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Research

Population Ecology

Inter- and Intrafield Distribution of Cereal Leaf Beetle Species (Coleoptera: Chrysomelidae) in Belgian Winter Wheat

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Abstract

Cereal leaf beetles (CLBs), a group of chrysomelid beetles of the genus *Oulema* (Coleoptera: Chrysomelidae), are well-known pest insects of small-grain cereals in many countries of the Northern hemisphere. Due to the small differences in morphology of species within this genus, classification up to species level remains a challenging task. Since an accurate view of species composition is important for developing targeted control strategies, the goal of this study was to unravel the *Oulema* species composition in Flanders' wheat fields. During three subsequent years at a series of different fields, *Oulema* species were collected and classified up to species level (2016: 28 fields, 2017: 30 fields, and 2018: 23 fields). This study reveals that the population consists of four different species: *Oulema melanopus, Oulema duftschmidi*, and *Oulema obscura* were most frequently encountered, while *Oulema rufocyanea* was only marginally present. Furthermore, the population was highly dynamic, as the population share of each species varied between different growing seasons and between the various sampling events within each season. The distance from the field edge had a minor influence on the species composition, but the abundance of beetles increased with the distance to the field edge. A discriminant analysis revealed that based on the measurements of various body parts, an accurate classification up to species level is possible. In conclusion, we observed that the population densities fluctuated within and between years, resulting in variable incidence of CLB in winter wheat fields in the Flanders region.

Key words: *Oulema melanopus*, population dynamics, morphometrics, sweep net, monitoring

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.): *Oulema melanopus* (Linnaeus 1758), *Oulema rufocyanea* (Suffrian 1847), *Oulema duftschmidi* (Redtenbacher 1874), *Oulema obscura* (Stephens 1831), *Oulema septentrionis* (Weise 1880), and *Oulema erichsonii* (Suffrian 1841) (Walczak 2005, Schmitt and Rönn 2011, Chapelin-Viscari and Maillet-Mezeray 2015). These species are often grouped into two species complexes, as determination up to species level is a challenging task because of their similar morphology. The first three species are referred to as the cereal leaf beetle (CLB) complex or the *O. melanopus*-complex, which are characterized by a red pronotum, while the latter three belong to the second species complex, which are characterized by a blue pronotum. In this work, species from the *O. melanopus*-complex are referred to as 'complex 1 species', while the other complex of species is referred to as 'complex 2 species'.

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 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 the CLB complex, causing damage to wheat crops in the United States 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). While *O. duftschmidi* is believed to appear sympatric to *O. melanopus*, the economic damage potential of this species is still unknown (Bechini et al. 2013). Although the damage potential of *O. melanopus* is better documented, Bezděk and Baselga (2015) argue that due to the sympatric appearance of both species in Eurasia, economic damage should be attributed to the complex of both species, rather than to *O. melanopus* only. The third species within the CLB complex, *O. rufocyanea*, is believed to be extremely rare and only appears in Central and Southern Europe (Bezděk and Baselga 2015). Concerning the distribution of the derivative species *Oulema mauroi* and *Oulema verae*, more studies are necessary to clarify their global distribution.

Oulema obscura, O. septentrionis, and *O. erichsonii* belong to the second species complex, which is characterized by a blue pronotum. *Oulema obscura* is widespread in Europe, causing yield losses in small grains in Central, Western, and Northern Europe (Walczak 2005). The other species within this complex are less widespread, only occurring in some parts of Europe, and are therefore rarely described as a pest insect in small-grain cereals. Host plants of *O. septentrionis* are oats (*Avena*), and bulrushes (*Typha*), while *O. erichsonni* feeds on floating sweet-grass (*Glyceria*). Both species favor plants grown in moist environments (Hubble 2012). Like species within complex 1, complex 2 species share a very similar morphology, although they can generally be differentiated without the need for a dissection of the aedeagus. According to Allen (1976) and Cox (2000), these species 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, and thus are not of economic importance. Moreover, this leaf beetle has been used as biocontrol organism of thistle (*Cirsium arvense* (L.) Scop.) in Canada (Peschken 1984).

Different techniques have been applied, with varying success, for classifying and identifying species within the genus *Oulema* (Bezděk and Baselga 2015). Molecular identifications of CLBs based on the sequence of the *cox1* gene are unreliable due to absence of clear differences between species (Bezděk and Baselga 2015). Another technique often used for classification up to species level is comparison of externally measured body parts of different species. However, Bezděk and Baselga (2015) showed that for the CLB complex, these measurements are variable, making this technique alone less useful. Many authors therefore conclude that for males, the shape of the internal flagellum of the aedeagus is the only exact method to classify up to species level. For females, the shape of the spermatheca, specifically the distal part of the ductus spermathecae to the bursa copulatrix seems to differ between these species. However, the latter can pose a problem in preserved specimens since this ductus is generally not sclerotized. Therefore, comparing the length of the spermathecal duct to the width of the terminal portion of the bursa copulatrix is another main characteristic for identification of the females within the CLB complex (Bezděk and Baselga 2015).

Within fields, population dynamics of 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. At the start of each season, when average daytime temperatures rise above 10–14°C, beetles migrate from the field edge to the center (Philips et al. 2011). Although absolute numbers of beetles can be highly variable within a field, studies show that the intrafield relative abundance of each species often is less variable than the interfield abundance (Ruesink and Haynes 1973). 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, than elsewhere in the field (Sawyer and Haynes 1986). Other empirical studies showed similar results (e.g., Lecigne and Roehrich 1977, Reay-Jones 2010).

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 integrated pest management-oriented strategy. As CLB incidence is caused by a complex of different species, insight into the species composition and dynamics within and between growing seasons is of paramount importance to develop accurate control strategies. In this light, the aim of this study was to gain insight into the intra- and interfield 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, Guppy and Harcourt 1978, Ali et al. 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.

Materials and Methods

Collection of CLBs

To assess the abundance of each species, CLBs were collected from several fields distributed throughout the different agro-climatic zones of Flanders. 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, according to the soil texture (SL, sandy loam; S, sand; P, polder; L, loam). Generally, wheat was cultivated following good agricultural practices, i.e., wheat was sown in October–November at a density of 350 kernels per m^2, fertilization rates were determined based on soil samples, herbicide applications were applied in April, and fungicide applications were applied in May and June. No insecticides were applied to any of the wheat fields before and during the period in which the beetles were collected.

For the investigation of CLB interfield 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 (10 and 50 m) from a selected field edge. 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 1 m length, this covers 30 m^2). 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 (Van De Vijver et al. 2018).

Another sampling scheme investigated the intrafield distribution and possible shifts during the growing season at five fields in 2018. 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 interfield distribution sampling.

Collection of Weather Data

Maximum, minimum, and mean temperatures, humidity, and wind speed were monitored using automated weather stations across Flanders (distance < 5 km from each field), directed by the Agricultural Centre for Potato Research (Kruishoutem). These data were used to find correlations with species presence or distribution in the field.

Identification of the Collected CLBs

In order to identify *O. melanopus*, *O. duftschmidi*, and *O. rufocyanea*, during initial dissection, we first determined beetle sex. Dissections were executed by means of a Zeiss Stemi 2000 0.8*10 (80× maximum magnification) and a standard dissection set. Only males were selected for further dissection and determination, while 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) (Fig. 1). 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), EL, elytra width, antenna length (AL), pronotum length (PL), and pronotum width (PW) of a random subset of 10 beetles per species were measured.

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 ($P < 0.05$), 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 $\alpha = 0.05$.

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 (LOOCV) was performed. LOOCV 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 Distribution

On most observation dates, male and female beetles had a similar share in the population. On most occasions, similar numbers of adult

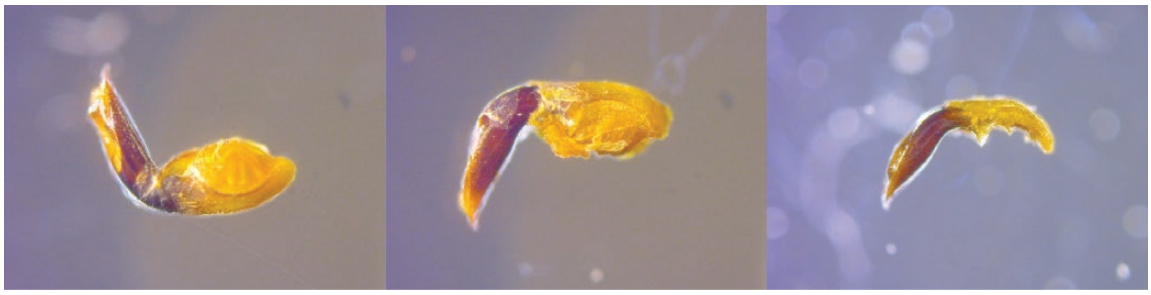

Fig. 1. Aedeagus of the main *Oulema* species. From left to right: *O. duftschmidi, O. melanopus, O. obscura*.

Table 1. Relative frequency (%) of the males (m) and females (f) from the species belonging to complex 1 (*O. melanopus, O. rufocyanea*, and *O. duftschmidi*) and the species belonging to complex 2 (*O. obscura*)

	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

CLB males and females were swept from the wheat fields (Table 1). Two exceptions were 1) April 2016, when the females of *O. obscura* outnumbered the males, and 2) the second half of May 2017, when the males of complex 1 outnumbered the females. Considerably more adults of complex 1 compared with complex 2 were present in 2016 and 2018, but similar numbers of adults of both complexes were present in 2017 (χ^2 = 106.39, df = 11, *P*-value < 0.001).

Results of the Chi-squared test indicated that the distribution of CLB species was dependent on the growing season and that shifts in population composition occurred during single growing seasons (χ^2 = 108.97, df = 12, *P*-value < 0.001; Fig. 2). 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.

Studying the species composition at different sampling locations (near the field edge or in the center of the field), revealed that in two of the sampled growth seasons, significant differences in the species composition were found (Fig. 3). In 2017 and 2018, the population composition was dependent on the position of sweeping. In May 2017, no *O. melanopus* adults were sampled near the field edge, whereas in the center of the field, the frequency of this species in the collected samples was 19.0%. In May 2018, an increased abundancy of both *O. duftschmidi* and *O. melanopus* was noted when going from the edge to the center of the field.

The geographical distribution of the different CLB species in Flemish wheat fields was determined as well (Figs. 4 and 5), according to the different agricultural regions. Pie charts present the population composition in the different regions during growth season 2018 (Fig. 5). In 2016 and 2018, the CLB population composition in the loamy region significantly differed from the composition in the other regions (*P*-values < 0.001). Within this region, the relative abundance of *O. obscura* was highest (36% in 2016; 80% in 2017; and 65% in 2018) in the collected samples. The relative abundance of *O. duftschmidi* was lower in the loamy region compared with 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.

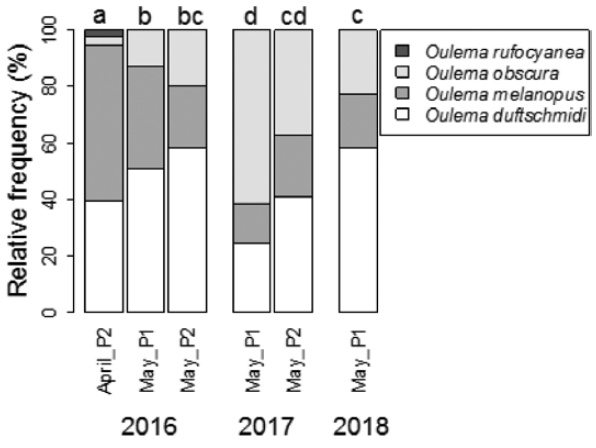

Fig. 2. Relative frequency (%) of the different *Oulema* species (males), on the sampling dates during the second part of April (April_P2), the first part of May (May_P1) and the second part of May (May_P2) during the seasons 2016–2018. Different letters above the bars point to significant differences between population distributions (Chi-squared test).

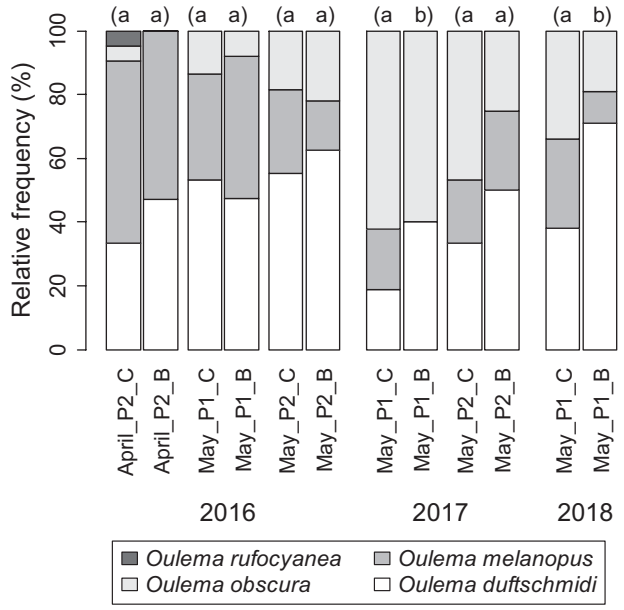

Fig. 3. Relative frequency (%) of the different *Oulema* species (males), during the second part of April (April_P2), the first part of May (May_P1), and the second part of May (May_P2) near to the border of the field (B) and in the center of the field (C) during the seasons 2016–2018. Different letters above the bars point to significant differences between population distributions (Chi-squared test).

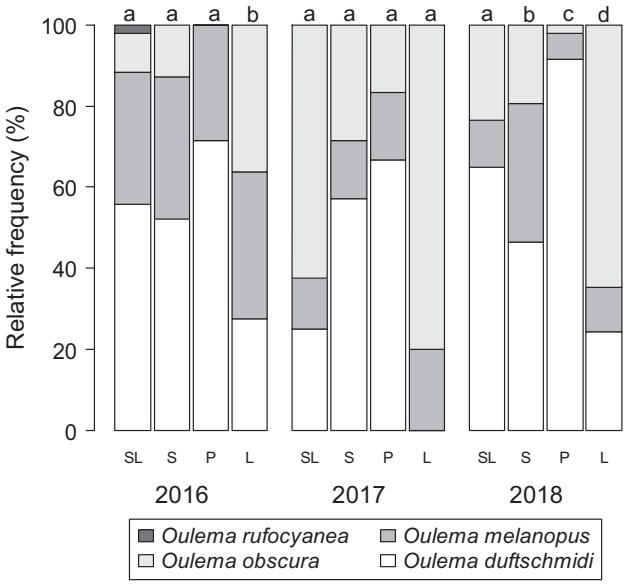

Fig. 4. Relative frequency (%) of the different *Oulema* species (males) for each agricultural region in Flanders during the seasons 2016–2018. Different letters above the bars point to significant differences between population distributions (Chi-squared test). SL = Sandy Loam; S = Sand; P = Polders; L = Loam.

Within-Field Distribution of *Oulema* Species

Presenting the average number of CLBs per sweep and the species distribution at different moments at various distances from the field edge (10, 20, 30, and 40 m) revealed significant differences in species distribution depending on the sampling position (Fig. 6). 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.

Except on May 2, more beetles (but not significantly) were found at 30 and 40 m than at 10 or 20 m from the field edge. Concerning the evolvement of the number of *Oulema* adults during the growing season, following normal phenology, a gradual increase followed by a decrease was expected. However, it can be seen that the 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. Weather conditions influenced the within season variations in the number of *Oulema* species; the number of *Oulema* species was negatively correlated with wind speed (–0.27, *P*-value = 0.12) and positively correlated with temperature (0.38, *P*-value = 0.044).

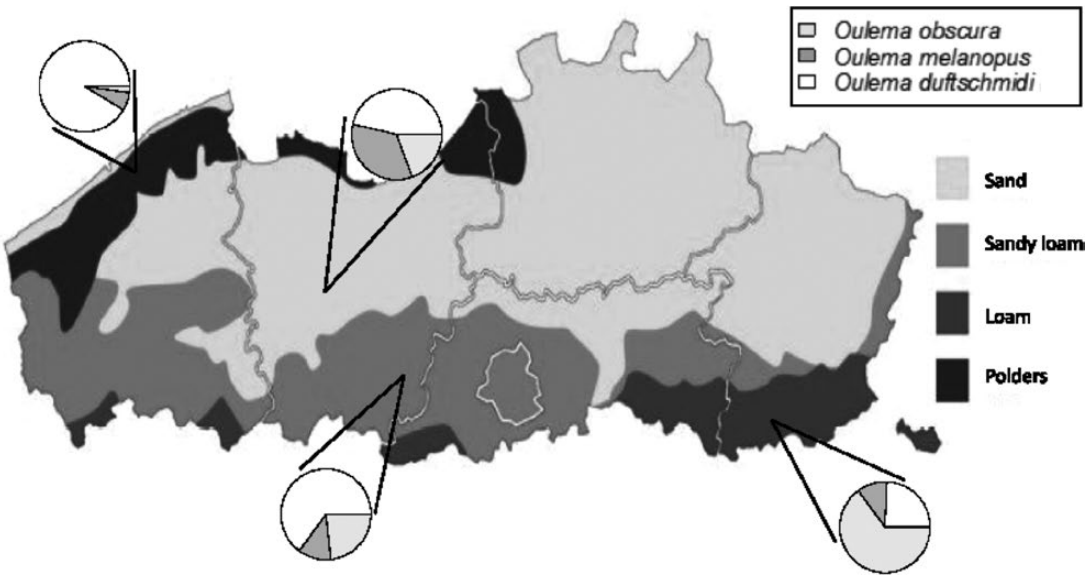

Fig. 5. *Oulema* species relative distribution (%, males) for each agricultural region in Flanders, 2018.

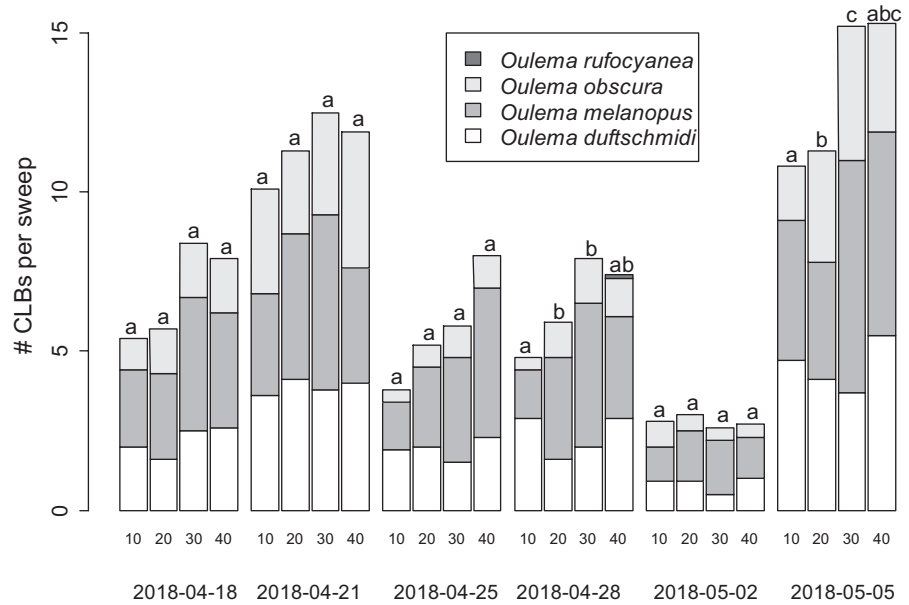

Fig. 6. Average number of CLBs per sweep at 10, 20, 30, or 40 m from the field border at different time points during the growing season (2018). Different letters per date point to significant differences in species composition between distances (Chi-squared test).

Table 2. Measurements (minimum–maximum) of the various body parts of different *Oulema* species (m = male, f = female) belonging complex 1 (*O. melanopus, O. rufocyanea*, and *O. duftschmidi*)

	Complex 1 (f)	*O. duftschmidi* (m)	*O. melanopus* (m)	*O. obscura* (m)	*O. obscura* (f)
BL	4.5–5.6 mm	4.3–4.8 mm	4.5–5.3 mm	3.8–4.2 mm	4–4.6 mm
EL	3.3–4.0 mm	3.2–3.5 mm	3.4–3.8 mm	2.8–3.1 mm	3–3.4 mm
EW	1.8–2.3 mm	1.6–2.0 mm	1.8–2.2 mm	1.7–2 mm	1.1–2.2 mm
AL	2.4–3.0 mm	2.5–3.0 mm	2.5–3.0 mm	2.2–2.5 mm	2.2–2.5 mm
PL	1.0–1.3 mm	0.9–1.1 mm	1.0–1.1 mm	0.8–1 mm	0.9–1.1 mm
PW	1.1–1.3 mm	1.0–1.1 mm	1.1–1.2 mm	0.9–1 mm	1–1.1 mm
EL/BL	0.71–0.78	0.70–0.76	0.7–0.79	0.71–0.78	0.70–0.81
EL/EW	1.59–1.83	1.60–2.12	1.64–2.00	1.46–1.67	1.45–3.09
AL/BL	0.50–0.58	0.54–0.68	0.51–0.62	0.55–0.62	0.52–0.61
PL/PW	0.83–1.00	0.90–1.10	0.91–1.00	0.89–1.11	0.90–1.00

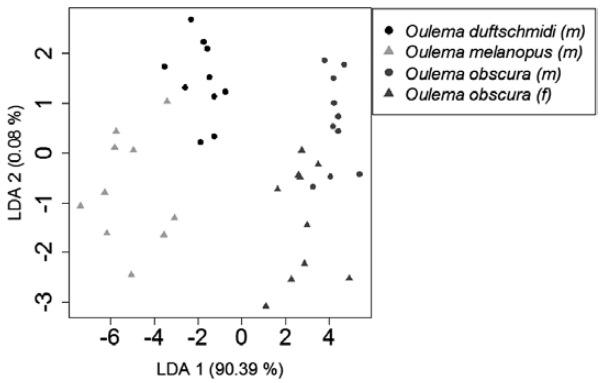

Fig. 7. Linear discriminant analysis plot of the first two discriminant functions showing the separation of the various *Oulema* species.

Classification Based on Morphometric Analysis

As mentioned above, *Oulema* determination up to species level is a challenging task due to their similar morphology. Observed size ranges for the BL, EL, EW, AL, PL, PW, and the ratios between these body parts, measured on ten individuals per species, are given in Table 2.

Linear combinations of various body measurements and their ratios for individual species (Table 2) enabled species discrimination. The first two LDA functions accounted respectively for 90.39 and 0.08% of the variance in an analysis for the three most abundant species (Fig. 7). The contingency table (Table 3) shows that all *O. duftschimidi* beetles were correctly predicted. Seven out of the 10 *O. melanopus* individuals were correctly assigned, while the remaining three were classified as *O. duftschimidi*. Two out of the 10 male *O. obscura* were classified as females, whereas three female *O. obscura* individuals were classified as males and one was assigned to the *O. duftschimidi* group.

Discussion

During the intrafield trial in 2018, we observed 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 wind speed, species are less easy to catch. This was also observed by Guttierez et al. (1974) and Stilmant et al. (1995).

When studying the within-field distribution of CLB complexes, we observed that the species distribution in the center and at the edge of the field was similar. Our work confirms that of Ruesink and Haynes (1973) and Chambon et al. (1983). However, our observations in 2018 suggested that species density was higher in the center of the field. In Flanders, *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 (e.g., 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 *O. melanopus* and *O. duftschmidi* is similar (Bechini et al. 2013), others have found different results, supporting our findings that *O. duftschmidi* is the 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 sampling 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). Ali et al. (1977) show that the developmental threshold temperature of adult *O. obscura* is lower than that of *O. melanopus*, suggesting that development starts earlier during the growth season. Moreover, Ali et al. (1979) found differences between mortality rates for both species. In their work, it was clear that *O. obscura* had a lower mortality compared with *O. melanopus*. Walczak (2005) also concluded that *O. obscura* was less tolerant 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 types of soils are more prone to variations in temperatures. *Oulema duftschmidi* was found to be more abundant in the polders, the heavier soil type. These soils are also closer to the North Sea, possibly subject to a slightly different climate. 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 distribution. 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.

We observed that species distribution shifts within the season. While growing seasons 2016 and 2018 clearly showed significant differences in the species composition between the different agricultural regions, no significant differences were found in 2017. In 2017, we observed the lowest CLB activity of the three growing seasons. Low overall population density of CLBs may have masked

Table 3. Contingency table obtained for the 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

differences in species composition. Indeed, in case the number of observations decreases, the power of the chi-test also decreases, so it becomes more difficult to find significant differences. In 2018, sampling was executed at only one time point. Therefore, caution is required when comparing these species compositions between seasons. As for the interfield distribution, on some fields, sampling happened only once within the season. Variable CLB densities between the growth seasons made sampling difficult. This makes comparing relative species abundances between different years difficult. Using traps with (*E*)-8-hydroxy-6-methyl-6-octen-3-one could be used to collect beetles more effectively as this agent acts as an aggregation pheromone for CLBs (Rao et al. 2003).

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 beetles are similar to those taken by Bezděk and Baselga (2015). An LDA analysis showed that by combining these body measurements, 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.

To conclude, it was shown that the *Oulema* population in Flemish wheat fields consisted mainly of *O. melanopus*, *O. duftschmidi*, and *O. obscura*. Our observations also demonstrated that the species distribution significantly differed between agricultural regions and was mainly affected by weather conditions. Within the field, no significant differences in species distribution were found. It is clear that more research is needed to gain a deeper insight into the driving factors influencing population density and composition and to develop control strategies accordingly. To study the species distribution in regions with a low overall population density, traps with pheromones could be used to collect beetles more effectively.

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