Special Issue on Pollination: Conference Reports from SCAPE

ESCAPED OILSEED RAPE: OCCURRENCE IN THE AGRICULTURAL LANDSCAPE AND POTENTIAL POLLEN-MEDIATED GENE FLOW FROM CROP OILSEED RAPE

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Journal of Pollination Ecology, 28(10), 2021, pp 127-137

DOI: 10.26786/1920-7603(2021)631

Received 12 October 2020, accepted 3 March 2021

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Abstract—To assess the role of feral oilseed rape (OSR) plants as resources for pollinators and avenues for gene flow, we compared occurrence of feral populations in standardized agricultural landscapes, using a landscape ecological approach. The occurrence of feral and volunteer populations was investigated in relation to differences in road length and width, number of OSR fields, and landscape scale. The potential for pollen-mediated gene flow from crop to feral oilseed rape was investigated with fluorescent dye in a field experiment. Moreover, greenhouse estimates of pollen germination rate and pollen tube growth rate were performed to get an indication of siring success in crop and feral plants. Escaped OSR occurred in 14 out of the 16 investigated landscapes, and feral populations were more common alongside large roads than small roads in large-scale landscapes. The number of plants in a habitat ranged from 1-160 individuals, with 1-19 habitats per landscape. In the field experiment with fluorescent dye, no transfer of dye was detected during early flowering in May. At the end of the flowering period in June, transfer of dye was found in 71.4% of the feral plants, showing that significant transfer, most likely by pollinators, occurred from the field to the feral plants. There was no difference in pollen germination rate between crop and feral plants. Pollen tube growth rate was significantly higher in feral oilseed rape than in the crop ($P < 0.001$). Our results contribute to increased understanding of i) the utilization of feral populations by pollinators in an intensively farmed agricultural landscape, and ii) crop-feral gene flow within OSR.

Keywords—Feral oilseed rape, *Brassica napus*, gene flow, dye transfer

INTRODUCTION

An estimated 87% of flowering plants worldwide benefit from animal pollination, with a large proportion of plants being pollinated by bees (Ollerton et al. 2011). In agricultural landscapes, pollinators perform important pollination services to crops (Garibaldi et al. 2013), and can also contribute to crop-wild gene flow as they distribute pollen from crops to their close relatives (Ellstrand 2003). Although crop-wild gene flow is well-studied in the *Brassica napus-rapa* complex (e.g. Jørgensen et al. 1994; 2007), spontaneous hybridization between crop and escaped oilseed rape (OSR) is less studied (Ellstrand 2003; Squire et al. 2010). Because feral OSR has viable pollen and is widespread along roads in Europe, it has received interest as a potential gene reservoir for OSR traits in agricultural areas (Squire et al. 2010). Insects and wind pollinate oilseed rape and this can make fertilization between crop and feral oilseed rape possible (Jørgensen 2007). The sticky pollen can also be transferred from plant to plant through physical contact between their flowers (Devos et al. 2004). Pollen dispersal in OSR has been found to be carried out by honeybees (*Apis mellifera*), pollen beetles (*Meligethes aeneus*) (Devos et al. 2004) and bumblebees (*Bombus terrestris*) (Devos et al. 2004).

Escaped oilseed rape has a particular characteristic of growing on natural and semi-natural habitats (Pivard 2008). These so-called feral populations are common in field margins, on roadsides and waste grounds (Crawley & Brown 1995; Pessel et al. 2001; Pivard et al. 2008) and are a result of seed spillage from farm machinery, trucks or directly from neighboring fields (Lutman, 2003). Whether the feral populations are results of persistence or immigration determines to which extent feral populations can be considered relays or reservoirs for genes, and contributes to their dispersal and persistence (Pivard et al. 2008).

During early spring, oilseed rape (OSR) *Brassica napus* is often seen turning up as solitaires or growing in clusters along road verges and field edges, or in arable fields where they grow as weedy volunteers. Feral populations of OSR are commonly found in areas with cultivation of OSR (Pivard et al. 2008). Ferals can originate from seed banks in the soil and from seeds produced by existing feral and volunteer populations or from human-mediated dispersal (Hodkinson & Thompson 1997; Pessel et al. 2001; Pivard et al. 2008). Several studies have shown long-lasting persistence of OSR in the ground (Schlink 1998; Pessel et al. 2001, D´Hertefeldt et al. 2008; Warwick et al. 2008). However, OSR has difficulty establishing and surviving in undisturbed vegetation (Crawley et al. 1993), and the numbers of feral plants can fluctuate between years (Crawley & Brown 199; 2004; Nishisawa et al. 2009). The significance of traffic-mediated dispersal of OSR has been pointed out in several studies (Crawley & Brown 1995; von der Lippe & Kowarik 2007; Nishizawa et al. 2009; Bailleul et al. 2012). Vehicles increase both the quantity and the distance of OSR seed dispersal (Bailleul et al. 2012). In addition, OSR feral populations were found to be more frequently occurring on the side of the road leading towards an oilseed processing factory than on the side leading away (Crawley & Brown 1995). The conclusion was that seed spillage from vehicles was the main source for the populations that established along the road. Additional factors affecting seed dispersal were road size and junctions (Crawley & Brown 1995; Bailleul et al. 2012), and the amount of seeds that was spilled was positively related to the area of OSR fields that a road connected to (Bailleul et al. 2012). However, the frequency of feral oilseed rape does not seem to be affected by the number of nearby oilseed rape fields (Crawley & Brown 1995; von der Lippe & Kowarik 2007; Nishisawa et al. 2009). Although road size has been suggested to be important for seed dispersal, this was mostly attributed to driver behaviour (Crawley & Brown 1995) or compared between 1- and 2-lane roads (Bailleul et al. 2012). From the pollinator's point of view, the type of road can also be important, since heavy traffic is suggested to be hazardous for insects (Phillips et al. 2019).

Studies on pollination of OSR have shown that a variety of pollinators visit the non-specialized flowers of OSR, including bees, sawflies and pollen beetles (cf. Chifflet et al. 2010). The role of insect pollinators in pollination of OSR is not fully resolved, and other vectors include wind or direct contact between plants (cf. Lindström et al. 2016; Ouvrard & Jacquemart 2019). For European crops, the dependency on pollinators has been shown to be higher in Southern than in Northern European countries (Ouvrard & Jacquemart 2019). We therefore assume that the potential role of feral OSR as a nectar and pollen sources for pollinators in intensively farmed agricultural habitats may differ, depending on flowering time and pollinator activity at a given geographic location. It is also well documented that large and homogenous agricultural fields are linked to lower bee species richness compared to areas rich in semi-natural habitats, that contribute nesting habitats and food resources (Öckinger & Smith 2007; Ricketts et al. 2008; Garibaldi et al. 2011; Senapathi et al. 2015).

Gene flow from a crop to related species or feral plants depends on two factors, gene dispersal and gene establishment in the natural gene pools (Endler 1977). Pollen dispersal in oilseed rape has been found to decrease rapidly with the distance from the source, with 60% of the pollen being deposited within 6 meters from an individual plant (Lavigne et al. 1998; Rieger et al. 2002). However, pollen has been detected up to 1000-3000 meters from the source (Timmons et al. 1995; Rieger et al. 2002; Devaux et al. 2005). Apart from genetic compatibility, ecological factors such as population structure and density have been found to influence hybridization rates (Jørgensen & Andersen (1994).

The crop oilseed rape has been bred for high seed production and improved oil quality (reviewed by Abbadi & Leckband 2011). Seed production is a direct result of fertilization, and pollen might be expected to be of higher quality in crop oilseed rape than in ferals. Feral oilseed rape may also need to allocate resources to other functions than pollen since they grow in less favorable conditions than the crop plants. However, when crop cultivars and ferals were compared, there was no difference in pollen viability (Lankinen et al. 2018).

The aim of this study was to investigate the occurrence of feral OSR populations (Fig. 1) and pollinator-mediated gene flow from crop to feral oilseed rape. We inventoried the occurrence of feral populations in standardized, circular landscapes placed in the agricultural region of Scania, South Sweden, and we compared landscapes with large fields to those with smaller-scaled agriculture. We investigated the potential role of roads for seed dispersal by measuring the distance of roads in the inventoried circular landscapes. To estimate which road types are most important for seed dispersal, we assessed traffic intensity, using the width of the road as a proxy for road categories (Fig. 2). We performed a comparison of all road categories within agricultural landscapes in order to specifically investigate the role of different types of roads for OSR seed dispersal and feral occurrence in agricultural landscapes of different scales. Although feral plants are known to occur in the area, it is not known if pollinator activity overlaps with flowering of the feral plants. To investigate if pollinators utilize feral OSR populations, we compared pollen dispersal from a crop field to potted feral receiver plants during early and late parts of the flowering season. Finally, we compared the germination rate and length of the pollen tubes of crop and feral oilseed rape pollen in the greenhouse.

Figure 1. Feral oilseed rape plants growing alongside roads in Scania, South Sweden. Photo: J. Malm.

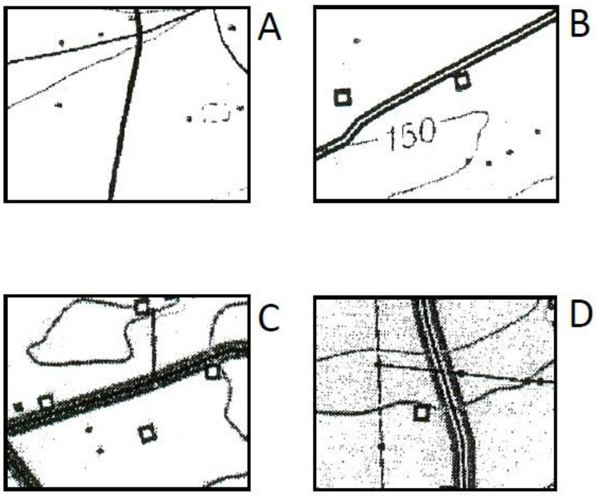

Figure 2. Symbols for roads of different sizes in the Swedish Kartex maps, used as a proxy for traffic intensity. The four categories according to how they appear on the Kartex maps (v. 5.32, The Swedish mapping, cadastral and land registration authority, Lantmäteriet) were:

1 – roads that are reproduced on the map as a straight black line (Fig: 2A). These are small roads not suitable for regular cars.

2 – roads reproduced as two black lines next to each (Fig: 2B). These roads are small with a speed limit of 50 km/h or occasionally of 70 km/h.

3 – roads that are reproduced as a black line with bold lines on the sides (Fig: 2C). Roads with a speed limit from 60 up to 80 km/h, larger country roads.

4 – roads that are reproduced as a black line with two bold lines next to it (Fig: 2D). This includes where the speed limit is 80 km/h or higher, including motorways.

MATERIALS AND METHODS

STUDY SPECIES

Oilseed rape (*Brassica napus*) is grown in large areas of Sweden, with Brassica oilseed crop covering 99, 300 ha, of which winter oilseed rape is the most important crop with 93, 000 ha (Virtuella floran 2008; Jordbruksverket 2020). The yellow flowers are 10 - 14 mm (Mossberg 2003), and in South Sweden flowering is in June-July (Virtuella floran 2008). Oilseed rape is a mass-flowering crop (Lindström et al. 2016). The cylindrical flowers are usually situated lower than the buds (Mossberg 2003; Virtuella floran 2008). The siliques can grow up to one decimeter long (Mossberg 2003; Virtuella floran 2008). Escaped plants from previous crops occur as volunteers in agricultural fields and as feral plants in natural and semi-natural areas (D'Hertefeldt et al. 2008) (Fig. 1).

INVENTORY OF FERAL OSR AND CATEGORIZATION OF ROADS IN THE LANDSCAPE

Selection of circular landscapes in small- and large-scale agricultural areas

We inventoried feral and volunteer oilseed rape, *Brassica napus*, in 8 circular landscape pairs in Scania, Sweden (Tab. 1). The circular landscapes had a diameter of 3,000 meters. We matched one landscape in an agricultural area with larger scale agricultural fields with a landscape in an agricultural area with smaller fields (Tab. 1). The maps for the study were made in the Kartex program for Swedish maps (v. 5.32, Lantmäteriet (the Swedish mapping, cadastral and land registration authority)). We placed the two landscapes in a pair as close to each other as possible, and we verified the accuracy of the division into landscapes with large-scale and small-scale agricultural fields by observations during the inventories in the field.

Inventory of volunteer and feral oilseed rape in circular landscapes

We investigated the occurrence of feral OSR populations in the circular landscapes by walking, or when walking was unsafe driving, along all roads in each landscape. We excluded small entrance roads leading to houses and small overgrown country roads. We counted all individual plants, except when we found high numbers of OSR as a volunteer in crop fields. For these cases we estimated the number of individuals. We assigned all individuals that grew within approximately 10 meters of each other and in the same habitat to the same location. We divided the habitats next to the roads into the four habitat categories field, road verge, field edge and ruderal ground. All oilseed rape that grew as a weed or volunteer in agricultural fields was placed in the category "field". We named the area beside the road the road verge. When we did not see any road verge, we named the location where oilseed rape grew outside the field a field edge. Finally, we called bare ground, for example where soil had been dumped, for ruderal ground. In addition, we noted the number of oilseed rape fields within each circular landscape.

Calculation of distance of roads and division into road categories in circular landscapes

We scanned the maps provided in Kartex (v. 5.32, Lantmäteriet) and used ImageJ v. 1,40g (National Institute of Health) to digitize and calculate the length of roads in each circular

Table 1.A compilation of the field inventories showing the number of feral oilseed rape (OSR) individuals per circular landscape pair (large vs. small), the number of habitats and cultivated oilseed rape fields, and the calculated total length of road within each circular landscape. Large- and small-scale landscapes are defined in the methods section.

Pair	Landscape pairs	Scale	Individuals of feral OSR (total nr.)	Habitats	Oilseed rape fields	Road length (km)
1	Dalby	Large	150	13	8	15.94
1	Esarp	Small	1	1	4	13.37
2	Getinge	Large	36	3	6	10.60
2	Södra Sandby	Small	160	5	4	10.17
3	Kattarp	Large	3	2	0	10.29
3	Kvidinge	Small	105	19	2	14.56
4	Bjärsjölagård	Large	130	12	5	16.33
4	Heinge	Small	61	4	5	15.57
5	Östra Vram	Large	83	5	0	10.91
5	Västra Vram	Small	1	1	2	18.94
6	Borlunda	Large	40	4	3	8.34
6	Bosarp	Small	116	13	2	18.38
7	Tirup	Large	0	0	2	8.97
7	Dagstorp	Small	0	0	2	11.89
8	Hörby	Large	48	6	5	11.46
8	Boarp	Small	11	2	1	11.32

landscape. We examined the occurrence and size of feral and volunteer populations of OSR in relation to road length, number of OSR fields, landscape scale, and traffic intensity, with width of the road as proxy. We assigned all the roads within a circular landscape into four size categories, according to how the roads were reproduced on the maps (Fig. 2). Roads that were not inventoried were excluded from the statistical analyses. We summed the distances of each road category to obtain a total road distance for each road category.

CROP TO FERAL DYE TRANSPORT IN THE FIELD AND CROP VS. FERAL POLLEN TRAITS IN THE GREENHOUSE

Collection and cultivation of feral plant material

We collected feral plants as rosettes in early March from four localities in Scania, South Sweden (Fig. 3, Appendix 1.). The feral rosettes were planted in potting compost and cultivated in the greenhouse, Dept of Biology, Lund University, from March to May. To provide a 12 h day, we added additional light when outside light levels fell below 12.000 lux. The plants were automatically watered every 3 days. The crop plants that we used for the comparison in pollen traits between crop and feral plants were collected at the beginning of May from the field in Alnarp where we also performed the field experiments with fluorescent dye (Fig. 3).

Field experiment on crop-feral transport of fluorescent dye

Fluorescent dye was used as a marker for insect-mediated pollen flow from crop to feral plants early and late in the flowering season of the OSR crop. We performed the early trial at the beginning of May and the late trial approximately four weeks later. We performed the two trials in the same OSR field in Alnarp (Fig. 3), but we placed the trial in different parts of the field to avoid potential traces of dye from the first trial (first trial: Lat N 55° 39′ 15″ Lon E 13° 3′ 38″; second trial: Lat N 55° 38′ 52″ Lon E 13° 3′ 48″). We used two sets of feral OSR receiver plants and all feral plants were in bloom.

We placed ten groups of five receiver plants at least 1.5 meters from the OSR field (Fig. 4). In the first trial, we first placed all receiver plants along one side of the OSR field, and in the second trial we placed them along a different side and continued around a corner of the field, resulting in ten groups of five plants and seven plants at the corner. The two sides of the field bordered to a dirt road and a grassy elevation, respectively. Thereafter, we dusted the flowers of the outermost plants in the field border opposite the receiver plants with yellow fluorescent powder dye, Radiant Color NV, type R-10, color Chartreuse (www.radiantcolor.com). To prevent accidental transfer of fluorescent dye, the person applying the dye stood between the potted plants and the field, facing the field.

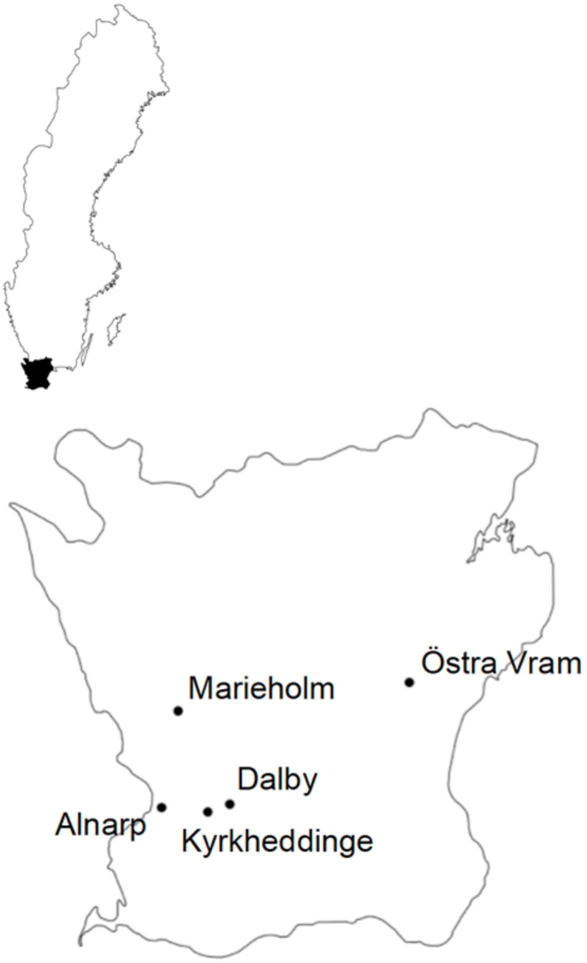

Figure 3. Map of the county Scania, South Sweden showing the four locations where the feral *Brassica napus* plants were collected (Marieholm, Östra Vram, Dalby, Kyrkheddinge). The field experiments took place at locality Alnarp. Map by C. Anderberg Haglund.

After two days we cut off the inflorescences of the feral receiver plants and placed them in individual paper bags to prevent loss of petals during the transport to the lab at Lund university. The inflorescences were illuminated with a portable ultraviolet lamp (Ultra-violet lamp for stamps, DL-01 2 LUVR) in a dark room to investigate traces of fluorescent dye.

As a precaution to accidental dye transfer, we used different clothes during dye application, UV readings and when collecting the inflorescences in the late-flowering trial.

Comparison of pollen traits between crop and feral plants in the greenhouse

We tested pollen viability and competitive ability in feral plants from three localities (Kyrkheddinge, Dalby and Marieholm, grown in the greenhouse), and in crop plants (collected from the experimental field in Alnarp) (Fig. 3, Appendix 1). We used pollen from six to eight plants per locality, depending on how many usable flowers that were available (Alnarp and Kyrkheddinge *N* = 8, Dalby *N* = 7 and Marieholm *N* = 6). Pollen germination rate and pollen tube growth rate was assessed following germination *in vitro* in Hoeksta medium (Hoekstra & Bruinsma 1975), modified as described in Lankinen et al. (2018). We germinated pollen from two flowers from each plant in a drop of the medium on a microscopic slide that was placed in the dark, at a constant temperature of 22°C for three hours. We thereafter added 100% glycerol to terminate germination. We evaluated the pollen traits under a light microscope. The ratio of germinated to non-germinated pollen grains was calculated for approximately 100 pollen grains in a representative area. Pollen tube growth rate was estimated as the average length of ten pollen tubes per sample. We conducted the germination tests at a similar time during three days to minimize environmental effects.

STATISTICAL ANALYSIS

We used linear regressions to test for potential relationships between the number of oilseed rape individuals and road length or the number of oilseed rape fields in the area, respectively. We performed the analyses for all oilseed rape individuals as well as for ferals (oilseed rape individuals growing in road verges and field edges). We used a t-test to compare the number of oilseed rape individuals in large-scale and small-scale landscapes. A Chi^2-test was performed to investigate any possible difference in the proportion of plants with fluorescent dye by the dirt road compared to the grassy elevation. We used a Mann-Whitney test to test for differences in germination percentage between crop varieties and ferals, and a t-test for pollen tube growth rate. All statistical analyses were made in SPSS 14 and 19 (ref).

RESULTS

PRESENCE OF FERAL OILSEED RAPE IN EIGHT LANDSCAPES IN SCANIA

The inventory of eight landscape pairs in Scania showed that feral and volunteer oilseed rape plants occurred in 14 out of the 16 areas (Tab. 1). The number of plants ranged from 1-160 individuals, and plants were found at 1-19 habitats per landscape (Tab. 1). Feral plants were most commonly found on road verges and rarely found on ruderal ground (Fig. 4).

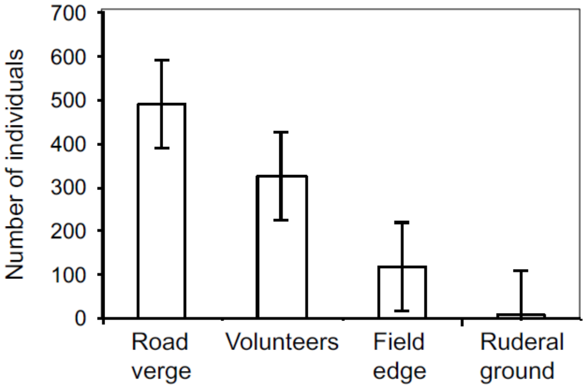

Figure 4. Mean number (±SD) of OSR individuals in different habitat categories. A description of each habitat category is given in the material and methods.

There was a positive relationship between the number of feral and volunteer OSR plants and road length in large-scaled landscapes (Fig. 5, Tab. 2), but not in small-scaled landscapes (Tab. 2).

There was no significant correlation between the number of oilseed rape fields in a circular landscape and the number of feral and volunteer plants that were found (Tab. 2). Neither the analysis including all landscapes, nor individual analyses within large scale or small-scale areas, showed any significant correlations (Tab. 2). There was no difference in the number of oilseed rape plants found in small-scale (56.88 ± 63.30) compared to large-scale landscapes (61.25 ± 55.40) ($P = 0.885$, $t = -0.147$, $df = 14$) (Tab. 1).

The number of feral plants growing on the habitats road verges and field edges was compared among road categories. Roads of categories 2 and

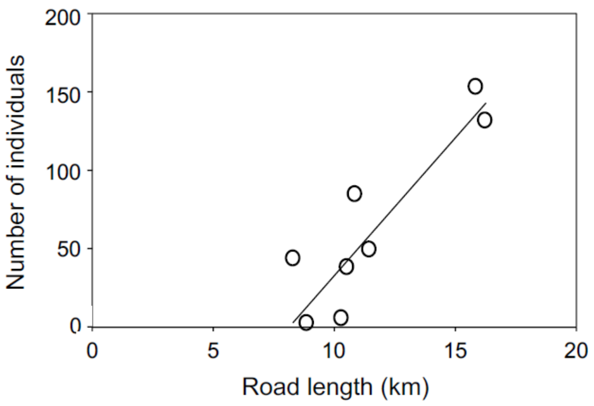

FIGURE 5. The relationship between number of feral and volunteer OSR individuals in relation to road length within large scale areas when counting all individuals. P = 0,003, R^2 = 0,796, F = 23,34 and df = 6.

3 were the most common roads in the landscapes (Tab. 3, Fig. 5). The mean number of ferals was highest along the largest roads (category 4) and lowest along roads of categories 1 and 3 (Tab. 3).

FIELD EXPERIMENT ON CROP-FERAL GENE FLOW USING FLUORESCENT DYE

During the setup of the first dye application, very little pollinator activity was observed in the field. Netting performed in the area during the same time found only queen bumble bees and no workers, typical for early-season activity (Annika Söderman, pers. com.). The UV-lamp analysis detected no fluorescent dye on the receiver plants.

During the setup of the second dye application four weeks later, pollinator activity in and nearby the field was observed to be much higher. The UV-analyses detected fluorescent dye on 40 out of 56 plants (71.4 %). When comparing the two different sides of the OSR field, more receiver plants along the dirt road received fluorescent dye than those on the border to the grassy elevation (Chi², $P < 0.05$) (Fig. 6).

COMPARISON OF POLLEN TRAITS BETWEEN CROP AND FERAL PLANTS

There was no significant difference in germination rate between crop (46 ± 9 %; $N = 8$) and feral oilseed rape (51 ± 14 %; 3 ferals with N = 6-8) (Mann-Whitney U-test, $P > 0.05$). When pollen tube lengths were compared after 3 h, feral OSR pollen tubes were significantly longer (0.029 mm; 4 ferals with N = 3-8) than crop OSR (0.021 mm; N = 8) (independent sample t-test, P < 0.001) (Fig. 7), suggesting a faster pollen tube growth rate in ferals than in crop plants.

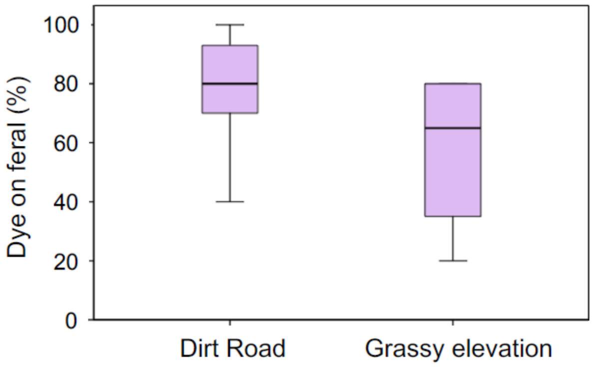

Figure 6. Box-plot of the percentages of fluorescent dye on feral receiver plants placed either on the border to the dirt road or in the grassy elevation.

Table 2. Linear regressions of the number of feral and volunteer oilseed rape plants related to landscape parameters. Significant (P < 0.05) parameters are in bold.

Landscape parameter	F	df	R²	P
Road length within small scale areas	0.030	6	0.005	0.869
Road length within large scale areas	23.34	6	0.796	0.003
Number of fields within small scale areas	0.394	6	0.062	0.553
Number of fields within large scale areas	3.084	6	0.340	0.130
Number of fields all areas	3.096	14	0.181	0.100

Table 3. The number of feral OSR individuals on road verges and field edges, road length and feral rape individuals/km divided into the different road categories.

Road category	Ferals (road verge and field edge)	Road length (km)	Ferals per km
1	19	21.70	0.88
2	242	86.10	2.81
3	62	72.79	0.85
4	287	26.49	10.83

DISCUSSION

In our inventory of feral and volunteer OSR along the roads of an intensive agricultural area in South Sweden, we found more feral and volunteer plants with increasing road length in the large-scaled landscapes, but not in the small-scaled landscapes. Previous studies have noted the importance of vehicles for seed dispersal (Crawley & Brown 1995; von der Lippe & Kowarik 2007; Nishisawa et al. 2009). We found that ferals were more common along the largest roads (motorways and other high-speed roads, i.e., category 4). This suggests that seeds were not primarily dispersed by farmland machinery, but rather by lorries during transport after harvest. Bailleul et al. (2012) has previously suggested that spillage along larger roads might be more common because of a combination of higher speed limits and poorly covered trucks, causing wind turbulence to result in seed dispersal. They further argued that different styles of driving on the different types of roads might affect seed loss levels, which agreed with our findings that the highest frequency of ferals occurred along roads in category 4, which had the highest speed limits. The finding that OSR ferals occurred in higher frequency along the largest roads may also be related to construction work. During the field inventory, we observed that road work had been done during recent years. Areas with soil disturbance in previous years often have a high representation of OSR plants, while fewer plants are typically found along road verges of older roads with mature vegetation (Crawley & Brown 1995). We conclude that the higher number of ferals along the largest roads in our landscapes may be a combination of large lorries and seeds lost from them, and disturbance of the soil during road works that stimulate germination of seeds in the seed bank.

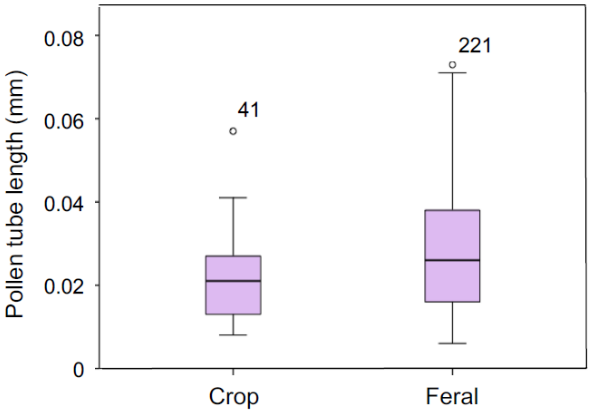

Figure 7. Box plot of pollen tube lengths after 3 h in crop and feral oilseed rape in the greenhouse.

The landscape perspective in our study adds information that can help to understand to what extent pollinators are likely to detect and utilize feral OSR plants. The large scale landscapes in our study contained larger agricultural fields, and if we assume that this corresponds to a lower presence of semi-natural habitats, feral OSR may contribute resources to pollinators in landscapes that are lower in such resources and where pollinator diversity is low (Öckinger & Smith et al., 2007; Ricketts et al., 2008; Garibaldi et al., 2011, Senapathi et al., 2015).

The result from the field experiment with fluorescent dye, as an indication of pollen transfer from crop to feral plants by pollinating insects, suggested that pollinators in the mass flowering OSR field visited individual feral receiver plants. This indicates a potential for pollinator-mediated gene flow from crop to feral OSR, a mechanism that has been shown with molecular methods (Jørgensen, 2007). Although we find it interesting that pollinators visit individual plants outside the mass flowering OSR field, records of dye deposition do themselves not confirm that pollen flow, and ultimately gene flow, have taken place.

In a comparative study of pollen and dye deposition, Thomson et al. (1986) demonstrated that pollen was consistently transported over shorter distances and in smaller amounts than dye and concluded that dye did not provide exact assessments of pollen transport.

Transfer of dye was only detected in the second trial that took place later in the OSR flowering season. In the first trial, no dye was detected on the receiver plants, and there was very low pollinator activity in and nearby the field. In the second field experiment (in the beginning of June), both crop and feral oilseed rape plants were towards the end of their flowering. Many flowers had already lost most of their petals, while pollinator activity was very high. During the first field experiment the opposite was true, all plants were in excellent condition but pollinator activity was low. Since we had four weeks between the trials, we did not detect if there was a short period of optimal conditions for both pollinator activity and flowering. It has previously been demonstrated that the occurrence of pollinators in *Brassica* crop fields is linked to flower phenology (Mesa et al. 2013), which suggests that the importance of pollinators differs between the earliest and latest flowering flowers on OSR feral plants.

The dye transfer showed that although the distance to feral plants was short, pollinators appeared selective in which direction to go, since more dye was detected on the ferals alongside the dirt road than in the grassy elevation. Because this was a relatively windy habitat, we had instead expected the pollinators to prefer the calmer circumstances towards the grassy elevation. Perhaps visibility matters; the potted ferals along the grassy elevation were not as visible as the ones by the dirt road, because they were partially obscured by high grass.

In a previous study on pollen germination rate and pollen tube growth rate, Lankinen et al. (2018) found larger variation within five feral populations and seven cultivars of OSR than between these two groups. They demonstrated that only one cultivar showed significantly higher pollen performance than one feral population. In contrast, we found that pollen tube growth rate was significantly higher in the feral oilseed rape than in crop oilseed rape. This finding could be a result of feral plants being grown for a longer time in the greenhouse compared to the crop plants they were compared with, but might also suggests that pollen traits of feral populations could differ depending on their cultivar origin.

CONCLUSION

Feral populations of OSR were common in most of the intensively farmed areas in our study, and can therefore have a role as nectar and pollen sources for pollinators in intensively farmed agricultural habitats. Although pollinator behaviour within crop fields or from crops to flower borders is well studied, less is known about how pollinators find and utilize common, but small and scattered, feral OSR populations. In addition, most ferals in the inventory were found on road verges, which are a problematic habitat for pollinators. Although road verges are increasingly appreciated as remnants of meadow vegetation and reservoirs of biodiversity in intensive production areas (cf. Manosterolo et al. 2020), they may be risky habitats for pollinators when resource plants grow close to busy roads (Phillips et al. 2019). Although fewer feral populations were found alongside the smallest roads, these calmer roads may be preferable as resource plants for pollinators early in the season. The possibility for pollinators to utilize this potential nectar and pollen source will therefore likely depend on the location, visibility, and flowering time of feral populations.

APPENDICES

Additional supporting information may be found in the online version of this article:

Appendix I. Feral oilseed rape localities.

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