Taylor & Francis
Taylor & Francis Group

Acta Agriculturae Scandinavica, Section B — Soil & Plant Science

ISSN: 0906-4710 (Print) 1651-1913 (Online) Journal homepage: https://www.tandfonline.com/loi/sagb20

Repellency of insecticides and the effect of thiacloprid on bumble bee colony development in red clover (*Trifolium pratense* L.) seed crops

Lars T. Havstad, John I. Øverland, Silja Valand & Trygve S. Aamlid

To cite this article: Lars T. Havstad, John I. Øverland, Silja Valand & Trygve S. Aamlid (2019) Repellency of insecticides and the effect of thiacloprid on bumble bee colony development in red clover (*Trifolium pratense* L.) seed crops, Acta Agriculturae Scandinavica, Section B — Soil & Plant Science, 69:5, 439-451, DOI: 10.1080/09064710.2019.1596301

To link to this article: https://doi.org/10.1080/09064710.2019.1596301

Published online: 20 Mar 2019.

ACTA AGRICULTURAE SCANDINAVICA, SECTION B — SOIL & PLANT SCIENCE
2019, VOL. 69, NO. 5, 439–451
https://doi.org/10.1080/09064710.2019.1596301

Taylor & Francis
Taylor & Francis Group

Repellency of insecticides and the effect of thiacloprid on bumble bee colony development in red clover (*Trifolium pratense* L.) seed crops

Lars T. Havstad[a], John I. Øverland[b], Silja Valand[b] and Trygve S. Aamlid[c]

[a]Department Grain and Forage Seed Agronomy, The Norwegian Institute of Bioeconomy Research (NIBIO), Grimstad, Norway; [b]Norwegian Agricultural Extension Service (NLR) Viken, Stokke, Norway; [c]Department for Urban Greening and Environmental Engineering, The Norwegian Institute of Bioeconomy Research (NIBIO), Grimstad, Norway

ABSTRACT
The study intended to compare repellency of three insecticides on bumble bees and honey bees in Norwegian red clover (Trifolium pratense L.) seed crops, and to examine effects of thiacloprid on bumble bee colony development in the field. The repellency study was carried out in a large-scale field trial in SE Norway in 2013. On average for observations during the first week after spraying, 17 and 40% less honey bees ($P = .03$) and 26 and 20% less bumble bees ($P = .36$) were observed on plots sprayed with the pyrethroids lambda-cyhalothrin and alpha-cypermethrin, respectively, than on unsprayed control plots. No pollinator repellency was found on plots sprayed with the neonicotinoid thiacloprid. Compared with unsprayed control the seed yield increases were 22% on plots sprayed with thiacloprid vs. 12–13% on plots sprayed with pyrethroids ($P = .10$). Follow-up studies in 2014–2016 focused on the effect of thiacloprid on bumble bee colony development in commercially reared nests of *Bombus terrestris* placed into red clover seed crops at the start of flowering. Unsprayed control crops were compared with crops sprayed either at the bud stage or when 18–44% of flower heads were in full bloom. Chemical analyses of adult bumble bees showed that thiacloprid was taken up in bees when crops were sprayed during flowering, but not detected when crops were sprayed at the bud stage. The bumble bees in late-sprayed crops also developed weaker colonies than in unsprayed crops. Dead bees with a high internal concentration of thiacloprid were found in one crop sprayed during the night at 35% flowering. This shows that thiacloprid is not bee-safe if sprayed after anthesis and that spraying has to be conducted at the bud stage to reduce its contamination of nectar and pollen.

ARTICLE HISTORY
Received 29 November 2018
Accepted 28 February 2019

KEYWORDS
Apion spp; bumble bees; honey bees; *Hypera nigrirostis*; pollination; pyrethroids; neonicotinoids; weevils

Introduction

The red clover (Trifolium pratense L.) flowers are self-sterile and depend on pollen from other plants to be fertilised and produce seed (Williams 1931). The pollen is mainly transferred by bumble bees and honey bees which collect pollen as forage for themselves and especially their larvae. The red clover flowers are also rich in nectar, which is the main energy source for the bees (Konzmann and Lunau 2014). However, the nectar is located at the bottom of a relatively long corolla tube and can normally only be reached by long-tongued species of bumble bees (Goulson 2010). Thus, the long-tongued bumble bees are considered the most efficient pollinators of red clover and also those that find the red clover flowers most attractive (e.g. Goulson 2010).

During the last decades, there has been a general decline in the number of pollinating insects (e.g. Potts et al. 2010). This recession has particularly affected many of the long-tongued bumble bees (Ødegaard et al. 2009; Dupont et al. 2011; Bommarco et al. 2012). In addition to less flower diversity, habitat fragmentation, climate change, diseases and loss of nest sites as reasons for the decline (Goulson et al. 2008; Goulson et al. 2015), the use of pesticides is often mentioned as one important factor.

The growing concern for pollinating insects has led to a discussion among red clover seed growers whether to use insecticides to control insect pests. The main insect pests in Norwegian red clover seed crops are the red clover seed weevils (*Apion apricans*, *A. trifolii* and *A. assimile*) and the lesser clover leaf weevil (*Hypera nigrirostris*), with the latter species causing the highest risk for crop damage and seed yield reduction (Aamlid et al. 2010). Besides the general concern about the insecticides having a negative long-term effect on bee fertility and overall health, many growers are also concerned about their repellent effect on pollinators. The latter

CONTACT Lars T. Havstad lars.havstad@nibio.no Department Grain and Forage Seed Agronomy, The Norwegian Institute of Bioeconomy Research (NIBIO), Grimstad, Norway

has led many seed growers, despite having pest problems, to not chemically control the weevils in their seed crops (Aamlid et al. 2010).

In previous studies, bees have been found to change their behaviour by avoiding sprayed plants (Shires et al. 1984) and by reducing feed intake (Mueller-Beilschmidt 1990) when their sensory organs catch the scent of insecticides. For pollinating insects, this behaviour will be beneficial since they avoid contact with the chemical (Thompson 2001), but from a seed production point of view, it is unfortunate since the sprayed area is avoided and the flowers not pollinated. Of today's insecticides, the pyrethroids are known to have the strongest repellent effect on the behaviour of the bees but the neonicotinoids can have similar properties as well (Thompson 2003). In a feeding trial where the neonicotinoid imidacloprid was mixed into a sucrose solution, it was found that the contaminated forage was avoided as long as the bees were not starving for a long time (Decourtye and Devillers 2010). According to Thompson (2001), the repellent effect, at least for pyrethroids, applies to both honey bees and bumble bees.

Most studies on insecticide repellency have been carried out in laboratories or under artificial growing conditions (e.g. plastic tunnels), which may affect the behaviour of the pollinators (e.g. Schmuck et al. 2003). In a field study under natural conditions where the pyrethroid alpha-cypermethrin was sprayed during the flowering of rapeseed (*Brassica napus*), Karise et al. (2007) found that the repelling effect was dependent on flower density. At full flowering, the access to pollen and nectar was so attractive to the bees they disregarded the scent of insecticide. The neonicotinoid fipronil was avoided by the bees in a laboratory experiment where it had been mixed into a sucrose solution, but no repellency was detected when the chemical was sprayed at recommended rate in a field trial with flowering rapeseed (Mayer and Lunden 1997).

The impact of neonicotinoids on overall bee health and fertility has also been much debated recently (e.g. Whitehorn et al. 2012; Woodcock et al. 2016, 2017). Their toxicity depends on whether the active substance contains nitro or cyano groups. The nitro group, which is present in e.g. imidacloprid, thiamethoxan, nitenpyram, clothianidin and dinotefuran, is the most harmful, while products with an active cyano group, as thiacloprid and acetamiprid, are considered less toxic to pollinating insect (Mommaerts et al. 2010). This difference in toxicity is primarily because the cyano group is quickly broken down into non-toxic end products (Mommaerts et al. 2010; Iwasa et al. 2004). Of all neonicotinoids, thiacloprid is considered to be the least toxic (Blacquière et al. 2012; Mommaerts et al. 2010). However, despite the perception of thiacloprid as a relatively benign neonicotinoid, laboratory studies have shown a reduction in the number of bumble bee male drones after forage uptake of increasing concentrations in sugar water (Mommaerts et al. 2010). The lowest thiacloprid concentration in this study was 12 ppb and the highest 120 ppm, with the latter being maximum field recommended concentration. In addition, a recent study in raspberries (*Rubus ideaus*), grown in plastic tunnels, showed that bumble bee colonies exposed to thiacloprid were more likely to die prematurely, and that surviving colonies exposed to thiacloprid-sprayed raspberries reached a lower final weight than unexposed colonies (Ellis et al. 2017) Apart from these studies, surprisingly little is known about the long-term effect of thiacloprid on colony development of bumble bees under realistic field conditions.

As of 1 May 2018, insecticides approved for use in red clover seed crops in Norway are the pyrethroids lambda-cyhalothrin (Karate 5 CS, 50 g a.i./l), alpha-cypermethrin (Fastac 50, 50 g a.i./l) and deltamethrin Decis Mega (EW 50, 50 g a.i./l), and the neonicotinoid thiacloprid (Biscaya OD 240, 240 g a.i./l), the latter through an off-label registration for members of the Norwegian Seed Growers Association (Aamlid 2018). The objectives of this research were (1) to compare repellency of three of these insecticides on bumble bees and honey bees in Norwegian red clover seed crops, and (2) to examine effects of thiacloprid on bumble bee colony development in the field.

Methods

Experiment 1. Repellency of insecticides

In 2013, the pyrethroids lambda-cyhalothrin (Karate 5 CS), alpha-cypermethrin (Fastac 50) and thiacloprid (Biscaya OD 240) were investigated for their repellency to pollinators (honey bees and bumble bees) in a large-scale field trial in a first-year seed crop of red clover, cv. Lea (diploid), in Vestfold, SE Norway (59.1°N, 10.2 °E). On 16 July, when about ca. 30% of the flower heads were in full bloom, the three insecticides were applied to plots (10 m x 10 m) at the recommended rates of 15, 5 and 96 g a.i./ha, respectively. In accordance with the label instructions, spraying was performed during the night, to avoid direct exposure of pollinating insects. A total of four treatments (the three insecticides and one unsprayed control treatment) were distributed randomly with 20 m borders between plots in each of six blocks located at different sites in the seed crop.

Both on sprayed and unsprayed plots, pollinator activity (density) was registered just before spraying (day 0) and then 1, 2, 3, 4 and 7 days after spraying. The density was evaluated by counting the number of bumble bees and honey bees on 500 random flower heads in each plot. The registrations were carried out between 10 AM and 2 PM. A total of 200 bumble bees were observed and identified to species (10, 15, 22, 27, 33 and 93 bumble bees identified on day 0, 1, 2, 3, 4 and 7, respectively). Individuals belonging to *B. terrestris* and the *B. lucorum* group (*B. lucorum*, *B. magnus* and *B. cryptarum*) were recorded as one species (hereafter *B. terrestris*). These species are difficult to distinguish in the field, but functionally similar.

On 26 August, just before the seed crop was desiccated with diquat (Reglone, 2.5 l/ha), twenty random flower heads per plot were harvested by hand and incubated in perforated plastic boxes at 20°C. The numbers of *Apion* spp. and *Hypera nigriostris* were counted after hatching. At seed harvest on 3 September, all plant material on a random subplot (1 m^2) near the centre of each plot was cut at soil level, air-dried and transferred to NIBIO Landvik for seed threshing, cleaning and analyses of purity and seed water content.

Experiment 2. Effects of thiacloprid on bumble bee colony development, weevil density and seed yield

On 4–5 July 2014 and 2–3 July 2015 between two and five boxes containing commercially reared colonies of *Bombus terrestris* (Bombus Pollinering AS, Klepp, Norway) were set out in selected red clover seed crops in SE-Norway. In each of the two years, a total of 10 first-year seed crops were divided into five pairs. The two crops within one pair had the same cultivar, were located in the same geographical region, were matched on field size and received the same management (growth regulation, fertilising, etc.). except for treatment with thiacloprid. Mean field size was 7.1 ha (variation 2.5–12 ha). All fields were located in typically farm land areas with no known sources or land use beyond the fields affecting colony dynamics. Application of thiacloprid was randomly assigned to one crop per pair, the other crop serving as the untreated control.

In both years, the seed crops were located in Vestfold (59.4 °N, 10.4°E, pair 1, cv. Lea), Telemark (59.2°N, 9.0°E, pair 2 and 3, cv. Yngve (diploid) and cv. Reipo (tetraploid), respectively), and Buskerud (60.0 °N, 10.2°E, pair 4 and 5, both cv. Lea). In addition to the pairwise crops, registrations were also carried out in a single seed crop of cv. Yngve at the research station NIBIO Landvik, (58.2 ° N, 8.5°E) (Figure 1). Mean aerial distance between fields within each pair, across years, was 8,9 km (variation 4.6–12.4 km). In the single seed crop at Landvik and in one seed crop within each pair in Vestfold, Telemark and Buskerud, thiacloprid (Biscaya OD 240) was sprayed at the recommended rate of 96 g a.i./ha during the periods 7–10 July 2014 and 23 June – 6 July 2015. While spraying in 2014 was performed when 23–44% of the flower heads were in full bloom, all crops were at the bud stage at the application of thiacloprid in 2015. In 2014, spraying was always performed during the night to avoid direct exposure of pollinating insects in the blooming crops. In both years and in all sprayed crops, an area of minimum 200 m^2 was always left unsprayed to obtain additional information on the effect of thiacloprid on clover seed weevils and seed yield.

Registrations of bumble bees and colony development included:

- Collection of five adult bumble bees from each crop, either two weeks after spraying (2014, when crops were sprayed during flowering) or two weeks after the onset of flowering (2015, when crops where sprayed before flowering, allowing bees to pollinate before being collected). The bees, that were collected when entering/exiting the colonies, were kept frozen until analysis for thiacloprid residues (more detailed description of the pesticide analysis is given below).
- At the end of the flowering period, all boxes were collected during the night (with adults inside) and then frozen. The nests inside the boxes were later taken out and dissected. The following characters were recorded: number of adult bumble bees per nest, nest weight, number of larvae chambers and number of nectar pots. Registrations were performed in the laboratory at NIBIO Landvik.
- In 2015, the weight of the closed boxes containing bumble bee was recorded just before setting them out in the crops and again after 2–3, 4–5 and 7 weeks, the latter corresponding to the end of the flowering period. On average for sprayed fields, the weighing dates corresponded to 0, 3, 5 and 7 weeks after thiacloprid spraying, respectively.

In the sprayed seed crops, registrations of weevils and seed yields were performed on three plots, both on the sprayed area and on the unsprayed control area, as earlier described for Experiment 1. The three plots were located 20, 40 or 60 m away from the bumble bee boxes, respectively. In 2015 we also harvested 50 seed heads from the same plots before desiccation with diquat for an independent determination of seed yield per flower head. No seed yield or pest registrations were performed in the unsprayed fields.

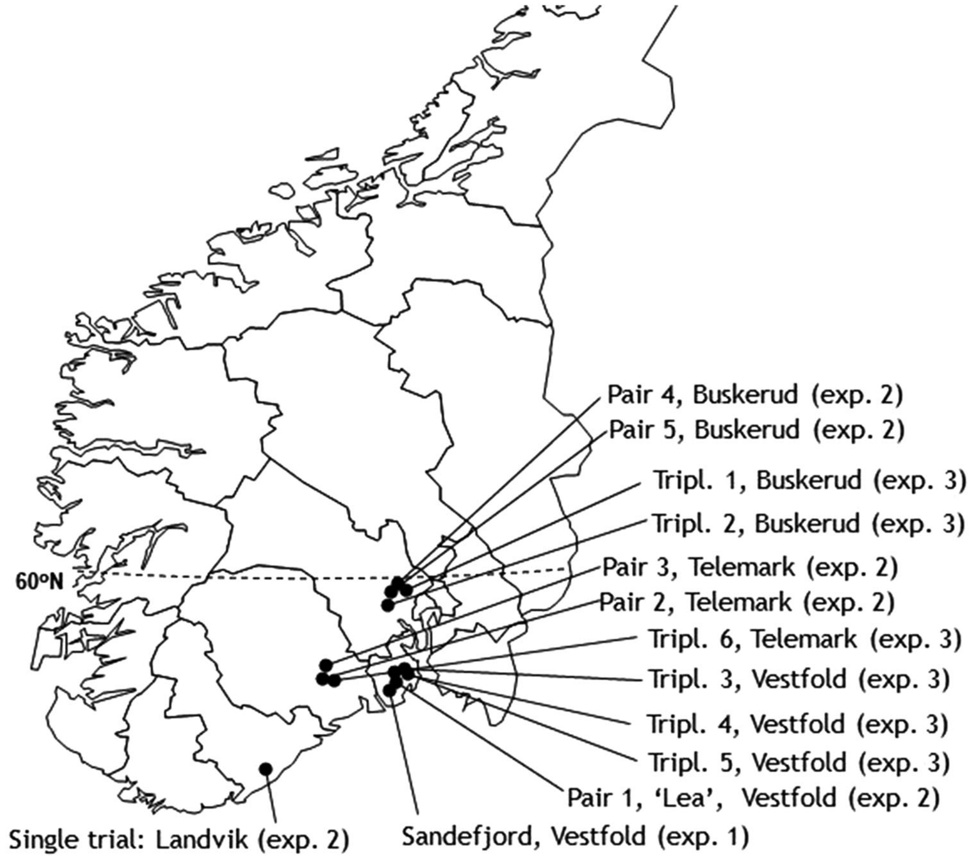

Figure 1. Map of South-Norway showing the trial locations in experiment 1 (2013), experiment 2 (2014 and 2015) and experiment 3 (2016).

Analysis of thiacloprid residues in bumble bees (procedure)

The analyses were performed at the Norwegian Institute of Bioeconomy Research, Division of Biotechnology and Plant Health, Department of Pesticides and Natural Products Chemistry, Ås, Norway, using the following procedure:

Samples of about 1 gram of the collected bumble bees were homogenised in 10 mL of acetonitrile using Polytron PT3100 (Fisher Scientific). For further sample preparation, the QuEChERS procedure was used (EN15662 2008; Christiansen 2016). The content of a Supel QuE Citrate Extraction Tube (Supelco cat. no. 55227-U) was added, the sample extracted and centrifuged. The extracts were further cleaned-up by freezing and adding calcium chloride (Norli et al. 2011). Two mL of the final extract was evaporated to dryness, re-dissolved in 200 µL acetonitrile and analysed by LC-MS/MS. The measurements were carried out using an Agilent 6410B triple quadrupole MS/MS with electrospray ionisation (ESI) in the positive mode coupled to an Agilent 1200 LC. The analytical column was a Zorbax Eclipse Plus C18 (Agilent), 1.8 µm particles, 100 mm long and 2.1 mm i.d., kept at 50°C. Injection volume was 2 µL and the flow rate was 0.3 mL/min. Mobile phase A was Milli-Q water with 5 mM ammonium formate and 0.01% formic acid. Mobile phase B was methanol with 5 mM ammonium formate and 0.01% formic acid. The gradient programme was 90% A for 1 min, ramped to 0% A at 18 min, hold for 2 min, ramped to 90% A at 20.1 min and hold for 12 min. The transitions used for thiacloprid were 253.0 -> 186.0 (quantifier, CE = 10 V) and 253.0 -> 126.0 (qualifier, CE = 20 V). The fragmentor energy was 90 V. A five level calibration curve in the range 0.01–1.0 µg/ml using external standard was applied for the quantitative calculations. Spiked control samples of blank bumble bees at 0.02 or 0.05 mg/kg were analysed together with each batch of samples and the recoveries were in the range 75–99%. The limit of quantification was 0.003 mg/kg.

Experiment 3. Timing of thiacloprid spraying on bumble bee colony development

In 2016, the effect of different timing of thiacloprid spraying was further examined using 18 red clover seed crops divided into six triplets. The crops within each triplet were as similar as possible except for the following insecticide treatments:

- No insecticide (control).
- Thiacloprid (Biscaya OD 240, 96 g a.i./ha) before flowering
- Thiacloprid (Biscaya OD 240, 96 g a.i./ha) after flowering had started

The seed crops, all of cv. Lea, were located in Buskerud (triplet 1 and 2), Vestfold (triplet 3, 4 and 5) and Telemark (triplet 6) (Figure 1). Between five (triplets 1, 2 and 6) and eight (triplets 3, 4, and 5) boxes containing commercially reared colonies of *B. terrestris* were set out in the crops during the period 17–24 June.

The early application of thiacloprid (treatment 2) was performed at the flower bud stage (between 11 and 25 June), while the late application (treatment 3) was performed between 6 and 17 July, when between 18 and 41% of the flower heads were in full bloom.

The boxes containing bumble bees were weighed on the day they were set out in the crop, and again after 2, 4 and 8–9 weeks. On average for sprayed fields the weighing dates corresponded to 0, 3, 4 and 7 weeks after early thiacloprid spraying (treatment 2), while in the late-sprayed fields (treatment 3) similar dates corresponded to 4 and 1 week before spraying and 1 and 4 weeks after spraying, respectively.

Weighing of boxes was performed during the night with adult bumble bees inside. Analysis of thiacloprid residues was performed on five bumble bees from each crop as described for Experiment 2, either two weeks after the start of flowering (treatment 1 and 2) or two weeks after spraying (treatment 3). At the end of the experiment (8–9 weeks after being set out), the boxes were frozen and colony development registered later as in Experiment 2.

No seed yield or pest registrations were performed in Experiment 3.

Weather conditions

During the repellency study (Experiment 1, 15–22 July 2013), all registration days were warm, sunny and with no rainfall. The mean and maximum daily temperature at the nearby weather station (Melsom, Sandefjord) varied from 17.8 to 21.6°C and from 23.1 to 26.9°C, respectively (EKLIMA 2019). Wind was not registered at Melsom, but at the neighbouring weather station Tjølling, average wind speed during all registration days (between 10 am and 2 pm) varied between 1.7 and 3.6 m/s (LMT 2019).

In 2014, the weather conditions for red clover pollination were also favourable. The mean temperature for July was 3–4°C higher and the precipitation 25–45% lower compared with the 30-year reference period (1961–1990) at all experimental sites.

Rainy weather during the flowering period made the conditions for pollination and bumble bee colony development less favourable in 2015 and 2016. In total for July and August 2016, the rainfall was 36 and 42% higher than the 30-year normal values in Vestfold (Ramnes) and Buskerud (Hønefoss), respectively.

Statistical analyses

All data were tested for normality of residuals before performing the analyses of variances, using the NORMALTEST option in the PROC CAPABILITY statement to request several tests of the hypothesis that the analysis variable values were random samples from a normal distribution (Shapiro–Wilk test, Kolmogorov–Smirnov test, Anderson–Darling test and Cramér-von Mises test) (SAS Institute 2015.).

For data failing the normality tests, including honeybee and bumblebee density (Table 1), Apion spp. and Hypera densities (Table 2), number of bumble bees per colony, nest weight, number of chambers with larvae and pots with nectar (Table 5) and *Apion* ssp. per seed head and *Hypera nig*. per seed head (Table 6), log10-transformations were carried out before performing any analyses of variances.

Analyses of variance for pollinator density in Experiment 1 were performed both individually for each registration day and collectively for all registration days after spraying (Table 1).

In Experiment 2 in 2014, all the boxes with bumble bees that had been set out in pair 5 (Buskerud; both the sprayed and the unsprayed field) and in the unsprayed field in pair 3 (Telemark), were destroyed by badgers. Also, the data from the unsprayed field in pair 4 (Buskerud) in the same year had to be deleted as all colonies were attacked by bumble bee wax moth (*Aphomia sociella*). For the remaining fields, overall analyses of variance for the various characters describing bumble bee colony development were performed collectively by year, according to an unbalanced design with each pair of fields being regarded as one replicate (Tables 4 and 5). Also in the experiments with the timing of thiacloprid application in 2016, similar analyses of variance were performed, always with each triplet being regarded as one replicate. For seed yield and pest characters (exp. 2, Table 6), each sprayed field with two treatments (sprayed and non-sprayed area) was regarded as one replicate.

All analyses of variances were performed using the procedure GLM of SAS (SAS Institute 2015). In addition to *p*-values, test criteria (*F*-value) and the degrees of freedom (DF) for error is given for each character in all tables. In Table 1, significant differences are indicated

Table 1. The effect of different insecticides on the density[1] of honey bees and bumble bees immediately before spraying (day 0) and 1, 2, 3, 4 and 7 days after spraying in a field trial in Vestfold, Norway, in 2013 (Experiment 1).

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 7	Average (days 1–7)	Rel.
Density of honeybees[1]								
A. No spraying (control)	0.3 (0.2)	0.5 (0.2)	3.0 (0.6)	2.0 (0.7)	8.2 (2.0)	5.8 (0.9) a	3.9 (0.6) a	100
B. Lambda-cyhalothrin (Karate)	0.0 (0.0)	0.2 (0.2)	3.2 (0.8)	1.5 (0.5)	8.7 (2.0)	2.7 (0.6) b	3.2 (0.6) ab	83
C. Alpha-cypermetrin (Fastac)	0.0 (0.0)	0.0 (0.0)	1.3 (0.4)	1.3 (0.5)	5.8 (0.9)	3.2 (0.7) b	2.3 (0.4) b	60
D. Thiacloprid (Biscaya)	0.3 (0.2)	0.0 0.0)	3.5 (0.7)	1.7 (0.4)	8.5 (0.7)	4.8 (0.9) a	3.7 (0.5) a	95
P	0.61	0.10	0.11	0.90	0.44	0.02	0.03	
F-value (DF in parentheses)	0.62	2.50	2.43	0.20	0.95	12.5	3.12	
DF	15	15	15	15	15	15	95	
Density of bumblebees[1]								
A. No spraying (control)	0.6 (0.3)	0.8 (0.3)	0.5 (0.3)	1.3 (0.6)	1.3 (0.6)	1.7 (0.5)	1.1 (0.2)	100
B. Lambda-cyhalothrin (Karate)	0.6 (0.3)	0.2 (0.2)	0.8 (0.3)	0.7 (0.3)	0.8 (0.5)	1.7 (0.3)	0.8 (0.2)	74
C. Alpha-cypermetrin (Fastac)	1.1 (0.3)	0.2 (0.2)	0.8 (0.3)	0.7 (0.3)	0.3 (0.3)	2.5 (0.9)	0.9 (0.2)	80
D. Thiacloprid (Biscaya)	0.8 (0.2)	0.2 (0.2)	1.3 (0.6)	0.8 (0.3)	0.8 (0.4)	2.8 (0.5)	1.2 (0.2)	106
P	0.66	0.14	0.70	0.63	0.29	0.53	0.36	
F-value (DF in parentheses)	0.54	2.13	0.48	0.58	1.36	0.75	1.07	
DF	15	15	15	15	15	15	95	

Note: Standard errors are given in parentheses.
[1]Density was assessed by counting the number of pollinating insects on 500 random flowers in each plot.

by different letters according to Duncan multiple comparison test at $P < .05$.

Results

Experiment 1. Repellency of insecticides

Pollinator density

The overall density of honey bees was low on the day before (Day 0) and directly after (Day 1) insecticide application but increased as flowering progressed and reached a peak on Day 4 (Table 1). Plots sprayed with alpha-cypermethrin had significantly less honey bees than unsprayed control plots on Day 7 and showed a similar tendency on Day 1 and Day 2. Lambda-cyhalothrin was less repellent with a significant decrease in honey bee density on Day 7 only. On average for five registration days, the honey bee densities on plots sprayed with the two pyrethroids were 40% and 17% lower than on unsprayed control plots, respectively (Table 1). Thiacloprid caused no significant reduction in honey bees density on any of the registration days; on average for days, the reduction relative to the unsprayed control was only 5%.

For bumble bees, the development of pollinator density from Day 0 to Day 7 was less evident than for the honey bees. The repellent effect of by insecticides was not significant on any of the registration days.

Of the 200 identified bumble bees, 83% were categorised within the *B. terrestris* group. Other species were *B. lapidarius* (10%), *B. hortorum* (6%) and *B. sylvarum* (2%).

Pest control and seed yield

The effect of insecticides on seed weevils was not significant although there was a tendency for the pyrethroids to be more efficient than thiacloprid in controlling *Hypera nigrirostis*. The highest seed yield ($P = .10$) was harvested on plots sprayed with thiacloprid (Table 2).

Experiment 2. Effects of thiacloprid on bumble bee colony development, weevil density and seed yield

Thiacloprid residues in bumble bees

In 2014, residues of thiacloprid were detected in bumble bees collected two weeks after spraying in three out of five seed crops (Table 3). In contrast, there were no detections in bumble bees collected from unsprayed crops in 2014 (Table 3) or from either unsprayed crops or crops sprayed before flowering in 2015 (data not shown).

Table 2. The effect of different insecticides on the number of weevils (*Apions* spp. and *Hypera nigrirostis*) hatched per flower head and seed yield in a field trial in Vestfold, Norway, in 2013 (Experiment 1).

	Number of weevils per flower head				Seed yield[1]	
Insecticide	(*Apion* spp.)	(*H. nigrirostris*)	Total	Total (rel.)	kg/ha	Rel
A. Unsprayed control	0.04 (0.02)	0.05 (0.03)	0.09 (0.05)	100	727 (57)	100
B. Lambda-cyhalothrin (Karate)	0.04 (0.04)	0.00 (0.00)	0.04 (0.04)	44	817 (35)	112
C. Alfacypermetrin (Fastac)	0.03 (0.02)	0.00 (0.00)	0.03 (0.02)	33	820 (32)	113
D. Thiacloprid (Biscaya)	0.01 (0.01)	0.03 (0.02)	0.04 (0.02)	44	886 (65)	122
P	0.66	0.08	0.47		0.10	
F-value (DF = 15)	0.54	2.70	0.88		2.44	

Note: Standard errors are given in parentheses.
[1]Adjusted to100% purity and 12% seed water content

Table 3. Residues of thiacloprid (mg / kg fresh weight) detected in bumble bees (*B. terrestris*) from sprayed and unsprayed seed crops in SE Norway in 2014.

	Residues of thiacloprid (mg / kg fresh weight)				
Treatment	Vestfold (pair 1)	Telemark (pair 2)	Telemark (pair 3)	Buskerud (pair 4)	Landvik
Unsprayed control	0	0	–[2]	0	–[2]
Thiacloprid	0.003	0	0.006	0.009	0

Note: The bumble bees were collected two weeks after thiacloprid spraying.
[1]Lower limit for detection: 0.003 mg/ kg.
[2]No bumble bees were collected.

Bumble bee colony development

In 2014, intact colonies from both crops within a pair were found in Vestfold (pair 1) and Telemark (pair 2) (Table 4). In both of these pairs, the number of bumble bees per colony and the nest weight was lower in the thiacloprid-sprayed crop than in the unsprayed control crop. On average for all observations across the two pairs, the reduction was 41 and 54%. The lower nest weight was due to a significantly lower number of chambers with larvae and pots with pollen and/or nectar.

The bumble bees in the sprayed crop in Buskerud (pair 4), only managed to develop a colony in one of the five boxes. This colony was very weak, and with no larvae in the nest (Table 4).

In 2015, the bumble bees produced intact colonies in both the unsprayed and sprayed crops within all five pairs. On average for pairs, there were no differences for any of the characters studied (Table 5).

Bumble bee box weight

Irrespective of spraying with thiacloprid, the average weight of boxes increased during the first 2–3 weeks after they had been set out on 2–3 July 2015 (Figure 2) The largest weight gain was found in boxes from sprayed crops (6%). Thereafter, the box weight was gradually reduced until the end of registrations on 20 August. There was no significant effect of thiacloprid on any date. The *p*-values (DF = 44) for weight analysis after 0, 2–3, 4–5 and 7 weeks were 0.59, 0.22, 0.77 and 0.25, respectively.

Pest control

Apion spp. were found in all crops in both years except in pair 1 in Vestfold in 2014 (Table 6). The highest density was found in Vestfold in 2015 (4.9 per flower head). In all infested crops, spraying with thiacloprid reduced the number of weevils compared to the unsprayed area. On average for all crops, the reduction in *Apion* spp. density was significant and amounted to 74% (Table 6).

Hypera nigriostris was found in all crops except at Landvik in 2014 (Table 6). However, although the mean values showed an overall reduction of 50% mostly due to the results from the sprayed crop in pair 5 in Buskerud

Table 4. The effect of thiacloprid on bumble bee colony development. Mean values of intact colonies / nests at the pairwise red clover seed crops in Vestfold, Telemark and Buskerud in 2014. Standard errors are given in parentheses.

Field location/ treatment	No of intact colonies at the end / No of colonies at the start	No of bumble bees per colony	Fresh weight per bumble bee (mg)	Nest weight (g)	No of chambers with larvae in the nest	No of pots with nectar and pollen in the nest
Vestfold (Pair 1, 'Lea'):						
Not sprayed	2 /2	111 (56)	91 (19)	76 (31)	11 (6)	18 (14)
Sprayed with thiacloprid	3[1] /5	81 (37)	77 (9)	56 (6)	24 (5)	0 (0)
Telemark (Pair 2, 'Yngve'):						
Not sprayed	5 / 5	112 (29)	117 (18)	187 (29)	87 (18)	153 (26)
Sprayed with thiacloprid	5 / 5	56 (12)	107 (21)	80 (18)	16 (6)	27 (13)
Telemark (Pair 3, 'Reipo')						
Not sprayed	–	–	–	–	–	–
Sprayed with thiacloprid	4[1]/5	44 (11)	62 (12)	47 (12)	9 (4)	4 (2)
Buskerud (Pair 4, 'Lea'):						
Not sprayed	–	–	–	–	–	–
Sprayed with thiacloprid	1[2]/5	84 (–)	77 (–)	30 (–)	0 (–)	11 (–)
Mean of all boxes (pair 1, 2, 3 and 4):						
Not sprayed	7/7	112 (23)	109 (14)	155 (29)	66 (19)	115 (31)
Sprayed with thiacloprid	13/20	59 (10)	82 (10)	80 (18)	14 (3)	13 (6)
P		0.07	0.54	0.04	0.01	0.009
F-value (DF = 15)		3.6	0.38	4.8	7.99	16.99

Note: Mean values of intact colonies/nests at the pairwise red clover seed crops in Vestfold, Telemark and Buskerud in 2014. Standard errors are given in parentheses.
[1]Remaining colonies destroyed by wax mouth.
[2]No colonies developed (empty boxes)

Table 5. The effect of thiacloprid on bumble bee colony development. Mean values of intact colonies / nests in the pairwise red clover seed crops in Vestfold, Telemark and Buskerud in 2015. Standard errors are given in parentheses.

Field location/ treatment	No of intact colonies at the end/no of colonies at the start	No of bumble bees per colony	Fresh Weight per bumble bee (mg)	Nest weight (g)	No of chambers with larvae in the nest	No of pots with nectar and pollen in the nest
Vestfold (Pair 1, 'Lea'):						
Not sprayed	5 / 5	14 (5)	206 (45)	38 (5)	18 (4)	1 (0)
Sprayed with thiacloprid	5 / 5	10 (4)	229 (42)	40 (6)	35 (11)	1 (0)
Telemark (Pair 2, 'Yngve'):						
Not sprayed	5 / 5	24 (8)	214 (32)	79 (21)	29 (10)	64 (35)
Sprayed with thiacloprid	5 / 5	37 (22)	212 (25)	86 (19)	62 (29)	30 (26)
Telemark (Pair 3, 'Reipo')						
Not sprayed	5 / 5	31 (9)	159 (30)	53 (15)	19 (9)	24 (13)
Sprayed with thiacloprid	5 / 5	17 (4)	191 (13)	59 (8)	29 (7)	46 (14)
Buskerud (Pair 4, 'Lea'):						
Not sprayed	5 / 5	11 (2)	190 (36)	64 (9)	30 (13)	17 (16)
Sprayed with thiacloprid	3[1] / 5	9 (2)	125 (5)	32 (6)	9 (5)	7 (1)
Buskerud (Pair 5, 'Lea'):						
Not sprayed	5 / 5	42 (8)	151 (1)	81 (11)	66 (6)	50 (18)
Sprayed with thiacloprid	4[1] / 5	47 (9)	119 (9)	45 (15)	46 (9)	29 (16)
Mean of all boxes (pair 1, 2, 3, 4 and 5):						
Not sprayed	25 / 25	25 (4)	188 (16)	56 (6)	32 (5)	29 (10)
Sprayed with thiacloprid	22 / 25	21 (6)	193 (15)	50 (6)	37 (9)	21 (8)
P		0.39	0.94	0.56	0.48	0.88
F-value (DF = 41)		0.75	0.01	0.35	0.47	0.02

Note: Mean values of intact colonies / nests in the pairwise red clover seed crops in Vestfold, Telemark and Buskerud in 2015. Standard errors are given in parentheses.
[1]Remaining colonies destroyed by wax mouth.

in 2015, the effect of thiacloprid on *Hypera nigriostris* could not be verified statistically.

Seed yield

The overall seed yield level was higher in the warm and dry season of 2014 than in the rainy season of 2015. However, irrespective of years, the seed yield was numerically higher on the sprayed than on unsprayed control area in nine out of ten crops studied (Table 6). The strongest positive effect of thiacloprid on seed yield (37% increase) coincided with the highest density of *Apion* spp. in Vestfold in 2015. On average for ten

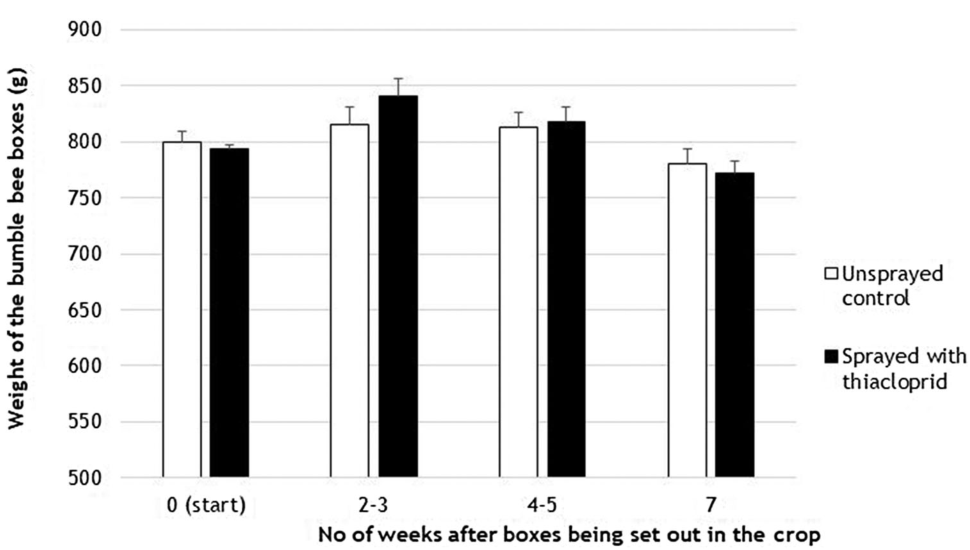

Figure 2. Weight of bumble bee boxes (g) 0, 2–3, 4–5 and 7 weeks after being set out in unsprayed crops and crops sprayed with thiacloprid in 2015. Bars represent 1 standard error (SE) ($n = 25$).

Table 6. The effect of thiacloprid on the number of weevils (*Apions* spp. and *Hypera nigirostis*) hatched per flower head and seed yield (kg/ha and mg/seed head) in 2014 and 2015.

	2014					2015						2014–2015
	Pair 1 Vestfold	Pair 2 Telemark	Pair 3 Telemark	Pair 4 Buskerud	Landvik	Pair 1 Vestfold	Pair 2 Telemark	Pair 3 Telemark	Pair 4 Buskerud	Pair 5 Buskerud	Landvik	Mean (all fields)
Apion spp./seed head												
Unsprayed	0	0.03	0.07	0.25	0.05	4.90	0.08	0.08	0.65	0.22	0.07	0.58
Thiacloprid	0	0	0	0.03	0	1.12	0.00	0.10	0.18	0.17	0.00	0.15
P												0.04
F-value (DF = 10)												5.93
H. nigirostris/seed head												
No spraying	0.02	0.02	0.03	0.05	0.00	0.02	0.02	0.03	0.05	0.12	0.03	0.04
Sprayed with thiacloprid	0.00	0.02	0.03	0.05	0.00	0.02	0.02	0.05	0.02	0.03	0.03	0.02
P												0.22
F-value (DF = 10)												1.68
Seed yield (kg/ha)												
No spraying	785	548	202	859	568	308	86	81	129	–[1]	334	390
Sprayed with thiacloprid	802	691	235	861	683	421	58	98	149	–[1]	350	435
P												0.04
F-value (DF = 9)												6.13
Seed yield (mg/seed head)												
No spraying	–	–	–	–	–	85	69	37	101	–	–	73
Sprayed with thiacloprid	–	–	–	–	–	103	100	47	103	–	–	88
P												0.09
F-value (DF = 3)												6.32

[1]Crop was by mistake seed harvested before seed from any of the experimental plots had been rescued.

crops, the seed yield increase amounted to 12% and was statistically significant (Table 6).

In 2015, the yield per seed head was always higher on the sprayed than on the non-sprayed area (Table 6). On average for four crops, the seed yield increase per seed head was 21% and almost significant ($P = .07$).

Experiment 3. Timing of thiacloprid spraying on bumble bee colony development

Thiacloprid residues in bumble bees

Residues of thiacloprid were detected in bumble bees from four of the six seed crops that had been sprayed after the start of flowering in 2016 (Table 7). In contrast, there were no residues detected in bumble bees from the unsprayed and early-sprayed crops. The highest uptake of thiacloprid was detected in dead bumble bees found in the crop sprayed at 35% flowering in Telemark (Table 7).

Box weight/bumble bee colony development

There was nearly no gain in box weight during the first two weeks of the flowering period, then the colony development started and a weight increase, regardless of treatments, was recorded after four weeks (Figure 3). The weight gain was highest in boxes from the late-sprayed crops. During the last part of the experiment (from 4 to 8–9 weeks), the box weight decreased, especially in the late-sprayed crops (Figure 3). The difference in box weight between the different spraying treatments was not statistically significant on any date. The *p*-values (DF = 99) for weight analysis after 0, 2, 4 and 8–9 weeks were all between .90 and .96.

When opening the boxes at the end of the experiment, dissecting the nests showed massive attacks of the bumble bee wax moth (*Aphomia sociella*). Larvae and adults were found in nests from all crops, regardless of spraying treatment. In many nests, the whole nest structure was degraded. Due to this damage, it was not possible to detect differences in colony development between treatments.

Discussion

Bumble bee species were only identified in Vestfold in 2013 (Experiment 1). The high number of species within the *B. terrestris* group found in this crop (83% of the individuals registered) is in accordance with other studies, both in red clover (Havstad et al. 2015) and other crops/landscape types in SE Norway (Ødegaard et al. 2009; Wermuth and Dupont 2010; Rundlöf et al. 2018). As this short-tonged species often bites a hole at the lower end of the corolla to get access to the

Table 7. Residues of thiacloprid (mg / kg fresh weight) detected in bumblebees (*B. terrestris*) from sprayed and non-sprayed seed fields in SE Norway in 2016.

	Residues of thiacloprid (mg/kg fresh weight)[1]					
Treatment	Buskerud (pair 1)	Buskerud (pair 2)	Vestfold (pair 3)	Vestfold (pair 4)	Vestfold (pair 5)	Telemark (pair 6)
1. Unsprayed control	0	0	0	0	0	–[2]
2. Thiacloprid at bud stage	0	0	0	0	0	–[2]
3. Thiacloprid at	0[4]	0[4]	0.013	0.009	0.009	0.31[3]

[1]Lower limit for detection: 0.003 mg/ kg.
[2]Bumblebees not collected.
[3]Analysis of dead bumblebees collected two days after spraying.
[4]Bumblebees collected four weeks after spraying.

nectar, its efficacy as red clover pollinator is debateable (e.g. Stout et al. 2000). However, several surveys during the last 50 years, including some in red clover, suggest that nectar robbers usually have a positive or neutral effect on pollination (Maloof and Inouye 2000). Of the long-tongued species, only a few *B. hortorum* and no *B. pascuorum* were observed. This was an unexpected result since both species are relatively common in Vestfold (Öberg et al. 2013).

The density of honey bees in Experiment 1 was high despite no beehives being set out in the red clover seed crop. However, depending on the food supply in the nearby area, honey bees may fly up to 12 km from their hive to collect pollen and nectar (Ratnieks 2000). Earlier studies have shown that honey bees are able to pollinate red clover despite their short tongues (Brødsgård and Hansen 2002; Havstad et al. 2015).

The number of honey bees in Experiment 1 was higher towards the end (days 4 and 7) than at the start of the registration period (Table 1). As the weather conditions were stable and optimal for pollinators (sunny and warm days), the increase in honey bee activity was mainly due to more flowering, from around 30% open flower heads at the start to nearly full flowering at the end of the week. The warm weather was probably especially beneficial for the honey bees, which normally require higher temperatures than the bumble bees before leaving the hive (Wratt 1968).

Although the density of honey bees varied among treatments during the registration period, their numbers were always low on plots sprayed with alpha-cypermethrin. Also, plots sprayed with the other pyrethroid lambda-cyhalotrin sometimes had less honey bees than the unsprayed control, especially on day 7. The reason why the pyrethroids lowered the honey bee activity more after 7 days than after 4 days is not known. Compared with the honey bees, the activity of the bumble bees was also less affected by the insecticides. However, on average for days 1–7, the overall density of pollinators was lower on plots sprayed with pyrethroids than on unsprayed control plots (Table 1). This is in concurrence with Thompson (2003) who found pyrethroids to be more repellent than other insecticides to pollinators.

As the pollinator density on average for days 1–7 was similar on unsprayed control plots and on plots sprayed

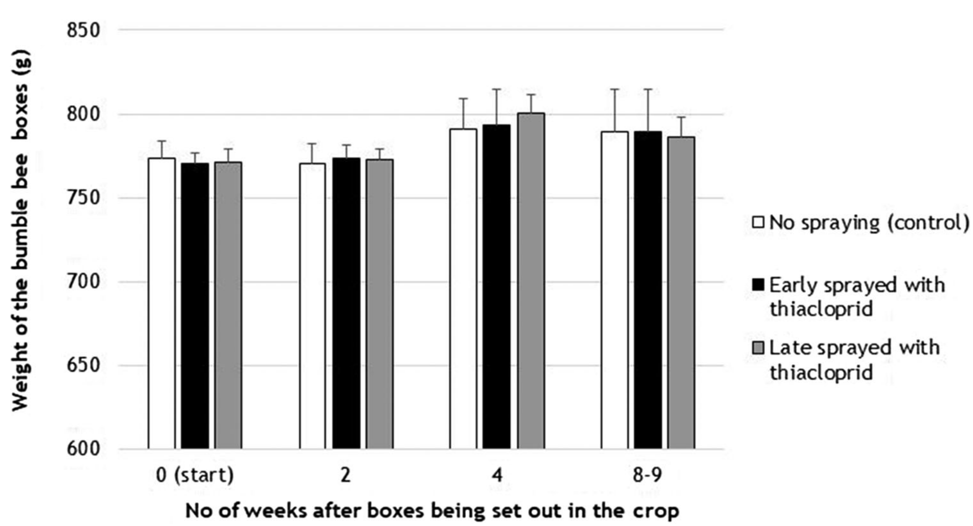

Figure 3. Weight of bumble bee boxes (g) 0, 2, 4 and 8–9 weeks after being set out in unsprayed crops and in crops sprayed early (before flowering) and late (after start of flowering) with thiacloprid in 2016. Bars represent 1 standard error (SE) ($n = 39$).

with thiacloprid, there was no tendency for this neonicotinoid to be repellent to either honey bees or bumble bees. Tison et al. (2016) came to a similar conclusion after studying visitation rate of honey bees on control feeders and feeders contaminated with the same neonicotinoid.

Despite less bees, the seed yield in Experiment 1 was 12–13% higher on the plots sprayed with pyrethroids than on the unsprayed control plots. This suggests that the positive effect of controlling weevils was stronger than the repellent effect of the two chemicals to the pollinators. The seed yield gain was highest on plots sprayed with thiacloprid, which in addition to reducing the density of weevils, was not repellent to the pollinators. This is in concurrence with a Swedish study (Pedersen 2010) that showed higher seed yields on plots sprayed with thiacloprid than on plots sprayed with the pyrethroid deltamethrin, with the lowest seed yield harvested on unsprayed plots. Together with this Swedish study, the results from Experiment 1 indicate that insecticide may have a positive effect on red clover seed yield, with thiacloprid being the most promising chemical. The positive effect of thiacloprid in reducing pest density and promoting seed yield was further demonstrated in Experiment 2, where the average seed yield was 12% higher on plots sprayed with thiacloprid than on unsprayed control plots.

One reason why thiacloprid and other neonicotinoids may be more efficient than mainly contact-acting pyrethroids in controlling weevils is that they have both contact and systemic activity. Thus they are able not only to control adult weevils, but also weevil larvae inside inflorescences (Lundin et al. 2012). However, both in Experiment 1 and Experiment 2, thiacloprid was more efficient in controlling the clover seed weevil (*Apion* spp.) than in controlling the lesser clover leaf weevil (*H. nigrirostris*). This is an important result that ought to be followed up by further studies as previous European investigations have mostly focused on *Apion* spp. (Lundin et al. 2012; Lundin 2013; Kolařík and Rotrekl 2013).

The overall density of weevils in unsprayed crops in Experiment 1 and 2 was relatively low compared to earlier studies in Norway (Aamlid et al. 2010), Sweden (Pedersen 2010) and the Czech Republic (Kolařík and Rotrekl 2013). The exception was Vestfold in 2015, where the control of weevils with thiacloprid increased seed yield by as much as 37%. The various *Apion* species found were not identified but earlier studies have shown that *A. apricans and Apion trifolii* are the most abundant species in the main red clover seed production areas in SE Norway (Aamlid et al. 2010). Although spraying had a positive impact on seed yield in most fields, the wide variation in pest density among locations suggests that spraying should not be conducted as a standard procedure, but rather based on the local experiences of each individual seed grower combined with observations of the leaf damage caused by adult weevils in the early season. Further investigations are therefore needed to construct economic thresholds for integrated pest management of red clover seed crops.

In Experiment 2, the development of the bumble bee colonies was significantly impaired by thiacloprid in 2014 but not in 2015. The pesticide analyses also detected residues of thiacloprid in adult bumble bees in 2014 only. As spraying was conducted when 23–44% of flower heads were open in 2014, but before flowering had started in 2015, the time for insecticide application is obviously critical. The residues detected after late spraying on flowers in full bloom in 2014 indicate that thiacloprid was transferred to nectar/pollen and later taken up by the bumble bees. The strongest negative impact on both pesticide concentration and colony development was found in pair 4 in Buskerud, which was also the crop that received the latest application (44% of the flower heads in full bloom).

Although unfavourable weather conditions weakened the bumble bee colonies, and a massive attack of *Aphomia sociella* erased possible differences in colony development between spraying treatments, the pesticide analyses in Experiment 3 confirmed that thiacloprid was taken up by the bumble bees in crops sprayed after the start of flowering. Here the high mortality and the high thiacloprid residues after the late spraying in Telemark in 2016 are rather alarming as thiacloprid has often described as ‘bee-safe’ and suitable for use in gardens and horticultural crops after anthesis (e.g. Jeschke et al. 2011). Even in red clover seed production, spraying with thiacloprid when 30% of flowers are in full bloom has sometimes been recommended (e.g. Pedersen 2010). In order to avoid uptake of thiacloprid from nectar and pollen during pollination, our results strongly suggest that thiacloprid should not be sprayed on flowering plants. This conclusion is concurrent with Ellis C et al. (2017) who in a plastic tunnel study with flowering raspberries found that thiacloprid-exposed colonies were more likely to die prematurely, and that surviving colonies reached a lower final weight. Other studies into the mechanisms on how neonicotinoids affect pollinators have shown disturbed navigation in honey bees (Henry et al. 2012) and reduced ability for bumble bees to collect pollen (Stanley et al. 2015) and lay eggs (Laycock et al. 2012).

In 2015, there was no indication that thiacloprid was harmful to the pollinators when sprayed on red clover seed crops at the bud stage. Such a practise is in agreement with the off-label registration for thiacloprid in Norwegian red clover seed production (Norsk frøavlerlag 2018).

Acknowledgements

Thanks are extended to May Rundlöf, Riccardo Bommarco, Jens Åström and Yoko Dupont for help with planning the experiments and to Karin Westrum, the staff at NIBIO Landvik and the Norwegian Agricultural Extension Service in Vestfold (NLR Viken), Telemark and Buskerud (NLR Østafjells) for skilful implementation of these experiments. Also, thanks to Nina Oseth Svendsen and Agnethe Christiansen for help with the pesticide analyses and to Torfinn Torp for statistical advices.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was funded by the Norwegian Seed Growers' Union (Norsk frøavlerlag), the Norwegian breeding company Graminor, the two Norwegian seed companies Felleskjøpet Agri and Strand Unikorn and a grant [grant number 225019/E40] from the Research Council of Norway.

Notes on contributors

Lars T. Havstad is a seed scientist (Ph.D.) at The Norwegian Institute of Bioeconomy Research (NIBIO) working with seed production of grasses and clovers and turf grass research.

John I. Øverland works for the Norwegian Agricultural Extension Service (NLR) Viken, with seed production extension in Vestfold county, which is the main seed producing area in Norway.

Silja Valand works for the Norwegian Agricultural Extension Service (NLR) Viken, with seed production extension in Vestfold county, which is the main seed producing area in Norway.

Trygve S. Aamlid is a seed scientist (Ph.D.) at The Norwegian Institute of Bioeconomy Research (NIBIO) working with seed production of grasses and clovers and turf grass research.

References

Aamlid TS. 2018. Frøavl av rødkløver. Dyrkingsveiledning Mai 2018. [Seed production of red clover. Grower manual May 2018]. Http://froavl.no. Norwegian.

Aamlid TS, Andersen A, Lindemark PO, Øverland JI, Breivik L, Kise S, Jørgensen S, Susort Å, Steensohn AA. 2010. Bekjemping av snutebiller i frøeng av rødkløver. [Control of weevils in red clover seed crops]. Bioforsk Fokus. 1:212–217. Norwegian.

Blacquière T, Smagghe G, van Gestel CAM, Mommaerts V. 2012. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. Ecotoxicology. 21:973–992.

Bommarco R, Lundin O, Smith HG, Rundlöf M. 2012. Drastic historic shifts in bumble-bee community composition in Sweden. Proc Royal Soc B Biol Sci. 279:309–315.

Brødsgård CJ, Hansen H. 2002. Bi-bestøvning af rødkløver. [Honey bee pollination of red clover]. Grøn Viden. Markbrug nr. 257. Juni 2002. 4 pp. Danish.

Christiansen A. 2016. Handbook of analytical methods for pesticide residues. Ås: Norwegian Institute of Bioeconomy Research.

Decourtye A, Devillers J. 2010. Ecotoxicity of neonicotinoid insecticides to bees. In: Thany SH, editor. Insect nicotinic acetylcholine receptors. 1st ed. New York: Springer; p. 85–95.

Dupont YL, Damgaard C, Simonsen V. 2011. Quantitative historical change in bumblebee (*Bombus* spp.) assemblages of red clover fields. PLoS ONE. 6(9):e25172. doi:10.1371/journal.pone.0025172.

Eklima. 2019. Meteorological data collected from http://eklima.met.no/.

Ellis C P, Whitehorn P KJ, David A, Goulson D. 2017. The neonicotinoid insecticide thiacloprid impacts upon bumble bee colony development under field conditions. Environ Sci Technol. 51:1727–1732.

EN15662. 2008. Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE – QuEChERS-method. ISBN 9780580584411. 84 pp.

Goulson D. 2010. Bumble bees behaviour, ecology and conservation. Second ed. Oxford: Oxford university press.

Goulson D, Lye GC, Darvill B. 2008. Decline and conservation of bumble bees. Annu Rev Entomol. 53:191–208.

Goulson D, Nicholls E, Botías C, Rotheray EL. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science. 347:1255957.

Havstad LT, Åström J, Øverland JI, Valand S, Hetland O, Susort Å. 2015. Bedre pollinering av rødkløver [Improved pollination of red clover]. Jord- og plantekultur. Bioforsk Fokus. 10 (1):224–232. Norwegian.

Henry M, Beguin M, Requier F, Rollin O, Odoux J, Aupinel P, Aptel J, Tchamitchian S, Decourtye AA. 2012. A common pesticide Decreases Foraging Success and Survival in honey bees. Science. 336:348–350.

Iwasa T, Motoyama N, Ambrose JT, Roe JR. 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. Crop Prot. 23:371–378.

Jeschke P, Nauen R, Schindler M, Elbert A. 2011. Overview of the status and global strategy for neonicotinoids. J Agric Food Chem. 59:2897–2908.

Karise R, Viik E, Mänd M. 2007. Impact of alpha-cypremethrin on honey bees foraging on spring oilseed rape Brassica napus flowers in field conditions. Pest Manage Sci. 63:1085–1089.

Kolařík P, Rotrekl J. 2013. Regulation of the abundance of clover seed weevils, *Apion* spp. (*Coleoptera: Curculionidae*) in a seed stand of red clover (*Trifolium pratense* L.). J Entomol Acarol Res. 45:105–109.

Konzmann S, Lunau K. 2014. Divergent rules for pollen and nectar foraging bumblebees – a laboratory study with artificial flowers offering diluted nectar substitute and pollen surrogate. PLoS ONE. 9(3):e91900. doi:10.1371/journal.pone.0091900.

Laycock I, Lenthall KM, Barratt AT, Cresswell JE. 2012. Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*). Ecotoxicology. 21:1937–1945.

LMT. 2019. Meteorological data collected from http://lmt.nibio.no.

Lundin O. 2013. Ecology and management of crop pollination and pest control. Insights from red clover seed production. Doctoral Thesis. Swedish University of Agricultural Sciences. SLU Service/Repro. 54 pp.

Lundin O, Rundlöf M, Smith HG, Bommarco R. 2012. Towards integrated pest management in red clover seed production. J Econ Entomol. 105:1620–1628.

Maloof JE, Inouye DW. 2000. Are nectar robbers cheaters or mutualists? Ecology, 81(10): 2651–2661.

Mayer DF, Lunden JD. 1997. Effects of imidacloprid insecticide on three bee pollinators. Horticultur Sci. 29:93–97.

Mommaerts V, Reynders S, Boulet J, Besard L, Sterk G, Smagghe G. 2010. Risk assessment for side-effects of neonicotinoids against bumble bees with and without impairing foraging behaviour. Ecotoxicology. 19:207–215.

Mueller-Beilschmidt D. 1990. Toxicology and environmental fate of synthetic pyrethroids. J Pestic Reform. 10:32–37.

Norli HR, Christiansen A, Deribe E. 2011. Application of QuEChERS method for extraction of persistent organic pollutants in fish tissue and analysis by gas chromatography mass spectrometry. J Chromatogr A. 1218: 7234–7241.

Norsk frøavlerlag. 2018. Tilleggsetikett Off-label. Biscaya OD 240 – tiakloprid [Additional label. Biscaya OD 240 – thiacloprid]. https://www.froavlerlaget.no/Off_label/Tilleggsetikett/Biscaya_OD_240_godkjent_tilleggsetikett.pdf. Norwegian.

Öberg S, Gjershaug JO, Staverløkk A, Åström J, Ødegaard F. 2013. Framdriftsrapport 2012 fra utviklingsprosjekt: Naturindeks; videreutvikling av kunnskapsgrunnlaget for humler og sommerfugler [Progress report 2012 from development project: Nature Index; extending the knowledge base for bumble bees and butterflies]. NINA Minirapport 418. 18 s. Norwegian.

Ødegaard F, Gjershaug JO, Öberg S, Mjelde A. 2009. Status for humler (Hymenoptera, Apidae, Bombus spp.) i Norge i 2010 [Status of bumble bees (Hymenoptera, Apidae, Bombus spp.) in Norway in 2010]. Fauna. 62(4):94–104. Norwegian.

Pedersen TR. 2010. Bekämpa och reglera i vallfrö [control and regulation in seed production]. Svensk Frötidning. 3 (10):35–36. Swedish.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol Evol. 25:345–353.

Ratnieks FLW. 2000. How far do bees forage. Bee Improv. 6:10–11.

Rundlöf M, Lundin O, Bommarco R. 2018. Annual flower strips support pollinators and potentially enhance red clover seed yield. Ecol Evol. 8:7974–7985.

SAS Institute. 2015. Base SAS® 9.4 procedures guide. Cary (NC): SAS Institute Inc.

Schmuck R, Stadler T, Schmidt HW. 2003. Field relevance of a synergistic effect observed in the laboratory between an EBI fungicide and a chloronicotinyl insecticide in the honeybee (*Apis mellifera* L, Hymenoptera). Pest Manage Sci. 59:279–286.

Shires SW, Le Blanc J, Murray A, Forbes S, Debray P. 1984. A field trial to assess the effects of a new pyrethroid insecticide, WL85871, on foraging honeybees in oilseed rape. J Apic Res. 23:217–226.

Stanley DA, Garratt MPD, Wickens JB, Wickens VJ, Potts SG, Raine NE. 2015. Neonicotinoid pesticide exposure impairs crop pollination services provided by bumble bees. Nature. 528:548–550.

Stout JC, Allen JA, Goulson D. 2000. Nectar robbing, forager efficiency and seed set: bumble bees foraging on the self-incompatible plant *Linaria vulgaris (Scrophulariaceae*). Acta Oecol. 21:277–283.

Tison L, Hahn ML, Holtz S, Rößner A, Greggers U, Bischoff G, Menzel R. 2016. Honey bees' Behavior Is impaired by Chronic exposure to the neonicotinoid thiacloprid in the field. Environ Sci Technol. 50:7218–7227.

Thompson HM. 2001. Assessing the exposure and toxicity of pesticides to bumble bees (*Bombus* sp. Apidologie. 32:305–321.

Thompson HM. 2003. Behavioural effects of pesticides in bees – their potential for use in risk assessment. Ecotoxicology. 12:317–330.

Wermuth KH, Dupont YL. 2010. Effects of field characteristics on abundance of bumblebees (*Bombus* spp.) and seed yield in red clover fields. Apidologie. 41:657–666.

Whitehorn PR, O'Connor S, Wackers FL, Goulson D. 2012. Neonicotinoid pesticide Reduces bumble bee colony growth and Queen production. Science. 336(6079):351–352.

Williams RD. 1931. Methods and technique of breeding red clover, white clover and lucerne. Imperial Bureau of Plant Genetics. Herbage Plants. 3:46–77.

Woodcock BA, Bullock JM, Shore RF, Heard MS, Pereira MG, Redhead J, Ridding L, Dean H, Sleep D, Henrys P, et al. 2017. Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. Science. 356:1393–1395.

Woodcock BA, Isaac NJ, Bullock JM, Roy DB, Garthwaite DG, Crowe A, Pywell RF. 2016. Impacts of neonicotinoid use on long-term population changes in wild bees in England. Nat Commun. 7:12459.

Wratt EC. 1968. The pollinating activities of bumble bees and honey bees in relation to temperature, competing forage plants, and competition from other foragers. J Apic Res. 7 (2):61–66.