	0.623	0.538
eGH.peak	2, 104	0.387	0.680
eGH.end	2, 106	0.826	0.441
eGH.duration	2, 106	0.209	0.812
eGH.PH	2, 107	3.948	0.022
lGH.start	2, 106	0.030	0.970
lGH.peak	2, 103	0.622	0.539
lGH.end	2, 106	0.429	0.652
lGH.duration	2, 106	0.369	0.692
lGH.PH	2, 107	2.386	0.097

Effect of crop rotation

Table 35: Results of a one-way ANOVA analysis comparing the effect of crop rotations over all monitored growth seasons (2014–2015, 2015–2016, 2016–2017 and 2017–2018). α = 0.05

	Df	F	p-value
eGH.start	2, 71	0.664	0.518
eGH.peak	2, 68	0.137	0.872
eGH.end	2, 70	0.074	0.929
eGH.duration	2, 70	1.149	0.323
eGH.PH	2, 74	9.767	< 0.001
lGH.start	2, 70	0.892	0.415
lGH.peak	2, 70	1.422	0.248
lGH.end	2, 70	0.245	0.784
lGH.duration	2, 70	0.023	0.977
lGH.PH	2, 74	0.739	0.481

Effect of sowing date

Table 36: Results of a one-way ANOVA analysis comparing the effect of different sowing dates over all monitored growth seasons (2014–2015, 2015–2016, 2016–2017 and 2017–2018). α = 0.05

	Df	F	p-value
eGH.start	2, 77	0.895	0.413
eGH.peak	2, 73	1.011	0.369
eGH.end	2, 76	2.298	0.107
eGH.duration	2, 76	0.906	0.409
eGH.PH	2, 80	0.441	0.645
lGH.start	2, 76	0.259	0.772
lGH.peak	2, 76	0.037	0.963
lGH.end	2, 76	1.188	0.310
lGH.duration	2, 76	0.618	0.542
lGH.PH	2, 80	0.504	0.606

Effect of sowing density

Table 37: Results of a one-way ANOVA analysis comparing the effect of different sowing densities over all monitored growth seasons (2014–2015, 2015–2016, 2016–2017 and 2017–2018). α = 0.05

	Df	F	p-value
eGH.start	2, 65	3.088	0.052
eGH.peak	2, 62	7.246	0.001
eGH.end	2, 64	0.624	0.539
eGH.duration	2, 64	0.977	0.382
eGH.PH	2, 68	0.081	0.922
lGH.start	2, 65	0.081	0.922
lGH.peak	2, 64	3.008	0.056
lGH.end	2, 65	0.404	0.670
lGH.duration	2, 65	0.373	0.690
lGH.PH	2, 68	0.101	0.904

Effect of soil tillage

Table 38: Results of an independent samples t-test comparing the effect of different soil tillage systems over all monitored growth seasons (2014–2015, 2015–2016, 2016–2017 and 2017–2018). α = 0.05

	Df	t	p-value
eGH.start	64	-0.188	0.851
eGH.peak	61	-1.472	0.146
eGH.end	63	-0.236	0.814
eGH.duration	63	0.003	0.997
eGH.PH	67	-0.365	0.716
lGH.start	64	-1.199	0.235
lGH.peak	63	-1.685	0.097
lGH.end	64	-1.401	0.166
lGH.duration	64	-0.259	0.797
lGH.PH	67	0.404	0.687

Effect of Fertilisation

Table 39: Results of a one-way ANOVA analysis comparing the effect of different fertilisation schemes over all monitored growth seasons (2014–2015, 2015–2016, 2016–2017 and 2017–2018). α = 0.05

	Df	F	p-value
eGH.start	2, 58	1.301	0.280
eGH.peak	2, 54	3.437	0.039
eGH.end	2, 58	2.843	0.066
eGH.duration	2, 58	2.403	0.099
eGH.PH	2, 61	0.679	0.511
lGH.start	2, 57	0.092	0.913
lGH.peak	2, 57	0.033	0.968
lGH.end	2, 57	0.392	0.677
lGH.duration	2, 57	0.466	0.630
lGH.PH	2, 61	0.108	0.898

Appendix 3: Cage trials

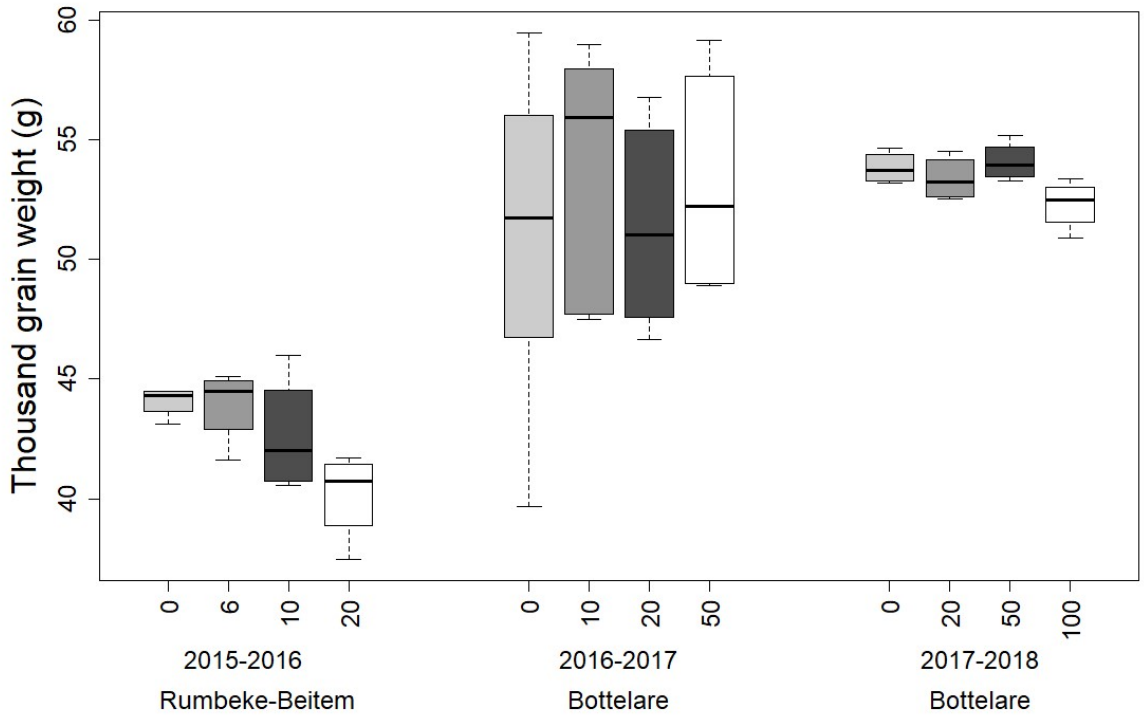

Figure 57: Thousand grain weight (g) in relation to the introduced number of Oulema adults for two locations and three growth seasons (winter wheat cage trials): 0 = control treatment, no beetles introduced; 6 = 6 beetles introduced per cage; 10 = 10 beetles introduced per cage; 20 = 20 beetles introduced per cage; 50 = 50 beetles introduced per cage; 100 = 100 beetles introduced per cage.

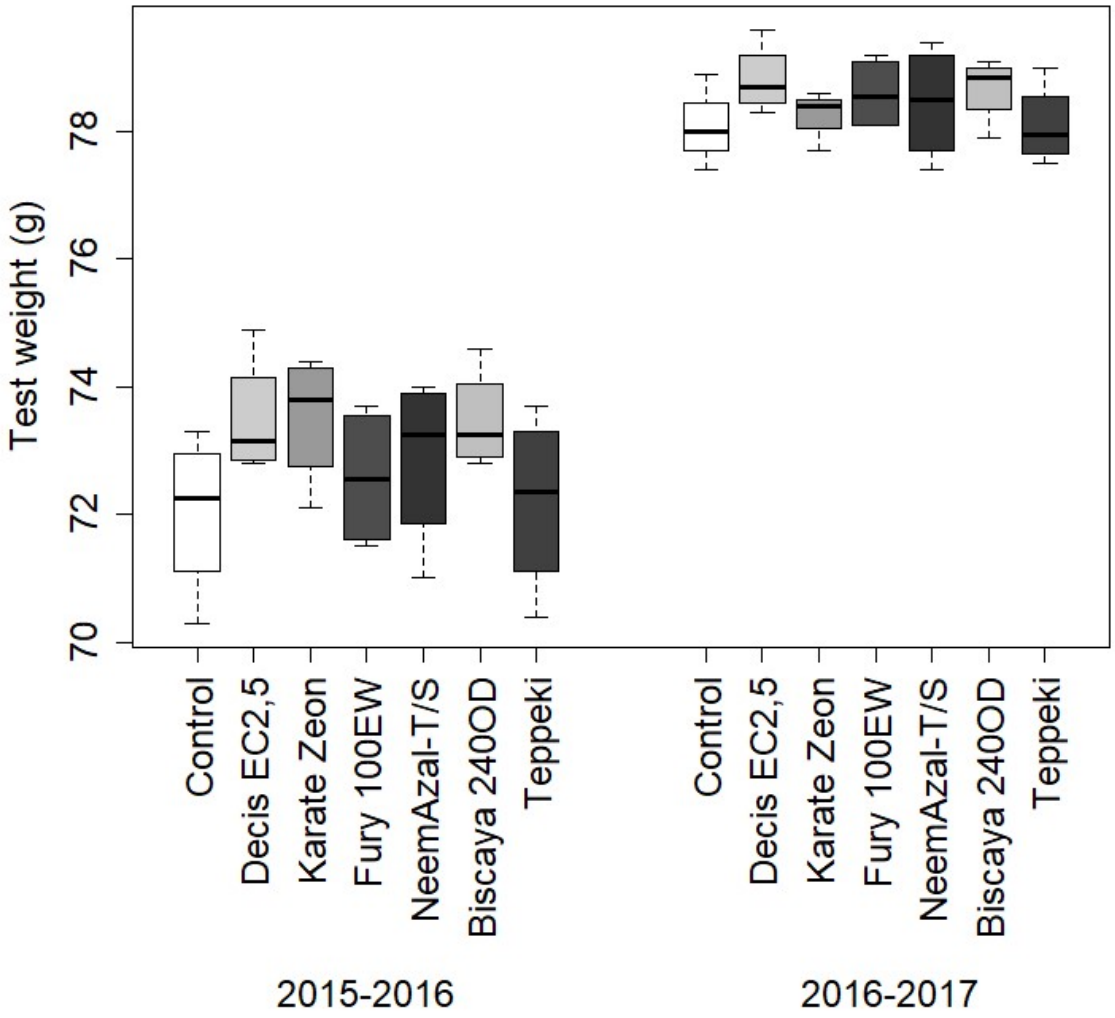

Figure 58: Test weight (g) for both growth seasons (2015–2016 and 2016–2017) and each treatment.

Curriculum vitae

Personal information

Name:	Elias Van de Vijver
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Place of birth:	Ghent
GSM:	+32 0475/61 94 93
E-mail:	elias.vandevijver@ugent.be eliasvandevijver@hotmail.com

Education

2018: Doctoral training programme
Doctoral Schools, Applied Bioscience engineering
Ghent University, 2014–2018

Followed courses in training programme:

Specialist courses

Module 1: Introduction to R
IPVW, 2015

Module 4: Introductory statistics. Basics of statistical inference
IPVW, 2015

Biodiversity based IPM in field crops
NOVA, 2015

Stacking biodiversity benefits for sustainable IPM
NOVA, 2017

Predictive modelling
Ghent University, 2016

Transferable skills

Schrijven voor niet-vakgenoten en pers
Doctoral Schools, (Bioscience) engineering, 2014

Advanced academic English: writing skills
Doctoral Schools, (Bioscience) engineering, 2014

Leadership foundation course
Doctoral Schools, (Bioscience) engineering, 2014

Conference skills: English proficiency for presentations
UCT, 2014

2014: Master of Science, Biosciences: Agri- and horticulture
Ghent University, 2010–2014
Specialisation: plant and animal production
Graduated with distinction

Professional and scientific experience

2014–2018: PhD research, Ghent University
'**Development of IPM tools for the management of *Oulema* beetles in winter wheat (*Triticum aestivum* L.)**'
Promotors: Prof. dr. ir. Geert Haesaert, Prof. dr. Bernard De Baets, Prof. dr. ir. Guy Smagghe

2013–2014: Master thesis, Ghent University
'**Influence of crop rotation in combination with fertilisation on the yield of maize (*Zea mays* L.)**'
Promotor: Prof. dr. ir. Geert Haesaert

Scientific output

Publications in international journals

Van de Vijver E., S. Landschoot, M. Van Roie, F. Temmerman, J. Dillen, K. De Ceuleners, G. Smagghe, B. De Baets, and G. Haesaert. 2018. **Inter- and intra-field distribution of cereal leaf beetle species (Chrysomelidae: Coleoptera) in Belgian winter wheat.** Environ. Entomol., doi: 10.1093/ee/nvz002

Van de Vijver E., S. Landschoot, G. Smagghe, B. De Baets, F. Temmerman, J. Dillen, and G. Haesaert. 2018. **Potentials and limitations of a growing degree day approach to predict the phenology of cereal leaf beetles**. Environ. Entomol. 47(4): 1039–1046.

Publications in valorising journals

Van de Vijver, E., N. De Geyter, and L. Haeck: **Adviestool voor bestrijding graanhaantje in de maak.** Published in VILT, 2017.

Van de Vijver, E., and N. De Geyter: **Waarschuwingssysteem voor graanhaantje in de maak**. Published in VILT, 2017.

Van de Vijver, E., F. Temmerman, and J. Claeys: **Bestrijding van het graanhaantje: een stap richting IPM.** Published in Landbouwleven, Drietand and Management and Techniek, 2016.

Participation to international scientific events

Van de Vijver, E., F. Temmerman, J. Claeys, G. Smagghe, B. De Baets, and G. Haesaert: **Review of a growing degree day approach to predict cereal leaf beetle.** 70th International Symposium on Crop Protection, May 22, 2018. Ghent, Belgium. Poster presentation.

Van de Vijver, E., F. Temmerman, J. Claeys, G. Smagghe, B. De Baets, and G. Haesaert: **L'importance de garder le criocère des céréales sous contrôle.** Ploème, 1ères biennales de l'innovation céréalière, January 24–25, 2018. Paris, France. Poster presentation and proceedings.

Van de Vijver, E., G. Haesaert, B. De Baets, and G. Smagghe**: Development of IPM-tools for the control of cereal leaf beetles and aphids in small grains: update on cage trials.** 69th International Symposium on Crop Protection, May 23, 2017. Ghent, Belgium. Poster presentation.

Van de Vijver, E., G. Haesaert, B. De Baets, and G. Smagghe: **Development of IPM-tools for the control of cereal leaf beetles and aphids in small grains: cage trials.** 68th International Symposium on Crop Protection, May 17, 2016. Ghent, Belgium. Poster presentation.

Van de Vijver, E., G. Haesaert, B. De Baets, and G. Smagghe: **Development of IPM-tools for the control of cereal leaf beetles and aphids in small grains: monitoring trials.** NJF seminar, August 28, 2015. Uppsala, Sweden. Oral presentation.

Van de Vijver, E., G. Haesaert, B. De Baets, and G. Smagghe: **Development of IPM-tools for the control of cereal leaf beetles and aphids in small grains: monitoring trials.** 67th International Symposium on Crop Protection, May 19, 2015. Ghent, Belgium. Poster presentation.

Van de Vijver, E., G. Haesaert, B. De Baets, and G. Smagghe: **Development of IPM-tools for the control of cereal leaf beetles and aphids in small grains: Introduction of project and trials.** IPM Innovation in Europe, January 14–16, 2015. Poznan, Poland. Poster presentation.

Supervision of students (bachelor and master)

Master students

Kevin De Ceuleners (2017–2018). Bepaling van de soortensamenstelling in de graanteelt in Vlaanderen. Promotor: Prof. dr. ir. Geert Haesaert.

Bart Laridon (2017–2018). Ontwikkeling van actuele economische schadedempels voor graanhaantje in wintertarwe. Promotor: Prof. dr. ir. Geert Haesaert.

Elien Van de Voorde (2016–2017). Ontwikkeling van actuele economische schadedrempels voor zomerbladluizen. Promotor: Prof. dr. ir. Geert Haesaert.

Linde Schouteeten (2016–2017). Levenscyclus van *Oulema* spp. en de gevolgen voor de opbrengst bij wintertarwe. Promotor: Prof. dr. ir. Geert Haesaert.

Anneleen De Zutter (2015–2016). Evaluatie van de natuurlijke vijanden van graanhaantje en hun tolerantie t.a.v. graaninsecticiden. Promotor: Prof. dr. ir. Geert Haesaert.

Bachelor students

Maarten Perneel, Michaël Devos, Gert-Jan Leers, Ellen Van De Velde (2017–2018). Studie van het effect van een handmatige ontbladering van tarwebladeren op de korrelopbrengst. Promotor: Prof. dr. ir. Geert Haesaert.

Gerben Meutermans, Kiani Vandewoestyne, Veerle Waeterloos (2017–2018). Populatiedynamiek van het adulte graanhaantje in Vlaanderen. Promotor: Prof. dr. ir. Geert Haesaert.