Project plan 2014:

Field assessment of impacts of neonicotinoids on bees

(English version 22 June 2014)

Background

The Swedish Board of Agriculture conducted a literature study in 2009 on economic consequences and possible interventions for colony losses in honey bees (Pedersen 2009). The investigation concluded that pesticides pose a risk to the health of honey bees. This together with e.g. Whitehorn et al. (2012) and Henry et al. (2012) have drawn attention to the need for new studies. Neonicotinoids have been used in the cultivation of spring and winter oilseed rape in Sweden, both as seed dressing (clothianidin, imidacloprid and thiamethoxam) and as direct spray (acetamiprid and thiacloprid). Seeds which were dressed with the neonicotinoids clothianidin were imported from e.g. Germany prior to the 2 year EU moratorium for clothianidin, imidacloprid and thiamethoxam in bee attractive crops (EU 2013). The effects of neonicotinoids on wild and managed bees under field conditions are poorly known. The existing Canadian field study evaluating the influence of clothianidin seed dressing in canola and effects on honey bees reported no difference in brood development, worker longevity, bee mortality, colony weight gain or honey yield between colonies in treated fields compared to untreated controls (Cutler & Scott-Dupree 2007). Clothianidin residues were found in both pollen and nectar from the colonies in treated fields, but in concentrations below the no observable effects concentration. The fields used in the study were however only 1 ha, which is 7 times smaller than the average spring oilseed rape field in Skåne during the years 2007-2011 (7.0 ± 7.3 ha (mean ± SD); data from the Integrated Administration and Control System). Another weakness of the study is the distance between treated and control fields, which could be down to 295 m (Cutler & Scott-Dupree 2007). The foraging range of honey bees is much larger than this (Steffan-Dewenter & Kuhn 2003, Greenleaf et al. 2007). Clothianidin residues were detected from a few of the nectar samples from colonies in control fields, indicating that bees from control fields foraged in treated fields at some sites (Cutler & Scott-Dupree 2007). Future studies should be designed to include large experimental fields, distant from other sources of clothianidin and with enough independent replications.

Aim

The aim of this project is to investigate the impact of clothianidin seed dressing in spring sown oilseed rape on honey bees, bumble bees and solitary bees under field conditions in Sweden.

Study system

The study system 2013 consisted of 16 fields with spring sown oilseed rape of the variety Majong (Rundlöf et al. 2013). We will as far as possible use the same study system as in 2013, but to switch the treatments, i.e. farms with clothianidin treated fields in 2013 will in 2014 be untreated control fields. This would reduce the relationship between treatment and the specific sites. The fields will not be the same as in 2013, due to crop rotation and to avoid cross-contamination via the soil between years.

Ten of the 16 farmers will grow spring sown oilseed rape also in 2014 and these will be included in our study (figure 1). An additional spring oilseed rape field, to form five field pairs used for the work on bumble bees and solitary bees. The fields will be inspected by Maj Rundlöf and others in the second half of May. At the same time, sites for placing honey bee hives, bumble bee houses and solitary bee poles will be located, noted with geographical coordinates (decimal degrees/lat long) and marked on maps.

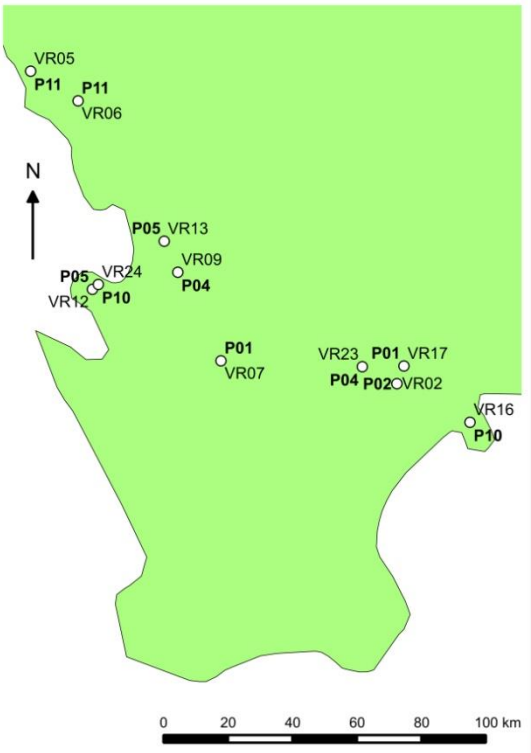

Figure 1. Location of selected spring oilseed rape fields included in the study (VR02-VR24) and matched into pairs (P01-P11) based on land use in the surrounding land use and geographical proximity.

Honey bees:

We will use the same honey bee (*Apis mellifera*) colonies as in 2013, but only colonies with one year old queens in 2013. The bees will be placed at the same treatment as in 2013, i.e. bees at control fields in 2013 will also be at control fields in 2014. Of the colonies used in 2013, 55 survived the winter satisfactory and had a one year old queen. These colonies will be equalized following the

same method as in 2013, but not mixing bees between treatments. This is done by Bengt Andréasson and Johan Bergstrand. The splits are created with 2 full honey combs (with bees), 2 combs with mainly sealed brood (with bees), 1 queen originating from the same colony as the split was taken from, bees from 2 combs shaken into the split, 1 drawn out empty comb and 5 combs with wax foundation. The comb size is full Langstroth. The 2 full honey combs were placed on one side, thereafter the drawn out empty comb and the 2 combs with sealed brood and finally the 5 combs with wax foundation. Colonies will be moved from the overwintering site to an organically managed winter oilseed rape field (figure 2) and from there to our ten spring oilseed rape fields at the onset of flowering.

The colonies were reduced and equalized a second time (8 June 2014), because they grew very large and some swarmed at the organically managed oilseed rape field. Each split now included 1 full honey comb (with bees), 3 combs with mainly sealed brood (with bees), 1 queen originating from the same colony as the split was taken from and 6 combs with wax foundation.

After the second equalization and the first colony strength assessment at the organically managed oilseed rape field, we have 43 colonies left. Four colonies will thus be placed at each of the ten experimental spring oilseed rape field, managed by the same farmers as last year. Estimated placement period is from the second week in June to the end of June, depending on the phenology of the oilseed rape. Estimated removal period is from mid to end of July. Colonies will be transported to and from the fields by Johan Bergstrand, after notification.

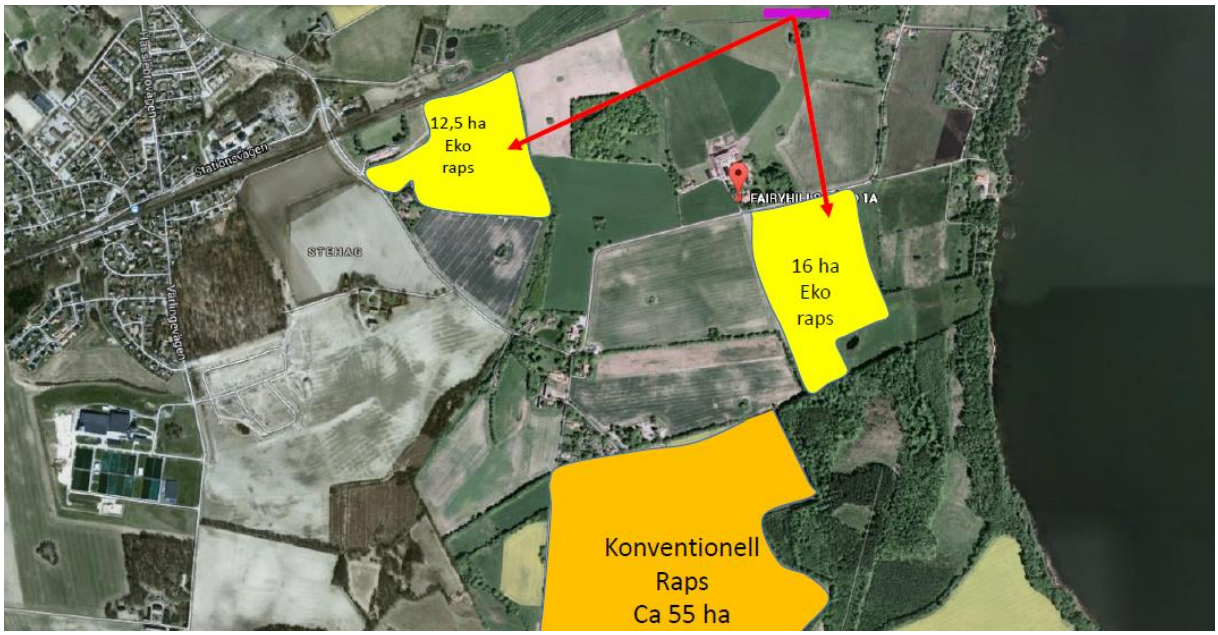

Figure 2. Location of honey bee colonies at the organically managed oilseed rape fields at Stehag (RT90 coordinates: 6181553, 1357267), before placement at the experimental fields.

Colony strength will be estimated using the same method as in 2013, the Liebefeld method (Imdorf et al. 1987, Delaplane et a. 2013), together with rating of sac and lime brood. The number of estimation occasions will be reduced to three, but will match three of the occasions in 2013. The first estimation will be done at the organically managed winter oilseed rape fields, just before the first colonies are transported to the experimental fields (matching the first occasions in 2013). The second

estimation of colony strength will be done at the spring oilseed rape fields at peak flowering, corresponding to BBCH 65-67 (Meier 2001) (matching the third occasion in 2013). The third and last estimation will be done at a common (overwintering) location, before the colonies are prepared for hibernation (matching the last occasion in 2013). Colonies are weighted at the first and third occasion to estimate honey production. The honey bee colonies are allowed to swarm and no actions are taken to prevent it, to follow the same procedure as last year. During the experiments, notes are, however, taken on swarming events and queen cells (noted in four classes: 1) egg, 2) open with larvae, 3) covered and 4) swarmed, and how many queen cells there are) in the comment field on the honey bee field protocol. Tomas Carling, assisted by Alanna Main, will do the colony strength estimation and weighting.

Samples of bees, pollen and nectar are collected for analyses of diseases, parasites and neonicotinoid residues. At the first and third survey occasions, at least 100 flight bees are collected from each colony. The bee samples are stored in paper boxes, frozen and sent to Ingemar Fries for analysis. After placement at the experimental fields, pollen traps are mounted on three hives at each site, but left open (i.e. not activated). The pollen traps are activated at the second survey occasion and left activated until at least 50 ml of pollen is collected. The pollen is stored frozen in 50 ml Falcon tubes until analysis and identification of plant species. At the second survey occasion, we will also collect 50 flight bees at the entrances of the nest boxes, 5 bees with pollen flying in the experimental fields (for pollen samples) and 5 bees without pollen flying in the experimental field (for dissection of the honey sacs, bee remains are stored in a separate tube). Number of bee and honey sacs is adjusted based on the volume of nectar. Total number of collected bees is noted on the prepared protocol. All samples are stored in individual plastic tubes and frozen. Samples are collected by Tomas Caring and Alanna Main.

Time line for honey bees (for details see the excel file):

Time	**Task**
9-11/6	Colony strength 1 + weight 1 + bee sample 1.
16-25/6	Honey bee colonies transported to oilseed rape fields.
23/6-11/7	Colony strength 2 + samples of pollen/nectar/bees + pollen trap sample.
14-22/7	Honey bee colonies transported home.
24-31/7	Colony strength 3 + weight 2 + bee sample 2.

Bumble bees:

We will use 66 commercially reared bumble bee (*Bombus terrestris*) colonies (Natupol N, Koppert Biological Systems) from Lindesro in Helsingborg. The colonies are of the same age as last year, approximately ten weeks old, with one queen and approximately 50 workers. The exposure time during 2013 was on average 31 days, ranging between 23 and 38 days. We will try to block the escape of queen bees from the nest boxes, to prolong the time we can follow the colonies under natural conditions. Six bumble bee colonies will be placed at all eleven experimental field, at the

onset of oilseed rape flowering. Colonies are placed three and three in two separate houses. We will focus our efforts on collecting foraging data using the RFID technique, with (at least) two rounds of data collection per field pair, one during oilseed rape flowering and one just after oilseed rape flowering. Colonies will also be weighted before placement at the fields, during the RFID data collection occasions and at the termination of the colonies. Julius Jonasson, assisted by Christopher Du Rietz, will work with the bumble bees and the RFID technique in his master thesis project, supervised by Maj Rundlöf and Georg Andersson.

Pollen collection frequency and amount will be registered by observing and catching bee returning to our hives. Pollen samples will be collected from individual bumble bees and stored in individual plastic tubes, for later analysis of plant origin and possibly also neonicotinoid/clothianidin content. We will also monitor bumble bee foraging behaviour in the oilseed rape fields. This will be done by Shuqi Chen, as part of her master thesis project, supervised by Maj Rundlöf and Sandra Lindström.

Terminating of the colonies will be done by freezing. After termination, the bumble bee colonies will be examined using the same protocol as in 2013.

Time line for bumble bees (for details see the excel file):

Time	**Task**
16-25/6	Bumble bee colonies weighted (1) and placed at the oilseed rape fields.
23/6-6/7	Colony weight 2 + RFID 1 (during flowering) + pollen samples 1 + bee behaviour 1.
7/7-21/7	Colony weight 3 + RFID 2 (after flowering) + pollen samples 2.
22-30/7	Alt. 1: bumble bee colonies terminated and transported home (36 days at field).
28/7-5/8	Alt. 2: bumble bee colonies terminated and transported home (42 days at field).
?	Colony examination.

Solitary bees:

We will use empty trap nests and solitary bees collected in Scania within the STEP project (stored in a fridge at 2-5°C in spring before the oilseed rape flowering). Five poles are placed at each experimental field and as many trap nests, both paper tubes and reed, as possible are mounted on the poles (see Albin As project plan). The opening of trap nests should be facing southwards and located so that vegetation shelter the northern side. The solitary bees collected in the STEP project are sorted by species to genus level, depending on what is possible. *Osmia bicornis* bees will further be sorted by sex. Bee groups and sexes are distributed evenly over trap nests at the ten experimental fields forming five field pairs. Poles, trap nests and bees are placed at the fields in mid to end of May, to allow for a control period at all sites before the onset of spring oilseed rape flowering. At the onset of flowering, tubes with nesting activity are marked with a permanent pen to separate the before and during flowering periods. Bee activity at the trap nests is observed by filming three traps at a time. Traps at a field pair are filmed during the same day. Traps nests are collected at the end of July and stored outside under shelter until they can be sorted through. Pollen samples are taken to

identify the proportion of rape pollen. Albin Andersson will work with the solitary bees in his master thesis project, supervised by Maj Rundlöf and Lina Herbertsson.

Persons involved and contact information

Albin Andersson (AA), master student working with solitary bees, 070-8883644, albinivik@hotmail.com

Bengt Andréasson (BA), professional beekeeper in Skåne, responsible for producing honey bee colonies, 0706-677397 (text only), bengt.andreasson@veberod.com

Georg Andersson (GA), researcher, Lund University, supervising Julius in his bumble bee work, 070-4478504, georg.andersson@biol.lu.se

Johan Bergstrand (JB), beekeeper in Skåne, responsible for maintaining and transporting honey bee colonies, professional 070-5109550, johan@bergstrand.net

Riccardo Bommarco (RB), researcher, responsible for the honey bee part, Swedish University of Agricultural Sciences, Uppsala, 073-0485878, riccardo.bommarco@slu.se

Tomas Carling (TC), beekeeper and bee inspector for the County Board, responsible for colony strength estimating and collection of honey bee related samples, 070-7306511, tomas.carling@hotmail.com

Shuqi Chen (SC), master student working with bumble bees, 076-7869671, shuqi.chen.716@student.lu.se

Christopher Du Rietz (CDR), assisting Julius in the work with bumble bees, biologist, 073-5806563, christopherr@luna.lu.se

Ingemar Fries (IF), researcher and honey bee guru, responsible for the honey bee part, Swedish University of Agricultural Sciences, Uppsala, 070-2253779, 018-672073, ingemar.fries@slu.se

Lina Herbertsson (LH), PhD-student, Lund University, supervising Albin A in his solitary bee work, 070-2964255, lina.herbertsson@cec.lu.se

Julius Jonasson (JJ), master student working with bumble bees, 0703-773381, gna10jjo@student.lu.se

Albin Gunnarson (AG), farmer contact and growing advice to the farmers, Svensk Raps AB, 070-5686027, albin@svenskraps.se

Björn Klatt (BK), researcher Lund University, 073-9317689, bjorn.klatt@cec.lu.se

Preben Kristiansen (PK), strategy for bee health, bee health consultant, 070-8901732, preben.kristiansen@biodlarna.se

Sandra Lindström (SL), Hushållningssällskapet Kristianstad/SLU/Lund University, supervising Shuqi in her bumble bee work, 070-8945358, sandra.lindstrom@hushallningssallskapet.se

Alanna Main (AM), assisting Tomas in the work on honey bees, biologist, 073-9648576, alanna.main@uqconnect.edu.au

Thorsten Rahbek-Pedersen (TRP), project leader for the honey bee and bumble bee parts, Swedish Board of Agriculture, Alnarp/Jönköping, 070-6943779, thorsten.pedersen@jordbruksverket.se

Maj Rundlöf (MR), researcher, project coordinator, Lund University, 070-9298524, maj.rundlof@biol.lu.se

Henrik Smith (HS), researcher, responsible for the bumble bee part, Lund University, 070-9782056, henrik.smith@biol.lu.se

References

Cutler, G.C. & Scott-Dupree, C.D. (2007) Exposure to clothianidin seed-treated canola has no long-term impact on honey bees. Journal of Economic Entomology 100: 765-772.

Delaplane, K.S., van der Steen, J. & Guzman-Novoa, E. (2013) Standard methods for estimating strength parameters of *Apis mellifera* colonies. Journal of Apicultural Research 52: Webbadress: http://dx.doi.org/10.3896/IBRA.1.52.1.03

EU (2013) Regulation (EU) No 485/2013. Official Journal of the European Union 139: 12-26. Webbadress: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:139:0012:0026:EN:PDF

Gill, R.J., Ramos-Rodriguez, O. & Raine, N.E. (2012) Combined pesticide exposure severely affects individual- and colony-level traits in bees. Nature 491: 105-109.

Greenleaf, S.S., Williams, N.M., Winfree, R. & Kremen, C. (2007) Bee foraging ranges and their relationship to body size. Oecologia 153: 589-596.

Henry, M., Beguin, M., Requier, F., Rollin, O., Odoux, J.-F., Aupinel, P., Aptel, J., Tchamitchian, S. & Decourtye, A. (2012) A common pesticide decreases foraging success and survival in honey bees. Science 336: 348-350.

Imdorf A., Bühlmann G., Gerig L., Kilchenmann V. & Wille H. (1987) Überprüfung der Schätzmethode zur Ermittlung der Brutfläche und der Anzahl Arbeiterinnen in freifliegenden Bienenvölkern, Apidologie 18: 137-146.

Meier, U. (ed.) (2001) Growth stages of mono-and dicotyledonous plants. BBCH Monograph. 2nd edition. Federal Biological Research Centre for Agriculture and Forestry, Berlin & Brunswick. Webbadress: http://www.bba.de/veroeff/bbch/bbcheng.pdf

Pedersen, T.R. (ed) (2009) Massdöd av bin – samhällsekonomiska konsekvenser och möjliga åtgärder. Swedish Board of Agriculture, Jönköping.

Steffan-Dewenter, I. & Kuhn, A. (2003) Honeybee foraging in differentially structured landscapes. Proceedings of the Royal Society of London. Series B: Biological Sciences 270: 569-575.

Whitehorn, P.R., O'Connor, S., Wackers, F.L. & Goulson, D. (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science 336: 351-352.