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Aphids and their natural enemies (Hymenoptera and Entomophthorales) in cereals - Population dynamics, prevalence and effect of insect pathogenic fungi

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Abstract

Aphids were sampled in 5 different cereal fields in southeast Norway in 2013 to investigate the population development through the growth season, and to evaluate whether counting of eggs from the bird cherry-oat aphid, *Rhopalosiphum padi,* during the winter gave good predictions on *R. padi*-population sizes the following growth season. Further, the prevalence of two groups of natural enemies of aphids, namely parasitoids and entomopathogenic fungi were recorded in the same fields. Results show that two cereal aphid species were recorded namely the English grain–aphid, *Sitobion avenae*, and *R. padi*. Aphid populations were low, however, especially for *R. padi.* Low numbers of *R. padi* eggs counted during February seemed to result in low numbers of *R. padi* aphids the following summer season. Whether this is a consistent correlation needs to be investigated further, however. Only one insect pathogenic fungal species, *P. neoaphidis*, was found, and it was observed in 3 out of 5 fields. It was recorded in *S. avenae only,* however. The total infection level in these fields ranged from 8-13 %. Several factors might have affected the low fungal infection level (e.g. climatic conditions, aphid population size etc.). Parasitoids from the Braconidae- family were recorded in all 5 fields. The Braconidae parasitoid *Aphidius avenae*, have not previously been documented in Norway. Parasitoids from Aphelinidae and hyperparasitoids from Figitidae were also recorded in some of the fields. The parasitoids were observed earlier in the season than entomopathogenic fungi, and before aphid population peak in all fields except one. In this study, laboratory bioassays were also conducted to investigate the efficacy of different *Pandora neoaphidis*-isolates at different temperatures, against two different pest aphid species; *Myzus persicae* and *R. padi*. The bioassays showed that *P. neoaphidis* resulted in a higher mortality of *M. persicae* than *R. padi.* Only one isolate of *P. neoaphidis* resulted in a lower nymph production by *M. persicae,* however, and no *P. neoaphidis* effect on *R. padi* nymph production was seen. Temperature affected nymph production of both aphid species, however, and at 12° C, nymph production was significantly lower for both species, than at 15 and 18° C.

1 Introduction

Aphids are common pests on several crops worldwide (Blackman and Eastop, 2000), and also vectors of a large number of plant viruses (REF). Natural enemies, like parasitoids and entomopathogenic fungi, are important regulators of aphid populations (Gullan and Cranston, 2005; Hawkins, 1994; Müller and Godfray, 1999; Tanada and Kaya, 1993) and may be used in different biological control strategies. Conservation biological control as defined by Eilenberg et al. (2001) may be one way of doing this. To be able to use these natural enemies in conservation biological control strategies, it is crucial, however, to have information on which enemies can be found in a cropping system, how prevalent they are, and in which part of the season they may be important for pest control.

Hymenopteran parasitoids are known to supress host populations, (Dixon, 1977; Hawkins, 1994), and two groups of parasitic wasps are found infecting aphids in Europe; Aphidiinae and Aphelinidae (Stary, 1970; Powell, 1982), and Norway (Hågvar and Hofsvang, 1991; Westrum et al., 2010). They can be highly effective and, in combination with insect predators, they may control aphid populations (Müller and Godfray, 1999).

Entomophthoralean fungi within the Class Entomophthoromycetes, order Entomophthorales, (Humber, 2012) are infecting aphids, as described by Hemmati et al. (2001b) and may also control aphid populations when conditions are right (e.g. microclimatic RH, temperature, population level etc) (Pell et al. 2001). Fungi are important entomopathogens, as they easily penetrate the insect cuticle and can supress insect populations (Hajek and St. Leger, 1994). Entomophthoralean conidia have a sticky surface, which attach to the host surface easily (Eilenberg et al., 1986). This is especially beneficial in decimating aphid populations, as they normally don't get infected through the mouthparts, since they are fixed on the host plant most of the time.

Pandora neoaphidis(Syn. Erynia neoaphidis) is an important fungal pathogen on aphids in temperate agroecosystems (Ekesi et al., 2005), and can cause epizootics in aphid populations in crops (McLeod et al., 1998; Elkassabany et al., 1992; Feng et al., 1992; Scorsetti et al., 2007; Barta and Cagan, 2006). Further, *P. neoaphidis* has been found to cause epizootics in aphids in Norway (Westrum and Klingen, 2007). The use of fungicides can have a negative effect on fungi in the Entomophthoromycetes, making them less efficient as control agents (Lagnaoui et Radcliffe 1998, Klingen & Westrum 2007).

P. neoaphidis has the ability to infect several aphid species on different host plants, including the green peach aphid*, Myzus persicae* (Reyes-Rosas et al., 2012, Elkassabany et al., 1992, Scorsetti et al., 2007, Lagnaoui and Radcliffe, 1998, McLeod et al., 1998), the english grain –aphid, *Sitobion avenae*, (Steenberg and Eilenberg, 1995; Dromph et al., 2002; Hemmati et al., 2001a; Feng et al., 1992; Barta & Cagáň, 2007) and the bird cherry-oat aphid, *Rhopalosiphum padi* Reyes-Rosas et al., 2012; Barta & Cagan, 2007).

M. persicae, *S. avenae* and *R. padi* are all important pest insects. *M. persicae* has the peach tree, *Prunus persicae* as primary host, but it can also feed on and is a pest on a wide variety of other host plants from over 40 plant families (Blackman and Eastop, 2000). Further, it is a vector for over 100 plant viruses, making it a threat to many different crops. *R. padi* and *S. avenae* are both considered major pests on cereal and are important vectors of the Barley Yellow Dwarf virus (BYDV) (Blackman and Eastop, 2000). *R. padi* lays eggs that overwinter on bird cherry (*Prunus padus*), and has all types of cereal and pasture grass as secondary hosts (Blackman and Eastop, 2000). *S. avenae* has cereals and pasture grasses as hosts in the growing season, and overwinters as eggs near the soil on the same plants (Blackman and Eastop, 2000).

Efficient control of aphid populations by parasitoids and entomopathogenic fungi requires that these natural enemies are well adapted to the life cycles of their hosts. Further, the aphid populations need to be big enough for natural enemies to be effective (Feng et al. 1991). Aphid species in non-crop plants (e.g. weeds) can act as important reservoirs for the natural enemies (Powell et al., 1986a). Climatic conditions (RH, temperature, rainfall) and light are suggested to be important factors affecting the performance of entomophthoralean fungi (Hemmati et al., 2001b, De Castro et al., 2013).

The object of this study was to assess how populations of aphid species in cereals develop during the growth season in Norway, with emphasis on the diversity and abundance of their natural enemies among parasitoids and entomopathogenic fungi. An applied aspect of the study was to see how well winter counting of *R. padi*-eggs on bird cherries (VIPS, 2013) predicts the number of aphids in cereals the following growing season. Further, isolates of the entomopathogenic fungus *P. neoaphidis* found in cereal fields were tested in laboratory assays, to investigate the effect of aphid species, temperature, and isolate on virulence of the fungus.

2 Materials and methods

2.1 Field survey on prevalence and population dynamic of aphids and their natural enemies (entomopathogenic fungi and parasitoids) in cereals

2.1.1 Survey locations

Field collections of aphids were done at five different study sites in southeast Norway, in three different counties in 2013 (Table 1). Three of the locations (Apelsvoll, Amagerjordet and Brandval), were selected because of annual samplings of *R. padi*- eggs on bird cherries. The locations consisted of two cereal fields with organic crops (Amagerjordet and Vollebekk organic), and three cereal fields with conventional crops (Apelsvoll, Vollebekk conventional and Brandval). Cereals included spring wheat, winter wheat and oats (Table 1). In addition to collecting aphids, Bird cherry trees located in the boundary vegetation of the cereal fields were observed for overwintering *R. padi* eggs. The organic fields were not treated with any pesticides. Sampling areas at the conventional fields

Apelsvoll and Brandval were kept untreated, but the surrounding areas were sprayed. At Vollebekk conventional, the sampling area got the same treatment as the surrounding fields, and was sprayed with herbicides (12 June), and fungicides (3 July). Pesticides used in the surrounding field at Apelsvoll included herbicides (applied 26 May), fungicides (applied 02 July and 12 July), and insecticides (applied 02 July). In the surrounding fields in Brandval only herbicides were applied (1 July).

Table 1. Aphid and *R. padi* egg sampling sites information.

Location/ ETRS89-coordinates	Cereal species and variety	Sow date (S) harvest date (H)	First and last sampling of Bird cherry and cereal	Fertilised with (date)
Apelsvoll, Østre Toten, Oppland N 60.70 E 10.87	*Triticum aestivum* Spring wheat 'Bjarne'	28.04.13 (S) 29.08.13 (H)	Bird cherries: 27.05.13 (first) 02.07.13 (last) Cereal: 11.06.13 (first) 27.08.13 (last)	NKP 19-4-12 (28.04.13)
Amagerjordet, Ås, Akershus, N 59.67 E 10.78	*Avena sativa* Oats 'Belinda'	15.05.13 (S) 27.08 (H)	Bird cherry: 11.06.13 (first) 09.07.13 (last) Cereal: 11.06.13 (first) 20.08.13 (last)	Cow manure (06.05.13)
Vollebekk organic, Ås, Akershus, N 59.40 E 10.47	*Triticum aestivum* Winter wheat 'Bjørke'	10.09.13 (S) 22.08.13 (H)	Bird cherries: 18.06.13 (first) 09.07.13 (last) Cereal: 18.06.13 (first) 20.08.13 (last)	Organic fertiliser with NKP 8-5-4 (21.05.13)
Vollebekk conventional, Ås, Akershus, N 59.40 E 10.47	*Triticum aestivum* Winter wheat 'Finans'	08.09.13 (S) 21.08.13 (H)	Bird cherries: 19.06.13 (first) 09.07.13 (last) Cereal: 19.06.13 (first) 20.08.13 (last)	NKP 22-3-10 (02.05.13)
Brandval kirke, Kongsvinger, Hedmark N 60.26 E 12.08	*Avena sativa* Oats 'Odal'	04.06.13 (S) 09.09.13 (H)	Bird cherry: 04.07.13 (first) 08.07.13 (last) Cereal: 04.07.13 (first) 16.09.13 (last)	NKP 22-3-10 (04.06.13)

2.1.2 Aphid and *R. padi* egg sampling

Bird cherry trees located in the boundary vegetation of the cereal fields were observed for overwintering *R. padi* eggs by collecting 10 branches from a Bird cherry tree in February. The branches had from 10-15 buds and were collected at 2 m above the ground on a tree in the border vegetation at Apelsvoll, Amagerjordet and Brandval. Branches were then transported to the laboratory for processing. In the growing season, sampling of *R. padi* aphids were done weekly from the bird cherry trees, starting as shown in table 1, until aphids were observed in the cereal field. At Apelsvoll, 5 bird cherries were sampled for *R. padi* aphids, 1 bird cherry was sampled at Amagerjordet, 2 at Vollebekk organic, and 2

at Vollebekk conventional. In the cereal fields, samplings were done weekly until harvest (Table 1). From the bird cherries, 10 leaves were collected from each tree and put in air-filled paper bags wrapped in plastic bags. They were kept in a Styrofoam box with a cooling device, and transported to the lab for processing the following day. The collection sites for aphids on cereal plants in the field was 10 m wide, and 60 m long and 10 cereal plants were collected at 3 different distances from the bird cherry trees and into the field; 0-5 m, 25-30 m and 55-60 m (Figure 1). This resulted in a total of 30 plants collected per location per sampling date. As Vollebekk organic was an experimental field, the gradients had to be adjusted, and plants here were collected from 0-5 m, at 30 m and 60 m. Plants were collected by cutting them at the plant basis and as close as possible to the soil. This was to secure that I would also be able to collect *S. avena* which is located were low on the plant at the beginning of the season. To avoid that this destructive sampling of plant would affect the aphid and natural enemy population, plants were collected at six different transects along the 10 m wide sampling area, starting at 3 m, the first week of collecting, and then at 4, 5, 6 and 7 m the following weeks, before starting at 3 m again (Figure 1). The plants from each gradient were kept in a Styrofoam box with a cooling device, and transported to the lab for processing the following day.

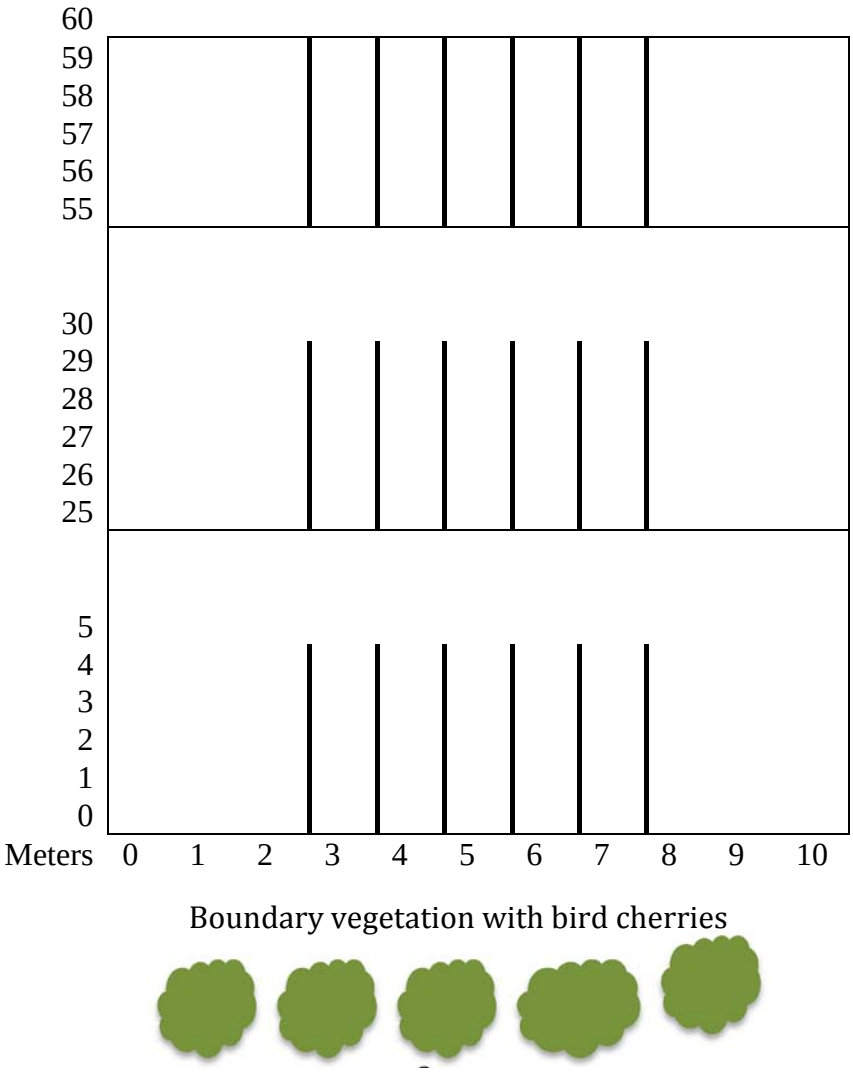


Figure 1. Aphid sampling design.

2.1.3 Observation of fungi and parasitoids in the laboratory

Branches collected from Bird cherry in February were observed under a compound microscope (40-80x) to quantify eggs per 10 buds. Cereal plants collected from the field during summer were observed under a compound microscope (40-80x) to identify and quantify adult aphids and nymphs. The adult aphids were then individually incubated on an oat leaf mounted in 30 mL plastic vials filled with 10 mL 1.5 % water agar. To avoid free water to develop in the vials, lids were perforated with 9 holes by the use of a thumbtack. The vials with the aphids were then kept in a climate chamber at 18° C, 65 % RH, L:D 18:6 and inspected daily except weekends, for mortality and development of entomopathogenic fungi and parasitoids. Vials with dead aphids were placed in a humidity chamber and examined daily, except weekends, for emergence of fungi within the Entomophthoromycota, or parasitoids. Parasitoids were put in glass vials in 80% ethanol for preservation and later identification. Sporulating aphid cadavers were squash mounted. Spore shape, size and number of kernels were measured, and species were identified according to relevant literature (Remaudière and Hennebert, 1980; Keller, 1991; Balazy, 1993).

2.1.4 Weather data

Weather data was provided by LMT (Agro Meteorology Norway), a service delivered by Bioforsk (Bioforsk 2014), and was collected from 3 different weather stations, near the sampling locations; Apelsvoll, Ås, (covering Amagerjordet, Vollebekk organic and Vollebekk conventional), and Roverud (Brandval). Two dates at Roverud had no registered data for temperature and RH (14 August and 15 August). For these two dates, data from Kongsvinger, roughly ten km from Roverud, was used (yr.no 2014).

2.2 Bioassays on the effect of temperature and isolate of *Pandora neoaphidis* on mortality and reproduction of two aphid species

2.2.1 Aphid stock cultures

Two aphid species; *M. persicae* and *R. padi,* were tested in the bioassay. They were collected and kept in a laboratory culture at Bioforsk Plant Health and Protection Centre at conditions shown in Table 2.

Table 2. Time and place of collection of the two aphid species used in the bioassay as well as conditions used in the laboratory culturing of them.

Aphid species	Year of collecting	Collection site	Collected on	Kept on	Climatic conditions
Green peach aphid *Myzus persicae*	2000	Jæren, Rogaland	Chinese cabbage *Brassica rapa* ssp	Pepper plants *Capsicum annuum*	LD: 16:8 Temp: 22 ° C RH: 60 %
Bird cherry-oat aphid *Rhopalosiphum padi*	2012	Toten, Oppland	Bird cherry *Prunus padus*	Oat plants *Avena sativa*	LD: 16:8 Temp: 21 ° C RH: 60 %

2.2.2 Isolating and culturing of the fungus *Pandora neoaphidis*

Two *P. neoaphidis* isolates were used in the bioassay, NCRI391/13(4) and NCRI393/13(6). Both isolates were obtained from *S. avenae*, collected in Nykirke, Horten, Vestfold, (WGS84 N 59° 26.083', E 10° 24.191') on 08.08.2013. The two *P. neoaphidis* isolates were grow on Saboraud Dextrose Milk Yolk Agar (SDAMY) on sterile petri dishes sealed with Parafilm, and transferred onto new petri dishes regularly to ensure maintenance of the culture. Fast-growing cultures of *P. neoaphidis* were selected for transfers. This was done by cutting three circular pieces (5 mm diameter) from the edges of the fungal mat and transferring these to new petri dishes with SDAMY. The petri dishes were then kept in dark plastic boxes lined with wet filter paper and placed at 18° C and 65% RH.

After 5 months, and 9 transfers, the isolates did not sporulate well. It was therefore made a decision to revitalize the isolate by letting it go through an insect and the isolates were therefore used to infect *M. persicae*, and the cadavers of fungi-infected aphids were used to make "new" revitalized and hopefully more virulent isolates. To isolate the spores from the infected *M. persicae*, a modified version of the "Ascending conidia shower method" (Keller, 1994; Barta, 2004), was used. One aphid cadaver was put on a piece of water agar in a big petri dish with wet filter paper in the bottom. A smaller petri dish with SDAMY was placed upside down over the aphid. The distance between the SDAMY with sporulating *P. neoaphidis* was not more than 3 mm, and within the conidial discharge range of *P. neoaphidis* (Hemmati et al., 2001a). The lid of the bigger petri dish was put on top, and everything was kept at room temperature in a sterile workbench in darkness. The lid with SDAMY was replaced every 30 min for 3 to 4 h after sporulation had started. After spores were collected, the SDAMY- dishes with *P. neoaphidis*-conidia were put in a dark plastic box with wet filter paper and left to grow, at 18° C and 65% RH. To get enough SDAMY- dishes with *P. neoaphidis* for the following assays, transfers were done when the fungal mat had grown to cover about 50 % of the petri dish.

2.2.3 Experimental set up

The *P. neoaphidis* isolates (NCRI391/13(old4), NCRI393/13(old6), NCRI391/13(new 4) and NCRI393/13(new 6)) (from now on called old4, old6, new4 and new6), were tested on adult females of the aphid species *M. persicae* and *R. padi*, at 3 different temperatures; 12° C, 15° C and 18° C. Twenty four h prior to use in the bioassay, the petri dishes with sporulating *P. neoaphidis* were put inside a dark plastic box with wet filter paper in the climate cabinet at the same temperature they would be tested at, to get acclimatised. Between 25 and 30 *M. persicae* or *R.padi* were placed on leaf dishes in a 55 mm petri dish with 1.5 % water agar, 24 h prior to the assay for the aphids to settle before exposure to the fungal isolates. *M. persicae* was placed on a 25 mm, circular paprika leaf disc and *R. padi* was placed on 4 pieces of oat leaf.

After 24 h, aphids were exposed to *P. neoaphidis* by placing sporulating cultures of the pathogen on SDAMY over the aphids. A fine plastic gauze with mesh size 1 mm x 0.5 mm was put in between the aphid dish and the lid, to avoid the aphids getting in direct contact with the inoculum (Shah et al. 2004; Barta 2004). The dishes with aphids and fungal cultures were put in dark plastic boxes with wet filter paper, and left to sporulate at 12° C, 15° C or 18° C, and 70 % RH Exposure time was 5 h. The lids with *P. neoaphidis* were rotated 90° every 75 min. An18x18 mm cover slip with grids of 0.6x0.6 mm was placed in each petri dish to check that spores were actually thrown and hence were present.
Aphids were incubated separately after fungal exposure, in 30 mL plastic vials filled with 10 mL 1.5 % water agar. To avoid the development of free water in the vials; lids were perforated with 9 holes by the use of a thumbtack. *M. persicae* were put on pepper leaf-discs, (15 mm in diameter) that were placed on top of the water agar. *R. padi* were put on an oat leaf that was mounted into the water agar. The incubated aphids were then placed at 12° C, 15° C or 18° C, 75% RH and 16:8 L:D.

The aphids were monitored daily for a maximum of 16 days after exposure, for number of nymphs produced and mortality of adult aphids. Nymphs were counted and removed. Dead aphids were kept in the plastic vial, but the plant was removed. The dead aphids were kept for up to 3 days, to confirm or exclude fungal infection. A total of 671 *M. persicae*, and 534 *R. padi* were tested in the assays. Number of aphids tested at the different temperatures and isolates are shown in table 3.

Table 3. Number of aphids of two different species exposed four *Pandora neoaphidis* isolates at 3 different temperatures.

Isolate	*Myzus persicae*			*Rhopalosiphum padi*		
	12° C	15° C	18° C	12° C	15° C	18° C
NCRI391/13(4)	24	48	48	42	19	20
NCRI393/13(6)	24	48	47	44	19	19
NCRI391/13(new 4)	48	48	48	48	48	48
NCRI393/13(new 6)	48	48	48	47	24	-
Control	48	48	48	68	44	44

2.2.4 Data analysis

Statistical analysis were done in R. Analysis of variance, ANOVA type III was used to compare reproduction of *M. persicae* and *R. padi* both exposed and non-exposed to *P. neoaphidis*. To compare mortality rates, Generalized linear mixed model (GLMM) fit by maximum likelihood (Laplace Approximation) was used. In addition, Chi square was used to test if the different variables (species, temperature and isolate), had effect separately. Tests were done with significance level 0.05. "p≈0" denotes $p<10^{-4}$.

3 Results

3.1 Field survey on prevalence and population dynamic of aphids and their natural enemies (entomopathogenic fungi and parasitoids) in cereals

The winter sampling of *R. padi*-eggs from bird cherries, done at Apelsvoll and Brandval, showed 4.1 and 0.4 eggs per 10 buds, respectively. No aphids were however found in bird cherry trees during the growth season. Two aphid species were found in the cereal fields, *S. avenae* and *R. padi*. All over the densities were low, but *S. avenae* was more numerous than *R. padi* in all locations (Figure 2 to 4). The *S. avenae* aphid density peaked in the field when the cereal was at the milk stage (BBCH75) in Apelsvoll, Amagerjordet (BBCH75) and Vollebekk organic (BBCH71). At Vollebekk conventional, the peak was observed when the cereal reached the dough state (BBCH85). At Brandval, the aphid population peak was when the cereal was in the flag leaf-opening stage (BBCH47). Since the number of collected aphids was low, numbers from the different distances were pooled.

Aphid parasitoids were found in all five cereal fields but only in *S. avenae*. They were from six genera; *Aphidius*, *Aphelinus*, *Ephedrus*, *Praon*, *Toxares* and *Ephedrus* (Table 6). Parasitoids were observed earlier in the season than entomopathogenic fungi, and before aphid population peak in all fields except Amagerjordet, were first observation of parasitoids was in the same week as the aphid population peak (Table 5).

Entomophthoralean fungi were observed in 3 out of 5 fields, namely at Apelsvoll, Vollebekk organic and Vollebekk conventional (Table 5). Only *S. avenae* were found infected with fungi, and *P. neoaphidis* was the only fungal species observed. The first observation of *P. neoaphidis* was at aphid population peak in Vollebekk organic and Vollebekk conventional, and two weeks after aphid population peak in Apelsvoll.

Table 5. Parasitoids from the order Hymenoptera found on *Sitobion avenae* on cereal plants in five locations.

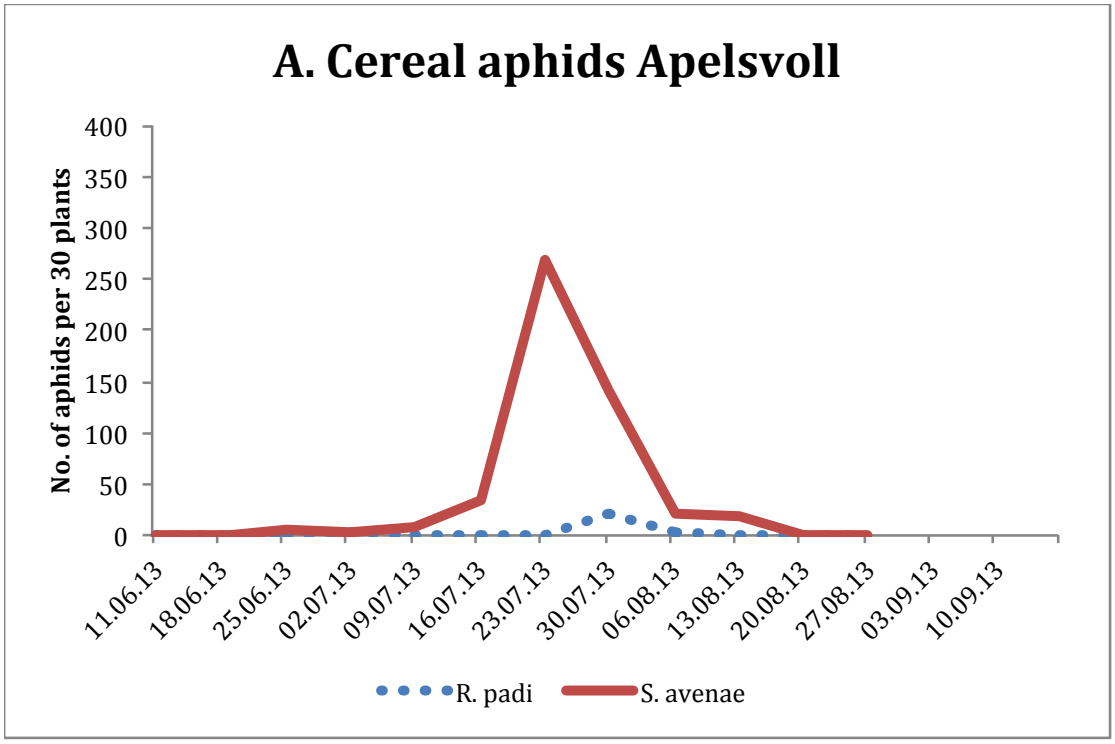
Family Subfamily (Primary or hyper-parasitoids)	**Genus**	**Species**	**Host plant**	**Location**	**First date of observation**
Aphelinidae Aphelininae (Primary parasitoids)	*Aphelinus*	-	*Triticum aestivum* Spring wheat 'Bjarne'	Apelsvoll	09.07.13
		-	*Triticum aestivum* Winter wheat 'Bjørke'	Vollebekk organic	30.07.13
		-	*Avena sativa* Oats 'Odal'	Brandval	08.07.13
Braconidae Aphidiinae (Primary parasitoids)	*Aphidius*	*Aphidius avenae*	*Avena sativa* Oats 'Belinda'	Amagerjordet	30.07.13
		Aphidius ervi	*Triticum aestivum* Spring wheat 'Bjarne'	Apelsvoll	23.07.13
		Aphidius ervi	*Triticum aestivum* Winter wheat 'Finans'	Vollebekk conventional	23.07.13
		Aphidius ervi	*Avena sativa* Oats 'Odal'	Brandval	08.07.13

Family Subfamily (Primary or hyper-parasitoids)	**Genus**	**Species**	**Host plant**	**Location**	**First date of observation**
	Ephedrus	*Ephedrus plagiator*	*Triticum aestivum* Spring wheat 'Bjarne'	Apelsvoll	16.07.13
		Ephedrus plagiator	*Triticum aestivum* Winter wheat 'Bjørke'	Vollebekk organic	09.07.13
	Praon	*Praon sp.* (*Praon gallicum* or *Praon necans*)	*Triticum aestivum* Winter wheat 'Finans'	Vollebekk conventional	09.07.13
	Toxares	*Toxares deltiger*	*Triticum aestivum* Winter wheat 'Finans'	Vollebekk conventional	16.07.13
		Toxares deltiger	*Avena sativa* Oats 'Odal'	Brandval	08.07.13
Figitidae Charipinae (Hyperparasitoids)	*Alloxysta*	-	*Triticum aestivum* Spring wheat 'Bjarne'	Apelsvoll	23.07.13
		-	*Triticum aestivum* Winter wheat 'Finans'	Vollebekk conventional	23.07.13

Table 6. Percentage of incubated adult females of *Sitobion avenae* dying from fungal infection or parasitoids.

Location	% *S. avenae* with *P. neoaphidis* (n*)	First sampling date with *P. neoaphidis*	% *S. avenae* with parasitoids (n*)	First sampling date with parasitoid
Apelsvoll	8 (38)	06.08.13	21 (38)	09.07.13
Amagerjordet	0 (18)	-	6 (18)	30.07.13
Vollebekk organic	13 (31)	16.07.13	6 (31)	09.07.13
Vollebekk conventional	7 (45)	23.07.13	13 (45)	25.07.13
Brandval	0 (12)	-	25 (12)	08.07.13

*n= numbers of adult *S. avenae* incubated for observation of fungi and parasitoids. All sampling dates were pooled.



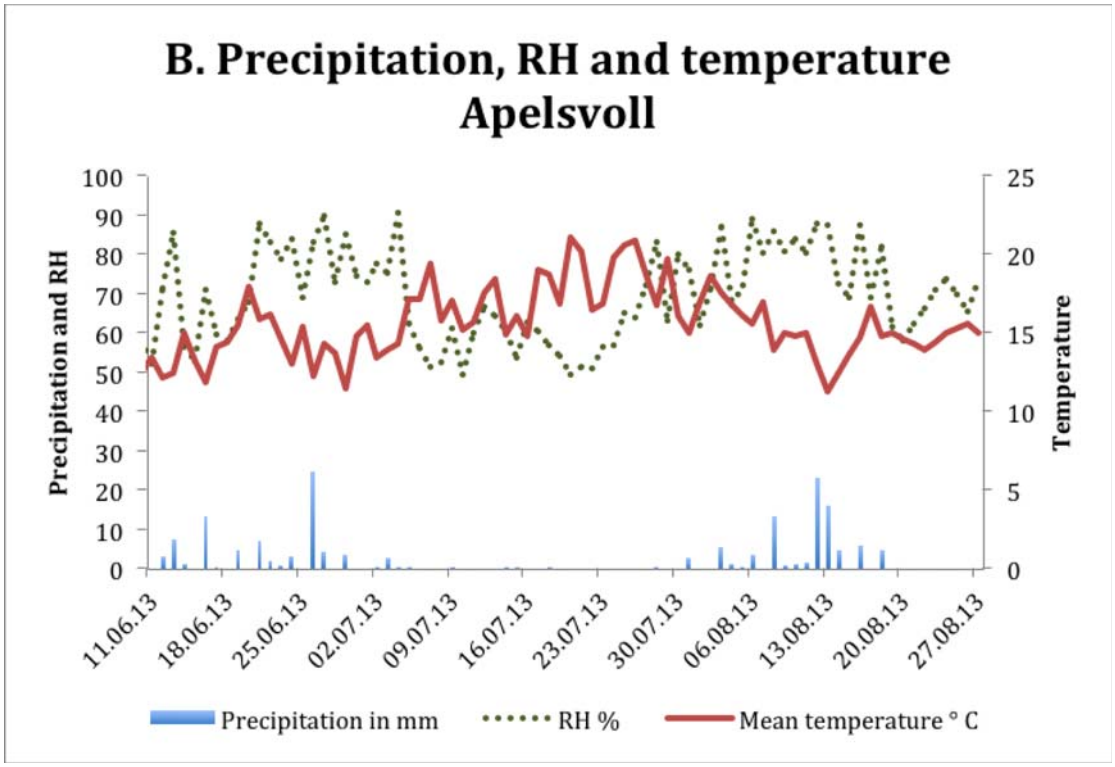
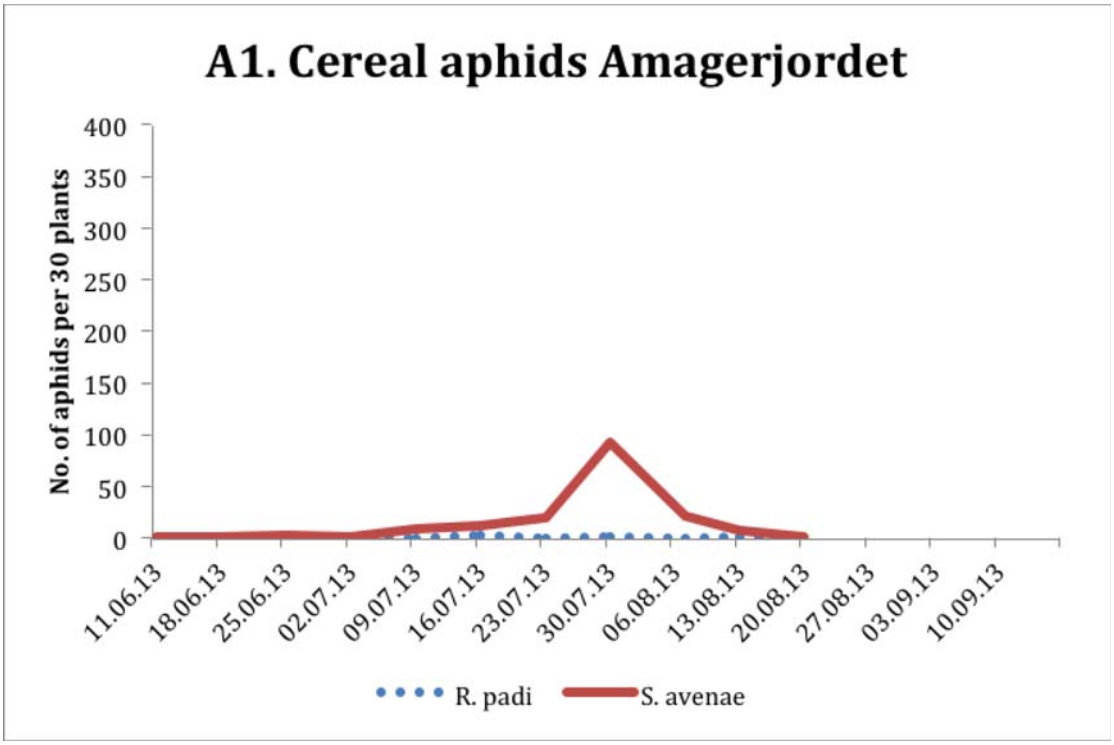
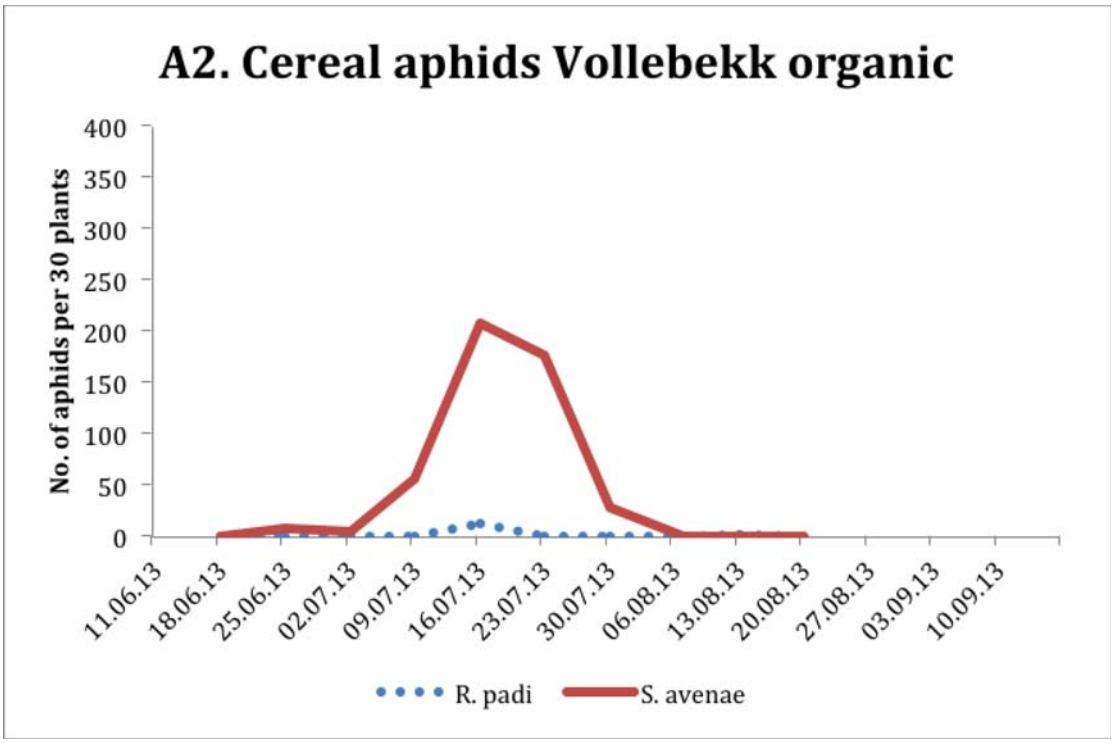


Figure 2. A) Number of aphid species *S. avenae* (solid line) and *R. padi* (dotted line) as a function of date in cereals surveyed at Apelsvoll. B) Weather data from Apelsvoll.


A1. Cereal aphids Amagerjordet
No. of aphids per 30 plants
400
350
300
250
200
150
100
50
0
11.06.13
18.06.13
25.06.13
02.07.13
09.07.13
16.07.13
23.07.13
30.07.13
06.08.13
13.08.13
20.08.13
27.08.13
03.09.13
10.09.13
R. padi
S. avenae


A2. Cereal aphids Vollebekk organic
No. of aphids per 30 plants
400
350
300
250
200
150
100
50
0
11.06.13
18.06.13
25.06.13
02.07.13
09.07.13
16.07.13
23.07.13
30.07.13
06.08.13
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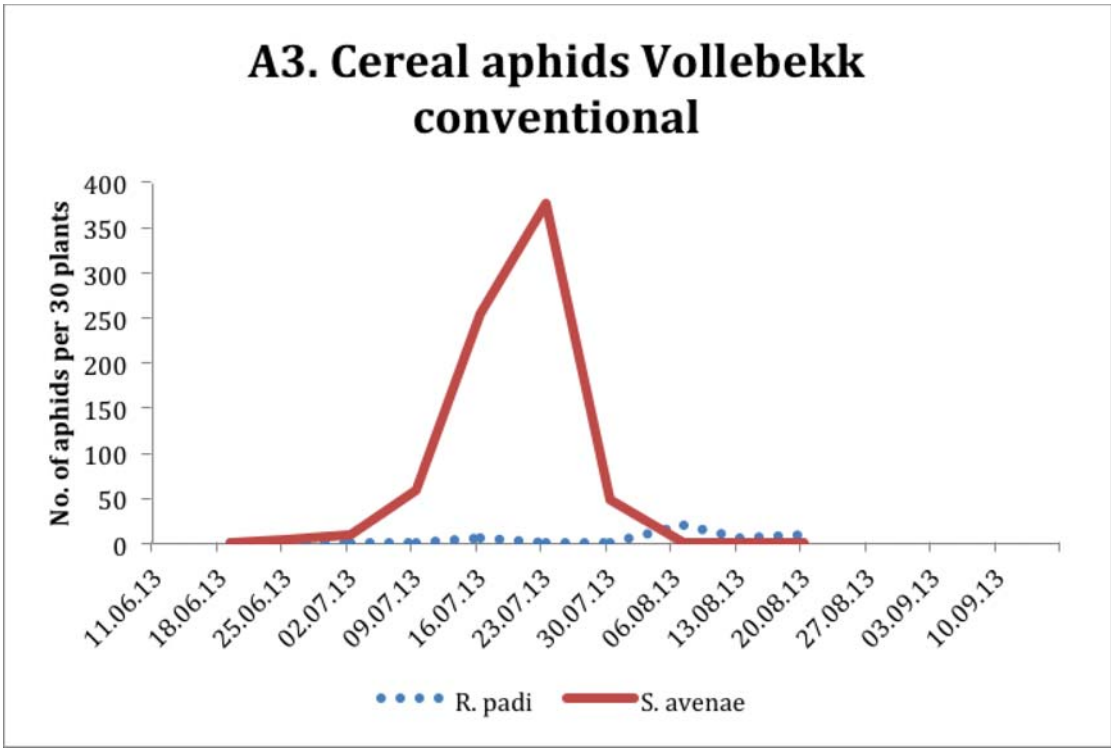
20.08.13
27.08.13
03.09.13
10.09.13
R. padi
S. avenae



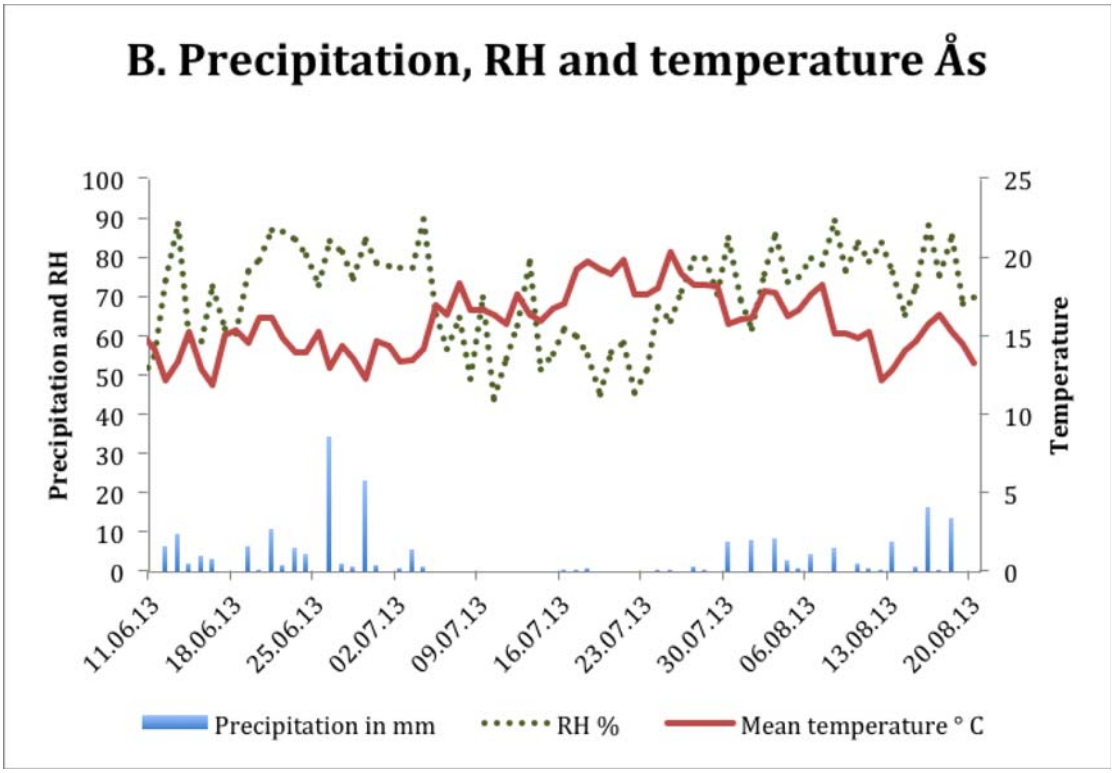


Figure 3. A) Number of aphid species *S. avenae* (solid line) and *R. padi* (dotted line) as a function of date in cereals surveyed at three locations in Ås; Amagerjordet (A1), Vollebekk organic (A2), and Vollebekk conventional (A3). B) Weather data from Ås.

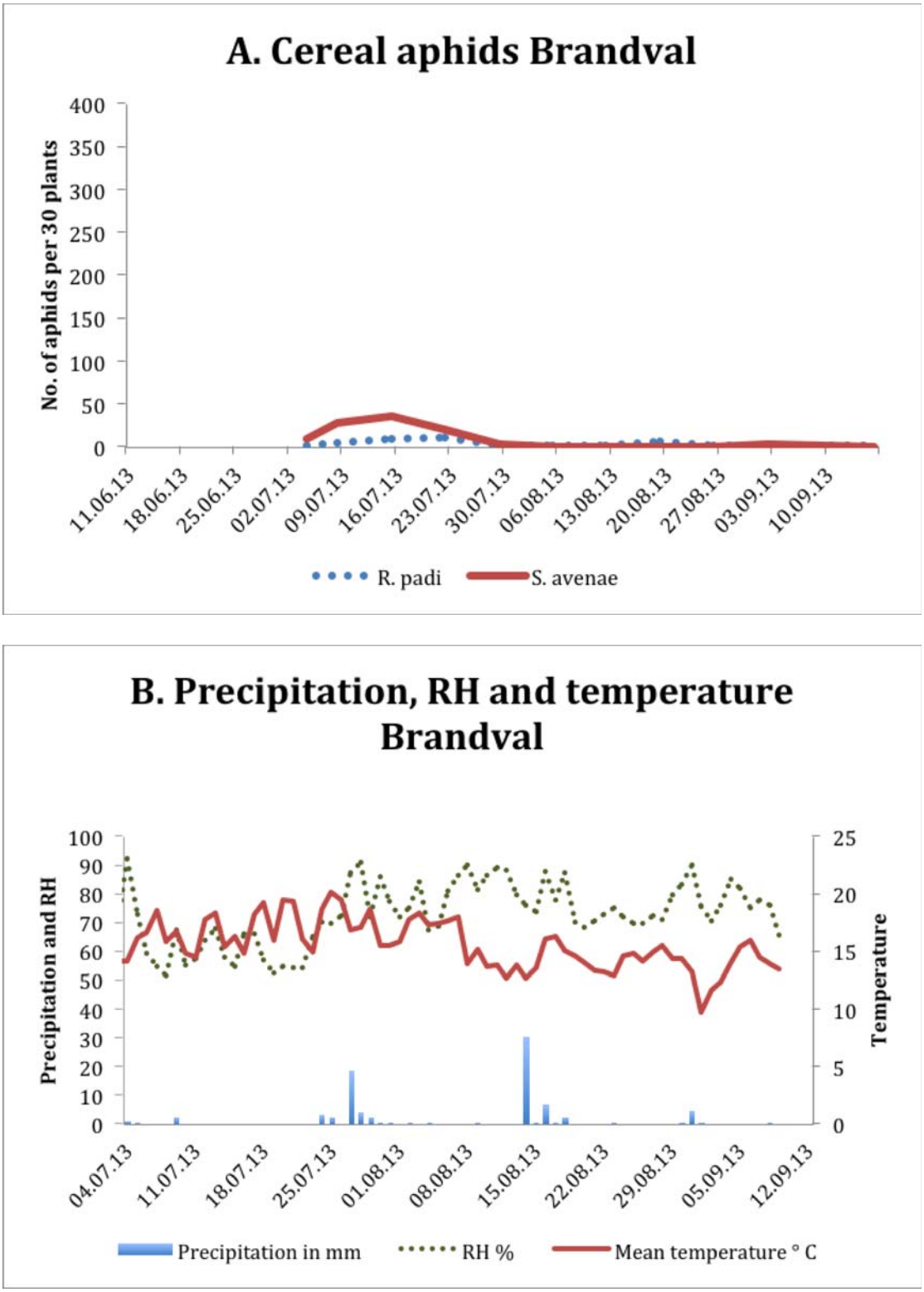


Figure 4. A) Number of aphid species *S. avenae* (solid line) and *R. padi* (dotted line) as a function of date in cereals surveyed at Brandval. B) Weather data from Brandval.

3.2 Bioassays on the effect of temperature and isolate of *Pandora neoaphidis* on mortality and reproduction of two aphid species

Two isolates obtained from *S. avenae* were initially used in the assays, but after passing them through *M. persicae* to see if this would revitalize them, a total of 4 isolates were tested on *M. persicae* and *R. padi;* Isolate NCRI 391/13(old4), Isolate NCRI 393/13(old6), Isolate NCRI 391/13(new 4), and Isolate NCRI 393/13(new 6).

Isolate NCRI 391/13(new 4) showed the highest mortality percentage in all treatments, except for *M. persicae* at 12 ° C, where isolates NCRI 391/13(new 4) and NCRI 393/13(old6) both resulted in a mortality of 42 % (Table 7). *R. padi* mortality was at 0% and lower in all assays, except for isolate NCRI 391/13(new 4) where there was some mortality at all temperatures (Table 7).

Table 7 (A-D). Mortality of *Myzus persicae* and *Rhopalosiphum padi* treated with two isolates of *Pandora neoaphidis* at three different temperatures.

A

Isolate NCRI 391/13(old4)			
Aphid species	12 ° C	15 ° C	18 ° C
	Percentage mortality ±SD (n)	Percentage mortality ±SD (n)	Percentage mortality ±SD (n)
M. persicae	0 (24)	6 ±3 (48)	6 ±3 (48)
R. padi	0 (42)	0 (20)	0 (20)

B

Isolate NCRI 393/13(old6)			
Aphid species	12 ° C	15 °C	18 ° C
	Percentage mortality ±SD (n)	Percentage mortality ±SD (n)	Percentage mortality ±SD (n)
M. persicae	42 ±10 (24)	6 ±3 (48)	26 ±6 (47)
R. padi	0 (44)	0 (20)	0 (19)

C

Isolate NCRI 391/13(new 4)			
Aphid species	12 ° C	15 °C	18 ° C
	Percentage mortality ±SD (n)	Percentage mortality ±SD (n)	Percentage mortality ±SD (n)
M. persicae	42 ±7 (48)	72.95 ±6 (48)	60.40 ±7 (48)
R. padi	16.65 ±5 (48)	16.65 ±5 (48)	8.33 ±4 (48)

D

Isolate NCRI 393/13(new 6)			
Aphid species	12 ° C	15 °C	18 ° C
	Percentage mortality ±SD (n)	Percentage mortality ±SD (n)	Percentage mortality ±SD (n)
M. persicae	0 (48)	4.15 ±3 (48)	10.40 ±4 (48)
R. padi	0 (48)	0 (24)	-

Mortality was significantly higher for *M. persicae* than *R. padi*. Pooling all assays, Chi-square testing showed an effect of species on total mortality ($p < 0.05$). Estimation in GLMM showed that *M. persicae* had 20 % total mortality, while *R. padi* only had 8 %, (Figure 5). There was no significant effect of temperature on total mortality (Figure 6). Chi-square of isolate effect on mortality showed that isolate had an effect ($p < 0.01$). Testing correlation with GLMM showed that there was a significant effect of Isolate new 4 on *M. persicae* (Figure 7). Control treatment had significantly lower mortality, except for *R. padi* treated with Isolate NCRI 393/13(new 6), where the control mortality was lower, but not significant (Figure 7).

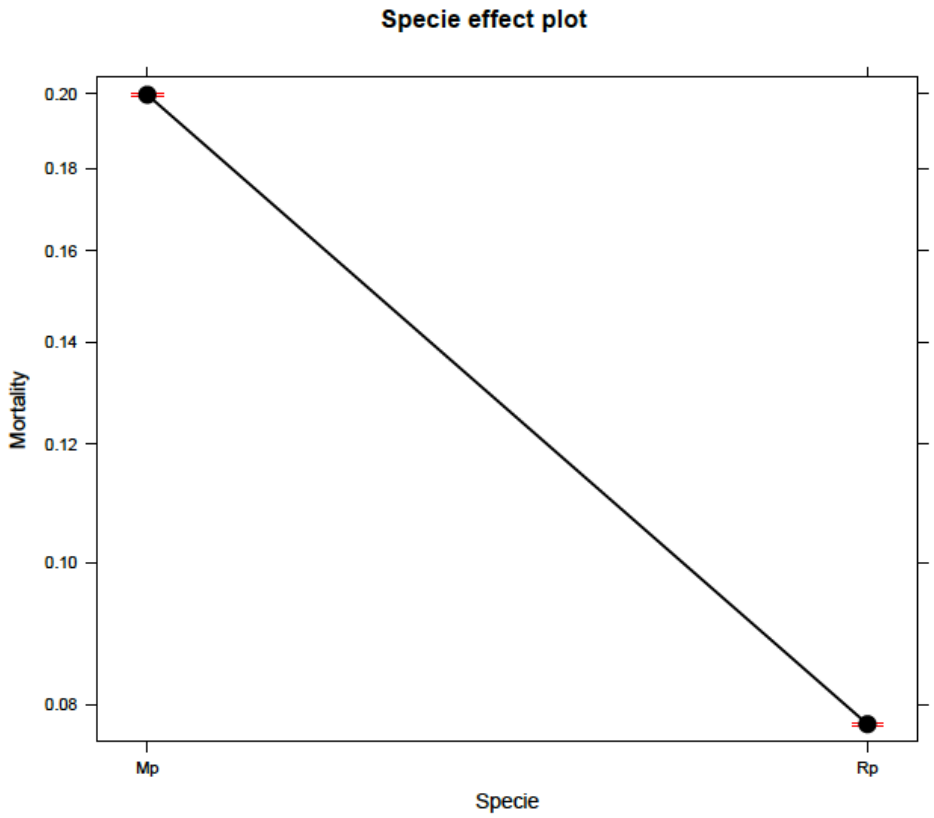


Figure 5. Effect plot showing overall mortality of *Myzus persicae* (=Mp) and *Rhopalosiphum padi* (=Rp) in the bioassays, (all data pooled).

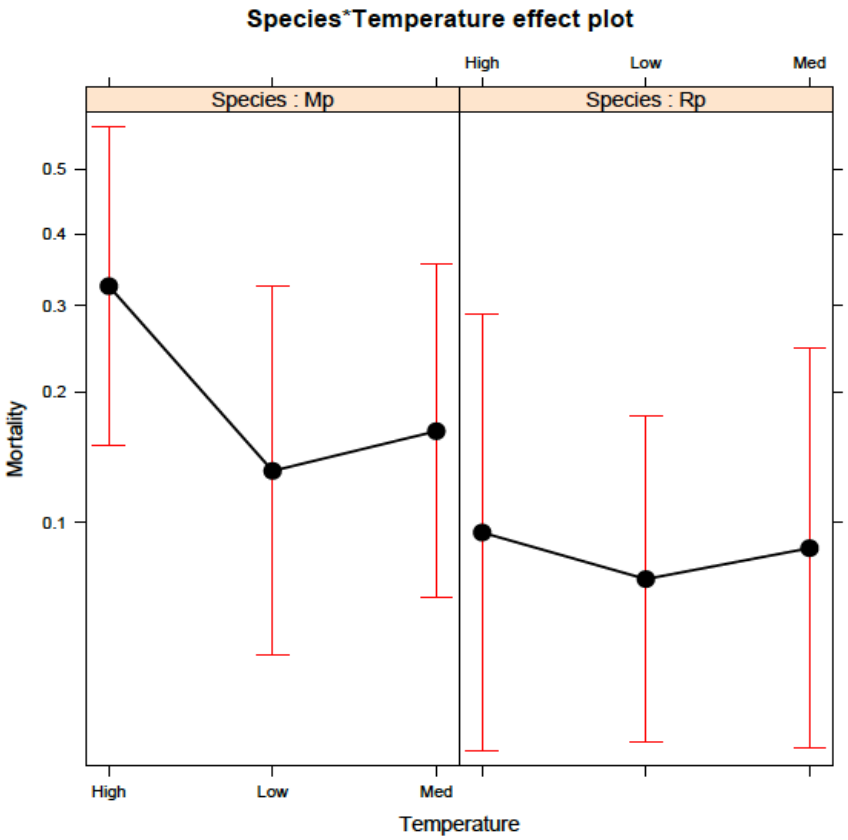


Figure 6. Mortality as a function of temperature in the two different aphid species (Mp=Myzus persicae, Rp=Rhopalosiphum padi).

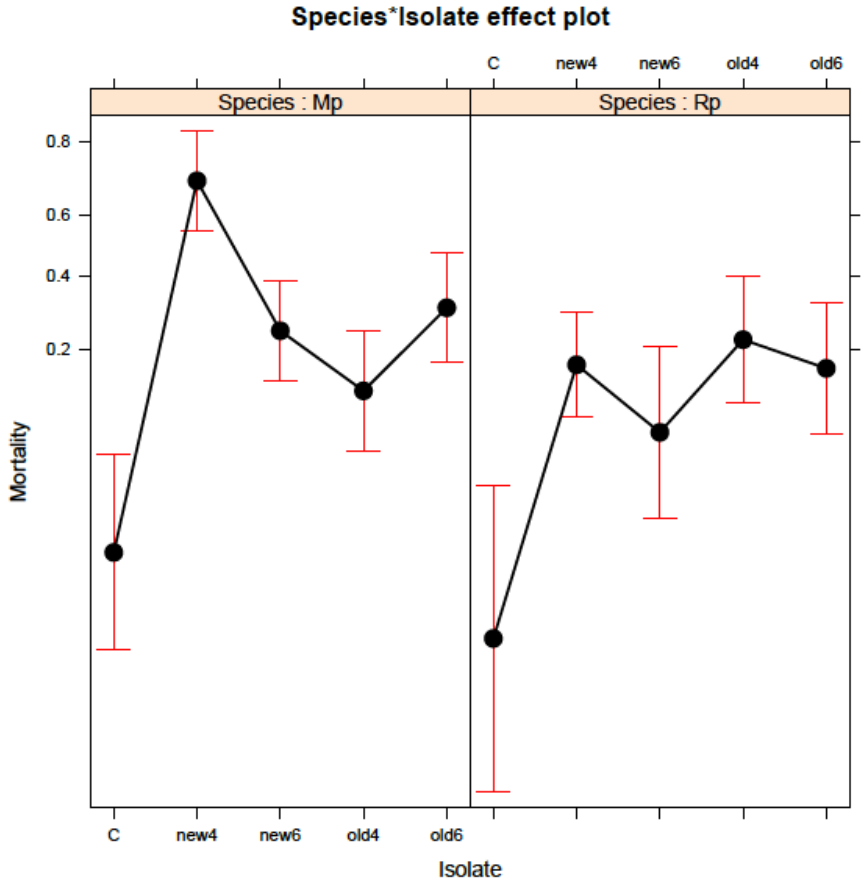


Figure 7.Mortality as a function of isolate in the two different aphid species (Mp=*Myzus persicae*, Rp=*Rhopalosiphum padi*).

Analysis of variance, ANOVA type “III” showed significant differences in reproduction, measured as mean total nymph production per isolate petri dish (n=24) for the observation period of 16 days. *R. padi* had significantly higher reproduction rate than *M. persicae* in general ($p \approx 0$). Reproduction rates were significantly lower at 12° C for both species ($p \approx 0$), (Figure 8). There were no significant differences within species between temperatures 15° C and 18° C. There was no significant interaction between the effect of species and temperature on reproduction (Figure 8).

Isolate NCRI 391/13(new 4) had significant effect on *M. persicae* reproduction ($p \approx 0$), (Figure 9), but it was not significantly different from the effect of reproduction of Isolate NCRI 393/13(new 6), (Figure 9). Reproduction rates in the control treatment of *M. persicae* was significantly higher than in all isolates except Isolate NCRI 391/13(old4), (Figure 9), with a p-value < 0.01. The control treatment of *R. padi* showed no significant difference in reproduction compared to the isolates.

There was no interaction between temperature and isolate (Figure 9, Figure 10), except for isolate new 4 and M. persicae, where reproduction rate was significantly lower than for the other isolates. This was an effect of high mortality, more than a decrease in fecundity.

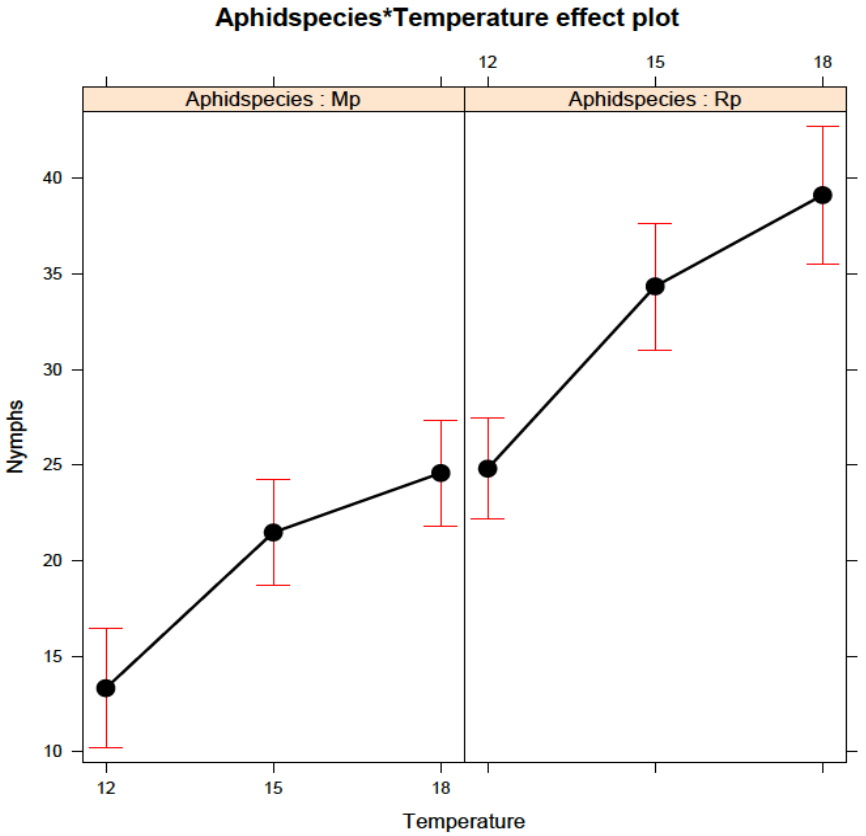


Figure 8. Temperature effect on reproduction for *Myzus persicae* and *Rhopalosiphum padi*.

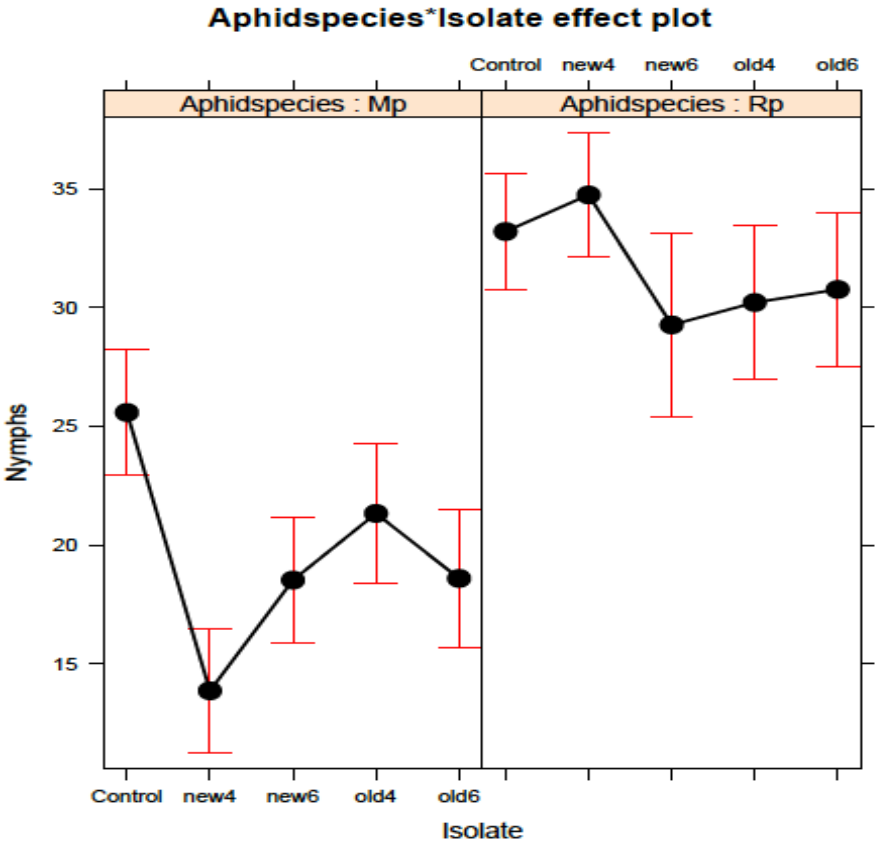


Figure 9. Isolate effect on reproduction for *Myzus persicae* and *Rhopalosiphum padi*.

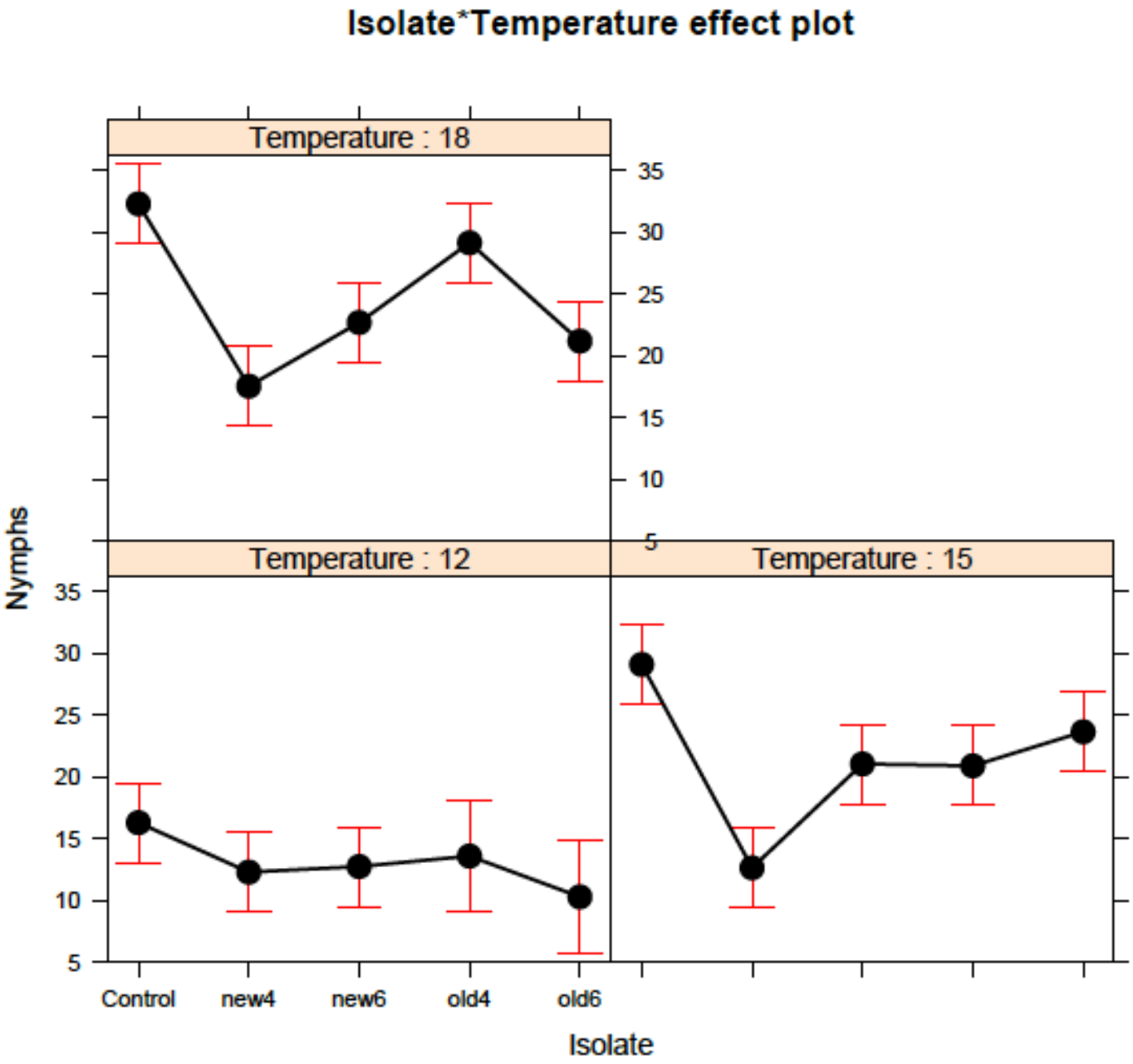


Figure 10. Correlation between isolate and temperature on *Myzus persicae*, with mean reproduction numbers as response.

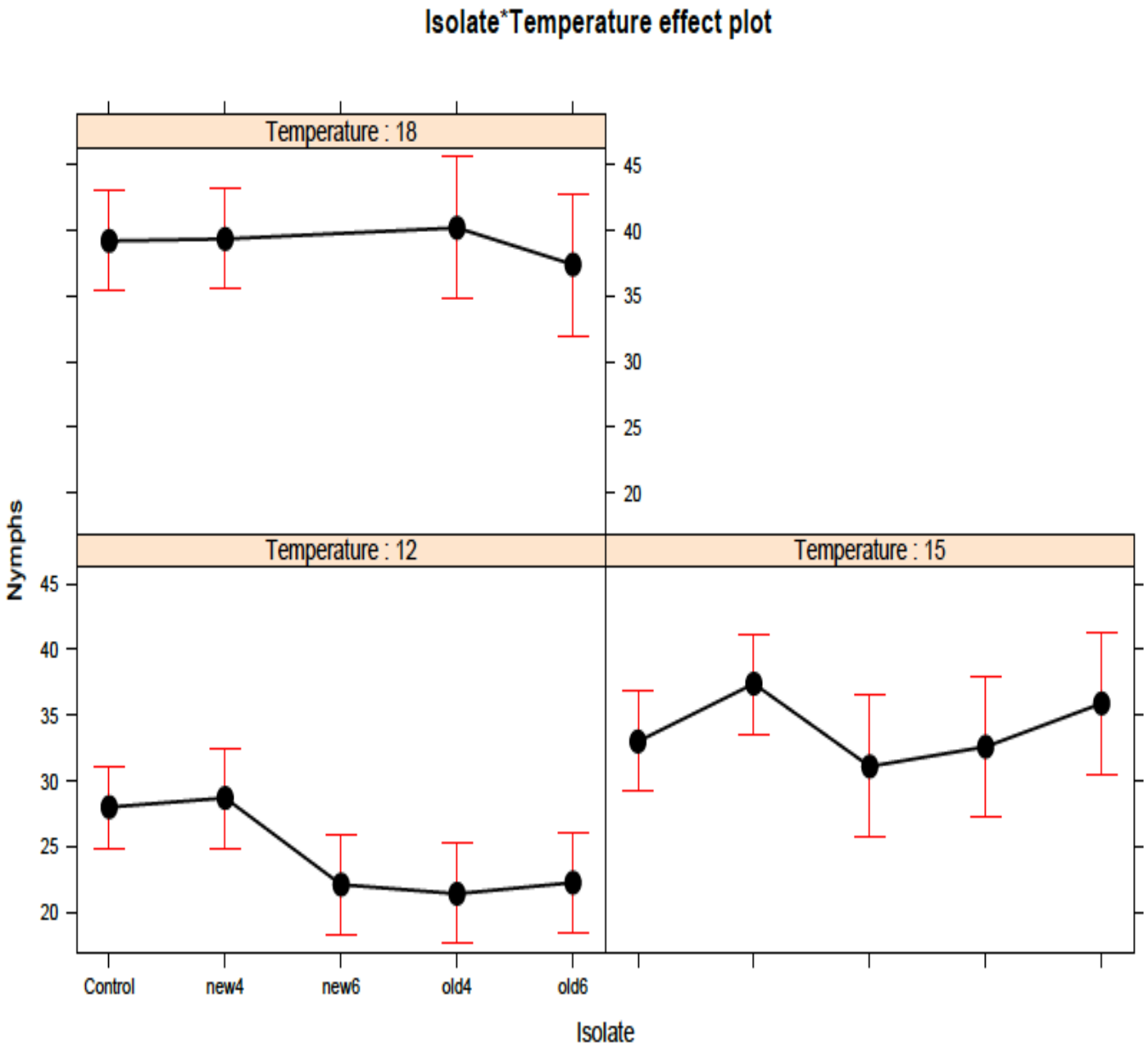


Figure 11. Correlation between isolate and temperature on *Rhopalosiphum padi*, with mean reproduction numbers as response.

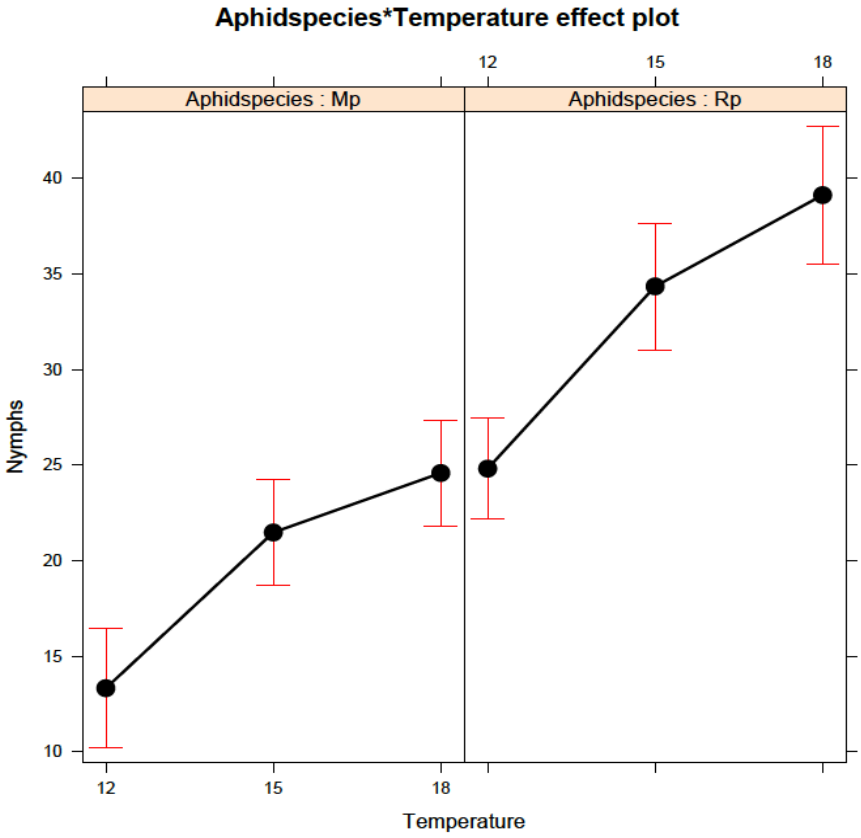


Figure 10. Temperature effect on reproduction for *Myzus persicae* and *Rhopalosiphum padi*.

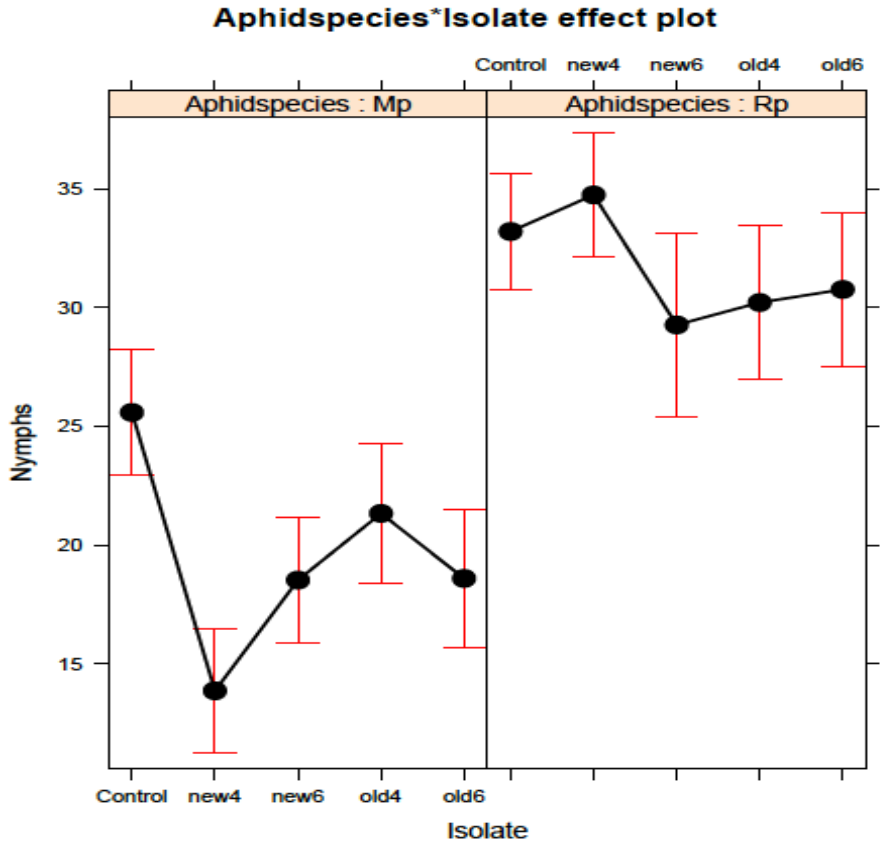


Figure 11. Isolate effect on reproduction for *Myzus persicae* and *Rhopalosiphum padi*.

4 Discussion

4.1 Field survey

According to the present warning system, the number of *R. padi*-eggs found on bird cherries in February (4.1 eggs per 10 buds at Apelsvoll, 0 eggs per 10 buds at Amagerjordet, and 0.4 eggs per 10 buds at Brandval), meant low risk of aphid crop damage at Amagerjordet and Brandval, and medium risk at Apelsvoll (Rygg, 1989). Of these three locations Apelsvoll had indeed the highest *R. padi* density in the cereal field, thus corresponding well with the egg density in February although numbers were all over quite low, and under the threshold level for crop damage (Klingen et al., 2008). Vollebekk organic and Vollebekk conventional had no winter samplings of *R. padi*-eggs, so no correlation could be made for those two locations. *S. avenae*-populations did not reach threshold levels for damage either, although the numbers were a lot higher than for *R. padi*.

All the parasitoids were found on *S. avenae*, and according to Westrum et al., (2010), none of these parasitoid species have previously been recorded on *S. avenae* in Norway. In addition, a species not recorded in Norway at all was found, namely *Aphidius avenae*. The most prevalent genus was *Aphidius*, and *Aphidius ervi* was the species with most observations. Although the numbers of

parasitoids were scarce, the list of parasitoid species resembled the one found in Denmark, where the most commonly found genus also was *Aphidius* (Sigsgaard, 2002), the most numerous species being *Aphidius rhopalosiphi* and *A. ervi*. The low number of parasitoids found in the present study could probably be linked to the low number of cereal aphids found that season.

At Apelsvoll, all parasitoid observations were after the surrounding fields were treated with insecticides, this did not seem to have a strong repelling effect on the parasitoids, but most of them appeared a bit later than in the other fields. It is possible that the sampling area served as a refuge for the parasitoids. Hyperparasitoids were observed after first observation of primary parasitoids at all locations, which were expected. Hyperparasitism can obviously influence the prevalence of primary parasitoids, but the main reason for this influence, may well be that they make the primary parasitoids leave the area when they are present (Höller et al., 1993).

Cereal aphids are only prevalent for a short period of the year, and for primary parasitoids specialising on aphids, alternative hosts can be important to complete their life cycle (Langer and Hance, 2004). This can make bordering vegetation important, as it can be a refuge for other species of aphids that the parasitoids can use as alternative hosts. Non-crop vegetation with alternative aphid species can increase the parasitism on cereal aphids (Langer and Hance, 2004).

Parasitoids were observed in the fields before fungi, which were in accordance to findings by Feng et al., 1991. Parasitoids can contribute to dispersal of *P. neoaphidis* (Baverstock et al., 2009; Fuentes-Contreras et al, 1998) but this was unlikely a contribution in these fields, as the fungal prevalence was low.

P. neoaphidis is a fungal species prevalent in most continents, and can be found in a number of different crops around the world (Feng et al., 1992; Hatting et al., 2000; Nielsen et al., 2001; Nielsen and Hajek, 2005; Powell et al., 1986b; Scorsetti et al., 2007). It can cause epizootics in aphids, and can effectively reduce aphid populations (Barta and Cagáň, 2006; Ekesi et al., 2005; Scorsetti et al., 2007). The most important factor for *P. neoaphidis* to work as an effective control agent on aphids is host density (Powell et al., 1986a). Non-crop aphid populations can act as reservoirs for the pathogen (Ekesi et al., 2005; Hatting et al., 2000; Powell et al., 1986a), but this requires the populations to be big enough to make conidia dispersal effective.

Climatic conditions (RH, temperature, rainfall) and light are suggested to be important factor affecting an effective performance of entomophthoralean fungi (Hemmati et al., 2001b, De Castro et al., 2013). Rain, especially early in the growing season, has been suggested to be important for the occurrence of epizootics (Feng et al., 1992; Hemmati et al., 2001b), and the low precipitation in all locations in June and July, could be an explanation for the few observations of fungal pathogens. At Brandval the RH and rainfall was quite low in the beginning of the season, which indicates that epizootics would be unlikely, even with a higher amount of aphids. Rain is especially important to create epizootics in *S.*

avenae, since it inhabits the upper parts of plants, where the microclimate is less humid than on the lower parts of the plants (Feng et al., 1991). Wilding, 1973 found that entomophthoralean species could survive at a RH of 50%, but did not produce infective conidia until the moisture level increased. De Castro et al. (2013) further suggest that evapotranspiration and hence microclimatic RH is more important than macroclimatic RH, but in this study microclimatic RH has not been recorded.

4.2 Bioassay

The big difference in *P. neoaphidis*-caused mortality to the two aphid species tested is in accordance to similar studies conducted by Shah et al. (2004) where *P. neoaphidis* resulted in a low *R. padi* mortality. In the present study, *M. persicae* was more susceptible to the isolates tested, and this resulted in both higher mortality, and lower reproduction by this aphid species when it had been exposed to *P. neoaphidis*. In other studies, *M. persicae* has shown a medium level of susceptibility compared to other aphid species (McLeod et al., 1998; Shah et al., 2004).

The isolates were obtained from *S. avenae*, but not tested on *S. avenae*, which can be a criticism of these assays. It is possible that the virulence would have been higher if the isolates were tested on the species they were obtained from. However, there are studies showing higher (Shah et al., 2004), as well as lower (Pell et al., 1993), virulence for entomopathogenic fungi if tested on other species than original host species .

The isolates were passed through *M. persicae*, and this could have contributed to the difference in virulence, between *R. padi* and *M. persicae,* since the "new" isolates were more virulent to *M. persicae* than *R. padi*.

The isolates were kept at 18° C; Baverstock et al. (2008) found that *P. neoaphidis*-conidia showed less activity when maintained on soil at 18° C, than at lower temperatures. It is possible that the isolates would have been more virulent if kept at lower temperatures. However, in the assays, there were no significant differences in mortality and reproduction between temperatures. Morgan et al., (1995) found that germination was higher for both primary and secondary conidia of *P. neoaphidis* between 18 and 21° C. Hemmati et al., 2001, found that conidial discharge distance was longest at 18° C, and lower at 10 and 15° C. If a new assay were to be conducted, it would be interesting to test isolates maintained at lower temperatures, but tested at the same temperatures (12, 15 and 18° C).

The virulence of two isolates of *P. neoaphidis* collected in the same field, was highly variable, both between isolates, and isolate subcultures. This should be considered when modelling management strategies for conservation biological control.

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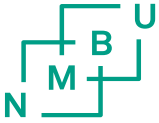

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