Eesti Maaülikool
Estonian University of Life Sciences

SUSTAINABLE APPROACHES TO OILSEED RAPE PEST CONTROL: STEPS TOWARDS INTEGRATED PEST MANAGEMENT

KAHJURITE JÄTKUSUUTLIKUD TÕRJESTRATEEGIAD RAPSI INTEGREERITUD TAIMEKAITSES

SILVA VILUMETS

A Thesis
for applying for the degree of Doctor of Philosophy
in Agricultural Sciences

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse erialal

Tartu 2024

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Doctoral Theses of the
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Institute of Agricultural and Environmental Sciences
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Opponent: **Michael Beckmann, PhD, professor**
Helmholtz Centre for Environmental Research (UFZ), Germany

Supervisor: **Eve Veromann, PhD, professor**
Estonian University of Life Sciences

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which are referred to by their Roman numerals in the text. All papers are reproduced with the kind permission of the publishers.

I **Sulg, S.**; Kovács, G.; Willow, J.; Kaasik, R.; Smagghe, G.; Lövei, L.G.; Veromann, E. 2023. Spatiotemporal distancing of crops reduces pest pressure while maintaining conservation biocontrol in oilseed rape. Pest Management Science, https://doi.org/10.1002/ps.7391

II **Vilumets, S.**; Kaasik, R.; Lof, M.; Kovács, G.; Holland, J.; Veromann, E. 2023. Landscape complexity effects on *Brassicogethes aeneus* abundance and larval parasitism rate: a two-year field study. Scientific Reports, 13, 22373, https://doi.org/10.1038/s41598-023-49690-1

III **Sulg, S.**; Kaasik, R.; Kallavus, T.; Veromann, E. 2023. Toxicity of essential oils on cabbage seedpod weevil (*Ceutorhynchus obstrictus*) and a model parasitoid (*Nasonia vitripennis*). Frontiers in Agronomy, 5: 1107201, https://doi.org/10.3389/fagro.2023.1107201

IV **Sulg, S.**; Kaasik, R.; Willow, J.; Veromann, E. 2022. Blue stem weevil (*Ceutorhynchus sulcicollis*) – a potential threat to oilseed rape production. Acta Agriculturae Scandinavica Section B – Soil & Plant Science, 72 (3): 1–3, https://doi.org/10.1080/09064710.2021.1977380

The contribution of Silva Vilumets (née Sulg) to the papers was as follows:

	Paper			
	I	**II**	**III**	**IV**
Idea and study design			*	*
Data collection	*		*	*
Data analyses and interpretation	*	*	*	*
Manuscript preparation	*	*	*	*

ABBREVIATIONS

BBCH . . . . . Decimal scale of plant growth stages
CBC . . . . . Conservation Biological Control
IPM . . . . . Integrated Pest Management
OSR . . . . . Oilseed rape
SOSR . . . . . Spring oilseed rape
WOSR . . . . . Winter oilseed rape

1. INTRODUCTION

Agriculture is one of the most important economic sectors in the world, accounting for approximately 37% of the Earth's mainland surface area in 2019 (FAO, 2021). The increase in global food demand has reshaped the management of agricultural landscapes. Small-scale farming has transformed into large-scale, intensively managed monocultural fields to meet the increased demand for food. This intensification and simplification of agricultural landscapes has led to the loss of natural and seminatural habitats (SNHs), making it a major contributor to global biodiversity loss (IPBES, 2019; Raven and Wagner, 2021). Understanding the importance of biodiversity has led to implementation of different agri-environmental schemes to compensate for biodiversity decline around agricultural fields (Boetzl et al., 2021; Marja et al., 2022). While adopting agri-environmental initiatives on farmland is novel and has its advantages, maintaining SNHs in agricultural areas is critical for effective biodiversity conservation (Bartual et al., 2019; Begg et al., 2017; Landis et al., 2000; Tscharntke et al., 2007). Diverse and heterogeneous landscapes encourage and provide critical resources for natural pest control providers and pollinators, that serve as key ecosystem service providers in agricultural landscapes (Holland et al., 2016).

Oilseed rape (OSR, *Brassica napus* L.) is a widely cultivated crop of great importance for industrial application, as well as in the food/feed sector. As a flowering crop cultivated in monocultural systems across vast landscapes, OSR attracts various invertebrates, both beneficial arthropods and pests. Several insect pests are attracted to OSR, all capable of causing damage to OSR plants at different developmental stages. In Europe, the pollen beetle *Brassicogethes aeneus* Fabricius (syn. *Meligethes aeneus* Fabricius) and the cabbage seed weevil *Ceutorhynchus obstrictus* Marsham (syn. *Ceutorhynchus assimilis* Paykull) are among the main pests, causing damage to flower buds before flowering or consuming seeds inside the seedpod, respectively (Williams, 2010).

These pests are commonly controlled by synthetic insecticides. However, both have developed resistance to the pyrethroid class of insecticides (Heimbach and Müller, 2013; Kann et al., 2021; Slater et al., 2011), and neonicotinoids, which were effective against them, have been banned in the European Union (European Commission, 2013) due to their adverse

impact on non-target and beneficial insects, such as pollinators and natural enemies of pests (Calvo-Agudo et al., 2019; Pisa et al., 2021; Raimets et al., 2018; Sánchez-Bayo et al., 2016; Sgolastra et al., 2018; Willow et al., 2019). With the absence of a reliable and efficient control method, there is an urgent need for natural and environmentally friendly pest management methods. For instance, plant-based essential oils have been explored as a new and non-synthetic approach in *B. aeneus* control (Pavela, 2011; Willow et al., 2020). The European Union aims to achieve a 50% reduction in overall pesticide use by 2030 through the Farm to Fork Strategy (European Commission, 2020), encouraging farmers to rely more on predatory arthropods and parasitoids as an alternative pest management approach. To promote and ensure naturally occurring pest control, integrated pest management (IPM) methods should be implemented in agricultural fields, prioritizing crop management practices that cause minimal disruption in production fields. It is crucial to include conservation biological control (CBC), a long-term pest management method, as a part of an IPM plan (Begg et al., 2017). CBC is based on the notion that preventing habitat loss and environmental disruption caused by intensive crop production aids in conserving natural enemies, thereby aiding in pest suppression (Begg et al., 2017). Methods like plant and habitat diversification (Letourneau et al., 2011), decreasing cropping intensity (Attwood et al., 2008; Garratt et al., 2011), and fostering more complex landscapes (Chaplin-Kramer et al., 2011; Veres et al., 2013) should all support and increase the abundance and species richness of natural enemies of agricultural pests. To implement effectively both IPM and CBC methods, a deep knowledge of pests and their natural enemies is required. This includes understanding the following:

i) the effect of distance between consecutive years' fields on abundance, damage, and parasitism rate of pests;
ii) the effect of different adjacent habitats around production fields and in-field distance from adjacent habitats on abundance and parasitism rate of pests; and
iii) understanding the potential effects of essential oils on a target pest and also on non-target organisms.

Brassicogethes aeneus and *C. obstrictus* have been extensively studied as OSR pests, focusing on their interactions with parasitoid wasps, feeding habits, and oviposition preferences. Climate change significantly impacts insect distributions and population dynamics. This is particularly significant because the altered climatic conditions may allow the growth of insects

that were previously unable to establish a stable population (Harvey et al., 2023). Such an increase in herbivorous insect populations could potentially lead to crop damage. For instance, *C. sulcicollis*, which has been present in Estonia (Veromann et al., 2006) but has not occurred in large numbers, might pose a threat to OSR plants in changing climatic conditions. Understanding the population size and occurrence of different insects in OSR fields would help farmers to make better and more environmentally friendly pest control decisions.

However, uncertainties persist regarding how landscape composition and different habitats within the agricultural landscape affect pest distribution and damage. Understanding the effects of landscapes on pests and their natural enemies is challenging, and in some cases, findings may differ between different studies. This study aims to assess the spatiotemporal distancing of production fields and examine surrounding habitats around focal fields, investigating their influence on pests and their natural enemies. Additionally, while essential oils have been previously studied on *B. aeneus*, research on *C. obstrictus* is lacking, and acquiring this knowledge is crucial to develop more sustainable methods to control *C. obstrictus*.

2. LITERATURE REVIEW

2.1. Structural effects of the agricultural landscape on insects

Long-lasting and sustainable food production requires effective agricultural landscape management. Integrated pest management (IPM) aims to reduce pest suppression by supporting natural enemies. Diverse landscapes surrounding agricultural areas have been shown to increase the abundance of natural enemies, consequently enhancing natural pest control (Begg et al., 2017; Bianchi et al., 2006; Chaplin-Kramer et al., 2011; Landis et al., 2000; Veres et al., 2013). The conservation biological control (CBC) approach focuses on enhancing the presence of natural enemies in the landscape by avoiding or reducing the negative impact on beneficial insects through reducing tillage, limiting pesticide use, and, at the same time, implementing measures that would benefit and support them, e.g. planting flower strips to provide nectar and shelter (Begg et al., 2017; Haan et al., 2021). Natural pest-control-providing insects move between different habitats, utilizing a diverse range of resources offered by various habitats (Haan et al., 2021). The composition (proportion of land-use type) and configuration (size, shape, and spatial composition of different habitats) of the landscape are important aspects of increasing biodiversity and ecosystem services, such as natural pest control, in agricultural landscapes (Haan et al., 2021; Martin et al., 2019). Enhancing the heterogeneity of the landscape composition and configuration at different spatial scales promotes biodiversity (Chaplin-Kramer et al., 2011; Haan et al., 2021; Martin et al., 2019; Veres et al., 2013). Generalist natural enemies respond positively to complex landscapes, irrespective of the scale at which landscape complexity is measured. In contrast, specialist natural enemies, like parasitoid wasps, respond to smaller-scale landscape complexity. Therefore, land management approaches aimed at enhancing natural enemies should depend on whether the dominant enemies are generalists or specialists (Chaplin-Kramer et al., 2011).

Studies have shown that the presence of *B. aeneus* in OSR fields is enhanced by a homogeneous landscape, whereas a more heterogeneous landscape increases the species richness, abundance, and fecundity of parasitoid wasps (Bianchi et al., 2006; Dainese et al., 2019; Rusch et al., 2010). Integrating non-cropped areas into agricultural landscapes, such as seminatural and natural habitats with diverse plant compositions, pro-

vides various benefits like shelter or overwintering habitats for insects, providing nectar and alternative prey, and supporting effective and stable natural pest control (Begg et al., 2017; Dainese et al., 2019; Gurr et al., 2017; Martin et al., 2019). In more complex landscapes, the parasitism rate of *B. aeneus* has been reported to be much higher compared to simpler landscapes (Dainese et al., 2019; Thies and Tscharntke, 1999). The spatial scale of non-cropped habitats or a previous year's crop fields is crucial when aiming to increase natural pest control while simultaneously reducing pest pressure. However, the primary consideration lies in the dispersal abilities of the insects (Haan et al., 2021; Miguet et al., 2016). Insects possess varying dispersal capacities, typically dependent either on their ability to fly or use the wind for passive dispersal, or on their reliance on ground-based dispersal (Haan et al., 2021).

Temporal heterogeneity is essential for both beneficial insects and pests. Crop rotation, a key IPM practice, involves temporal changes in the same field between different crops to prevent damage caused by pests that potentially overwinter in fields or field edges (Schneider et al., 2015; Skellern and Cook, 2018). Temporal heterogeneity in the surrounding non-crop habitats is also important, as they provide food and shelter for natural enemies over the whole season, not only during the high peak of the season (Haan et al., 2021; Iuliano and Gratton, 2020). It is important to consider the complexity and connectivity of the landscape to ensure biodiversity and important ecosystem services like natural pest control. Understanding the relationship between landscape–herbivore–natural enemy dynamics is complicated but crucial and valuable for effective and environmentally sustainable pest management.

2.2. Oilseed rape (*Brassica napus* L.)

Oilseed rape (OSR), *Brassica napus* L., is one of the most important and widely grown crops, used as biofuel, vegetable oil, and animal feed. In 2022, the EU produced around 19.4 million tonnes of OSR and turnip rape seeds, which is 2.5 million tonnes more than in 2021 (Eurostat, 2023a). Estonia produced around 86 448 tonnes of oilseed and turnip rape seeds in 2022, which was 8.8% more than in 2021 (Eurostat, 2023b). The growth area of OSR and turnip rape increased in 2022, covering around 5.9 million hectares in the EU; in Estonia, the cultivated area increased approximately 1.2% between 2021 and 2022 (Figure 1; (Eurostat, 2023a, 2023b)).

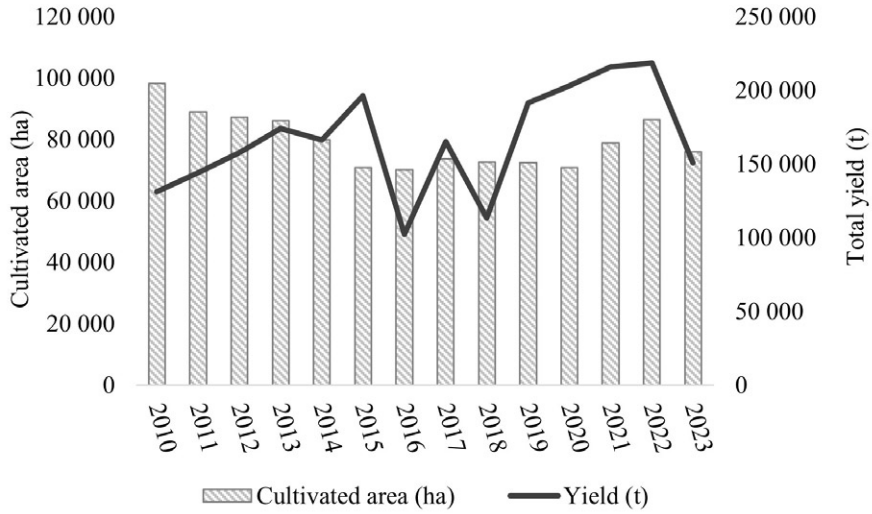

Figure 1. Sown area of oilseed rape/turnip rape in Estonia, 2010–2023 (Eurostat, 2023b).

There are two varieties of OSR grown – winter and spring varieties. Winter varieties are sown during the autumn season and need vernalization to promote the growth of stems and flowering. Winter varieties typically bloom in the spring. Spring cultivars are sown during the spring and bloom approximately one month after the winter varieties. In Europe, OSR is commonly cultivated using a four-year rotation scheme. This means that the same field is used to produce other crops such as cereals or legumes to limit the spread of disease and pests (Alford, 2003). As a mass-flowering crop, it is an appealing cultivar for a vast number of insect species. However, the plant itself does not rely on insect-based pollination, as it is mostly pollinated by the wind (Fairhurst et al., 2022). Nevertheless, research has demonstrated that bee pollination significantly enhances seed quality and quantity (Fairhurst et al., 2022; Lindström et al., 2016).

As an attractive crop to pollinators, OSR is also attractive to multiple insect pests; some of them can cause severe damage. The extent of damage and the insect causing the main harm depends on the OSR growth region. Insects can harm OSR plant roots, stems, flowers, buds, and seedpods, implying that the pests are present throughout the plant's life cycle. Europe has the greatest diversity of OSR insect pests (17 distinct insects), whereas the rest of the globe has a smaller diversity of such pests and more insect-specific problems (Zheng et al. 2020).

2.3. Pollen beetle (*Brassicogethes aeneus* Fabricius)

The pollen beetle (*Brassicogethes aeneus* Fabricius) is considered one of the main pests of OSR across Europe (Skellern and Cook, 2018; Zheng et al., 2020). The pollen beetle is univoltine, overwintering as an adult in different habitats underneath leaf litter, soil in herbaceous areas, or forest edges (Büchi, 2002; Rusch et al., 2011). In spring, after overwintering, the adult beetles feed on the pollen of various wild plants and migrate to OSR fields in order to feed, mate, and oviposit (Free and Williams, 1978; Mauchline et al., 2018). Before and during ovipositing, they destroy green flower buds to feed on pollen, causing the main damage to the crop (Free and Williams, 1978; Mauchline et al., 2018). Females lay their eggs into 2–3 mm long flower buds, where the eggs hatch in few days (Nilsson, 1988; Williams, 2010). The larvae develop inside the buds and the flower, feeding on the flower pollen (Free and Williams, 1978; Nilsson, 1988). Once matured, the larvae drop from the flower to the soil and dig into the soil to pupate (Williams, 2010). The new generation of adult beetles emerges during the summer and feeds on different plant species before seeking a suitable site for overwintering (Free and Williams, 1978).

The management of *B. aeneus* relies on insecticide spray applications. As of now, farmers across Europe mostly rely on pyrethroid-based insecticides, but there are a couple of neonicotinoid insecticides also available for use. The economic threshold for *B. aeneus* varies across Europe: in Estonia, per plant, 1–2 beetles during the flower bud stage or 4 beetles right before flowering (Maaelu Teadmuskeskus, 2023). The extensive use of pyrethroid-based insecticides has led to widespread resistance development among *B. aeneus* populations, not only in Estonia (Kann et al., 2021), but across Europe (Riggi et al., 2016; Slater et al., 2011; Zimmer et al., 2014). There is an urgent need to find suitable alternatives to control *B. aeneus* that would not be harmful to the beneficial and non-target organisms that are also present in agricultural landscapes during insecticide application.

2.4. Cabbage seed weevil (*Ceutorhynchus obstrictus* Marsham) and blue stem weevil (*Ceutorhynchus sulcicollis* Paykull)

The cabbage seed weevil (*Ceutorhynchus obstrictus* Marsham) is a univoltine species that exclusively feeds on cruciferous plants (Hussain et al., 2023; Williams, 2010). It overwinters under the leaf litter and perennial

plants in forest and field edges (Dosdall et al., 2006; Free and Williams, 1979; Hussain et al., 2023; Kovács et al., 2019). Overwintered adults emerge in the spring, with males often appearing before females; however, both male and female adults typically migrate to OSR fields during the plant flowering stage (Dmoch, 1965; Hussain et al., 2023). In the field, they feed on various plant parts (buds, flowers, pods, stem tops) in order to mature and initiate egg laying (Free and Williams, 1979; Hussain et al., 2023; Williams, 2010). The female weevil usually lays only one egg per pod, and to oviposit, they use their rostrum to penetrate the pod. Once the egg is in place, the female marks the pod with a pheromone to avoid ovipositing into the same pod more than once (Dosdall and Moisey, 2004; Ferguson et al., 1999; Hussain et al., 2023). After the larvae have hatched from the egg inside the seedpod, they begin consuming the developing seeds, typically around five to six seeds from the pod, that is about 20% of seeds (Dmoch, 1965; Hussain et al., 2023). Once mature, the larvae chew a hole through the pod to exit and drop to the ground, where they dig into the soil to pupate (Dmoch, 1965; Hussain et al., 2023). After two to three weeks, the new generation emerges and seeks different cruciferous plants to feed on before migrating to overwintering habitats (Buntin et al., 1995; Hussain et al., 2023).

Similarly to management of *B. aeneus*, *C. obstrictus* is primarily controlled by using pyrethroid-based insecticides. The economic threshold for *C. obstrictus* in Estonia is 1–2 weevils per plant at the bud stage, with at least 10% of plants at this level of infestation. In the case of *C. obstrictus*, application of insecticide shortly after flowering is also allowed (when petals have fallen), when the pods are beginning to develop (Maaelu Teadmuskeskus, 2023).

The blue stem weevil (*Ceutorhynchus sulcicollis* Paykull) is found across Europe. It feeds on wild cruciferous plants e.g. *Sisymbrium officinale* L. and *Alliaria petiolata* (M. Bieb.) Cavara & Grande (Morris, 2008). Occasionally, they are found feeding on and laying eggs into OSR plants (Alford et al., 2003). As it is not the main pest of OSR, this insect is not so well studied, and knowledge regarding its biology is not so well described. Dieckmann (1972) and Hayn (1970) have shown that this weevil is active throughout the winter, laying eggs inconsistently from autumn to spring, depending on winter conditions. Eggs are oviposited into the stem epidermis and/or the underside of the leaf. As the larvae hatch, they start to mine the stem, which results in stem damage. Once it is time to

pupate, the larvae bore out of the stem to pupate in the soil. The new generation of *C. sulcicollis* emerges around April and starts its maturation feeding before aestivating at the forest edge before reappearing in September, when the whole cycle starts again. Previously, in 2005, Veromann et al. (2006) found ten specimens in WOSR water traps and no specimens in the period 2002–2003 (Veromann et al., 2006a, 2006b), but it has been indicated by Grantina (2012) that under favourable conditions, *C. sulcicollis* may possibly increase in abundance. As of now, *C. sulcicollis* is not considered a pest of OSR, either in Estonia alone or at the European level; therefore, neither the threshold level nor its control methods are currently established.

2.5. Parasitoids of *Brassicogethes aeneus* and *Ceutorhynchus obstrictus*

Both *B. aeneus* and *C. obstrictus* have natural enemies among hymenopteran parasitoid wasps, which play a vital role in providing biological control and significantly influence the population size of pests in later years. *Brassicogethes aeneus* is attacked by at least nine species of egg and/or larval hymenopteran parasitoid wasps, with the most widespread being univoltine, koinobiont endoparasitoids, namely *Phradis interstitialis* Thomson, *Phradis morionellus* Holmgren, and *Tersilochus heterocerus* Thomson, all belonging to the Ichneumonidae family (Ulber et al., 2010). They overwinter as adults, diapausing within pupal cocoons in the soil of fields where OSR was cultivated (Ulber et al., 2010). They emerge from their overwintering habitats in the spring and migrate to a new OSR field. *Phradis interstitialis* is the earliest to emerge, typically appearing 1–2 weeks before other parasitoids, during the green bud stage of OSR plants. Both *P. morionellus* and *T. heterocerus* colonize the field at the beginning of the flowering stage (Ulber et al., 2010; Williams, 2006). The choice of host size and life stage for oviposition varies among parasitoid species. *Phradis interstitialis* prefers to oviposit into eggs or first-instar larvae, while *P. morionellus* and *T. heterocerus* favour small larvae within the bud; however, they target larger second-instar larvae once the flowers have opened (Berger et al., 2015; Nilsson, 2003).

The key parasitoid wasps of *C. obstrictus* larvae are ectoparasitoids, belonging to the Pteromalidae family: *Trichomalus perfectus* (Walker), *Stenomalina gracilis* (Walker), and *Mesopolobus morys* (Walker). They arrive in the OSR field later in the season, typically when the seedpods have started

to develop and the larvae have reached at least the second-instar stage inside the pod (Dmoch, 1975; Williams and Ferguson, 2010). They lay eggs by piercing through the pod wall and deposit a single egg on top of the host (Ulber et al., 2010). After the parasitoid larva has hatched, they feed externally on the larva and eventually kill the host (Ulber et al., 2010). The parasitoid wasps pupate inside the seedpod, and upon completing pupation, chew an exit hole, leaving the seedpod to mate and find a sheltered overwintering location (Dmoch, 1975; Graham, 1969; Murchie, 1996; Williams, 2003).

The parasitism rates of both *B. aeneus* and *C. obstrictus* can vary depending on the study region and the year. The biological control threshold for a parasitoid wasp to effectively manage pest population size is set at 32–36% (Hawkins and Cornell, 1994). The parasitism rate for *B. aeneus* has reportedly ranged from 17% to 48% in Estonia (Kaasik et al., 2014a; Veromann et al., 2013), and for *C. obstrictus*, there have been cases of parasitism rates reaching as high as 96% (Veromann et al., 2011).

2.6. Sustainable pest management strategies for oilseed rape

Implementing sustainable pest management for OSR involves the application of integrated pest management (IPM) approaches to control pests while minimizing the environmental impact. In the study conducted by Ortega-Ramos et al. (2022), although the main focus is on controlling the cabbage stem flea beetle (*Psylliodes chrysocephala* L.), the general principles of IPM also apply to *B. aeneus* and *C. obstrictus*. These approaches include key principles such as prevention and suppression through crop rotation, cultivation methods that promote beneficial insects, and the use of companion plants (Kaasik et al., 2014a, 2014b; Kovács et al., 2017). Furthermore, these approaches involve monitoring economic damage levels and making decisions based on established economic thresholds. The employment of non-synthetic control methods is encouraged before using synthetic insecticides, which should be considered only as the last option. These IPM approaches are equally relevant in effectively managing both *B. aeneus* and *C. obstrictus*.

Implementing various prevention methods or manipulating surrounding landscape elements can effectively control OSR pests and reduce the reliance on synthetic pesticides. Enhancing populations of beneficial predatory arthropods, such as parasitoid wasps, lacewings, spiders, ladybirds,

carabid beetles, and rove beetles, can result in efficient pest management within agricultural landscapes (Albrecht et al., 2020; Begg et al., 2017). For instance, parasitoid wasps have the capability to significantly reduce a *C. obstrictus* population by parasitizing up to 90% of the population (Kovács et al., 2019; Veromann et al., 2011). It is important to understand that pests like *B. aeneus* and *C. obstrictus* overwinter in habitats nearby to fields (e.g. in sheltered areas such as grassy field edges, forest edges, meadows, and grassland (Dosdall et al., 2006; Free and Williams, 1979; Kovács et al., 2019; Nilsson, 1988; Rusch et al., 2012)), but occasionally also in the OSR fields (Sutter et al., 2018), and immigrate from these habitats to production fields. In contrast, parasitoid wasps of *B. aeneus* overwinter in the same OSR field that the host larva pupates (Ulber et al., 2010). Meanwhile, parasitoid wasps of *C. obstrictus* leave the pupation site and overwinter in sheltered areas (Dmoch, 1975; Graham, 1969). Both *B. aeneus* and *C. obstrictus* can disperse relatively long distances from their overwintering sites to find their host crop (Juhel et al., 2017; Williams and Cook, 2010). However, there is limited understanding of the species-specific dispersal ability of beneficial insects, particularly hymenopteran parasitoids. Essential knowledge on dispersal ability is crucial to make informed landscape management decisions following conservation biological control approaches (Haan et al., 2021). If the spatial distance is unmanageable for the parasitoid wasps, it could lead to a delayed natural pest control service, lowering the overall effectiveness of natural pest control (de Lange et al., 2018; Thies and Tscharntke, 2010). By promoting the natural enemies, the dependency on synthetic insecticides diminishes. With the European Union's Farm to Fork Strategy aiming to minimize agriculture's adverse environmental impacts, there is a significant need to identify ecologically sustainable pest management methods (European Commission, 2020).

Essential oils also receive attention in the field of pest research due to their potential as a substitute for conventional plant protection products. Multiple investigations have emphasized the lethal effects of these substances on important agricultural pests (Das et al., 2021; Devrnja et al., 2022). There are studies on the efficiency of essential oils in managing *B. aeneus*, where it has been shown that *Carum carvi* L., *Thymus vulgare* L., and *Foeniculum vulgare* Miller have insecticidal effects against the pest (Pavela, 2011), and *Cinnamomum verum* J. Presl showed only a moderate effect at a high dosage (Willow et al., 2020). *Lavandula angustifolia* Miller has been shown to have a repellent and lethal effect against *B. aeneus* (Dorn et al.,

2014; Mauchline et al., 2013, 2005), but it elicits no behavioural response from *P. interstitialis* and *P. morionellus*, which are the main parasitoid wasps of *B. aeneus* (Cook et al., 2007). The efficiency of essential oils against *C. obstrictus* has not yet been studied, but if proven efficient, it may contribute to the simultaneous control of these two important pests. The biosafety of any plant protection product is an important aspect to consider before the product can be used, as they should not pose a threat to non-target organisms. Some studies have indicated that essential oils have no sublethal or lethal effects, and that they do not influence the parasitism or emergence success of next generation wasps on parasitoid wasps (Turchen et al., 2016; Werdin González et al., 2013), yet there is still evidence of essential oils decreasing parasitism rate (Boeke et al., 2003; Stenger et al., 2021), and therefore, not being safe for beneficial insects.

3. HYPOTHESES AND AIM OF THE STUDY

The objective of this study was to provide new insights into sustainable and environmentally safer integrated pest management approaches for controlling *B. aeneus* and *C. obstrictus* (**I**, **II**, **III**). The first approach focuses on assessing the impact of spatiotemporal separation among OSR fields (**I**) and examines the influence of various types of adjacent habitat and their distance on both pest abundance and parasitism rates (**II**). The second approach investigates the effects of different essential oils on the mortality and mobility of *C. obstrictus* (**III**); this study's objective was to comprehend the biosafety of these products by evaluating their impact on mortality and mobility of the model organism *N. vitripennis*, as well as their effects on the emergence of next-generation parasitoid wasps (**III**). Furthermore, this thesis provides an overview of findings concerning *C. sulcicollis* in Estonia and discusses the potential causes of the findings and further implications (**IV**).

The aims of the study were:

a. To assess whether the distance from the closest previous year's OSR field influences the abundance of *B. aeneus* and *C. obstrictus*, the percentage of damaged pods by *C. obstrictus*, and the parasitism rates of both species (**I**).

   Hypothesis: The abundance of pests and percentage of damaged pods and parasitism rates decrease when the consecutive year's fields are more distantly spaced in the landscape.

b. To determine the impact of different habitat types within a 1 km radius of the focal field and the impact of adjacent habitat type and in-field distance from the adjacent type on the abundance and parasitism rate of *B. aeneus* (**II**).

   Hypothesis: The adjacent habitat to the focal field or the within-field distance from it and the proportion of seminatural habitats around the focal fields increases the abundance and parasitism rates of *B. aeneus*.

c. To determine the lethal effects of essential oils on *C. obstrictus* and its parasitoid (**III**).

   Hypothesis: The tested essential oils are lethal and effective against *C. obstrictus* but safe for its parasitoid.

d. To provide an overview and examine the potential reasons for the increased abundance of *C. sulcicollis* in Estonia (**IV**).

   Hypothesis: Milder winters, relative to historical patterns, have created favourable conditions for the population increase of *C. sulcicollis.*

4. MATERIALS AND METHODS

4.1. Study area and experimental design

The study areas were located in Tartu County, southern Estonia, in conventionally managed winter oilseed rape (WOSR) fields (**I**, **II**). The field sizes and sampling methods varied depending on the studies' aims. In 2014 and 2015, each focal field had a non-overlapping landscape circle with a fixed radius of 1 km (**II**). Standardized methods from the EU FP7 project QuESSA were employed for general research site selection, utilizing a landscape circle radius of 1 km, SNH categorization, and experimental design, all of which were previously described in earlier studies (Fusser et al., 2017; Holland et al., 2020; Kovács et al., 2019; Pfister et al., 2017; Picchi et al., 2016). In total, there were 18 WOSR focal fields selected for both 2014 and 2015, and these were categorized into three groups: six fields bordered with woody linear element, six with herbaceous linear element, and six with another crop field (**II**). In a study conducted from 2016 to 2018, a total of 12 WOSR fields were chosen for each study year and divided into two groups: six located within 500 metres (< 500 m) of the previous year's nearest OSR field, and six beyond 500 metres (> 500 m) (**I**).

The composition of the landscape surrounding the study fields was a key aspect of both studies. This surrounding landscape primarily comprised agricultural land, forests, wetlands, rivers, residential areas, and peat bogs. The proportion of OSR fields in both groups (< 500 m and > 500 m) was similar across all study years (**I**). In 2018, one study field was excluded for the *C. obstrictus* study (**I**), as it was severely damaged by the cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsham). The landscape within a 1 km radius around the focal field was mapped in detail, and seminatural habitats (SNHs) were classified based on dominating vegetation, such as herbaceous areal, woody areal, cover crop, herbaceous linear, and woody linear (II). The landscape analysis was carried out using ArcGIS (**I**, **II**).

4.2. Insect sampling

4.2.1. *Brassicogethes aeneus* and its key parasitoids

To sample the abundance of overwintered *B. aeneus* in WOSR fields, the plant tapping method was used at the plant's most susceptible growth

stage (the green bud stage) (**I**, **II**), which is the most common way for farmers to assess the pest threshold. For this, the main raceme of each WOSR plant was tapped over a tray, in which all dislodged *B. aeneus* were counted. In each WOSR field, ten randomly chosen plants were tapped. In 2014 and 2015 there were four distances set from the field edge towards the field centre (2, 25, 50, and 75 m) (**II**), and in the period 2016–2018, there were five sampling points running parallel to the field edge (I). Sampling started at the green bud stage (BBCH 50 (Lancashire et al., 1991)) (**I**) and ended when the main raceme started to flower (BBCH 61–63 (**I**) and BBCH 57–60 (**II**)). Using yellow pan traps, flight activity of *B. aeneus* and its key parasitoids was observed (**I**). Yellow pan traps were filled with water containing a couple of drops of organic dishwashing liquid to avoid surface tension.

The parasitism rate of *B. aeneus* larvae was investigated during the full flowering stage of plants using funnel traps (**I**, **II**). For the funnel traps, plastic vials were used, that were placed into the soil, half-filled with water to prevent fallen larvae from escaping, and on top of each plastic vial, a 31 cm wide funnel was placed. Funnel traps were placed in the field next to yellow pan traps at the same distances (**I**), except in study **II**, where the funnel traps where only placed at 2, 25, and 75 m. Traps were emptied weekly from the full flowering stage (BBCH 65) until the beginning of the pod stage (BBCH 71–72), as this is the main time at which second-instar larvae are dropping from plants to pupate in the soil. Traps were emptied and the samples were sorted in the laboratory on the collection day, and all *B. aeneus* larvae (identified using the key by Osborne (1965)) were preserved in Eppendorf tubes filled with distilled water at -20 °C for the upcoming identification of parasitoids. To examine the parasitism rates of *B. aeneus* larvae, all larvae were dissected under a stereo microscope (Olympus, Tokyo, Japan) and identified using the key of Osborne (1960).

4.2.2. *Ceutorhynchus obstrictus* damage and parasitism rate

To evaluate the pod damage and parasitism rates of *C. obstrictus*, OSR pods were collected from the fields at the beginning of the pod ripening stage (BBCH 81–82). In each field, from 50 plants, a total of 1000 seedpods were collected (20 pods from each plant: 10 pods from the main stem and 10 from side branches). Each bundle of 20 seedpods was placed into a plastic labelled bag brought to the lab, where it was placed into a

cardboard emergence box (described in detail in Veromann et al. (2011) and Kovács et al. (2013)). Emergence boxes were kept at room temperature for a month. After that, each seedpod was opened and individually carefully inspected. All emerged parasitoids and *C. obstrictus* larvae were counted and preserved in ethanol for later identification. Each seedpod was individually examined to find and count the exit holes of larvae or parasitoids to calculate the damage rate using the formula previously described in Kovács et al. (2019). Additionally, yellow pan traps were used to monitor flight activity of *C. obstrictus* (**I**).

4.2.3. *Ceutorhynchus sulcicollis* abundance and damage rate

During the stem elongation growth stage (BBCH 30–31), 30 randomly selected WOSR plants were collected from untreated WOSR fields (**IV**). In the laboratory, plants were individually potted into 5 L pots and covered with fine mesh fabric to prevent contamination by other insects. For one month, plants were kept in a laboratory environment at 20–22 °C with a 16:8 h light–dark cycle and regular watering. After one month, all 30 WOSR plants were dissected, damaged areas measured, and stem-mining larvae counted and collected. As identification of stem-mining larvae to species level is not possible, collected larvae were placed into boxes filled with moist soil for pupation. The boxes were covered with fine mesh fabric and placed into a climate chamber at 20 °C, with a relative humidity of 60% and a light–dark cycle of 16:8 hours. Water was sprayed on the soil daily to prevent the soil from drying. Emerged adult weevils were collected and stored at -20 °C for identification. For comparison of stem damage between insecticide-treated and untreated plants, 30 randomly chosen WOSR plants were collected from the same field after Proteus OD (application rate of 0.6–0.75 l/ha with 200–300 l/ha water; active ingredients thiacloprid and deltamethrin) was used. Plants were dissected, damaged areas measured, and larvae handled as described above.

4.2.4. Examining the effect of essential oils on *Ceutorhynchus obstrictus* and *Nasonia vitripennis*

Six essential oils were tested on *Ceutorhynchus obstrictus* through treated WOSR leaves and flowers to examine mortality and immobility effects (**III**). The essential oils of thyme (*Thymus vulgaris*), fennel (*Foeniculum vulgare*), cumin (*Cuminum cyminum*), cinnamon (*Cinnamomum verum*),

caraway (*Carum carvi*), and hemp (*Cannabis sativa*) were used at a 1.5% concentration, using acetone as the solvent and Tween80 at a 0.05% concentration as a wetting agent. The negative controls were acetone and Tween80. Lambda-cyhalothrin at the recommended field concentration (7.5 g active compound/ha) dissolved in acetone and Tween80 was used as the positive control. Both WOSR leaves and flowers were treated with the test solutions. WOSR leaves were individually treated with 1000 µl of treatment solution, applied using a pipette, and the flowers were dipped into the solution. After the treatment, the flowers and leaves were left to dry before they were placed in testing cages. Each cage contained one leaf and four flowers, to which eight *C. obstrictus* adults were added. There were five cages per treatment, which were kept in a ventilated room with an ambient air temperature of 22±2 °C, away from direct sunlight. Survival and mobility were checked 3 h and 24 h after residue exposure. The experiment had two replicates, with a total of 10 cages per treatment, and a total of 80 weevils per treatment.

After the initial experiment with six different essential oils, the two most effective essential oils, *C. verum* and *C. cyminum*, were further evaluated in four different concentrations (0.5%, 1%, 1.5%, and 2%). The experimental setup was the same as described above; the main difference was that the experiment was repeated only once (a total of 5 cages per treatment; in total, 40 weevils per treatment).

To examine the impact of essential oils on a non-target organism, the parasitoid species *Nasonia vitripennis* was used. Six essential oils were used in this study at four concentrations: 0.1%, 0.3%, 0.5%, and 1.5%. The essential oil treatments were prepared similarly as described above, using Tween80 and acetone. The negative Tween80 and acetone control and positive lambda-cyhalothrin control were also used in this study. The primary distinction between studying the effects of these essential oils on *C. obstrictus* and on *N. vitripennis* was that in the *C. obstrictus* study, WOSR leaves were treated, but for *N. vitripennis*, filter paper was used. Similarly, the filter paper was left to dry for one hour and placed into a cage, into which 10 *N. vitripennis* adults were introduced. Mortality and mobility were monitored after 3 h and after 24 h. This experiment was repeated twice, with a total of 10 cages per treatment and a total of 80 *N. vitripennis* adults per treatment.

The impact of the essential oils on the development success of *N. vitripennis* was tested on already-parasitized blow fly pupae. *N. vitripennis* adults were allowed to freely parasitize the blow fly pupae for seven days, after which the parasitoids were removed. Parasitized blow fly pupae were dipped into 1.5% concentration solutions of the six different essential oils. This study had untreated pupae as a negative control and lambda-cyhalothrin as a positive control.

4.3. Statistical analysis

Generalized linear mixed-effects models with Wald statistic type III empirical standard error were used to analyse the abundance, the flight activity and the parasitism rate of *B. aeneus*, but also to analyse the flight activity of *B. aeneus* parasitoids and the parasitism rates and the pod damage rates caused by *C. obstrictus* in the period 2016–2018 (**I**). Generalized linear mixed-effect models were used to analyse 2014 and 2015 data to examine the effects of adjacent habitat type and the distance from the field edge on *B. aeneus* abundance and the parasitism rate of *B. aeneus* larvae (**II**).

The study focused on field as the independent variable, which was categorized into two groups: closer than 500 metres (< 500 m) and further than 500 metres (> 500 m) from the previous year's WOSR field. Depending on the data, either Gaussian or negative binomial distributions were used. Pairwise comparisons were conducted using least-squares means with Tukey adjustment. The sampled fields were used as a random factor (**I**).

In the analysis of the 2014 and 2015 data, either binomial or Poisson distributions (with a log link function) were used to examine the abundance of *B. aeneus* and the parasitism rate. Both years were analysed separately, with either *B. aeneus* abundance or the parasitism rate as a predictor variable. The fixed factors in the model were adjacent habitat type, distance, and their interaction. Additionally, the model had random factors such as sampling round and the number of days since the last insecticide application. The type II Wald statistic was used to evaluate the individual and combined effects of adjacent habitat type and distance from the edge. To compare the means, Tukey adjustment was used (**II**). The DHARMa package was used to assess the fit of generalized linear mixed-effects models (**I**, **II**).

To analyse the residuals effect of essential oils on *C. obstrictus* analyses, generalized linear models (GLMs) with a Poisson distribution, log link function, type III Wald statistics empirical standard error were used. A Tukey test was applied for post hoc comparison (**III**).

Pearson's correlations were used to examine the relationship between the habitats within a 1 km radius of the focal field and *B. aeneus* abundance (**II**). Additionally, a Welch's t-test was used to compare the quantity of weevil larvae found in the stems of treated and untreated WOSR plants (**IV**), and a Student's t-test was used to examine the proportions of WOSR between the two distinct groups (**I**). Non-normally distributed *N. vitripennis* data were analysed using the nonparametric Kruskal–Wallis test, followed with Bonferroni–Dunn's test for post hoc pairwise comparison (**III**).

For paper **II**, random forest analysis was carried out to determine the variance that can be explained by the landscape types surrounding the focal fields. After the random forest analysis, a decision tree was built. Annual landscape data for both 2014 and 2015 were used to build the random forest and decision tree, in which the dependent variable was the parasitism rate of *B. aeneus* larvae. For the landscape data, proportions of different landscapes within a 1 km radii were used. The random forest model was set to generate 5000 trees per run and was re-run 10 times (creating a total of 50 000 trees) to see the variation in the variable importance. A cross-validation technique was used to evaluate the performance of the models. For the decision tree, the anova method was used, which is used for a regression task in which the target variable is continuous or numeric.

All statistical analyses were performed in Rstudio, using the following R packages: randomForest (**II**), rpart (**II**), partykit (**II**), lme4 (**I**, **II**), car (**I**, **II**, **III**), emmeans (**I**, **II**, **III**), MASS (**I**, **II**, **III**), DHARMa (**I**, **II**, **III**), and dunn.test (**III**). For data visualization, ggplot2 (**I**, **II**), tidyverse (**I**, **II**), ggpubr(**I**, **II**), and rpart.plot (**II**) were used.

5. RESULTS

5.1. The spatiotemporal distancing and adjacent habitat effects on the abundance and flight activity of *Brassicogethes aeneus*

Plant tapping experiments in the period 2014–2018 showed that overwintered *B. aeneus* was present during the most damage-susceptible OSR growth stage and after this stage too (**I**, **II**). In all study years, 2014–2018, plant tapping experiments revealed that the abundance of *B. aeneus* was low and did not exceed the economic threshold (**I**, **II**). In 2016 and 2018, the abundance of *B. aeneus* was lower in fields > 500 m from the previous year's OSR field compared to < 500 m from the previous year's OSR field (χ^2 = 15.12, df = 1, P < 0.001; χ^2 = 16.23, df = 1, P < 0.001, respectively) (**I**). Only in 2017, was there no significant difference between these two groups and their impact on *B. aeneus* abundance (χ^2 = 3.45, df = 1, P = 0.06). In each research year, there was a substantial difference between the two observation groups at various sampling points, with the biggest difference shown at the beginning of the season (Figure 2.; **I**).

In 2014 and 2015, adjacent habitat did not have a significant effect on *B. aeneus* abundance during the green bud stage (**II**). In 2014, the highest mean number of *B. aeneus* individuals per plant was observed in fields adjacent to woody linear element (0.32±0.79), followed by another crop (0.26±0.56) and herbaceous linear element (0.19±0.39) (Figure 3.; **II**); however, in 2015 there was more *B. aeneus* in fields adjacent to herbaceous linear element (0.59±0.84), followed by another crop (0.56±1.54) and woody linear element (0.34±0.41). The distance from the adjacent habitat and the combined effect of adjacent habitat and distance within the field had a significant effect on *B. aeneus* abundance in both 2014 and 2015 (2014: χ^2 = 57.39, df = 3, P < 0.0001; 2015: χ^2 = 216.72, df = 3, P < 0.0001; 2014: χ^2 = 65.35, df = 6, P < 0.0001; 2015: χ^2 = 43.76, df = 6, P < 0.0001, respectively; **II**). Surrounding habitats within 1 km of the focal fields influenced *B. aeneus* abundance only in 2014, when a significant positive relationship between herbaceous linear element and *B. aeneus* abundance was found (r = 0.68; P = 0.002).

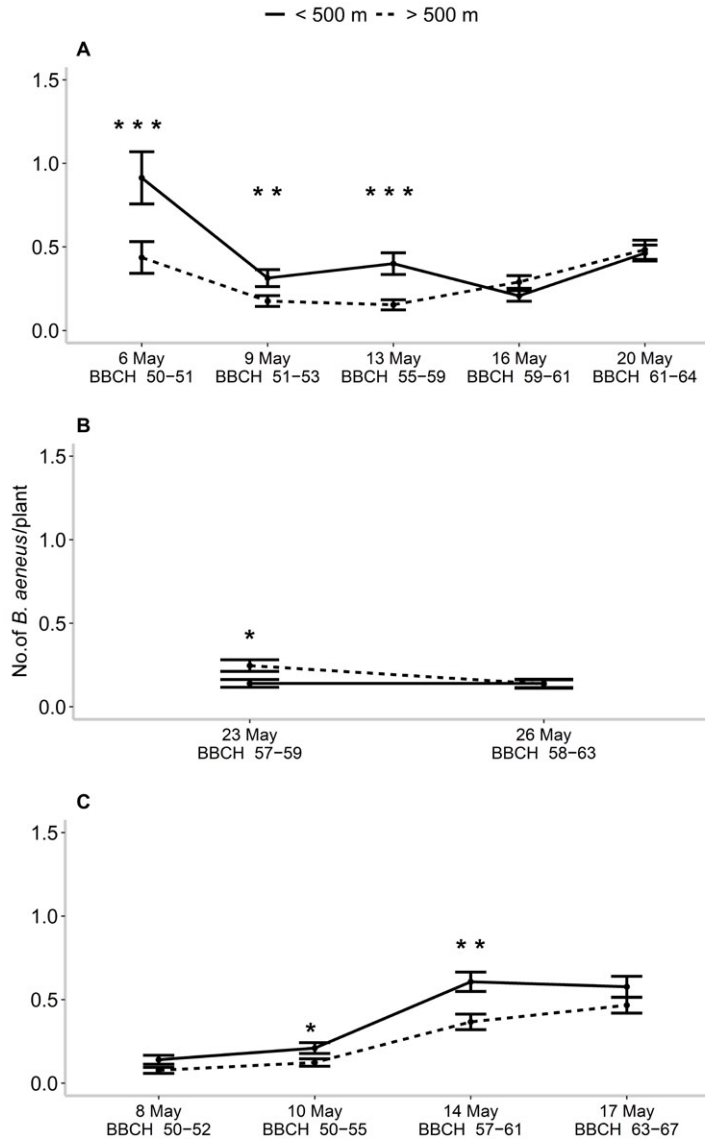

Figure 2. Mean (±SE) number of adult *Brassicogethes aeneus* per oilseed rape plant (OSR) in fields less than 500 m from the previous year's nearest oilseed rape field (< 500 m) and further than 500 m from the previous year's nearest oilseed rape field (> 500 m); results for a three-year study ((A) 2016, (B) 2017, and (C) 2018) (**I**; Sulg et al., (2023)). Asterisks (*) indicate level of statistically significant difference (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) between < 500 m and > 500 m fields.

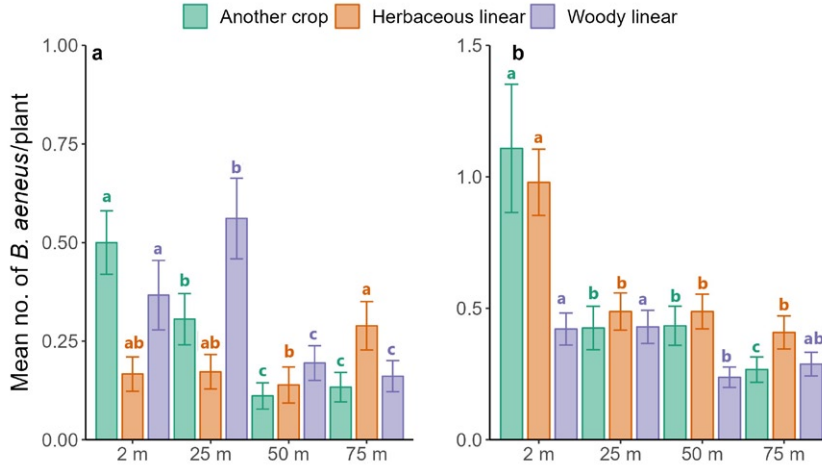

Figure 3. Mean (±SE) number of *Brassicogethes aeneus* adults per plant during the winter oilseed rape (WOSR) bud stage (BBCH 50–59) collected from WOSR fields with different adjacent habitat types and at different distances from the edge of fields in (a) 2014 and (b) 2015 (**II**; Vilumets et al., (2023)). Statistically significant differences between distances (within groups) in the mean number of *Brassicogethes aeneus* adults per plant (with Tukey adjustment) are indicated with different letters ($P < 0.05$). Colour indicates the grouping variable: another crop is green, herbaceous linear element is orange, and woody linear element is purple.

5.2. The spatiotemporal distancing effect on the flight activity and damage rate of *Ceutorhynchus obstrictus*

The flight activity and the damage rate by *C. obstrictus* were observed during a three-year field study (2016–2018) (I). The number of *C. obstrictus* individuals caught by yellow water traps was low during the study years. However, in 2016 and 2018, significantly more *C. obstrictus* adults were caught in traps < 500 m from the previous year's WOSR fields, compared to > 500 m from the previous year's OSR fields ($\chi^2 = 7.04$, df = 1, $P = 0.008$; $\chi^2 = 19.28$, df = 1, $P < 0.0001$, respectively). In 2017, no difference between these two groups was observed. The pod damage rate caused by the weevil larvae was significantly different between study years ($\chi^2 = 29.16$, df = 2, $P < 0.001$). However, only in 2017 and 2018 were the pod damage rates significantly different between these two groups. Notably, the fields located < 500 m from the previous year's WOSR fields had significantly more weevils ($\chi^2 = 37.49$, df = 1, $P < 0.001$; $\chi^2 = 27.57$, df = 1, $P < 0.001$, respectively, Figure 4.; I).

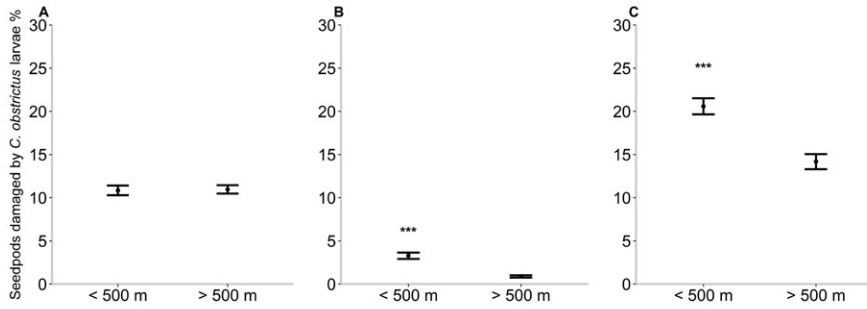

Figure 4. Mean percentage (±SE) of seedpod damage caused by *C. obstrictus* in winter oilseed rape (WOSR) fields less than 500 m from the previous year's nearest oilseed rape crop (< 500 m), and in oilseed rape crops further than 500 m from the previous year's nearest oilseed rape crop (> 500 m) in 2016 (A), 2017 (B), and 2018 (C) (**I**; Sulg et al., (2023)). Asterisks (*** P < 0.001) indicate statistically significant differences between < 500 m and > 500 m fields.

5.3. Spatiotemporal and adjacent habitat effects on parasitoid wasps and the parasitism rate

Overlapping flight activity between *B. aeneus* and its parasitoids was observed in all study years (2016–2018); however, the number of parasitoids caught by yellow pan traps was relatively low compared to their host (**I**). Both in 2017 and 2018, there were significantly more parasitoid wasps in yellow pan traps in fields that were located less than 500 metres from the previous year's WOSR (**I**).

Generally, the mean parasitism rates of *B. aeneus* and *C. obstrictus* were high and varied between the study years and groups, reaching up to 79% (**I**). Only in 2017 did the parasitism rate of *B. aeneus* differ between observation groups, with a considerably greater parasitism rate identified in the group where fields were < 500 m from the previous year's WOSR field ($\chi^2 = 5.40$, df = 1, P = 0.02; Figure 5). There were no significant impacts on *B. aeneus* in 2016 and 2018 or *C. obstrictus* in any of the observation years 2016–2018 (Figure 5; **I**).

Additionally, the parasitism rate of *B. aeneus* was not affected by the adjacent habitat, distance from the adjacent habitat, or the combined effect of them (**II**). The parasitism rate for *B. aeneus* varied between 2014 and 2015, reaching a mean value of 73% in 2014 in fields adjacent to another crop and 81% in 2015 for fields adjacent to woody linear element. The

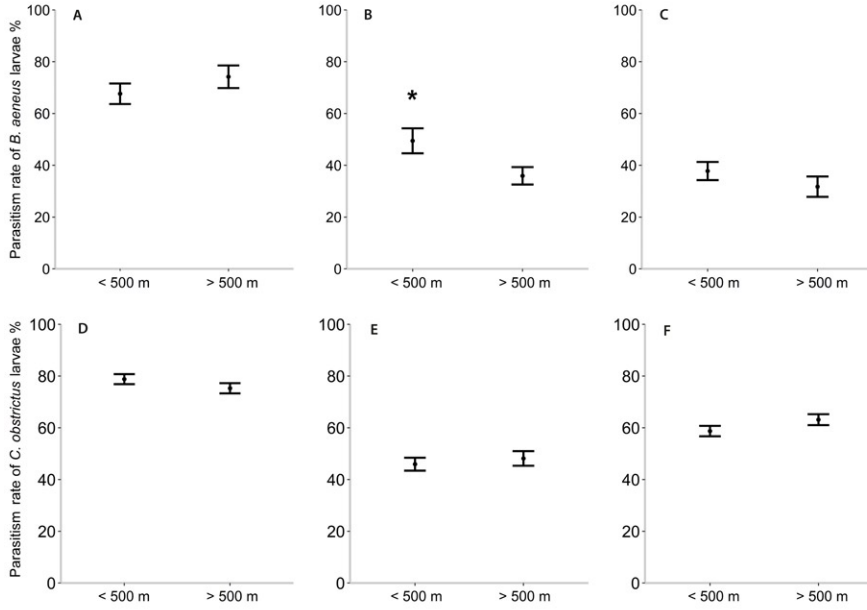

Figure 5. Mean (±SE) parasitism rate of *Brassicogethes aeneus* larvae (A–C) and *Ceutorhynchus obstrictus* (D–F) in oilseed rape fields less than 500 m from the previous year's nearest oilseed rape field (< 500 m), and in fields further than 500 m from the previous year's nearest oilseed rape field (> 500 m), in 2016 (A, D), 2017 (B, E), and 2018 (C, F) (**I**; Sulg et al., (2023)). Asterisk * indicates statistically significant ($P < 0.05$) difference between < 500 m and > 500 m fields.

parasitism rate of *B. aeneus* was also affected by the proportion of different landscape characteristics surrounding the focal field. If the proportion of seminatural habitats around the focal fields was more than 18%, the predicted parasitism rate was 70%, whereas if it was lower than 18%, the predicted parasitism rate was only 24% (Figure 6; **II**). It was also discovered that if the proportion of SNHs in the surrounding landscape surpassed 43% and the proportion of herbaceous area was greater than 3.9%, the parasitism rate of *B. aeneus* was predicted to be 87%.

5.4. The effect of essential oils on *Ceutorhynchus obstrictus*

There was no difference in survival rates of *C. obstrictus* between the essential oil treatment and the negative control treatment after 3 h of exposure to the dry residues (**III**). Nonetheless, we saw a substantial impact on survival 24 h after treatment ($\chi^2 = 132.41$, df = 7, $P < 0.0001$). After 3 h, *C. obstrictus* mortality and immobility rates were significantly affected by

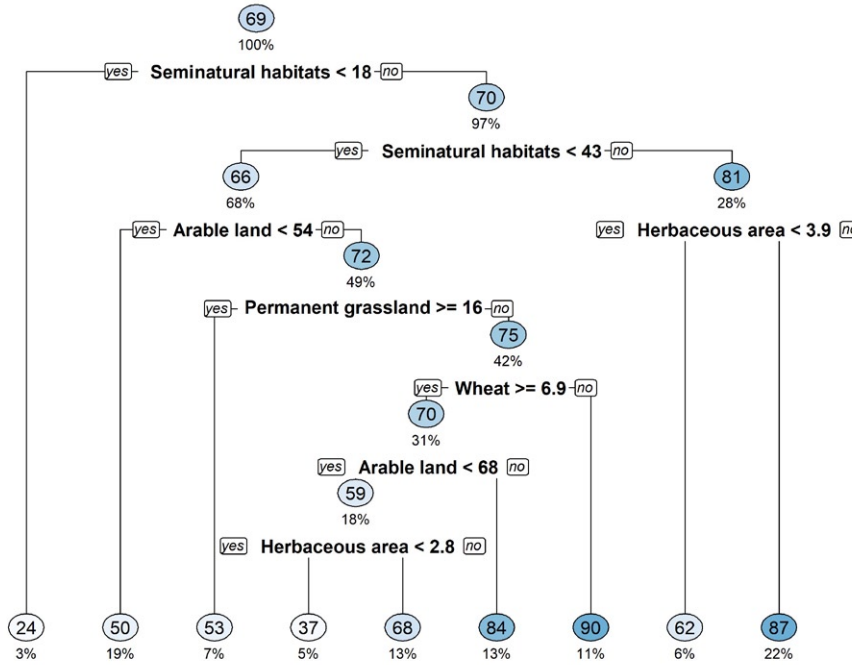

Figure 6. Decision tree based on the 2014 and 2015 landscape characteristics surrounding the focal fields to determine the median parasitism rate of *Brassicogethes aeneus* (pooled data 2014 and 2015) (**II**; Vilumets et al., (2023)). Each terminal node (inside the circle) gives a predicted median parasitism rate of *B. aeneus*, and under the node the value (percentage (%)) represents the proportion of data used to predict the parasitism rate. The values next to the landscape variable represent the percentage used for the division setpoint.

essential oil treatment (χ^2 = 90.32, df = 7, P < 0.0001), yet the positive control treatment had the most impact on *C. obstrictus* mortality and immobility (76.3±6.3%). All treatments, aside from *C. carvi*, increased the mortality and immobility rates of *C. obstrictus* 24 h after exposure to dry residues. Pairwise comparison of all treatments showed that two essential oil treatments (*T. vulgaris* and *C. sativa*) did not differ from the negative control. The two most effective essential oils causing high mortality and immobility were from *C. cyminum* and *C. verum*, for which the mortality was the same as the positive lambda-cyhalothrin control.

Both *C. cyminum* and *C. verum* were further examined at four different concentrations, with *C. verum* at a 1% concentration showing a significant effect on *C. obstrictus* survival after 3 h, but there was no effect of *C. cyminum* at this time point. After 24 h, both essential oils had a significant

lethal effect on *C. obstrictus*; however, *C. verum* showed higher mortality rates compared to *C. cyminum* (*C. verum* $\chi^2 = 126.49$, df = 4, $P < 0.0001$; *C. cyminum* $\chi^2 = 87.29$, df = 4, $P < 0.0001$). Both *C. verum* and *C. cyminum* essential oils, at the four different concentrations observed, had a significant effect on the mortality and immobility of *C. obstrictus* ($\chi^2 = 300.77$, df = 4, $P < 0.0001$) compared to the negative control, for which the mortality was zero.

5.5. The effect of essential oils on *Nasonia vitripennis*

The mortality and immobility rates of *Nasonia vitripennis* were significantly affected by essential oils at the 0.1% concentration after 3 h of exposure to residues ($\chi^2 = 20.96$, df = 6, $P = 0.0019$), with more parasitoids affected by the *T. vulgaris* and *F. vulgare* treatments (**III**). Interestingly, lambda-cyhalothrin (used as a positive control) did not cause a high mortality. After 24 h of exposure to dry treatment residues, no significant effect on mortality and immobility was observed for any of the essential oil treatments applied at the 0.1% concentration ($\chi^2 = 7.49$, df = 6, $P = 0.28$). At the 0.3% concentration, a significant difference in *N. vitripennis* mortality and immobility was observed at both 3 h and 24 h after exposure ($\chi^2 = 24.98$, df = 6, $P < 0.001$; $\chi^2 = 27.78$, df = 6, $P < 0.0001$, respectively) compared to the positive control. Essential oils at a concentration of 0.5% increased the mortality and immobility at both 3 h and 24 h post-exposure ($\chi^2 = 59.54$, df = 6, $P < 0.0001$; $\chi^2 = 53.84$, df = 6, $P < 0.0001$, respectively). Already after 3 h, *C. verum* and *T. vulgaris* caused 100% mortality in *N. vitripennis*, and *F. vulgaris* caused 97±2.1% mortality. In contrast, the negative control treatment and lambda-cyhalothrin did not significantly reduce *N. vitripennis* survival or mobility. Additionally, after 3 h, all essential oils at a concentration of 1.5% caused 100% mortality to *N. vitripennis*.

The residues of essential oils affected the emergence of the next generation of *N. vitripennis*. Pupae treated with *C. verum* (176±11.3 specimens) and *F. vulgare* oils (171.8±12.5 specimens) had the highest average number of emerging next-generation *N. vitripennis* adults compared to the untreated control group. However, these differences were not statistically significant. The number of parasitoids that emerged from *C. sativa*, *C. cyminum*, and *T. vulgaris* treatments was much lower than for untreated pupae; the number was more similar to pupae treated with lambda-cyhalothrin, for which only 85.7±16 parasitoids emerged.

5.6. *Ceutorhynchus sulcicollis* abundance

Out of 30 dissected untreated WOSR plants, larvae of stem-mining weevils were found in 29 of them, and the number of weevils collected from the untreated plants was significantly higher compared to the insecticide-treated plants ($P < 0.0001$) (**IV**). On average, 9.33 ± 1.41 weevils per plant were found in the untreated group, while in the insecticide-treated group, on average, only 1.17 ± 0.28 larvae per plant were found. Out of the 280 larvae gathered from untreated plants, the majority (90%, total of 251 specimens) successfully completed pupation, whereas in the insecticide-treated plants, only two out of 35 collected larvae completed pupation. The subsequent generation of weevils collected from the untreated plants was identified as *C. sulcicollis* (204 specimens out of 251) and *C. pallidactylus* (47 specimens out of 251). In the insecticide-treated plants, out of the two successfully emerged specimens, one was *C. sulcicollis* and the other was *C. pallidactylus*.

6. DISCUSSION

6.1. The effect of spatiotemporal distancing and landscape elements on pest abundance and control by natural enemies

Even though the overall abundance of *B. aeneus* was low from 2014 to 2018 during the green bud stage, a distinct difference in the dependent landscape factors was observed between the two studies (**I**, **II**). *B. aeneus* abundance during the most damage-susceptible stage of OSR growth did not exceed the threshold level (**I**, **II**). As the most susceptible growth stage of OSR is during the green bud stage, it is important to inspect *B. aeneus* abundance at this time (**I**, **II**), as their high abundance may lead to substantial damage levels. Throughout these studies, it was found that at the focal field level, the adjacent habitat did not influence the abundance of *B. aeneus* (**II**). However, at the landscape scale, when the distance from the previous year's OSR field exceeded 500 metres, there was an overall positive trend in reducing pest suppression during the plant's most susceptible stage of development (**I**). Similar findings were noted for flea beetles (Coleoptera, Alticinae), which also cause severe damage to cruciferous plants; it was revealed that the pest density and crop damage to spring OSR plants decreased as the spatial scale distance increased between the previous year's fields (Boetzl et al., 2023).

After *B. aeneus* has completed its overwintering in field edges, hedgerows, forest edges, meadows, and grasslands (Nilsson, 1988; Rusch et al., 2012), and occasionally also in a previous year's OSR field (Sutter et al., 2018), individuals begin searching for OSR fields, guided by volatile organic compounds emitted by the plants (Cook et al., 2002; Mauchline et al., 2018; Smart and Blight, 2000). The implementation of spatiotemporal separation between consecutive years' fields (**I**) and having a diverse and complex surrounding landscape that includes natural and seminatural habitats (**II**) has provided evidence that these measures can reduces pest density (Albrecht et al., 2020; Boetzl et al., 2023; Haan et al., 2021; Martin et al., 2019; Thies and Tscharntke, 1999).

Understanding the relationship between pests and landscape elements is challenging due to conflicting findings in various studies. The role of non-crop habitats and their impact on pests is not always easy to comprehend, as these interactions are often species-specific. This complexity makes it challenging to develop landscape-scale plans, as approaches need

alignment with the ecological traits of the target pest, while considering the effects on species-specific natural enemies (Boetzl et al., 2023; Haan et al., 2021; Martin et al., 2019; Tscharntke et al., 2016).

The spatiotemporal distancing also had an impact on *C. obstrictus* damage level, showing lower damage rates in two out of three study years when the OSR fields were located further than 500 m from the previous year's fields (**I**). This distancing lowered the damage caused by *C. obtrictus* at the landscape scale. However, at the focal field level, Kovács et al. (2019) found that the herbaceous linear adjacent habitat had the highest damage rate caused by *C. obstrictus* compared to the focal fields adjacent to woody linear or another crops. This supports the hypotheses and findings that *C. obstrictus* tends to overwinter near to those fields in which they recently completed pupation (Dosdall et al., 2006; Free and Williams, 1979; Kovács et al., 2019). Implementing distancing between consecutive years' OSR fields could alleviate the damage caused by *C. obstrictus*. Similar findings emerged for the white clover seed weevil *Protapion fulvipes*, which also prefers overwintering near a previous year's clover field. This suggests that sowing a new clover field closer than 2–3 km would result in higher pest density and damage (Hederström et al., 2022). Landscape elements significantly impact *C. obstrictus* damage extent, as Kovács et al. (2019) found, showing that grasslands and wheat fields are the most important landscape elements affecting damage. However, D'Ottavio et al. (2023) showed that neither canola crops, hay/pasture, nor forests increased the level of infestation. Instead, they revealed that open roads and cereals positively influenced *C. obstrictus* abundance, while Langer and Jensen (2023) demonstrated that the forest edge positively influenced *C. obstrictus* abundance. Cereal crops typically follow OSR in crop rotation plans; therefore, if the cereal crop is nearby, it can increase *C. obstrictus* damage (D'Ottavio et al., 2023; Kovács et al., 2019). Interannual changes in OSR resource can also influence *C. obstrictus* abundance and damage (D'Ottavio et al., 2023; Kovács et al., 2019; Langer and Jensen, 2023). The landscape elements affecting insect movement are often species-specific and largely depend on dispersal abilities (Haan et al., 2021; Miguet et al., 2016). The influence of different landscape elements on *C. obstrictus* abundance and damage varies based on the study region due to distinct climatic conditions and landscape management methods, highlighting the complex relationships between the presence of pests and landscape elements (D'Ottavio et al., 2023; Kovács et al., 2019; Langer and Jensen, 2023).

Considering studies **I** and **II** in conjunction with Kovács et al. (2019), it is evident that two pests sharing the same host crop are affected at both landscape and focal field levels by the surrounding landscape. In studies **I** and **II**, as well as in Kovács et al. (2019), it was observed that the pest's natural enemies were not significantly influenced by the adjacent habitat or spatiotemporal distancing, as parasitism rates exceeded the required level for effective natural pest control (at least 32% (Hawkins and Cornell, 1994)). The temporal dynamics of landscape components, such as the percentage of OSR grown in one year, can impact the parasitism rate in the following year, ultimately affecting the pest population size in the third year (Beduschi et al., 2015). The parasitism rate in OSR fields increases if the field is adjacent to flowering fields or calcareous grassland, indicating the beneficial effects of these landscape elements on ecosystem services, and allowing a reduction in overall intensive pesticide use (Krimmer et al., 2022). Decision trees also highlight the importance of seminatural habitats within a 1 km radius of the focal field, emphasizing the difference between the landscape-level effects on the parasitism rate. If the proportion of seminatural habitats is over 18%, there is an almost 50% increase in the parasitism rates (**II**). Thies and Tscharntke (1999) demonstrated that if the proportion of seminatural habitats around the focal fields is less than 20%, it affects the parasitism rate of *B. aeneus*, and the natural pest control threshold is not reached. Functioning ecosystem services require a substantial proportion of seminatural habitats around the production fields to function correctly (DeClerck, et al., 2023; Martin et al., 2019; Thies and Tscharntke, 1999). Managing OSR fields with an ecofriendly approach (such as perennial flower strips and avoiding insecticide applications in spring) has been shown to increase the parasitism rate of *B. aeneus* compared to using conventional methods (Serée et al., 2023).

These findings highlight the complexities involving pest–parasitoid interactions at the landscape level. It largely depends on species, and when dealing with multiple pests in the same host crop, the measures implemented at the focal field level might work for one pest but not as effectively for another. Farmers face challenges in implementing measures such as creating distance between fields in consecutive years or managing the surrounding habitats, as field managers are often renters rather than owners. Finding available farmland for rent has become increasingly challenging due to high demand. Additionally, making changes to the landscape requires obtaining the owner's approval, which applies to adjacent habitats typically owned by someone else. Thus, well-managed landscape ele-

ments depend not only on one farmer but rather on a larger community working together to maintain a functioning agroecosystem (Zheng et al., 2020). The landscape does not have walls separating the forests, hedgerows, and grasslands from production fields – everything is interconnected and influences everything else, even when not always visible.

6.2. The influence of essential oils on the target pest and beneficial insects

The effects of essential oils on *C. obstrictus* have not been extensively studied according to published literature, especially in comparison with *B. aeneus*. This scarcity limits the number of comparable studies available. The presence of essential oil residues from treated OSR leaves and flowers resulted in mortality and immobility of *C. obstrictus* adults. The use of essential oils also impacted the mortality and immobility of a model organism of parasitoid, *N. vitripennis*. Furthermore, it was also revealed that essential oils affected the emergence of the next generation of adult parasitoid wasps from their hosts (**III**). Across different concentrations, the most promising essential oils against *C. obstrictus* were *C. verum* and *C. cyminum*. Willow et al. (2020) suggested that among seven tested essential oils, *C. verum* exhibited the most potential for controlling *B. aeneus*, while Pavela (2011) indicated the potential of *C. carvi*, *T. vulgaris*, and *F. vulgare*.

B. aeneus and *C. obstrictus* show an overlap in their flight activity and arrival at OSR fields (**I**). This suggests the possibility of simultaneous control of these two pests. Synchronizing the use of plant protection when both pests are present could ideally reduce the number of applications of plant protection products throughout the growing season, thereby lowering the environmental impact. However, it is also important to use plant protection products that are safe for non-target organisms. Unfortunately, the residues of *C. verum* essential oils tested at a 1.5% concentration resulted in 100% mortality of *N. vitripennis*. Essential oils also affected the hatching of new *N. vitripennis* generations, significantly reducing emergence when the parasitized pupae were treated with *C. sativa* and *C. cyminum*. The fact that *N. vitripennis* experienced a 100% mortality rate when directly exposed to essential oils, but this effect was not observed in the treated pupae, suggests that the fly pupae might act as a protective barrier, mitigating harmful impacts. Previous studies Werdin González et al. (2013) have demonstrated negative effects of essential oils on another parasitoid

species, *Trissolcus basalis* Wollaston (Platygastridae). Even though these parasitoid species can survive after contact with essential oils, it remains unclear if these oils have any sublethal effects, such as affecting their ability to locate hosts or their efficiency in egg laying (**III**).

The results of the study **III** highlighted that essential oils could be as lethal to both pests and beneficial insects as synthetic insecticides that are still used in the fields. Further investigations should consider variations in the compounds contributing to the primary causes of mortality in both *C. obstrictus* and *N. vitripennis* when exposed to essential oils. Moreover, the methods used to test the essential oils often depend on a study's specific objectives, making it challenging to compare studies. Further research should aim to include all possible pests and the non-target organisms associated with the same host crop, implementing consistent conditions and experimental setups to better understand the effects of essential oils.

6.3. *Ceutorhynchus sulcicollis* as a new potential pest

Study **IV** underscores that *C. sulcicollis*, currently not considered a pest of WOSR, has the potential to become one in favourable conditions, as previously suggested by Grantina (2012). The mild winter conditions of 2019–2020, with an average temperature around 2.5 °C, are believed to have been favourable for the reproduction and survival of *C. sulcicollis*. However, the scarcity of literature and knowledge on *C. sulcicollis* poses a challenge in drawing conclusions and identifying the specific conditions influencing changes in pest population sizes. This emphasizes the critical importance of conducting fundamental research in the agricultural sector, especially as our knowledge gaps become more apparent due to changing climatic conditions. Additionally, this lack of information contributes to the challenge of understanding the reasons behind the significant findings in this experiment.

In contrast to *B. aeneus* or *C. obstrictus*, the lifecycle of *C. sulcicollis* is entirely different. Adults of *C. sulcicollis* are active throughout the winter and aestivate from April until September (Dieckmann, 1972; Hayn,1970), unlike *B. aeneus* and *C. obstrictus*, which emerge from overwintering sites in spring to infest OSR fields (Dmoch, 1965; Free and Williams, 1978; Hussain et al., 2023; Mauchline et al., 2018). While controlling *B. aeneus* and *C. obstrictus* together due to their overlapping immigration to OSR

fields (**I**) might be feasible, controlling *C. sulcicollis* would require a different approach. Using essential oils on *C. sulcicollis* could be a viable option, considering the promising results in controlling *C. obstrictus* (**III**), which belongs to the same genus. Based on the results from study **IV**, synthetic insecticide proved effective on *C. sulcicollis*, as only one *C. sulcicollis* larvae was found in plants that were collected from treated fields. However, understanding the population status of *C. sulcicollis*, whether it requires any prevention methods or requires control, is essential before classifying it as an economically important pest of OSR.

CONCLUSIONS

This study aimed to investigate the impact of spatiotemporal separation of OSR fields on the abundance of *B. aeneus*, the damage rate caused by *C. obstrictus*, and parasitism rates of both pests (**I**). Additionally, it aimed to examine how different types of adjacent habitats surrounding the focal fields and the distance from these habitats influence the abundance of *B. aeneus* and its parasitism rates (**II**), seeking to understand the landscape management effects on both the pest and its natural enemies.

The study revealed that the abundance of *B. aeneus* and percentage of damaged pods caused by *C. obstrictus* decreased significantly when consecutive years' fields were spaced further apart than 500 m in the landscape (**I**) partially confirming the first hypothesis. Moreover, the study showed that the habitat adjacent to OSR fields did not increase the abundance of *B. aeneus* in the focal during the most susceptible stage of OSR plants development (**II**). This partially confirms the second hypothesis, indicating no significant reduction in abundance, yet no increase either. The abundance and damage rate of these two pests are more likely influenced by the landscape composition (the percentage of different habitats in the surrounding area) and the spatial separation of the fields in consecutive years, rather than solely depending on adjacent habitats.

The study also revealed that the parasitism rate of *B. aeneus* was affected by the composition of the landscape (**II**); additionally, *B. aeneus* and *C. obstrictus* were also affected by the spatial separation of consecutive years' OSR fields (**I**). Specifically, the proximity of the previous year's fields did not decrease or increase the parasitism rate, rather it was maintained at a sufficient level required for effective natural pest control (**I**). Furthermore, the proportion of seminatural habitats in the surrounding areas influenced the parasitism rate, with a low proportion leading to an almost 50% rate decrease (**II**). These findings partially reject the first hypothesis, emphasizing the need for spatiotemporal separation (**I**) and confirming the second hypothesis, emphasizing the importance of a supportive and diverse surrounding landscape – at least 18% seminatural habitat around focal fields (**II**).

Using essential oils might be a promising method for controlling *C. obstrictus*, as two out of the seven oils tested showed comparable effects to

synthetic insecticides (**III**), partially confirming the third hypothesis; this study also indicated the need for further investigation into essential oils and their biosafety towards non-target organisms, as it revealed lethal effects on both the adults and developing larvae of *N. vitripennis*, which partially rejects the third hypothesis.

Our knowledge concerning how other insects potentially damage OSR, particularly their biology and suitable conditions for successful reproduction, is limited. The high quantity of *Ceutorhynchus sulcicollis* found in study **IV**, is something that was not previously described in Estonia or neighbouring countries. The potential increase in the abundance of this species may be due to the milder climate conditions, especially winter conditions, which influence insect population sizes, confirming the fourth hypothesis. These findings are interesting, and monitoring the population size of *C. sulcicollis* is important; however, as of now, there is no reason to be concerned about *C. sulcicollis* causing significant damage to OSR plants in Estonia. To make further conclusions, a more in-depth knowledge of *C. sulcicollis* would be required, and this means more research is needed on this species.

In conclusion, this thesis underscores the importance of interplay between landscape composition and configuration and the impact of these on both pests and their parasitoids. Furthermore, it highlights the potential of essential oils to control OSR pests but indicates the need to study their biosafety as well. Lastly, it emphasizes the need to understand dynamics of insect populations and their responses to changing climatic conditions in our efforts to ensure long-lasting and sustainable food production.

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SUMMARY IN ESTONIAN

KAHJURITE JÄTKUSUUTLIKUD TÕRJESTRATEEGIAD RAPSI INTEGREERITUD TAIMEKAITSES

Ülemaailmse toidunõudluse kasvust tingituna on põllumaade majandamine muutunud intensiivsemaks. Majandamisviis on muutnud ka põllumajandusmaastikke, kus on vähenenud looduslike ja poollooduslike alade osakaal ning suurenenud põllumassiivide mõõtmed. Kõik see on kaasa toonud üldise elurikkuse vähenemise põllumajandusmaal. Looduslike ja poollooduslike alade tähtsus seisneb muu hulgas selles, et nad aitavad põldudel tagada niisuguste oluliste loodushüvede toimimist nagu looduslik kahjuritõrje ja tolmeldamine ning annavad sel viisil oma panuse, et põllumajandus oleks jätkusuutlik ja keskkonnasõbralik.

Raps on üks olulisi põllukultuure, mille saadusi kasutatakse nii tööstus-, toidu- kui ka söödasektoris. Samas on raps atraktiivne toidutaim paljudele lülijalgsetele, sh tolmeldajatele, röövtoidulistele lülijalgsetele ja kahjurputukatele. Euroopas, sh Eestis on põhilised rapsikahjurid naeri-hiilamardikas (*Brassicogethes aeneus*) ja kõdra-peitkärsakas (*Ceutorhynchus obstrictus*), kelle tõrjeks kasutatakse peamiselt sünteetilisi insektitsiide. Paraku on mõlemal kahjuril kujunenud nende suhtes resistentsus, mistõttu on oluline leida uusi tõhusaid looduslikke ja keskkonnasõbralikke tõrjemeetodeid. Järjest rohkem tuntakse huvi looduslike eeterlike õlide kasutuse vastu, mis võiksid olla alternatiiviks sünteetilistele insektitsiididele.

Kahjurite arvukuse vähendamisel etendavad tähtsat osa looduslikult esinevad röövtoidulised lülijalgsed, sh parasitoidid. Loodusliku kahjuritõrje edendamiseks ja tagamiseks tuleb rakendada integreeritud taimekaitse põhimõtteid, mille alustalaks on bioloogiline mitmekesisus. Oluline on säilitada olemasolevaid looduslikke ja poollooduslikke elupaiku, mis pakuvad kahjurite looduslikele vaenlastele talvitus-, varje-, paljunemis-, elu- ja toitumispaiku. Taimede ja elupaikade mitmekesistamine, sünteetilise põllumajanduskeemia kasutamise vähendamine, keerukamate ja sidusate maastike säilitamine ja taastamine – kõik need meetmed aitavad suurendada röövtoiduliste lülijalgsete arvukust ja liigirikkust. Tähtis on ka mõista, et muutuvates kliimatingimustes, eriti pehmemate ja lühemate talvede mõjul võib kahjurite liigiline koosseis teiseneda ja arvukus suureneda ning liigid, mis tavapäraselt kahju ei tekita, võivad muutuda

arvukaks ning tõusta kahjuristaatusse. Seetõttu on oluline jälgida putukate levikut ja arvukuse dünaamikat põllumajandusmaal.

Käesoleva doktoritöö eesmärgiks oli uurida jätkusuutlikke integreeritud taimekaitse lähenemisviise naeri-hiilamardika ja kõdra-peitkärsaka tõrjes. Esiteks keskenduti rapsipõldude aegruumilise eraldatuse uurimisele järjestikustel aastatel ning analüüsiti selle mõju kahjurite arvukusele, kahjustustele ja parasiteerituse tasemele (**I**). Teiseks uuriti tootmispõldudega külgnevate alade ning põllul ja seda ümbritsevas maastikus asuvate elupaikade mõju naeri-hiilamardikate arvukusele ja parasiteerituse tasemele (**II**). Kolmas eesmärk oli uurida eeterlike õlide mõju kõdra-peitkärsaka suremusele ja mudelorganismina kasutatud parasitoidi suremusele (**III**). Lisaks eelöeldule antakse väitekirjas ülevaade peitkärsaka *C. sulcicollis* kahjustusest rapsil ning selle võimalikest põhjustest (**IV**).

Materjal ja metoodika

Rapsipõldude aegruumilise eraldatuse ning tootmispõldudega külgnevate alade ja maastikus asuvate elupaikade mõju uurimiseks naeri-hiilamardikale (**I**, **II**) ja kõdra-peitkärsakale (**I**) ning nende parasiteerituse tasemele (**I**, **II**) viidi läbi välitööd Tartumaa tavatootmispõldudel aastatel 2014–2015 (kokku 36 põldu) (**I**) ja 2016–2018 (kokku 36 põldu) (**II**). Aastatel 2014–2015 olid katsepõllud jagatud kolme gruppi: kuus põldu piirnesid teise tootmispõlluga, kuus rohtse põlluservaga ja kuus puiskoridoriga. Peale selle kaardistati iga põllu ümber 1 km raadiuses maastik, et hinnata erinevate elupaikade mõju (**I**). Aastatel 2016–2018 olid põllud jagatud kahte gruppi: kuus põldu asusid eelmise aasta rapsipõllule lähemal kui 500 meetrit ja kuus põldu kaugemal.

Naeri-hiilamardika arvukust hinnati raputusmeetodiga. Vastsete parasiteerituse taseme väljaselgitamiseks koguti vastsed lehterpüüniste abil ja lahati laboris (**I**, **II**). Kõdra-peitkärsaka tekitatud kahjustuse ja vastsete parasiteerituse taseme hindamiseks koguti katsepõldudelt kõtru, mida hoiti laboris väljakasvatuspüünistes umbes kuu aega. Pärast seda loendati kõik väljunud vastsed ja parasitoidid ning vastsete või parasitoidide väljumisaugud (**I**). Lisaks hinnati nii kahjurite kui ka parasitoidide lennuaktiivsust, kasutades kollaseid vesipüüniseid (**I**).

Eeterlike õlide mõju kõdra-peitkärsaka suremusele hinnati laborikatses (**III**). Selleks töödeldi rapsi lehti ja õisi eeterlike õlidega 1,5% kontsent-

ratsioonis järgmistelt liikidelt: *Thymus vulgaris* (aed-liivatee), *Foeniculum vulgare* (apteegitill), *Cuminum cyminum* (harilik vürtsköömen), *Cinnamomum verum* (tseiloni kaneelipuu), *Carum carvi* (harilik köömen) ja *Cannabis sativa* (harilik kanep). Negatiivse kontrollina kasutati katses ühendit Tween 80 ja positiivse kontrollina insektitsiidi lambda-tsühalotriin. Pärast töödeldud lehtede ja õite kuivamist asetati neile kõdrapeitkärsakad ning registreeriti nende suremus ja liikumisaktiivsus. Kuna *C. cyminum* ja *C. verum* olid kõige letaalsemad 1,5% kontsentratsiooni juures, testiti neid ka 0,5%, 1% ja 2% kontsentratsiooniga.

Eeterlike õlide mõju parasitoidi *Nasonia vitripennis* suremusele hinnati samade eeterlike õlidega töödeldud filterpaberi abil. Ühtlasi hinnati eeterlike õlide mõju parasitoidide uue põlvkonna arengule. Selleks töödeldi õlidega eelnevalt parasiteeritud kärbse nukke (parasitoidi peremees) (**III**). Kuna 1,5% kontsentratsioon oli 100% letaalne parasitoididele kolme tunni möödudes, kasutati kõige väiksemat võimalikku kontsentratsiooni 0,1% ja peale selle veel 0,3% ning 0,5%. Parasiteeritud kärbse nukke töödeldi ainult 1,5% lahusega.

Tulemused ja arutelu

Kõikidel katseaastatel (2014–2018) oli naeri-hiilamardika arvukus rapsitaimede kõige haavatavamal perioodil, rohelise punga staadiumis väike ega ületanud tõrjekriteeriumi. Naeri-hiilamardika arvukus ja kõdra-peitkärsaka kahjustatud kõtrade osakaal vähenesid oluliselt, kui kahe järjestikuse aasta põllud olid üksteisest maastikul rohkem kui 500 m kaugusel (**I**). Lisaks selgus, et rapsipõldudega külgnevad alad ei suurenda naeri-hiilamardikate arvukust rapsi rohelise punga faasis (**II**). See näitab, et kahjuri arvukust või kahjustuse osakaalu mõjutab pigem rapsipõldude aegruumiline eraldatus maastikul (**I**) ning see ei sõltu ainult põlluga külgnevast alast (**II**).

Parasiteerituse tase oli kõrge kõikidel aastatel, ulatudes 90%-ni (**II**) ja ületades igal aastal efektiivse loodusliku kahjuritõrje taseme (vähemalt 32%). Töös leiti, et naeri-hiilamardika parasiteerituse taset mõjutab mitteharitavate alade hulk põllumajandusmaastikul (**II**), kusjuures rapsipõldude aegruumiline eraldatus ei mõjutanud naeri-hiilamardika ja kõdra-peitkärsaka parasiteerituse taset, mis oli alati kõrgem efektiivsest kahjuritõrje tasemest (**I**). Mitmekesine looduslike ja poollooduslike aladega maastik toetab kasulike putukate esinemist ja parasiteerituse taset ning põldude aegruumiline eraldatus ei alanda parasiteerituse taset järgmisel aastal.

Katsetulemused näitasid, et kuuest uuritud eeterlikust õlist on kõdra-peitkärsaka tõrjes kõige tõhusam kasutada *C. cyminum*'i ja *C. verum*'i õli, kuid ühtlasi leiti, et eelnevalt tuleb kontrollida nende ohutust sihtrühma mitte kuuluvatele liikidele. Eeterlikud õlid avaldasid parasitoidi valmikutele mõju juba 0,1% kontsentratsiooni juures, olles 100% letaalsed 1,5% kontsentratsiooni juures. Parasitoidi uuele populatsioonile avaldasid enim mõju *C. verum*'i ja *F. vulgare* õlid, mistõttu katses kasutatud eeterlikud õlid ei olnud ohutud sihtrühma mitte kuuluvatele liikidele (**III**).

Põllumajandusmaastikul on oluline jälgida ka herbivoorsete putukate ehk potentsiaalsete kahjurputukate levikut ja dünaamikat. Käesolevas töös leiti peitkärsaka *C. sulcicollis* kahjustatud rapsitaimi. Kahjustused olid tingitud sellele kahjurile soodsatest talvistest temperatuuridest, mis võimaldasid tal edukalt talvituda ja paljuneda, mida varem pole Eestis täheldatud. Kuigi see leid on huvitav ja harukordne, pole *C. sulcicollis* veel oluliste kahjurite staatusse jõudnud. Tema tekitatud kahjustuste avastamine rõhutab eelkõige vajadust uurida ka teisi liike, mis võivad rapsi kahjustama hakata, arvestades eriti muutuvaid kliimatingimusi (**IV**).

Kokkuvõte

Kokkuvõtteks rõhutab käesolev töö mitteharitavate alade osakaalu olulisust põllumajandusmaastikus ja tootmispõldude ajalis-ruumilise paiknemise tähtsust ning toob esile nende tegurite mõju nii kahjuritele kui ka nende parasitoididele. Samuti tõstab väitekiri esile eeterlike õlide potentsiaali alternatiivsete tõrjevahenditena kahjurite vastu, kuid osutab vajadusele uurida nende ohutust sihtrühma mitte kuuluvatele liikidele. Lõpetuseks rõhutab doktoritöö vajadust uurida ja jälgida putukapopulatsioonide dünaamikat muutuvates kliimatingimustes, mis on oluline jätkusuutliku toidutootmise tagamiseks.

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Lastly: "Give yourself some credit for how far you've come"
– author unknown.

ORIGINAL PUBLICATIONS

I **Sulg, S.**; Kovács, G.; Willow, J.; Kaasik, R.; Smagghe, G.; Lövei, L.G.; Veromann, E. 2023. Spatiotemporal distancing of crops reduces pest pressure while maintaining conservation biocontrol in oilseed rape. Pest Management Science, https://doi.org/10.1002/ps.7391

II **Vilumets, S.**; Kaasik, R.; Lof, M.; Kovács, G.; Holland, J.; Veromann, E. 2023. Landscape complexity effects on *Brassicogethes aeneus* abundance and larval parasitism rate: a two-year field study. Scientific Reports, 13, 22373, https://doi.org/10.1038/s41598-023-49690-1

III **Sulg, S.**; Kaasik, R.; Kallavus, T.; Veromann, E. 2023. Toxicity of essential oils on cabbage seedpod weevil (*Ceutorhynchus obstrictus*) and a model parasitoid (*Nasonia vitripennis*). Frontiers in Agronomy, 5: 1107201, https://doi.org/10.3389/fagro.2023.1107201

IV **Sulg, S.**; Kaasik, R.; Willow, J.; Veromann, E. 2022. Blue stem weevil (*Ceutorhynchus sulcicollis*) – a potential threat to oilseed rape production. Acta Agriculturae Scandinavica Section B – Soil & Plant Science, 72 (3): 1–3, https://doi.org/10.1080/09064710.2021.1977380

I

ORIGINAL PUBLICATIONS

I **Sulg, S.**; Kovács, G.; Willow, J.; Kaasik, R.; Smagghe, G.; Lövei, L.G.; Veromann, E. 2023. Spatiotemporal distancing of crops reduces pest pressure while maintaining conservation biocontrol in oilseed rape. Pest Management Science, https://doi.org/10.1002/ps.7391

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Spatiotemporal distancing of crops reduces pest pressure while maintaining conservation biocontrol in oilseed rape

Silva Sulg,[a*] Gabriella Kovács,[a] Jonathan Willow,[a,b] Riina Kaasik,[a] Guy Smagghe,[b] Gabor L Lövei[c,d,e] and Eve Veromann[a*]

Abstract

BACKGROUND: Agricultural landscapes provide resources for arthropod pests as well as their natural enemies. To develop integrated pest management (IPM) practices, it is important to understand how spatiotemporal location influences crop colonization and damage severity. We performed a 3-year (2016–2018) field experiment in winter oilseed rape (OSR, *Brassica napus*) fields in Estonia, where half of the fields were within 500 m of the location of the previous year's winter OSR field and half were outside this zone. We investigated how distance from the previous year's OSR crop influences the infestation and parasitism rates of two of its most important pests: the pollen beetle (*Brassicogethes aeneus*) and the cabbage seed weevil (*Ceutorhynchus obstrictus*).

RESULTS: When the distance from the previous year's OSR crop was >500 m, we recorded significantly reduced pest pressure by both *B. aeneus* and *C. obstrictus* in the study fields. Biocontrol of both pests, provided by parasitic wasps, was high in each study year and commonly not affected by distance. Mean parasitism rates of *B. aeneus* were >31%, occasionally reaching >70%; for *C. obstrictus*, mean parasitism was >46%, reaching up to 79%, thereby providing effective biocontrol for both pest species.

CONCLUSION: Spatiotemporal separation of OSR fields can reduce pest pressure without resulting in reduced parasitism of OSR pests. This supports a spatiotemporal field separation concept as an effective and sustainable technique for IPM in OSR.
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Supporting information may be found in the online version of this article.

Keywords: agricultural land management; biological control; cabbage seed weevil; integrated pest management; pollen beetle; rapeseed

1 INTRODUCTION

Agriculture is one of the most important economic sectors globally, with cultivated crops occupying around 11% of the Earth's land area.[1] Global increase in food demand led to substantial intensification of agricultural practices and associated loss of natural and seminatural habitats in agricultural landscapes, becoming an important driver of global biodiversity loss.[2,3] Agricultural intensification has also led to dependence on agrochemicals, as well as to the simplification of landscapes.[4] However, simplified landscapes with large monocultures do not sustain ecosystem service providers, resulting in reduced natural pest control, pollination, and nutrient cycling, each being vital for sustainable crop production.[5–8] Monocultures are even more widespread for crops grown for industrial use, such as oilseed rape (OSR, *Brassica napus* L.). OSR is a widely cultivated cruciferous crop, with numerous insect pests that can cause damage during different growth stages.[9] Two of the main pests of OSR in Europe are the pollen beetle *Brassicogethes aeneus* Fabricius (syn. *Meligethes aeneus* Fabricius), which causes damage when adult beetles feed on the developing flower buds, and the cabbage seed weevil *Ceutorhynchus obstrictus* Marsham (syn. *Ceutorhynchus assimilis* Paykull), whose larvae consume developing seeds within seed pods.[10]

The oil obtained from OSR seeds can be used for human consumption, but its global production has increased mainly due to its use to produce biofuel.[11,12] OSR is attacked by multiple insect

* *Correspondence to: S Sulg or E Veromann, Chair of Plant Health, Estonian University of Life Sciences, 51006 Tartu, Estonia, E-mail: silva.sulg@emu.ee (Sulg); E-mail: eve.veromann@emu.ee (Veromann)*

a *Chair of Plant Health, Estonian University of Life Sciences, Tartu, Estonia*

b *Department of Plants and Crops, Ghent University, Ghent, Belgium*

c *Department of Agroecology, Aarhus University, Slagelse, Denmark*

d *ELKH-DE Anthropocene Ecology Research Group, University of Debrecen, Debrecen, Hungary*

e *Department of Zoology & Ecology, Hungarian University of Agricultural and Life Sciences, Godollo, Hungary*

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pests that are managed by intensive use of insecticides (mainly pyrethroids), which has also triggered the widespread pyrethroid resistance in *B. aeneus*.[13–17] For *C. obstrictus*, pyrethroid resistance was reported in Poland and Germany.[16,18,19] As problems of resistance increase and research continues to document pyrethroids as unsafe for the environment,[20,21] farmers started to use alternative insecticides (e.g. neonicotinoids). Although no neonicotinoid resistance in *C. obstrictus* has yet been reported, the first indications of reduced susceptibility to thiacloprid appeared in *B. aeneus* populations.[22,23] Additionally, multiple studies have highlighted the negative effects of neonicotinoids on beneficial insects,[24–29] leading to an EU-wide ban on their outdoor use.[30] There is now a pressing need to find alternative methods of OSR pest management, raising renewed interest in nonchemical methods.

Crop rotation, a foundation of integrated pest management (IPM), can also be effective in preventing damage caused by pests overwintering in fields or field edges.[31] Crop rotation confers the direct benefit of reduced insecticide use,[32] and a temporal rotation, together with maintaining spatial separation between fields of a given crop, can maximize this benefit.[33] To be effective, rotational growing requires knowledge on the dispersal abilities of the relevant pests and beneficial insects, but our knowledge of this area is surprisingly limited. For the Colorado potato beetle (*Leptinotarsa decemlineata* Say), a distance of >400 m from the previous year's crop reduces infestation during the subsequent spring.[33] To limit the spread of the western tarnished plant bug (*Lygus hesperus* Knight) between cotton fields, a minimum distance of 1500 m from the source field is recommended.[34] Although no similar data on spatiotemporal field distancing for OSR pests exist, *B. aeneus* has a 1.2-km average dispersal range,[35] with a majority of marked beetles within 500–1000 m of the release point.[36]

While crop rotation and spatiotemporal distancing can reduce pest colonization, generalist natural enemies like ground beetles, rove beetles and spiders contribute to lower pest abundance in fields.[37] Specialist parasitoid wasps are effective natural enemies, and the parasitism rate for OSR pests can reach 90%.[38,39] However, natural enemies are not always able to keep up with the dispersal of their hosts. In some cases, parasitoid wasps arrive late to the fields and the control effect is delayed.[40] This results in pest density increase and ongoing damage to the crop, therefore large distances between OSR fields in consecutive years may create dispersal barriers, thus diminishing biocontrol services.[41]

Here we examined the effects on pest density and parasitism of spatiotemporal separation between OSR fields in a 3-year study. We aimed to determine what role distance plays in reducing infestations of two primary OSR pests and the consequences for biocontrol services provided by their specialist hymenopteran parasitoids. As there is no reliable evidence about the dispersal ability of these parasitoid wasps, a model distance of 500 m was chosen based on a previous German study on interannual changes in OSR field area and its effect on parasitism rate.[41–43] We examined (i) whether the abundance of both *B. aeneus* and *C. obstrictus* and the proportion of pods damaged by *C. obstrictus* were influenced by distance from the nearest previous year's OSR field and (ii) whether the parasitism rates of *B. aeneus* and *C. obstrictus* were influenced by distance from the nearest previous year's OSR fields.

2 MATERIALS AND METHODS

2.1 Study area and experimental design

The study was carried out in conventional winter OSR fields in Tartu County, Estonia, 2016–2018. The total study region (covering all study fields in a given year, plus an additional buffer radius of 500 m from the outermost study fields) varied between 10 567 ha in 2017 to 28 195 ha in 2018 (Table S1). The landscape in the study region was predominantly agricultural land (50–61% during the 3 years), followed by forest (26–32%), wetlands and rivers (9%), residential areas (15%), and peat bogs (18%). Agricultural land in the study region also contained other OSR fields, with total area, including all study fields, varying between 388 ha in 2018 and 1604 ha in 2016 (Table S1). Each year, 12 OSR fields were selected: six were located <500 m from the previous year's nearest OSR field and the other six were >500 m from the previous year's nearest OSR field (Table S2). To understand whether the proportion of OSR grown in the two field groups (<500 and >500 m) was similar, we measured the amount of OSR grown within a 500 m radius from sampling points in each focal field and statistically compared the group means. We concluded that the amount of OSR growing around the focal fields was similar between the two treatment groups (<500 and >500 m) in all study years (Table S3). All fields were bordered by narrow grassy strips. In 2018, data on *C. obstrictus* in one <500 m field were excluded due to significant damage caused by the cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsham). Crops were managed conventionally, with insecticides used during the green bud stage (following the decimal code by Lancashire *et al.*[44]; BBCH 50–51), or right after flowering (BBCH > 61; Tables S4–S6). ArcGIS 10.6[45] was used to analyse landscape coverage and to measure distances between study fields.

2.2 Insect sampling

2.2.1 B. aeneus *and its key parasitoids*

To estimate the abundance of overwintered adult *B. aeneus* in OSR fields, the plant tapping method was used,[46,47] which is also the main method used by farmers to assess the threshold level. The main raceme of the selected plants was tapped three times over a tray to dislodge the beetles from the buds and flowers,[47] and all *B. aeneus* that fell onto the tray were counted. In each field, 10 plants were randomly selected at each of five sampling points, 25 m apart, along a linear transect running parallel from the field edge, 12 m from the crop edge. The field edge sampled always faced the nearest previous year's OSR field. Sampling started at the beginning of the green bud stage (BBCH 50) and ended when the main raceme started to flower (BBCH 61–63). Five sampling occasions were performed in 2016, two in 2017 (in 2017, OSR plants developed fast from the bud to the flowering stage, preventing further sampling), and four in 2018. Weather data for the plant tapping experiment were obtained from the Estonian Weather Service database.[48] The mean temperature in all years varied between 12.4 and 16.3 °C. Precipitation was between 0.01 and 2.13 mm (for details see Fig. S1).

Flight activity and immigration of *B. aeneus* and their key parasitoids were monitored using yellow water traps. Each water trap consisted of a 500-mL plastic bowl painted UV-bright yellow (Sparvar Leuchtfarbe, Spray Color, Merzenich, Germany), installed at canopy height, and adjusted at each sampling occasion in accordance with crop growth. Each water trap was filled with ~300 mL water and a drop of unscented dishwashing liquid to break the surface tension. Five water traps per field, 60 in total, were installed during the OSR yellow bud stage (BBCH 57–59) 12 m from the edge of the field; all sampling points were 25 m from the adjacent sampling point(s). Traps were emptied twice a week until the end of OSR flowering (BBCH 70) by sieving the collected arthropods through a fine mesh and placing the sample into plastic bags. Samples were stored at −20 °C until identification.

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To estimate the parasitism rate, second-instar larvae dropping from plants to pupate in the soil were collected using funnel traps. Traps were 31-cm diameter funnels with plastic vials fixed to their bottom which were half-filled with water to prevent falling larvae escaping. Funnel traps were installed close to plant stems (vials were dug into the ground to stabilize the funnel). There were five funnel traps per field, i.e. a total of 60 funnel traps per study year. Traps were placed close to the yellow water traps. Traps were emptied weekly from full flowering (BBCH 65) until the beginning of the pod stage (BBCH 71–72); this covered the whole *B. aeneus* larval dropping period. The contents of the funnel traps were sieved through a fine mesh and placed into labelled plastic bags. Samples were sorted in the laboratory immediately after collection and only *B. aeneus* larvae (identification key by Osborne[49]) were subsequently stored in distilled water in Eppendorf tubes at −20 °C. All *B. aeneus* larvae were counted and dissected under a stereo microscope (Olympus, Tokyo, Japan), using green food colour dye as a contrast medium to detect parasitoid eggs and larvae. Over the entire study, 3707 *B. aeneus* larvae were dissected (805 in 2016, 870 in 2017, and 2032 in 2018). All parasitoid eggs and larvae were counted and, when possible, identified to species or genus (identification key by Osborne[50]).

2.2.2 C. obstrictus *and its key parasitoids*

C. obstrictus flight activity and immigration were monitored using yellow water traps (described in Section 2.2.1). To assess the parasitism rate of *C. obstrictus* and the crop damage caused by this pest, seed pod samples were collected at the beginning of the seed-ripening stage (BBCH 76–84). Examining seed pods allowed us to assess crop damage and parasitism rate at the same time. In each field, seed pods were collected from five sampling points, 25 m apart, along a linear transect running parallel from the field edge, 12 m from the crop edge. The field edge sampled always faced the nearest previous year's OSR field. At each sampling point, 20 seed pods per plant (10 from the main raceme and 10 from side branches) were collected from 10 randomly chosen plants. In total, 1000 seed pods per study field were collected. The sample of 20 seed pods, collected from each plant, were placed in separate plastic bags. In the laboratory, seed pods were placed in cardboard emergence boxes,[39] each box containing 20 seed pods collected from the same plant. Boxes were kept at room temperature for 1 month, after which the boxes were opened and emerged parasitoid adults and *C. obstrictus* larvae were counted. All pods were carefully examined and dissected to find *C. obstrictus* or parasitoid remains and their exit holes. Based on the number of exit holes, the damage rate of pods caused by *C. obstrictus* was calculated. The parasitism rate was calculated for each sample using the formula provided by Kovács *et al.*[51]:

$$\%\text{parasitism of } C.\ obstrictus = \frac{\sum_1^n \text{parasitoids}}{\sum_{\text{larvae}}\text{healthy} + \sum_{\text{larvae}}\text{dead} + \sum_{\text{larvae}}\text{parasitized} + \sum_{\text{parasitoid}}\text{alone} + \sum\text{holes}} \times 100$$

Parasitoid adults were stored in 70% ethanol at −20 °C and identified using a stereo microscope according to identification keys.[52–56]

2.3 Statistical analysis

Statistical analyses were performed in R v1.2.1335[57] using the R packages 'lme4',[58] 'car',[59] 'emmeans'[60] and 'MASS'.[61] For all analyses, generalized linear mixed-effects models (GLMMs) with Wald statistic Type III empirical standard error were used, including the sampling point as the random factor. The location of the field (<500 or >500 m) was used as the independent variable. The model with the best fit was chosen based on Akaike information criteria values. Gaussian distribution was used for *B. aeneus* funnel trap data as well as for *C. obstrictus* parasitism data. Negative binomial distribution was used to analyse plant tapping data, the abundance of *B. aeneus*, key parasitoids of *C. obstrictus* and *B. aeneus*, and seed pod damage caused by *C. obstrictus*. Pairwise comparison was performed via least-squares means with Tukey adjustment. Student's *t*-test was used to analyse differences between OSR proportions in the two landscape groups (>500 and <500 m).

3 RESULTS

3.1 *B. aeneus* abundance

In general, adult *B. aeneus* abundance was low. Plant tapping indicated a 3-year average of 0.29 *B. aeneus* adults per plant. Despite this low abundance, significantly more *B. aeneus* adults were found in <500 m fields than in >500 m fields in both 2016 (season mean of <500 m fields = 0.46 *B. aeneus* adults per plant; season mean of >500 m fields = 0.31 *B. aeneus* adults per plant; $\chi^2 = 15.12$, df = 1, $P < 0.001$) and 2018 (season mean of <500 m fields = 0.38 *B. aeneus* adults per plant; season mean of >500 m = 0.26 *B. aeneus* adults per plant; $\chi^2 = 16.23$, df = 1, $P < 0.001$). In 2016, at the beginning of the green bud stage (BBCH 50–51), *B. aeneus* adult abundance in <500 m fields was significantly greater than in >500 m fields ($\chi^2 = 9.67$, df = 1, $P = 0.002$; Fig. 1(A)) and remained so during the second (BBCH 51–53) ($\chi^2 = 4.95$, df = 1, $P = 0.02$) and the third sampling occasions (BBCH 55–59) ($\chi^2 = 11.27$, df = 1, $P < 0.001$) (Fig. 1(A)).

In 2017, the abundance of *B. aeneus* adults was extremely low, and no significant differences were detected between the <500 and >500 m fields (season means of 0.14 and 0.19 *B. aeneus* adults per plant, respectively; $\chi^2 = 3.45$, df = 1, $P = 0.06$). In 2017, due to particularly warm weather conditions, OSR plants developed very quickly from bud to flowering growth stage, allowing only two plant tapping surveys to be performed, making the *B. aeneus* data in 2017 unsuitable for comparison with the other years. Nonetheless, on the first sampling occasion, *B. aeneus* adult abundance in >500 m fields was significantly higher than in <500 m fields ($\chi^2 = 6.36$, df = 1, $P = 0.01$; Fig. 1(B)). No difference was observed on the second sampling date ($\chi^2 = 0.01$, df = 1, $P = 0.93$).

In 2018, in the bud stage (BBCH 50–61), the abundance of *B. aeneus* adults in <500 m fields was significantly higher in the second (BBCH 50–55) and third (BBCH 57–61) sampling occasions

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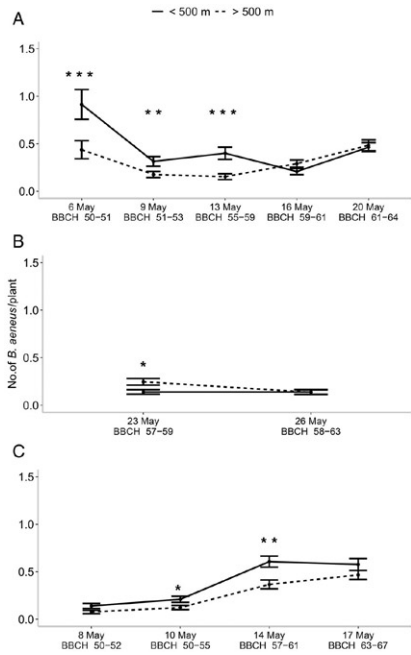

Figure 1. Mean (±SE) number of adult *Brassicogethes aeneus* per oilseed rape plant (OSR) in OSR fields closer than 500 m from the previous year's nearest oilseed rape field (<500 m) and OSR fields further than 500 m from the previous year's nearest oilseed rape field (>500 m) during and following the most damage susceptible stage of OSR growth stage BBCH 50–61 in a 3-year study: 2016 (A), 2017 (B) and 2018 (C). Asterisks (*) indicate significant differences (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) between the <500 and >500 m fields.

($\chi^2 = 4.87$, df = 1, $P = 0.03$ and $\chi^2 = 9.96$, df = 1, $P < 0.001$, respectively; Fig. 1(C)), but no significant difference in abundance between the two groups was recorded in the first and fourth sampling occasions ($\chi^2 = 3.75$, df = 1, $P = 0.06$ and $\chi^2 = 1.99$, df = 1, $P = 0.16$, respectively).

3.2 Flight activity of *B. aeneus* and its key parasitoids

The number of *B. aeneus* adults and the key larval parasitoids of *B. aeneus* that were caught in yellow water traps from inflorescence emergence until the pod ripening stage (BBCH 50–81) showed overlapping flight activity (Fig. 2(A)–(F)). Both in 2016 and 2017 the abundance of *B. aeneus* adults did not differ between <500 and >500 m fields (2016, season mean of 2.77 and 2.22 *B. aeneus* adults per trap, respectively; $\chi^2 = 3.20$, df = 1, $P = 0.07$; 2017, season means of 9.5 and 9.48 adult *B. aeneus* per trap, respectively; $\chi^2 = 0.001$, df = 1, $P = 0.98$). The main parasitoid species caught in yellow water traps were *Phradis interstitialis* Thomson, *Phradis morionellus* Holmgren, and *Tersilochus heterocerus* Thomson (Table S7). As expected, the number of *B. aeneus* parasitoids collected was relatively low compared to that of their host (Fig. 2(D)–(F)). No significant difference was detected in 2016 between <500 and >500 m fields (season means 0.49 and 0.41, respectively; $\chi^2 = 1.22$, df = 1, $P = 0.27$), whereas in 2017 there were more parasitoids in <500 than >500 m fields (season means 0.48 and 0.21 parasitoids per trap, respectively; $\chi^2 = 12.49$ df = 1, $P < 0.001$). There were significantly more *B. aeneus* adults and parasitoids in <500 than in >500 m fields in 2018 (season means 11.23 and 7.95 *B. aeneus* adults per trap, respectively; $\chi^2 = 9.89$ df = 1, $P = 0.001$; 2018 season means 0.11 and 0.05 parasitoids per trap, respectively; $\chi^2 = 4.84$ df = 1, $P = 0.02$).

3.3 *C. obstrictus* flight activity and damage

Overall, the number of *C. obstrictus* adults caught by yellow water traps was low in all study years (Fig. 3). Both in 2016 and 2018, there were significantly more *C. obstrictus* adults in <500 than >500 m fields (2016, means of 0.42 and 0.22 *C. obstrictus* adults per trap, $\chi^2 = 7.04$, df = 1, $P = 0.008$; Fig. 3(A); 2018, mean; of 2.14 and 0.69 *C. obstrictus* adults per trap, $\chi^2 = 19.28$, df = 1, $P < 0.0001$, respectively; Fig. 3(C)). In 2017, there were no significant differences in the numbers of *C. obstrictus* adults caught in yellow water traps between <500 and >500 m fields (means 1.58 and 2.35 *C. obstrictus* adults per trap, respectively; $\chi^2 = 2.36$, df = 1, $P = 0.12$; Fig. 3(B)). Yellow water trap data indicated that both *B. aeneus* (Fig. 2(A),(B)) and *C. obstrictus* occurred in the fields at the same time, *C. obstrictus* were captured in the first sampling assessment, at the early bud growth stage (BBCH ≥50, when the main inflorescence is visible but still closed; Fig. S2). The proportion of damaged pods caused by *C. obstrictus* varied between years ($\chi^2 = 29.16$, df = 2, $P < 0.001$; Fig. 4). In 2016, the mean pod damage rate was not affected by the closeness of the previous year's nearest OSR field (10.85% pod damage in <500 m fields; 10.97% pod damage in >500 m fields; $\chi^2 = 0.02$, df = 1, $P = 0.87$; Fig. 4(A)). In 2017 and 2018, a larger distance from the previous year's nearest OSR field significantly decreased seed pod damage (2017, <500 m fields, 3.28% pod damage; >500 m fields, 0.88% pod damage; $\chi^2 = 37.49$, df = 1, $P < 0.001$; Fig. 4(B); 2018, <500 m fields, 20.58% pod damage; >500 m fields, 14.18% pod damage; $\chi^2 = 27.57$, df = 1, $P < 0.001$; Fig. 4(C)).

3.4 Effect of spatial distance on parasitism rates

The mean parasitism rates of *B. aeneus* varied from 31% in 2018 to 74% in 2016, and were significantly different between years ($\chi^2 = 81.59$, df = 2, $P < 0.001$; Fig. 5(A)–(C)). In both 2016 and 2018, the mean parasitism rate of *B. aeneus* was similar between the >500 and <500 m fields (2016: $\chi^2 = 0.88$, df = 1, $P = 0.35$; 2018: $\chi^2 = 1.31$, df = 1, $P = 0.25$). Only in 2017 was the mean parasitism rate of *B. aeneus* significantly lower in >500 m fields (35.9%) compared to <500 m fields (49.5%; $\chi^2 = 5.40$, df = 1, $P = 0.02$; Fig. 5(B)). The main parasitoid species identified from larval dissections was *T. heterocerus* (Table S8).

Similar to the parasitism of *B. aeneus*, the parasitism rate of *C. obstrictus* varied between years ($\chi^2 = 28.49$, df = 2, $P < 0.001$; Fig. 5(D)–(F)) and was notably high in all years, reaching 78.8% in 2016 (Fig. 5(D)). There were no significant differences between >500 and <500 m fields (2016, $\chi^2 = 2.22$, df = 1, $P = 0.14$; 2017, $\chi^2 = 0.12$, df = 1, $P = 0.73$; 2018, $\chi^2 = 1.29$, df = 1, $P = 0.26$). The most common parasitoid species of *C. obstrictus* reared from pods were *Trichomalus perfectus* Walker, *Stenomalina gracilis* Walker and *Mesopolobus morys* Walker. The most dominant species in all study years was *T. perfectus*, while the abundance of both *S. gracilis* and *M. morys* varied between years (Table S9).

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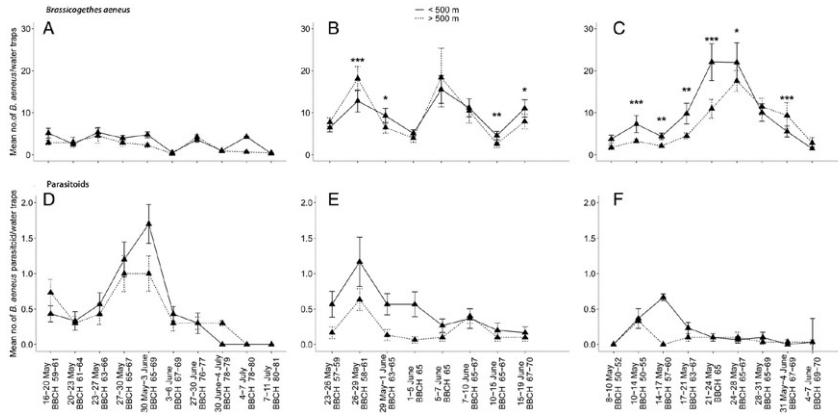

Figure 2. Mean (±SE) number of adult *Brassicogethes aeneus* (A–C) and its key parasitoids (D–F) caught by yellow water traps in oilseed rape crops closer than 500 m from previous year's nearest oilseed rape crop (<500 m) and in oilseed rape crops further than 500 m from the previous year's nearest oilseed rape crop (>500 m) on different sampling dates and crop growth stages (BBCH 50–81) in 2016 (A, D), 2017 (B, E) and 2018 (C, F). Asterisks (*) indicate significant differences (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) between <500 and >500 m fields.

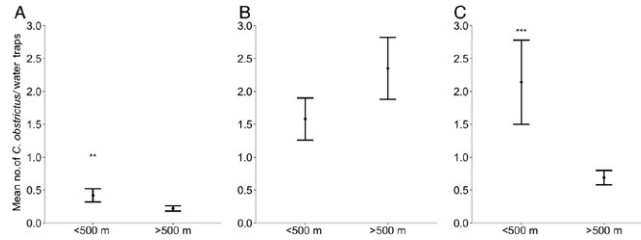

Figure 3. Mean (±SE) number of adult *Ceutorhynchus obstrictus* caught by yellow water traps in oilseed rape crops closer than 500 m from previous year's nearest oilseed rape crop (<500 m) and in oilseed rape crops further than 500 m from the previous year's nearest oilseed rape crop (>500 m) in 2016 (A), 2017 (B) and 2018 (C). Asterisks indicate significant differences (**$P < 0.01$, ***$P < 0.001$) between <500 and >500 m fields.

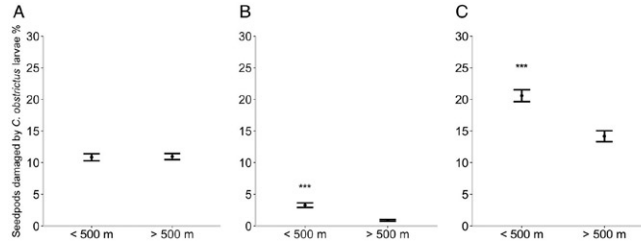

Figure 4. Mean percentage (±SE) of seedpod damage caused by *Ceutorhynchus obstrictus* in oilseed (OSR) rape fields closer than 500 m from previous year's nearest oilseed rape crop (<500 m) and in oilseed rape crops further than 500 m from the previous year's nearest oilseed rape crop (>500 m) in 2016 (A), 2017 (B) and 2018 (C). Asterisks indicate significant differences (***$P < 0.001$) between <500 and >500 m fields.

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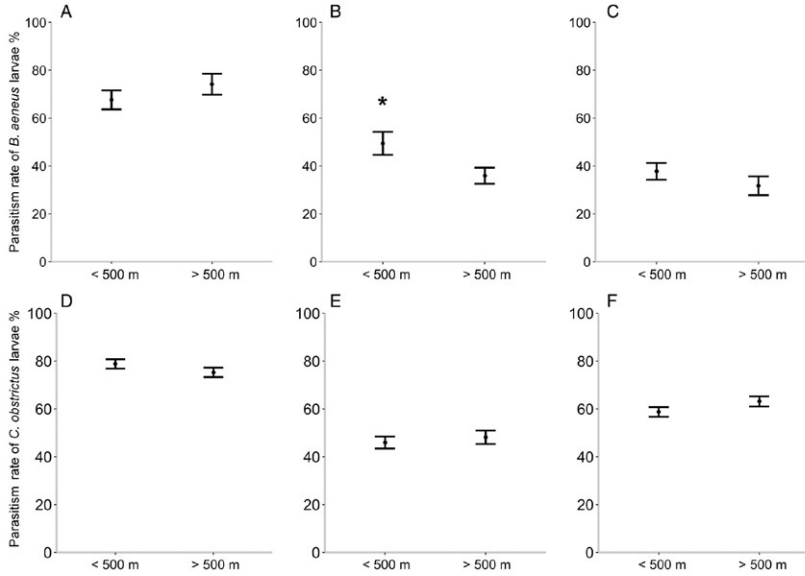

Figure 5. Mean (±SE) parasitism rate of *Brassicogethes aeneus* larvae (A–C) and *Ceutorhynchus obstrictus* (D–F) in oilseed rape fields closer than 500 m from the previous year's nearest oilseed rape field (<500 m) and in fields further than 500 m from the previous year's nearest oilseed rape field (>500 m) in 2016 (A, D), 2017 (B, E) and 2018 (C, F). Asterisks indicate significant differences (*$P < 0.05$) between <500 and >500 m fields.

4 DISCUSSION

In this study, we examined the effects of spatiotemporal separation between OSR fields on pest abundance and parasitism rate. The study demonstrated a significant negative effect of spatiotemporal distancing of OSR on pest abundance at a landscape scale, for both *B. aeneus* and *C. obstrictus*, with significantly higher *B. aeneus* adult abundance in <500 m than in >500 m fields. The number of overwintered *B. aeneus* adults at the green bud stage were also influenced by mean daily temperature (Fig. S1). Temperatures below 12 °C are not optimal for *B. aeneus*'s migratory activities[62–66] and as the temperature increased and OSR plants started to flower, the abundance of *B. aeneus* increased and distance from the previous year's nearest OSR field no longer influenced pest abundance. Volatile organic compounds emitted by plants during flowering are host-seeking cues for *B. aeneus* searching for oviposition sites.[67–70] Interplay between the rising air temperature and plant volatile cues may facilitate long-distance *B. aeneus* dispersal. However, our results indicate that 500 m represents a sufficient distance to reduce intercrop infestation of *B. aeneus* during the most susceptible OSR growth stage (BBCH 50–59). When *B. aeneus* adults emerge from their overwintering habitats (e.g. field edges, hedgerows, forest edges, meadows, grasslands,[10,71–73] but also from previous OSR fields[74]), they seek food plants in the proximity of their emergence sites.[66] The availability of overwintering habitats in the study area, as well as their distance from OSR fields, can influence the beetles' migration to the host crop,[74] but the current study did not focus on overwintering habitats and their influence on pest abundance; this should be investigated in future studies.

In 2017 and 2018, the pod damage rate caused by *C. obstrictus* was significantly greater in <500 m than in >500 m fields. This was also supported by water trap data, where in 2016 and 2018, more adults of *C. obstrictus* were caught in traps from fields that were <500 m than >500 m from the previous year's nearest OSR field. *C. obstrictus* adults prefer to overwinter at field edges,[62,75,76] and sowing an OSR crop near the previous year's crop may facilitate the pest's immigration. Our results support the effectiveness of spatiotemporal isolation to reduce the subsequent year's infestation by *C. obstrictus*. In 2018, the first adults of *C. obstrictus* were caught in the water traps during the early green bud stage (BBCH 50–52), with the highest number caught during bud development (BBCH 50–55; Fig. S2), indicating that they arrived at the inflorescence emergence stage. Williams[10] previously stated that adults of both sexes migrate to OSR crops during the flowering stage. Our study in Estonia shows that *C. obstrictus* adults migrated to OSR much earlier than previously reported and that the highest abundance was recorded before the flowering stage. Insecticide treatment targeted against *B. aeneus* was carried out only in two fields in 2016 (one <500 m and one >500 m field), but the majority of study fields were treated against *C. obstrictus* between full flowering (BBCH 65) and pod development (BBCH 70–71; Tables S4–S6). As insecticides were

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applied to both treatments (<500 and >500 m) relatively equally, we presume a similar impact on both <500 and >500 m fields. Our study was carried out in conventionally managed fields to study the spatiotemporal effect on pest and parasitoid abundances, and we did not ask farmers to change their common field management practice. Future studies should be carried out where insecticide treatment is a part of the experimental design to assess the impact of insecticide use concurrently with spatiotemporal distancing between OSR fields.

The primary parasitoids of *B. aeneus* are larval endoparasitoids that complete their metamorphosis below the soil surface of OSR fields, next to the remains of their hosts, and overwinter at the same location in the following crop.[38] Adult parasitoids emerge from the soil in spring and migrate to new OSR crops to parasitise *B. aeneus* larvae or eggs.[68,77] We observed a significantly higher parasitism rate of *B. aeneus* in <500 m than in >500 m fields in 2017. The parasitoids of *C. obstrictus* are ectoparasitoids, and adults emerge from OSR seed pods, subsequently overwintering as adults in sheltered areas.[78,79] The parasitism rate of *C. obstrictus* was very high across all years in all fields, regardless of the distance to the previous year's OSR. These results suggest that the parasitoids of *C. obstrictus* have more efficient host-seeking abilities or are stronger fliers than parasitoids of *B. aeneus*. However, adults of *C. obstrictus* and *B. aeneus* are attracted to various cruciferous plants, therefore their parasitoids can also parasitise their host developing on other cruciferous plant species.[51,80–83] Consequently, parasitoids could have reached the OSR field from other habitats containing cruciferous plants (e.g. natural or seminatural habitats) and not necessarily from the previous year's OSR crop. This may be an additional reason why pest abundance, as well as seed pod damage rate, were lower in >500 m fields. Furthermore, parasitoids of *C. obstrictus* can parasitise other *Ceutorhynchus* species,[53,84] so it is also possible that the key parasitoids of *C. obstrictus* have emerged from alternative hosts. To establish whether the presence of the key parasitoids of both *C. obstrictus* and *B. aeneus* parasitoids in the studied fields is a consequence of their general presence in the landscape, or because of their superior dispersal capacity, further studies are needed on possible alternative hosts, parasitoid dispersal power, and the spatiotemporal distancing effect. Distance from seminatural habitats and other OSR fields, and the proportion of each in the landscape, can have an effect on parasitoid community composition.[42,85,86] Practices for enhancing populations of different parasitoid species may include reducing disturbances in agricultural fields,[87] and providing suitable feeding and overwintering sites in the surrounding natural and seminatural habitats, where numerous natural enemies occur.[88,89]

An increasing proportion of monocultural land and the decline in agro-biodiversity[90–95] may lead to increased crop damage in many agroecosystems. Agricultural landscape management needs to combine multiple ecologically sustainable techniques to maintain biocontrol services while simultaneously minimizing crop damage. Habitat heterogeneity in agricultural landscapes can benefit biocontrol.[7,8,42,96] Spatiotemporal crop distancing should be based on pest phenology, life history, and dispersal abilities, and when needed spatiotemporal crop distancing should be modified accordingly.

Based on our findings, the damage caused by *B. aeneus* and *C. obstrictus* could potentially be reduced by appropriate spatial distancing between the previous and the subsequent year's OSR fields. The efficacy of this management practice may increase if practised over a landscape scale, which would support the view that landowners and agricultural practitioners need to cooperate.[96] Furthermore, maintaining a sufficient spatiotemporal aspect to crop production on a landscape scale could potentially reduce pest pressure without affecting biocontrol service provision, thereby reducing the need for applying insecticides.

5 CONCLUSION

Managing agricultural pests is becoming more challenging because conventional agricultural management practices are not ecologically sustainable. This study was conducted to determine whether between-year spatial distancing between OSR crops could potentially reduce pest pressure by two of its main pests, *B. aeneus* and *C. obstrictus*, and whether this influences the biocontrol services provided by their specialist parasitoids. Our results demonstrate the ability to maintain biocontrol service provision when implementing spatial distancing between crop locations in consecutive years, and this can help to achieve sustainable crop production. We suggest that similar spatiotemporal distancing of crops, especially when implemented on a landscape scale, can work at the core of an IPM framework and can be applied to a broad variety of crop–pest systems. This practice would require planning and cooperation between landowners and agricultural practitioners, and could result in reducing the need for agrochemical inputs.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

EV, GK, and RK designed the study. SS, GK, RK, JW, and EV participated in the fieldwork and data collection. SS, GK, EV, and JW identified the pest species and parasitoid wasps. SS wrote the original draft of the manuscript and analysed the data. SS and GL visualized the data. RK analysed the landscape data. All authors contributed to the writing and revision, and approved the manuscript. Authorship is by the first-and-last principle.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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II

II **Vilumets, S.**; Kaasik, R.; Lof, M.; Kovács, G.; Holland, J.; Veromann, E. 2023. Landscape complexity effects on *Brassicogethes aeneus* abundance and larval parasitism rate: a two-year field study. Scientific Reports, 13, 22373, https://doi.org/10.1038/s41598-023-49690-1

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Landscape complexity effects on *Brassicogethes aeneus* abundance and larval parasitism rate: a two-year field study

Silva Vilumets[1]✉, Riina Kaasik[1], Marjolein Lof[2], Gabriella Kovács[1], John Holland[3] & Eve Veromann[1]

Global biodiversity has suffered a decline primarily attributed to landscape simplification and intensified agricultural practices. Agricultural environments, characterized by homogeneity and frequent disturbances, are often suboptimal habitats for various insect species. While agricultural fields do favour pests, they generally fail to provide suitable habitats for natural enemies. The inclusion of diverse supporting habitats, such as semi-natural habitats, grassy and woody field margins etc. surrounding agricultural fields, play a crucial role in fostering effective biodiversity conservation. Moreover, determining the influence of different adjacent habitat types is essential in elucidating their influence on pest abundance and parasitism rates. Our two-year field study focused on assessing the abundance of *Brassicogethes aeneus* and its parasitism rate. The findings revealed that the adjacent habitat type did not significantly increase pest abundance and the parasitism rate of *B. aeneus* larvae consistently stayed over the threshold for effective biological control throughout the fields. This was attributed to the high proportion (35 and 38% in the 2 study years) of semi-natural habitats within most of the 1 km radius study areas. While our study did not identify any specific adjacent habitat type or habitat within a 1 km radius that directly impacted *B. aeneus* abundance, it emphasises the intricate interplay between the pests, parasitism and the surrounding environment because the interactive effect of distance from the crop edge and habitat type had a significant influence on *B. aeneus* infestation levels but not on parasitism. Decision tree analysis suggests that > 18% semi-natural habitat is needed to ensure sufficient levels of parasitism for effective biological control. A comprehensive understanding of habitats that influence not only *B. aeneus* but also other pests is critical for the successful implementation of IPM strategies and conservation initiatives within the agricultural sector.

The global biodiversity crisis has worsened in the last decades, mostly due to landscape simplification and the intensification of the agricultural sector[1–3]. To compensate for the biodiversity decline surrounding agricultural fields, targeted agri-environment schemes have been implemented, supporting farmers in adopting organic farming practices, by adding more flowering strips and grassy field margins to the agricultural landscape[4,5]. While implementing agri-environmental schemes on farmland offers advantages, it is crucial to retain semi-natural habitats (SNH) around the agricultural fields for effective biodiversity conservation. SNHs provide necessities, like food resources, shelter, and hibernation habitats, for various insect taxa[6], including pollinators and predatory arthropods. Pollinators and predatory arthropods play vital roles in agricultural production by increasing yield through pollination and reducing pest density through biocontrol[7–11].

Oilseed rape (*Brassica napus* L.) (OSR) is an economically important crop around the world, mainly used to produce vegetable oil and biofuel feedstock, grown in large monocultural fields, and attracts various herbivorous insects specialised on cruciferous plants. One such insect that causes damage to OSR plants is the pollen beetle (*Brassicogethes aeneus* Fabricius syn. *Meligethes aeneus* Fabricius), damaging both winter (WOSR) and spring oilseed rape plants at the green bud stage that may cause yield reduction. *Brassicogethes aeneus* overwinter in woody and sheltered habitats and in spring, they seek food sources and oviposition sites[12]. They oviposit into

[1]Plant Health Chair, Estonian University of Life Science, Kreutzwaldi 1, 51006 Tartu, Estonia. [2]Environmental Systems Analysis Group, Wageningen University and Research, 6708PB Wageningen, the Netherlands. [3]Game and Wildlife Conservation Trust, Fordingbridge, Hampshire SP6 1EF, UK. ✉email: silva.vilumets@emu.ee

the buds, and while first instar larvae develop inside the buds, the second instar larvae exclusively feed on pollen from open flowers[13]. However, the main damage is done by adults feeding on the buds, leading to bud abortion and eventual cessation of seed development[14], resulting up to 80% loss in yield on spring oilseed rape[15]. The control of *B. aeneus* in conventional OSR fields relies on synthetic insecticides, which not only reduces the abundance of the target pest but also affects the natural pest control providers and pollinators[16–19]. In line with the European Union's Farm to Fork Strategy, there is a target to reduce overall pesticide use by 50% by 2030[20]. This urges farmers to adopt alternative pest control methods and rely on the predatory arthropods and parasitoid wasps. Parasitism by parasitoids play a significant role in natural pest control. *Brassicogethes aeneus* is attacked by at least nine species of hymenopteran parasitoids, with three key species: *Tersilochus heterocerus* (Thomson), *Phradis interstitialis* (Thomson) and *Phradis morionellus* (Holmgren)[21]. These three parasitoid species are univoltine and koinobiont endoparasitoids. They overwinter in pupal cocoons in the soil, close to their hosts, and emerge in spring to migrate to the OSR fields[22]. It is known that these parasitoids differ in their host-finding behaviours and attack their hosts at different growth stages, suggesting niche separation among them[23]. Despite the host-finding behaviours, studies have shown that the parasitism rates of *B. aeneus* can vary widely from 0 to 63%[24–26], and can depend on the distance from adjacent habitat[27] and farmland management methods[28–30]. Landscape structure and composition significantly affect both *B. aeneus* and its parasitoids. One alternative pest control method is conservation biological control. This aims to reintroduce beneficial insects into crop systems to improve natural pest management. Previous studies have highlighted the links between conserving natural habitats and reducing pest pressure in farmland[31,32]. Whereas homogeneity in the landscape enhances the presence of *B. aeneus* in OSR field, more heterogenic landscape enhances species richness, abundance and fecundity of parasitoid wasps[7,32,33]. Additionally, complex landscapes, as opposed to simple ones, provide a higher parasitism rate of *B. aeneus*[7,34]. *Brassicogethes aeneus* abundance is influenced by changes in total OSR area size and the distance from the previous year's OSR fields, which can effectively lower *B. aeneus* abundance while maintaining sufficient parasitism rates[26]. In this two-year study, we examined the landscape effects on *B. aeneus* abundance and parasitism of *B. aeneus* larvae in OSR fields. Our objectives were to determine the influence of adjacent habitat type and the in-field distance from the adjacent type on the abundance and parasitism rate of *B. aeneus*. Additionally, we sought to examine how the surrounding habitat types within a 1 km radius affect both *B. aeneus* abundance and the parasitism rate of *B. aeneus* larvae.

Results

Landscape characteristics

In 2014 and 2015, across the studied landscape circles the average coverage of arable land was 53.8% and 58%, respectively, with a minimum coverage of 20.2% and a maximum of 82.7%. The average SNH proportion across landscape circle coverage was – 37.7% in 2014 and 34.7% in 2015, the minimum cover of SNH was 11.3% (in 2015) and maximum of 52% (in 2015), of which woody areal elements typically occupied the largest area. The average coverage of OSR fields was 14.7% in 2014 and 15.7% in 2015. The mean proportion of SNH in landscape circles of focal fields with different adjacent elements was quite similar in both study years—herbaceous linear element [containing narrow (1.5–12 m width) grassy crop margin] 33.8%, woody linear element (containing hedge or line of trees) 40.9%, and another crop field (either bordering with OSR, barley, wheat, potato, oat, or pea) 38.3% in 2014 and 33.8%, 36.4% and 34.2% respectively in 2015. Also, the mean proportion of arable land in landscape circles was relatively similar in both years. In 2014, arable land constituted 54.0% on landscape circles where focal field was bordered by herbaceous linear element, 51,6% on landscape circles where focal field was bordered by woody linear elements and 55.9% on landscape circles where focal field was bordered by another crop field In 2015, the proportions changed slightly – 59.3%, 54.6% and 60.1%, respectively. The full list of landscape characteristics is presented in the supplementary materials (Table S1–S2).

Effects of adjacent habitats and distance from the edge of the field on B. aeneus abundance

Generally, throughout the study period, the mean number of *B. aeneus* per WOSR plant was low and stayed firmly under the economic threshold level (in Estonia 1–2 beetles per plant at BBCH 50–51 and 3–5 at BBCH 55–59[35]). The mean number of *B. aeneus* adults was high and surpassed the economic threshold level (mean 5.72 ± SE 1.29) in only one of the fields, during the last sampling round (BBCH 55–59) in 2015. There were three sampling rounds in 2014, during which a total of 2160 WOSR plants were tapped, and four sampling rounds in 2015, during which a total of 2880 WOSR plants were tapped. Observing the yearly means of *B. aeneus* per study field, shows that only one field in 2015 was close to the economic threshold, when the mean number of beetles per plant was 1.86 (SE ± 0.37) (Table S3). In 2014, the mean number of *B. aeneus* per plant was highest in fields adjacent to woody linear (0.32 ± SE 0.79), followed by another crop (0.26 ± SE 0.56) and herbaceous linear habitat (0.19 ± SE 0.39) (Figure 1). Whereas in 2015, the greatest mean number per plant was found in fields adjacent to herbaceous linear (0.59 ± SE 0.84), followed by another crop (0.56 ± SE1.54) and woody linear (0.34 ± SE 0.41) (Figure 1). The combined effect of distance and adjacent habitat type (2014: χ^2= 65.35, df= 6, $p < 0.0001$; 2015: χ^2= 43.76, df= 6, $p < 0.0001$) had a significant effect on the estimated mean abundance of *B. aeneus* adults in both study years. Generally, the estimated mean value of *B. aeneus* per ten plants was higher at 2 m from the adjacent habitat and the abundance declined with distance from the boundaries (Table 1).

Effects of habitats surrounding the focal field on B. aeneus abundance

We analysed the correlations between *B. aeneus* abundance in focal fields and various landscape characteristics within 1 km radius from the focal field in pooled data of 2014–2015. In our study, we did not find any significant correlations between them. Furthermore, we also carried out random forest tree analyses but none of the forests could describe representative proportion of the samples. The best model described only 16% from the samples

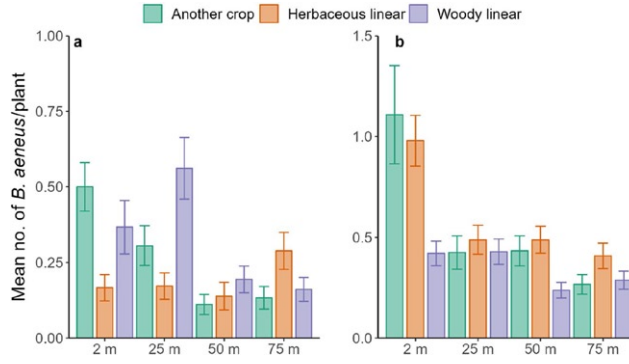

Figure 1. Mean (± SE) number of *Brassicogethes aeneus* adults per plant during the bud stage of WOSR (BBCH 50–59) collected from WOSR fields with different adjacent habitat types and from different distances from the edge of fields in 2014 (**a**; N = 216) and 2015 (**b**; N = 288). The probabilities from the variance analysis: 2014: $P_{distance} < 0.0001$, $P_{adjacent\ habitat} = 0.28$, $P_{distance*adjacent\ habitat} < 0.0001$; and 2015: $P_{distance} < 0.0001 P_{adjacent\ habitat} = 0.09$, $P_{distance*adjacent\ habitat} < 0.0001$.

	Another crop				Herbaceous linear			Woody linear		
Year	Contrast	df	t	*P*	df	t	*P*	df	t	*P*
2014	2 m versus 25 m	201	2.877	**0.0219**	201	− 0.128	0.9992	201	− 2.688	**0.0376**
	2 m versus 50 m	201	6.084	**< .0001**	201	0.673	0.9064	201	3.034	**0.0138**
	2 m versus 75 m	201	5.753	**< .0001**	201	− 2.407	0.0784	201	3.691	**0.0015**
	25 m versus 50 m	201	3.874	**0.0007**	201	0.800	0.8532	201	5.403	**< .0001**
	25 m versus 75 m	201	3.390	**0.0044**	201	− 2.288	0.1034	201	5.923	**< .0001**
	50 m versus 75 m	201	− 0.602	0.9288	201	− 3.017	**0.0153**	201	0.749	0.8742
2015	2 m versus 25 m	273	8.230	**< .0001**	273	6.164	**< .0001**	273	− 0.140	0.9990
	2 m versus 50 m	273	8.120	**< .0001**	273	6.164	**< .0001**	273	3.453	**0.0034**
	2 m versus 75 m	273	10.232	**< .0001**	273	7.273	**< .0001**	273	2.440	0.0714
	25 m versus 50 m	273	− 0.139	0.9990	273	0.000	1.0000	273	3.584	**0.0025**
	25 m versus 75 m	273	2.923	**0.0189**	273	1.294	0.5644	273	2.575	0.0506
	50 m versus 75 m	273	3.056	**0.0129**	273	1.294	0.5644	273	− 1.067	0.7082

Table 1. Differences in the estimated mean number of *Brassicogethes aeneus* adults per plant during the bud stage of WOSR (BBCH 50–59) collected from WOSR fields with different adjacent habitat types and from different distances from the edge of fields in 2014 and 2015. *df* Degrees of freedom, *t* t statistic, *P P* value. *P* values in bold show statistically significant differences ($p < 0.05$).

that is not enough to extrapolate these results to the whole population and to draw fundamental conclusions. However, only for 2014 a positive correlation between herbaceous linear elements and the abundance of *B. aeneus* was found (Pearson correlation r = 0.68; $p = 0.002$; Table S4). In 2014 the abundance of *B. aeneus* increased significantly with increasing proportion of herbaceous linear habitats within the 1 km radius of focal field however, in the next year, 2015, the correlation between herbaceous linear habitats and *B. aeneus* abundance was opposite, meaning the abundance of *B. aeneus* decreased with increasing proportions of herbaceous linear habitats but relationship was not statistically significant. Therefore, we cannot conclude that herbaceous linear habitats may increase *B. aeneus* abundance as our results were inconsistent. Other habitats within the 1 km radius of focal field did not have significant correlations with *B. aeneus* abundance, in both 2014 and 2015 (Tables S4–S5).

Effects of adjacent habitats and distance from the edge of the field on parasitism rate

The parasitism rate of *B. aeneus* larvae in both study years was very high and even reached up to 100% at field level on three fields adjacent to herbaceous linear and woody linear habitats, while in two focal fields adjacent to herbaceous linear habitat no parasitism was observed (Table S3). The parasitism rate in both study years was unaffected by adjacent habitat, distance from the adjacent habitat nor the combined effect of them (all *p* values > 0.05)

(Figure 2). The mean parasitism rate in 2014 and 2015 was high with 66.24% (SE ± 2.71) and 71.85% (SE ± 3.87), respectively. The mean parasitism rate was high across all fields regardless of the adjacent habitat, both in 2014 and 2015. The highest parasitism rate was observed in focal fields adjacent to another crop 73.04% (SE ± 4.73) in 2014 and in 2015 in focal field adjacent to woody linear habitat 81.02% (SE ± 7.89).

Effects of landscape characteristics on B. aeneus parasitism rate

The two years parasitism rate of *B. aeneus* was very high and reached up to 69%. The random forest analysis showed an overall R-squared value of 0.45 suggests that approximately 45.3% of the variance in the *B. aeneus* parasitism percentage can be explained by the predictor variables selected by the model. A decision tree (Figure 3) revealed that the parasitism rate of *B. aeneus* varied based on the landscape characteristics surrounding the focal field. When the semi-natural habitat proportion (SNH) around the focal field was below 18%, the parasitism rate was only 24%. When the SNH proportion around the focal field was over 18%, the predicted parasitism rate was 70%. It is important to highlight that 97% of data connected with parasitism rate were sampled from landscape circles where SNH proportion was greater than 18% and only 3% of data was collected from landscape circles with SNH percentage below 18%. In addition, almost 30% of parasitism rate data was collected from areas where SNH proportion was greater than 43%. Thus, our data reflect an agricultural landscape, that is heterogeneous due to its very high SNH coverage, which is important for conservation biological control. The decision tree also revealed that if the proportion of SNHs exceeded 43% and the proportion of herbaceous area was over 3.9% in the surrounding landscape then the median of parasitism rate of *B. aeneus* was 87%. When proportion of SNHs was lower than 43% then the proportion of arable land influenced the parasitism rate—resulting in higher parasitism rate (72%), if the proportion of arable land was more than 54%, whereas if the proportion of arable land was smaller than 54%, the parasitism rate was 50%.

Discussion

The field study conducted over two consecutive years showed very low abundance of *B. aeneus* during the damage susceptible growth stage of WOSR and even though no relation between the bordering habitat and their abundance was found. The mean larval parasitism rate of *B. aeneus* was extremely high with 69% and occasionally reaching up to 100%, indicating an effective natural pest control in the landscape. This is confirmed by the decision tree that showed that landscapes with more than 18% SNH coverage have a high (70%) predicted parasitism rate.

The study showed that the abundance of *B. aeneus* during WOSR bud stage, that is the growth stage most susceptible to damage by *B. aeneus*, was very low in both years and the bordering habitat had no significant impact on their abundance. Also, it is important to highlight that in our study area the agricultural landscape surrounding study fields had very high proportion of SNHs within 1 km radius around the focal fields (Table S3). Therefore, our study shows that in diverse agricultural landscapes comprised of woody and herbaceous linear elements there is a lower risk of *B. aeneus* infestations in WOSR. This was despite the presence of a multitude of overwintering sites near the focal fields. Thus, the number of *B. aeneus* was low and did not exceed the economic threshold during bud stage of plants. Studies on the *B. aeneus* density, plant damage, and local or regional landscape effects were shown to be inconsistent. Several studies suggest that complex landscapes may reduce

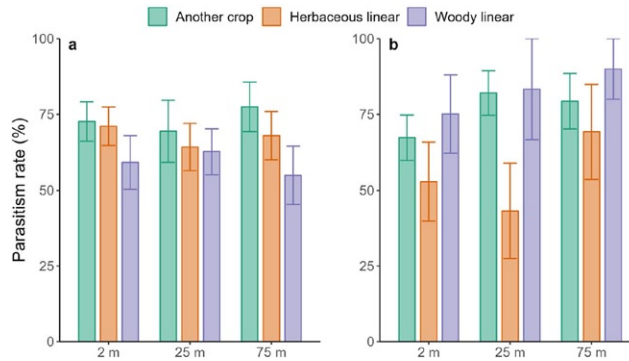

Figure 2. Mean (± SE) parasitism rate of *Brassicogethes aeneus* on WOSR field, depending on the distance from the field edge and the adjacent habitat type in 2014 (**a**; N = 162) and in 2015 (**b**; N = 162). The probabilities from the variance analysis: 2014: $P_{distance} = 0.917$ $P_{adjacent\ habitat} = 0.467$, $P_{distance*adjacent\ habitat} = 0.128$; and 2015: $P_{distance} = 0.407$ $P_{adjacent\ habitat} = 0.583$, $P_{distance*adjacent\ habitat} = 0.108$.

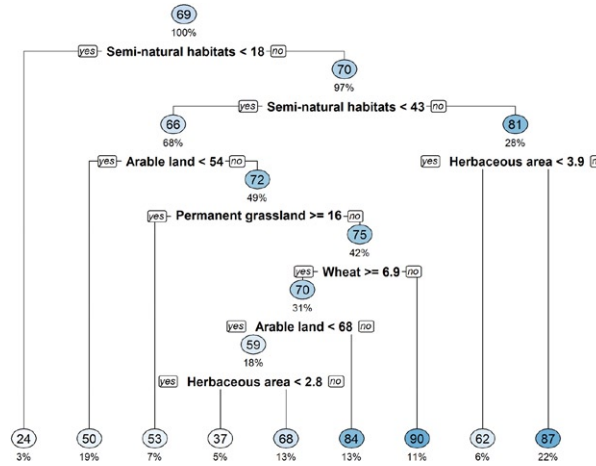

Figure 3. Decision tree based on the 2014 and 2015 landscape characteristics surrounding the focal fields to determine the median parasitism rate of *Brassicogethes aeneus* (pooled data 2014 and 2015). Each terminal node (inside the circle) gives a predicted median parasitism rate of *B. aeneus* and under the node the value [percentage (%)] represents the proportion of data used to predict the parasitism rate. The values behind the landscape variable represent the percentage, which was used for the division setpoint.

pest density[34,36–41], although there are also evidence of increasing pest pressure[42,43]. The proportion of woodland in the landscape is usually associated with an increase of *B. aeneus* abundance[43–45], whereas for grasslands there were contrasting results, indicating both increases and decreases in abundance[34,42]. As woody areas provide a suitable overwintering habitat for *B. aeneus*, this can increase pest density in the spring[46], however, regardless of presence of quite large woodland areas in the study sites, the results of our study did not confirm this finding as we did not find any significant correlations between beetles abundance and woodland proportions. Pest density and its relationships with the landscape configurations have complicated and multilevel ties. There are many direct and indirect factors at large and small scale that influence pest abundance such as crop rotations, pesticide usage, tillage regimes, abundance, and species richness of naturally occurring enemies of pests, pest pathogens, amount, quality and connectivity of landscape elements, soil health, weather conditions etc. In our study, the adjacent habitat of WOSR did not influence *B. aeneus* abundance, but the distance from the adjacent habitat and the combined effect of distance and adjacent habitat type had a significant impact on the estimated mean abundance of *B. aeneus* throughout the study years. The abundance of *B. aeneus* during inflorescence emergence stage (BBCH 50–59) was greater at 2 m and 25 m from the edge of the WOSR and decreased at 50 m and 75 m from edge. This suggests that local adjacent habitats were the source of infestation. It was not possible to identify which habitat type led to greater infestations because results were inconsistent between years. Other studies also showed that *B. aeneus* tends to aggregate on the field edge during bud stage of plants and expand more into the field once the plants start to flower[47–49]. This shows that there are opportunities to use Integrated Pest Management (IPM) tools such as a precision agriculture techniques and/or trap cropping to manage *B. aeneus* abundance and use insecticide treatments only along WOSR headlands to reduce the agrochemical inputs and preserve natural enemies[24,25,48,50–52]. However, aggregation at the edges of fields is not common for all insect pests of oilseed rape, for instance cabbage seedpod weevil (*Ceutorhynchys obstrictus* Marsham), does not have similar in-field immigration pattern and is evenly distributed at different distances from the edge[53], also *Psylliodes chrysocephala* (L.) is non-uniformly distributed within the crop and does not aggregate along field edges[54]. This may because of differences in overwintering sites and/or dispersal abilities.

In both years, the parasitism rate of *B. aeneus* larvae was extremely high with average rate of 69%, which is close to previous finding in Estonia[26] and over the minimum effective biological control level (at least 32%)[55]. In our study, we found that the parasitism rate was not influenced by the adjacent habitat or by distance from the field edge. Interestingly, the mean parasitism rate was consistently high across almost all focal fields regardless of the adjacent habitat type, indicating a strong and efficient level of natural pest control against *B. aeneus* in both years. Our findings are comparable to Kovács et al.,[53], where they also showed a similar effect on *C. obstrictus*; the adjacent habitat type did not affect the parasitism rate which was likewise outstandingly high. In our study, in 33 of 36 fields the parasitism rate was above the threshold value of effective biological control (32–36%)[55], even reaching up to 100% in three fields. Thus, the population size of beetles was efficiently controlled by

hymenopteran parasitoids. Unfortunately, it was not possible to identify the impact of adjacent habitats on levels of parasitism in focal fields. The high proportion of different semi-natural and other non-cropped habitats can potentially provide suitable habitats for parasitoids and created an abundance of parasitoids at a landscape scale, thereby masking any field-scale effects. Landscape complexity and the amount of SNHs in agricultural landscape have previously shown to have a positive impact on pollen beetle (*Brassicogethes* ssp) parasitism[34,37,41,56,57]. Semi-natural habitats around and nearby arable fields provide alternative host plants[32,58–60], and as SNHs tend to have lower disturbance, parasitoids can sustain a proficient population at landscape level and therefore provide the pest control service to agricultural fields. Furthermore, the study by Le Clec'h et al.[61] indicated a dilution of the additional effect of linear elements on ecosystem services supply when linear elements were implemented in a landscape where the supply of ecosystem services was already high, compared to a landscape where the initial supply was low. The decision tree created in our study, based on the landscape and parasitism data, revealed that if the SNH percentage was below 18% in the 1 km radius landscape circle the parasitism rate was low. Thies and Tscharntke[60] showed that the SNH coverage below 20% led to the decrease of *B. aeneus* parasitism below the threshold necessary for effective biological control. The decision tree based on our data confirms their results—our model predicted that if the percentage of SNHs was below 18% then the predicted parasitism rate was only 24% and if it was over 18% then predicted parasitism rate was 37–90%. Thus, our results show that having a complex landscape, it is possible to provide a consistent parasitism rate over the two years. Furthermore, two main parasitoid species of *B. aeneus*, *Phradis morionellus* and *P. interstitialis* are oppositely affected by the woodland coverage around OSR fields[62] and may therefore contribute differently on the overall parasitism of *B. aeneus* based on woody habitat abundance and location. To ensure functioning ecosystem, pollination, biological pest control service at least 10–20% SNH is recommended per km^2, although our results suggest that 20% would be the minimum level in 1 km radius of a focal field[63]. Our results also support the previous findings showing that the landscape with a low proportion of oilseed rape had a negative effect on the parasitism rate[37]. In the same study region, Kovács et al.,[53], stated that the landscape in the study area is still sufficiently diverse and complex, providing different habitats for both the pest and the natural enemies. Our results classified by the decision tree highlight the importance of landscape characteristics, particularly the presence of SNH as these types of areas are the necessary for conservation biocontrol.

In conclusion, our study highlights the complicated relationship between pest, parasitoids and landscape and highlights that a specific adjacent habitat type or the habitat within the 1 km radius did not increase *B. aeneus* abundance. Understanding the habitats and factors that influence not only *B. aeneus* but also other pests, is vital for implementing successful integrated pest management strategies and conservation efforts in agricultural landscapes. Future study should investigate the processes that drive parasitism rates and evaluate other factors that may contribute to the observed trends. Such knowledge will be useful in designing efficient strategies to improve natural enemy populations and facilitate biological control of *B. aeneus* in agricultural landscapes.

Methods

The study was carried out in 18 non-overlapping landscape circles with a radius of 1 km around the focal fields in Tartu County, Estonia in 2014 and 2015. General study site selection, landscape circle radius of 1 km, classification of SNH-s and experimental design were based on the standardized protocols of the EU FP7 project QuESSA across participating regions and crops and are previously described in detail:[53,64–67]. For the project as well as our study, the 1 km radius was chosen, as it aligns with the recommended spatial scale for the dispersal of oilseed rape parasitoids in landscape sectors[41,62]. In both study years, 18 WOSR focal fields were selected; six bordered with woody linear, six fields with herbaceous linear and six with another crop. The minimum size of the focal field was 3 ha, the width for both woody linear and herbaceous linear was between 1.5 and 25 m and at least 50 m long. In 2014, the bordering crop was spring oilseed rape (1 field), barley (2 fields), wheat (2 fields), potato (1 field), and in 2015 we had barley (1 field), wheat (2 fields), oat (1 field), pea (2 fields). The landscape surrounding the focal field within 1 km radius for both years (2014 and 2015), was mapped separately with ArcMap 10.1 (ESRI 2012) using data from the Estonian Land Board and the Agricultural Registers and Information Board with any remaining gaps filled during field visits. The minimum distance between fields was larger than 1 km, ensuring that the landscape in the 1 km radius around the focal field did not overlap. Based on the dominating vegetation, semi-natural habitats (SNH) were classified as: herbaceous areal (semi-) natural hayfields or abandoned fields that have not developed > 30% shrub/tree canopy cover), woody areal (woodland), cover crop, herbaceous linear (narrow grassy crop margin) and woody linear (hedge, line of trees), their area and proportion within 1 km radii of the focal field was calculated using ArcMap 10.1. The focal fields in this study were managed conventionally. Each sampling transect was set at 90° from the focal field bordering element progressing into the crop. Within each field they were located at least 25 m away from other field edges, that were not the focal adjacent habitat type.

B. aeneus abundance assessment

The abundance of overwintered adult *B. aeneus* in WOSR fields was assessed using the plant tapping method[68–70]. The abundance of *B. aeneus* adults was the assessed along the sampling transect at four distances: 2, 25, 50, and 75 m from the edge of the crop. At each sampling point ten random WOSR plants were selected, the plants' main raceme was tapped over a tray to dislodge *B. aeneus* from the buds and flowers. Dislodged *B. aeneus* which fell onto the tray were counted. In 2014, sampling started at the bud stage of plants (growth stage BBCH 52–53; following the decimal code by Lancashire et al.[71]), and ended when the plant growth stage was BBCH 57–60 (end of bud stage and beginning of flowering stage). In total, three sampling occasions were performed in 2014. In 2015, there were four sampling occasions, sampling started when the plant growth stage BBCH 50–51(bud stage of plants) and sampling ended when the plant growth stage was BBCH 60–61 (start of flowering stage).

Collection of the B. aeneus larvae and parasitism rate

The parasitism rate of *B. aeneus* larvae was assessed using the funnel trap method[27]. Funnel traps were placed along the sampling transect at three distances: 2 m, 25 m, and 75 m from the edge of the field. The trap consisted of a plastic funnel (diameter 31 cm) and a plastic container (50 ml) attached to the funnel end. The upper part of the funnel trap remained above the ground below the side shoots while the lower part with collection cup was dug into the ground. The trap was placed close to the WOSR plants at the end flowering stage of plants (BBCH 67) and were kept in the field until the end of flowering (BBCH 70). In each year a total of 162 samples were collected. The larvae of the last growth stage were collected as they were ready to leave from flowers and drop to the ground to pupae in soil. The area of which the larvae dropping from the plants were collected was equal to the diameter of the funnel (754.4 cm^2). Funnel traps were emptied once a week for a period of three weeks. The content of the funnel traps was strained through a fine mesh fabric and placed into labelled plastic bags. In the laboratory, samples were sorted, all *B. aeneus* larvae were counted, placed in labelled Eppendorf tubes, containing distilled water, and stored in the freezer at – 20 °C for later dissection. All larvae were dissected using green food colouring as contrast medium to detect parasitoid eggs and larvae. All parasitoid eggs and larvae were counted and, when possible, identified to species or genus using identification key by Osborne[72].

Statistical analyses

Statistical analyses were carried out in R statistical software (version RStudio 2022.02.3 + 492)[73]. To determine the effects of adjacent habitat type and the distance from the field edge on *B. aeneus* abundance (count data), generalized linear mixed model (package "glmmTMB"[74]), with Poisson distribution and log link function was used. We generated a model for both 2014 and 2015, were the adjacent habitat type (categorical variable), distance (categorical variable), and their interaction as fixed-factors and the field site (in total 18 fields per year), sampling round and the number of days from the last insecticide application as random factors in model. Analysis of deviance of Wald statistic Type II was used to test the individual and combined effect of adjacent habitat type and the distance from the edge. We applied estimated marginal means (package "emmeans"[75]) to compare marginal mean of *B. aeneus* abundance in the interaction model. Pearson's correlation to examine the relationship between the habitats within the 1 km radius of focal field and *B. aeneus* abundance both in 2014 and 2015 were performed. The parasitism rate of *B. aeneus* larvae was determined for each sampling point by multiplying the total number of parasitized larvae by 100 and dividing the result by the total number of larvae captured with the funnel trap at a specific sampling point. To analyse the adjacent habitat type and the distance from the field edge effect on parasitism rate, generalized linear mixed model with binomial distribution was performed, followed by analysis of deviance applying Wald statistic Type II. Adjacent habitat type, the distance from the edge and the combined effect of them were used as fixed effects, and the field number as random factor. Missing values of parasitism rates were removed prior to the analysis. We used DHARMa package[76] to assess the model fit.

Random forest analysis was carried out to determine the variance that can be explained by the landscape types surrounding the focal fields and afterwards a decision tree was built. For this analysis we combined both 2014 and 2015 dataset of landscape characteristics and the parasitism rate to have complete dataset. For random forest, we used previously described proportions of different landscapes within 1 km radii. These variables were used as predictors in the random forest model. Model was set to generate 5000 trees at one run and re-run ten times (creating in total 50,000 trees) to see the variation in the variable importance. We used cross-validation technique to evaluate the performance of the models. The R-squared value, which measured the proportion of variance in the observed parasitism explained by our model predictions. The decision tree was built on the predictors and target variable. We used "anova" method, which is used to solve regression task where the target variable is continuous or numeric. For the analysis, we used the 'randomForest'[77], 'rpart'[78] and 'partykit'[79] pacakages in Rstudio.

Data availability

Data used in the study is available from the authors on a request.

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Author contributions
E.V., R.K., G.K., J.H. conceived the experiment(s), E.V., R.K., G.K. conducted the experiment(s), S.V. and M.L. analysed the data. S.V. wrote the first draft. R.K., M.L., G.K., J.H., E.V. reviewed and made changes to the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to S.V.

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EDITED BY
Agnieszka Synowiec,
University of Agriculture in Krakow, Poland

REVIEWED BY
Mirza Abdul Qayyum,
Muhammad Nawaz Shareef University of Agriculture, Pakistan
Jozsef Kiss,
Szent István University, Hungary

*CORRESPONDENCE
Silva Sulg
silva.sulg@emu.ee
Eve Veromann
eve.veromann@emu.ee

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Toxicity of essential oils on cabbage seedpod weevil (*Ceutorhynchus obstrictus*) and a model parasitoid (*Nasonia vitripennis*)

Silva Sulg[1]*, Riina Kaasik[1], Triin Kallavus[1,2] and Eve Veromann[1]*

[1]Chair of Plant Health, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia, [2]Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Plant essential oils are being increasingly studied as a potential environmentally friendly alternative to synthetic insecticides. The insecticidal efficacy of essential oils on the cabbage seedpod weevil (*Ceutorhynchus obstrictus*), an important oilseed rape pest, has not been previously tested. We examined the impact of six essential oils on *C. obstrictus via* contact with dry residues on leaf and flower surfaces. We also examined the effect of these essential oils on a model non-target parasitoid wasp, *Nasonia vitripennis*. Exposure to dry residues of cumin (*Cuminum cyminum*) and cinnamon (*Cinnamomum verum*) essential oils (applied to oilseed rape leaves) resulted in significant loss of mortality and immobility in *C. obstrictus* adults. Treatment with *C. cyminum* essential oil at 1.5% resulted in 50.71% mortality and 87.3% combined mortality and immobility in *C. obstrictus*. *Cinnamomum verum* oil, at 1.5% concentration, resulted in 88.8% mortality and immobility among *C. obstrictus* 24 h post-treatment. All treatments studied with essential oil dry residues at 0.3% concentration caused high mortality and immobility in *N. vitripennis*. The greatest mortality and immobility were observed at 0.3% concentration in *F. vulgare* and *C. verum* treatments (54 and 53% loss respectively). At 0.1% concentration, *F. vulgare* and *T. vulgaris* significantly reduced parasitoids mobility and at 1.5% concentration all essential oils resulted in 100% mortality of *N. vitripennis* after 3 h. Our study revealed that *C. cyminum* and *C. verum* essential oils may have potential in the management of *C. obstrictus*. However, their impact on non-target organisms, including parasitoids, needs to be studied more thoroughly to determine the potential of essential oil main compounds in integrated pest management.

KEYWORDS

***Ceutorhynchus obstrictus*, Hymenoptera, biopesticide, biosafety, endoparasitoid, *Brassica napus*, IPM**

1 Introduction

The cabbage seedpod weevil (*Ceutorhynchus obstrictus* Marsham) is widely distributed and one of the most important oilseed rape pests in Europe (Williams, 2010) and North America (Buntin, 1998; Dosdall et al., 2006; Dosdall and Mason, 2010). Adult *C. obstrictus* feeds on flower buds and young pods of oilseed rape plants, but the main economic loss is caused by larvae feeding within the seedpods (Bonnemaison, 1957; Williams and Free, 1978). Management of *C. obstrictus* is mostly based on synthetic insecticides that have harmful effects on biodiversity, pollute the environment (Geiger et al., 2010) and may leave pesticide residues on products (Yigit and Velioglu, 2020). Broad-spectrum insecticides have fatal effects on naturally occurring predatory arthropods such as lacewings, spiders, ladybirds, carabid beetles, rove beetles and parasitoids, which otherwise can effectively control the abundance of agricultural pests and reduce the need to apply insecticides (Tschumi et al., 2016; Begg et al., 2017; Albrecht et al., 2020). For example, key parasitoids of *C. obstrictus* can substantially contribute to biocontrol services, as the parasitism rate of *C. obstrictus* can reach up to 90% (Veromann et al., 2011; Kovács et al., 2019). In the light of the European Union's Farm to Fork Strategy which aims to diminish the negative impacts of agriculture on the environment, there is a great need to find environmentally sustainable pest control measures (European Commission, 2020).

Essential oils are of interest in pest science, as a possible alternative to synthetic plant protection products (Menossi et al., 2021; Devrnja et al., 2022). Several studies have highlighted their insecticidal effects on important agricultural pests (Das et al., 2021; Devrnja et al., 2022). An added complexity of essential oil studies is due to the variable chemical composition, even within the same plant species, as a result of different cultivars, growing conditions, production methods, plant parts used and harvesting times (Figueiredo et al., 2008; Baser and Buchbauer, 2009; Turek and Stintzing, 2013). Previously, several studies have examined the potential of essential oils to control another oilseed rape pest – pollen beetle (*Brassicogethes aeneus* Fabricius) (Mauchline et al., 2005; Pavela, 2011; Mauchline et al., 2013; Dorn et al., 2014; Willow et al., 2020). Pavela (2011) found that *Carum carvi* L., *Thymus vulgare* L. and *Foeniculum vulgare* Miller had an insecticidal effect against the pollen beetle, while Willow et al. (2020) showed only a slight insecticidal effect of residual exposure using very high dosage of *Cinnamomum verum* J. Presl oil; Mauchline et al. (2005); Mauchline et al. (2013) and Dorn et al. (2014) found repellent and lethal efficacy with *Lavandula angustifolia* Miller while Cook et al. (2007a) indicated no behavioural response from the main parasitoids *Phradis interstitialis* Thomson and *P. morionellus* Holmgren. However, it is unknown whether these essential oils are effective against another oilseed rape pest, *C. obstrictus*, as well which could potentially aid in the control of these two major pests simultaneously. While botanical insecticides are considered to be less toxic to humans and the environment compared to synthetic pesticides, their impact on the natural enemies of pests should be assessed, as they can interfere with their behavior or biology (Rampelotti-Ferreira et al., 2017; Parreira et al., 2018; Lima et al., 2020; Stenger et al., 2021).

Biosafety of plant protection products is a major concern in agriculture. Ideally, plant protection product applications should not be at the expense of non-target organism populations, especially those contributing to biological control of the target pest. Some essential oils have been evidenced as safe for such beneficial insects. For instance, essential oil residues of *Origanum vulgare* L. and *T. vulgaris* showed no sublethal or lethal effects in the parasitoid *Trissolcus basalis* Wollaston (Platygastridae) in direct contact and fumigation bioassays (Werdin González et al., 2013). Essential oil from *Piper aduncum* L. showed promising results to control the stink bug *Euschistus heros* Fabricius, while parasitism and emergence of the egg parasitoids *Telenomus podisi* Ashmead (Platygastridae) and *Trissolcus urichi* Crawford (Scelionidae) were unaffected (Turchen et al., 2016). However, decreases in parasitism rate have been observed in other species (Boeke et al., 2003; Stenger et al., 2021), leaving the biosafety profile of essential oils questionable with regard to parasitoids.

In this study, we aimed to investigate whether the dry residues of six plant essential oils affect the mortality and immobility of *C. obstrictus*, as well as mortality and immobility, and next generation development of the model parasitoid *Nasonia vitripennis* Walker (Hymenoptera: Pteromalidae). We show that *Cuminum cyminum* L. and *C. verum* essential oils have potential for use in *C. obstrictus* management, but also that their impact on parasitoids needs further study to determine the actual potential of these essential oils within integrated oilseed rape protection.

2 Materials and methods

2.1 Insects

We collected *C. obstrictus* adults from an untreated oilseed rape field (58.36377°N, 26.66145°E, Tartu County, Estonia) using a plant tapping method and collecting insects into a ventilated plastic bottle. In the laboratory we identified *C. obstrictus via* Morris (2008) and allowed weevils to feed *ad libitum* on oilseed rape leaves and flowers also collected from the same field. As the key parasitoids of *C. obstrictus* are of the family Pteromalidae, we used *N. vitripennis* as a model non-target biocontrol species in this study. *Nasonia vitripennis* were reared in a climate chamber (Sanyo MLR-351H, Japan) at 20 ± 2°C, 60% RH and 16:8 h light:dark cycle, using blow fly (*Calliphora* sp.) pupae as the host. Blow fly larvae were bought from a commercial fishing store and were allowed to pupate in the laboratory. To produce new *N. vitripennis* adults, we placed approximately 20 blow fly pupae into transparent, polystyrene, ventilated insect breeding dishes (diameter 10 cm x height 4 cm; SPL Life Sciences, Gyeonggi-do, South Korea; hereafter referred to as cages), and introduced approximately 20–50 fast moving (a proxy for insect health) *N. vitripennis* adults into the cages. Prior to introducing the parasitoids to the cages, parasitoids were fed 50% sugar water to optimize their reproductive potential.

2.2 Examining the effect of six essential oils on *C. obstrictus via* contact with dry treatment residues on leaf and flower surfaces

To examine the effect of essential oil applications on *C. obstrictus*' mortality and immobility, we treated oilseed rape leaf and flower

surfaces with *T. vulgaris*, *F. vulgare*, *C. cyminum*, *C. verum*, *C. carvi* and *Cannabis sativa* L. essential oils. Pure essential oils were ordered from Talia (Rome, Italy; www.taliaessenze.com) in 2019, and once received stored in a refrigerator at +4°C, in small separate boxes in darkness. Details regarding the origin of plants, plant parts used, extraction method, and the major relevant compounds in each essential oil used, are described in detail in Willow et al. (2020). The gas chromatography–mass spectrometry (GC-MS) used for analyzing the essential oils, is described in detail in Kännaste et al. (2014). In the present study, essential oils were used at a concentration of 1.5%, with acetone as the solvent and polysorbate Tween80 (0.05%) as a wetting agent. The negative control treatment contained only acetone and Tween80; the positive control treatment was analytical grade lambda-cyhalothrin applied at the recommended field concentration (7.5 g active compound/ha) in acetone and Tween80. For each treatment, oilseed rape leaves were individually placed on a petri dish and using a pipette, at 1000 µl of treatment solution were applied onto each oilseed rape leave (~ 12 cm x 9 cm). In each petri dish we included four oilseed rape flowers dipped in the respective [acetone + Tween80 + essential oil] solution. After that, treated leaves and flowers were allowed to air dry for 1 h, and then one leaf and four flowers were placed into each cage, followed by the introduction of eight *C. obstrictus* adults into each cage. Five cages per each treatment were prepared. Cages were kept in a ventilated room with an ambient air temperature of 22 ± 2°C, away from direct sunlight. Survival and mobility of weevils were assessed after 3 h and 24 h of exposure to dry residues of each treatment. In each sample, the number of weevils displaying immobility effects, including erratic movements or loss of mobility, was recorded, and all dead weevils were counted. This experiment was repeated twice, in total ten cages per treatment (N=10), total of 80 weevils per treatment.

The two most effective essential oils from the abovementioned tests were then evaluated for *C. obstrictus* control efficacy at four different concentrations. Here, we examined the dry residues of *C. verum* and *C. cyminum* essential oils applied at 0.5%, 1%, 1.5% and 2% concentrations. The experimental setup was the same as previously described, but the experiment was not repeated (N=5), total of 40 weevils per treatment.

2.3 Examining non-target effects of six essential oils on *N. vitripennis*

To examine the effects of essential oils on *N. vitripennis*, we treated filter paper, *via* pipette, with the same six plant essential oils as were tested on *C. obstrictus*, using acetone as the solvent, as well as Tween80 (0.05%). The positive controls were the same as used in the *C. obstrictus* assays. Here, all six essential oils were applied at four concentrations: 0.1%, 0.3%, 0.5% and 1.5%. For each treatment, we pipetted 1000 µL of treatment solution onto five pieces of filter paper (~ 7 cm x 3 cm) individually on a petri dish. After treatment, the filter papers were allowed to air dry for 1 h, and subsequently placed into cages, one piece of filter paper per cage. Eight *N. vitripennis* adults were introduced to each cage, and the cages were kept in a ventilated room with an ambient temperature of 22 ± 2°C. At 3 h and 24 h post-exposure to treatment residues, *N. vitripennis* mortality and immobility were monitored. This experiment was repeated twice, in total ten cages per treatment (N=10), total of 80 parasitoids per treatment.

To assess the potential impact of essential oils on the developmental success of *N. vitripennis*, we used already-parasitized blow fly pupae. For that, the parasitoids we allowed freely to lay eggs into the blow fly pupae for 7 days. After 7 days, adult parasitoids were removed, and blow fly pupae were dipped into the treatment solutions for 2 seconds, allowed to air dry for 1 h, and then placed into cages, 10 pupae per cage. Cages were kept in a ventilated room with an ambient temperature of 22 ± 2°C, away from direct sunlight, for three weeks. After that, all emerged parasitoids were counted, and all pupae were dissected to indicate the presence of unemerged parasitoids. The experiment was repeated twice and in total ten cages per treatment (N=10), total of 80 pupae per treatment.

2.4 Statistical analysis

Statistical analyses were performed in R v3.6.1 (R Core Team, 2018), using the R packages "car", "emmeans", "MASS", "DHARMa" and "dunn.test" (Venables et al., 2002; Dinno and Dinno, 2017; Hartig and Hartig, 2017; Fox and Weisberg, 2019; Lenth, 2022). For *C. obstrictus* analyses, Generalized Linear Models (GLMs) with Poisson distribution and log link function and Wald statistics Type III empirical standard error were used. For the *post-hoc* comparisons, the Tukey test was used. To analyze *N. vitripennis* data, as the residuals of the model were not normally distributed, we used the nonparametric Kruskal-Wallis test, followed by Bonferroni-Dunn's test for *post-hoc* pairwise comparisons.

3 Results

3.1 Effect of essential oil residues on *C. obstrictus* mortality and immobility

After 3 h of contact with dry residues of essential oil treatments, survival rates did not differ from the negative control treatment (Figure 1A). At 24 h post-treatment, however, we observed a significant effect on survival ($\chi 2$ = 132.41, df=7, p<0.0001). The highest mortality (82.5 ± 6.8%) was observed in the *C. verum* oil treatment, followed by the positive control (53.75 ± 3.75%) and *C. cyminum* oil (50.71 ± 8.77%) treatment, while the mortality of weevils exposed to *T. vulgaris*, *C. carvi* and *C. sativa* essential oil treatments did not differ significantly from the negative control (Figure 1A). Negative control treatment had no effect on survival.

Treatment with essential oils had a significant impact on *C. obstrictus* mortality and immobility rates after 3 h ($\chi 2$ = 90.32, df=7, p <0.0001; Figure 1B). The greatest effect on *C. obstrictus* mortality and immobility was observed in the positive control treatment (76.3 ± 6.3%), followed by *C. carvi* oil (33.8 ± 11.9%) and *C. cyminum* oil (22.5 ± 7.9%). The mortality and immobility rate increased at 24 h post-exposure to dry residues, in all treatments except *C. carvi*. The greatest losses of mortality and immobility in *C. obstrictus* were observed in the *C. cyminum* (87.3 ± 4.9%) and *C. verum* (88.8 ± 4.7%) treatments, which did not differ significantly from the positive control treatment (98.8 ± 1.3%) with lambda-cyhalothrin. Treatments with *T. vulgaris* and *C. sativa* did not

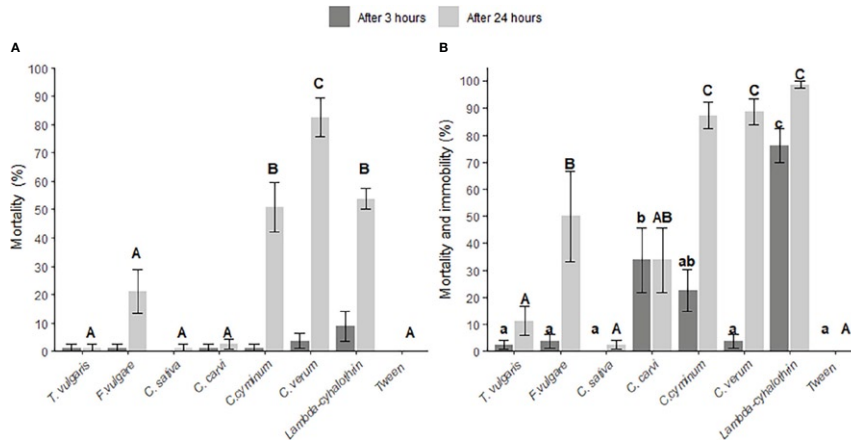

FIGURE 1
Effect of six plant essential oils (*Thymus vulgaris*, *Foeniculum vulgare*, *Cuminum cyminum*, *Cinnamomum verum*, *Carum carvi* and *Cannabis sativa*), lambda-cyhalothrin (positive control) and Tween80 (negative control) on *Ceutorhynchus obstrictus* mortality **(A)** and mortality and immobility **(B)**, at 3 h and 24 h post-exposure to treated oilseed rape leaves and flowers, N=10 (80 weevils per treatment). All treatments were compared using Generalized Linear Models (GLMs) with Wald statistic Type III, *post-hoc* comparisons with Tukey test, error bars: ± SE. Different lowercase and uppercase letters indicate significant differences (p<0.05) between treatments at 3 h and 24 h post-exposure, respectively.

differ from the negative control (p>0.05), but *F. vulgare* differed significantly from both the positive and negative control (p<0.05). No mortality nor immobility effects were observed in the negative control treatment.

3.2 Effect of different concentrations of *C. cyminum* and *C. verum* essential oil residues on *C. obstrictus* mortality and immobility

As *C. cyminum* and *C. verum* represented the most effective essential oils against *C. obstrictus*, we examined the effects of their residues against *C. obstrictus* after applying these two essential oils at increasing concentrations (0.5%, 1%, 1.5% and 2%) (Figures 2A-D). At 3 h *C. verum* showed a significant effect on *C. obstrictus* survival (χ^2 = 12.93, df=4, p=0.012) (Figure 2A), where concentrations of 1% and higher caused mortality; however no significant effect on *C. cyminum* was observed after 3 h (Figure 2B). At 24 h post-treatment, both *C. cyminum* and *C. verum* essential oil residues significantly increased *C. obstrictus* mortality (χ^2 = 126.49, df=4, p<0.0001; χ^2 = 87.29, df=4, p<0.0001, respectively). *Ceutorhynchus obstrictus* mortality rates in *C. cyminum* treatments were significantly greater in 1.5% and 2% compared to the 0.5% and 1% solutions (Figure 2B). The mortality rates of *C. obstrictus* treated with *C. verum* essential oil at 1%, 1.5% and 2% solutions exceeded 80% (82.5 ± 3.1%, 90 ± 10% and 90 ± 4.7%, respectively), each differing significantly from the *C. verum* 0.5% and control treatments. Mortality and immobility rates of *C. obstrictus* reached up to 90% at 24 h post-exposure to dry residues of *C. cyminum* essential oil applied at 1.5% and 2.0% concentrations (χ2 = 300,77, df=4, p<0.0001; Figure 2D).

3.3 Effect of essential oil residue concentrations on *N. vitripennis* mortality and immobility

After 3 h of exposure to essential oil residues applied at 0.1% concentration, there was a significant effect on *N. vitripennis* mortality and immobility (χ2 = 20.96, df=6, p=0.0019), more parasitoids were affected in *T. vulgaris* and *F. vulgare* treatments, 43.5 ± 11.9% and 15 ± 8.3%, respectively (Figure S1I). After 3 h we observed under 10% mortality and immobility for *C. sativa* (2 ± 0.42%), *C. carvi* (0%), *C. verum* (7.0 ± 1.89%), *C. cyminum* (6 ± 1.07%). For insecticide (lambda-cyhalothrin) treated group, a small mortality was observed (1.5 ± 0.26%), same for negative control (tween) (4.5 ± 0.63%). At 24 h post-exposure of dry treatment residues, no significant effect on mortality and immobility was observed for any of the essential oil treatments applied at 0.1% concentration (χ2 = 7.49, df=6, p=0.28). The lowest mortality and immobility rates were observed in the *C. carvi* treatment (3 ± 1.5%) and the highest in the *T. vulgaris* treatment (29 ± 12.5%), but no significant differences between treatments were found. The mortality and immobility rates at 24 h post-exposure to *T. vulgaris* oil residues were lower than at 3 h post-exposure, indicating that some specimens were able to recover from knockdown effects.

In all essential oil treatments applied at 0.3% concentration, *N. vitripennis* mortality and immobility were significantly decreased at both 3 h and 24 h (χ2 = 24.98, df=6, p<0.001; χ2 = 27.78, df=6,

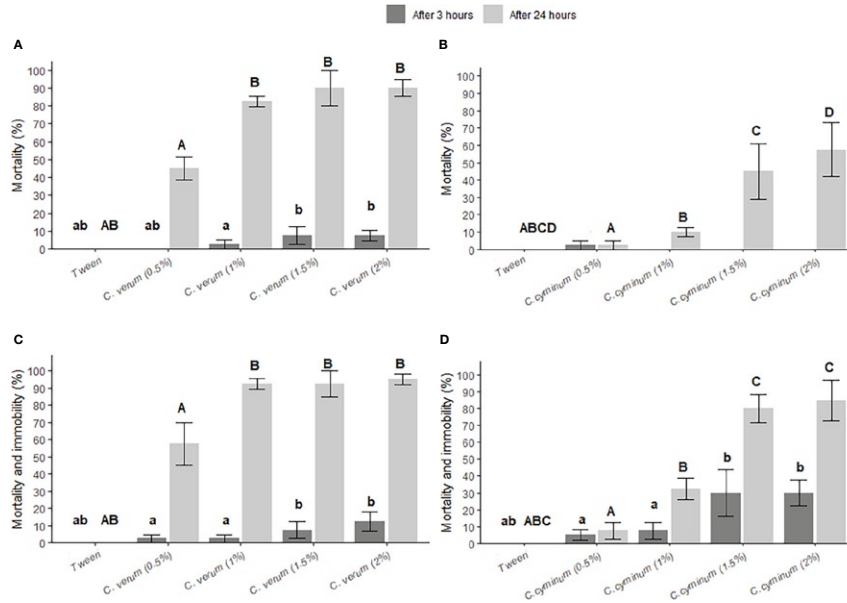

FIGURE 2
Effect of *Cuminum cyminum* essential oil residues, at different concentrations, on *Ceutorhynchus obstrictus* mortality (± SE) **(A)** and mortality and immobility (± SE) **(C)**, at 3 h and 24 h post-exposure to treated oilseed rape leaves and flowers. Effect of *Cinnamomum verum* essential oil residues, at different concentrations, on *C obstrictus* mortality (± SE) **(B)** and mortality and immobility (± SE) **(D)**, at 3 h and 24 h post-exposure to treated oilseed rape leaves and flowers, N=10 (80 weevils per treatment). All treatments were compared using Generalized Linear Models (GLMs) with Wald statistic Type III and *post-hoc* comparisons with Tukey test. Different lowercase and uppercase letters indicate significant differences ($p<0.05$) between treatments at 3 h and 24 h post-exposure, respectively. No significant difference was observed for *C cyminum* mortality after 3 h.

$p<0.0001$, respectively) post-exposure to essential oil dry residues, compared to the control treatment. At 3 h, the *F. vulgare* treatment at 0.3% concentration resulted in the greatest mortality and immobility (54 ± 14.3%), followed by the *C. verum* treatment (53 ± 15.7%), *T. vulgaris* (50 ± 5.27%), *C. sativa* (3 ± 0.48%), *C. carvi* (15 ± 2.06%), *C. cyminum* (10 ± 3.16%), compared to the insecticide treatment lambda-cyhalothrin (1.5 ± 0.26%) and negative control (tween) (4.5 ± 0.63%). At 24 h, *C. verum* oil at 0.3% concentration resulted in the greatest loss of mobility (63.5 ± 4.1%), followed by *T. vulgaris* oil (60 ± 4.8%) (Figure 3A).

In all essential oil treatments applied at 0.5% concentration, *N. vitripennis* mortality and immobility were significantly decreased at both 3 h ($\chi2 = 59.54$, df=6, $p<0.0001$) and 24 h ($\chi2 = 53.84$, df=6, $p<0.0001$). At 3 h post-exposure to the *C. verum* treatment at 0.5% concentration, the mortality and immobility rate of *N. vitripennis* was 100%, followed by the *T. vulgaris* and *F. vulgare* treatments (100% and 97 ± 2.1%, respectively), *C. cyminum* (44 ± 4.48%), *C. carvi* (21 ± 3.31%) and *C. sativa* (24 ± 2.95%). At 24 h, there was a significant loss of mortality and immobility in all 0.5% concentration essential oil treatments. The greatest loss of mortality and immobility at 24 h was observed in the *C. verum* treatment (100%) (Figure 3B). Contact with dry residues of the insecticide lambda-cyhalothrin did not result in a significant loss of *N. vitripennis* mortality and immobility, compared to the negative control treatment. All essential oils, at 1.5% concentration, resulted in 100% mortality of *N. vitripennis* after 3 h.

3.4 Effect of essential oils on the number of next generation *N. vitripennis*

After allowing *N. vitripennis* to parasitize untreated blow fly pupae for 7 days, we treated the parasitized pupae to determine the post-parasitism mortality of developing parasitoids. Compared to the untreated control group, the average number of next generation *N. vitripennis* adults that emerged was greatest in the group consisting of untreated pupae (223 ± 16.3 specimens), followed by pupae treated with *C. verum* oil (176 ± 11.3 specimens) and *F. vulgare* oil (171.8 ± 12.5 specimens), although the differences were not significant (Figure 4). Compared to untreated pupae, significantly less parasitoids emerged from *C. sativa, C. cyminum and T. vulgaris* treatments decreasing the number of emerging parasitoids similar to lambda-cyhalothrin where only 85.7 ± 16 next generation parasitoids emerged.

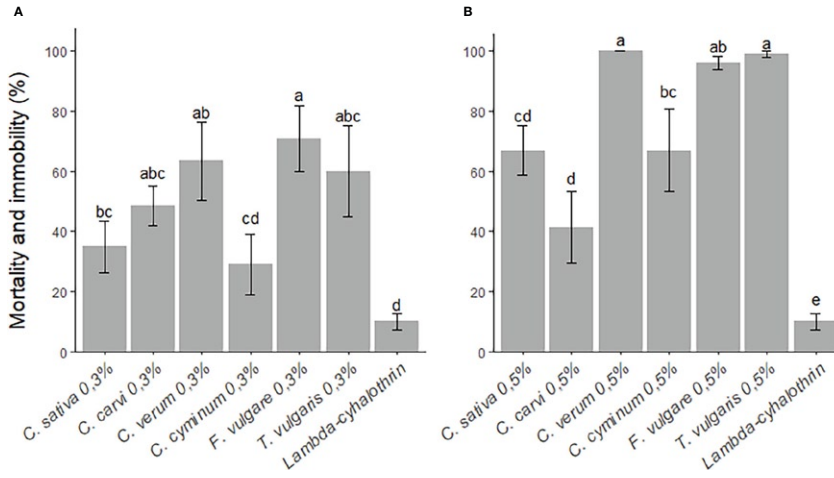

FIGURE 3
Mortality and immobility rate (± SE) of *Nasonia vitripennis* adults at 24 h post-exposure to essential oil (*Thymus vulgaris*, *Foeniculum vulgare*, *Cuminum cyminum*, *Cinnamomum verum*, *Carum carvi* and *Cannabis sativa)* dry residues [**(A)** 0.3% and **(B)** 0.5% concentrations] on filter paper. All treatments were compared using Kruskal-Wallis test, followed by Bonferroni-Dunn's test for *post-hoc* pairwise comparisons. Different letters indicate significant differences ($p<0.05$) between treatments.

4 Discussion

The present study showed that contact with essential oil residues *via* treated oilseed rape leaves and flowers caused both mortality and immobility in *C. obstrictus* adults. In addition to *C. obstrictus*, essential oil treatments showed an impact on the mortality and immobility of a model pteromalid parasitoid, *N. vitripennis*, and furthermore influenced the number of next generation adult parasitoids that emerged from their hosts. Our results showed that at 24 h post-treatment with *C. cyminum* and *C. verum* essential oils at 1.5% concentration they were as effective as the synthetic insecticide lambda-cyhalothrin, mortality and immobility of *C. obstrictus* adults reached 82.5% for *C. verum* and 50.7% for *C. cyminum*. There is an overlap in the occurrence of *B. aeneus* and *C. obstrictus* in oilseed rape fields (Veromann et al., 2006; Sulg et al., Under Review); *B. aeneus* arrives a little bit earlier than *C. obstrictus*, as its flight threshold temperature is 12°C (Williams, 2010), whereas for *C. obstrictus*, it is 13–15°C (Free and Williams, 1979; Lerin, 1991). However, they are both present in oilseed rape fields from the green bud stage (BBCH 51) (Veromann et al., 2006; Veromann et al., 2012). Therefore, it is possible that treatments targeting *B. aeneus* may also contribute to *C. obstrictus* control and *vice versa*. Our new findings show the potential of *C. verum* essential oil to manage *C. obstrictus*, but as it greatly exceeds that of previously reported for *B. aeneus* (17.5% combined immobility and mortality) (Willow et al., 2020), the two species are unlikely to be managed simultaneously using only *C. verum*. Based on previous results and our new findings, the treatment of oilseed rape with *C. verum* affects two of its main pests to some extent, therefore indicating the need for further investigations. The essential oils used in our study were almost the same (excluding anise) as in Willow et al. (2020). Gas chromatography–mass spectrometry results of the *C. verum* oil used in the present study are reported in detail in Willow et al. (2020). The primary active compound in the *C. verum* oil used was reported to be (E)-cinnamaldehyde (46%), followed by caryphyllene (15%), linalool (12%) and D-limonene (8%).

According to our best knowledge, the toxicities of essential oils for *C. obstrictus*, or other species in the genus *Ceutorhynchus*, have not previously been assessed. However, there are previous studies examining other members of the family Curculionidae, where essential oil treatment efficiencies have been examined. For instance, essential oils isolated by hydrodistilling the dried fruit of *Trachyspermum ammi* (L.) Sprague ex Turrill (Apicaceae) and *Nigella sativa* L. (Ranunculaceae) have shown repellent activity and toxic effects against the rice weevil (*Sitophilus oryzae* L.) (Chaubey, 2012). The rice weevil was also examined by Saad et al. (2018), where they found that, from all examined compounds contributing to acetylcholinesterase inhibition, the most promising was trans-cinnamaldehyde. Different essential oils, including *C. verum*, were studied against the stored product pest *Sitophilus zeamais* Motschulsky by Ramlal et al. (2020). They observed repellent and lethal effects of *C. verum* oil, resulting in 78% and 97% mortality at concentrations of 75 and 100 µL/mL, respectively. Similar to our study, the main constituent of *C. verum* essential oil in their study was cinnamaldehyde (62%) confirming that this compound can be potentially exploited against weevil pests.

The mortality rates in other essential oil treatments in our study were under 50% at 24 h, suggesting that their efficacy was insufficient for use in controlling *C. obstrictus* abundance in oilseed rape crops. In the present study, we did not examine other effects than mortality and immobility of these essential oils on *C. obstrictus* adults, e.g. repellence

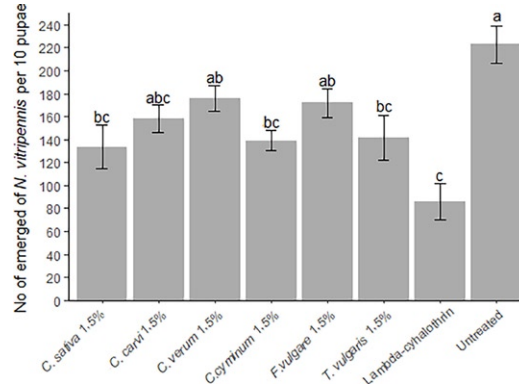

FIGURE 4
Mean (± SE) number *Nasonia vitripennis* specimens emerged from ten blow fly (*Calliphora* sp.) pupae treated with different essential oils (*Thymus vulgaris, Foeniculum vulgare, Cuminum cyminum, Cinnamomum verum, Carum carvi* and *Cannabis sativa*) (1.5% concentration) and lambda-cyhalothrin (positive control), as well as in untreated pupae, 7 days after first generation *N. vitripennis* were introduced to their hosts. Differences between treatments were compared using Kruskal-Wallis test, followed by Bonferroni-Dunn's test for *post-hoc* pairwise comparisons. Different letters indicate significant differences between treatments ($p<0.05$).

etc. For instance, previous studies have shown the repellent effects of essential oils on insects (reviewed in Lee (2018)) and also their potential to be used in storage facilities as pest management approaches (Cook et al., 2007b; Nerio et al., 2010; Campolo et al., 2018; Xu et al., 2018; Bandeira et al., 2021), but the use for managing agricultural pests have gained less attention. Essential oils can also be used to manage agricultural pests, but in order to use them in agricultural fields, the essential oils need to be more stable, preventing them from evaporating or biodegrading (Oladipupo et al., 2022). Future studies on *C. obstrictus* should examine behavioral effects in addition to the effects examined in the present study as exposure to essential oils, as well as treatment residuals, can result in behavioral changes, for example deterring egg laying or acting as antifeedant (Lazarević et al., 2020; Magierowicz et al., 2020; Stenger et al., 2021). Examining our two most effective (against *C. obstrictus*) essential oils, *C. verum* and *C. cyminum*, at four different concentrations, showed *C. verum* oil at 1% concentration to be the most efficient, resulting in almost 90% mortality and immobility at 24 h post-exposure, whereas *C. cyminum* oil at 2% concentration resulted in 90% morality and immobility at 24 h post-exposure. We presented mortality and immobility rates, since in nature, immobility is likely to result in mortality. Immobile insects are easier prey, as well as they may also die of dehydration, starvation, cold or heat stress, etc., as a result of being in contact with toxic substances.

Similar to other insecticides, the effects of essential oils on non-target organisms, including economically beneficial insects, should always be assessed. It is necessary to assess the impact of essential oils on the natural enemies of target pest species (e.g. relevant model parasitoids). The present study demonstrated that *C. verum* essential oil has the potential to control abundance of *C. obstrictus*, but at the same time it resulted in almost 100% mortality in the model pteromalid parasitoid *N. vitripennis* at 3 h post-exposure to treatments at 1.5% concentration of all studied essential oils except *C. sativa*. Testing the oils at 0.1% concentration showed that at 3 h post-exposure, *T. vulgaris* oil residues resulted in the highest mortality and immobility rates in *N. vitripennis*, although some parasitoids were able to recover from immobility by the 24 h time point. Similar to our results, Werdin González et al. (2013) found that one day old residues of *T. vulgaris* essential oil resulted in 100% mortality in parasitoid *Trissolcus basalis* Wollaston (Platygastridae) while one week old residues did not result in any mortality. When targeting a pest species, knowledge of parasitoid distribution in or arrival to the crop is crucial, as pesticide application times can be planned in a manner where the pesticide residues represent an insignificant threat to target pest-relevant biocontrol agents. Essential oil residues of *C. verum* show promising results for controlling *C. obstrictus*. However, *C. verum* oil treatment, at 0.1% concentration, resulted in an immobility rate of 17% in *N. vitripennis*. Increasing the concentration of *C. verum* oil to 0.3% resulted in almost four times this immobility rate in *N. vitripennis*, and residues from a 0.5% concentration application of *C. verum* oil resulted in 100% immobility. Thus, residues of *C. verum* essential oil, even when applied in low concentrations, are not safe for the parasitoid *N. vitripennis*. It remains unclear whether the essential oils could cause side effects in the next generation of *N. vitripennis*.

When treating parasitized pupae with *C. sativa* and *C. cyminum* it lowered hatching of the new generation of *N. vitripennis* to the same level as treatment with insecticide. But as direct exposure to essential oil residues, in the concentration of 1.5%, caused 100% mortality after 3 h among *N. vitripennis*, we can only assume that fly pupae served as protective shield mitigating the toxic effect of treatments. It has been reported that lambda-cyhalothrin can alter the ability of the parasitoids to find and infest their hosts, even when mortality is not observed among next generation female parasitoids (Desneux et al.,

2004). Even though parasitoids developed in our study, their following parasitism efficacy remains unknown, and should be investigated in future studies. Whether the pods concealing parasitoids of *C. obstrictus* provide similar protection from developing parasitoids needs investigating. It also remains unknown whether the essential oil treatments in the present study had sublethal effects on next generation adult parasitoids, representing a crucial knowledge gap that is in need of assessment. Undetected sublethal effects, could result in death or decreased fecundity or jeopardize host location abilities. Our results show the potential of essential oils use in controlling *C. obstrictus*. However, much more research is needed before essential oils could be recommended for pest control.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

SS, RK, and EV conceived the study. SS, TK, and RK performed the experiments. SS performed data analyses and visualization. SS and EV wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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IV

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Blue stem weevil (*Ceutorhynchus sulcicollis*) – a potential threat to oilseed rape production

Silva Sulg [a], Riina Kaasik [a], Jonathan Willow [a,b] and Eve Veromann [a]

[a]Chair of Plant Health, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia; [b]Laboratory of Agrozoology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Oilseed rape (*Brassica napus*) has numerous insect pests, some of which are stem-miners. Currently, blue stem weevil (*Ceutorhynchus sulcicollis*) is not considered a pest of oilseed rape. In the present study, a total of 60 (30 untreated, 30 insecticide-treated) oilseed rape plants were dissected; and stem-mining larvae were collected, and subsequently allowed to pupate in soil. After pupation, all emerged adult weevils were identified as either blue stem weevil (*C. sulcicollis*) or cabbage stem weevil (*Ceutorhynchus pallidactylus*). We report that under favourable conditions *C. sulcicollis* was capable of reaching pest status, and was significantly more abundant than *C. pallidactylus*, indicating a critical need for future studies regarding *C. sulcicollis*.

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Introduction

Oilseed rape (*Brassica napus* L.) is a valuable food resource for insects. Most of them are not harmful to oilseed rape production, but rather beneficial by pollinating the crop. However, some are considered as pests, and can considerably decrease yield, or even destroy the plants (reviewed in Alford et al. 2003). Blue stem weevil (*Ceutorhynchus sulcicollis* Paykull) is found all over Europe, but rarely recorded on oilseed rape (Alford et al. 2003). Its primary food plants are wild cruciferous plants like *Sisymbrium officinale* L. and *Alliaria petiolata* (M. Bieb.) Cavara & Grande (Morris 2008). The biology of *C. sulcicollis* is poorly described, but as Dieckmann (1972) and Hayn (1970) report, this weevil species is active throughout winter and lays eggs variously in autumn and spring, depending on winter conditions. They oviposit on the underside of leaf petioles, into the stem epidermis; and larvae mine into the stem, causing stem damage, eventually boring out of the stem to pupate under the soil surface. Next generation adults emerge from April onwards, and after a brief period of maturation feeding they aestivate in forest edges, reappearing in September. *Ceutorhynchus sulcicollis* shows similar behaviour to its intragenus relative *Ceutorhynchus picitarsis* Gyllenhal, which also undergoes summer aestivation until September, and lays eggs through autumn and winter (Barari et al. 2005). In Estonia, Veromann et al. (2006a) had detected only ten *C. sulcicollis* specimens in 2005 (using yellow water traps) on winter oilseed rape and zero in their previous studies in 2002–2003 in winter and spring oilseed rape (Veromann et al. 2006b, 2006c). However, Grantina (2012) indicated that the abundance of *C. sulcicollis* in Latvia can differ 11.2-fold between years, and that under certain conditions abundance may increase. The present study provides an overview of *C. sulcicollis* findings in Estonia, from 2020; and we discuss potential causes and implications of our findings.

Material and methods

Thirty randomly chosen winter oilseed rape plants were collected from an untreated oilseed rape field (58.37389° N, 26.33114°E) in Nasja, Tartu County, Estonia, on 4 May 2020, during the stem elongation growth stage (BBCH 30–31; Meier 2001). Plants were transported to the lab, replanted in 5 L pots, and individually covered with fine mesh fabric bags in order to prevent contamination by other insects. Covered plants were maintained in the lab for one month at 20–22 °C, under a 16:8 h light:dark cycle; plants were watered regularly. After one month, plants began to wilt, and we investigated the cause of wilting by dissecting all 30 plants on 1 June 2020. Dissections revealed severe stem damage and an abundance of stem-mining larvae. Since the identification of insect larvae is problematic, collected larvae were pooled into

CONTACT Silva Sulg silva.sulg@emu.ee Chair of Plant Health, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, F. R. Kreutzwaldi 5, Tartu, 51006, Estonia

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a box of moist soil to facilitate pupation. The box was covered with fine mesh fabric, and placed into a climate chamber (Sanyo MLR-351H, Osaka, Japan) at 20°C, 60% relative humidity and 16:8 h light:dark cycle. Soil was kept moist by spraying with water daily. Emerged adult weevils were collected and kept at −20°C until later identification. Species identification was performed via Kirk (1992) and Morris (2008) and confirmed via comparison to voucher specimens from the entomological collection of the Estonian University of life Sciences.

To compare stem damage between untreated- and insecticide [Proteus OD (application rate of 0.6–0.75 l/ha with 200–300 l/ha water) active ingredients deltamethrin 10 g/L and thiacloprid 100 g/L (Bayer CropScience)]-treated plants, 30 randomly chosen winter oilseed rape plants were collected from the same field as the untreated plants, on 2 June 2020, after insecticide application (field was treated on 5 May 2020). Plants were transported to the lab and dissected as described above. To compare the number of weevil larvae found in stems of untreated vs. treated winter oilseed rape plants, Welch's t-test was performed in R v1.2.1335 (R Foundation for Statistical Computing, Vienna, Austria). The total number of emerged weevils per treatment was recorded.

Results and discussion

Stem-mining weevil larvae damaged 29 of the 30 collected untreated winter oilseed rape plants. The number of weevil larvae collected from stems of untreated winter oilseed rape plants was significantly greater than that collected from insecticide-treated plants ($t = 5.67$, $df = 31.2$, $p < 0.0001$); respective averages of 9.33 ± 1.41 and 1.17 ± 0.28 larvae per plant being found. The majority (90%) of the 280 larvae collected from untreated plants successfully completed pupation. Regarding weevil larvae collected from insecticide-treated plants (total of 35 larvae), only two specimens emerged as next generation adults. Of all emerged next generation adults from specimens collected from untreated oilseed rape plants, 204 of them were identified as *C. sulcicollis*, and 47 as *C. pallidactylus*. Of the two adult specimens from larvae collected from treated oilseed rape plants, one was identified as *C. sulcicollis*, and the other as *C. pallidactylus*. Growing conditions between untreated- and treated plants were different, as treated plants were in the field longer. Plants used for dissection were collected from the same field, and the weevils likely oviposited while the plants were still in the field. Collecting larvae from dissected plants can be problematic, and if the plants would have been dissected immediately after collection (early May), chances for first-instar larval survival and successful pupation would have been low. Larvae collected from treated plants may have been more vulnerable due to different environmental conditions, compared to untreated plants, possibly affecting the survival rate. Seemingly healthy larvae collected from treated plant stems were not able to successfully complete pupation, suggesting a possible effect of insecticide to the larvae, representing a potential reason for very low abundance in previous years.

Currently, *C. sulcicollis* is not considered an important pest of oilseed rape. Our findings support the hypothesis of Grantina (2012), that under favourable conditions they may reach pest status. We suggest that our findings are likely linked to the relatively mild winter conditions of 2019–2020, which may have been favourable for *C. sulcicollis* reproduction and survival. According to the Estonian Weather Service, there have been no winters with a mean temperature as warm as that of 2019–2020 since 1961–1962 (the beginning of systematic weather data collection). In previous years, the mean winter temperature has been approximately −3.3°C, whereas the winter of 2019–2020 had a mean temperature of 2.5°C (Estonian Weather Service 2020). *Ceutorhynchus sulcicollis* is known in Northern Europe, yet literature regarding this insect for international readers is inadequate, indicating the importance of these findings for the broader scientific community. The outcome of this experiment was unexpected and previously not detected in our region. Therefore, supporting data from previous years is non-existent. Our results indicate the need for field studies regarding *C. sulciollis*, as this species which is currently not considered to be of economic importance may prove to be more widespread and abundant with sufficient monitoring.

Winter oilseed rape, in autumn, forms a group of low-hanging leaves that wither under typical Estonian winter conditions (Ilumäe 2013); and thus, eggs laid during the autumn before winter 2019–2020 may not have survived. Therefore, oilseed rape may not have been a suitable host plant for *C. sulcicollis* during past years in Estonia, because the plant parts suitable for oviposition perished during winter. Weevil larvae collected from untreated vs. treated oilseed rape plants, together with the emergence rates of next generation *C. sulcicollis* adults reared from the same treatments, suggest that treating oilseed rape fields in spring may have reduced the abundance of *C. sulcicollis*. However, further studies considering resistance and lethality of insecticides should be carried out, as there are no studies evaluating the efficacy of insecticides on *C. sulcicollis*.

In order to understand whether population sizes – or distributional area of *C. sulcicollis* have increased, and whether there is indeed a new oilseed rape pest in

northern Europe, further studies are needed. Literature regarding this weevil is scarce and outdated. Our results suggest that, under favourable winter conditions, *C. sulcicollis* populations may increase, thus representing a potential threat to oilseed rape production.

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Disclosure statement

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Notes on contributors

Silva Sulg is a PhD student focusing on interactions between plants, pests and parasitoids in agricultural landscapes.

Riina Kaasik (PhD) is a researcher focusing on integrated pest management (IPM) and functional agrobiodiversity.

Jonathan Willow (PhD) is a researcher and his primary research is focusing on RNAi and its role in crop protection.

Eve Veromann (PhD) is a professor in applied entomology in the Plant Health Chair, Estonian University of Life Sciences. She is also an expert in the EFSA GMO Panel where she works in environmental risk assessment workgroup. She is focused on the studies of sustainable and environmentally sound plant protection strategies both at the focal and landscape level, integrated pest management; RNAi approach in agricultural pest control, biological pest control, tritrophic interactions between plant-pest-natural enemies, trap-cropping systems and other related topics.

ORCID

Silva Sulg http://orcid.org/0000-0002-4060-9596
Riina Kaasik http://orcid.org/0000-0002-8090-2199
Jonathan Willow http://orcid.org/0000-0003-2377-4917
Eve Veromann http://orcid.org/0000-0002-0977-5270

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CURRICULUM VITAE

First name: Silva
Last name: Vilumets (née Sulg)
Date of birth: November 21st, 1994
Address: Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 5, Tartu
E-mail: silva.vilumets@emu.ee

Professional employment:

2020–2024 Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Chair of Plant Health, Junior Researcher (1.0)

2020 Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Chair of Plant Health, Specialist (0.25)

2018–2019 Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Chair of Plant Health, Laboratory assistant (0.50)

Education:

2019–2024 Doctoral studies in Agriculture, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences

2017–2019 Master's studies in Landscape Management and Protection, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences

2014–2017 Bachelor's studies in Environmental Protection, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences

2001–2013 Tartu Art Gymnasium

Academic Degree:

2019 MSc in Landscape Management and Protection for the thesis "Damage and parasitism rate of seed and stem weevils in winter oilseed rape" (Estonian University of Life Sciences).

Foreign languages:

English

Research interests:

Agricultural entomology, natural enemies, ecosystem services, sustainable plant protection

Scientific organizational and administrative activities:

2021	Estonian Plant Protection Society
2019	Estonian Female Students' Society

Honours & awards:

2019	Estonian National Academy Contest for University Students, III prize for Master's thesis in the category of Bio and Environmental Sciences.
2021	AS Baltic Agro scholarship
2021	SA Archimedes DoRa short-term visit to Superiore Sant'Anna in Pisa, for laboratory and fieldwork
2023	Estonian University of Life Sciences' best research publication scholarship for a young scientist

Participation in research projects:

2021–2025	Estonian Research Council Funding no. PRG1056, "Investigating the potential for RNA interference-based management of oilseed rape pests", senior research staff
2020–2025	Horizon 2020 Programme, project no. V200023PKTE FRAMEwork: Farmer clusters for Realising Agrobiodiversity Management across Ecosystems, senior research staff

Professional training:

2023	Workshop on Bee Identification of the XII European Congress of Entomology – ECE2023, Heraklion, Crete
2022	ArcGIS Pro: Essential Workflows, ERSI, Estonian University of Life Sciences, Tartu, Estonia
2021	Workshop on Multivariate Analysis in Ecology (& Other Sciences), using PRIMER version 7 (online)
2019	Time management training, Aeternum, University of Tartu, Estonia
2019	Scientific Writing and Communication, Estonian University of Life Sciences, Tartu, Estonia

ELULOOKIRJELDUS

Eesnimi:	Silva
Perenimi:	Vilumets (eelmine nimi: Sulg)
Sünniaeg:	21. November 1994
Aadress:	Põllumajandus- ja keskkonnainstituut, Eesti Maaülikool, Fr. R. Kreutzwaldi 5, Tartu
E-post:	silva.vilumets@emu.ee

Teenistuskäik:

2020–2024	Eesti Maaülikool, Nooremteadur (1.0)
2020	Eesti Maaülikool, Spetsialist (0.25)
2018–2019	Eesti Maaülikool, Laborant (0.25)

Hariduskäik:

2019–2024	Eesti Maaülikool, Põllumajandus ja keskkonnainstituut, doktoriõpe
2017–2019	Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, maastikukaitse ja -hoolduse eriala, magistriõpe
2014–2017	Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, keskkonnakaitse eriala bakalaureuseõpe
2001–2013	Tartu Kunstigümnaasium

Teaduskraad:

2019	Silva Sulg, magistrikraad, (juh) Eve Veromann, Kõdra- ja varre-peitkärsaka kahjustuse ja parasiteerituse tase talirapsil, Eesti Maaülikool.

Võõrkeelte oskus:

Inglise keel

Teadustöö põhisuund:

Põllumajandusentomoloogia, looduslikud vaenlased, ökosüsteemiteenused, jätkusuutlik taimekaitse

Teadusorganisatsiooniline ja -administratiivne:

2021	Eesti Taimekaitse Selts
2019	Eesti Naisüliõpilaste Selts

Teaduspreemiad ja stipendiumid:

2019	Üliõpilaste teadustööde riikliku konkursi III preemia bio- ja keskkonnateaduste valdkonnas magistritöö eest
2021	AS Baltic Agro stipendium
2021	Dora pluss lühiajalise õpirände stipendium osalemaks välitöödel Itaalias, Pisas, Scuola Superiore Sant'Anna
2023	Eesti Maaülikooli Mahestipendium parimale teaduspublikatsioonile

Uurimisprojektides osalemine:

2021–2025	PRG1056 "Uudne jätkusuutlik RNAi-tehnoloogial põhinev lahendus rapsikahjurite tõrjeks", täitja
2020–2025	Horizon 2020 V200023PKTE "Põllumeeste ühendused elurikkuse haldamiseks agroökosüsteemides", täitja

Täiendkoolitused:

2023	XII Euroopa entomoloogiakongressi mesilaste määramise töötuba – ECE2023, Heraklion, Kreeta
2022	ArcGIS Pro: Essential Workflows, ERSI, Eesti Maaülikool
2021	PRIMER-e koolitus
2019	Ajajuhtimise koolitus, Aeternum, Tartu Ülikool
2019	"Scientific Writing and Communication", Eesti Maaülikool, 2019

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VIIS VIIMAST KAITSMIST

TRIIN RILANTO

EESTI PIIMALEHMADE PRAAKIMINE JA ELUIGA – LOOMAPÕHISED, KARJA NAKKUSLIKUD JA LOOMAPIDAJAGA SEOTUD RISKITEGURID

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Kaasprofessor **Kerli Mõtus**
12. detsember 2023

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Professor **Petras Rimantas Venskutonis**, professor **Rajeev Bhat**, dr **Reelika Rätsep**
14. detsember 2023

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THE EFFECT OF FERTILIZING AND MODIFIED ATMOSPHERE STORAGE ON BLUEBERRY (*VACCINIUM* SPP) FRUIT QUALITY

Professor **Kadri Karp**, dr. **Marge Starast**, professor **Ulvi Moor**
16. jaanuar 2024

ARGO ORUMAA

PÕLENGUJÄRGNE HARILIKU MÄNNI METSAÖKOSÜSTEEMIDE TAASTUMISE DÜNAAMIKA HEMIBOREAALSES VÖÖNDIS

POST-FIRE RECOVERY DYNAMICS OF HEMIBOREAL SCOTS PINE FOREST ECOSYSTEMS

Professor **Marek Metslaid**
6. märts 2024

NIKKI SJULANDER

LIGNOTSELLULOOSSE BIOMASSI KEMIKAALIVABA EELTÖÖTLUS STRUKTUURSETE POLÜMEERIDE KONVERSIOONIKS

CHEMICAL-FREE PRETREATMENT OF LIGNOCELLULOSIC BIOMASS FOR THE CONVERSION OF STRUCTURAL POLYMERS

Professor **Timo Kikas**
22. märts 2024

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