

Partners in crime – How cabbage seed weevil assists brassica pod midge in damaging pods of winter oilseed rape in fields in Southern Sweden

How is insect abundance related to crop damage?

Vapendragarna – Hur rapsviveln möjliggör skidgallmyggan att skada höstrapskidor i fält i södra Sverige.

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Abstract

The brassica pod midge (*Dasineura brassicae*) has emerged as an important pest causing problems in Winter Oilseed Rape (WOSR) (*Brassica napus* ssp. *napus*) production in Southern Sweden during recent years. Adult female *D. brassicae* oviposit into WOSR pods. After hatching from the eggs, the *D. brassicae* larvae feed on the inside of the pod, causing deformation and premature opening of the pod, which can result in dramatic yield losses. As the ovipositor of the adult female *D. brassicae* is too weak to pierce WOSR pod walls, oviposition is mostly done in pre-damaged WOSR pods. Most of these damages that are utilized for oviposition by *D. brassicae* are caused by the cabbage seed weevil (*Ceutorhynchus obstrictus*). The weevil feeds on the WOSR pods, thereby causing damages that facilitate oviposition by *D. brassicae*. Pest control measures are therefore directed against *C. obstrictus* as the brassica pod midge is extremely difficult to control. More information about the phenology and the combined effect of the two insect species is needed in order to create pest control as sustainable as possible.

In this study, *C. obstrictus* and *D. brassicae* were monitored during six weeks from May until June 2019 in 20 different WOSR fields around Scania, the southernmost province of Sweden. Different active (visual count) and passive (yellow pan traps, yellow sticky traps) monitoring methods were used to assess the abundance of *C. obstrictus* and *D. brassicae* in the 20 WOSR fields and the effect of their presence on the amount of pod damage by *D. brassicae*. Trap samples were collected weekly and analyzed in the laboratory. At the end of the study period, pod damage was assessed in each field.

The number of captured insects of the two species was very low in comparison with previous studies from the last two years, so was the percentage of pod damage. Phenology of *C. obstrictus* deviated from the expected pattern: Usually the cabbage seed weevil can be observed in a WOSR field earlier in the season than the pod gall midge. In this study *C. obstrictus* captures peaked 2-3 weeks later than *D. brassicae* captures, presumably creating less oviposition possibilities for *D. brassicae*. Nonetheless, the brassica pod midge could benefit from the presence of the cabbage seed weevil – significant correlations between abundance of *C. obstrictus* monitored in the field border and pod damage caused by *D. brassicae* inside the field were found. Insecticide treatment showed no effect as the amount of pod damage inside an insecticide-free control zone did not differ from the amount of pod damage outside the insecticide free control zone. Nonetheless, insecticides had been used by the farmers, indicating the need for development of more refined and more rapidly available monitoring and decision tools for farmers to improve IPM strategies for pest control of *C. obstrictus* and *D. brassicae* and to reduce insecticide use.

Sammanfattning

Skidgallmyggan (*Dasineura brassicae*) har nyligen blivit en viktig skadegörare på raps (*Brassica napus* ssp. *napus*) och har de senaste åren orsakat stora skador i höstrapodlingar i Södra Sverige. *D. brassicae*-honor lägger ägg i höstrapsskidor och de nykläckta larverna äter på insidan av rapsskidan. Larvernas gnag påverkar rapsskidan så att den blir deformerad och spricker i förtid. Detta kan resultera i stora skördeföruster. *D. brassicae*-honans äggläggningsrör är för svagt för att genomborra rapsskidans vägg och äggen läggs därför oftast i redan skadade rapsskidor. De flesta av dessa skador på rapsskidor som kan utnyttjas för äggläggning av *D. brassicae*-honor är gjorda av blygrå rapsviveln (*Ceutorhynchus obstrictus*). När viveln äter på rapsskidorna uppstår gnagskador som kan gynna äggläggningen av *D. brassicae*. Växtskyddsåtgärder riktas därför mot *C. obstrictus*, eftersom skidgallmyggan är extremt svårkontrollerad. Det behövs mer kunskap om fenologin och den kombinerade påverkan av de två insektsarterna för att kunna utforma växtskyddsåtgärder så hållbart som möjligt. I denna studie blev *C. obstrictus* och *D. brassicae* övervakad i 20 olika höstrapsfält över hela Skåne under 6 veckor från maj till juni 2019. Olika aktiva (räkna vivlar på plantan) och passiva (gulskålar och gula klisterskivor) övervakningsmetoder användes för att uppskatta abundans av *C. obstrictus* och *D. brassicae* i de 20 höstrapsfälten och för att undersöka hur abundans av de båda insektsarterna hänger ihop med omfattningen av skador på höstrapsskidor orsakad av *D. brassicae*. Fällfångster samlades veckovis och räknades på labb. I slutet av undersökningsperioden genomfördes en skadegradering i varje fält. Antal fångade insekter från de två undersökta arterna samt omfattningen av skador på höstrapsskidor orsakad av *D. brassicae* var väldigt få i jämförelse med studier från de två föregående år. *C. obstrictus* fenologi avvek från det förväntade mönstret: Vanligtvis observeras blygrå rapsvivel i ett höstrapsfält tidigare på säsongen än skidgallmyggan. I denna studie nådde fångsterna av *C. obstrictus* sin topp 2–3 veckor senare än *D. brassicae* fångster. Förmodligen förvärrade detta möjligheter till äggläggning för *D. brassicae*. Skidgallmyggan verkar ha kunnat dra nytta av blygrå rapsvivelns närvaro ändå: signifikanta korrelationer hittades mellan förekomsten av *C. obstrictus* i fältkanten och skador på höstrapsskidor orsakad av *D. brassicae* inuti fältet. Kemisk bekämpning med insekticider verkar inte ha gett effekt, eftersom skadorna på rapsskidorna inuti och utanför en insekticidfri kontrollruta inte skiljde sig åt. Att kemisk bekämpning med insekticider ändå har utförts av jordbrukarna tyder på att övervakningsmetoder kan behöva förfinas för att ge jordbrukarna beslutsunderlag för att förbättra IPM strategier mot *C. obstrictus* och *D. brassicae* och minska insekticidanvändningen.

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1 Introduction

1.1 Oilseed rape (*Brassica napus* ssp. *napus*)

Oilseed rape (*Brassica napus* ssp. *napus*) is an annual herbaceous plant from the *Brassicaceae* family. The oilseed rape plant usually grows up to 1-2 m high, developing a long, thin tap root and elongated, branching stems (Alford 2003). Leaves are bluish-green with incised margin and a waxy coating, and the yellow flowers are four-petaled and grow in racemes (Figure 1). The fruits are elongated pods (siliqua) that turn from green to increasingly brown color during ripening (Alford 2003).

Oilseed rape is an important crop in central and northern Europe (Alford 2003). Since 2000 the area on which oilseed rape is grown in Europe has increased by approximately 60% (Slater et al. 2011). In Sweden, some oilseed rape cultivation started during the 19th century, mostly to produce oil for lamps (Fogelfors 2015). But in the late 19th century oilseed rape production was crushed by the competition of cheap mineral oil and severe damage from the pollen beetle (*Meligethes aeneus*) (Fogelfors 2015). Oilseed rape cultivation in Sweden increased after insecticides became available in the 1950s (Fogelfors 2015).



Figure 1: Flowering winter oilseed rape plant in a field in Scania in May 2019. (Picture by Sarah Heithausen 2019)

Oilseed rape can either be sown in late summer (early August to early September) for harvest in the following summer, or it can be sown in spring for harvest in the summer of the same year. In the former case it is referred to as Winter Oilseed Rape (WOSR) and in the latter as Spring Oilseed Rape (SOSR) (Alford 2003). After seed treatments containing neonicotinoid insecticides were restricted in the EU, attacks of the flea beetle (*Phyllotreta* spp.) on the early SOSR crop have

increased, posing an obstacle for SOSR cultivation. Although alternative cultural control measures against *Phyllotreta* spp. such as altering of tillage regime and seeding date are currently being tested (Lundin et al. 2018), most farmers choose to cultivate WOSR because it has a greater ability to survive attacks by the flea beetle in the spring. And therefore, 98,5% of oilseed rape cultivated in Scania in 2018 was WOSR (SCB 2019).

In 2018 WOSR was cultivated in Sweden on an area of 88136 ha – thereof 36002 ha (40.8%) in Sweden's most southern province Scania (SCB 2019). Even though Scania makes up only 2.5% of Sweden's area, 17% of Sweden's arable land is located in the province. About 40% of Scania's surface is used as arable land. On average WOSR yields are about 5-6% higher in Scania than in the rest of Sweden (SCB 2019). The oilseed rape yield in Sweden is on average 25% lower than in other countries like Germany or France (Fogelfors 2015). Cultivation of oil seed rape has an important function in cereal-dominated crop rotations, increasing the yield of following crops by 5-25% as the tap root of oilseed rape loosens the soil and improves growing conditions for the following crops (Fogelfors 2015). Cultivation of oilseed rape in a cereal-dominated crop rotation can also reduce cereal-attacking pathogens. Including oilseed rape in a crop rotation can help the grower to distribute the work load more evenly over the growing season as oilseed rape matures earlier in the season than cereals (Fogelfors 2015). Furthermore, cultivation of oil seed rape provides better economic profitability than cereal cultivation (Fogelfors 2015). Rapeseed flowers can even be beneficial for pollinators such as bees and bumblebees and oilseed rape fields can provide shelter for breeding birds (Naturskyddsföreningen 2015).

After harvest the oilseed rape seeds are pressed into oil which is mostly used for human consumption, as biofuel and in technology (e.g. in the food industry or as a coating on plastic bags). The plant residues that are left after oil production contain high amounts of protein and are used as animal feed (Fogelfors 2015). Rapeseed oil has a high proportion of unsaturated fatty acids, even some fatty acids that are essential for humans and rape seed oil can lower the level of cholesterol in the human bloodstream (Fogelfors 2015). In recent years the demand for biofuel and fodder made from oilseed rape has increased in Sweden (Naturskyddsföreningen 2015).

When oilseed rape cultivation grew in Sweden in the 1940s the brassica pod midge (*Dasineura brassicae* Winnertz) soon became an important pest starting in Scania and progressing further north. Oftentimes damage was severe (Stephansson & Åhman 1998). Since the 1970s damage by *D. brassicae* decreased, even if severe damage occurred locally in some years, e.g. 1992 (Stephansson & Åhman 1998). In recent years (2015 – 2018) severe damages from *D. brassicae* were observed in Scania (Aiéro et al. 2019). As oilseed rape cultivation serves several important functions, it would be devastating if WOSR

cultivation decreased because of insect pests like *D. brassicae*. To prevent this, knowledge about the most important WOSR pests is needed in order to develop appropriate control strategies.

1.2 Insect – Host Interaction

The cabbage seed weevil (*Ceutorhynchus obstrictus* (Marsham) syn. *C. assimilis* (Paykull)) and the brassica pod midge (*Dasineura brassicae* Winnertz) are two of the six most relevant pests on European WOSR (Williams 2010). These phytophagous pests use olfactory and visual cues to locate a WOSR host plant. At longer distances olfactory stimuli are most important to attract a pest to a host plant and at closer range the olfactory stimuli can help the insect to become aware of visual cues and to locate the plant (Williams & Cook 2010). These olfactory stimuli (kairomones) guiding the insects are chemicals that are emitted by plants and that can influence behavior in a way that is advantageous only to the receiver (insect) but not to the emitter (plant) (Williams & Cook 2010). Visual cues, for example size, shape and color of the plant, are less specific than olfactory cues. Visual stimuli that are attractive to WOSR pests are the blue-green color of the vegetative parts and the yellow color of the petals during flowering (Williams & Cook 2010). *C. obstrictus* is attracted to the yellow color of oilseed rape flower petals (Figure 2) (Williams & Cook 2010). An individual insect's reaction to visual and olfactory stimuli is influenced by several factors such as generation, age, sex, nutritional status, previous experience and time since last oviposition (Williams & Cook 2010).

Once an insect has landed on a potential host plant it examines the plant's chemosensory and tactile characteristics to decide if the plant is suitable for feeding and oviposition (Williams & Cook 2010).



Figure 2: Adult *C. obstrictus* on WOSR flower
(left picture by Sarah Heithausen 2019 ; right picture by Jonatan Sundelin 2019)

1.3 Cabbage seed weevil (*Ceutorhynchus obstrictus* syn. *C. assimilis*)

The cabbage seed weevil (*Ceutorhynchus obstrictus*) is an important pest on WOSR and SOSR in Europe and North America (Williams 2010). The 2 – 3 mm long, lead-grey colored *C. obstrictus* has a pronounced rostrum (snout-like projection from the head), grey-black legs and seven-segmented antennae (Figure 3). It has longitudinal furrows on its elytra (hardened forewing) and between the furrows are rows of whitish hair. (Williams 2010). With its rostrum *C. obstrictus* perforates the pod wall and eats young seeds (Stephansson & Åhman 1998).



Figure 3: Adult *C. obstrictus*
"A *Ceutorhynchus assimilis* imago 2-3 mm long" by Entomart 2007 (@entomart)

Biology

C. obstrictus is oligophagous on *Brassica* species. Adults feed on buds, flowers, pods and stem tips of *Brassica* plants (Williams 2010). Upon emergence from hibernation sites in springtime, the adult *C. obstrictus* first feed on wild *Brassicaceae* species and migrate to WOSR when it starts to flower (Hiiesaar et al. 2003). Under this migration *C. obstrictus* can fly considerable distances of up to 6 km in 10 days (Williams & Cook 2010), following the odor of the WOSR kairomones upwind towards the crop (anemotaxis) (Williams & Cook 2010). Flight activity is affected by weather conditions - cool and rainy weather creates unfavorable flying conditions for *C. obstrictus* (Hiiesaar et al. 2003). Migration occurs when temperatures rise above the weevil's flight threshold temperature of 13-15°C (Williams 2010), with high flight activity at temperatures above 18°C (Stephansson & Åhman 1998).

C. obstrictus reproduces with one generation per year. Upon emergence from overwintering sites, the ovaries of female *C. obstrictus* need to mature ca two weeks before they can oviposit (Williams 2010, Jordbruksverket 2019a). For oviposition the female *C. obstrictus* bites a hole in the WOSR pod wall, preferably into small young pods (2 – 4 cm

long). One single egg is placed through this hole into the pod and a deterring pheromone is deposited on the pod wall to prevent further oviposition into the same pod (Williams 2010). Larvae hatch after 1-2 weeks (Williams 2010). They are legless, creamy white with a yellow-brown head capsule (Stephansson & Åhman 1998). The newly hatched larvae scale and feed on the developing seeds inside the pod, each larva consuming ca five seeds in the pod (ca 8-15% of the seeds present) (Williams 2010). The larvae stay inside the pods for 3-5 weeks, going through three larval phases (Williams 2010) and growing to 5 mm long (Stephansson & Åhman 1998). A third instar larva exits the pod through a self-chewed hole in the pod wall and drops to the ground where it burrows itself down to a depth of 13 cm and pupates for 9-23 days (Williams 2010). The adults of the old *C. obstrictus* generation die during June and from late July a new generation of *C. obstrictus* emerges from pupation sites (Hiiesaar et al. 2003). The adults of the new generation feed for 1-2 weeks on *Brassica* plants (unripe pods of WOSR or wild cruciferous plants) (Hiiesaar et al. 2003) until they start searching for hibernation sites in August - either in the same field or further away, in perennial vegetation, leaf litter of field margins and woodlands (Williams 2010). The ovaries of the females of the new generation are not mature and mating occurs first after diapause (Williams 2010).

Damage

If the level of infestation is as high as one adult *C. obstrictus* per WOSR plant, yield can be reduced by ca 4% (Williams 2010). Larval feeding is more devastating to WOSR production and can reduce yield by ca 18% (Williams 2010). But the plant damage caused by adult *C. obstrictus* through feeding and oviposition can cause severe indirect problems as the lesions facilitate oviposition of *D. brassicae* and can even be an entry point for fungal diseases like *Phoma lingam*, especially in rainy weather (Williams 2010).

The spatial distribution of *C. obstrictus* in a WOSR field is uneven, complex and dynamic, varying in the different phases of the season like immigration, oviposition and emigration from the crop (Hiiesaar et al. 2003; Williams & Ferguson 2010). *C. obstrictus* abundance is usually higher in the field border than in the field center, especially during the immigration phase and when infestation is low in a field (Williams & Ferguson 2010). After the immigration phase in April and May the proportion of *C. obstrictus* adults in the field border gradually diminishes as adults move further into the field in June and July and pods with *C. obstrictus* larvae are more evenly distributed over the crop than the adults (Williams & Ferguson 2010). In severely infested fields, the center can be more infested than the edge, presumably because *C. obstrictus* adults invading the field from different sides converge at the center (Williams & Ferguson 2010).

1.4 Brassica pod midge (*Dasineura brassicae*)

The brassica pod midge (*Dasineura brassicae*), a small gall midge (dipteran family Cecidomyiidae), is an important pest of both WOSR and SOSR in most of Europe (Williams 2010). *D. brassicae* is mostly active during the day and especially during warm and calm weather (Gunnarsson 2016). The male is about 0.7-1.5 mm long and somewhat smaller than the female that is ca 0.9-2.2 mm long. The two sexes can be distinguished by the color of their abdomen, with the male having a yellow-grey abdomen and the female a pinkish-red abdomen (Williams 2010). The sex of *D. brassicae* can also be determined by the shape of the antennae. Both sexes have many-segmented antennae, but the antennae of the female are shorter than the antennae of the male. Furthermore, while the antennae of the females have less space and distinct whorls of hair in between the almost rectangular segments, the antennae of the males are longer and have more space in between the rounder segments. The male antennae have a characteristic pearl-necklace shape (Figure 4).



Figure 4: Description of how to determine *D. brassicae*'s sex by looking at the shape of the antennae

Biology

D. brassicae (Figure 5) has several generations per year (Ekbohm 2010), usually two generations on WOSR (Williams et al 1987; Williams 2010). The larvae overwinter in cocoons in the soil of fields in which WOSR was cultivated the previous year (Williams et al. 1987; Ekbohm 2010). The temperature threshold for development of the larvae in the soil is 141 DD (degree days) above 8,1°C, but can vary with weather conditions (Axelsen 1992). Pupation happens in spring and the adults (males and females emerge under several weeks from the middle of May to the middle of July (Ekbohm 2010). Time and duration of emergence depends on soil temperature and moisture and varies for

different years and places (Williams et al. 1987). In Sweden emergence lasts about 29-43 days (Williams et al. 1987).

Males are attracted to females with the help of a sex pheromone (Williams 2010). Mating is said to occur shortly after emergence at the emergence site. Shortly afterwards the mated females migrate to a WOSR field, while males stay at the emergence site and die soon after mating (Williams et al. 1987; Williams & Ferguson 2010). As *D. brassicae* is small, fragile and has a short life-span (1-3 days) the WOSR field they migrate to should be as close as possible to the site of emergence (Williams et al. 1987; Williams 2010; Ekbohm 2010). Even some males might move to WOSR fields if the distance is close and wind conditions favorable (Williams et al. 1987). At the emergence site more males than females might be caught in sticky traps and pan traps because the males show greater flight activity as they search for females (Williams et al. 1987).

D. brassicae are weak flyers, mainly dispersing with the wind (Williams & Ferguson 2010). Adults can migrate 100-500 m (Williams et al. 1987). Wind conditions (strength and direction) during migration of the mated female from the site of emergence influences her ability to reach a WOSR crop and infestation occurs mainly in field borders of fields downwind from the emergence site through anemotaxis (Nilsson 2009; Williams & Cook 2010). At closer range the yellow WOSR flowers are an attractive visual stimulus for *D. brassicae* (Williams & Cook 2010).



Figure 5: Female adult *D. brassicae*

The big picture shows an adult female *D. brassicae* with an extended ovipositor ("Dasineura brassicae adult" by Gilles San Martin licensed under CC BY-SA 2.0).

The small picture shows an adult female *D. brassicae* that has hatched in the laboratory (Picture by Sarah Heithausen 2019)

Mated female *D. brassicae* can start to oviposit directly upon arrival to the WOSR field (Williams et al. 1987). After landing on a pod, the *D. brassicae* female assesses if the pod can be a suitable oviposition site by walking back and forth over the pod surface to encounter punctures in it. A hole is first examined with the mouthpart of the female, before the ovipositor is inserted through it into the pod (Åhman 1987). The tip of the ovipositor of the female *D. brassicae* bears long, innervated bristles and can help the female to perceive mechanical and chemical characteristics of the oviposition site but does not function as a bore to pierce the wall of an undamaged pod (Åhman 1987). Therefore, eggs are usually laid in pods that are pre-damaged in some way (Hughes & Evans 2003), mainly in pods pre-damaged by *C. obstrictus* (Williams 2010). Under favorable conditions, if the pods are very young and tender, female *D. brassicae* are able to oviposit into undamaged pods (Hughes & Evans 2003; Gunnarsson 2016).

One female *D. brassicae* lays eggs in three pods on average (Stephansson & Åhman 1998), placing batches of 20-30 eggs per pod in the narrow spaces between the seeds and the pod wall (Åhman 1987). One single pod can be used for oviposition by several females (Åhman 1987; Williams 2010). The larvae hatch after 3-4 days and develop through three larval instars (Åhman 1987; Williams 2010; Gunnarsson 2016). The 2 mm long, legless, whitish-yellow larvae (Figure 6 and Figure 7) feed on the inner pod wall and even on the seeds (Åhman 1987; Williams 2010). Larvae-infested pods split open prematurely and the larvae drop to the ground, burrow themselves in the soil and spin themselves in white cocoons (Williams 2010; Gunnarsson 2016). Third instar larvae usually start emerging from pods between the beginning and middle of June, 16-27 days after the first females arrive to the WOSR field, and until late July to early August there is a continuous daily drop of larvae from pods to the ground (Williams et al. 1987). The larvae can burry themselves up to 20 cm below the soil surface, but most larvae (94%) do not descend further down than 5 cm (Williams et al. 1987). Some larvae pupate immediately upon arrival in the soil and emerge as second-generation adults 4-6 weeks later, usually in late June, depending on temperature, and cause a second infestation of the crop in the same season (Williams et al. 1987; Williams 2010; Ekbom 2010). The time required for pupal development varies over different seasons and places and has been estimated to 13-33 days in Sweden. Generation time has been estimated to 29-48 days in Sweden (Williams et al. 1987). Second-generation adults can establish a third generation by laying eggs in pods of the WOSR field of emergence or even migrate to nearby SOSR fields for oviposition (Ekbom 2010). Otherwise *D. brassicae* is rarely found on SOSR if it is not cultivated in close proximity to WOSR as *D. brassicae* emergence in spring is too early to find sufficient pod resources in the SOSR crop (Ekbom 2010). Other larvae enter diapause after cocooning themselves in the soil and emerge the following year or even up to 5 years later (Williams 2010). Cocooned larvae can survive in diapause in the soil for many years (Gunnarsson 2016). Low emergence of second-generation adults or

even a complete stop of emergence might be a survival strategy of *D. brassicae* as a species under adverse weather conditions, like a period of drought (Graora et al. 2015). In a place where WOSR infested with *D. brassicae* has been grown before, the soil can serve as a reservoir for re-infestation for nearby WOSR fields for at least 3 years, probably longer (Williams et al. 1987; Gunnarsson 2016).

Damage

Larvae infesting WOSR pods enzymatically dissolve the inner tissue of the pod, causing deformation and color change in the pods (Figure 6) that swell up (Figure 7), turn first yellow and then brown, then dry and crack and split prematurely so that larvae and seeds fall to the ground. Up to 82% of the seed weight from infested pods can be lost and yield is drastically reduced (Williams 2010; Graora et al. 2015).



Figure 6: Pod damage by *D. brassicae* and *D. brassicae* larvae (Picture by Jonatan Sundelin 2019)

Infestation is often more severe in the field border (Stephansson & Åhman 1998; Nilsson 2009; Williams 2010; Gunnarsson 2016; Jordbruksverket 2019a) as larvae and adults are usually strongly edge-distributed in WORS fields (Williams & Ferguson 2010) and its poor ability for flight prevents *D. brassicae* from migrating further into the field (Gunnarsson 2016). Infestation of a crop by *D. brassicae* and spatial distribution of the damage probably depends on several factors such as the distance to fields with infestations in previous years, distance to the site of emergence, strength and direction of wind during migration, and spatial distribution of pods that are pre-damaged by *C. obstrictus* in the WORS field (Williams & Ferguson 2010; Jordbruksverket 2019a).



Figure 7: Brassica pod midge larvae in oilseed rape silique. The larvae and seeds are more mature than in figure 6. (Picture by Sarah Heithausen 2019)

1.5 Interaction between *C. obstrictus* and *D. brassicae*

The ovipositor of the female *D. brassicae* is not adapted to function as a bore or to penetrate the pod surface (Åhman 1987; Hughes & Evans 2003). Therefore, *D. brassicae* utilizes feeding holes made by *C. obstrictus* for its oviposition (Figure 8) (Åhman 1987; Hughes & Evans 2003). The perforations made by the weevil can be used for oviposition by *D. brassicae* up to 13 days after they were created (Åhman 1987). But even other types of pod damage (e.g. damage by other phytophagous insects, fungal diseases, plant growth disturbances or mechanical damage) can be utilized for oviposition (Hughes & Evans 2003), although *C. obstrictus* damage is presumably the biggest source of oviposition sites for *D. brassicae* in nature (Åhman 1987). *D. brassicae* populations are unlikely to reach economically damaging levels without a source of sufficient quantities of pre-damaged pods (Hughes & Evans 2003). Laboratory studies have shown that pods damaged by *C. obstrictus* are preferred over lesions made by other sources, for example by Lygus bugs (*Lygus rugulipennis*) (Hughes & Evans 2003). But in case *C. obstrictus* damage is missing, lesions from other sources are used for oviposition, although it is unclear if other sources of pod damage occur in WOSR fields in sufficient quantities to replace the amount of oviposition sites provided by *C. obstrictus* (Hughes & Evans 2003). Furthermore, other pod-damaging insects should even have a phenology and spatial distribution matching those of *D. brassicae* in order to provide oviposition sites for the midges (Hughes & Evans 2003).

Usually *D. brassicae* females oviposit into the feeding holes, but not the oviposition holes of *C. obstrictus* (Åhman 1987). Presumably, the holes

are either too small for oviposition (they are smaller than feeding damage) or sharing a pod might be a reciprocal disadvantage for larvae of both species (Åhman 1987). When *D. brassicae* larvae feed on the pod walls, growth of the seeds, which the *C. obstrictus* larvae feed on, is impaired (Åhman 1987). On the other hand, feeding of *C. obstrictus* larvae on the seeds interferes with growth of the pod wall (Åhman 1987). Furthermore, since larval development is faster in *D. brassicae*, *C. obstrictus* larvae might fall out of the pods 2-3 weeks prematurely when the pods split open due to infestation with *D. brassicae* larvae in the same pod (Åhman 1987). However, larvae of the two species can sometimes be found in the same pod chamber, but in these instances *D. brassicae* eggs might have come in through weevil feeding damage on the same pod (Åhman 1987).

Dependence on other insects for finding oviposition sites is a disadvantage for *D. brassicae* that is probably balanced by the advantages of an endophytic larval development site that offers protection from adverse weather conditions and natural enemies (Åhman 1987).



Figure 8: WOSR pod showing damage by *D. brassicae* as well as damage by *C. obstrictus* (Picture by Sarah Heithausen 2019)

1.6 Pest control of *C. obstrictus* and *D. brassicae*

D. brassicae can establish big populations, especially in places where both WOSR and SOSR are cultivated (Stephansson & Åhman 1998). The number of pests can vary between different WORS fields depending on several environmental factors that influence formation of insect fauna, such as distance to hibernation sites, direction of prevailing winds, vegetation surrounding the field, weather conditions, agricultural practices, etc. (Hiiesaar et al. 2003). Damages are usually more severe in small fields in sheltered locations. Therefore, cultivation of WOSR in big, wind-exposed fields at great distances from infested fields of previous years and SOSR fields are recommended (Stephansson & Åhman 1998). *D. brassicae* might be controlled with parasitoids, but more research is needed (Ekbohm 2010). After the larvae fall to the ground and before they bury themselves in the soil, they are exposed to predators that live on the ground (e.g. carabids, spiders), but little is known about the impact of those predators or impact of other soil pathogens (e.g. fungi) on *D. brassicae* (Ekbohm 2010). The potential for biological control of *D. brassicae* is unclear. As a cultural control measure, ploughing soil that contains *D. brassicae* cocoons in diapause can be a mortality factor for *D. brassicae* (Williams 2010). As *D. brassicae* use odor-mediated upwind anemotaxis to locate a WOSR

field, planting the crop downwind from the previous year's field where pests overwinter makes it less likely that olfactory stimuli from the crop reach the pests and attract them to the crop during their migration phase (Williams & Ferguson 2010). Even management of weeds from the *Brassicaceae* family that might serve as alternative host for *D. brassicae* might be important (Hiiesaar et al. 2003), but larval development is worse in those wild *Brassicaceae* species than on the preferred host *Brassica napus* and populations will probably remain small (Åhman 1988; Stephansson & Åhman 1998).

It is difficult to estimate abundance of *D. brassicae* in a field as they are so small and short-lived as egg-laying adults (Stephansson & Åhman 1998). *D. brassicae* has two generations in WOSR and can migrate into the field during a long-time interval during flowering, which makes it hard to control (Jordbruksverket 2019a). No economic threshold has been defined to estimate necessity of control measures for *D. brassicae* (Nilsson 2009, Aiéro et al. 2019; Jordbruksverket 2019a). To control *D. brassicae*, focus is therefore put on controlling *C. obstrictus* as damages caused by the weevil facilitate *D. brassicae* oviposition (Gunnarsson 2016; Aiéro et al. 2019). Economic threshold for *C. obstrictus* in Sweden is defined as 1 individual per plant if only *C. obstrictus* is present in the field and 0,5 individual per plant if even *D. brassicae* is present in the field (Gunnarsson 2016).

Insecticide treatment during late flowering has given best results (Jordbruksverket 2019a). As *C. obstrictus* and *D. brassicae* are very edge-distributed, especially during the immigration phase, and damage is often more severe in the field border, treatment of the field border only might be sufficient to reduce a majority of the adult population in case infestation is low (Williams & Ferguson 2010; Aiéro et al. 2019). If *C. obstrictus* abundance is great, several insecticide treatments might be necessary, especially in areas with severe infestations in the previous years (Jordbruksverket 2019a). Insecticide treatment should preferably be performed at the end of flowering and treatment in full bloom should be avoided to protect beneficial insects (Aiéro et al. 2019; Jordbruksverket 2019a). As WOSR continuously grows new pods, insecticide treatment has only short-term effect (Jordbruksverket 2019a) and effectiveness of insecticide treatment is often below 50% (Aiéro et al. 2019). If infestation is low and growing conditions are good no treatment is necessary as WOSR has a good compensation capacity (Aiéro et al. 2019). The Swedish Board of Agriculture (SBOA) recommends one insecticide treatment with a neonicotinoid (Biscaya (0,3l/ha) or Mospilan (0,2 kg/ha)) or pyrethroid (Mavrik (0,2 l/ha) or Fastac 50 (0,2-0,3 l/ha)) at the end of flowering, but only if necessary, i.e. if abundance of *C. obstrictus* is above the economic threshold (Aiéro et al. 2019, Jordbruksverket 2019a).

1.7 Relevance of monitoring and economic threshold

Using insecticides before an economic threshold has been reached is unnecessary as damaged plants can compensate pest attacks by themselves without yield loss (Williams & Ferguson 2010). Another disadvantage is that broad spectrum insecticides kill many natural enemies, directly and indirectly (by killing their hosts on the crop), especially if they are applied during or after flowering, when parasitoid populations are generally very vulnerable (Williams & Ferguson 2010). Insecticides applied during flowering on WOSR pose the highest risk of damaging populations of beneficial insects and thereby reducing the level of parasitoid activity and parasitism (Nilsson 2009; Ulber et al. 2010). Parasitoid wasps are the most important natural enemies for *C. obstrictus* and *D. brassicae* (Gunnarsson & Nilsson 2017). At the beginning of the 1970s there were severe problems with *C. obstrictus* in France and despite intensive chemical control with insecticides, yield was reduced by 50%. After insecticide treatments were reduced in number and more focused on the field border, the degree of parasitoid activity increased and problems with *C. obstrictus* were minimized (Gunnarsson & Nilsson 2017).

Chemical control of the pollen beetle (*Meligethes aeneus*), which can be a very devastating pest in WOSR crops, is usually applied before the onset of flowering (Aiéro et al. 2019). But chemical treatment against *C. obstrictus* and *D. brassicae* has to be done in late bloom, which poses a greater risk of damaging beneficial insect populations and of disturbing agroecological balances in the field. Therefore, gaining knowledge about phenology and abundance of *C. obstrictus* and *D. brassicae* and their influence on pod damage is essential for developing reliable economic thresholds and monitoring routines to establish sustainable IPM control measures that are as environmentally sound as possible. Pesticide use can be more than halved by using control thresholds (Nilsson et al. 2015). Information about the phenology and abundance of insect pests allows for conservation of beneficial insect populations and reduction of insecticide use through temporal and spatial targeting of insecticide application in “spray windows” at times and places where parasitoids are less active, and the pests are more active (Williams & Ferguson 2010). Optimization of insecticide use to protect beneficial insects is especially important in WOSR cultivation where prophylactic application of broad-spectrum insecticides is common (Ulber et al. 2010). Reducing insecticide use as much as possible can also have economic benefits for the grower (Nilsson et al. 2015), because even if WOSR is self-pollinating, yield can increase by 15% in the presence of wild pollinators (Fogelfors 2015).

Another danger with overusing insecticides is the development of insecticide resistance that often leads to an increase in insecticide application while productivity is still negatively affected (Thieme et al. 2010). In WOSR crops an especially high risk of resistance development is created as several pests are present in the same crop and need to be controlled within a few weeks and this results in a high selection

pressure for resistance (Thieme et al. 2010). For many years the pollen beetle (*Meligethes aeneus*) has been controlled in WOSR crops with prophylactic pyrethroid treatments and in recent years an increasing insecticide resistance is becoming apparent: In Germany the percentage of *M. aeneus* that are highly resistant against pyrethroids has increased from 7% in 2005 to 94% in 2017 (Brandes & Heimbach 2018). Even in Sweden *M. aeneus* shows increasing resistance against pyrethroids and therefore the SBOA recommends that the same type of insecticide (e.g. pyrethroid or neonicotinoid) should not be used more than once per growing season in the same crop (Aiéro et al. 2019). 100% of *M. aeneus* samples collected in Sweden were pyrethroid resistant during two of the three years of a study (Slater et al. 2011). As pod damage by *D. brassicae* has been a problem in WOSR cultivation from 2015 - 2018 (Aiéro et al. 2019), insecticide use against *C. obstrictus* and *D. brassicae* is foreseen to increase accordingly. In this situation it seems important to learn from the development of insecticide resistance in the case of *M. aeneus* and to use insecticide treatment against *C. obstrictus* and *D. brassicae* with precision to prevent similar development of insecticide resistance in those insect populations. Especially since there are already signs for development of pyrethroid resistance in *C. obstrictus* populations as well (Brandes & Heimbach 2018).

To target and time insecticide treatments more precisely, exact information about spatio-temporal distribution of a pest and the factors determining it- phenology, times for emergence and migration etc.- is needed. This knowledge might also be used to develop biological control measures and prediction models that assess at which time specific areas of the crop are under highest risk of pest attack (Williams & Ferguson 2010). Monitoring is used to gather this kind of important data. Monitoring is an essential part of IPM strategies and is also needed to determine pest abundance in order to control if an economic threshold has been reached and for evaluation of control measures (Williams 2010).

Monitoring can be done “active” or “passive”. Active monitoring is done by visiting the crop and looking for and counting the pest (e.g. counting adult individuals of the pest on a certain number of plants or on part of plants). Passive monitoring on the other hand involves placing insect traps in the crop, examining their contents at intervals and recording the presence and abundance of pests caught (e.g. yellow sticky traps or pan traps) (Williams 2010). Type and color of a trap influence sampling as different trap types differ in insect trapping efficacy (Östrand 2011). Sampling might be more difficult in a blooming crop, because the flowers of the crop can compete with the color of a trap and reduce insect capture (Östrand 2011).

1.8 Objectives

The aim of this study was to monitor the abundance of *C. obstrictus* and *D. brassicae* in 20 WOSR fields in Scania, Southern Sweden, and to investigate if insect abundance has an influence on damage on WOSR pods caused by *D. brassicae*. Furthermore, it was assessed if the abundance of the insects in a field was related to the distance to the closest field in which WOSR had been cultivated the previous year. The objective was further to investigate if the amount of pod damage caused by *D. brassicae* was influenced by pest control with chemical insecticides. In order to test this, pod damages inside a 12x15 m large insecticide-free control zone (IFCZ) were compared with pod damages in the insecticide-treated part of the WOSR field.

Another objective was to compare sampling capability of different types of traps and visual count to investigate which different sampling methods are helpful in monitoring and if the different traps and visual count complement each other or are mostly redundant. The study was built on earlier studies from previous years (Rös vik 2017; Henriksson 2019; Johansson 2019). A challenge with field studies are the many uncontrollable confounding variables like weather conditions. This study was partly replicating a study from 2018, a growing season with unusual weather conditions that probably influenced study results (Henriksson 2019; Johansson 2019).

Study questions and hypotheses

Can correlations be found between different sampling methods for *C. obstrictus*?

Is it important to use different types of traps and visual count when monitoring *C. obstrictus* or it is sufficient to use only one trap type? Do the different sampling methods complement each other?

How is the phenology of the two insect species, *C. obstrictus* and *D. brassicae* during the period of monitoring?

The expectation is that *C. obstrictus* migrates into the field when WOSR starts to flower and that *D. brassicae*, which is very short-lived, arrives later when pods are starting to appear and *C. obstrictus* has pre-damaged the pods. For both insects, abundance of adults is expected to decline later in the season.

Are there any correlations between the amount of *C. obstrictus* trapped and the amount of trapped *D. brassicae*?

C. obstrictus abundance should facilitate *D. brassicae* oviposition. Therefore, it should be expected that the abundance of *C. obstrictus* positively correlates with the abundance of *D. brassicae*, at least with second-generation adults of *D. brassicae*. But even abundance of first-generation adults should positively correlate as it can be assumed that environmental conditions that facilitate *C. obstrictus* abundance will even facilitate *D. brassicae* abundance.

Does trap location influence monitoring?

Is there a significant difference between the abundance of *C. obstrictus* and *D. brassicae* trapped on the four different sides of the field? Is it sufficient to set up traps on only one side of the field? *D. brassicae* is a weak flyer and can't fly long from the WOSR field where it emerges. Therefore, it should be expected that more *D. brassicae* fly into the field from the side that is closest to last year's WOSR field. No such pattern is to be expected for *C. obstrictus* as they can fly longer and have more variation in overwintering sites.

Are trap catchment of the two trap sets on the same side of the field correlated? In case migration happens evenly, they should be correlated.

Are there significant differences in the amount of pod damage by *D. brassicae* between the insecticide-free control zone (IFCZ) and the insecticide-treated area of the field?

If chemical pest control with insecticides is efficient, damage should be lower in the insecticide-treated area of the field.

Are there any correlations between the abundance of trapped insects of the two species, *C. obstrictus* and *D. brassicae* and pod damage caused by *D. brassicae* in a field?

There should be a positive correlation between *D. brassicae* captures and damage done by *D. brassicae*. There should also be a positive correlation between *C. obstrictus* captures and damage done by *D. brassicae*, as damage done by *C. obstrictus* facilitates oviposition by *D. brassicae*.

Is there a correlation between distance to last year's WOSR field and abundance of and pod damages by *D. brassicae*?

As *D. brassicae* are short-lived and weak flyers a negative correlation was expected, meaning that abundance of captured *D. brassicae* should increase when distance to last year's WOSR field is shortened.

Likewise, it was expected that damage by *D. brassicae* would increase in severity with decreasing distance to the closest WOSR field from the previous growing season.

2 Method

From April 17th until April 30th, 2019 insect traps were placed in 20 WOSR fields in Sweden's southernmost province Scania, in order to monitor abundance of *C. obstrictus* and *D. brassicae* and to examine how abundance of these two insect species influences damages on WOSR pods caused by *D. brassicae*. The traps were surveyed and emptied weekly until June 20th, 2019. During these occasions of trap inventory, sampling was complemented with a visual count of adult *C. obstrictus* present at the trapping sites. From June 17th – June 21st pod damage by *D. brassicae* was estimated in the 20 WOSR fields.

2.1 Field trial location

In the recruitment process of finding WOSR fields for the present study, farmers that had participated in previous studies were contacted and asked for permission to set up insect traps in their field. Selection criteria were whether the farmer in question was growing WOSR during the 2019 growing season and the geographical location of the field. The ambition with trap distribution was to cover as much of the area of Scania as possible. Another purpose with field selection was to achieve a variation in the surrounding landscape, topography, microclimate and weather conditions (e.g. closeness to the ocean, wind conditions etc.) between the different test sites in order to exclude confounding variables through randomization. Finally, 20 WOSR fields were selected for the study (Appendix, Table X1).

The climate in Scania is on average milder than in the rest of Sweden. The average temperature varies from -2 - 0°C in January to 15 -17°C in July. Average annual precipitation varies from 500 mm to 1000 mm (SMHI 2019a).

The different field trial sites were distributed over almost all of Scania (Figure 9) with less farms in northeastern Scania and most farms located in southeast Scania. The reason why less trial sites were located in northeastern Scania being that less WOSR was grown in this region, therefore it was difficult to find trial sites there.

In 16 of the 20 field trial locations, henceforth called "standard fields" the present study was the only trial in the field. But in four out of the 20 field trial locations, Sveriges frö- och oljeväxtodlare (the association of Swedish rape seed growers) was conducting a field experiment in cooperation with the Swedish Hushållningssällskapet (The Rural Economy and Agricultural Societies) about the effect of different insecticides on *C. obstrictus* abundance (Gunnarson 2018). In these four fields, henceforth called "HHS fields", insect traps for the present study were set up alongside Hushållningssällskapet's field trials.

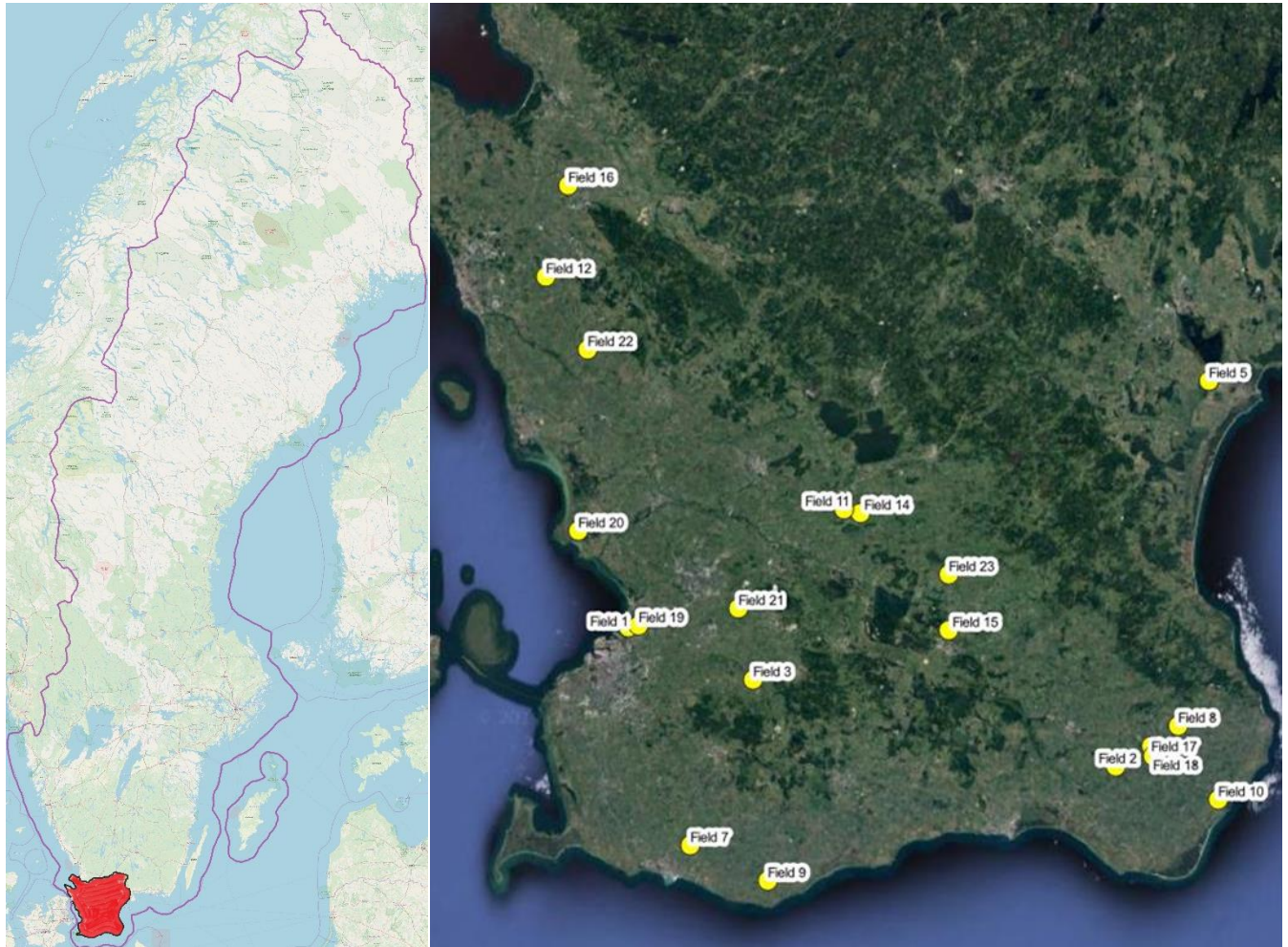


Figure 9: The left map shows Sweden, the red-colored part is Scania, the southernmost province of Sweden. The map to the right shows Scania and the 20 different field trial sites. Field 17, 21, 22 and 23 were HHS fields while the rest were standard fields. Both maps are produced with QGIS (version 3.6) by Sarah Heithausen.

2.2 Material

The present study was part of an ongoing research project at the department of plant protection biology at the Swedish University of Agricultural Sciences in Alnarp. The research project is aimed at the development of selective odor traps for *D. brassicae* and at finding an economic threshold for *D. brassicae*. The present study continued previous research of Rösvik (2017), Henriksson (2019) and Johansson (2019). The field equipment (traps and accessories) from previous field trials during the 2017 and 2018 growing seasons was repaired, reused and completed with additional material if necessary.

Traps used in the field trial

Different types of traps were used in the field trial. All traps were placed at the field edge. In all 20 field trial sites two yellow sticky traps (Figure 10) and two yellow pan traps (Figure 11) were set out. In addition to these, one yellow sticky trap and one yellow pan trap were set up on each of the other four sides of the four HHS fields. In summary 16 fields

were equipped with two pan traps and two sticky traps (Figure 12, 13) and four fields were equipped with five pan traps and five sticky traps (Figure 14). Thus 52 pan traps and 52 sticky traps in 20 different field trial locations were surveyed and emptied weekly.



Yellow sticky traps manufactured by the company Borregaard Bio Plant with a size of approximately 23 x 20 cm were set up on wooden sticks placed at the height of the canopy of the WOSR plants. The yellow sticky traps were surrounded by a metal net to prevent bycatch of unwanted animals (Figure 10).

*Figure 10: Picture of yellow sticky trap.
(Picture by Sarah Heithausen 2019)*

Yellow pan traps were filled with approximately 1740 ml of water and a small amount of detergent was added to reduce surface tension and increase the probability that insects would drown. Pan traps had a circumference of 70 cm and were 8 cm deep. They were set up on round wooden sticks. Those traps were also placed at the height of the canopy of the WOSR plants. The liquid-filled pan traps were covered with a yellow plastic grid to prevent larger insects or other larger animals from gaining access to the trap (Figure 11). As the traps could be shifted on the round wooden sticks, they were adjusted accordingly to the development of the plants so that they were always sitting at the height of the canopy of the crop.



*Figure 11: Picture of yellow pan trap from above.
(Picture by Sarah Heithausen 2019)*

2.3 Set up of field trials

The insect traps were set up on the side of the field with the closest distance to the WOSR field of the previous year. This was done, because according to the literature as discussed in the introduction it was expected that the migration of *D. brassicae* into the field should be higher from the direction of previous WOSR cultivation.

Placement of traps

Placement of traps in standard fields

Traps were placed at the edge of the field (Figure 12 and Figure 13). In front of every field two sets of traps were placed. One of them in front of an insecticide free control zone (IFCZ, with an area of approximately 12x15m) that was located ca 20 m inside the field and marked with flags and was not treated with insecticides as the rest of the field. A trap set consisted of a flag marking the location of the trap set and making it visible for growers working in the field so that the traps would not get damaged by tractors during field work. A yellow pan trap and a yellow sticky trap were placed on either side of the flag (Figure 12). The distance between the two traps was approximately 6-7 meters. The distance between the two different trap sets varied between 10-40 meters.



Figure 12: Trap set with sticky trap in the foreground and pan trap in the background, in the middle the flag that marks the trap set. (Picture by Jonatan Sundelin 2019)

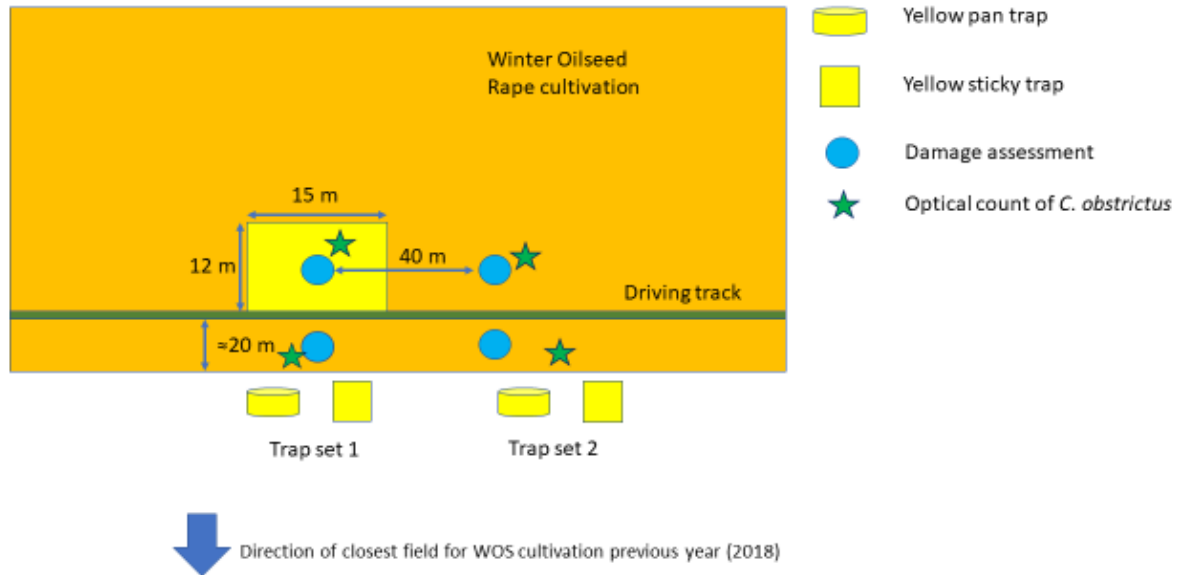


Figure 13: experimental set up in all "standard fields"
figure created with Power Point by Jonatan Sundelin and Sarah Heithausen 2019

Placement of traps in HHS fields

On the side of the field that was closest to the nearest WOSR field of the previous growing season, the set up with trap set 1 and trap set 2 and an IFCZ was just like in the standard fields. The difference in the HHS fields were that one additional trap set was also placed on each of the other three sides of the field (trap set 3-5 in Figure 14) to enable comparison of migration of *C. obstrictus* and *D. brassicae* into the field from different sides of the field.

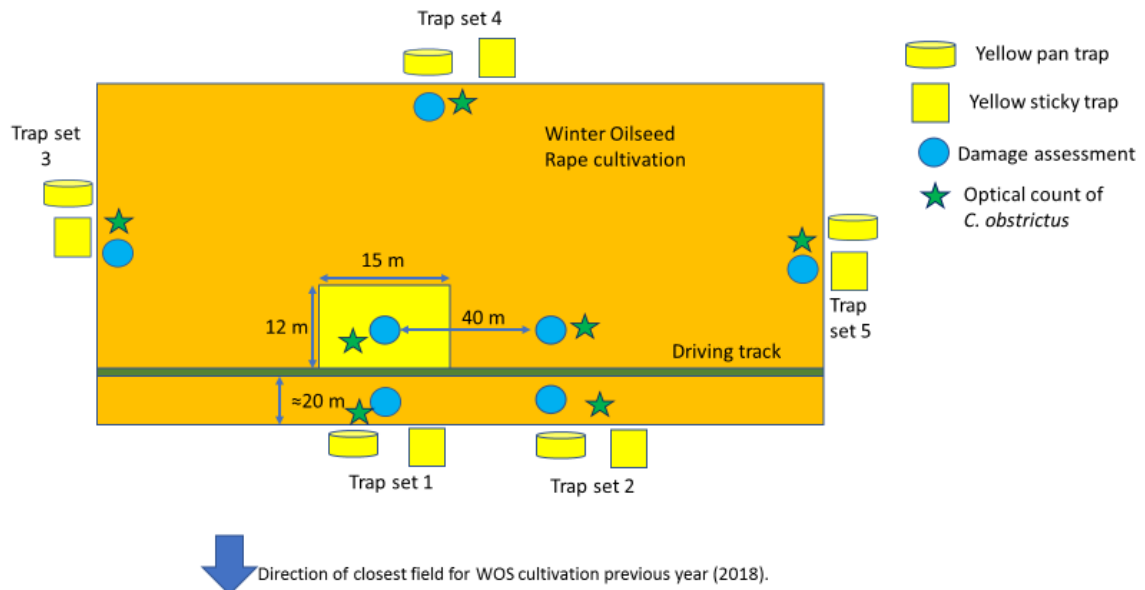


Figure 14: experimental set up in "HHS fields"
figure created with Power Point by Jonatan Sundelin and Sarah Heithausen 2019

2.4 Insect monitoring

As the 20 field trial locations were distributed over almost the entire province of Scania, it was logistically impossible to visit all the different locations and empty all traps on the same day. Each trap was emptied weekly during the study period (May 13th – June 21st, 2019).

Table 1: Overview over assessment of developmental stage of WOSR plants during the study period. Assessment was done according to BBCH decimal system (Meier 2001) after Jordbruksverket (2011).

week	Sampling once in each field during time period	Developmental stage (according to BBCH decimal system)
20	May 13 th – 17 th	65 (full bloom) in all 20 fields
21	May 20 th – 25 th	65 (full bloom) in all 20 fields
22	May 27 th – 31 st	65 in 5 fields, 67 in 13 fields, 69 in 2 fields → average: 66.7 (flowering decreases)
23	June 3 rd – 7 th	67 in 5 fields, 69 in 3 fields, 71 in 5 fields, 73 in 7 fields → average: 70,4 (flowering finished, pods are developing)
24	June 10 th – 14 th	71 in 2 fields, 73 in 5 fields, 75 in 2 fields, 77 in 4 fields, 79 in 7 fields → average: 75,9 (pods are maturing, 50% of pods have matured to maximum size)
25	June 17 th – 21 st	79 in 10 fields and 80 in 10 fields → average: 79,5 (almost all pods have grown to full size) 80: incipient seed maturity, seeds are full size but still green



Figure 15: *C. obstructus* in rape seed flower. (Picture by Jonatan Sundelin 2019)

On the occasions when the samples were collected from the traps, the developmental stage of the WOSR crop was assessed according to the guidelines of the Swedish Board of Agriculture (Jordbruksverket 2011) (Table 1). These guidelines are based on the BBCH decimal system for description of growth stages of mono- and dicotyledonous plants (Meier 2001).

Furthermore, on occasions of trap inventory, the abundance of *C. obstructus* was assessed by visual count (active monitoring). In each standard field visual count was performed at four sampling points: close to each trap set and within the field, inside and outside of the IFCZ (Figure 13). In the HHS fields visual count was performed at seven sampling points: in the same sampling

points as in the standard fields and in addition to that *C. obstructus* individuals were also counted close to the trap sets on the other three field sides (Figure 14). In summary each week *C. obstructus* was counted at 85 sampling points.

In each of these locations *C. obstrictus* individuals were counted in the flower canopy of 25 randomly chosen plants (Figure 15). As *C. obstrictus* sitting on a plant easily falls to the ground when the plant moves (Hiiesaar et al. 2003; Jordbruksverket 2019a), it was important to progress cautiously while doing visual count and not cause any unnecessary plant movements.

When the traps were surveyed and emptied weekly, the contents of the yellow pan trap were drained through a funnel into a tea filter bag that was put into a tube (50 ml) that was filled with 85% ethanol to preserve the captured insects (Figure 16, left picture). The yellow sticky traps were wrapped in plastic film and replaced with a new sticky trap (Figure 16, right picture). All samples were stored in a refrigerator (ca 8°C) before they were evaluated in the laboratory.



Figure 16: Emptying of pan trap (left picture) and sticky trap (right picture).
(Pictures by Sarah Heithausen 2019)

In the laboratory the contents of the pan traps were taken out of the tubes and the captured insects were spread out on a petri dish and *C. obstrictus* individuals were sorted out with the help of a stereo microscope and calculated (Figure 17). From the pan trap samples only *C. obstrictus* individuals were counted. The sticky traps were observed under a stereo microscope and individuals of *C. obstrictus* and *D. brassicae* were counted. *D. brassicae* males and females were counted separately using the shape of the antennae for sex determination (Figure 4). Insects were only counted on the side of the sticky trap that was facing outward from the field.

The evaluation of the insect samples at the laboratory was performed by student research assistants that had been instructed in differentiating between different insect species and between *D. brassicae* of different sex.

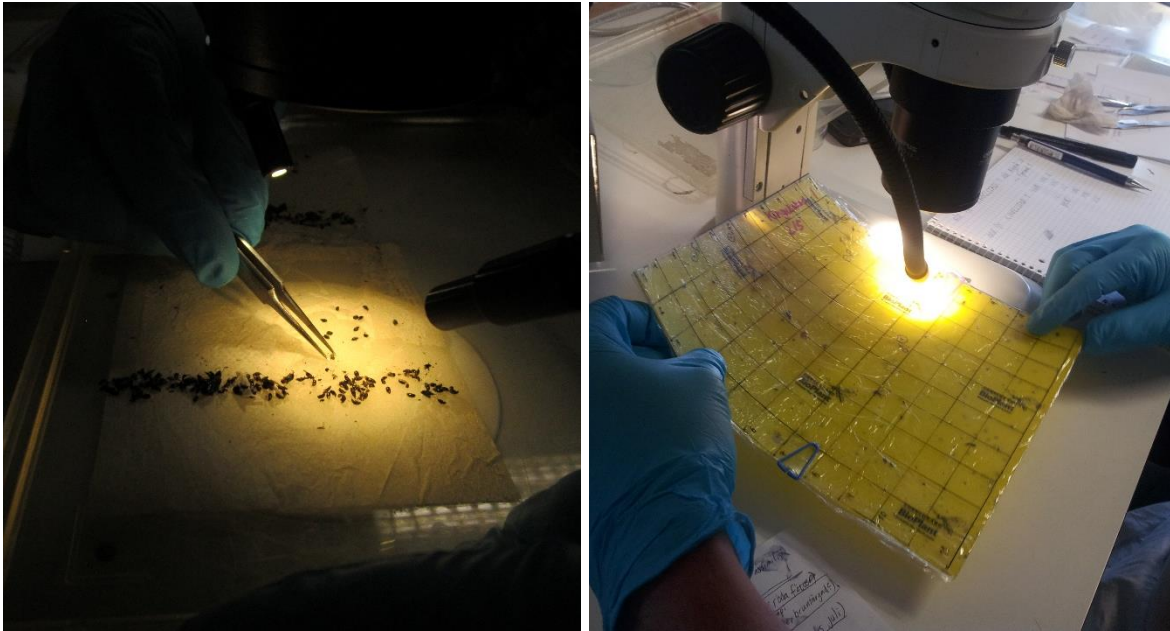


Figure 17: Counting insects caught in pan trap (left picture) and sticky trap (right picture) at the laboratory. (Pictures by Sarah Heithausen 2019)

2.5 Damage assessment

The damages caused by *D. brassicae* in the 20 WOSR fields studied were assessed once in each field between June 17th - June 20th, 2019. During the period of damage assessment WOSR plants were in the development stage DC 79-80. The pods had grown to full size but were still green, which was essential to be able to differentiate pods damaged by *D. brassicae* that had turned yellow from the undamaged green pods. Later in plant development it would have been difficult to estimate if yellowing of pods was due to *D. brassicae* damage or pod maturity. Damage was assessed in four different locations in the 16 standard fields (at each trap set and inside and outside the IFCZ) and in seven locations (at each of the 5 trap sets and inside and outside the IFCZ) in the four HHS fields (Figure 13 and Figure 14).

At each sampling point the pod damage was assessed on 10 randomly chosen plants. On these plants all the pods of the three topmost shoots of the plant were counted (Figure 18). Then the number of damaged siliques (yellowed, swollen by *D. brassicae* infestation, Figure 6) was assessed to enable calculation of average percentage of pod damage for each plant. Finally, a mean damage for each sampling point was calculated by combining results from the ten randomly chosen plants there. Pods that were damaged or missing because of attack by pollen beetle (*Meligethes aeneus*) were not counted in damage assessment.



Figure 18: Damage assessment in a WOSR field. (Picture by Sarah Heithausen 2019)

2.6 Statistical calculations

Data that was included in the statistical analyses was collected from May 13th to June 21st, 2019. Only for this period of six weeks data from all the different types of traps and from visual count was available, a prerequisite to enable comparisons over different trap types. Due to logistical reasons, intervals between occasions of trap survey varied between 4-9 days in some cases. Therefore, estimates of abundance were based on a mean value of insects caught per day of trapping. Insects per day caught in a trap were calculated by dividing the number of captured insects with the number of days the trap had been in the field. This was done so that catchment of different traps should be comparable even if intervals between emptying of traps slightly varied.

To calculate average captures for the whole sampling season, average weekly captures were summarized for each trap type and the result divided with the number of the six study weeks.

Even total abundance of insects caught weekly with the different trap types was calculated by summarizing all the individuals of *C. obstrictus* and *D. brassicae* captured in a trap type in all 20 WOSR fields for each week. This data was also used to visualize the insects' phenology.

Distance between the current WOSR field trial location and the closest WOSR field from the 2018 growing season was measured from the edge of last year's WOSR field to the edge of this year's WOSR field with the help of GIS programs ArcGIS and QGIS.

The original data was collected in a Microsoft Excel file. Correlations between different variables were calculated with Spearman rho

correlation coefficient as data was not parametric. Calculations were done with Minitab 18.

Migration into the field from different sides was measured at the four “HHS fields” by comparing insect abundance in the traps with a non-parametric Friedman-test, calculated with Minitab18.

As every field is unique in its locality, surrounding landscape, size, trap placement etc., it did not seem reasonable to calculate mean values over different fields. Thus, migration into the field from different sides was compared separately for each of the four HHS fields. On field sides where more than one trap was set up, mean values from the two traps were calculated for each of the six weeks that are included in calculations.

Efficiency of insecticide treatment was evaluated with Minitab 18 with a paired t-test that compared if average pod damage by *D. brassicae* in the insecticide-treated field and average pod damage by *D. brassicae* in the IFCZ differed significantly. Information about insecticide treatment was only available for 12 fields and therefore only those 12 fields were included in the calculations. Difference between average within-field pod damage by *D. brassicae* and damages by *D. brassicae* in the field border were also compared with a paired t-test.

3 Results

3.1 Comparison of different sampling methods

Numbers of *C. obstrictus* caught with different sampling methods

The pan traps in the 20 different WOSR fields captured 0,25 *C. obstrictus* individuals/trap/day on average during the study period (standard deviation (SD) was 0,20). Maximum capture was 1 individual/field/trap/day.

The sticky traps in the 20 different WOSR fields captured 0,12 *C. obstrictus* individuals/trap/day on average (SD: 0,10) during the study period. Maximum capture was 0,37 individuals/field/trap/day.

During the weekly visual count of *C. obstrictus* in the 20 different WOSR fields 0,35 individuals of *C. obstrictus* were on average encountered on 25 WOSR plants (0,014 individual/plant). In order to simplify comparison with captures from pan traps and sticky traps, even a daily average for visual count was calculated: 0,002 *C. obstrictus* individuals/plant/day were found on average (SD: 0,003) during the study period. Maximum capture was 9 individuals on 25 plants or 0,36 individuals/plant. In other words, the amount of *C. obstrictus* assessed visual count never came close to the economic threshold of 1 or 0,5 individual per plant.

[Within-field comparison of different sampling methods for *C. obstrictus* in the same trap set](#)

Calculations of Spearman rho correlation coefficients between the mean number of *C. obstrictus* captured on average over the whole sampling season per day with pan trap, sticky trap or visual count (Appendix, table X2) showed no significant correlations between the number of *C. obstrictus* caught in the pan trap and the sticky trap in the same location

(the same trap set) (Table 2). And no correlations were found between sticky trap catchment and visual count from the same trap set. However, a highly significant correlation was found between pan trap catchment and visual count at trap set 2 ($sr=0,704$, $p=0,001^{***}$). The average daily catchment of pan trap 1+2 and the average visual count in the same area correlate significantly ($sr=0,563$, $p=0,010^{**}$).

Within-field comparison of the same sampling method for *C. obstrictus* in two different trap sets

Results showed no significant correlations for average daily *C. obstrictus* capture from trap set 1 and trap set 2 in the same field (spearman $\rho = 0,000$, $p=1,000$). Neither average daily captures of sticky traps from trap set 1 and trap set 2 were significantly correlated (spearman $\rho = 0,333$, $p=0,152$). However, the average number of *C. obstrictus* counted visually at trap set 1 and trap set 2 of the same field was significantly correlated ($sr=0,525$, $p=0,018^*$) (Table 2).

Table 2: Within-field correlations (Spearman rho) between different sampling methods for *C. obstrictus* in the 20 different WOSR fields

The seasonal average number of *C. obstrictus* individuals caught daily in each trap type in one field (Appendix, table X2) was correlated between different trap types in the same location in a field border of the same field (PT 1 vs ST1; PT1 vs VC 1; ST1 vs VC1) and between the same trap type in 2 different locations (trap set 1 and trap set 2) in a field border of the same field (PT1 vs PT2; ST1 vs ST2; VC1 vs VC2).

	PT 1	PT 2	ST 1	ST 2	VC 1
PT 2	$sr= 0,000$ $p= 1,000$				
ST 1	$sr= -0,045$ $p= 0,850$				
ST 2		$sr=0,027$ $p=0,909$	$sr= 0,333$ $p= 0,152$		
VC 1	$sr= 0,236$ $p= 0,317$		$sr= 0,404$ $p= 0,077$		
VC 2		$sr= 0,704$ $p= 0,001^{***}$		$sr= 0,237$ $p= 0,314$	$sr= 0,525$ $p= 0,018^*$

PT 1 = pan trap of trap set 1, PT 2 = pan trap of trap set 2, ST 1 = sticky trap of trap set 1, ST 2 = sticky trap of trap set 2, VC 1 = visual count around trap set 1, VC 2 = visual count around trap set 2, sr = Spearman rho, p= p-value

There was no correlation between the abundance of *C. obstrictus* assessed through visual count in the edge of the field (data from sampling point 1 and 2 were averaged) with the number of *C. obstrictus* counted within the field (neither inside nor outside the IFCZ). It seems that the spatial distribution of *C. obstrictus* in the field was uneven.

3.2 Phenology and migration patterns of *C. obstrictus* and *D. brassicae*

Figure 19 shows the phenology for *C. obstrictus* and *D. brassicae* over the six weeks of the study period. The diagram shows the number of trapped *C. obstrictus* and *D. brassicae* per day for each sampling method accumulated over the 20 fields for each week (Appendix, Table X3). In other words, phenology of the two insect species was investigated summarized over the 20 different WOSR fields in contrast to the within-field focus of the other study questions.

The number of *D. brassicae* caught was much higher than the amount of *C. obstrictus* caught during the whole study period. Even the amount of female *D. brassicae* caught was much lower than the amount of male *D. brassicae* caught in the sticky traps during the whole duration of the study. Abundance of female *D. brassicae* peaked in week 21 (23,7 individuals per day summarized from the 40 sticky traps in the 20 fields), when WOSR was in full bloom and declined steadily in the following weeks. Abundance of male *D. brassicae* peaked one week later in week 22 (95,7 individuals per day summarized from the 40 sticky traps in the 20 fields) and declined steadily in the following weeks. The abundance of *C. obstrictus* peaked ca 2-3 weeks later than the abundance of *D. brassicae*.

The amount of *C. obstrictus* caught in the traps was very low in the beginning of the study period, when the WOSR crop was in full bloom (0.17 - 2.29 individuals per day summarized from the 40 sticky traps in the 20 fields and 1.38 - 2.86 individuals per day summarized from the 40 pan traps in the 20 fields) and peaked around week 24 after flowering of WOSR (8.57 individuals per day summarized from the 40 sticky traps in the 20 fields and 14.57 individuals per day summarized from the 40 pan traps in the 20 fields). The number of *C. obstrictus* counted in the fields visually was highest in the beginning of the study period when the crop was flowering (0.57 – 4.57 individuals counted per day on 500 plants (25 plants x 20 fields = 500 plants). During the first two weeks the daily average number of *C. obstrictus* sampled with visual count was higher than the daily average number of insects trapped with either pan trap or sticky trap, and decreased later in the season, the last two weeks declining to 0. The pan traps caught the highest numbers of *C. obstrictus* per day and caught more than the sticky traps.

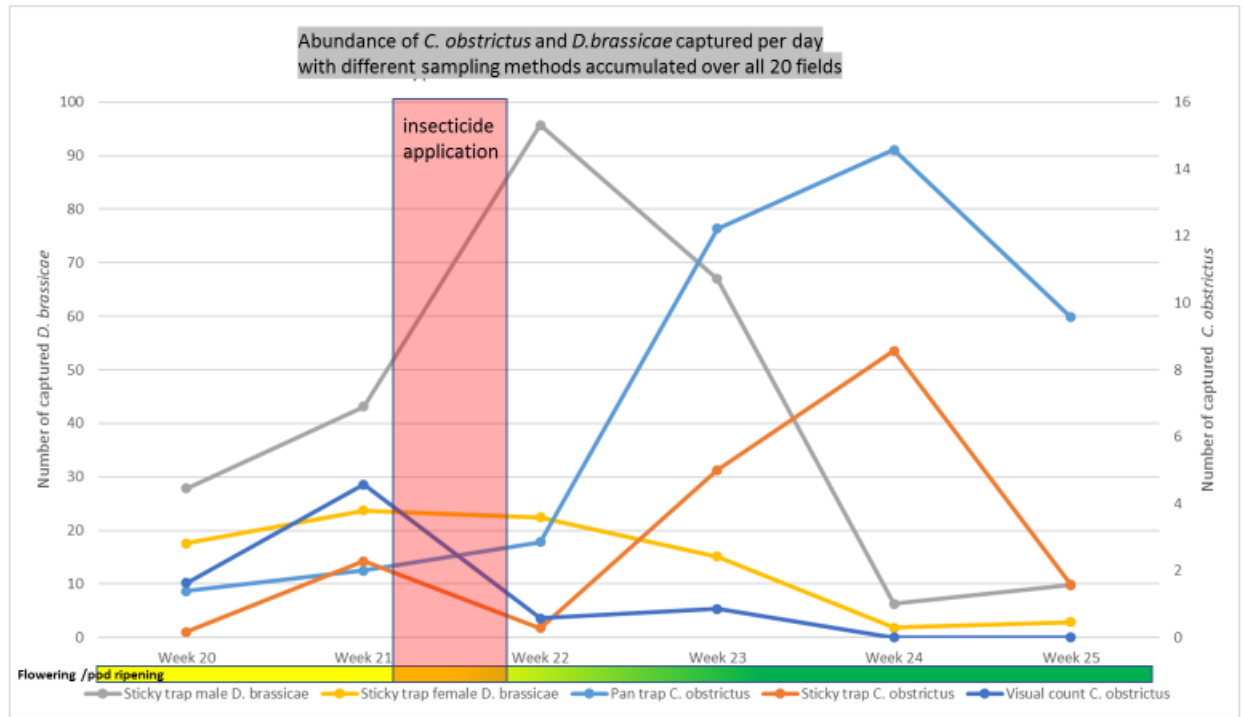


Figure 19: Daily insect captures from different trap types accumulated weekly over all 20 WOSR fields (Appendix, Table X3). The yellow-green colored gradient represents the plants' development, with yellow indicating flowering and green indicating pod ripening after flowering is completed (Table 1). The red bar indicates the recommended time for insecticide treatment against *C. obstrictus* at the end of the flowering of the WOSR crop.

Correlation between abundance of *C. obstrictus* and *D. brassicae* in the same field

No significant correlation was found between the abundance of *C. obstrictus* and the abundance of *D. brassicae* in the traps of the same trial site. No significant correlation could be found for any of the trap sets for any of the possible combinations between abundance of male or female *D. brassicae* and the different types of sampling for *C. obstrictus*.

Correlation between abundance of male and female *D. brassicae* caught in sticky traps in the same field

The correlation between number of male and female *D. brassicae* caught on average per day in the sticky traps of trap set 1 and trap set 2 of the same field over the whole six-week sampling period (Appendix, Table X4) was statistically significant ($sr=0,526$, $p=0,017^*$) (Table 3). Even the correlations between seasonal average abundance of male and female *D. brassicae* at each individual sampling point were statistically significant (trap set 1: $sr=0,590$, $p=0,006^{**}$; trap set 2: $sr=0,519$, $p=0,019^*$). This indicates that if average abundance of males is high at a sampling point, even the average abundance of females is high in the same place. In other words, male and female *D. brassicae* visit in the same places.

Table 3: Correlations between average daily abundance of male and female *D. brassicae* in the sticky traps of trap set 1 and trap set 2 of the same field, averaged over the whole six-week study period

	ST 1 male	ST 2 male	Mean of ST1+ST2 male
ST 1 female	sr= 0,590 p= 0,006**		
ST 2 female		sr= 0,519 p= 0,019*	
Mean of ST1+ST2 female			sr= 0,526 p= 0,017*

ST1 male = abundance of male *D. brassicae* caught in sticky trap of trap set 1, **ST2 male** = abundance of male *D. brassicae* caught in sticky trap of trap set 2, **ST1 female** = abundance of female *D. brassicae* caught in sticky trap of trap set 1, **ST2 female** = abundance of female *D. brassicae* caught in sticky trap of trap set 2, **mean of ST1+ST2 male** = average number of male *D. brassicae* caught in sticky traps summarized over both trap sets, **mean of ST1+ST2 female** = average number of female *D. brassicae* caught in sticky traps summarized over both trap sets

3.3 Migration into the field and trap location

Positioning of the traps might influence the monitoring results in case insect migration varies from different sides of the WOSR field. In order to test if migration into the field was even, in the four “HHS fields” traps were set up on each of the four sides of the fields and results were compared.

Comparison of insect abundance in traps on different sides of the same HHS field

Results of trap catchment comparison with Friedman-test (Table 4) showed that no significant differences between the insects trapped on different sides of the field (Figure 14) with any of the different sampling methods were found. The only exception is that abundance of female *D. brassicae* in field 21 differed significantly on the different sides of the field ($\chi^2=8,56$, $p=0,036$). Interestingly it was the side that was closest to the WOSR field of the previous year that had the lowest amount of trapped *D. brassicae* females. (Average daily captures on the different sides of each HH field with the different sampling methods for each of the six sampling weeks can be found in table X5 in the appendix).

Table 4: Comparison of insect abundance in traps on different field sides with Friedman test, calculated separately for each of the four HHS fields. Each cell represents comparison captures from one trap type for the six weeks of the study period for the different sides of one single HHS field, represented by the different trap sets (Figure 14). Each cell shows in the upper part the results from the Friedman test, with number of trap set in first column, median in second column and sum of ranks in third column. The lower part of each cell shows the χ^2 -value and the p-value for the Friedman test.

Field	<i>C. obstrictus</i> pan trap			<i>C. obstrictus</i> sticky trap			<i>C. obstrictus</i> visual count			<i>D. brassicae</i> male sticky trap			<i>D. brassicae</i> female sticky trap		
Field 21	1+2	0,01	10,0	1+2	0,03	18,5	1+2	0	12,5	1+2	0,81	15,0	1+2	0,10	9,0
	3	0,21	17,5	3	0,01	14,5	3	0	16,0	3	1,02	17,5	3	0,24	12,5
	4	0,17	17,5	4	-0,002	14,5	4	0	16,5	4	0,83	13,5	4	0,28	19,0
	5	0,12	15,0	5	-0,002	12,5	5	0	15,0	5	0,83	14,0	5	0,51	19,5
	$\chi^2=6,43$ p=0,093			$\chi^2=4,38$ p=0,223			$\chi^2=2,11$ p=0,550			$\chi^2=1,04$ p=0,792			$\chi^2=8,56$ p=0,036*		
Field 22	1+2	0,05	12,5	1+2	0,04	9,5	1+2	0	12,0	1+2	0,26	13,0	1+2	0,24	13,0
	3	0,02	11,5	3	0,30	14,5	3	0	11,0	3	0,29	13,5	3	0,21	12,5
	4+5	0,04	12,0	4+5	0,04	12,0	4+5	0	13,0	4+5	0,17	9,5	4+5	0,19	10,5
	$\chi^2=0,11$ p=0,946			$\chi^2=3,33$ p=0,189			$\chi^2=2,00$ p=0,368			$\chi^2=2,00$ p=0,368			$\chi^2=0,74$ p=0,692		
Field 23	1+2	0,19	17,5	1+2	0	15,5	1+2	0	16,5	1+2	0,21	20,0	1+2	0,23	14,0
	3	0,13	14,0	3	0	16,0	3	0	17,5	3	0,10	12,0	3	0,24	15,5
	4	0,15	15,0	4	0	16,5	4	0	13,0	4	0,12	14,0	4	0,29	19,0
	5	0,12	13,5	5	0	12,0	5	0	13,0	5	0,12	14,0	5	0,17	11,5
	$\chi^2=1,27$ p=0,737			$\chi^2=2,50$ p=0,475			$\chi^2=4,71$ p=0,194			$\chi^2=5,68$ p=0,128			$\chi^2=3,93$ p=0,269		
Field 17	1+2	0	17,0	1+2	0	14,0	1+2	0	14,5	1+2	1,05	17,5	1+2	0,98	19,0
	3	0	14,0	3	0	15,0	3	0	14,5	3	0,60	11,0	3	0,42	14,5
	4	0	14,5	4	0	17,5	4	0	16,5	4	0,99	17,0	4	0,39	14,0
	5	0	14,5	5	0	13,5	5	0	14,5	5	0,84	14,5	5	0,31	12,5
	$\chi^2=2,20$ p=0,532			$\chi^2=3,17$ p=0,367			$\chi^2=3,00$ p=0,392			$\chi^2=2,74$ p=0,433			$\chi^2=2,47$ p=0,480		

Comparison of insect abundance in traps at two different points on the same side of the same field

In order to test if migration into a field happens evenly at different points at the same side of the same field, correlations for captures from trap set 1 and trap set 2 (from all the 20 WOSR fields), that were both on the same side of the field (Figure 13), were calculated. Results showed no significant correlations for mean *C. obstrictus* capture between pan trap 1 and 2 or sticky trap 1 and 2, but significant correlations were found for visual count (Table 2). For *D. brassicae* the correlations between captures from sticky trap 1 and 2 were statistically significant for males (Spearman rho=0,714, p=0,000***) and females (Spearman rho=0,763, p=0,000***). Even damage assessment around trap set 1 and trap set 2 showed significant correlation (Spearman rho=0,860, p=0,000), further indicating evenly distribution of *D. brassicae* migration and oviposition.

3.4 Effect of insecticide use on average *D. brassicae* pod damage

Damage level was very low across all studied fields (Appendix, Table X6). The average percentage of pod damage by *D. brassicae* in the 20 WOSR fields was approximately 2%. The maximum damage level was 7%. The average pod damages caused by *D. brassicae* inside the IFCZ were compared with average *D. brassicae* pod damages inside the same field outside of the IFCZ where the crop had been treated with

insecticides (sampling points for damage assessment are shown in figure 13.).

Results of paired t-test showed no significant difference (T-value= -1,08 and p=0,303) between the average pod damages by *D. brassicae* inside the IFCZ (mean: 1,67; SD: 2,59) and the insecticide-treated part of the field outside of the IFCZ (mean: 2,00; SD: 2,29) (Figure 20).

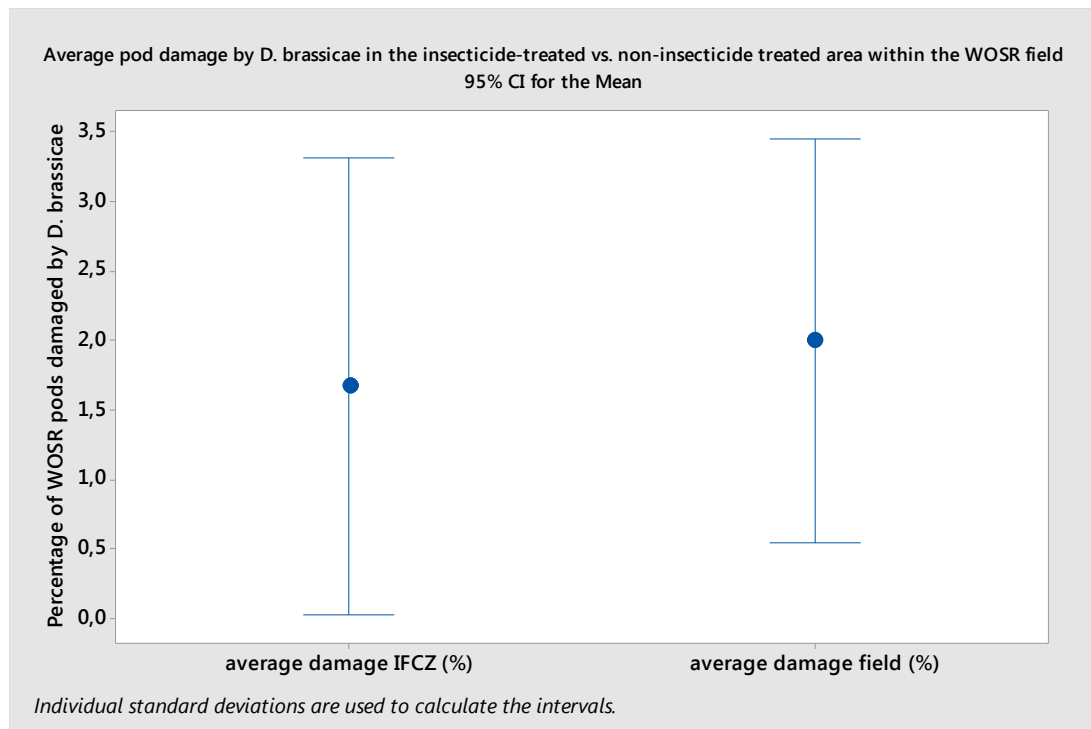


Figure 20: Results of paired t-test showed no significant difference between the average pod damage by *D. brassicae* inside the IFCZ (mean: 1,67, SD: 2,59) and the average pod damage by *D. brassicae* in the insecticide-treated part of the field outside of the IFCZ (mean: 2,00, SD: 2,29)

As no significant difference in average pod damage by *D. brassicae* could be found between the IFCZ and the insecticide-treated part of the inside of the same field, it was concluded that insecticide treatment did not affect average pod damage by *D. brassicae* and that assessed pod damage inside and outside the IFCZ can be combined to an average value for within-field pod damage by *D. brassicae* for further calculations.

Another paired t-test was performed to investigate differences between pod damage by *D. brassicae* within the field (inside and outside the IFCZ combined) and in the field border (at trap set 1 and 2). Results showed no significant difference (T-value= 2,11 and p=0,058) between the within-field pod damages by *D. brassicae* (inside and outside the IFCZ combined) (mean:1,83; SD:2,39) and pod damages by *D. brassicae* in the field border (trap set 1 and 2 combined) (mean: 2,69; SD: 3,05) (Figure 21).

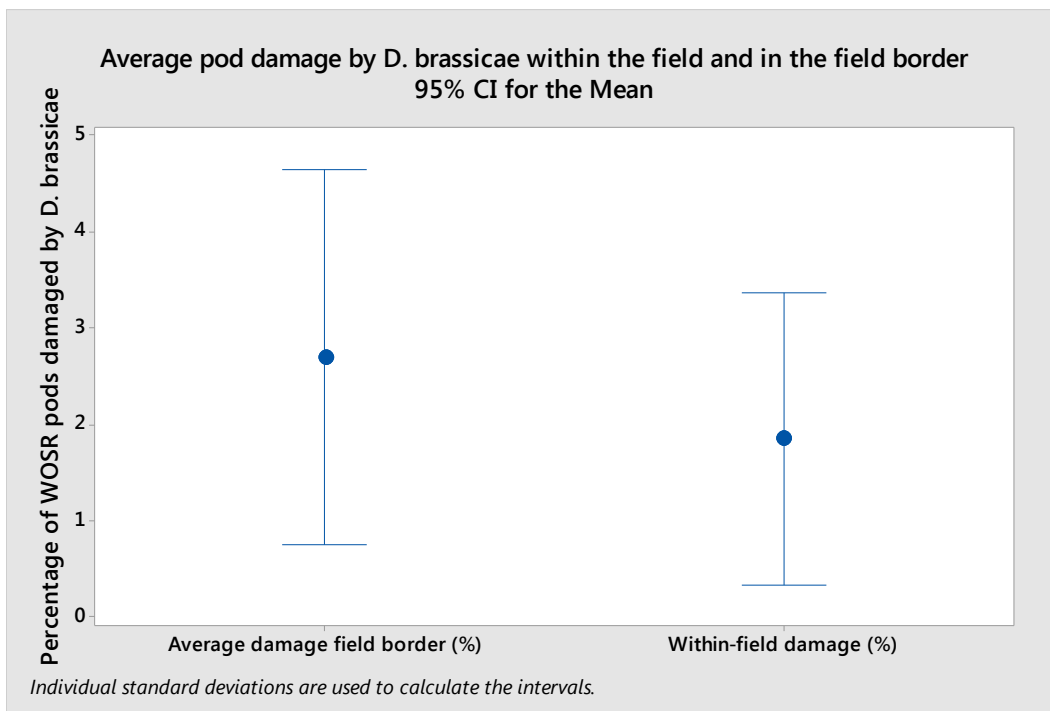


Figure 21: Results of paired t-test showed no significant difference between the average percentage of damage by *D. brassicae* within the field (inside and outside the IFCZ combined) (mean: 1,83; SD: 2,39) and the average percentage of pod damage by *D. brassicae* in the field border (trap set 1 and 2 combined) (mean: 2,69; SD: 3,05).

There is a highly significant correlation between average damages inside the field (inside and outside the IFCZ) and average damage at the field edge (trap set 1+2) ($sr = 0,743$, $p=0,000^{***}$).

3.5 Relation between insect abundance and amount of pod damage by *D. brassicae*

Correlations between abundance of *C. obstrictus* and amount of pod damage by *D. brassicae*

Average *C. obstrictus* abundance in pan traps in the field border (mean of trap set 1 and 2) correlated significantly with pod damage inside the IFCZ ($sr= 0,590$; $p= 0,006^{**}$) in the same field. Average *C. obstrictus* abundance in pan traps in the field border correlated even significantly with average within-field pod damage by *D. brassicae* (inside and outside the IFCZ) ($sr= 0,571$; $p= 0,009^{**}$) in the same field (Figure 22). This correlation remains significant even when it is calculated without the outlier-value (marked with a red circle in figure 22): $r= 0,499$; $p= 0,030^*$.

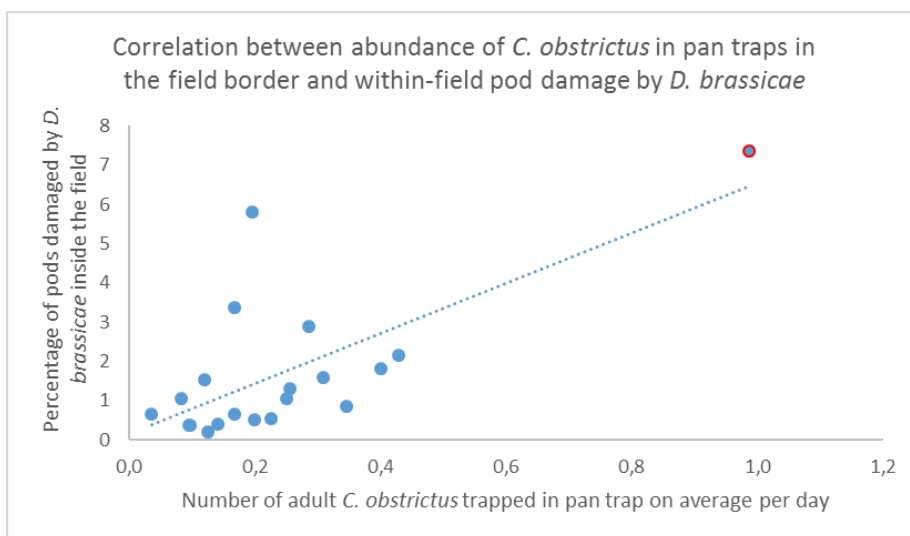


Figure 22: Significant correlation between average *C. obstrictus* abundance in the two pan traps in the field border and pod damage by *D. brassicae* within the same field ($sr= 0,571$; $p= 0,009^{**}$) Correlation is even significant without the red-circled outlier value ($sr= 0,499$; $p=0,030^*$).

No significant correlation was found between sticky traps in the field border and average within-field pod damage by *D. brassicae* in the same field (Figure 23).

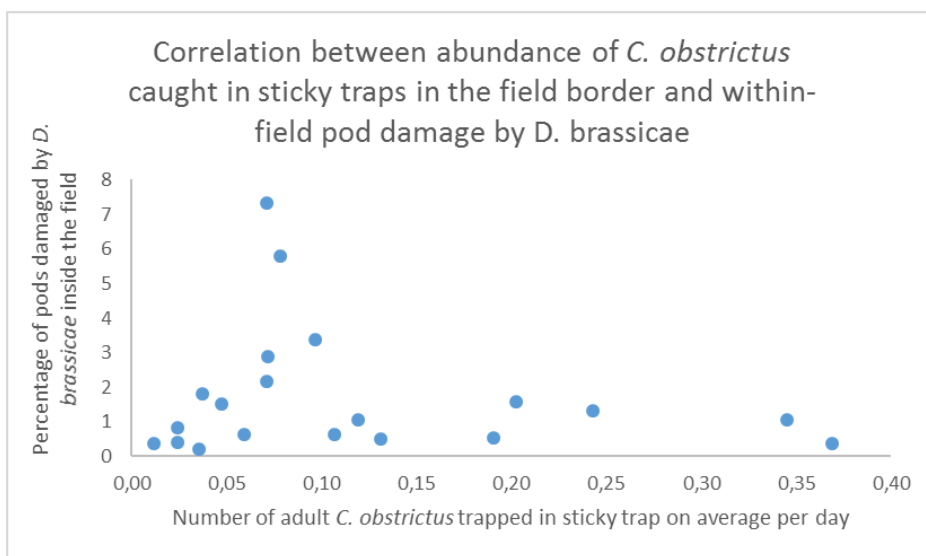


Figure 23: Correlation between sticky traps in the field border and within-field pod damage by *D. brassicae* is not statistically significant ($sr=0,111$; $p= 0,642$).

Average number of *C. obstrictus* individuals counted in the field border (around trap set 1 and 2) correlated significantly with average pod damage by *D. brassicae* in the same location ($sr= 0,457$; $p= 0,043^*$), but also with average pod damage by *D. brassicae* within the same field (inside and outside the IFCZ) ($sr= 0,540$; $p= 0,014^*$) (Figure 24).

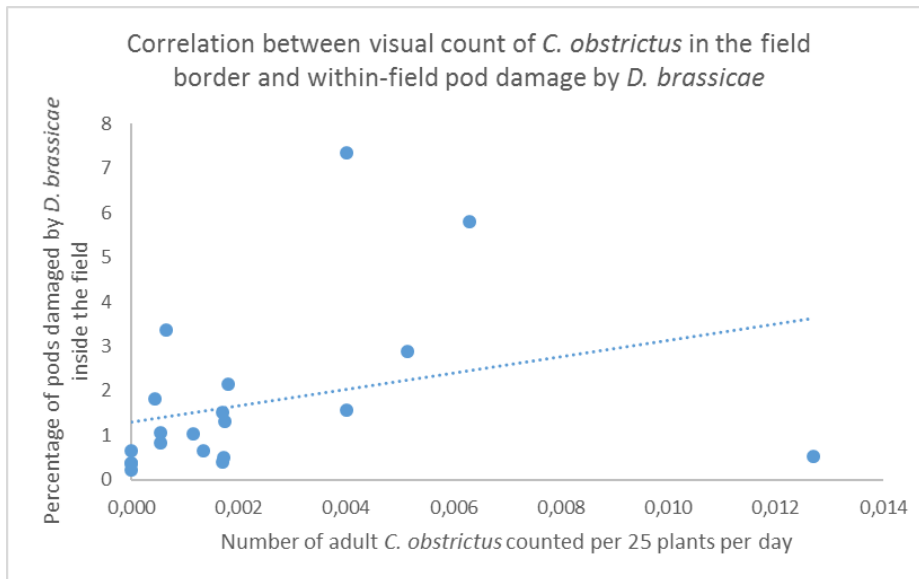


Figure 24: Significant correlation between average number of *C. obstrictus* individuals counted in the field border and pod damage within the field ($sr= 0,540$; $p= 0,014^*$)

Correlations between abundance of *D. brassicae* and pod damage by *D. brassicae*

Correlations between abundance of male D. brassicae and pod damage by D. brassicae

No significant correlations were found between abundance of male *D. brassicae* in the sticky traps in the field border (average from trap set 1 and 2) and average pod damage by *D. brassicae* inside the same field (inside and outside the IFCZ combined) (Figure 25).

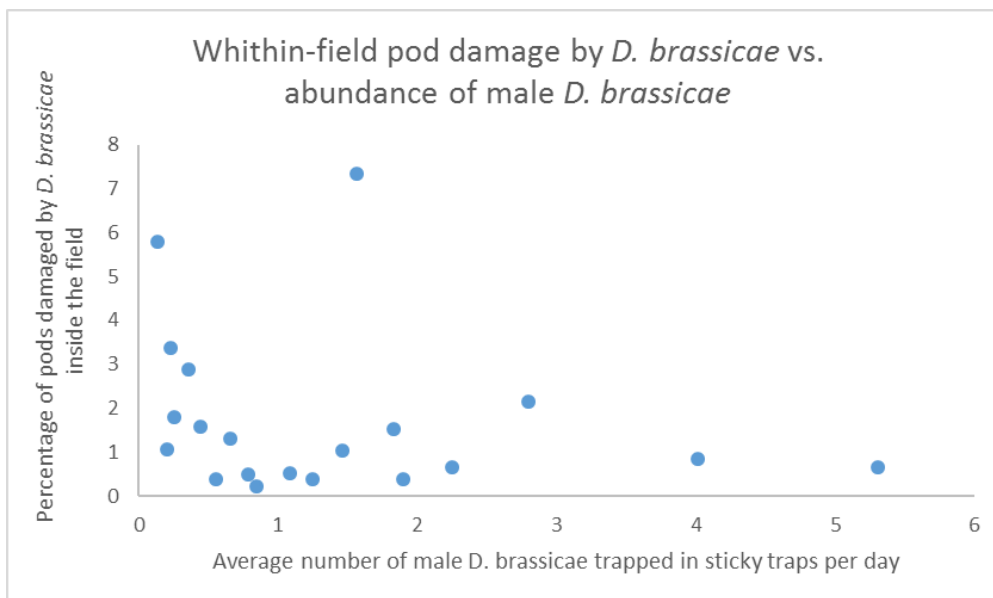


Figure 25: Correlation between abundance of male *D. brassicae* in the field border (average of trap set 1 and 2) and within- field pod damage (inside and outside the IFCZ combined) is not statistically significant ($sr=-0,335$; $p=0,149$).

Correlations between abundance of female D. brassicae and pod damage by D. brassicae

The correlation between average abundance of female *D. brassicae* in the field border (average of trap set 1 and 2) and average pod damage by *D. brassicae* inside the same field (inside and outside of the IFCZ combined) is not statistically significant ($r = -0,324$; $p = 0,163$) (Figure 26).

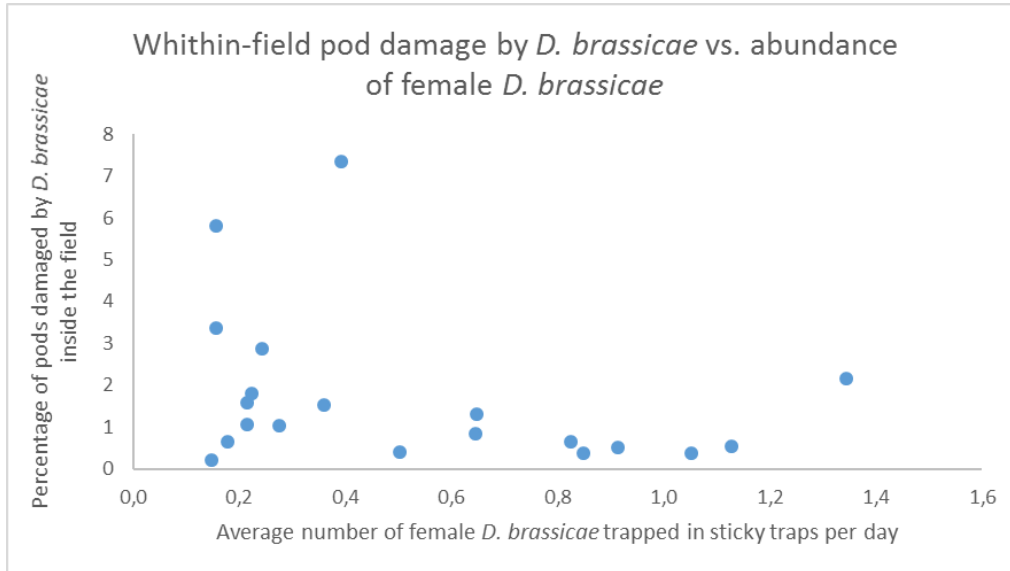


Figure 26: Correlation between abundance of *D. brassicae* in the field border (average of trap set 1 and 2) and pod damage by *D. brassicae* inside the same field (inside and outside of the IFCZ combined) is not statistically significant ($r = -0,324$; $p = 0,163$).

3.6 Importance of distance to previous year's WOSR field.

No significant correlation was found between average pod damage by *D. brassicae* inside the field (inside and outside of the IFCZ combined) and distance to the closest WOSR field from the previous year ($r = -0,036$; $p = 0,882$) (Figure 27).

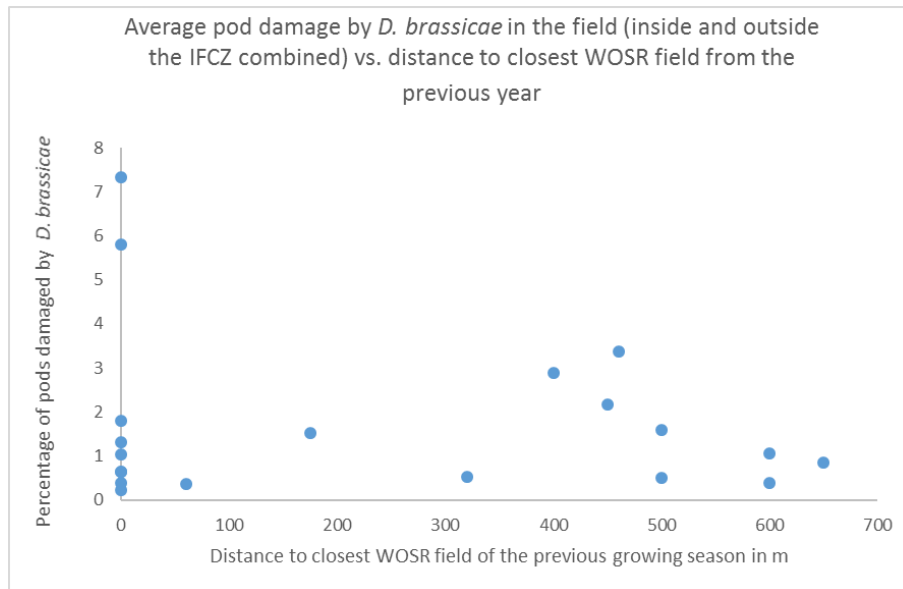


Figure 27: Correlation between average pod damage by *D. brassicae* inside the field and distance to closest WOSR field from the previous season is not statistically significant ($sr = -0,036$; $p = 0,882$).

No significant correlation was found between average pod damage by *D. brassicae* in the field border (mean of trap set 1 and 2) and distance to the closest WOSR field from the previous year ($sr = -0,020$; $p = 0,934$) (Figure 28).

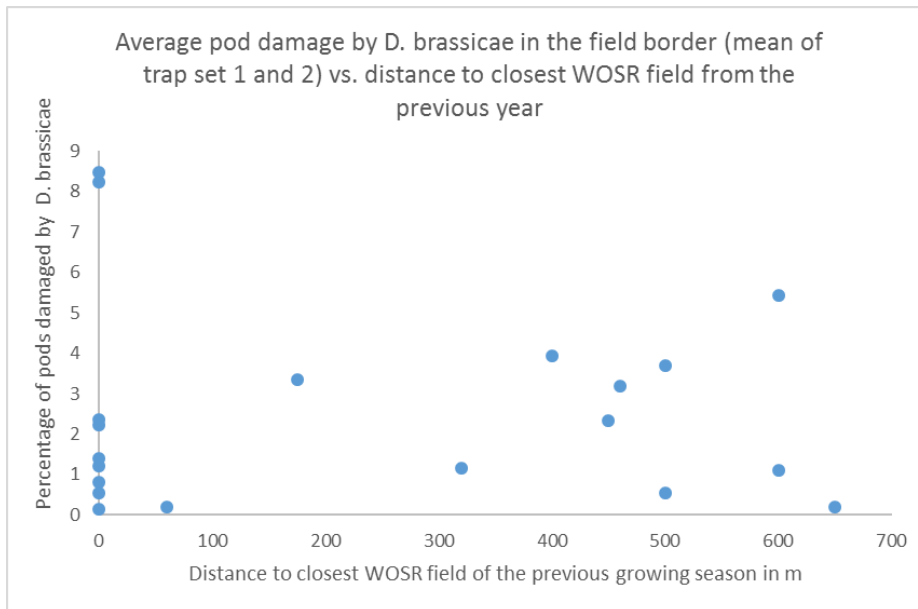


Figure 28: Correlation between average pod damage by *D. brassicae* in the field border and distance to closest WOSR field from the previous season is not statistically significant ($sr = -0,020$; $p = 0,934$).

No significant correlation was found between average abundance of male *D. brassicae* in the sticky traps at trap set 1 and 2 and distance to the closest WOSR field from the previous year (Figure 29). No effect of distance to last year's WOSR field on abundance of male *D. brassicae* was found.

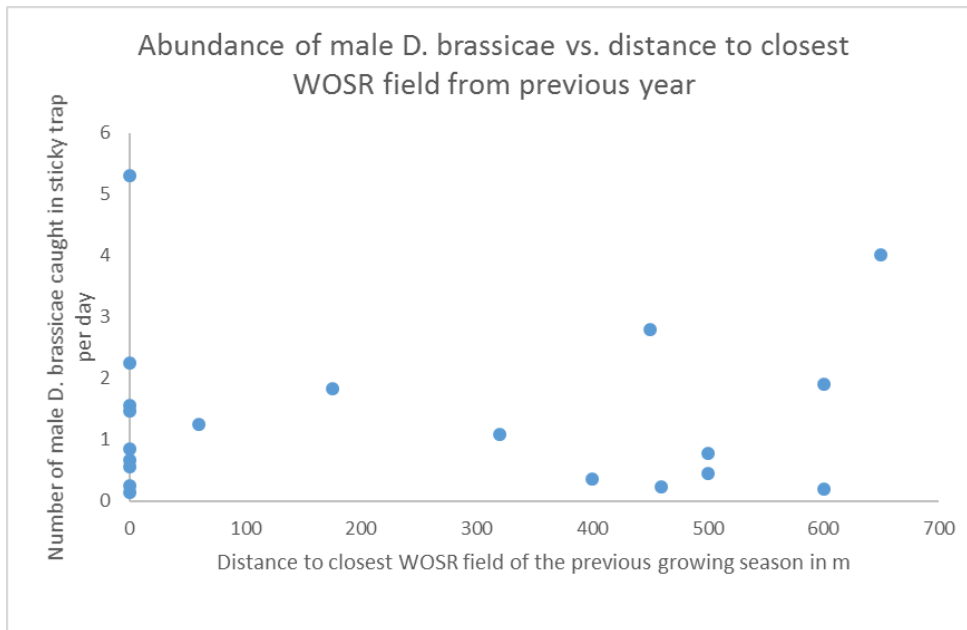


Figure 29: Number of male *D. brassicae* caught in the sticky traps per day vs. distance to closest WOSR field of the previous year. No significant correlation ($sr=0.007$, $p=0.976$)

No significant correlation was found between average abundance of female *D. brassicae* in the sticky traps at trap set 1 and 2 and distance to the closest WOSR field from the previous year (Figure 30). No effect of distance to last year's WOSR field on abundance of female *D. brassicae* was found.

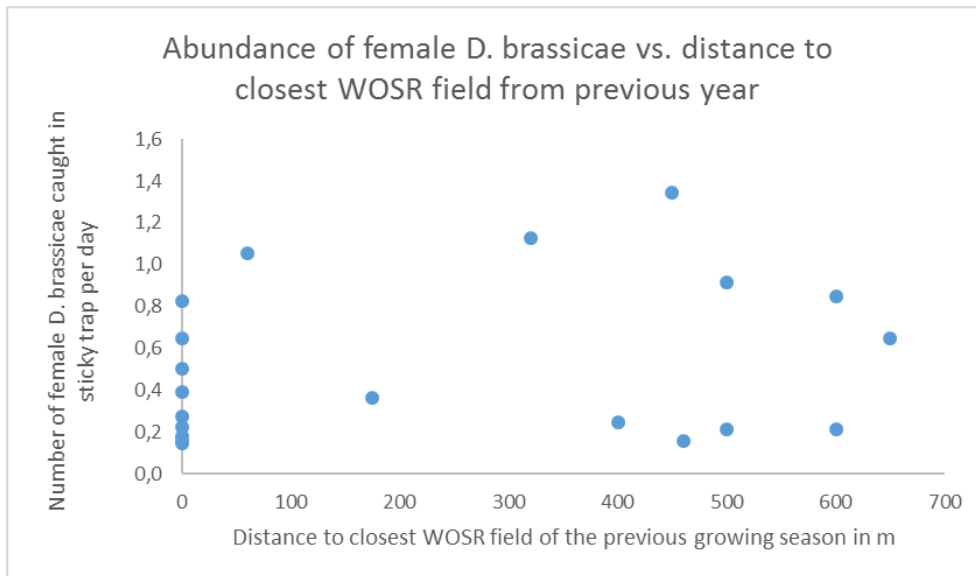


Figure 30: Number of female *D. brassicae* caught in the sticky traps per day vs. distance to closest WOSR field of the previous year. No significant correlation ($sr=0.216$, $p=0.360$)

4 Discussion

Overall it is noteworthy that abundance of *C. obstrictus* and *D. brassicae* was much lower than in earlier studies (Rösvik 2017; Henriksson 2019; Johansson 2019). The average capture of *D. brassicae* over the 20 study locations for the whole study period of 2019 was 41% lower for females and 48% lower for males than the captures from the study of 2018 (Henriksson 2019; Johansson 2019).

The average captures of *C. obstrictus* over the 20 study locations for the whole study period of 2019 was 77% lower for pan traps, 67% lower for sticky traps and 92% lower for visual count than the captures from the study of 2018. Compared with the study of 2017 (Rösvik 2017) the average captures of *C. obstrictus* in the 2019 season were even 98% lower for pan traps and 96% lower for sticky traps. The reductions in *C. obstrictus* and *D. brassicae* captures from 2017 to 2018 might be explained through a cold, wet winter 2017/2018 and an extremely dry summer in 2018 (Johansson 2019). The season of 2019 was not influenced by drought and even though the year of 2019 was on average a little warmer and rainier than the average year in Sweden, it was not considered a year of extreme weather (SMHI 2020). Therefore, weather conditions of the season of 2019 cannot be used as an explanation for the low captures of the two studied insect species. Other possible reasons for observed low levels of *C. obstrictus* and *D. brassicae* might be that populations of natural enemies have grown and reduced populations of the two studied insect species (Aldén et al 2019).

Even the average pod damage by *D. brassicae* was very low in comparison with other years (Aldén et al 2019). Average percentage of pods damaged by *D. brassicae* per field was 50% lower than in 2018 and 82% lower than in 2017. While the highest percentage of pod damage by *D. brassicae* in a field was 54% in 2017 and 10% in 2018, it was only 7% in this study.

The low insect pressure might have been a contributing factor to the good WOSR yield in the season of 2019 that was 72% higher than in 2018 and 21% higher than the average yield of the past five years (Jordbruksverket 2019b). Even the cool weather in May was presumably disadvantageous for *C. obstrictus* and might have reduced activity of the weevil (Aldén et al. 2019).

4.1 Comparison of different sampling methods

Sampling of *C. obstrictus* with different methods

No significant correlations were found between abundance of *C. obstrictus* in sticky traps and pan traps. These results are in contrast to earlier studies that have found statistically significant positive correlations between abundance of *C. obstrictus* in pan traps and sticky traps (Rösvik 2017; Johansson 2019). This might be a result of the very low number of insects caught in the traps in this study compared with studies from previous years.

As the captures from different traps do not correlate, they seem to assess abundance of *C. obstrictus* in different ways and complement

each other. Monitoring with several sampling methods in the same place helps to gain a better understanding of the insect's abundance and phenology. As mentioned in the introduction the spatial distribution of *C. obstrictus* in a field is uneven (Hiiesaar et al. 2003; Williams & Ferguson 2010) and that might explain why captures of the same trap type at trap sets in the same field do not correlate. That there was no correlation found between visual count of *C. obstrictus* at the field border and within the field was also expected because of their uneven spatial distribution and because there is consensus in the literature that pest abundance is often greater at the field border than in the field center (Williams & Ferguson 2010).

It is interesting that visual count is the sampling method that correlates best between sampling points, because visual count is often the only sampling method available to a grower and a method that gives instant results. From the significant correlations between visual count at trap set 1 and at trap set 2 it seems that it would be sufficient to count *C. obstrictus* in one of the places to gain information about current abundance. A reduction of sampling points of visual count could make monitoring more economic for the grower.

When using visual count to assess *C. obstrictus* abundance, some methodological considerations might be useful: During data collection in the field the author noticed that no adult *C. obstrictus* were observed on the plants during windy and/or rainy weather conditions. Unfortunately, these observations are merely anecdotal as no systematic, empirical record of weather conditions was kept during the field study. However, according to the literature, weather conditions might not have been favorable for *C. obstrictus* as flight activity has shown to be highest when relative humidity is low and mean temperature is above 12°C (Tansey et al. 2010). The average temperature in Scania in May 2019 was only 11°C (SMHI 2019b). Relative humidity usually increases before rainstorms that can cause adult mortality and as an adaptation to that flight activity is decreased under higher relative humidity (Tansey et al. 2010). Even other studies have found trap captures of *C. obstrictus* to be low in cool and rainy weather (Hiiesaar et al. 2003; Tansey et al. 2010). The Swedish Board of Agriculture noticed in the beginning of May 2019 that the weather was rather cold and immigration and abundance of adult *C. obstrictus* was rather low in comparison with previous years (Jordbruksverket 2019a). Even data from SMHI (2019c) (Figure 31) shows that the average daily temperature in the beginning of May was slightly below average. Future research should include weather monitoring to investigate the influence of weather as a confounding variable on weevil abundance.

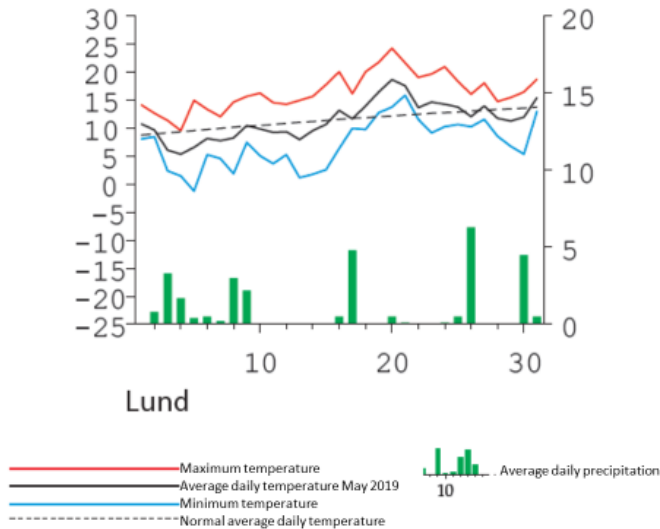


Figure 31: Average daily temperature and average daily precipitation in May 2019 for the city of Lund in Scania. (“Maj 2019 – Lufttemperatur och nederbörd” by SMHI 2019c licensed under CC 4.0 SE)

Another methodological consideration about visual count is that *C. obstrictus* individuals were only counted once a week (six times in total during the study period) when traps were emptied (active monitoring). Results from weekly visual count were even divided by the number of days in between visual count occasions to obtain a value for average number of individuals per sampling point per day in order to enable comparison between assessment of *C. obstrictus* abundance with different sampling methods and in different fields. Comparability with the traps that were constantly present in the studied fields (passive monitoring) could be questioned, as visual count only gave insight into a brief moment of a week and is probably highly influenced by weather situation. Therefore, visual count during unfavorable weather conditions might underestimate *C. obstrictus* abundance.

4.2 Phenology and migration patterns of *C. obstrictus* and *D. brassicae*

Usually *C. obstrictus* is expected to migrate into WOSR host fields during the onset of flowering (Hiiesaar et al. 2003) and create feeding damage on WOSR pods before adults of *D. brassicae* start arriving to the same crop in May (Ekbohm 2010). As figure 19 shows, trap captures of *C. obstrictus* peaked 2-3 weeks later than trap captures of *D. brassicae*, well beyond the time of flowering and into the time of pod maturation. This result is opposite to the expected pattern of migration and has several possible explanations. Weather conditions were unfavorable for *C. obstrictus* as it was quite cold in May 2019 before it became warmer in June (Aldén et al. 2019). These weather conditions might have delayed migration of *C. obstrictus* into the WOSR host crop as the weevil is most active at warmer temperatures (Stephansson & Åhman 1998; Hiiesaar et al. 2003; Williams 2010).

Traps can measure insect abundance only indirectly as only flying, active insects can be captured (Murchie et al. 1999). In other words, *C. obstrictus* might have been present in the WOSR crop but not have

shown much active flying behavior due to low temperatures. Therefore, only very few *C. obstrictus* individuals could be captured in the pan traps and sticky traps during the first three weeks of the study period. Passive behavior of *C. obstrictus* under cold weather conditions might also explain why the number of weevils assessed with visual count (active monitoring) is higher than the number of weevils assessed through trap captures (passive monitoring). During visual count the passively sitting individuals of *C. obstrictus* could be assessed while they could not be caught in the traps as they showed very little flight activity.

Another explanation for the late peak of *C. obstrictus* captures is that sampling is more difficult in a blooming crop as the yellow color of the flowers can compete with the yellow color of the traps (Östrand 2011). During the first three weeks of the study the WOSR crop was flowering and *C. obstrictus* was surrounded by huge amounts of attractive yellow-colored WOSR flowers on plants exuding attractive kairomones and therefore the temptation to fly into the traps might have been low as they were possibly overlooked in the yellow sea of WOSR flowers, especially since the flowers were offered in combination with kairomones.

Interestingly, in accordance with this explanation, pan traps and sticky traps catch less *C. obstrictus* individuals than visual count while the WOSR crop is blooming and after blooming this is reversed and captures of *C. obstrictus* is higher in the traps than with visual count as the attractive yellow color of the trap becomes more visible to the insects after flowering of the crop has ended. These potential disadvantages of pan traps and sticky traps for monitoring, that they attract *C. obstrictus* and *D. brassicae* only by color and can easily be outcompeted in a blooming crop, calls for the development of traps that are attractive to the target insect species in more ways than just color. The development of selective pheromone traps could be a solution. Selective pheromone traps with female sexual pheromones could be used for mass trapping of *D. brassicae* males or possibly even for mating disruption. And pheromone traps with floral volatiles or other kairomones from the WOSR plant could even be used to attract and kill or monitor *D. brassicae* of both sexes.

The number of *C. obstrictus* assessed with visual count declines drastically after flowering, this is because it was mostly the canopy that was being examined during visual count and without the presence of the yellow flowers the canopy *C. obstrictus* might have visited more on other parts of the WOSR plants. And the weevils were also much easier to see in front of the yellow color of the petals than the green color of the stem and leaves.

As there is significant correlation between *C. obstrictus* abundance and pod damage by *D. brassicae* (Figure 21), pods pre-damaged by *C. obstrictus* must have been available to *D. brassicae* females for oviposition, especially since the short-lived *D. brassicae* females do not like to oviposit into very freshly made holes (Åhman 1987). Figure 19 shows that *C. obstrictus* and *D. brassicae* were definitely present during the same time. However, as the numbers of *C. obstrictus* were very low during the first three weeks of the study it can be assumed that weevil

feeding damage facilitated *D. brassicae* oviposition even during these weeks, but that oviposition might have been even more successful and resulting damage more severe if *C. obstrictus* abundance had peaked before *D. brassicae* abundance peaked, because populations of *D. brassicae* cannot increase dramatically without sufficient quantities of available pre-damaged pods (Hughes & Evans 2003). Future research should start monitoring *C. obstrictus* earlier as Aldén et al. (2019) recorded the highest abundance of trapped *C. obstrictus* in 2019 around April 26th, when the WOSR crop just started flowering, approximately two weeks before data collection for this study started.

Numbers of male *D. brassicae* captured are higher than number of female *D. brassicae* captured (Figure 19). As traps assess abundance indirectly through flight activity (Murchie et al. 1999), more males are captured as they show more active flying behavior while searching for females (Williams et al. 1987). It is noteworthy that male capture is high even though, according to the literature, the males die shortly after mating at the emergence site (Williams et al. 1987; Williams & Ferguson 2010). But since Scania is highly agriculturally exploited, the distance to the closest WOSR crop of last season is oftentimes not far. As cocooned *D. brassicae* can survive for several years in the soil, emergence presumably happens in many cases in close proximity to a WOSR field. Johansson (2019) also found that male *D. brassicae* were overrepresented in the traps.

As expected, abundance of the adults of both insect species declined towards the end of the data collection period as the adult generation of *C. obstrictus* and even the first adult generation of *D. brassicae* die after oviposition (Hiiesaar et al. 2003; Ekbom 2010; Williams 2010).

Correlation between abundance of *C. obstrictus* and *D. brassicae* in the same field

No significant correlation was found between the abundance of *C. obstrictus* and the abundance of adult *D. brassicae* in the traps of the same trial site. This indicates that *C. obstrictus* and *D. brassicae* have different spatial distribution. This is a little surprising as both insect species are attracted to the crop through anemotaxis (Williams & Cook 2010) and both species have a reputation of visiting predominantly in the field border (Williams & Ferguson 2010). But distinctions in the biology of the two insect species might explain the different spatial distribution: The differences in overwintering habits (Williams 2010) and the more advanced flying skills of *C. obstrictus* (Williams & Cook 2010; Williams & Ferguson 2010), might broaden the weevil's flexibility to distribute over the landscape and a host crop in comparison to the less mobile *D. brassicae*. Another difference between the two insect species is the duration of the life-span as adults. While the longer-living *C. obstrictus* typically migrates into the crop during the onset of flowering (Hiiesaar et al. 2003) and then has time the whole season to spread out over the crop, newly-emerged adults of the very short-lived *D. brassicae* species arrive to the crop under several weeks from May to July and die shortly after oviposition (Ekbom 2010). While *D. brassicae* adults mate

at the site of emergence before migrating to the host crop, the adults of *C. obstrictus* mate in the host crop and the spatial distribution is uneven because of the aggregation flight of the weevils (Hiiesaar et al. 2003). Another difference in biology that could contribute to uneven spatial distribution of the two insect species are differences in oviposition behavior: While several *D. brassicae* females can oviposit into the same pre-damaged pod, *C. obstrictus* females place only one egg in each pod and other females are deterred from oviposition with a pheromone (Åhman 1987; Williams 2010). Another indication for different spatial distribution of *C. obstrictus* and *D. brassicae* are also the results described in section 3.3 that the captures of trap set 1 and trap set 2 are correlated for *D. brassicae* but not for *C. obstrictus*, which indicates that flight activity is more evenly distributed for *D. brassicae* compared with *C. obstrictus*.

Numbers of male and female *D. brassicae* caught in the sticky traps

Abundance of male and female *D. brassicae* captured with sticky traps was significantly correlated in a field over the whole season. It seems not surprising that males are attracted to the same places as females, because the females produce a sex pheromone that is attractive to the males (Williams 2010). But according to the literature mating happens at the emergence site before the females migrate to the WOSR host field alone while the males die shortly after mating (Williams et al. 1987; Williams & Ferguson 2010). Therefore, it is surprising that more males than females are captured in the WOSR host crop. There is indication in the literature that even some males might move from the site of emergence to nearby WOSR fields in case of favorable wind conditions (Williams et al. 1987). And male captures are usually higher at the emergence site than female captures, which is explained through greater flight activity of the males that are searching for the females (Williams et al. 1987). As approximately 40% of Scania's area is used as arable land and WOSR production is often clustered in favorable areas (Figure 9), the migration distance from emergence site to the nearest WOSR field might oftentimes be quite short in Scania. As cocooned *D. brassicae* can survive in diapause in the soil for several years (Williams et al. 1987; Williams 2010; Graora et al. 2015; Gunnarsson 2016), the site of emergence might in many cases be near to or even overlap with the closest WOSR field of the season. The captured males may therefore represent males that have migrated from nearby emergence sites as well as males that have emerged from the soil in the WOSR field after several years in diapause. And the higher abundance of males in the sticky traps compared to female abundance can be explained by higher flight activity of the males in search of females for mating. However, future research could investigate if *D. brassicae* males and females are similarly attracted to yellow sticky traps. The positive correlation between the abundance of the two sexes indicates also that conditions that are favorable for males probably are also favorable for females.

4.3 Migration into the field and trap location

No differences for migration into the crop from different sides of the same HHS field could be found for either of the two studied insect species. The only exception was that abundance of female *D. brassicae* caught in the sticky traps in field 21 differed significantly on the different sides of the field. Interestingly it was the side that was closest to the WOSR field of the previous year that had the lowest amount of trapped *D. brassicae* females (trap sets 1+2, Table 4). A possible explanation could be that there was an overgrown shrubbery grove precisely outside the field on the side closest to last year's WOSR field. This variation in the otherwise completely open and flat landscape might have influenced wind currents and migration patterns in such a way that less insects were trapped.

The results were expected for *C. obstrictus* but unexpected for *D. brassicae*. That abundance of captured *D. brassicae* was not higher on the side closes to last year's WOSR field might be explained with the quantity of arable land in Scania resulting in the situation that distance to last year's WOSR is often short. And as *D. brassicae* larvae can survive in the soil for several years (Williams et al. 1987; Williams 2010; Graora et al. 2015; Gunnarsson 2016), they can migrate towards the crop from different fields in the landscape that were previously cultivated with WOSR. The emergence of *D. brassicae* adults in several fields close to the studied WOSR field could lead to migration into the crop from different sides of the field. As a methodological reflection it should be mentioned that distance from last year's WOSR field to the current WOSR crop was only measured in a straight line in this study, while predominant wind directions or surrounding vegetation were not evaluated at all. Wind conditions around the field should be important as *D. brassicae* adults are weak flyers getting dispersed with the assistance of wind currents (Williams & Cook 2010). As wind conditions and even the influences of surrounding vegetation on wind conditions are relevant for the dispersal of *D. brassicae* (Nilsson 2009; Williams & Cook 2010; Williams & Ferguson 2010; Gunnarsson 2016) and it is often windy in Scania's open agricultural landscape, future research should include evaluation of the influence of predominant wind directions and surrounding vegetation on the ability of *D. brassicae* to spread and infest a WOSR field.

As no significant difference in abundance of study organism trapped on the different sides of the same field was found in the data from the four HHS fields, it was assumed that it was sufficient to position traps on only one side of the field in order to capture a representative sample of insect migration into the field. While collecting data for this study it became apparent that monitoring with one trap set on each side of the field is time-consuming. Moving in uneven terrain with research equipment around the perimeters of a large WOSR field through increasingly higher vegetation to count *C. obstrictus* and to empty and exchange traps could take up to one hour per HHS field. If an experimental set up with traps on only one field side (as in the 16 standard fields) is sufficient for

monitoring, this makes monitoring less difficult and more economic for farmers.

Abundance of the insects caught at the 20 trial sites in the traps of the two different trap sets on the field side that was closest to last year's WOSR field (trap set 1 and trap set 2) was highly significantly correlated for both male and female *D. brassicae*. This suggests that the migration of the midges into the field is even and that one trap set might have been sufficient for monitoring if monitoring should be done more economic. *C. obstrictus* abundance on the other hand was not correlated between the different trap types. But optical count was correlated between trap set 1 and 2. The spatial distribution of *C. obstrictus* is more complex than the spatial distribution of *D. brassicae* and flowering of the crop might have interfered with weevil trapping. Future research should start monitoring earlier in the season before WOSR starts to bloom in order to investigate if *C. obstrictus* captures are influenced by flowering of the crop.

4.4 Effect of insecticide use on average *D. brassicae* pod damage

No difference between average pod damage by *D. brassicae* in the IFCZ and the insecticide-treated inside of the field (outside of the IFCZ) could be found (Figure 20). This indicates that insecticide treatment had no effect. It might have been timed wrong and applied too early - in late bloom of the crop as recommended (Jordbruksverket 2019a), but before *C. obstrictus* migrated into the field - as weevil abundance peaked unusually late in 2019 (Figure 19). As timing of insecticide application and peak in *C. obstrictus* abundance was mismatched, efficacy of insecticide treatment might have been reduced.

Another possible explanation is that insect abundance was so low in general (Aldén et al. 2019) that insecticide treatment showed no effect. In 2018 significant differences were found between the average percentage of pod damage by *D. brassicae* in an IFCZ and the average percentage of pod damage by *D. brassicae* in parts of the field that had been treated with insecticides. The average difference was 3.2% and the average percentage of pod damage was still 2% in areas of the field where insecticides had been used (Johansson 2019, Henriksson 2019). As the average percentage of pod damage by *D. brassicae* in the 20 WOSR fields of the present study was shown to be only 2% on average, regardless of pesticide use or not, it can be assumed that these damage levels are so low that effects of insecticides could not become visible. Effects of insecticide application on average pod damage by *D. brassicae* seem to vary even in the literature (Nilsson 2009). Gunnarsson & Nilsson (2017) showed a significant effect of insecticides, reducing pod damages by *D. brassicae* with 50-80%, although no significant differences in amount of yield could be found.

It is noteworthy that insecticide was used on at least 12 of the WOSR fields, even though insect pressure was extremely low compared with other years (Rösvik 2017; Aldén et al. 2019; Henriksson 2019; Johansson 2019) and *C. obstrictus* observations were never above the economic threshold (Aldén et al. 2019). Insecticide application despite

low insect pressure, far below the economic threshold, and with questionable efficacy can be considered unnecessary. It is noteworthy that insecticides apparently are still overused without consideration of the economic threshold, although IPM is mandatory in the EU since 2014 and one of the most important objectives with IPM is to reduce pesticide use. This situation shows that there is still possibility for improvement of IPM implementation and that farmers need better tools for monitoring and decision making. It is of high relevance to growers to quickly gain contemporary information about pest status in a field. There might be a discrepancy between time-consuming scientific procedures and the need for quickly available decision-supporting information for daily agricultural work. It could be observed during data collection (not through any empirical assessment, only through some spontaneous conversations in the field) that farmers were interested in monitoring data in order to be able to decide about insecticide treatment. And due to the logistic routines of this research project farmers could only be provided with data that was oftentimes already one week old. It takes time to count insects under the microscope, but farmers need fast information. Hopefully in the future cooperation between scientists and farmers can be improved to reduce unnecessary insecticide applications.

As data analysis takes time it is helpful that a significant correlation was found between visual count and damage (Figure 24). Despite some inherent methodological weaknesses (section 4.1), visual count is a fast, economic monitoring method for the farmer to control if *C. obstrictus* populations are currently below the economic threshold.

No significant differences between average percentage of pod damage by *D. brassicae* inside the field and average percentage of pod damage by *D. brassicae* in the field border was found. This result was not in line with the expectation that pod damage by *D. brassicae* is usually more severe in the field border (Stephansson & Åhman 1998; Nilsson 2009; Williams 2010; Gunnarsson 2016). Maybe the unusual phenology of *C. obstrictus* has influenced spatial distribution of pod damage by *D. brassicae* in the field.

There is a highly significant correlation between average pod damages inside the field and average damages at the field border. This indicates that it might be sufficient to assess pod damage at the field border in order to predict damage severity inside the field. This is very suitable for making damage assessment more economic, as it can be time-consuming and troublesome to walk inside a full-grown WOSR field.

Reflections about the experimental design with an insecticide-free control zone (Figure 13) might lead to other explanations for the missing difference in average percentage of pod damage by *D. brassicae* inside and outside the IFCZ. In case insecticides have been dispersed with the wind into the IFCZ during insecticide application in the surrounding parts of the field, the missing insecticide effect might be due to the fact that the IFCZ was not free from insecticides and insects were killed there as well. Another possibility might be that insects from the IFCZ have re-

infested the insecticide-treated part of the field after insecticide application. Despite these concerns, experimental designs with insecticide-free control zones or several parcels with different insecticide treatments for comparison of treatment efficacy are very common in agricultural research. In the four HHS fields, independent from the present study, a different study was conducted with a study design including many different parcels that were each much smaller than the IFCZ used in this study, to compare efficacy of different insecticides against *C. obstrictus* (Gunnarsson 2018). It might be an interesting research project to evaluate the advantages and disadvantages of experimental designs with an IFCZ and systematically evaluate dispersal of pesticides and migration pattern of different insects into and out of the IFCZ.

4.5 Relation between insect abundance and amount of pod damage by *D. brassicae*

Correlations between abundance of *C. obstrictus* and amount of pod damage by *D. brassicae*

Average *C. obstrictus* abundance in pan traps correlated significantly with average pod damage by *D. brassicae* inside the field (Figure 22). This means that monitoring the field with pan traps on the field border might help to predict pod damage by *D. brassicae*. Even visual count of *C. obstrictus* in the field border correlated significantly with pod damage inside the field (Figure 24). This means that the number of *C. obstrictus* counted on plants at the edge of the field should be a good predictor for pod damage by *D. brassicae* inside the field. The monitoring data is useful to control if pest abundance is under the economic threshold and even to further develop models that explain the influence of different factors on pod damage by *D. brassicae*.

In accordance with theoretical expectations the results showed that abundance of *C. obstrictus* facilitates pod damages by *D. brassicae* and that controlling the weevil might be a successful pest control measure against the extremely difficult controllable *D. brassicae*.

C. obstrictus abundance is usually higher in the field border than in the middle of the field, especially when the pest migrates into the field (Williams & Ferguson 2010). Thus, caution must be taken to not overestimate insect abundance if sampling is restricted to the field border (Williams 2010). Insect sampling is quite time-consuming and therefore monitoring has to be balanced between accuracy and efficacy in order to be functional. In this study insect traps were only placed at the field border, an experimental set up that contains the risk of overestimation of insect abundance. But on the other hand, the objective of the study is to increase knowledge about the correlations between pest abundance and crop damage and to contribute to development of applicable IPM decision tools for farmers. Therefore, the experimental set up in the field border is justified as an economic, pragmatic monitoring method for investigations about economic thresholds. Furthermore, *C. obstrictus* abundance in the field border correlates with

within-field pod damage by *D. brassicae*, confirming that monitoring in the field border only, may be an economic and feasible damage prediction tool for the farmer.

Correlations between abundance of *D. brassicae* and pod damage by *D. brassicae*

No significant correlations were found between abundance of male *D. brassicae* in the sticky traps in the field border and pod damage by *D. brassicae* inside the field (Figure 25). Maybe the presence of males has no influence on pod damage by *D. brassicae* as it is the oviposition of the females and the resulting larvae that damage the pod and therefore the presence of males has no influence as mating happens at the site of emergence before the females migrate towards the WOSR crop (Williams et al. 1987; Williams & Ferguson 2010). However, a significant correlation was found between abundance of male and female *D. brassicae* (Table 3), suggesting that in a field with many females, that can potentially damage pods, there should also be many males and therefore abundance of male *D. brassicae* should be correlated with pod damage by *D. brassicae*.

Monitoring in this study covered only the period of activity of the first adult generation. It would be interesting to continue monitoring while the second adult generation emerges inside the WOSR field approximately in late June (Williams et al. 1987; Williams 2010; Ekbohm 2010) and starts mating and oviposition. A second damage assessment later in the season, after the females of the second generation have oviposited, might help to investigate the influence of male *D. brassicae* abundance on pod damage done by females of the second adult generation. A drawback might be caused by difficulties with differentiating between pod damages done by the first or second adult generations.

There were no significant correlations found between abundance of female *D. brassicae* in the sticky traps at the field border and pod damage by *D. brassicae* inside the field (Figure 26). This result was unexpected, as it is the oviposition by the females and the resulting larvae that cause WOSR pod damage. Therefore, higher abundance of female *D. brassicae* should cause higher amounts of WOSR pod damage. This result might also explain why abundance of male *D. brassicae* is not correlated with pod damage by *D. brassicae*, even though correlation between abundance of male and female *D. brassicae* in the same location is significant (Table 3).

The results might be caused by the extremely low number of trapped insects and the very low levels of damage in comparison to previous years (Rösvik 2017; Aldén et al. 2019; Henriksson 2019; Johansson 2019). The results might also have been influenced by unknown confounding variables that could gain influence as numbers of trapped insects and pod damage were unusually low. Interestingly, Johansson (2019) found no statistically significant effect between abundance of *D. brassicae* or *C. obstrictus* and pod damage at the field edge or 20 m into the field. Apparently, study results can differ between different seasons even if the experimental design is quite similar. The reason might be

that abundance of the two insect species and amount of pod damage might be moderated by more than the investigated variables (e.g. abundance of natural enemies). Future research and variations in study results might help to clarify in more detail which variables influence the relationship between abundance of *C. obstrictus* and *D. brassicae* and WOSR pod damage by *D. brassicae*.

4.6 Importance of distance to previous year's WOSR field

No significant correlation was found between pod damage by *D. brassicae* and distance to the closest WOSR field from the previous year. There was also no significant correlation between abundance of male or female *D. brassicae* and the distance to last year's WOSR field. As *D. brassicae* is a weak flyer (Williams & Ferguson 2010), it was expected that pod damage severity should increase with decreasing distance to the nearest WOSR field from the previous growing season. This effect might not have become visible as the pod damages were very low. Another explanation is the long survival of larvae in the soil for up to five years (Williams et al. 1987; Williams 2010; Graora et al. 2015; Gunnarsson 2016) and the omnipresence of arable land in Scania. The distance to a field where WOSR has been grown sometime during recent years oftentimes is not long and therefore *D. brassicae* adults can migrate into a WOSR field from different sides, originating from different nearby previous WOSR fields. As wind conditions are important for migrating females, further research should include wind measurements to investigate if favorable wind conditions could be a confounding variable influencing migration of *D. brassicae* and blurring the correlation between abundance of and damage by *D. brassicae* and distance to last year's WOSR field.

5 Conclusion

It can be concluded that the number of *C. obstrictus* and *D. brassicae* captured in the two trap types and assessed through visual count was very low compared to earlier studies. Even the percentage of pod damage was very low in comparison with previous years. Phenology of the two insect species showed that abundance of *C. obstrictus* peaked unusually late in the season, disfavoring *D. brassicae* oviposition. Nonetheless, significant correlations could be found between *C. obstrictus* abundance in the field border and pod damage caused by *D. brassicae* inside the field. Consistent with earlier research, it seems that feeding damage on the pods by *C. obstrictus* facilitates oviposition into the pod by *D. brassicae* and resulting pod damage. These results indicate potential to increase feasibility of monitoring and to develop more economic monitoring routines. Especially since visual count, the most instant, cheapest and simplest monitoring measure, despite having some drawbacks, was significantly correlated with pod damage inside the field. As pest abundance was very low in the field and no significant difference between pod damage inside and outside an insecticide free control zone could be found, it can be assumed that the insecticide treatment in the fields had no effect - probably due to deviating *C. obstrictus* phenology. As insecticides were used in at least 12 of the 20

fields, despite the low numbers of *C. obstrictus*, that never rose above the economic threshold, it can be concluded that IPM methods for control of *C. obstrictus* and *D. brassicae* have to be refined further in order to assist farmers in decision making about pest control in order to decrease pesticide use. Economic thresholds are worthless if they cannot gain trust from the farmers and are not considered in pest control decisions.

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8 Appendix

Table X1: Overview over the 20 trial sites

Table X1: Overview over the different 20 WOSR fields that were trial sites in this study. The table shows which trial site were HHS fields and which were standard fields. Distance to the closest WOSR field from the previous growing season (2018) in m is given for every trial site.

Trial site	Type of field	Distance to last year's WOSR field in m
Field 15	standard	600
Field 14	standard	0
Field 2	standard	0
Field 12	standard	0
Field 9	standard	450
Field 5	standard	0
Field 11	standard	500
Field 8	standard	320
Field 10	standard	0
Field 7	standard	500
Field 1	standard	0
Field 19	standard	460
Field 3	standard	175
Field 16	standard	600
Field 17	HHS	60
Field 18	standard	650
Field 20	standard	0
Field 21	HHS	0
Field 22	HHS	0
Field 23	HHS	400

Table X2: Average daily captures of *C. obstructus* with different sampling methods

Table X2: Average daily captures for the different *C. obstructus* sampling methods

For every sampling method captures from trap set 1 and trap set 2 (Figure 13 and Figure 14) from each of the 20 WOSR fields are presented. The values represent average number of *C. obstructus* individuals captured on average per day over the whole research period (six weeks).

Trial site	pan trap 1	pan trap 2	sticky trap 1	sticky trap 2	visual count 1	visual count 2
Field 15	0,143	0,024	0,190	0,500	0,001	0
Field 14	0,200	0,190	0,086	0,071	0,006	0,007
Field 2	0,095	0,186	0,048	0	0,002	0,001
Field 12	0,251	0	0,048	0,024	0	0
Field 9	0,214	0,643	0,071	0,071	0,001	0,002
Field 5	1,405	0,571	0,143	0	0,002	0,006
Field 11	0,095	0,524	0,167	0,238	0,003	0,005
Field 8	0,262	0,190	0,143	0,238	0,012	0,014
Field 10	0,286	0,048	0,119	0,095	0,003	0
Field 7	0,238	0,161	0,119	0,143	0,001	0,002
Field 1	0,029	0,484	0,200	0,286	0,002	0,002
Field 19	0,286	0,048	0,194	0	0,001	0
Field 3	0,143	0,095	0,024	0,071	0	0,003

Field 16	0,107	0,086	0,714	0,024	0	0
Field 17	0,167	0,024	0	0,024	0	0
Field 18	0,286	0,405	0	0,048	0	0,001
Field 20	0,452	0,048	0,143	0,095	0,002	0
Field 21	0,071	0	0,048	0,071	0	0
Field 22	0,147	0,655	0,051	0,024	0	0,001
Field 23	0,238	0,333	0,048	0,095	0,002	0,008

Table X3: Average daily captures of *C. obstrictus* and *D. brassicae* with different sampling methods accumulated over all 20 trial sites for each week of the study period

Table X3: Average Daily captures of C. obstrictus and D.brassicae with different sampling methods, accumulated over all 20 WOSR fields for each of the six weeks of the study period.

Sampling method	Week 20	Week 21	Week 22	Week 23	Week 24	Week 25
Pan trap <i>C. obstrictus</i>	1,38	2	2,86	12,21	14,57	9,57
Sticky trap <i>C. obstrictus</i>	0,17	2,29	0,29	5	8,57	1,57
Sticky trap male <i>D. brassicae</i>	27,85	43,14	95,71	67	6,29	9,86
Sticky trap female <i>D. brassicae</i>	17,62	23,71	22,43	15,14	1,86	2,86
Visual count <i>C. obstrictus</i>	1,63	4,57	0,57	0,86	0	0

Table X4: Average daily captures of male and female *D. brassicae* at the 20 trial sites

Table X4: Average daily captures of male and female D. brassicae in the 20 WOSR fields. Captures of males and females in sticky traps of trap set 1 and trap set 2 are shown as well as the mean of the two trap sets. The average values were calculated as mean of the whole six-week study period.

Trial site	D. brassicae male 1	D. brassicae male 2	D. brassicae male 1+2	D. brassicae female 1	D. brassicae female 2	D. brassicae female 1+2
Field 15	0,119	0,286	0,203	0,167	0,262	0,215
Field 14	0,057	0,214	0,136	0,171	0,143	0,157
Field 2	0,610	0,500	0,555	0,681	0,324	0,503
Field 12	0,884	0,807	0,845	0,161	0,132	0,147
Field 9	2,053	3,543	2,798	1,610	1,076	1,343
Field 5	1,810	1,310	1,560	0,333	0,452	0,393
Field 11	0,476	0,405	0,440	0,167	0,262	0,214
Field 8	1,157	1,019	1,088	0,890	1,367	1,129
Field 10	7,114	3,490	5,302	0,552	1,095	0,824
Field 7	0,776	0,785	0,781	1,033	0,795	0,914
Field 1	0,491	0,827	0,659	0,467	0,829	0,648
Field 19	0,281	0,167	0,224	05.10	0,095	0,155
Field 3	2,351	1,304	1,828	0,214	0,506	0,360
Field 16	2,714	1,074	1,894	1,164	0,532	0,848
Field 17	0,914	1,571	1,243	0,767	1,338	1,052
Field 18	3,281	4,743	4,012	0,714	0,576	0,645
Field 20	0,456	2,464	1,460	0,238	0,313	0,276
Field 21	0,357	4,143	2,250	0,238	0,119	0,179
Field 22	0,193	0,312	0,253	0,267	0,180	0,223
Field 23	0,286	0,429	0,358	0,200	0,286	0,243

Table X5: Average daily *C. obstrictus* and *D. brassicae* captures from different sampling methods on each side of the four HHS fields for each week of the study period

Table X5: Average numbers of C. obstrictus and D. brassica captured per day with the different sampling types on each side of the four different HHS fields for each of the six weeks of the sampling period

Trial site	Trap set	Sampling method	insect species	week 20	week 21	week 22	week 23	week 24	week 25
Field 21	1+2	pan trap	<i>C. obstrictus</i>	0	0	0	0	0,143	0,071
Field 21	3	pan trap	<i>C. obstrictus</i>	0	0	0	1	0,571	0,857
Field 21	4	pan trap	<i>C. obstrictus</i>	0	0	0	1,143	0,571	0,286
Field 21	5	pan trap	<i>C. obstrictus</i>	0	0,286	0	0,286	0,286	0,143
Field 21	1+2	sticky trap	<i>C. obstrictus</i>	0	0,071	0	0,071	0,286	0
Field 21	3	sticky trap	<i>C. obstrictus</i>	0	0	0	0	0,286	0
Field 21	4	sticky trap	<i>C. obstrictus</i>	0	0	0	0	0	0,286
Field 21	5	sticky trap	<i>C. obstrictus</i>	0	0	0	0	0	0
Field 21	1+2	visual count	<i>C. obstrictus</i>	0	0	0	0	0	0

Field 21	3	visual count	<i>C. obstrictus</i>	0	0,143	0	0,286	0	0
Field 21	4	visual count	<i>C. obstrictus</i>	0	0	0,143	0	0,286	0
Field 21	5	visual count	<i>C. obstrictus</i>	0	0,429	0	0	0	0
Field 21	1+2	sticky trap	<i>D. brassicae</i> male	0,143	0,786	11,571	0,857	0,071	0,071
Field 21	3	sticky trap	<i>D. brassicae</i> male	0,571	2,429	2,857	2,571	0	0
Field 21	4	sticky trap	<i>D. brassicae</i> male	0,286	1,143	3	1,571	0	0
Field 21	5	sticky trap	<i>D. brassicae</i> male	0,143	1,714	2,714	1,714	0	0,143
Field 21	1+2	sticky trap	<i>D. brassicae</i> female	0,143	0,143	0,286	0,357	0	0,143
Field 21	3	sticky trap	<i>D. brassicae</i> female	0	1	0,857	0,7143	0	0
Field 21	4	sticky trap	<i>D. brassicae</i> female	0,143	0,286	1	3	0,143	0,143
Field 21	5	sticky trap	<i>D. brassicae</i> female	0,857	0,571	1,571	0,714	0	0,426
Field 22	1+2	pan trap	<i>C. obstrictus</i>	0	0,071	0	0	0,423	2,286
Field 22	3	pan trap	<i>C. obstrictus</i>	0	0	0,143	1,5	0,286	0
Field 22	4+5	pan trap	<i>C. obstrictus</i>	0	0,143	0	0,083	0,357	0
Field 22	1+2	sticky trap	<i>C. obstrictus</i>	0	0	0	0	0	0,143
Field 22	3	sticky trap	<i>C. obstrictus</i>	0	0	0	1	1,429	0,571
Field 22	4+5	sticky trap	<i>C. obstrictus</i>	0,056	0	0	0,083	0,214	0
Field 22	1+2	visual count	<i>C. obstrictus</i>	0,056	0	0	0	0	0
Field 22	3	visual count	<i>C. obstrictus</i>	0	0	0	0	0	0
Field 22	4+5	visual count	<i>C. obstrictus</i>	0,167	0	0	0	0	0
Field 22	1+2	sticky trap	<i>D. brassicae</i> male	0,667	1,571	0,426	0	0,143	0,429
Field 22	3	sticky trap	<i>D. brassicae</i> male	1,444	1,714	0,5		0	0,143
Field 22	4+5	sticky trap	<i>D. brassicae</i> male	0,556	0,929	0		0	0
Field 22	1+2	sticky trap	<i>D. brassicae</i> female	0,5	0,786	0,286	0	0,286	0,571
Field 22	3	sticky trap	<i>D. brassicae</i> female	0,444	1,714	0,5		0	0,143
Field 22	4+5	sticky trap	<i>D. brassicae</i> female	0,389	0,929			0	0
Field 23	1+2	pan trap	<i>C. obstrictus</i>	0	0	0,2143	0,286	0,714	0,5
Field 23	3	pan trap	<i>C. obstrictus</i>	0	0	0	0,143	2,571	0,286
Field 23	4	pan trap	<i>C. obstrictus</i>	0	0,143	0	0,143	0,571	0,857
Field 23	5	pan trap	<i>C. obstrictus</i>	0	0	0,143	0,857	0	0
Field 23	1+2	sticky trap	<i>C. obstrictus</i>	0	0,286	0	0	0,143	0
Field 23	3	sticky trap	<i>C. obstrictus</i>	0	0,571	0	0		0,143
Field 23	4	sticky trap	<i>C. obstrictus</i>	0	0	0	0,286		0,286
Field 23	5	sticky trap	<i>C. obstrictus</i>	0	0	0	0		0
Field 23	1+2	visual count	<i>C. obstrictus</i>	0,143	0,5	0	0	0	0
Field 23	3	visual count	<i>C. obstrictus</i>	0,286	0	0	0,143	0	0
Field 23	4	visual count	<i>C. obstrictus</i>	0	0	0	0	0	0
Field 23	5	visual count	<i>C. obstrictus</i>	0	0	0	0	0	0
Field 23	1+2	sticky trap	<i>D. brassicae</i> male	0,214	0,286	1	0,5	0	0

Field 23	3	sticky trap	<i>D. brassicae</i> male	0,143	0	0,286	0,286		0
Field 23	4	sticky trap	<i>D. brassicae</i> male	0,429	0,143	0,143	0		0
Field 23	5	sticky trap	<i>D. brassicae</i> male	0,143	0	0,429	0,429		0
Field 23	1+2	sticky trap	<i>D. brassicae</i> female	0,429	0,214	0,214	0,429	0	0,143
Field 23	3	sticky trap	<i>D. brassicae</i> female	0	0,286	0,857	0,714		0
Field 23	4	sticky trap	<i>D. brassicae</i> female	0,571	0,286	0,286	0,714		0,143
Field 23	5	sticky trap	<i>D. brassicae</i> female	0	0,571	0,143	0,286		0
Field 17	1+2	pan trap	<i>C. obstrictus</i>	0	0	0	0	0,286	0,286
Field 17	3	pan trap	<i>C. obstrictus</i>	0	0	0	0	0,143	0
Field 17	4	pan trap	<i>C. obstrictus</i>	0	0	0	0	0	0,286
Field 17	5	pan trap	<i>C. obstrictus</i>	0	0	0	0	0	0,286
Field 17	1+2	sticky trap	<i>C. obstrictus</i>	0	0	0	0	0,071	0
Field 17	3	sticky trap	<i>C. obstrictus</i>	0	0	0	0	0	0,286
Field 17	4	sticky trap	<i>C. obstrictus</i>	0	0	0	0	0,286	0,286
Field 17	5	sticky trap	<i>C. obstrictus</i>	0	0	0	0	0	0,143
Field 17	1+2	visual count	<i>C. obstrictus</i>	0	0	0	0	0	0
Field 17	3	visual count	<i>C. obstrictus</i>	0	0	0	0	0	0
Field 17	4	visual count	<i>C. obstrictus</i>	0	0	0,286	0	0	0
Field 17	5	visual count	<i>C. obstrictus</i>	0	0	0	0	0	0
Field 17	1+2	sticky trap	<i>D. brassicae</i> male	0,6	0,571	2,286	3,5	0,429	0,357
Field 17	3	sticky trap	<i>D. brassicae</i> male	0,8	1,714	1,714	1,143	0,286	0
Field 17	4	sticky trap	<i>D. brassicae</i> male	0,8	0,429	8,286	0,571	0,429	0,429
Field 17	5	sticky trap	<i>D. brassicae</i> male	0,8	2,286	1,143	2,423	0	0,143
Field 17	1+2	sticky trap	<i>D. brassicae</i> female	1,1	2,214	1,426	1,357	0,071	0,143
Field 17	3	sticky trap	<i>D. brassicae</i> female	0,2	1,143	1,857	0,429	0,286	0
Field 17	4	sticky trap	<i>D. brassicae</i> female	0,2	0,286	4,143	0,429	0	0,286
Field 17	5	sticky trap	<i>D. brassicae</i> female	0	0,571	0,286	0,857	0,286	0

Table X6: Average pod damage by *D. brassicae* in % for the different sampling points in each of the 20 WOSR fields

Table X6: Average pod damage by *D. brassicae* in % for the different sampling points (trap set 1, trap set 2, inside the IFCZ, inside the field outside of the IFCZ) for each of the 20 different WOSR fields. Average values for damage in the field border (mean from trap set 1 and trap set 2) and within-field damage (mean from IFCZ and field) are also given. At each sampling points percentage of damage on the three topmost shoots of 10 randomly chosen WOSR plants was assessed and a mean percentage of damage was calculated for every sampling point.

Trial site	pod damage trap set 1 (%)	pod damage trap set 2 (%)	pod damage 1+2 mean (%)	pod damage IFCZ (%)	pod damage field outside IFCZ (%)	pod damage IFCZ + field outside IFCZ mean (%)
Field 15	3,63	7,23	5,43	0,51	1,61	1,06
Field 14	8,97	7,50	8,24	5,89	5,71	5,80
Field 2	0,07	0,23	0,15	0,21	0,58	0,40
Field 12	0,59	2,18	1,39	0,14	0,29	0,22
Field 9	2,22	2,42	2,32	2,96	1,36	2,16
Field 5	7,82	9,13	8,48	8,10	6,58	7,34
Field 11	4,06	3,33	3,70	2,84	0,32	1,58
Field 8	2,07	0,24	1,16	0,53	0,54	0,54
Field 10	0,41	0,67	0,54	0,88	0,42	0,65
Field 7	0,35	0,71	0,53	0,51	0,49	0,50
Field 1	1,85	2,87	2,36	1,48	1,15	1,32
Field 19	3,54	2,86	3,20	2,15	4,58	3,37
Field 3	2,21	4,48	3,35	0,72	2,33	1,53
Field 16	1,27	0,93	1,10	0,34	0,43	0,39
Field 17	0,21	0,19	0,20	0,48	0,27	0,38
Field 18	0,29	0,10	0,20	0,66	1,03	0,85
Field 20	2,68	1,77	2,23	0,19	1,9	1,05
Field 21	0,68	1,74	1,21	0,65	0,65	0,65
Field 22	1,14	0,50	0,82	1,25	2,37	1,81
Field 23	2,68	5,18	3,93	4,02	1,75	2,89