MTT REPORT 157

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MTT

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Interim report

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Abstract

The Neomehi project is studying how neonicotinoid-based plant protection products used in the cultivation of oilseed rape and turnip rape affect honey bees in Finnish oilseed cultivation. The interim report brings together the main results of the first growing season.

The experimental protocol included four trial fields where spring turnip rape was cultivated. Each trial field was treated in a different way with neonicotinoid insecticides: without neonicotinoids, foliar spraying with neonicotinoids (thiacloprid) against pollen beetles and/or seed treatment with neonicotinoids (thiametoxam) against flea beetles. The plant density and crop growth was determined in the trial fields. The number of honey bees and other pollinators was assessed with the applied line transect method during the growing season. Five test bee hives were located at the edge of each trial field. The condition of the bee hives was checked and the amount of bees and brood was counted 4-5 times during the summer season. Census was done also in autumn and in spring to get overwintering data. Bees and bee hive products from all test bee hives of the trial fields were analysed for residues of neonicotinoids. In addition, residues were analysed from samples collected as a survey from forty other bee hives in South-West Finland. Half of those bee hives were located close to oilseed cultivation and the other half far from oilseed cultivation.

The crop growth was normal in three of the trial fields. In one trial field (seed treatment with neonicotinoids) the crop growth suffered probably because of too much varied drilling depth. According to the main results of the counting of pollinators, the number of honey bees in the trial fields was high when the crop growth was good and lower when crop growth was poor. In the field which was treated with foliar spraying with a neonicotinoid (thiacloprid) the number of honey bees decreased after the treatment. However, the number of honey bees clearly increased 2-3 days after the foliar treatment.

The results of the first growing season did not show, that the use of neonicotinoids affects the success of bee colonies, which were located at the edge of the trial fields. Both the adult and the brood population dynamic curves showed typical levels and shape of bee and brood population development. The average range of food consumption of the bees during overwintering and the overwintering index (the relation of the number of adult bees in spring compared to the number of adult bees in the beginning of overwintering) showed also typical levels compared to normal bee colonies in South-West Finland. Two bee hives lost their queen during fall and winter. One of those hives was located in the field without use of neonicorinoids and the other in the field in which a seed treatment neonicotinoid was used. The winter losses of the test bee colonies did not differ from the average winter losses (7 %) in the South-West of Finland.

The results of the residue studies showed that residues of neonicotinoids migrate with pollen and nectar into bee hives. The total residue levels of seed treatment neonicotinoids, thiametoxam and chlothianide, were at such a low level, that acute harm to bees is unlikely. However, the residue levels, especially in nectar, resulted in an estimated exposure which is close to the chronic and acute sublethal risk limits presented in literature. Therefore, that kind of risk cannot fully be excluded. The neonicotinoid (thiacloprid) used as a foliar spray resulted in higher residue levels in certain samples than the seed treatment neonicotinoids, and on the other hand in higher estimated exposures. However, the toxicity of the neonicotinoids used in foliar sprayings is only a hundredth of the toxicity of the ones used in seed treatment and therefore the exposure is estimated to be clearly below the risk limits.

The Neomehi project continued with the new field experiments in this summer. Final report based on wider research data will be published in 2015.

Tiivistelmä

Neomehi-hankkeessa tutkitaan minkälaisia vaikutuksia rypsinviljelyssä käytettävillä, neonikotinoideja sisältävillä, torjunta-aineilla on mehiläisiin suomalaisessa öljykasvin viljelyssä. Väliraportti kokoaa yhteen kaksivuotisen Neomehi-hankkeen keskeisimmät tulokset ensimmäiseltä kasvukaudelta.

Koejärjestely sisälsi neljä kenttäkoetta, joissa viljeltiin rypsiä. Neonikotinoideja sisältäviä insektisidejä käytettiin eri tavoin kullakin pellolla. Koepellolla joko ei käytetty neonikotinoideja tai ruiskutettiin neonikotinoidilla kirppoja vastaan ja/tai käytettiin neonikotinoidilla peitattua siementä rapsikuoriaisia vastaan. Kasvien kasvua ja kasvutiheyttä seurattiin ja pelloilla vierailevien mehiläisten ja muiden pölyttäjien lukumäärä määritettiin kasvukauden aikana. Kunkin pellon laidalla pidettiin viittä mehiläispesää. Mehiläispesien kuntoa seurattiin ja mehiläisten ja niiden jälkeläisten lukumäärä laskettiin vähintään neljällä eri tarkastuskäynnillä kesän aikana. Vahvuuslaskentoja tehtiin myös syksyllä sekä seuraavana keväänä jotta saatiin tarkempaa tietoa talvehtimisesta. Mehiläisiin ja mehiläispesän tuotteisiin kerääntyviä neonikotinoidien jäämiä analysoitiin kaikista kenttäkokeen pesistä. Lisäksi jäämiä tutkittiin neljästäkymmenestä näytteestä, jotka kerättiin otantana Lounais-Suomessa sijaitsevilta mehiläistarhoilta. Otantatutkimukseen pesät valittiin siten, että puolet pesistä sijaitsi lähellä rypsinviljelyä ja puolet kaukana.

Kasvien kasvu ja kukintojen tiheys oli normaalia kolmella koepellolla. Yhdellä pellolla kasvu ei ollut niin hyvää johtuen todennäköisesti väärästä kylvösyvyydestä. Pölyttäjälaskennat osoittivat, että pääsääntöisesti mehiläisten lukumäärä pellolla oli korkea kun kasvin kasvu oli hyvä ja kukintoja runsaasti ja toisaalta taas pölyttäjien lukumäärä alhainen kun kasvin kasvu heikkoa. Koekentällä, joka käsiteltiin neonikotinoidi ruiskutuksella, ei mehiläisiä juuri havaittu heti ruiskutuksen jälkeen. Muutama päivä käsittelystä mehiläisten lukumäärä pellolla oli kuitenkin palautunut ruiskutusta edeltäneeseen tilaan.

Ensimmäisen kauden tulosten perusteella ei voitu havaita, että neonikotinoideilla olisi ollut vaikutuksia koekenttien läheisyydessä olevien mehiläisyhdyskuntien kuntoon. Mehiläisten sekä niiden jälkeläisten lukumäärän kehittyminen kasvukauden aikana oli normaalia kaikilla koekentillä. Myös talvenaikainen ruoankulutus sekä talvehtimisindeksi (mehiläisten ja niiden jälkeläisten lukumäärän suhde syksyllä ja keväällä) asettuvat tyypillisiin arvoihin, joita mehiläisyhdyskunnille on mitattu Lounais-Suomessa. Talven aikana kaksi pesää menetti kuningattaren. Toinen pesä oli koekentällä, jota ei käsitelty neonikotinoideilla ja toinen kentällä, jossa neonikotinoideja oli käytetty siementen peittaukseen. Talvehtimistappiot eivät eroa koko Suomen keskiarvosta (7%).

Jäämätutkimusten perusteella neonikotinoidien jäämiä siirtyy siitepölyn ja meden mukana mehiläispesään. Peittausaineiden (tiametoksaamin, klotianidiinin) yhteenlasketut jäämätasot ovat niin alhaisia, että akuutti haitta mehiläisille on epätodennäköistä. Kuitenkin, mitatut jäämäpitoisuudet etenkin medessä johtavat arvioon altistumistasosta, joka on lähellä kirjallisuudessa esitettyjä kroonisia ja akuutteja subletaaleja riskirajoja. Jäämätulosten perusteella ei voida siis täysin pois sulkea tämäntyyppistä riskiä. Ruiskutteena käytetyn neonikotinoidin (tiaklopridi) jäämäpitoisuudet ovat näytteissä huomattavasti korkeampia kuin peittausaineiden pitoisuudet ja näin ollen myös mehiläinen altistuu suuremmille pitoisuuksille. Kuitenkin, johtuen ruiskutteiden sisältämien neonikotinoidien huomattavasti alemmasta myrkyllisyydestä, tässä tutkimuksessa arvioitu altistuminen jää selkeästi alle riskirajojen.

Contents

1 Introduction

NEOMEHI Project was launched in 2013 by MTT Agrifood Research Finland (MTT) and Finnish Food Safety Authority Evira (Evira). The Project is studying how neonicotinoid-based insecticides used in the cultivation of spring oilseed crops (oilseed rape and turnip rape) affect honey bees (*Apis mellifera*).

The aim of Neomehi project is to provide answers to the following questions:

1. Do the neonicotinoids influence the number of pollinators in the field environment?
2. Do the neonicotinoids influence the performance of beehives?
3. Are there differences in the impact of neonicotinoids due to the use pattern (seed treatment and/or foliar sprayings)?
4. Are there residues of neonicotinoids in the honeybee colonies (worker honey bees, brood, bee bread, nectar, honey, pollen) used in the pollination service in the oilseed fields?
5. What are the influences on oil seed crop cultivation if the use of neonicotinoids are limited or banned?

In addition, within the framework of the project, reliable analytical methods will be built for the determination of pesticide residues in plant material, in bees and different bee hive matrices.

2 Experimental set-up

The project consisted of two parts; a field study (part A) and a sample survey (part B). The project was established in spring 2013 and will last two growing seasons, to the end of 2014.

2.1 Part A. Field study

The field study was carried out in co-operation with local beekeepers and local farmers. A research unit consisted of four trial sites where spring turnip rape was cultivated. A trial site in the text consisted of a trial field (spring turnip rape) and five test bee hives.

The identification of trial sites in 2013, dates of activities, plant protection data, cultivation methods and transportation of bee hives to and from the trial sites are presented in Appendix 1.

The trial fields were either drilled using direct drilling or conventional drilling. The drilling of trial fields 1 and 4 was delayed in order to decrease the risk for damage on young turnip rape plants caused by flea beetles (*Phyllotreta* sp.). Trial fields 2 and 3 were treated against flea beetles with pyrethroids Sumi alpha 5 FW (esfenvalerate 50 g/l a.i.) and/or Decis Mega EW 50 (deltamethrin 50 g/l a.i.) from one to three times (Table 1). The plant density was counted once per each trial site at four randomly chosen places in each field just after the flowering of turnip rape.

Each trial field had a different protocol for the use of neonicotinoid insecticides (table 1). In trial fields 2, 3 and 4 neonicotinoids (thiametoxam and/or thiacloprid) were used as seed treatment and/or foliar sprayings. No neonicotinoids were used in trial field 1. The seed was treated with thiametoxam in trial fields 2 and 3. The foliar sprayings with thiacloprid against pollen beetles in trial fields 3 and 4 were made when the crop stage at the foliar sprayings was near the full flowering stage, BBCH 64-65, in both places.

The neonicotinoid product used for seed treatment of spring turnip rape was Cruiser OSR (thiamethoxam 280 g/l, metalaxyl-M 32,3 g/l, fludioksonil 8 g/l). It was used at the product rate 15 ml/kg seed. Amount of active ingredients per kg seed were thiamethoxam 4.2 g a.i., metalaxyl-M 0.48 g a.i. and fludioksonil 0.12 g a.i. The variety of spring turnip rape seed was Apollo (Batch code 357-01059B). According to the seed treatment analysis report the rate of thiamethoxam was 92 % of target rate, which is acceptable (Appendix 2). The foliar sprayings with a neonicotinoid were done with Biscaya OD 240 (thiacloprid 240 g/l) at the product rate 0,35 l/ha. Amount of active ingredient of thiacloprid was 84 g a.i. The plant protection products used were approved by Finnish Safety and Chemicals Agency TUKES and applied at the approved product rates.

Table 1. Insecticide treatments in the field study.

Trial Site	Treatments with neonicotinoids	Treatment with other insecticides
1	- no seed treatment - no foliar spraying	- synthetic pyrethroid (product Sumi alpha 5 FW) as foliar application against pollen beetles until beginning of flowering of turnip rape
2	- seed treatment with thiametoxam 280 g/l+metalaxyl_M 32.3 g/l+fludioxonil 8 g/l (product Cruiser OSR) 15 ml product/1 kg turnip rape seed - no foliar spraying	- synthetic pyrethroid (product Sumi alpha 5 FW) as foliar application against flea beetle until 2-4 true leave stage - synthetic pyrethroid (product Sumi alpha 5 FW) as foliar application against pollen beetles until beginning of flowering of turnip rape
3	- seed treatment with thiametoxam 280 g/l+metalaxyl_M 32.3 g/l+fludioxonil 8 g/l (product Cruiser OSR) 15 ml product/1 kg turnip rape seed - foliar application of thiacloprid (product Biscaya OD 240) 0.35 l product/ha against pollen beetles at the beginning of flowering of turnip rape	- synthetic pyrethroids (products Sumi alpha 5 FW and Decis) as foliar application against flea beetle until 2-4 true leave stage - synthetic pyrethroids (product Sumi alpha 5 FW) as foliar application against pollen beetles until beginning of flowering of turnip rape
4	- no seed treatment - foliar application of thiacloprid 240 g/l (product Biscaya OD 240) 0.35 l product/ha against pollen beetles at the beginning of flowering of turnip rape	- synthetic pyrethroids (product Sumi alpha 5 FW) as foliar application against pollen beetles until beginning of flowering of turnip rape

The cultivated crops around each trial site were taken into consideration by choosing the trial fields so that there would not be other oil seed crops close to the trial field. The bee colonies examined in this field study were located at least 1 km from other oilseed fields. In addition the cultivation of oilseed crops on fields closer than 3 kilometers from the test beehives in each trial site were charted (Appendix 3). In appendix 4 more detailed information of proximity to oilseed cultivation of hives of trial site 1 (control site). Due to the logistic reasons the hives of trial site 1 were temporarily held during 24-28.6.2013 at MTT before being moved to trial field 1. Seed treatment and foliar sprayings against pollen beetles with neonicotinoid products were made in the turnip rape fields at MTT. Therefore it is possible, that exposure of hives to the neonicotinoids during their temporary stay might have happened in some blooming turnip rape fields. Later the distance of trial site 1 from other oilseed cultivation areas was 1.4 km and 1.9 km.

The area of each trial site was targeted to be between 1 and 2 hectars. In the practice the areas were 1.7 ha in the trial sites 1-3 and 1.4 ha in the trial site 4.

Five bee hives were placed at the edge of each trial field. The trial beehives were owned and managed by the same beekeeping company Hunajaluotsi Ltd.

In order to estimate comprehensively the exposure of the honeybees to the neonicotinoids the occurrence of neonicotinoid residues (chlothianidin, thiacloprid) was studied in the plant and hive samples of each trial site. In addition, residues of some other pesticides included in the seed treatment (fludioxonil, metalaxyl-M) and foliar spraying products (esfenvalerate, deltamethrin) were monitored from hive samples.

Different types of samples were collected for the residue analyses (Table 2) The plant samples during flowering stage were collected and the floral parts of plants were removed for the analyses. Nectar samples from turnip rape flowers were not collected in the field because of low volume rate of nectar in turnip rape flowers in 2013. The samples from the bee hives included worker bees and pollen from the posterior legs of the bees. The pollen was collected by pollen traps. Furthermore, nectar and bee bread (fermented bee pollen) were collected from the bee hives. All the samples were frozen after collection.

Sampling times were scheduled in a way that both the blooming of the vegetation and the spraying times of the neonicotinoids were taken into consideration. The plan was to collect dead bees from each hive for residue analysis especially if increased death of bees would have been observed. Increased death of bees was not observed in the field study in 2013. The pollen and nectar samples were identified microscopically to get information of the relative amount of turnip rape pollen/nectar in the samples.

The number of honey bees and other pollinators like bumble bees, flower flies and butterflies in turnip rape at all four NEOMEHI trial sites were assessed between the start of flowering (BBCH 59) and full flowering (BBCH 65) in June and in July in 2013. The line transect method[1] was applied to monitoring the honey bees and some other pollinators. The numbers of insects were count along a line of 50 meters length. The line was placed at the same place in the trial fields during all countings. The 50 m line for counting pollinators was placed in the centre of the spring turnip rape area in the trial field.

GEP (Good Experimental Practice) standards were followed in assessments and samplings in the NEOMEHI trial sites.

Table 2. The table for the samplings in the field study of NEOMEHI Project in 2013.

Time of sampling, BBCH* crop stages when possible	Counting of pollinators	Plant	Bee	Pollen	Samples from hives
plant stage, when plants are at 2-4 true leaves BBCH 12-16	Counting of bees in crop stand	Whole plant Two samples with 15 subsamples per trial field. Frozen. Not analysed.			
bud stage (foliar spraying) BBCH 50-59	Counting of bees on crop stand	Flowerbuds Two samples with 15 subsamples per trial field. Frozen. Not analysed.	Control samples* if there are bees in the counts collection of bees entering hives		
flowering stage BBCH 65-69	Counting of pollinators (line method)	Flowers Two samples both with 15 subsamples per trial field, 3-4 timepoints per field	crop stand and from apiaries	pollen collection from flight entrance (2 hives/field); at minimum four time points per field	nectar, bee bread (4-5 hives/field); one time point
After season					honey (4-5 hives/field); one time point

*BBCH Phenological growth stages of turnip rape: BBCH 63-64 Principal growth stage 6: Flowering 30%-40% of main raceme open, BBCH 65 Full flowering i.e. 50 % flowers on main raceme open, older petals falling

2.1.1 Production and management of beehives

Twenty honey bee colonies and 10 spare colonies were produced by shaking 25 kg of young bees to big swarm box from ordinary bee hives on 28.5.2013.[2] After fasting 24 hours in a dark 17 C temperature room 750 g of bees was placed in a five frame new Farrar hive body on vax foundation frames. Each swarm was situated in one half of the divided hive body. Young queens and 2 liters of 50 % sugar syrup was given to every swarm with a top feeder. The swarms were kept in a dark temperature room for 48 hours after the establishment. The colonies were moved to an isolated forest apiary Perho, located in

[1] Krebs J.C. (1989) - Ecological Methdology. 2nd Edition. 113-120.
[2] Sandrock C, Tanadini M, Tanadini LG, Fauser-Misslin A, Potts SG, et al.
(2014) Impact of Chronic Neonicotinoid Exposure on Honeybee Colony Performance and Queen Supersedure. PLoS ONE 9(8): e103592.
doi:10.1371/journal.pone.0103592

Tammela, were the nearest cultivation areas were at least 5 km from the beehives. Queens and egg laying was checked after one week and the feeding was continued. After two weeks all the colonies were moved to their own hive body by giving five more vax foundation frames to each of the colonies. At the same time 350 g of young bees were given to the colonies. The hives of trial site 1 were temporarily held during 24-26.6 at MTT (MTT Lypsyasema) before being moved to trial field 1. The beehives were moved to test fields when the first turnip rape flowers were open and taken back when the blooming was over. The amount of bees and brood was counted four times during the season.

The bee colonies were fed in the fall with 67 % sugar syrup and varroa treatment was done in august with Thymol and later in fall with oxalic acid trickling. The beehives were weighed in fall and in spring. The number of winter bees was estimated during the oxalic acid treatment. The number of dead bees during the winter was measured from downfall immediately after the cleansing flight. The first spring census was done on 22-24.3.2014. The winter food consumption and over wintering index (number of adult bees in spring / number of adult bees in autumn) was calculated.

2.1.2 Pollen analysis of nectar, honey and bee bread

The nectar flow and honey samples were prepared by the methodology recommended by the International Commission of Bee Botany and the International Honey Commission [3] Pollen analyses were performed using 400 * magnification and all the pollen grains of each plant species, families and groups were counted separately until the total number of 300 grains was exceeded. The percentage of each species, family or group was calculated.

Pollen loads collected from the entrance of bee hives were classified by color to unique groups and the plant species representing each group was identified by microscope inspection. The percentage of each plant species was calculated.

The bee bread samples were homogenized and 5 g of the sample were put in a centrifuge tube with 10 ml of distilled water. 0.1 ml of homogenized sample was taken with micropipette to an object glass and prepared after Loveaux et al. 1997. Pollen analyses were performed using 400* magnification and all the pollen grains of each plant species, families and groups were counted separately until the total number of 300 grains was exceeded. The percentage of each species, family or group was calculated.

2.2 Part B. Sample Survey

Part B research protocol of NEOMEHI project is connected to another project coordinated by The Finnish Food Safety Authority Evira in which epidemiologic information on the mortality and infectious diseases in the beehives is obtained (EU-project directed by the EU reference laboratory).

The effects of the neonicotinoids on honeybees were analysed in two parts:

1) Relationship between proximity of oilseed cultivation and bee health, statistical analysis

The first part included 322 of the apiaries that were followed in the EU project in 2012-2013 and 2013-2014. Locations of turnip rape fields nearby the surveyed apiaries were taken into consideration, when assessing the beehive mortality and the occurrence of diseases. The information on the nearby turnip rape fields was obtained from the national field plot register (official database of field crop cultivation by MAVI - Agency for Rural Affairs). The effects of the nearby fields on the mortality and the diseases were assessed statistically. Using the coordinates of the apiary, the amount of turnip rape and oilseed rape fields within 1km or 3km and a distance to the nearest field were calculated. Relationship between mortality and cropping intensity was tested using linear or non-linear regression analysis. This analysis is not yet finished.

[3] Louveaux J., Maurizio A., Vorwohl G.
(1978) - Methods of Melissopalynology. Bee World, 59(4): 139-157.

2) Residue analysis

Samples for residues analyses were also collected from 10 apiaries of the EU project from the South-West of Finland near the cities of Jokioinen and Salo in 2013. The choice of the apiaries was done so that two beehives from the same beekeeper were chosen. One beehive was to be situated close to an oilseed field and the other was to be situated far from an oilseed field. The inspectors of the EU project collected nectar and bee bread samples from the bee hives during turnip rape blooming. The neonicotinoid compounds used in Finland for turnip rape cultivation (thiamethoxam, chlotianidin, acetamiprid, thiacloprid) were prioritized in the residue analyses. In addition, also residues of pyrethroids (lamda-cyhalotrin, esfenvalerate, tau-fluvalinate, deltamethrin) and fungicides (iprodione, fludioxonil, metalaxyl-M) were analysed. These active substances are commonly used in turnip rape cultivation in Finland and might have synergistic effects with neonicotinoids. Data on residues and the survival of beehives will be compared to/with the distance to oilseed and turnip rape fields. This analysis is not yet finished.

3 Results

3.1 Crop growth in the field study

The cultivation of spring turnip rape is challenging and there are many issues, which need to be in good order to produce a good growth of the plant. Overall, the aim of maintenance of the trial fields was to get as good a crop growth and rich flowering of spring turnip rape as possible in order to attract honey bees and other pollinators as much as possible.

Results of cultivation of spring turnip rape in 2013 are described in Table 3. The plant density varied quite a lot between the trial fields. The crop growth of turnip rape was normal in the Trial sites 1, 3 and 4 in 2013. In the trial site 2 the crop growth suffered probably because of too high drilling depth in some places of field. The crop density was too low which resulted in a varying crop growth.

The drilling of spring turnip rape with untreated turnip rape seeds worked out well in the trial sites in 2013. There was a rather good germination of the seed and proper leaf development in trial fields 1 and 4. A rather good crop stand of turnip rape was gained in both places in 2013. The insect pest pressure (both flea beetles and pollen beetles) was lower than normal in 2013.

Harvesting was done by the farmers in the trial sites 1-3 and by MTT in the trial site 4. The estimated seed yield was between 1.1 and 2.0 tn/ha in trial sites 1-3. In trial Site 2 the average yield was 0.730. tn/ha (in ⅓ of area the yield was 1.6 tn/ha, ⅓ of area 0.6 tn/ha and in ⅓ of area 0 tn/ha). In trial site 4 the seed yield was 2.26 tn/ha SD±0.20.

Table 3. Number of drilled seeds, flowering plants, plant density, yield results and tsw (thousand seed weight) of NEOMEHI Trial fields in 2013. The plant density and the overall crop growth of spring turnip rape in trial site 2 was estimated after the decline of flowering as follows: ⅓ of field area was slightly normal, ⅓ was thin and ⅓ of area was open without hardly any spring turnip rape plants.

Trial Sites, Location, Cultivated area of turnip rape, Seeding rate in ha	**Number of drilled seeds x10^6 in ha**	**Number of flowering plants in m^2**	**SD**	**Plant density. Percent of normal 150 plants per m^2**	**Number of flowering plants x10^6 in field**	**Number of flowering plants x10^6 in ha**	**Seed yield kg in ha**	**SD**	**TSW of seed yield g**	**SD**
Site 1, Somero, 1.7 ha, 13 kg	9.09	68.25	±12.87	45.5	1.16	0.68	1700		2.43	±0.04
Site 2, Forssa, 1.7 ha, 10 kg	6.83	70.50 36.75 0 Mean 35.75	±11.0 ±6.08 Mean 5.69	47.0 24.5 0 Mean 23.8	0.61	0.36	1600 600 0 Mean 733		2.49	±0.01
Site 3, Koski, 1.7 ha, 6 kg	4.10	109	±24.09	72.7	1.85	1.09	2000		2.49	±0.01
Site 4, MTT, 1.4 ha, 10 kg	5.76	150.75	±29.03	100.5	2.11	1.51	2259	±203.61	2.43	±0.04

3.2 Counting of pollinators in the field study

The number of pollinators like honey bees, bumble bees, flower flies and butterflies foraging in the turnip rape crop stand were counted on 50 meters line in each trial site from the start of flowering to the flowering decline (Figure 1.) According to the results a spring turnip rape crop in good growth will attract clearly more pollinators like honey bees than a poor crop stand. The proper and good maintenance of a crop stand will improve and lead to a rich flowering of rape plants, which will result in the honey bees having better foraging surroundings. The number of other pollinators than honey bees was quite low in the NEOMEHI trial sites. This might more likely have been due to the environment or the habitat around the trial fields than to the treatments made in the trial fields. However, the number of honey bees and bumblebees were low 1-2 days after foliar spraying with thiacloprid, but after the decline period the number of pollinators increased clearly. The number of honey bees in trial site 3 at 5 DAT (days after treatment) and the number of honey bees and bumble bees in trial site 4 at 3 DAT and at 4 DAT were the highest of the whole period. The number of honey bees was high, when the crop growth was good as in trial site 1 (mean 122.5) and it was low, when the crop growth was poor like in trial site 2 (mean 34). There was one collapse in the number of honey bees in trial site 1, when the weather was cloudy and a bit rainy. Data on the number of pollinators in the NEOMEHI Trial sites in 2013 are presented as graphs and tables in Figure 1.

After counting the number of pollinators in the flowering turnip rape crop it is concluded that the foliar spraying with the neonicotinoid thiacloprid during the flowering resulted in a significant decrease in the number of honey bees for 2 days. However, the number of honey bees clearly increased back after 2-3 days from the foliar treatment. In addition, in trial site 2, where the maintaining of a proper crop stand failed, the number of honey bees was lower compared to trial fields 1, 3 and 4 with the normal crop growth. Conclusions are not unambiquous since the number of honey bees might vary in relation to the density of crop growth, i.e., the better the crop growth is during the flowering the more it will attract the honey bees and vice versa: a poor crop growth and a lower number of flowering plants resulted in a lower number of honey bees.

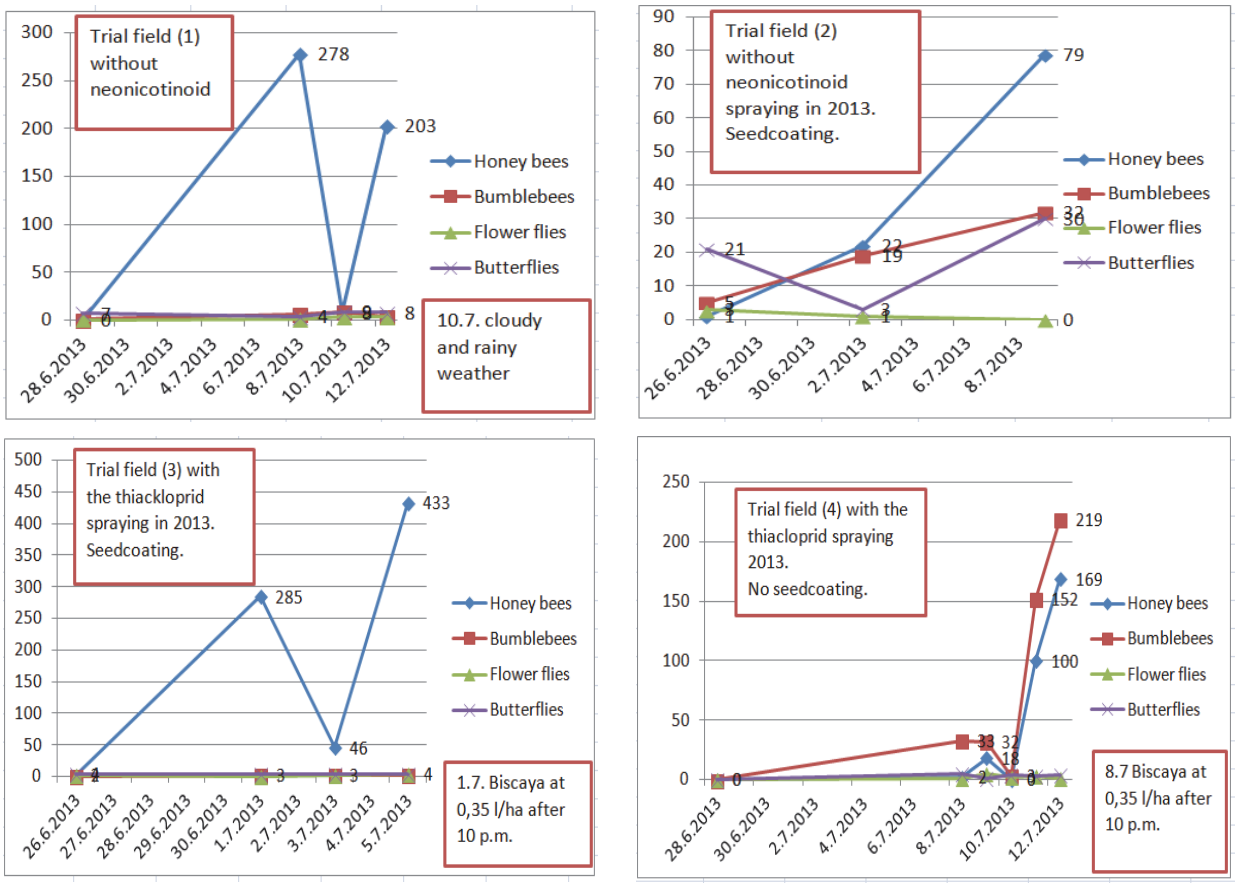


Figure 1. Number of honey bees, bumble bees, flower flies and butterflies in the field study during the flowering of turnip rape in 2013.

3.3 Weather data in April-September in Jokioinen area in 2013

The growing conditions in the growing season of 2013 were favorable for spring turnip rape. The mean temperatures of May, June and August were higher than normal, and by the end of August the effective temperature sum was 1284.6 °C, which is 165.8 °C higher than the long-term (1981-2010) average. May was dry, but in the rest of the growing season the precipitation was about normal and the dry periods were rather short. No late spring frosts or early autumn frosts occurred. Graphs of daily temperature and precipitation are presented in Appendix 5 and overall weather data in the Appendix 6.

3.4 Condition of honey bee colonies, number of honey bees and broods, bee colony development

The bee hives were inspected four times during the summer season and once late in fall at the beginning of over wintering in 2013. The timing of the inspections during the summer was synchronized with the flowering rhythm of the test site vegetation. The first census was done on 25.6.- 3.7., the second on 1.7 – 19.7., the third on 17.7. – 2.8. and the fourth on 2.8. – 20.8. depending of the growth stage of the test field. In the trial site 3 test hives were also inspected 24.8. The last census for all test hives was done on 28.10. at the same time when the oxalic acid trickling was done. The average number of adult bees and brood in the test colonies during the summer 2013 are presented as population dynamic curves in Figures 2 and 3. Both the adult and the brood population dynamic curves show typical levels and shape of bee and brood population development. The average percentages in Figure 4 shows how many of the August bees and brood remains in the winter cluster. This indicates a possible weakening of the colony during fall. [4]

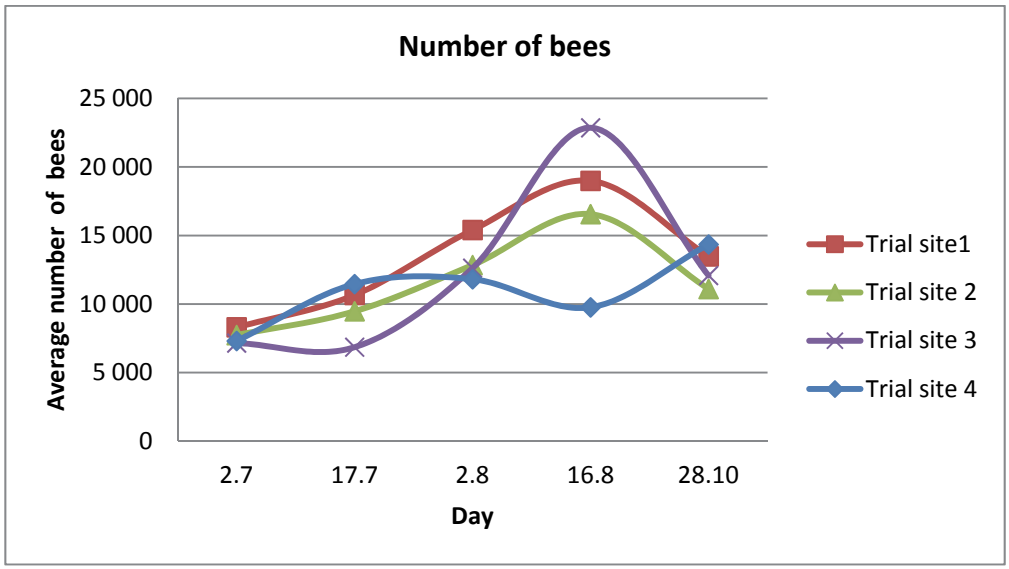


Figure 2. Average number of bees in the hives of each trial site

[4] Delaplane, K. S. *et al*. 2013. Standard methods for estimating strength parameters of *Apis mellifera* colonies. Colossa BEEBOOK I.

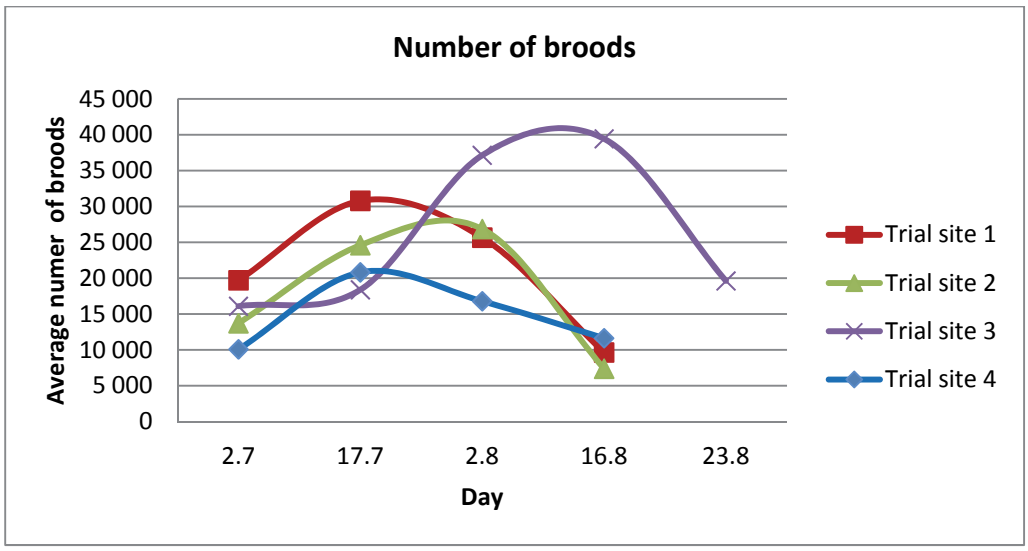


Figure 3. Average number of broods in the hives of each trial site

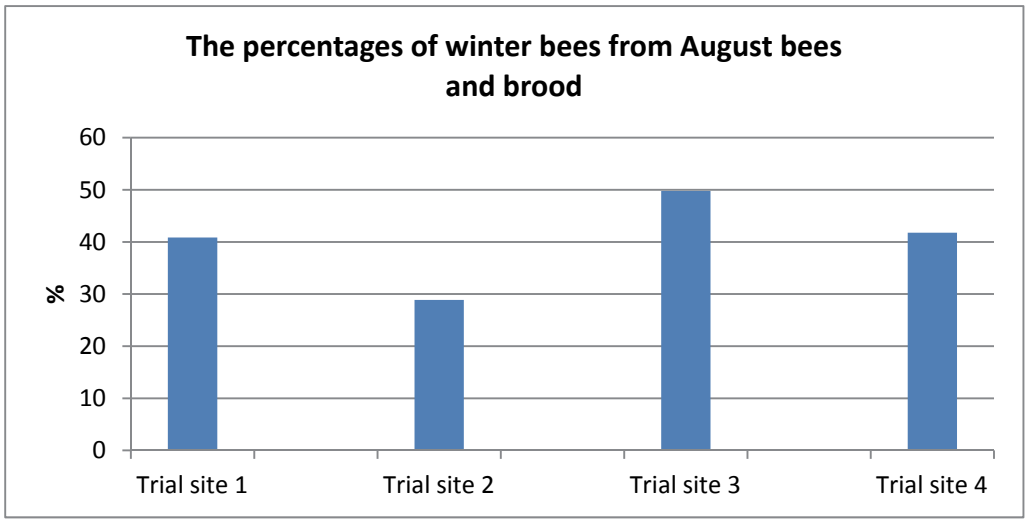


Figure 4. The percentages of bees in October from August bees and brood.

Spring census in 2014 was done on 22-23.4 when the bees had done the first cleansing flights after the over wintering. Two bee hives were lost during the fall and winter. These were hive number 3 from trial site 1 and hive number 5 from trial site 2. Both hives lost their queen. All things considered the winter losses in spring 2014 in the field study do not differ from the winter losses on average of 7 per cent in the whole Finland (Coloss survey 2014).

The average range of food consumption during over wintering was 13-18 kg per beehive in each trial site (Figure 5). Food consumption is typical to normal bee colonies in South-West Finland. Figure 6 shows average overwintering indexes in each trial sites. Over wintering index (OWI) describes the relation of adult bees in spring compared to the number of adult bees in the beginning of over wintering[5].The average of OWI-index in the researched areas ranges between 0.2-0.6. The spring evaluation has been done when the number of adult bees is lowest and brood rearing has begun.

[5] Hatjina *et al*. 2014 Population dynamics of European honey bee genotypes under different environmental conditions. Journal of Apicultural Research 53(2): 233-247 (2014) © IBRA 2014. DOI 10.3896/IBRA.1.53.2.05

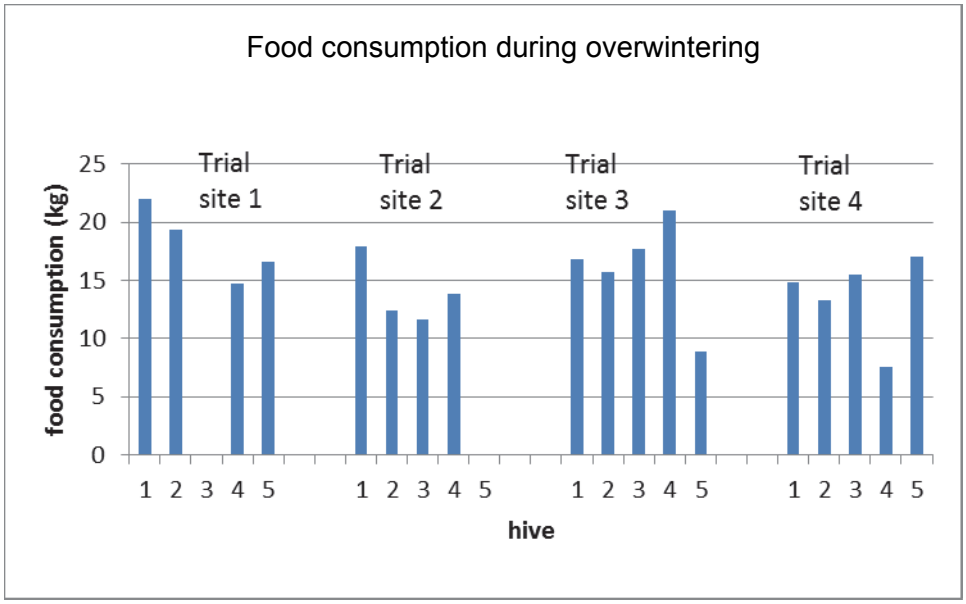


Figure 5. Food consumption during over wintering.

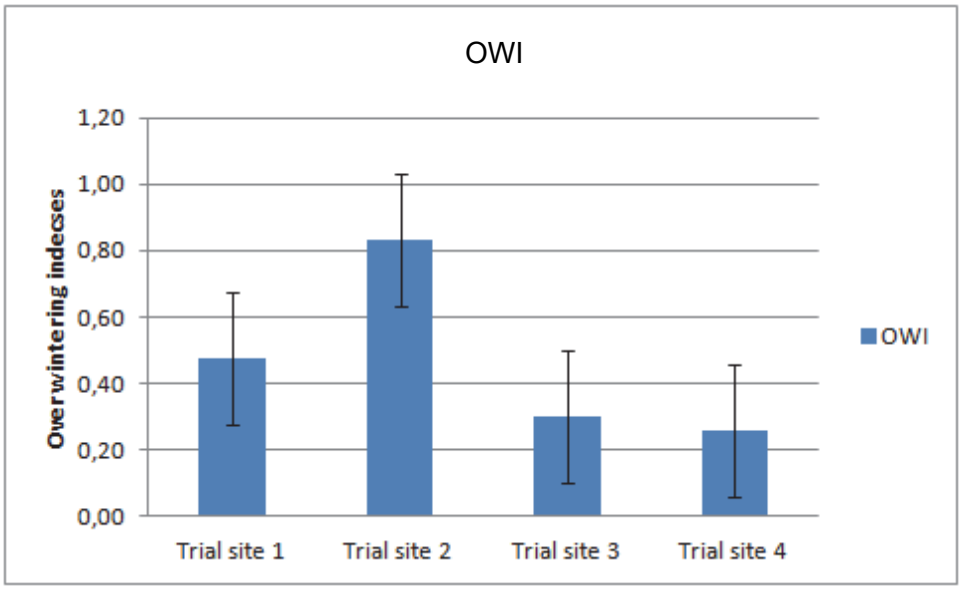


Figure 6. Average overwintering indexes

3.5 The origin of honey, pollen loads and bee bread in trial sites

The average content of *Brassica* pollen in pollen loads, bee bread, nectar and honey samples are presented in table 4. The results indicate that bees have visited and gained yield from *Brassica*.

Table 4. Average content of Brassica pollen grains in the beecolonies at trial sites in 2013.

	Average content of Brassica pollen grains (% sd)			
	Pollen loads	**Perga**	**Nectar**	**Honey**
Trial site 1	81,39 (±9,84)	84,7	58,98 (±31,30)	82,6 (±10,68)
Trial site 2	88,69 (±14,95)	86,3	79,1 (±14,26)	69,93 (±1,77)
Trial site 3	40,17 (±20,23)	48,6 (±19,37)	67,5 (±13,54)	65,46 (±16,44)
Trial site 4	72,36 (±17,55)	36,65 (±7,71)	53,92 (±14,08)	43,6 (±33,98)

3.6 Residue analysis

The interim report summarises the residue data of neonicotinoids in samples collected during the first summer of the project. All neonicotinoids approved in Finland are monitored and all of them are approved for turnip rape cultivation either for foliar spraying (thiacloprid, acetamiprid) or for seed treatment (chlothianidin, thiametoxam, imidacloprid). From these imidacloprid has not really been in use in the past years when approximately half of the oil plant seeds in Finland have been treated with thiametoxam and the other half with clothianidin[6].

Residue data of other pesticides which are used for turnip rape cultivation and which are also monitored in this study are not yet fully processed and therefore not included into this interim report.

3.6.1 Analytical methods (neonicotinoids)

Analytical methods developed for the determination of pesticide residue levels in honey, bee bread, pollen bees and turnip rape flowers were based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) sample preparation method originally introduced by Anastassiades et al 2003. No pretreatment was needed for the pollen samples, whereas honey samples were heated less than 35 ^{0}C in a water bath. The bee bread was grounded in a mortar before the extraction, whereas bees were lyophilized and grounded as a pretreatment. The turnip rape flowers were freeze-dried and homogenized in a small laboratory mill before analysis. The neonicotinoid residues (acetamiprid, clothianidin, imidacloprid, thiacloprid and thiametoxam) and their main metabolites (6-chloronicotinic acid and acetamiprid-N-desmethyl) were extracted with water-acetonitrile mixture by dispersive solid phase extraction (dSPE). The resulting extract from the hive samples was further cleaned with primary and secondary amine (PSA) and octadecyl silane (C18) absorbents. Plant samples were cleaned with PSA and carbon (ENVI-Carb) absorbents in order to remove plant pigments. Cleaned extracts were concentrated by evaporation, reconstituted to methanol-water and filtered for the UPLC-MS/MS analysis.

The UPLC-MS/MS analyses were performed on Waters Xevo UPLC coupled with a Waters TQMS triple quadrupole tandem mass spectrometry. The chromatographic separation was performed on a Waters Acquity BEH C18 column (1.7 µm, 2.1 mm x 100 mm) equipped with a precolumn (Waters, VanGuard). Electron spray ionization (ESI) operating on positive mode was used on the mass spectrometric analysis. For each analyte, at least two MRM transitions were measured; for acetamiprid four and for clothianidin and thiametoxam three MRM transitions.

The analytical methods for bees and hive products were validated based on DG Sanco guidance (DG Sanco 12571/2013). Matrix-matched calibration was used in the quantification of the compounds. The limit of quantification (LOQ) was determined as the lowest standard point. The LOQs for neonicotinoid residues were determined as 0.05 µg/kg honey, 0.1 µg/kg pollen or bee bread and 0.3 µg/kg bees for all neonicotinoid compounds except for 6-chloronicotinic acid which had LOQs of 7.5; 15 and 45 µg/kg respectively. In addition, the separation of acetamiprid-N-desmethyl confirming ion was not satisfactory in less than 1.2 µg/kg bee.

Matrix-matched calibration was used also for analysis of flowers for thiametoxam and clothianidin: Quantitative areas were 0.25 -35 ng/g as dry weight. The separate calibration for thiacloprid was needed, because concentrations of thiacloprid were so high in some flower samples: Different amounts of thiacloprid were added in blank flower extracts, with quantitative area 0.25 -50 µg/g (as dry weight). Thiacloprid results have been confirmed with recovery tests (recovery 93 -100 %).

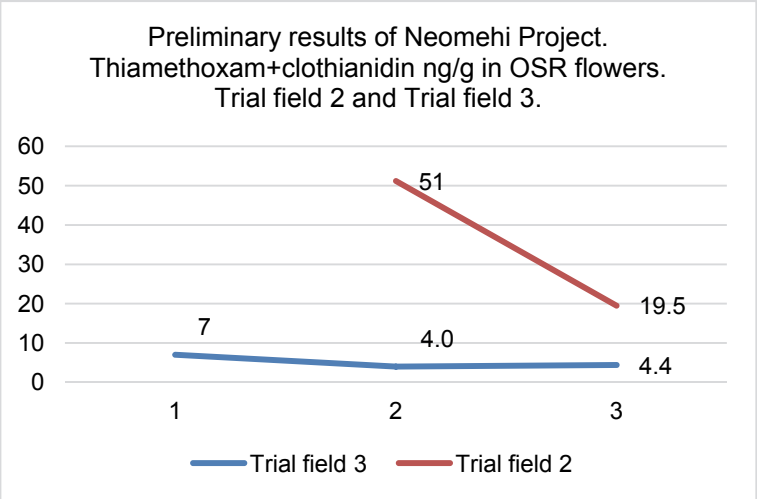
3.6.2 Residues of neonicotinoids in turnip rape flowers in the field study

Three neonicotinoids (clothianidin, thiacloprid, thiametoxam) which were applied in the trial sites were analysed from flower samples. Residue amounts of clothianide + thiametoxam in samples collected from trial fields 2 and 3 and residue amounts of thiacloprid in samples from trial fields 3 and 4 are presented in Figure 7. Seed treatment was used in trial fields 2 and 3 and foliar sprayings with neonicotinoids were done in trial fields 3 and 4. The sum concentrations of clothianide + thiametoxam varied between differ-

[6] Markkula, A. 2014. Interview 1.9.

ent sampling points from 4 to 51 ng/g and the differences between the fields were significant. The concentration of thiacloprid immediately after spraying was similar in both trial fields; concentration being 26.7 and 29.5 µg/g. The thiacloprid amounts decreased logically after the spraying.

Residues of neonicotinoids in the turnip rape flowers in 2013



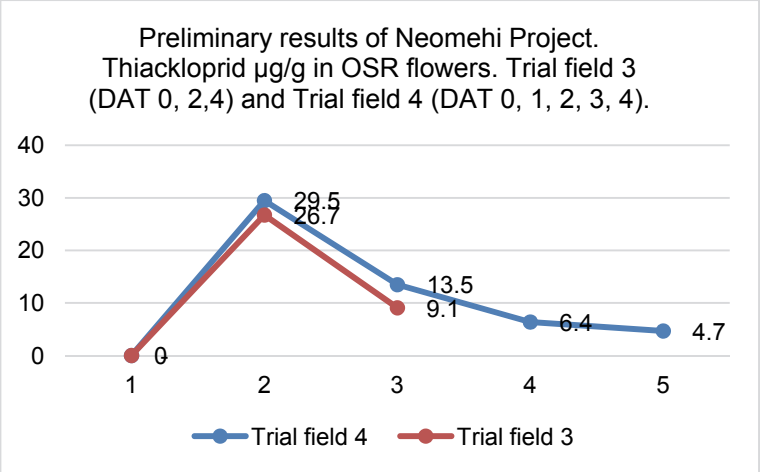


Figure 7 Residues of neonicotinoids in turnip rape flowers in 2013. Concentrations are in dry-weight.

3.6.3 Residues in the honey bee colonies (nectar, honey, bee bread, pollen, worker honey bees)

Residues of thiametoxam and chlothianidin

Thiametoxam was used in trial fields 2 and 3. Clothianidin is a main metabolite of thiametoxam and residues of both thiametoxam and chlothianidin were expected to appear in the samples. Residues were measured in all types of hive samples including nectar, honey, bee bread and pollen. In the bees itself residues were ≤loq. In this report residues of thiametoxam and clothianidin are mainly discussed as a sum of concentrations ($c_{thia+clo}$) which is a relevant procedure because thiametoxam and clothianidin own similar toxicity and LD50 values.

Residues of imidacloprid were not found in any of the samples.

Residues of clothianidin and thiametoxam in the field study (Part A)

Figure 8 shows residue levels of thiametoxam and clothianidin in nectar collected from the hives in each test site. Sampling was done during turnip rape flowering in the middle of June. Sum of concentrations of thiametoxam and clothianidin ($c_{thia+clo}$) in nectar are between 0,05-4,45 ng/g. Highest concentration, 4,45 ng/g, was suprisingly found in the field with no use of neonicotinoids. Seed treatment neonicotinoids were detected in all samples even though the trial field was not treated with thiametoxam. It can be infered that the test assembly was not succesfull in all field trials and propably bees have flown further for feed. Average $c_{thia+clo}$ and relative standard deviation of all 20 nectar samples is 0,92±0,98 ng/g.

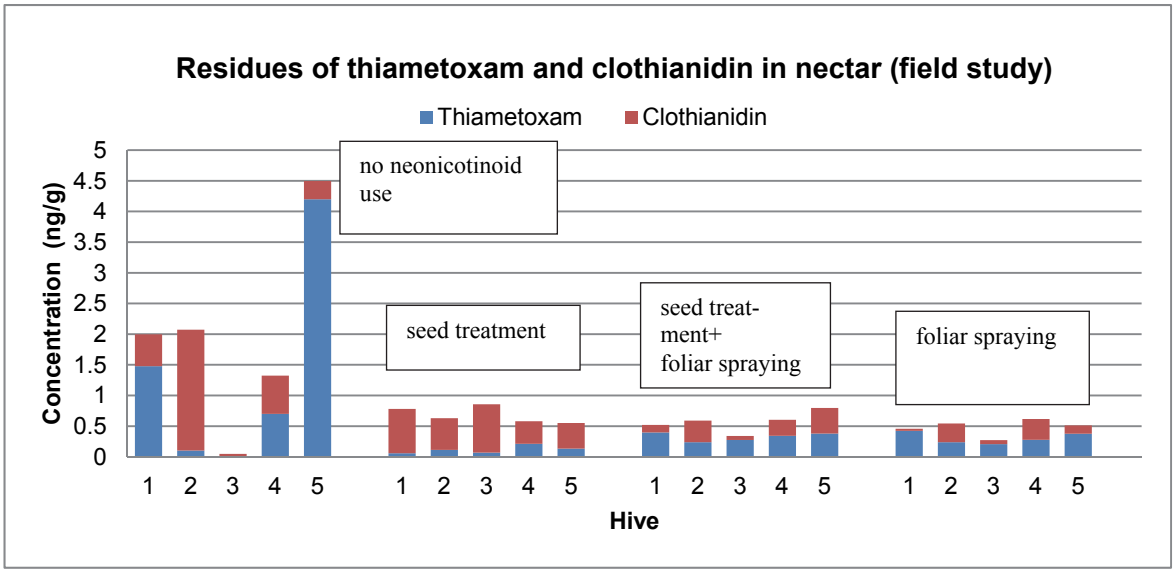


Figure 8. Field study. Residues of thiametoxam and clothianidin in nectar.

Honey samples were collected from hives on the 21-23th of August. Thiametoxam and clothianid concentrations in honey were between 0,64-2,98 ng/g .

As nectar samples, also bee bread samples contained residues of thiametoxam and clothianidin even though neonicotinoids were not used for seed treatment in the corresponding test field. Sum of concentrations of thiametoxam and clothianid were between 0-2,44 ng/g. Average $c_{thia+clo}$ and relative standard deviation of all samples (4 fields, 4-5 hives per each field) was 0,97±0,70ng/g.

Table 6 shows residues of thiametoxam and clothianidin in pollen collected by pollen traps from the posterior legs of worker bees entering the hives. Residue levels of thiametoxam and clothianidin in pollen are not comparable to findings in nectar (Figure 8 vs Table 6) when highest residues were found in the field in which neonicotinoids were not used. However, only a limited number of samples were analysed for residues since the amount of collected pollen from several sampling points was insufficient for analysis. More comprehensive sampling is needed for reliable estimations of residues in pollen.

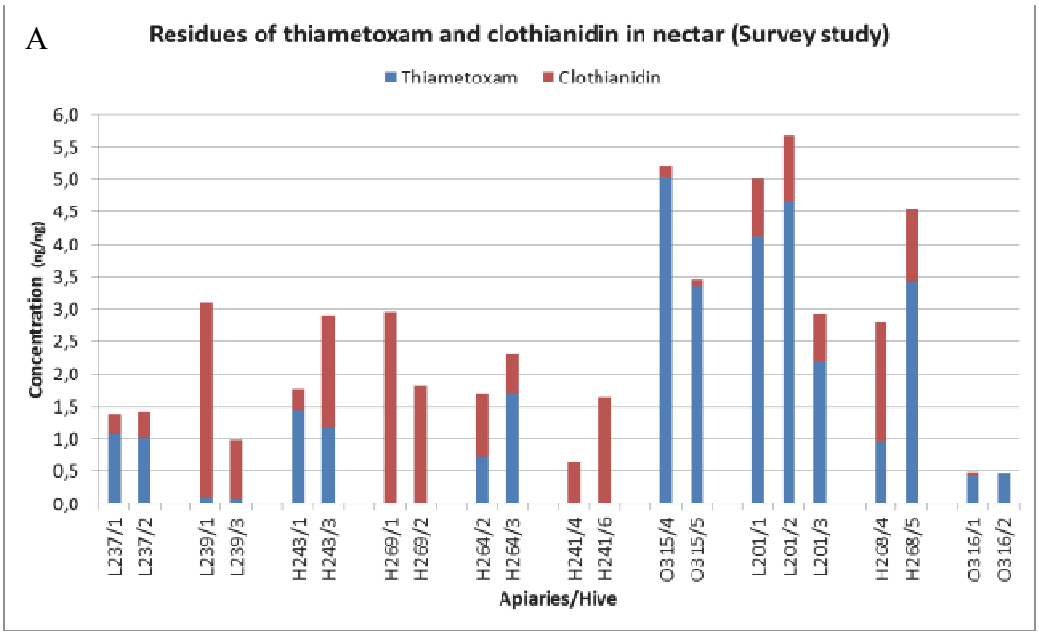
Table 6. Residues of thiametoxam and clothianidin in pollen in the field study. Sampling points are time points during flowering.

Trial site	Neonicotinoid treatment of field	Number of hives	Number of sampling points per hive	Average sum of thiametoxam and clothianidin (ng/g)±rsd%	Min/Max sum thiametoxam and clothianidin (ng/g)
1	no neonicitinoid use	1	4	3,04±3.74	0,51/ 8,57
2	seed treatment	2	4,4	3,03±0.75	1,96 / 4,14
3	seed treatment and foliar spraying	2	6,6	0,84±0.79	0,00/ 2,92
4	foliar spraying	2	1,4	≤0.1 (LOD)	0,00/ 0,42

In worker bees residues of thiametoxam and clothianidin were ≤loq.

Residues of clothianidin and thiametoxam in Sample survey (Part B)

In Part B of NEOMEHI project nectar and bee bread samples were collected as a sample survey. Figures 9 a) shows residues of thiametoxam and clothianidin in nectar samples collected from hives which were located near the fields. Average $c_{thia+clo}$ and the relative standard devion of results from nine apiaries was 2.75±1.45 ng/g (organic farm not included). The highest measured concentration $c_{thia+clo}$ was 5.67 ng/g. Figures 9 b) shows residues of thiametoxam and clothianidin in nectar samples collected from hives which were not near oilseed fields. Concentrations were between 0-1 ng/g, with the expection of one apiary in which $c_{thia+clo}$ was at the highest 3.5 ng/g.



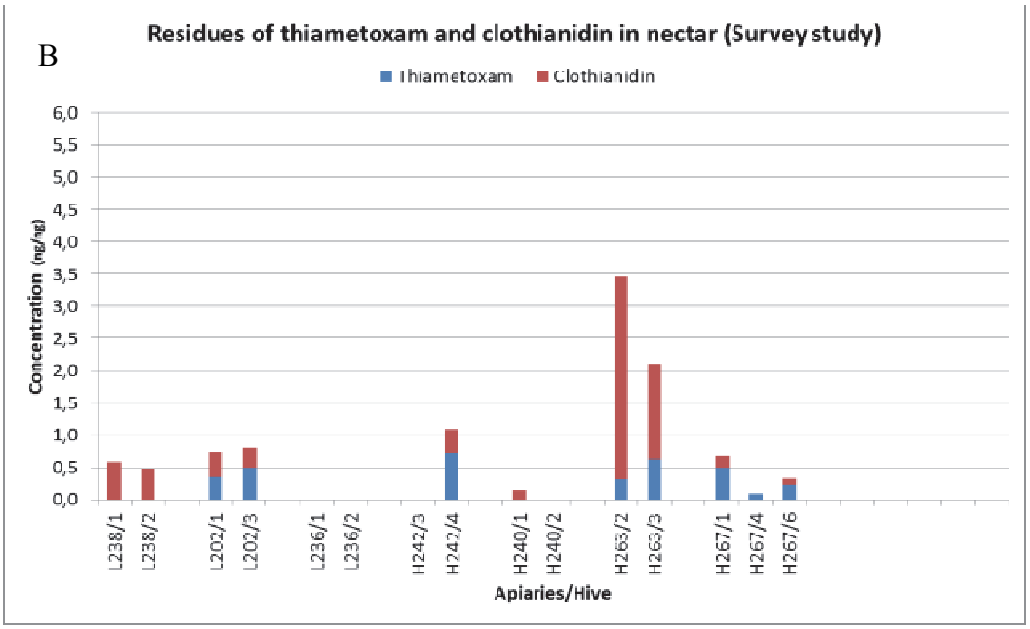


Figure 9 a) Residues of thiametoxam and clothianidin in nectar from hives which were located near oilseed fields. O316 is organic farm. b) Residues of thiametoxam and clothianidin in nectar from hives which were not located near oilseed fields.

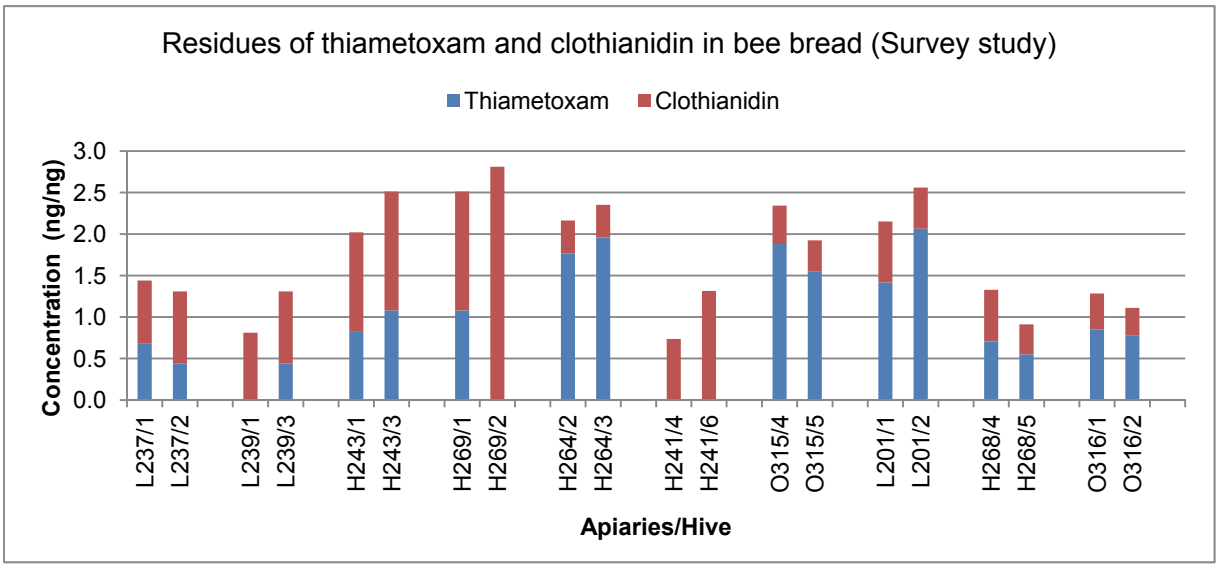


Figure 10. Residues of thiametoxam and clothianidin in bee bread from hives which were located near oilseed fields. O316 is an organic farm.

Figure 10 shows residues of thiametoxam and clothianidin in bee bread samples collected from hives which were located near oilseed fields. Average $c_{thia+clo}$ and the relative standard deviation of results from ten apiaries was 1.75±0.70 ng/g. Concentrations were between 0.7-2.6ng/g. Because of insufficient amount of sample only seven control bee bread samples from five apiaries which were not located near oilseed fields were analysed for neonicotinoid residues. Average $c_{thia+clo}$ in control bee bread samples was 0.66±0.57 ng/g.

Residues of thiacloprid

Residues of thiacloprid in field study (Part A)

In the field study the first sampling point for nectar followed the day of the sprayings. Honey samples were collected four weeks later. Thiacloprid concentrations varied from 26-130 ng/g in nectar and 40-114 ng/g in honey (Figure 11). Residues of thiacloprid was not found or the level was low ≤0.2 ng/g in the samples collected from fields where thiacloprid sprayings were not applied.

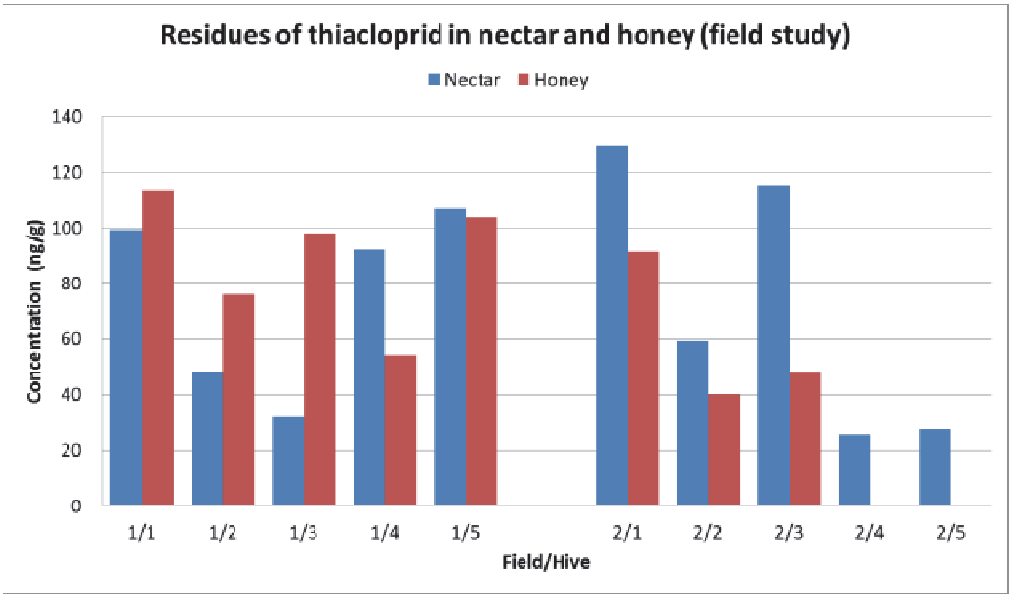


Figure 11. Residues of thiacloprid in nectar and honey in trial fields 3 and 4. Both fields were treated with thiacloprid sprayings. Honey samples were not collected from hives 2/4 and 2/5.

Pollen samples collected from the hives contained thiacloprid more than 150 ng/g immediately after the spray application (Figure 12). As predicted, the thiacloprid amounts decreased as the time from the spraying point elapsed. The residue amounts of hives on field 1 showed a correspondence. From the other field all the pollen, which was available, was collected from one hive. Because of the unrepresentative sampling, the highest thiacloprid concentration measured, 482 ng/g, is only an approximative result. Confirmation of thiacloprid levels in nectar will be achieved from the field experiment of next summer.

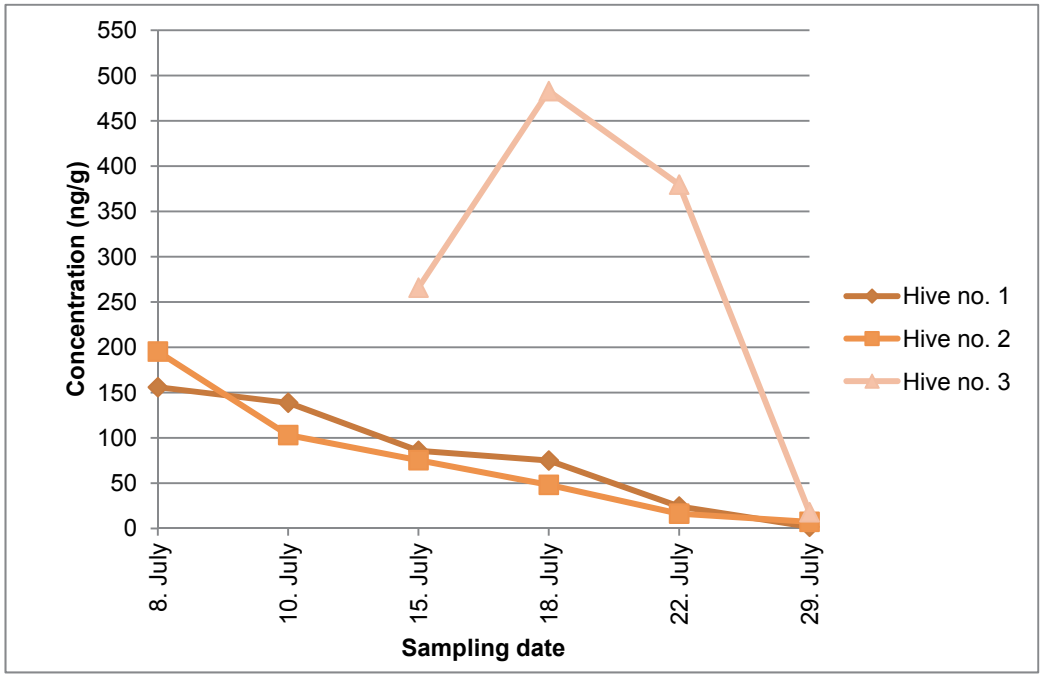


Figure 12. Thiacloprid levels in pollen samples. Hive no 1 and 2 are located in the trial field 1, whereas hive no 3 is in trial field 2.

Bee samples collected from the trial fields contained at maximum 4.6 ng/g thiacloprid residues. In corresponding bee bread samples thiacloprid residue levels were between 30-666 ng/g (9 hive samples together from two fields).

Residues of thiacloprid in Sample survey (Part B)

Nectar samples which were collected as a survey contained thiacloprid at maximum ca 5 ng/g. In the hives of three apiaries (near oilseed fields) thiacloprid levels were 3.7-6.2 ng/g. Sampling dates of sample survey were not optimised and fell mostly at the end of flowering which explains why as high tiacloprid levels as in the field study were not determined. As nectar, also those bee bread samples which were collected from the above mentioned apiaries and hives contained highest amount of thiacloprid, concentrations being between 14-163 ng/g.

Residues of acetamiprid

Residues of acetamiprid and its des-methyl metabolite were found only at very trace levels in some of the samples collected from the field study or as sample survey. Concentrations of acetamiprid were less than 3.5 ng/g in all nectar samples and less than 2.6 ng/g in all bee bread samples. 6-chloronicotinic acid, which is a metabolite of both acetamiprid and thiacloprid, was not detected or residue levels were ≤LOQ in nectar, bee bread or bee samples. In pollen collected from trial fields at maximum 24 ng/g of 6-chloronicotinic acid was measured.

4 Conclusions

A great interest will be focused on the results of thiametoxam and chlothianidin for which the EU introduced a partial ban in 2013 and for which new research data is expected. This study provides for the first time research data from Finnish oilseed cultivation conditions.

As a conclusion of the residue studies in both the field study and the sample survey the sum of the thiametoxam and clotianidin residue ($c_{thia+clo}$) in nectar collected from beehives was ≤ 5.7 ng/g. In 50% of all nectar samples $c_{thia+clo}$ was 0.05-3 ng/g. Results of the first trial summer gives a good estimation of residue levels in nectar of seed treatment neonicotinoids in Finnish oilseed cultivation. This can be concluded even if it seems that the selection of a control trial field with no neonicotinoid treatment failed in this study. The highest residue levels were found in nectar samples collected from the field that was not treated with neonicotinoids. Also in the sample survey residues were found in the hives which were located far from oilseed fields though a clear difference occurred between samples taken from fields close to and far from oilseed fields (Figure 9 a and b). Probably bees have in this case flown further for feed. Residues are surely originating from other oilseed fields because the use of thiametoxam and clothianidin is limited and oilseed crops are the only crops attracting bees for which thiametoxam and clothianidin are approved. In addition, results from analysis of origin of pollen supports the conclusion (Table 4). Subsequent analysis of the other oilseed cultivation areas near the control field showed that there were two oilseed cultivation areas in a distance less than three kilometers (1.359 km and 1.928 km) from the control field (Appendix 4). In both fields thiametoxam products were used for seed treatment. The distance was clearly not long enough to get control hives with no exposure to neonicotinoids. However, the fact that residues appeared also in control samples is known and a previously reported problem in this type of field studies.[7]

A rough estimate of exposure to neonicotinoids can be calculated based on the residue results for worker bee, queen bee and larva. Maximum consumption of sugar and pollen used in risk assesment[8] is for worker bee 128 mg sugar/bee/day; for queen bee 12 mg pollen+50mg sugar/bee/day and for larva 2mg pollen+59.4mg sugar/larva. Using above value of consumption, 60 % sugar content of nectar and highest cthia+clo measured in nectar (5.7 ng/g) exposure to thiametoxam +clothianidin as worst case situation is for worker bee 1.2 ng/day. If exposure is compared to the toxicological end point values (table 6) the concentration is close to the risk limits and especially chronic and acute sublethal risks cannot be excluded. Results are in line with the EFSA conclusion regarding thiametoxam and chlothianidin[9],[10].

Table 6. Toxicological end point values for chlothianidin and thiametoxam. (LD50=median lethal dose, NOEC=No observed effect level).

Substance	Toxicolocical end point	
thiametoxam	acute oral LD_{50}	5 ng/ bee
clothianidin	acute oral LD_{50}	3.79 ng/ bee
thiametoxam	sublethal dose	1.34 ng /bee
clothianidin	sublethal dose	0.5 ng/ bee
thiametoxam	chronic 10-dayLC_{50}	> 0.2 ng/ bee/day
clothianidin	chronic 10-day $NOEC_{bee}$	8.13 ng/g food

[7] Pohorecka et. al, Journal of Apicultural Science (2012), 56 (2):115
[8] EFSA Journal 2013; 11 (7): 3295
[9] EFSA Journal 2013; 11 (1): 3066
[10] EFSA Journal 2013; 11 (1): 3067

The corresponding calculations for thiacloprid used as foliar sprayings results in an exposure of 25.6 ng/bee/day (max concentration in nectar 130 ng/g) which is 500 times lower than LD50. LD50 for thiacloprid is 14.6 ug/bee. The case of thiacloprid is clearly below the acute risk limit. However, mixture toxicity of several active compounds applied at the same time should be taken into account, eg. in this case simultaneous exposure to thiacloprid, thiametoxam and chlothianidin.

The calculations must be viewed with caution at this stage due to many possible sources of errors such as a limited number of test fields, lack of control samples and especially in the case of pollen the amount of collected samples which was not enough for representative sample and analysis. Therefore, at least results from next summer are needed to make more reliable conclusions.

The condition of honey bee colonies and colony development were normal in all of trial sites and use of neonicotinoids or residues in hive products did not have clear impact on the condition of the colonies. However, as hives in all trial sites were exposed to seed treatments neonicotinoids no comparison between exposed and non-exposed can be done. Two bee hives were lost because of loss of queen during the fall and winter. This does not differ from the normal winter losses in the whole of Finland (Coloss survey 2014).

5 Appendices

Appendix 1. The table for the activities in the field study of NEOMEHI Project carried out in 2013.

	Trial site 1	Trial site 2	Trial site 3	Trial site 4
Farm, farmer name, Postal code and gps-location of NEOMEHI Trial site	1.Trial site: Laurila, S. Raiskio, FI-31600 Jokioinen Location: Somero N 6738372 E 303039	2.Trial Site: T. Jaska, FI-30100 Forssa, N 6747940 E 318711	3. Trial Site: Mäkelä, H. Jalli, FI-31500 Koski, N 6729954 E 289281	4. Trial Site: MTT Agrifood Research Finland, FI-31600 Jokioinen, N 6745860 E309922
Planting method, previous crop, machinery	2013: Glyphosate before drill (2012 timothy seed grass), direct drilling (VM)	2013: Glyphosate before drill (2012 barley), direct drilling (Tume)	2013: Conventional tillage, (spring wheat 2012), conventional drilling (Juko)	2013: Glyphosate before drill (2012 barley), direct drilling (VM)
Seed treatment/variety/Lot code / Germination rate	Uncoated / Apollo / BOR 357-01059B / 98%	Cruiser OSR 15 ml/kg /Apollo / BOR 357-01059B / 96%	Cruiser OSR 15 ml/kg/ Apollo / BOR 357-01059B / 96%	Uncoated / Apollo / BOR 357-01059B / 98%
Sowing date, Seeding rate kg/ha (real seeding-rate may change from target rate according to drilling method, soil moisture etc.)	29/05/13, 13 kg/ha (10 kg/ha)	17/05/13, 10 kg/ha (8-10 kg/ha)	18/05/13, 6 kg/ha (8-10 kg/ha)	29/05/13, 10 kg/ha (10 kg/ha)
Planting method, seed bed / soil moisture at sowing	Direct drilling VM, fine / good	Direct drilling Tume Nova Combi, coarse / above normal	Conventional drilling, fine / good	Direct drilling VM, fine / good
Foliar spraying against flea beetles, (*Phyllotreta* sp.).	-	Pyrethroid (Sumi alpha 5 FW) 06/06/13,	Pyrethroid (Decis Mega EW 50) 07/06/13, 10/06/13 (Sumi alpha 5 FW)	-
Foliar spraying against pollen beetles (*Meligethes aneus*)	Pyrethroid Sumi alpha 5 FW 28/06/13	Pyrethroid Sumi alpha 5 FW 19/06/13	Pyrethroid Sumi alpha 5 FW 20/06/13 Biscaya OD 240 0.35 l/ha 01/07/13	Pyrethroid (Sumi alpha 5 FW) 28/06/13 Biscaya OD 240 0.35 l/ha 08/07/13
Principal growth stage at foliar spraying	-	-	Flowering stage BBCH 63-64 (at minimum late bud stage)	Flowering stage BBCH 63-64 (at minimum late bud stage)
haFungicide	-	-	-	-
Harvesting date	09/09/13	23/09/13	17/09/13	09/09/13
Bee hives transported to trial field dd-mm, gps location	06/07/13 N6738362 E318747	29/06/13 N6748018 E318747	17/06/13 N6729948 E289287	29/06/13-mm, N6745832 E310021
Bee hives transported away from trial field and placed to overwintering sites dd-mm, gps location	13/08/13	12/08/13	09/08/13	13/08/13

Appendix 2. Seed Treatment Analysis Report of treated seed used in 2013. Syngenta Seedcare Institute 14.4.2014

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Site de St Pierre la Garenne
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To:

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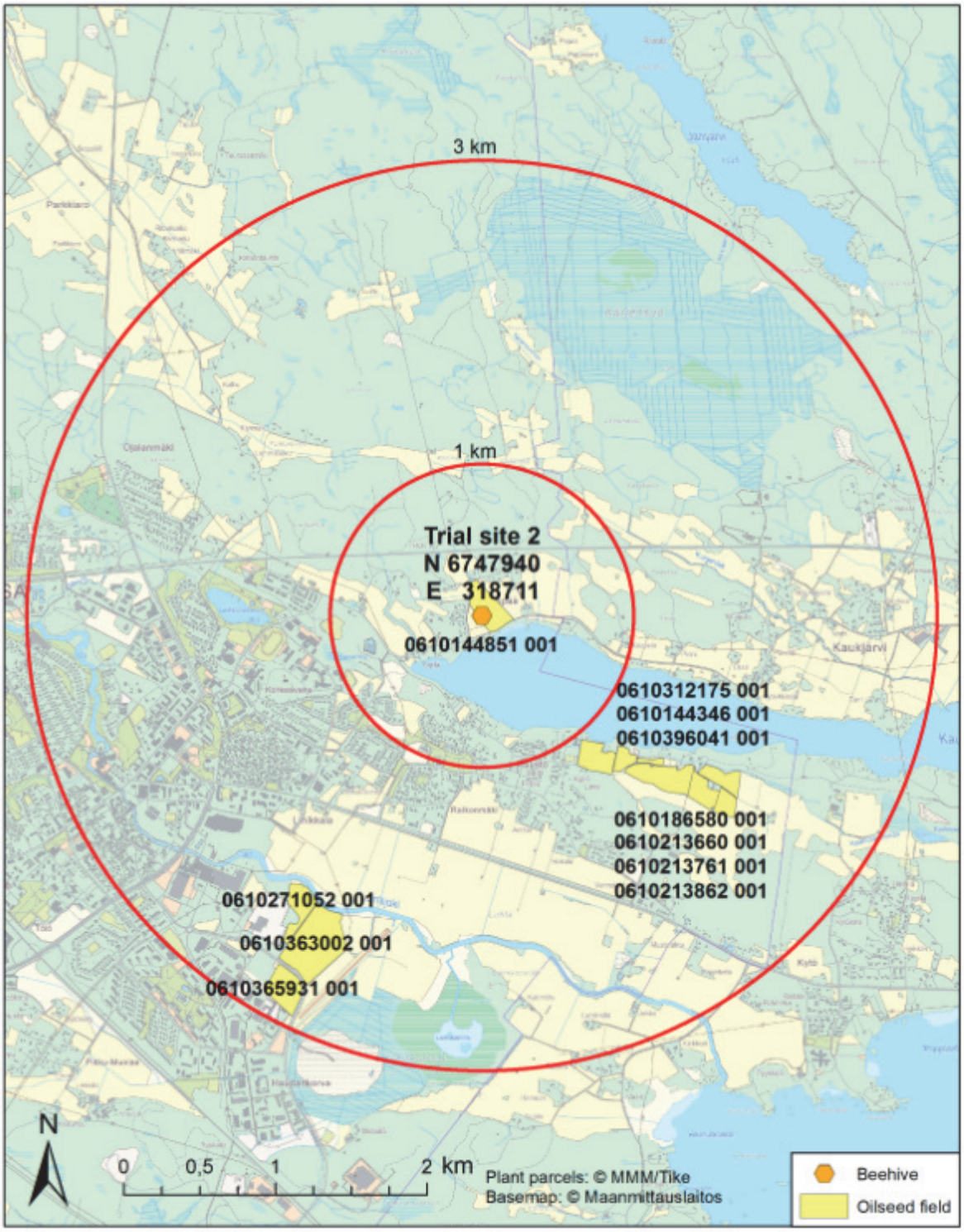
Seed Treatment Analysis Report 14.04.2014

Site	**BOREAL KASVINJALOSTUS OY /**	Customer	**Others**
Seedcare Case	**478-1401-E-FI-OTH-OIL-CRO**	Crop	**Oil seed rape**
Main Product	**Cruiser OSR**	Date of Delivery	
Define analysis and method	**DUST / Heubach 2 min**	**SEED LOADING / HPLC**	**Thiamethoxam**
Remarks	**14.133**		

Sample		**357-01-059B**
ST Batch Id		357-01-059B
Variety		APOLLO
Weight of Samples		279.00
Reception Date		08.04.2014
TGW (g)		2.4
Date of Analysis		11.04.2014
General Remarks		
Dust		
Limit		0.5000 g/700000 seeds
Dust Result		0.0376 g/700000 seeds
Comment		
Dust Remarks		
Seed Loading (SLA)		357-01-059B
AI Analyzed	Cruiser OSR	Thiamethoxam
Target Rate	Cruiser OSR	420 g a.i./100 kg
SLA Rate	Cruiser OSR	386 g a.i./100 kg
% of Target	Cruiser OSR	92 %
Comment	Cruiser OSR	
SLA Remarks	Cruiser OSR	

Appendix 3. Oilseed fields around 1 and 3 kilometres from Neomehi Trial sites 1-4. Oilseed fields are coloured with bright yellow in the maps.


3 km
1 km
Trial site 2
N 6747940
E 318711
0610144851 001
0610312175 001
0610144346 001
0610396041 001
0610186580 001
0610213660 001
0610213761 001
0610213862 001
0610271052 001
0610363002 001
0610365931 001
N
0
0,5
1
2 km
Plant parcels: © MMM/Tike
Basemap: © Maanmittauslaitos
Beehive
Oilseed field

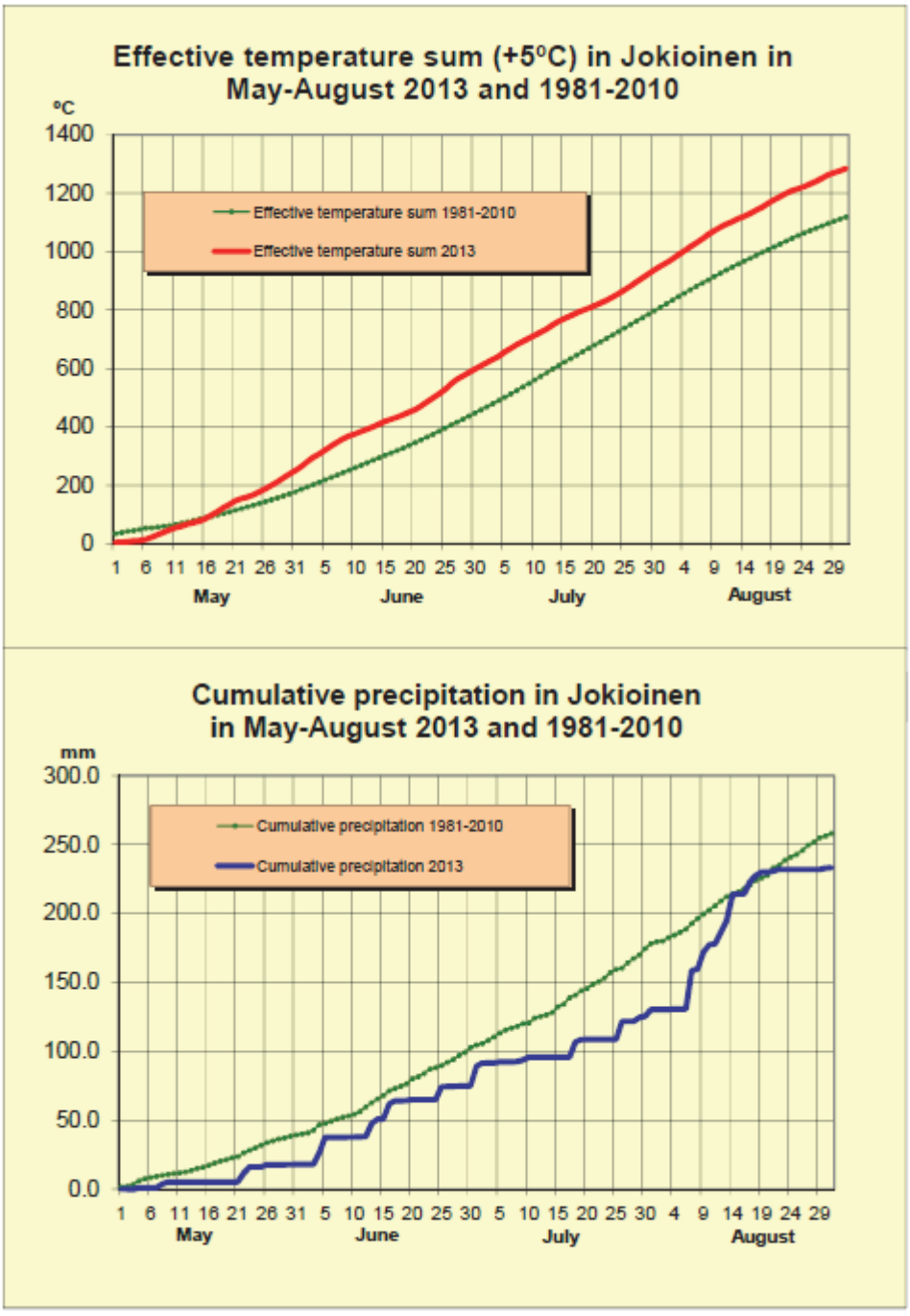

3 km
1 km
Trial site 3
N 6729954
E 289281
2840202032 001
2840204052 001
2840283571 001
2840228708 003
2840228708 002
2840234465 001
2840205365 001
2840248714 001
2840268922 001
2840085430 002
2840202436 001
2840308732 001
2840061683 001
2840061885 002
2840133829 001
2840093413 001
2840093514 001
2840093615 001
N
0
0,5
1
2 km
Plant parcels: © MMM/Tike
Basemap: © Maanmittauslaitos
Beehive
Oilseed field

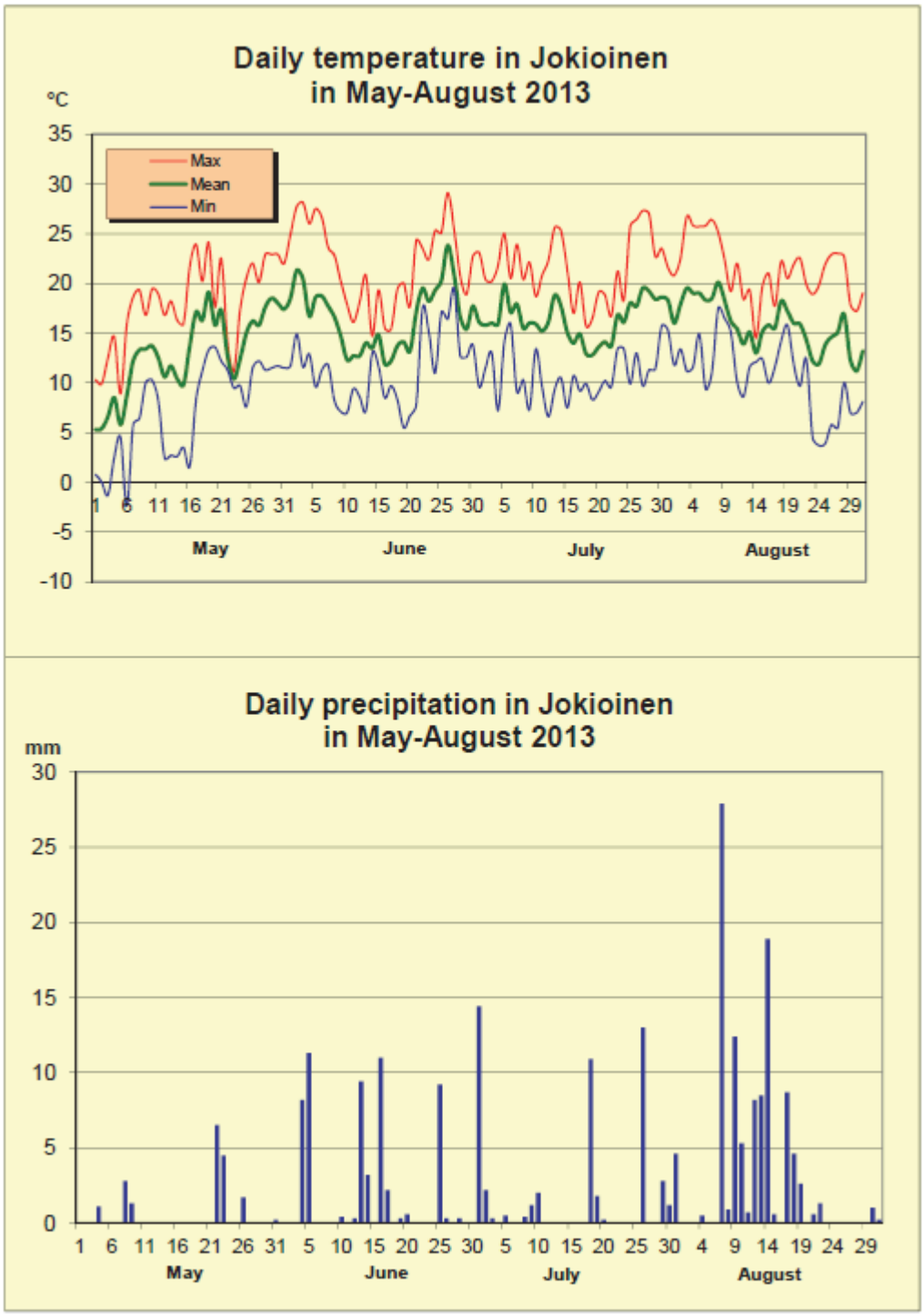

3 km
Lypsyasema
N 6747952
E 309449
1690344632 005
1690260261 004
1690260261 006
1690260261 005
1690276833 002
3 km
1690236821 007
Trial site 4
N 6745860,2434
E 309922,4622
1690237932 003
N
0
0,5
1
2 km
Plant parcels: © MMM/Tike
Basemap: © Maanmittauslaitos
Beehive
Oilseed field

Appendix 4 Plant protection products used in the neighboring oilseed fields of trial site 1 and MTT Lypsyasema. Bee hives were temporarily placed in the MTT lypsyasema before being moved to trial site 1.

Field	Location/village of the neighboring OSR- field near the test bee hives	Distance between OSR-field and test bee hive m, area of OSR field ha	plant protection products for foliar applications, active ingredients	Foliar application dd/mm/yy	Seed coating of osr seed, product, drilling in May 2013	Beehives in the Trial site during dd/mm-dd/mm/yy
Trial site 1	Lehtimäenkulma	1359m	Mospilan (acetamiprid)	11/06/13	Cruiser OSR (thiametoxam, metalaxyl, fludioxonil)	28/06-31/07/13
			Avaunt (indoxacarb) +Cyberkill (cybermethrin)	22/06/13		
	Vähäsuo	1928m, 7.44 ha	Cyberkill	30/05/13	Cruiser OSR	
			Focus Ultra + Decis (deltamethrin)			
Väliparkki MTT lypsyasema	OSR trial fields	around 1000m, total area 2 ha	Biscaya OD 240 (thiacloprid)	24/06/13, 26/06/13 01/06/13-20/06/13	Cruiser OSR, Elado FS 480 (clothianidine, betacyfluthrin)	24/06-28/06/13
			Karate 2.5 WG (lambdacyhalothrin)	03/06/13, 27/05/13, 30/05/13, 03/06/13, 25/07/13	Cruiser OSR	
			Sumi alpha 5 FW (esfenvalerate)	06/06/13, 11/06/13, 19/06/13, 26/06/13		
	Lamminkylä	>1000m	Galera Avaunt Decis	01/06/13 04/06/13 15/06/13	Modesto (clothianidine, betacyfluthrin)	

Appendix 5. Weather data (daily) in Jokioinen in May-August 2013.




Daily temperature in Jokioinen
in May-August 2013
°C
35
30
25
20
15
10
5
0
-5
-10
Max
Mean
Min
1 6 11 16 21 26 31 5 10 15 20 25 30 5 10 15 20 25 30 4 9 14 19 24 29
May
June
July
August
Daily precipitation in Jokioinen
in May-August 2013
mm
30
25
20
15
10
5
0
1 6 11 16 21 26 31 5 10 15 20 25 30 5 10 15 20 25 30 4 9 14 19 24 29
May
June
July
August

Appendix 6. Weather data (daily) in Jokioinen in April-September 2013.

WEATHER CONDITIONS IN JOKIOINEN 2013. DATA FROM THE OBSERVATORY OF JOKIOINEN

(location 60.81402°N, 23.49829°E according to map datum WGS 84**, altitude 104 m).** *Data source: Finnish Meteorological Institute.*

April

Date	Temperature					Precipitation		Relative humidity
	Mean	Effective temp. sum	Max	Min	Surface Min		Sum	(mean)
	° C	° C	° C	° C	° C	mm	mm	%
1	-4.5	0.0	0.0	0.0	.	0.0	0.0	57
2	-2.5	0.0	5.1	-11.9	.	0.0	0.0	55
3	-1.9	0.0	5.0	-10.5	-16.3	0.0	0.0	55
4	-0.7	0.0	7.1	-8.8	.	0.0	0.0	59
5	-0.4	0.0	5.9	-9.5	-11.1	0.0	0.0	43
6	-2.1	0.0	3.3	-7.1	-11.3	0.0	0.0	42
7	-3.2	0.0	1.3	-9.2	-12.3	2.7	2.7	97
8	-4.7	0.0	1.4	-10.5	.	0.0	2.7	87
9	-3.1	0.0	4.5	-11.6	.	0.0	2.7	73
10	-2.7	0.0	5.6	-11.1	-11.4	0.0	2.7	55
11	-0.4	0.0	4.3	-7.6	-12.7	0.0	2.7	83
12	2.6	0.0	5.4	0.6	0.1	2.3	5.0	90
13	2.0	0.0	3.3	1.9	0.8	2.7	7.7	99
14	3.1	0.0	8.1	0.2	0.0	0.0	7.7	80
15	3.0	0.0	5.2	0.3	-1.5	4.4	12.1	94
16	4.5	0.0	6.0	3.4	2.0	0.4	12.5	85
17	5.8	0.0	9.6	3.9	3.1	0.3	12.8	80
18	5.4	0.0	8.3	2.2	1.3	8.7	21.5	96
19	4.7	0.0	5.7	4.1	3.1	0.6	22.1	96
20	4.3	0.0	7.9	1.9	0.9	0.0	22.1	46
21	4.6	0.0	11.1	-2.8	-6.1	0.0	22.1	45
22	5.6	0.6	11.0	-1.0	-4.9	0.0	22.1	41
23	5.1	0.7	8.7	1.4	2.2	1.4	23.5	95
24	6.5	2.2	11.6	1.4	0.0	0.1	23.6	47
25	5.7	2.9	11.2	0.7	-2.6	0.0	23.6	42
26	2.8	2.9	6.4	-1.1	-4.6	3.8	27.4	94
27	4.2	2.9	9.7	1.4	1.1	0.2	27.6	70
28	3.9	2.9	9.7	-1.0	-3.4	0.0	27.6	56
29	6.2	4.1	10.5	3.1	-0.5	4.8	32.4	80
30	5.2	4.3	7.7	3.3	2.8	1.0	33.4	74
Month	2.0					33.4		
Normal 1981-2010	*3.5*					*30.0*		

May

Date	Temperature					Precipitation		Relative humidity
	Mean	Effective temp. sum	Max	Min	Surface Min		Sum	(mean)
	° C	° C	° C	° C	° C	mm	mm	%
1	5.3	4.6	10.3	0.8	-1.2	0.0	0.0	32
2	5.4	5.0	9.9	0.0	-3.9	0.0	0.0	48
3	6.6	6.6	12.5	-1.3	-4.5	0.0	0.0	30
4	8.5	10.1	14.6	2.5	1.3	1.1	1.1	41
5	5.8	10.9	8.9	4.5	3.8	0.0	1.1	79
6	8.7	14.6	15.9	-2.4	-6.2	0.0	1.1	52
7	12.2	21.8	18.6	5.8	2.9	0.0	1.1	57
8	13.3	30.1	19.3	6.4	3.1	2.8	3.9	44
9	13.4	38.5	16.8	10.0	5.6	1.3	5.2	80
10	13.7	47.2	19.4	10.3	10.5	0.0	5.2	72
11	12.5	54.7	18.9	8.2	5.1	0.0	5.2	49
12	10.6	60.3	16.8	2.4	-0.8	0.0	5.2	48
13	11.7	67.0	18.2	2.7	-1.0	0.0	5.2	61
14	10.4	72.4	16.3	2.6	-1.9	0.0	5.2	87
15	9.8	77.2	15.9	3.5	-0.9	0.0	5.2	45
16	13.7	85.9	21.7	1.6	-2.4	0.0	5.2	28
17	17.1	98.0	23.9	8.3	2.6	0.0	5.2	80
18	16.3	109.3	20.2	11.2	7.2	0.0	5.2	78
19	19.1	123.4	24.1	13.3	10.7	0.0	5.2	65
20	15.8	134.2	17.6	13.6	10.4	0.0	5.2	78
21	17.3	146.5	22.5	12.2	7.6	0.0	5.2	47
22	12.7	154.2	14.1	11.3	7.9	6.5	11.7	96
23	10.4	159.6	11.1	9.5	9.5	4.5	16.2	95
24	12.4	167.0	17.3	9.7	9.4	0.0	16.2	75
25	15.0	177.0	20.5	7.6	2.4	0.0	16.2	40
26	16.2	188.2	22.0	11.5	6.0	1.7	17.9	76
27	15.8	199.0	20.1	12.2	11.2	0.0	17.9	65
28	17.6	211.6	22.9	11.3	6.3	0.0	17.9	40
29	18.5	225.1	.	11.5	4.8	0.0	17.9	41
30	18.0	238.1	22.9	11.7	6.9	0.0	17.9	76
31	17.4	250.5	22.0	11.5	7.1	0.2	18.1	57
Month	12.9					18.1		
Normal 1981-2010	*9.8*					*41.0*		

WEATHER CONDITIONS IN JOKIOINEN 2013. DATA FROM THE OBSERVATORY OF JOKIOINEN

(location 60.81402°N, 23.49829°E according to map datum WGS 84**, altitude 104 m).** *Data source: Finnish Meteorological Institute.*

June

Date	Temperature					Precipitation		Relative humidity
	Mean	Effective temp. sum	Max	Min	Surface Min		Sum	(mean)
	° C	° C	° C	° C	° C	mm	mm	%
1	18.5	264.0	24.9	11.7	7.5	0.0	0.0	55
2	21.3	280.3	27.7	14.9	9.8	0.0	0.0	43
3	20.4	295.7	28.1	11.6	7.7	0.0	0.0	58
4	16.7	307.4	26.0	12.9	12.3	8.2	8.2	96
5	18.6	321.0	27.5	9.6	6.6	11.3	19.5	64
6	18.7	334.7	26.6	11.3	8.8	0.0	19.5	72
7	17.7	347.4	23.6	11.8	8.6	0.0	19.5	43
8	16.6	359.0	22.8	8.2	4.5	0.0	19.5	38
9	14.7	368.7	20.2	7.1	2.7	0.0	19.5	69
10	12.3	376.0	17.9	7.0	3.7	0.4	19.9	71
11	12.7	383.7	16.1	9.4	8.3	0.0	19.9	63
12	12.7	391.4	18.1	8.6	5.7	0.3	20.2	66
13	14.0	400.4	20.8	7.2	3.0	9.4	29.6	95
14	13.5	408.9	14.7	13.0	12.8	3.2	32.8	90
15	14.8	418.7	19.3	11.7	11.4	0.0	32.8	68
16	11.9	425.6	15.5	8.5	5.8	11.0	43.8	84
17	12.2	432.8	15.4	9.7	8.8	2.2	46.0	72
18	13.7	441.5	19.4	8.3	4.6	0.0	46.0	51
19	14.1	450.6	20.0	5.5	2.1	0.3	46.3	41
20	13.2	458.8	17.7	6.7	3.3	0.6	46.9	77
21	17.1	470.9	24.3	7.8	4.2	0.0	46.9	58
22	19.5	485.4	23.5	17.5	16.5	0.0	46.9	73
23	18.2	498.6	22.4	15.1	14.6	0.0	46.9	63
24	19.3	512.9	25.3	11.0	7.4	0.0	46.9	53
25	20.3	528.2	25.1	17.1	15.5	9.2	56.1	78
26	23.8	547.0	29.1	16.4	13.6	0.3	56.4	57
27	20.9	562.9	25.5	19.5	18.5	0.0	56.4	79
28	16.6	574.5	20.7	12.7	11.0	0.3	56.7	60
29	15.4	584.9	18.9	12.6	11.1	0.0	56.7	78
30	17.7	597.6	22.7	13.8	13.7	0.0	56.7	59
Month	16.6					56.7		
Normal 1981-2010	*14.0*					*63.0*		

July

Date	Temperature					Precipitation		Relative humidity
	Mean	Effective temp. sum	Max	Min	Surface Min		Sum	(mean)
	° C	° C	° C	° C	° C	mm	mm	%
1	16.1	608.7	23.1	9.6	5.2	14.4	14.4	92
2	15.8	619.5	20.4	11.5	10.8	2.2	16.6	64
3	16.0	630.5	20.2	13.0	10.9	0.3	16.9	53
4	15.9	641.4	21.8	7.2	3.4	0.0	16.9	89
5	19.9	656.3	25.0	14.1	11.2	0.5	17.4	77
6	17.1	668.4	20.5	15.9	15.3	0.0	17.4	63
7	17.9	681.3	23.9	9.1	5.7	0.0	17.4	49
8	15.5	691.8	20.4	10.3	7.1	0.4	17.8	58
9	16.0	702.8	22.1	7.3	3.5	1.2	19.0	45
10	15.9	713.7	18.7	13.4	12.5	2.0	21.0	96
11	15.2	723.9	20.8	9.6	7.3	0.0	21.0	59
12	16.1	735.0	22.3	6.6	3.0	0.0	21.0	48
13	18.8	748.8	25.6	9.3	5.9	0.0	21.0	47
14	17.7	761.5	25.3	10.5	7.3	0.0	21.0	85
15	15.1	771.6	21.4	7.5	3.9	0.0	21.0	62
16	14.0	780.6	17.0	10.7	9.9	0.0	21.0	51
17	14.9	790.5	20.1	9.2	7.7	0.0	21.0	55
18	12.9	798.4	15.7	9.9	7.3	10.9	31.9	90
19	12.8	806.2	16.5	8.3	4.9	1.8	33.7	77
20	13.6	814.8	19.1	9.1	7.6	0.2	33.9	72
21	14.1	823.9	18.8	10.2	9.7	0.0	33.9	60
22	13.7	832.6	16.7	9.6	8.4	0.0	33.9	81
23	16.8	844.4	21.2	13.4	13.2	0.0	33.9	74
24	16.1	855.5	18.3	13.4	11.9	0.0	33.9	83
25	18.0	868.5	25.7	9.9	6.7	0.0	33.9	66
26	17.7	881.2	26.4	13.0	9.7	13.0	46.9	94
27	19.5	895.7	27.3	9.7	7.2	0.0	46.9	60
28	19.2	909.9	27.0	11.3	8.4	0.0	46.9	70
29	18.4	923.3	22.7	11.4	6.8	2.8	49.7	94
30	18.6	936.9	23.5	15.7	15.3	1.2	50.9	75
31	18.3	950.2	21.6	15.3	15.1	4.6	55.5	71
Month	16.4					55.5		
Normal 1981-2010	*16.7*					*75.0*		

WEATHER CONDITIONS IN JOKIOINEN 2013. DATA FROM THE OBSERVATORY OF JOKIOINEN

(location 60.81402°N, 23.49829°E according to map datum WGS 84**, altitude 104 m).** *Data source: Finnish Meteorological Institute.*

August

Date	Temperature					Precipitation		Relative humidity
	Mean	Effective temp. sum	Max	Min	Surface Min		Sum	(mean)
	° C	° C	° C	° C	° C	mm	mm	%
1	16.0	961.2	20.8	11.8	8.1	0.0	0.0	79
2	17.9	974.1	22.5	13.4	12.1	0.0	0.0	75
3	19.5	988.6	26.7	11.2	7.9	0.0	0.0	60
4	19.0	1002.6	25.8	11.6	8.7	0.5	0.5	65
5	19.0	1016.6	25.7	14.9	14.4	0.0	0.5	46
6	18.3	1029.9	25.8	9.4	5.8	0.0	0.5	43
7	18.5	1043.4	26.4	11.1	6.3	27.9	28.4	99
8	20.1	1058.5	25.1	17.5	15.7	0.9	29.3	84
9	18.3	1071.8	22.4	16.5	15.6	12.4	41.7	99
10	16.2	1083.0	19.2	15.1	14.8	5.3	47.0	96
11	15.4	1093.4	22.0	10.1	7.9	0.7	47.7	91
12	13.9	1102.3	18.4	8.6	6.5	8.2	55.9	75
13	15.1	1112.4	19.3	11.6	9.0	8.5	64.4	75
14	13.0	1120.4	14.5	12.1	11.7	18.9	83.3	97
15	15.1	1130.5	19.6	12.4	11.8	0.6	83.9	82
16	15.8	1141.3	21.0	10.0	8.2	0.0	83.9	64
17	15.5	1151.8	17.7	11.6	7.6	8.7	92.6	97
18	18.2	1165.0	22.2	14.2	12.2	4.6	97.2	79
19	17.2	1177.2	20.5	15.8	15.2	2.6	99.8	75
20	16.0	1188.2	21.9	11.9	7.7	0.0	99.8	61
21	15.9	1199.1	22.5	9.7	5.8	0.6	100.4	65
22	14.3	1208.4	19.9	12.4	9.4	1.3	101.7	66
23	12.2	1215.6	18.9	4.5	0.5	0.0	101.7	74
24	11.9	1222.5	19.8	3.7	0.7	0.0	101.7	74
25	13.8	1231.3	21.9	3.9	1.0	0.0	101.7	67
26	14.6	1240.9	22.9	5.8	2.2	0.0	101.7	68
27	15.1	1251.0	23.0	5.5	1.8	0.0	101.7	64
28	16.9	1262.9	22.7	10.0	3.9	0.0	101.7	73
29	12.3	1270.2	17.9	7.0	3.0	0.0	101.7	83
30	11.2	1276.4	17.2	7.0	4.4	1.0	102.7	77
31	13.2	1284.6	19.0	8.1	4.0	0.2	102.9	94
Month	15.8					102.9		
Normal								
1981-2010	*15.0*					*80.0*		

September

Date	Temperature					Precipitation		Relative humidity
	Mean	Effective temp. sum	Max	Min	Surface Min		Sum	at 3 p.m.
	° C	° C	° C	° C	° C	mm	mm	%
1	13.2	1292.8	18.5	11.0	5.9	3.0	3.0	93
2	9.8	1297.6	15.1	3.8	0.0	0.1	3.1	62
3	11.7	1304.3	19.2	4.5	0.3	0.0	3.1	60
4	12.6	1311.9	20.6	4.8	1.9	0.0	3.1	54
5	13.1	1320.0	21.3	4.4	1.4	0.0	3.1	46
6	13.4	1328.4	20.7	6.3	2.4	0.0	3.1	.
7	13.6	1337.0	21.4	5.1	1.1	0.0	3.1	52
8	14.5	1346.5	23.2	6.2	2.7	0.0	3.1	53
9	14.4	1355.9	21.0	7.7	4.9	5.6	8.7	60
10	13.2	1364.1	15.2	11.7	11.3	1.2	9.9	83
11	14.6	1373.7	18.9	11.3	8.9	0.0	9.9	87
12	14.0	1382.7	19.9	10.5	7.1	0.0	9.9	79
13	13.9	1391.6	20.9	7.8	5.3	0.0	9.9	67
14	12.8	1399.4	19.9	6.9	3.7	0.0	9.9	59
15	11.1	1405.5	15.9	5.6	0.8	0.0	9.9	66
16	12.0	1412.5	15.3	7.8	2.2	0.0	9.9	61
17	13.3	1420.8	15.4	11.5	10.6	0.0	9.9	65
18	13.6	1429.4	15.5	11.6	10.4	5.3	15.2	86
19	14.5	1438.9	15.9	13.4	12.9	1.6	16.8	95
20	13.4	1447.3	15.4	13.2	13.1	0.7	17.5	89
21	11.6	1453.9	15.8	8.6	5.6	0.0	17.5	72
22	9.8	1458.7	15.0	4.9	0.9	0.9	18.4	65
23	8.5	1462.2	12.2	6.3	5.0	2.0	20.4	84
24	5.6	1462.8	8.6	3.8	1.8	0.0	20.4	65
25	2.6	1462.8	7.3	-0.4	-2.0	0.0	20.4	54
26	2.8	1462.8	7.6	-1.3	-4.2	2.1	22.5	67
27	6.1	1463.9	10.0	3.8	1.0	0.0	22.5	82
28	6.4	1465.3	10.5	2.2	-2.7	0.0	22.5	71
29	5.8	1466.1	7.9	5.5	5.1	0.3	22.8	71
30	2.9	1466.1	6.0	-1.8	-5.7	0.0	22.8	56
Month	10.8					22.8		
Normal								
1981-2010	*9.9*					*58.0*		

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