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Trifolium pratense L.

Camilla J. Brødsgaard & Henrik Hansen

Pollination of red clover in Denmark

Bestøvning af rødkløver i Danmark

Ministry of Food, Agriculture and Fisheries

Danish Institute of Agricultural Sciences

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Summary

In Denmark, in 1998 and 1999, a study of red clover pollination was conducted. The aim of the project was to study the bees visiting Danish red clover fields and the pollination of red clover by *Apis mellifera* and *Bombus terrestris* in cage plots.

In the first year, the following activities were carried out: The distribution of wild bees and honeybees in red clover fields with diploid and tetraploid cultivars was compared. Furthermore, the distribution of wild bees and honeybees in conventional, large scale and organic smaller scale red clover fields was compared as well as the corolla length of different current Danish cultivars of red clover. In addition, examination of the *B. terrestris* hole biting of the corolla was carried out. In both years, the tongue lengths of selected Danish honeybees were examined. In the second year, comparative caged plot studies of *B. terrestris* and honeybees as pollinators of red clover were carried out, and pollen loads from honeybees adjacent to red clover fields were collected.

Our results suggest that it does not seem to be a problem for neither honeybees nor bumblebees to reach the middle of a large diploid red clover field (16-17 ha). The results indicate that bee pollination, independent of bee species (*A. mellifera* or *B. terrestris*), clearly increases the yield, and that honeybees are important as pollinators of red clover. But, as the natural *B. terrestris* population size is unpredictable and the cost of *B. terrestris* hives high it is not recommended to rely only on *B. terrestris* as pollinator. In fact, the results indicate that it is more important now than ever to place honeybee colonies near crops that need bee pollination and to ensure nesting possibilities for bumblebees.

The current Danish recommendation for red clover pollination using honeybee colonies (4-5 colonies/ha) is supported. In hot, dry summers honeybee colonies placed up to 1 km from a red clover field are able to provide satisfactory pollination of red clover and, even two strong colonies/ha could provide satisfactory pollination under such conditions.

The caged plots study showed that though there was a significant difference in the tongue lengths of the three honeybee groups this could not be correlated to differences in seed yield. Furthermore, though *B. terrestris* had significantly longer tongues than honeybees, and this bumblebee is known to rob flowers, there were no differences in yield between honeybee pollinated plots, *B. terrestris* pollinated plots with or without sugar sirup feeding, or uncaged field whatsoever.

Finally we found a positive correlation between corolla length and the degree of corolla biting of red clover flowers, but no significant difference between the corolla length of the diploid and tetraploid cultivar.

Resumé

Resultaterne, som præsenteres i denne rapport 'Pollination of red clover in Denmark' er en del af projektet "Biernes rolle i agerlandskabet", der indgik i forskningsprogrammet "Arealanvendelse - jordbrugeren som landskabsforvalter". Forskningsprogrammet blev finansieret af Miljøministeriet og Ministeriet for Fødevarer, Landbrug og Fiskeri.

I Danmark er det hovedsagelig diploid rødkløver, som dyrkes til frøproduktion. I 2000 var det totale dyrkningsareal 912 ha. På grund af et større foderudbytte af tetraploide sorter er der hos frøavlerne en øget interesse for at producere frø fra disse sorter, som på dette tidspunkt dyrkedes på ca. 40 ha.

Frøavlere med både diploid og tetraploid rødkløver har rapporteret om dårlige frøudbytter. I den forbindelse er det diskuteret, om de danske honningbier overhovedet kan bestøve de sorter, som dyrkes i øjeblikket. Det er også nævnt, at der mangler viden om forekomsten af humlebier, som er egnet til bestøvning, og at den intensive dyrkning måske ikke giver redemuligheder for humlebierne. Der er endvidere gisnet om, at markerne er for store til, at humle- og honningbier kan besøge midten af dem.

Nærværende studier af rødkløverbestøvning blev udført i 1998 og 1999. Formålet med projektet var at undersøge, hvilke bier som besøger forskellige rødkløversorter, og bestøvning af rødkløver i burforsøg med honningbier (*Apis mellifera)* og jordhumler (*Bombus terrestris*).

Det første år indeholdt følgende aktiviteter: Forekomsten af vilde bier og honningbier i rødkløvermarker med diploid og tetraploid rødkløver, en sammenligning af forekomsten af vilde bier og honningbier i konventionelt, i stordrifts og i økologisk dyrket mark, en sammenligning af kronrørslængden i forskellige danske rødkløversorter samt en undersøgelse af jordhumlens gennembidning af kronrøret. I begge år blev tungelængden af udvalgte danske honningbier undersøgt. I det andet år blev der foretaget sammenlignende undersøgelser i bure af jordhumlers og honningbiers bestøvning af rødkløver. Herudover blev pollenindsamlingen hos bifamilier, som var placeret nær rødkløvermarker, undersøgt.

Følgende biarter blev observeret i rødkløvermarkerne: Agerhumle (*B. agrorum*), *Bombus soroeënsis*, havehumle (*B. hortorum*), honningbi (*A. mellifera*), hushumle (*B. hypnorum*), jordhumle (*B. terrestris*), kløverhumle (*B. distinguendus*), moshumle, (*B. muscorum*) og stenhumle (*B. lapidarius*). Der blev ikke observeret solitære bier.

Vores resultater viser, at det ikke er noget problem for hverken honningbier eller humlebier at nå midten af en stor diploid rødkløvermark (16-17 ha). Resultaterne viser også, at bibestøvning, uanset om det drejer sig om honningbier eller jordhumler klart øger frøudbyttet, og at honningbier er vigtige bestøvere af rødkløver. Det kan ikke anbefales kun at satse på jordhumler til bestøvningen, da den naturlige populations størrelse er uforudsigelig, og da udgifterne til et tilstrækkeligt antal små stader med jordhumler er meget store. Vores resultater tyder på, at det er mere vigtigt end tidligere at placere honningbifamilier nær afgrøder, som kræver bibestøvning, samt at sikre gode redemuligheder for humlebier.

På nuværende tidspunkt anbefales det at placere 4-5 bifamilier pr. ha for at sikre en god bestøvning af rødkløver. Vi kan støtte denne anbefaling. I varme og tørre somre kan bifami-

lier, som er placeret i op til 1 km fra rødkløvermarken, sikre en tilfredsstillende bestøvning, og endog 2 stærke bifamilier/ha kan under disse forhold sikre bestøvningen.

Burforsøgene viser, at selv om der var en signifikant forskel i tungelængden hos tre forskellige grupper af honningbier, var der ikke en signifikant forskel i frøudbytterne. Forsøgene viser også, at jordhumlen havde en signifikant længere tunge end honningbien. På trods af dette, og på trods af at jordhumlen kan foretage negativt besøg i rødkløverblomsterne, var der ingen forskel i udbytterne fra honningbi-bestøvede parceller, jordhumle-bestøvede parceller (jordhumle-familier med eller uden sukkerfodring) og i udækkede parceller.

Herudover fandt vi en positiv sammenhæng mellem kronrørslængden og graden af gennembidning af kronrøret. Men vi fandt ingen signifikant forskel imellem kronrørslængden hos den diploide og tetraploide sort.

Frøproduktionen hos den tetraploide sort "Kvarta" har ikke været tilfredsstillende i Danmark. Selv når honningbifamilier blev placeret ved marken, var udbyttet meget lavere end hos den diploide sort. Årsagen til dette fænomen er ikke klarlagt og forbliver et åbent spørgsmål.

Preface

The results presented in this report 'Pollination of red clover in Denmark' are part of the project 'The role of bees in the agricultural landscape' which again is a part of a larger project 'Novel strategies in weed control providing for environment, economy, and landscape resources'. The project is a cooperation between four departments of the Danish Institute of Agricultural Sciences (DIAS), the Danish Forest and Landscape Research Institute, and the University of Copenhagen. The project is included in the research programme 'Land use - farmers as landscape managers (1997-2001)', financed by the Ministries of Environment & Energy and of Food, Agriculture and Fisheries.

The aim of the project 'Pollination of red clover in Denmark' presented in this report was to study the bees visiting red clover fields of different cultivars grown under different conditions and to study the pollination of red clover by *Apis mellifera* and *Bombus terrestris* in cage plots.

Figure 1. *Apis mellifera* in red clover. Honningbi i rødkløver.

Introduction

The cultivated clover probably originate from Southwest Asia (Svendsen 1994). Red clover (*Trifolium pratense*) is self-sterile and therefore needs insect pollination to set seed (Darwin 1889 from McGregor 1976). Darwin thought that the bumblebee (*Bombus sp.*) was the only insect which could perform the necessary cross-pollination. Pollination of red clover has been studied world-wide during the past century and has been reviewed by Free (1993).

The New Zealand story - pollination or robbing?

In the last part of the 19th century, red clover was cultivated intensively in New Zealand (N.Z.) as fodder crop. Cultivation of seeds was not possible which was believed to be because bumblebees were not present on the islands. European honeybees (*Apis mellifera*) had been introduced but they were at that time not believed to be able to pollinate red clover. Therefore, in 1885 bumblebees were imported to N.Z. from England. During the next ten years, the bumblebees spread all over N.Z. to such an extend that the honeybee-keepers were worried that the bumblebees might oust the honeybees (Stapel 1933).

Along with the spread of bumblebees a satisfactory pollination of red clover was achieved. In the following years, studies were made on the bumblebee population in N.Z. and it was revealed that it mainly consisted of *Bombus terrestris* and only to a lesser extend of *B. hortorum.* Moreover, these studies showed that *B. terrestris* because of its relative short tongue (cf. figure 2) had difficulties in collecting nectar from the long corolla of the red clover. *B. terrestris* therefore bit a hole in the corolla (cf. figure 3) to get access to the nectar (termed primary robbers by Free 1993). In that way, the visit to the flower was negative regarding to pollination (Stapel 1933). Furthermore, though honeybees are not able to make holes in the corolla some of them used the existing holes made by *B. terrestris* resulting also in 'negative visits' (termed secondary robbers by Free 1993)(Pedersen & Sørensen 1935).

All of this lead to believe that *B. terrestris* was a pest. Therefore, in 1906 the decision was made to import more bumblebee queens of *B. hortorum* and *B. lapidarius* to N.Z. (Stapel 1933). These species are known for their longer tongues (cf. figure 2).

Later it was shown that the mulitilation of the corolla does not prevent the flower from being pollinated and producing seed (Hansen 1934) and pierced flowers were visited to the same extent as non-pierced flowers (Skovgaard 1936). However, results from field tests with small caged plots indicated that short-tongued bumblebees are less efficient in pollinating than long-tongued bumblebees or honeybees (Palmer-Jones *et al*. 1966). On the other hand, in Denmark, Pedersen (1945) found that *B. terrestris* was the most important bumblebee species visiting red clover, which was confirmed from N.Z. by Forster & Hadfield (1958) and Hawkins (1961).

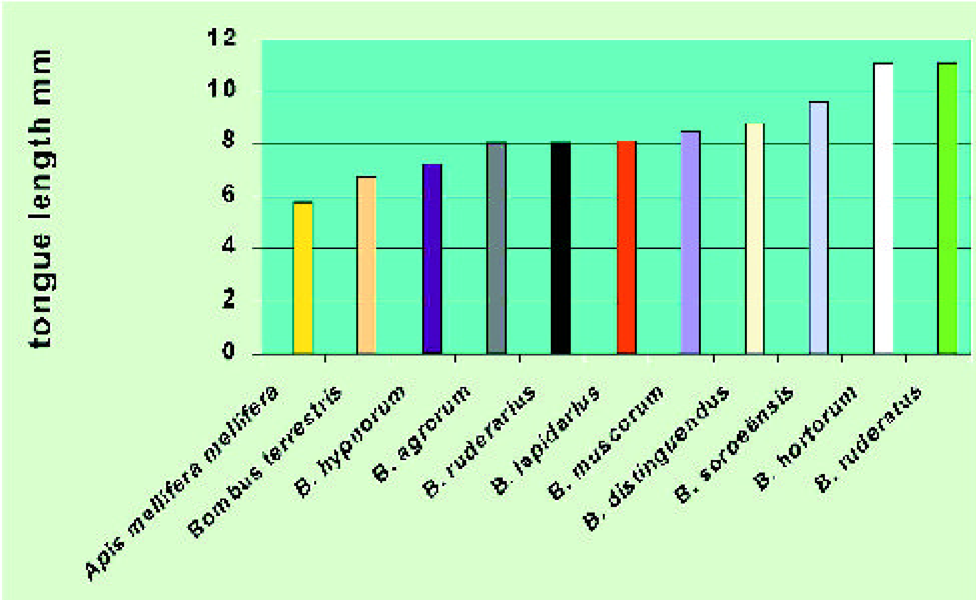


Figure 2. Tongue lengths in mm of Danish *Apis mellifera* and *Bombus* species (Hammer & Holm 1970, Henriksen 1957, Stapel 1936). Danske honning- og humlebiers tungelængder. Agerhumle (*B. agrorum*), havehumle (*B. hortorum*), honningbi (*A. mellifera*), hushumle (*B. hypnorum*), jordhumle (*B. terrestris*), kløverhumle (*B. distinguendus*), moshumle, (*B. muscorum*), stenhumle (*B. lapidarius*).

Figure 3. Red clover flower with a hole in the corolla bitten by *B. terrestris* (Stapel 1936). Rødkløverblomst med hul på kornrøret efter bid af jordhumler.

The Danish story - pollination by honeybees

In Denmark in 1750, red clover was introduced as fodder plant (Svendsen 1994) and approximately 70 years ago, the red clover in rotation constituted 1/4 of the total cultivated area i.e. 700.000 ha (cf. figure 4). At that time, the Danish red clover cultivars originated from Danish Institute of Agricultural Sciences= (DIAS) experimental breeding station in Tystofte. These cultivars were very productive but apparently seed breeding was a problem in Denmark. Thus, the seed breeding was conducted in Czechoslovakia and Poland while red clover seed growing was carried out in Spain and the seed for fodder production imported from Spain to Denmark (Stapel, pers. comm.). In 1933, Christian Stapel, scientist at DIAS, was making studies in Czechoslovakia. These studies and his subsequent research became extremely important to the Danish red clover growing and started the apicultural research in Denmark at DIAS.

In Czechoslovakia, Stapel realized that the only difference between the growing of red clover in Denmark and Czechoslovakia was that in Czechoslovakia honeybee colonies were placed in the red clover fields to ensure pollination. Subsequently in Denmark, Stapel a.o. studied the presence of bumble- and honeybee in red clover observation fields. He found that both the scolded *B. terrestris* and honeybees were important pollinators of these red clover fields. Thus, he found that up to 67% of the visiting *B. terrestris* was pollinators and that honeybees constituted 60-70% of the pollination effect in the fields (Stapel 1933). Later studies by Stapel (1935) showed that *A. mellifera ligustica* had tongue lengths 0.31 mm longer than the ordinary Danish bee at that time (*A. mellifera mellifera*) and thus were shown to be even better pollinators of red clover.

Tetraploid red clover

In the 1960s, the tetraploid cultivar was introduced into Denmark because of the better fodder yield. However problems with inadequate pollination were reported and in 1967, Dennis and Haas conducted comparative experiments with pollination of diploid and tetraploid red clover. Their studies showed that the tetraploid cultivar had a significantly longer corolla and a greater diameter than the diploid, and in the cold, wet summer of 1967 the number of bumblebees visting the experimental site was significantly larger in diploid field than in a tetraploid field. If honeybees were included in the counts there were no differences in the total number of visiting bees in the two types of red clover. In addition, it has been suggested that the lower seed yield in tetraploid cultivar may not be caused by poor pollination but could be due to general fertility problems (Wexelsen & Vestad 1954).

The deeper corolla of the tetraploid red clover lead to the assumption that honeybees were poor pollinators of this type of red clover but as honeybees are easier bred than bumblebees they are preferred as pollinators. Thus, in the 1980s, experiments with the growth regulatory substance Alar 85 that shorten the corolla of red clover were conducted (see Svendsen 1986). The Danish experiments confirmed that it was possible to reduce the length of the corolla, to increase the number of flowers, to reduce the number of negative visits, and to increase the seed yield (Svendsen 1986).

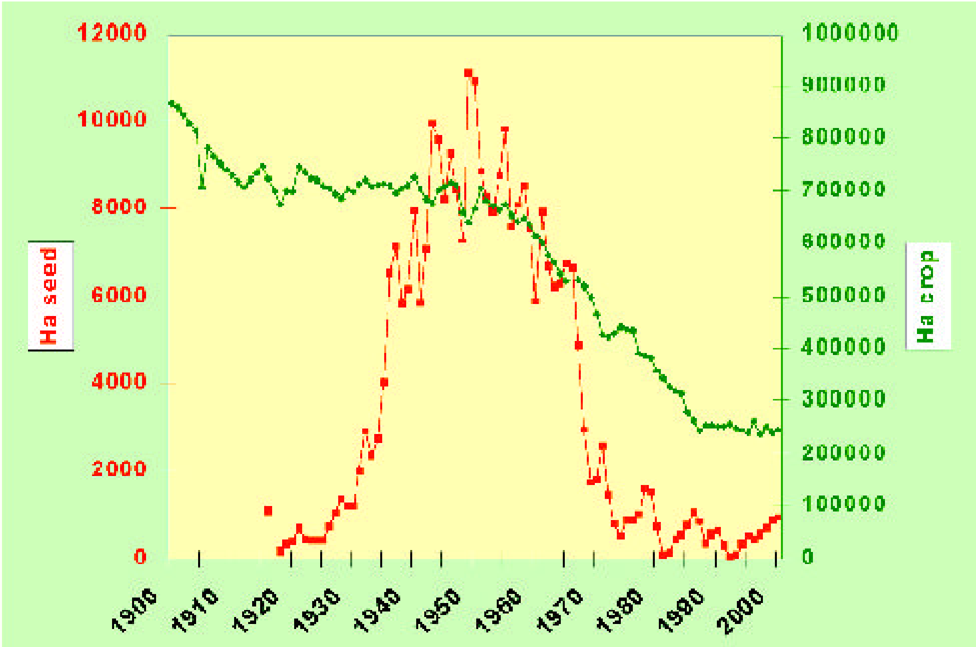


Figure 4. The red clover area in Denmark 1900-2000. *Red curve*: Seed in ha [1900-1945: Danmarks statistik (1965), 1946-1961: Tidsskrift for frøavl (1950, 1954, 1959 & 1964), 1962-1987: Landbrugsministeriets udvalg vedr. ind- og udførsel af frø (1972, 1979, 1987 & 1993), 1988-2000: Plantedirektoratets frøavlsstatistik (1998 & 2001)]. *Green curve*: Crop in rotation in ha [(1900-1965: Danmarks statistik (1965), 1967-1978: Olesen (1971, 1974, 1979),1979-1983: Bennetzen (1984), 1984-88: Skriver (1988, 1990), 1989-2000: Pedersen (1994, 2000)]. Rødkløver arealet i Danmark 1900-2000. Rød kurve: Kløverfrø i ha. Grøn kurve: Kløvergræs i omdrift.

The current Danish situation

In Denmark, the presently primary grown red clover cultivars for seed production are diploid with totally 912 ha in 2000 (figure 4). However, because of the better fodder yield of tetraploid cultivars, there is increasing interest among growers for the tetraploid cultivar, now grown on approximately 40 ha (A. Larsen, DLF-Trifolium, pers. comm.). In Denmark, Alar 25 is now only registered for ornamentals and the fertility/pollination problem seems now to be relevant for the tetraploid growers.

In 1998, when the first years activities were started, some growers both of tetraploid (DLF-Trifolium, pers. comm.) and diploid cultivars (B. Søgård, pers. comm.) reported lower yields than the national mean and previous years. The question then arose whether the Danish honeybees were not able to pollinate the current cultivars. The tongue lengths of the present Danish honeybees and the abundance of suitable bumblebees were not known.

In addition to the possible pollination problems caused by the flower morphology, it has been argued that some of the pollination problems might arise from intensive grown, large field without nesting possibilities for wild bees and too large fields for both the honey- and the bumblebees to visit the center of the fields.

The aim of the project

The aim of the project was to study the bees visiting Danish red clover fields and the pollination of red clover by *A. mellifera* and *B. terrestris* in cage plots.

Activities in the project

Comparison of the distribution of wild bees and honeybees in red clover fields with diploid and tetraploid cultivars.

Comparison of the distribution of wild bees and honeybees in conventional large scale and organic smaller scale red clover fields.

Examination of the tongue lengths of selected Danish honeybees.

Comparison of the corolla length of different current Danish cultivars of red clover.

Examination of the *B. terrestris* hole biting of the corolla.

Comparative caged plot studies of *B. terrestris* and honeybees as pollinators of red clover.

Collection of pollen loads from honeybees adjacent to red clover fields.

Comparative studies in large scale, organic and conventional red clover fields

Materials and Methods

Fields

In 1998, three farmers on Sealand hosted our experiments and made their ordinary red clover fields available for our studies: The large scale grown field with the diploid cultivar ‘Rajah’ had an area of 17.4 ha. The organic grown field was also the cultivar ‘Rajah’. This field had an area of 3 ha. The conventionally grown field with the tetraploid cultivar ‘Kvarta’ had an area of 4.3 ha. In the vicinity of these fields 18, 5, and 10 honey bee colonies, respectively, were placed by beekeepers.

Counting of bees

In the middle of June, five counting plots of 20 m × 1 m were defined in each field by placing four plastic sticks with rope winded around in each corner of the plots (see figure 5). The plots were distributed evenly diagonally across the fields. The plots were numbered 1-5, 1 and 5 being the plots closest to the borders of the field. The bees in the plots were counted and determined to species *in situ* according to Hammer & Holm (1970) seven times in the ‘Kvarta’ field and four times in the two ‘Rajah’ fields from the beginning of the bloom (late June to mid July) to desiccation or harvest of the field (mid August). The relative proportion of bees in each plot as well as the average number of bees in the five plots per field was calculated. Observations were carried out between 10.30 am and 4.00 p.m. on days without rain.

Figure 5. The counting plots were defined by placing four plastic sticks with rope winded around in each corner of the plots. The bees in the plots were counted and determined to species *in situ.* Tællefelterne var afmærket med plasticpinde i hvert hjørne og med en snor, som forbandt pindene. Bierne i tællefelterne blev artsbestemt på stedet.

Tongue length

The tongue lengths of honeybee workers from four colonies at the ‘Kvarta’ field, two at the ‘Rajah’ organic field and four colonies at the ’Rajah’ large scale field were measured by a Glossometer (Henriksen 1957) (see figure 6). This instrument indirectly measures the tongue length by the bees’ ability to reach the sugar food in the instrument by licking. The Glossometer was placed in the colonies for 24 hours. Four repetitions were made in each colony.

Corolla length

In total, the corolla lengths of 10 red clover flowers in full bloom from each of 30 plants were measured from each field. The plants were chosen randomly in each of the counting plots of the fields. The length of the corolla tube was measured from the floral receptacle to the point where the free edge of the standard merges with the corolla (Pedersen 1933) (see figure 7).

Biting of the corolla

The number of corollas that was bitten (see figure 3) was calculated by studying corollas in full blossom from 20 different plants from each field. The plants were chosen randomly in each of the counting plots of the fields. Totally, 2,129 corollas from the ‘Kvarta’ field, 2,124 corollas from the ‘Rajah’ organic field and 2,217 corollas from the ‘Rajah’ large scale field were studied and the biting percent calculated.

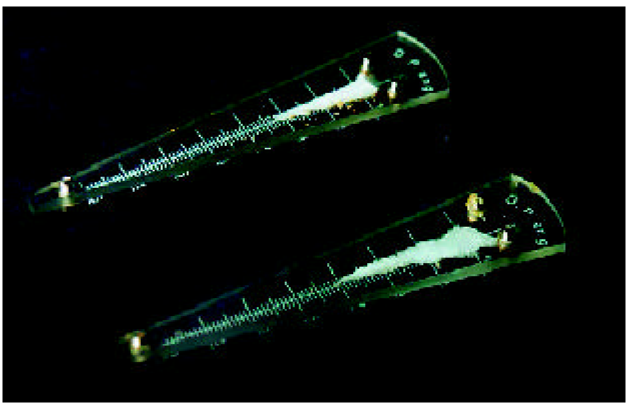


Figure 6. A Glossometer measured the tongue lengths of honeybee workers. Honningbi-arbejdernes tungelængde blev mål med et Glossometer.

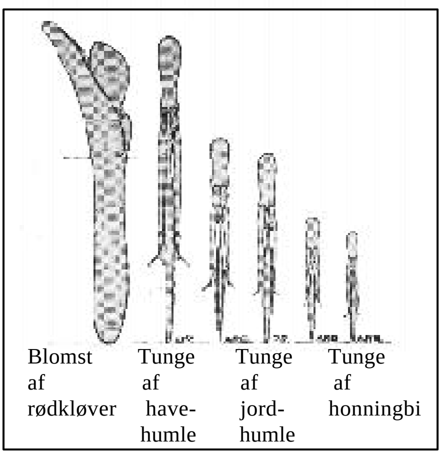


Figure 7. The length of the red clover flowers’ corolla and the length of the tongue of *B. hortorum* (= Havehumle), *B. terrestris* (= Jordhumle) and *A. mellifera* (= Honningbi). DR = Queen (dronning), ARB = worker (arbejder). Kronrørets længde hos rødkløver og tungelængde hos ovennævnte bier.

Red clover yield
After harvest, the experimental hosts informed us of the yield as a mean of their total red clover crop. Furthermore, the yield of the red clover field grown in large scale was computed for plots of 20 m × 20 m by means of GPS (Agro-Map).

Results
The honeybees and the bumblebees are evenly distributed in all three red clover fields (figure 8 and 9). However, there is a tendency towards more bumblebees (up to quite 30%) in section four and five in the organic grown field with the diploid cultivar and lesser bumblebees (approximately 10%) in section four and five in the large scale field grown with the diploid cultivar.

Figure 10 shows the average number of bees in five plots of the three red clover fields. In all three fields honeybees (figure 11) which constitute between 30 to 70% of the total bee population and *B. terrestris* (figure 12) which constitute between 20 to 50% of the total bee population are predominant. *B. lapidarius* (figure 13) constitute between 3 to 13% and *B. agrorum* (figure 14) between 2 and 5%. Each of the other bumlebees *B. hortorum* (figure 15), *B. muscorum* (figure 16) and *B. ruderarius* constitutes less than 2%. There is no significant difference in the total number of bees between the three fields (χ^2, P>0.05). No solitary bees were observed.

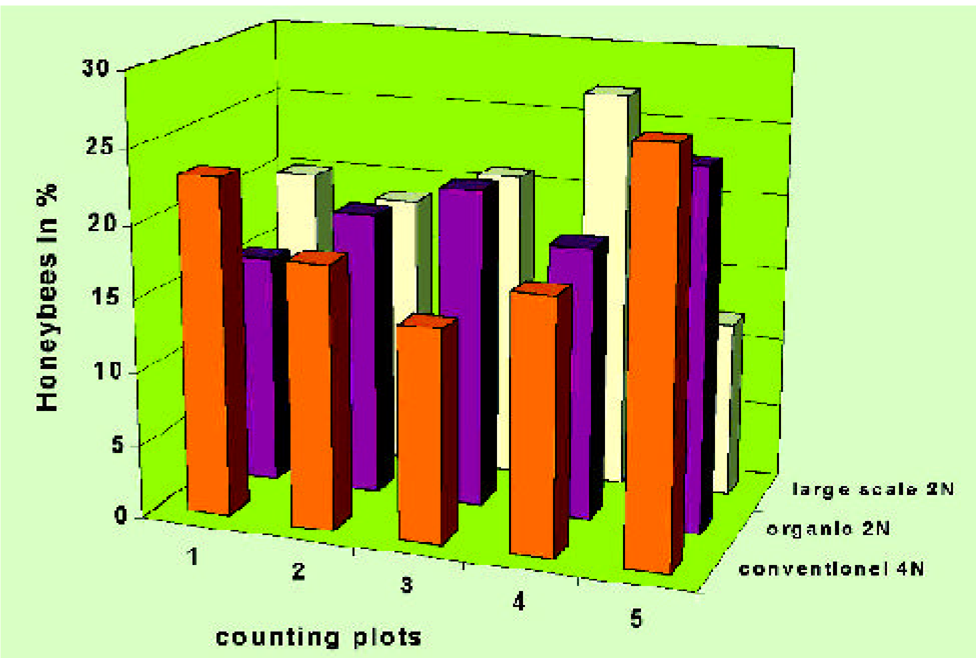


Figure 8. Distribution of honeybees in different plots of three red clover fields conventionally grown with a tetraploid cultivar, organic grown with a diploid cultivar, or large scale grown with a diploid cultivar. Fordeling af honningbier i forskellige tællefelter i tre rødkløvermarker med henholdsvis en konventionelt dyrket tetraploid sort, en økologisk dyrket diploid sort og en konventionel stordiftsform med en diploid sort.

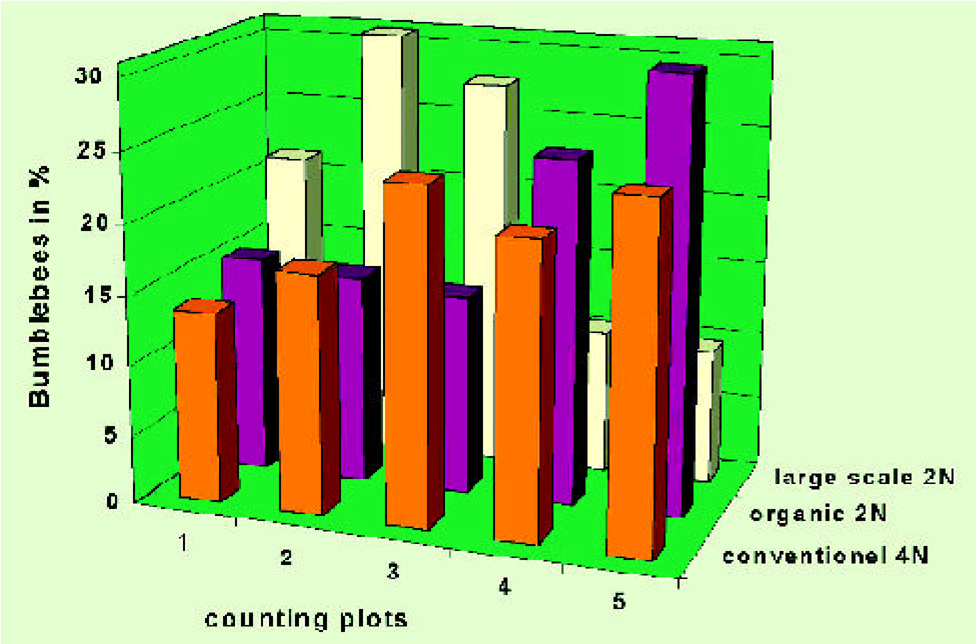


Figure 9. Distribution of bumblebees in different plots in the three red clover fields. Fordeling af humlebier i forskellige tællefelter i de tre rødkløvermarker.

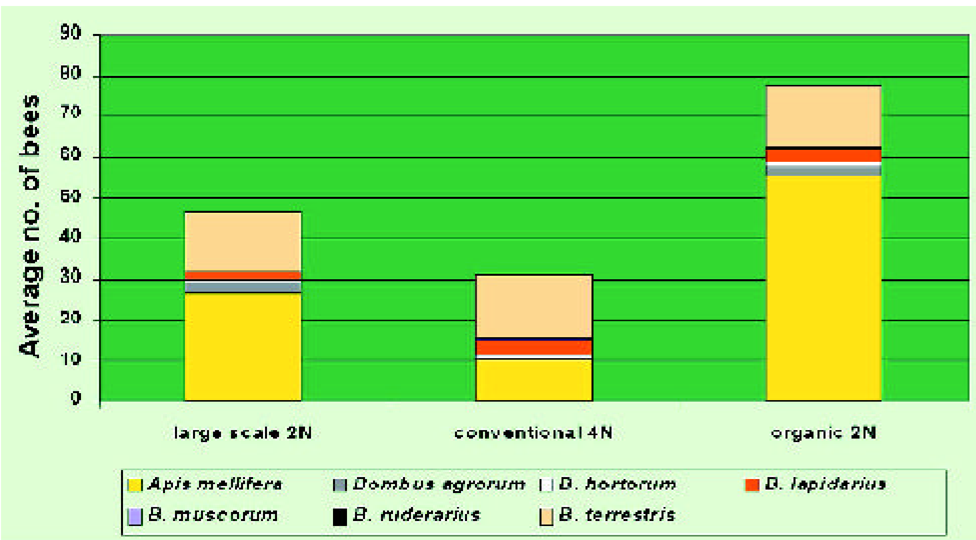


Figure 10. The average number of bees in five counting plots separated into species in the three red clover fields. Det gennemsnitlige antal bier fordelt på arter i de fem tællefelter i de tre rødkløvermarker. Agerhumle (*B. agrorum*), *B. soroeënsis*, havehumle (*B. hortorum*), honningbi (*A. mellifera*), hushumle (*B. hypnorum*), jordhumle (*B. terrestris*), moshumle, (*B. muscorum*), stenhumle (*B. lapidarius*).

Figure 11. ***A. mellifera*.** Honningbi.

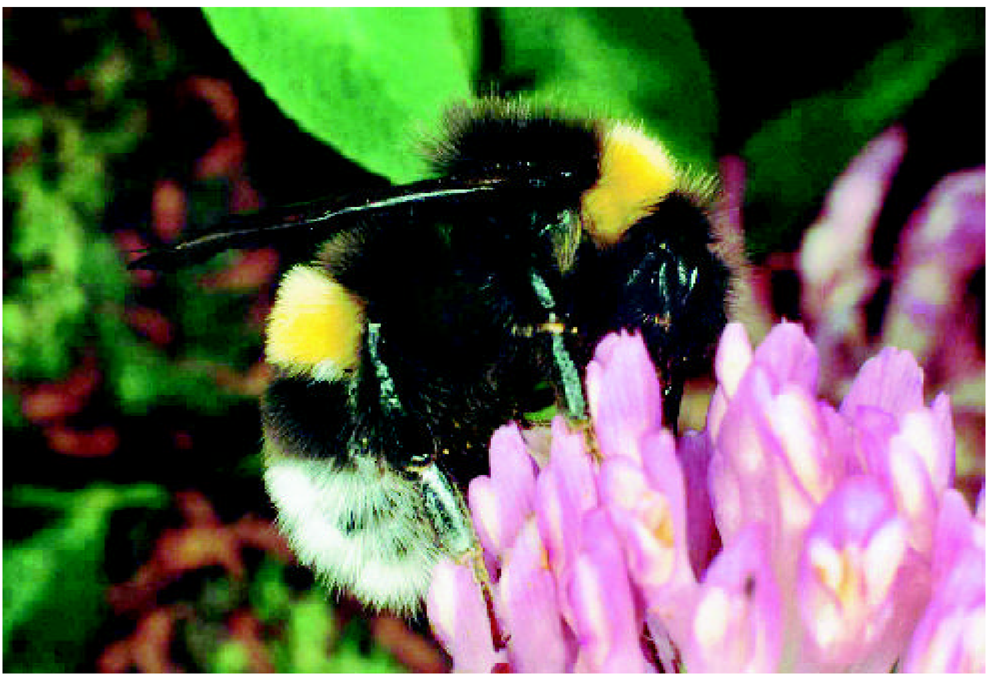


Figure 12. ***B. terrestris*.** Jordhumle.

Figure 13. ***B. lapidarius***. Stenhumle.

Figure 14. ***B. agrorum***. Agerhumle.

Figure 15. ***B. hortorum.***
Havehumle.

The tongue length of the honeybees vary from an average of 5.05 ± 0.69 mm in a Hawaiian hybrid colony placed nearby the organic field to 8.48 ± 0.26 mm in a Buckfast colony placed nearby the conventionally grown field (figure 17). There is a rather large variation in the tongue lengths of the Buckfast bees, between 5.48 ± 0.61 mm and 8.48 ± 0.26 mm. Thus, one of the Buckfast colonies placed in the large scale field has a significant shorter tongue than the others but it is not significantly shorter than the tongues of the Hawaiian hybrids in the organic field (5.05 ± 0.69 - 5.80 ± 0.67 mm). The tongue lengths of the *ligustica* bees in the two colonies in the conventionally grown field are significantly shorter than the Buckfast colony with bees with the longest tongues, and significantly longer from the Buckfast and Hawaiian colonies with the shortest tongues (Kruskal-Wallace, $P<0.05$).

Figure 16. ***B. muscorum***. Moshumle.

The corolla length varies from 8.71 ± 0.04 mm in the diploid cultivar from the large scale field to 8.82 ± 0.04 mm in the diploid cultivar from the organic grown field (figure 18). There is a significant difference between these two cultivars (Kruskal-Wallace, P<0.001) while the tetraploid cultivar is not significantly different from any of them (Kruskal-Wallace, P>0.05).

The percentage of corollas that were bitten (figure 19 and 20) varies from 3.6 ± 1.3% in the diploid cultivar from the large scale field to 17.2 ± 2.4% in the diploid cultivar from the organic grown field (figure 21). The percentage of bitten corollas in the large scale field is significantly different from the percentage of bitten corollas in the organic and the tetraploid cultivar (Kruskal-Wallace, P<0.05). Furthermore, there is a positive correlation (r^2=0.90) between the length of the corollas and degree of corolla biting.

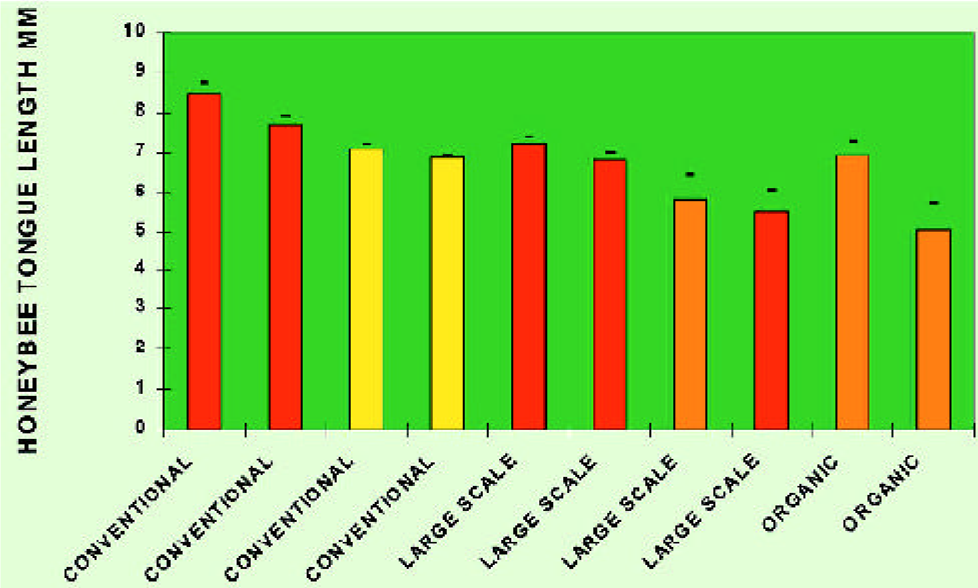


Figure 17. The tongue length in mm (+S.E.M.) of honeybees from colonies placed nearby the three red clover fields. Red columns are Buckfast colonies, yellow are *ligustica* colonies, and orange are Hawaiian hybrid colonies. Tungelængde (+S.E.M.) hos honningbier fra bifamilier, som var placet op ad de tre rødkløvermarker. Røde søjler er Buckfast bifamilier, gule er *ligustica* bifamilier og orange er Hawaii hybrider.

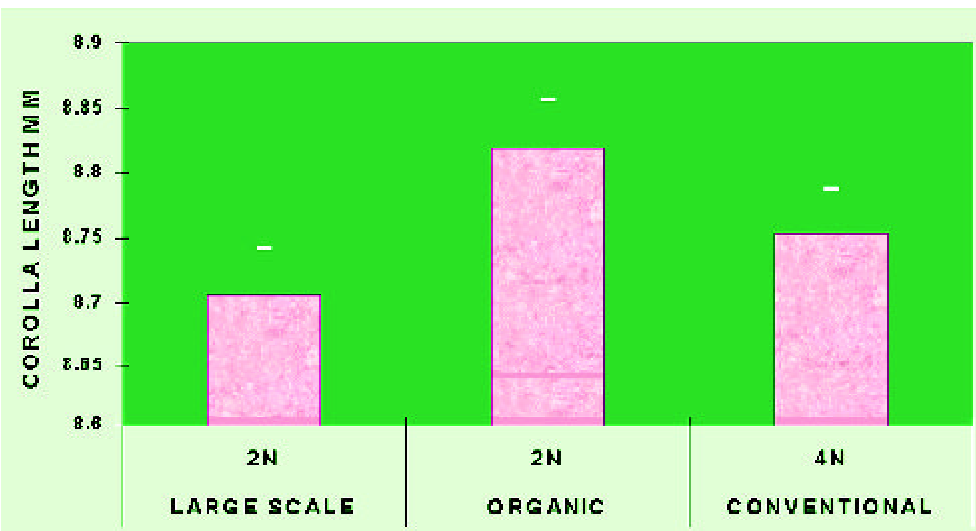


Figure 18. The length in mm (+S.E.M.) of red clover corollas in full blossom in the three red clover fields. Længden af kronrør i mm (+S.E.M.) fra rødkløverhoveder i fuld blomst i de tre rødkløvermarker.

Figure 19. Bitten corollas in a red clover head. Gennembidte kronrør i et rødkløverhoved.

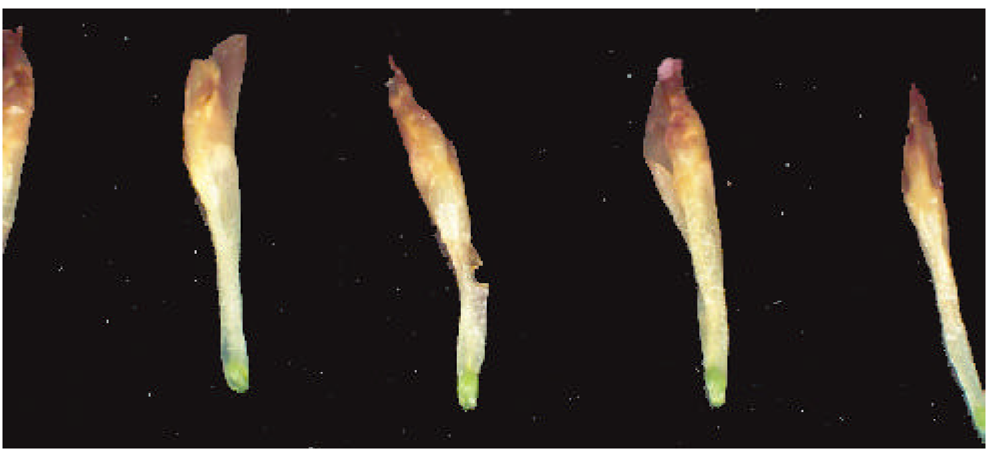


Figure 20. Bitten corolla. Gennembidt kronrør.

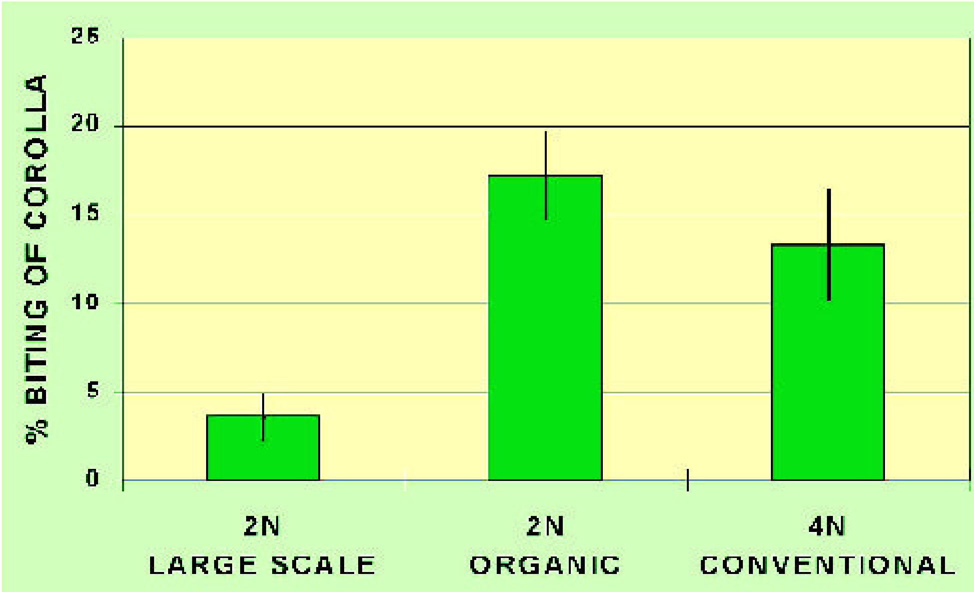


Figure 21. The percentage (± S.E.M.) of corollas that had holes bitten in the three red clover fields. Procentdelen (± S.E.M.) af kronrør, som var gennembidte, i de tre rødkløvermarker.

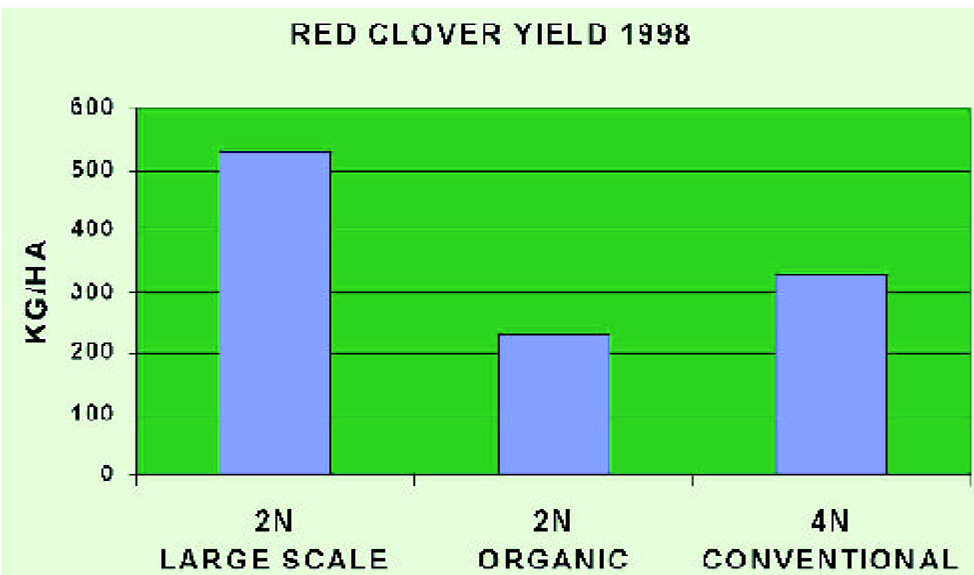
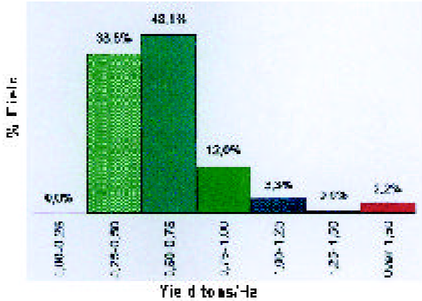


Figure 22. The red clover yield in kg cleaned seed/ha of the three fields. Rødkløverudbytte i kg renvare/ha i de tre marker.



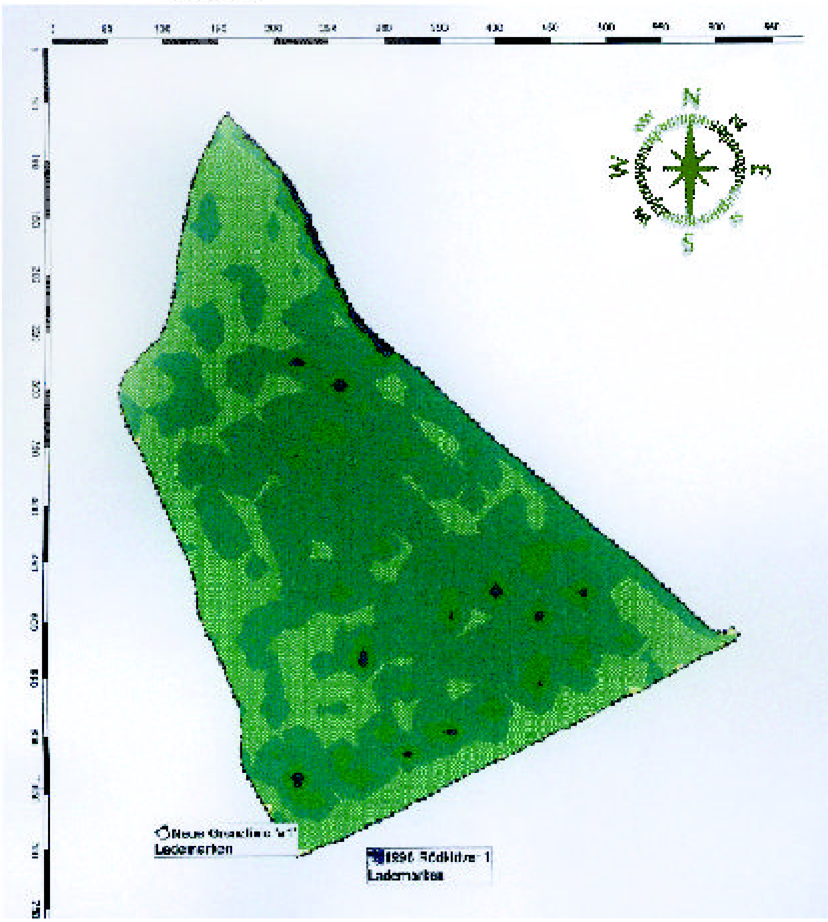


Figure 23. Yield distribution in the large scale field 2N (Lademarken) 1998 in tons/ha. The counting plots were placed across the field from west to southeast. Eight honeybee colonies were placed on the the southeast side of an afforestation on the northwest side of the field. Five honeybee colonies were placed in the south-southeast corner of the field (H.H. Kofoed, pers. comm.). Høstudbytte af 2N stordriftsmarken (Lademarken) 1998 i tons/ ha udtrykt som procent af marken. Tællefelterne blev placeret diagonalt i marken fra vest til sydøst. Otte bifamilier var placeret på sydøst siden af en beplantning på nordvest siden af marken. Fem bifamilier var placeret i det syd-sydøstlige hjørne af marken.

Figure 22 shows the yield of the three red clover fields. The yield is lowest in the organic grown field (205 kg cleaned seed/ha) and highest in the large scale field (540 kg cleaned seed/ ha). There is no significant difference between the yields in the three fields (χ^2, P>0.05).

The yield of the large scale field is shown in figure 23. Approximately 43% of the field had a yield of 0.50 to 0.75 tons/ha. This yield was distributed all over the field. Approximately 39% of the field had a yield of o.25 to 0.50 tons/ha. Only approximately 6% of the field had a yield higher than 1 ton/ha. This yield was distributed as small plots in the field.

Studies of bumble- and honeybees - caged vs. non-caged - in a red clover field

Materials and methods

Fields

In 1999, one farmer on Sealand made two of his red clover fields available for our studies. These conventionally grown fields with the diploid cultivar ‘Rajah’ had sizes of 6 ha (termed ‘east’) and 5.7 ha (termed ‘west’). They were placed 1 km apart.

Honeybee colonies

Totally 33 honeybee colonies were placed at the edges of the east field (see figure 24) at the beginning of the red clover bloom. The bees were not restricted in their flight. Two days after the colonies were moved to the site a pollen trap was placed on each colony. Pollen was collected each working day from mid July to mid August.

Counting of bees

Five counting plots of 20 m $\times$ 1 m were defined diagonally across each field as described for material and methods for 1998. The bees in the plots were counted and determined to species *in situ* according to Hammer & Holm (1970) eleven times in the east field and eight times in the west field from the peak of bloom (mid July) to desiccation or harvest of the field (late August). The relative distribution of bees among plots as well as the average number of bees in the five plots was calculated. Observations were carried out between 8.00 am and 2.30 p.m. on days without rain.

Figure 24. Honeybee colonies at the edge of the east field. Honningbifamilier i kanten af østmarken.

Cages

In the east field, 12 mesh cages with an area of 12 m^2 and a height of 1.8 m were established with iron bars as framework (see figure 25). The bars were covered with mesh net with a mesh size of 12.25 mm^2. The cages were placed in the field from middle of July until harvest at the end of August.

Bees in cages

The 12 cages were divided into 3 experimental groups. In each of the groups the following subgroups were established: 1. without bees, 2. honeybees (see figure 26), 3. bumblebees with sugar feeding (see figure 27), 4. bumblebees without feeding. The honeybees in each group were of different maternal strains: *A. m. ligustica* (strain 06-03), *A. m. ligustica* (strain 10-15), *A. m.'Buckfast'* (strain 06-22). The colonies were placed in multiple-storey hives (366 mm × 260 mm) consisting of one box. The honeybees were given the opportunity to fly both to the inside of the cage and into the open field. The bumblebees were *B. terrestris* medium size colonies with queens (see figure 28) from Borregaard BioPlant. These colonies consisted of approximately 60-80 individuals (S. Borregaard, pers. comm.). The bumlebees were restricted to flight inside the cages. In case of too many bumblebee workers in the cage compared to the number of flowers available the cages were opened shortly to let some of the workers out of the plot, thus, lowering the worker/flower ratio to a sustainable level.

Tongue length

The tongue lengths of *B. terrestris* and honeybee workers in the cages were measured as described for 1998. In the cages, the tongue lengths were measured one time in each of the honeybee colonies and two times in each of the bumblebee colonies. Furthermore, the tonguelengths were measured three times in 18 randomly chosen honeybee colonies placed at the east field.

Figure 25. Mesh cages in the field. Netbure i marken.

Figure 26. Honeybee colony in a multiple storey hive. The honeybees were given the opportunity to fly both inside the cage and into the open field. Honningbifamilie i opstablingsstade. Honningbierne havde mulighed for at flyve både inde i netburet og udenfor.

Red clover yield

After harvest, the experimental host informed us of the yield of the two red clover crops. Furthermore, the yield of the red clover grown in cages were harvested experimentally by us and the yield was calculated.

Climatic data

Data for the daily maximum temperature, daily precipitation, and daily wind velocity in the red clover bloom period were obtained from the recordings by a DIAS' weather station in the experimental area.

Figure 27. Bumblebee colony sheltered by a box from a multiple storey hive. The bumblebees were restricted to flight inside the cage. Humlebifamilie beskyttet af en kasse fra et opstablingsstade. Humlebierne havde kun mulighed for at flyve inden i netburet.

Figure 28. *B. terrestris* queen in the bumblebee colony. Jordhumledronning i humlebifamilien.

Results

The honeybees and the bumblebees are evenly distributed in the red clover fields (figure 29 and 30). In both fields *A. mellifera* which constitutes almost 70%, *B. terrestris* that constitutes around 14%, and *B. lapidarius* which constitutes between 12-15% are predominant species (figure 31). Each of the other bumblebee species *B. agrorum, B. distinguendus, B. hortorum, B. hypnorum, B. muscorum,* and *B. soroeënsis* constitute less than 3.4% . Most honeybees are observed in the west field.

Pollen collected from honeybee colonies placed nearby flowering red clover fields is totally dominated by red clover (figure 32). In the first week after the beginning of the bloom the amount of collected pollen decreased. At the peak of the bloom it increased suddenly. At that time the red clover pollen constituted approximately 90% of the total collected pollen and even just before harvest, when the field had nearly finished flowering, the red clover pollen constituted approximately 50%.

In the beginning of the flowering period, the maximum daily temperature decreased from approximately 27°C to approximately 17°C (figure 33). At the peak of the flowering the maximum temperature increased again to same level as in the beginning. At the end of the flowering period the temperature decreased again. At the beginning and at the end of the flowering period the precipitation was up to 3-5 mm pr. day. During the period of peak of the bloom no precipitation was measured. During the period of flowering, the average wind velocity was between 2.5 and 6 m/s. At the period of full flowering the lowest values were measured.

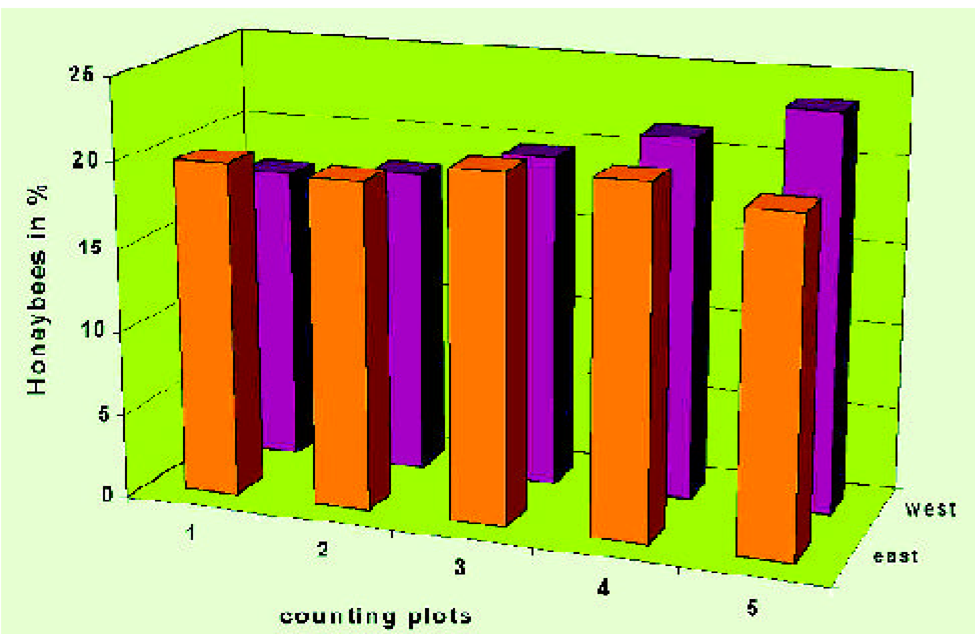


Figure 29. Distribution of honeybees in different plots of two red clover fields. Fordeling af honningbier i forskellige tællefelter i to rødkløvermarker.

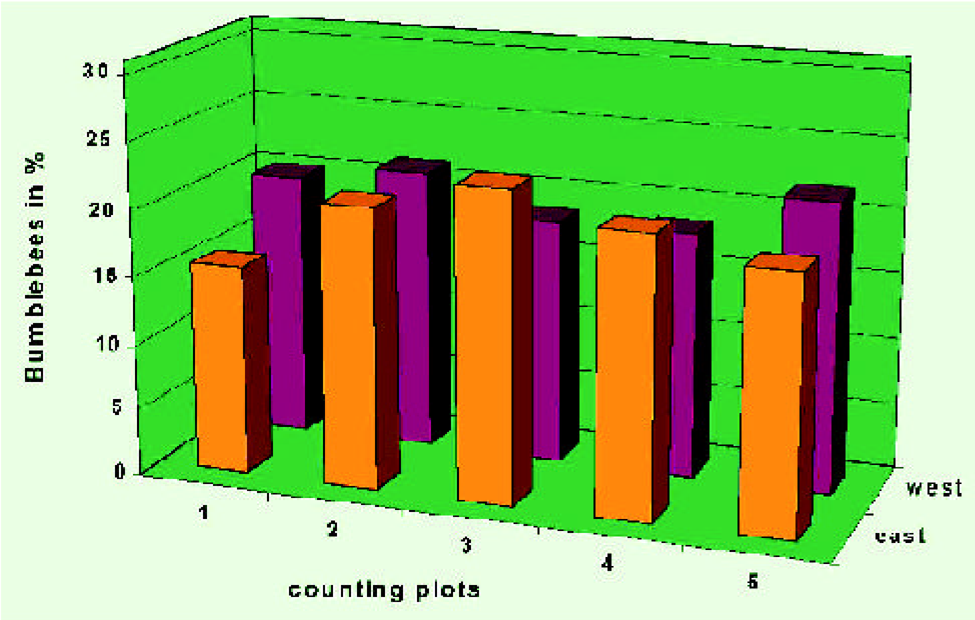


Figure 30. Distribution of bumblebees in different plots of two red clover fields. Fordeling af humlebier i forskellige tællefelter i to rødkløvermarker.

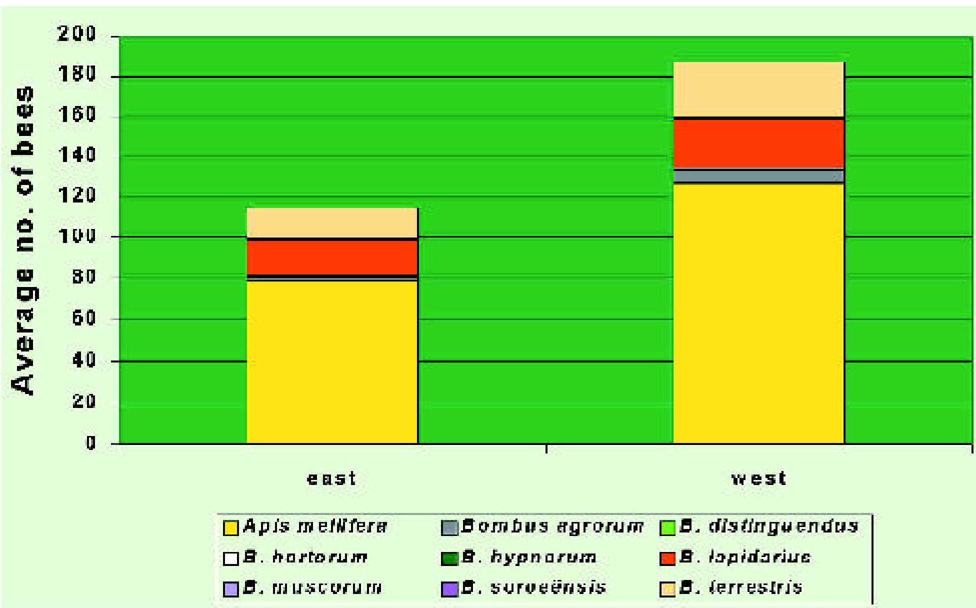


Figure 31. The average number of bees, separated in species, in five plots in the two red clover fields. Det gennemsnitlige antal bier fordelt på arter i de fem tællefelter i de to rødkløvermarker. Agerhumle (*B. agrorum*), *B. soroeënsis*, havehumle (*B. hortorum*), honningbi (*A. mellifera*), hushumle (*B. hypnorum*), jordhumle (*B. terrestris*), kløverhumle (*B. distinguendus*), moshumle (*B. muscorum*), stenhumle (*B. lapidarius*).

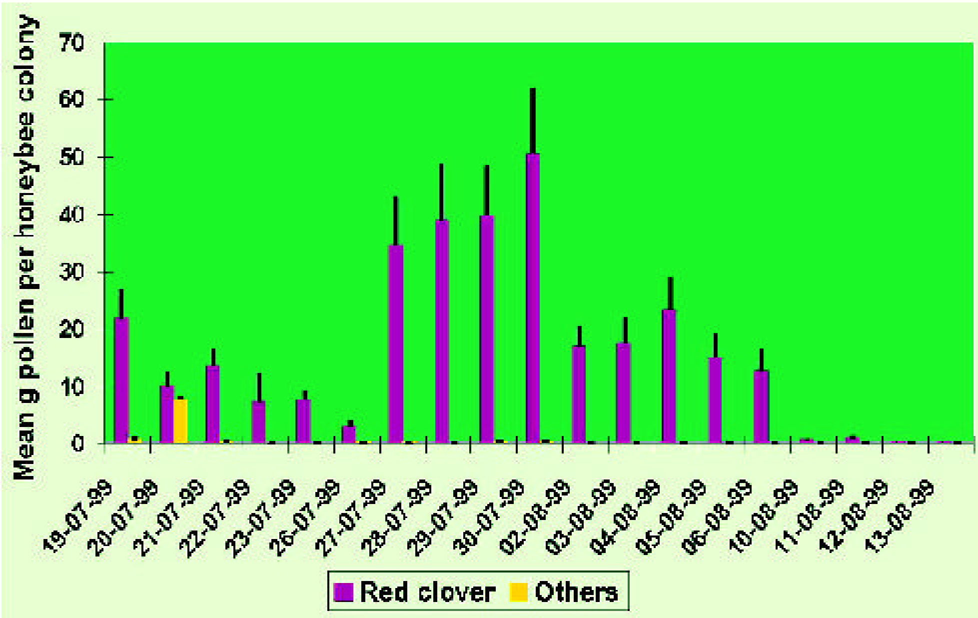


Figure 32. The mean (+ S.E.M.) amount of pollen collected by honeybee colonies on different dates during red clover flowering. Honningbifamiliers gennemsnitlige pollen-indsamling (+ S.E.M.) på forskellige datoer i løbet af rødkløverblomstringen.

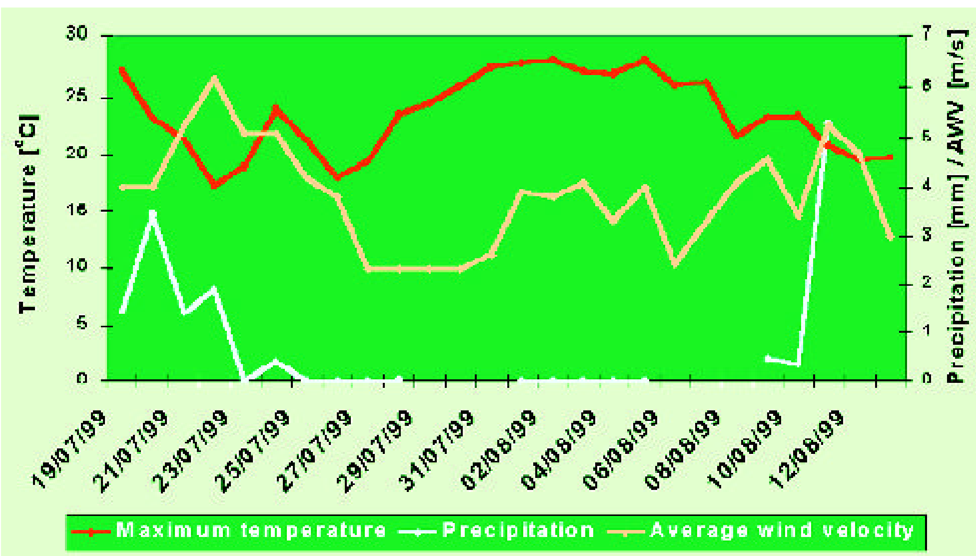


Figure 33. Selected climatic parameters (maximum temperature, precipitation, and average wind velocity) during the period of pollen collection shown in figure 32. Udvalgte klimatiske parametre (maksimum temperatur, nedbør og middel vindhastighed) i den periode, hvor der blev indsamlet pollen.

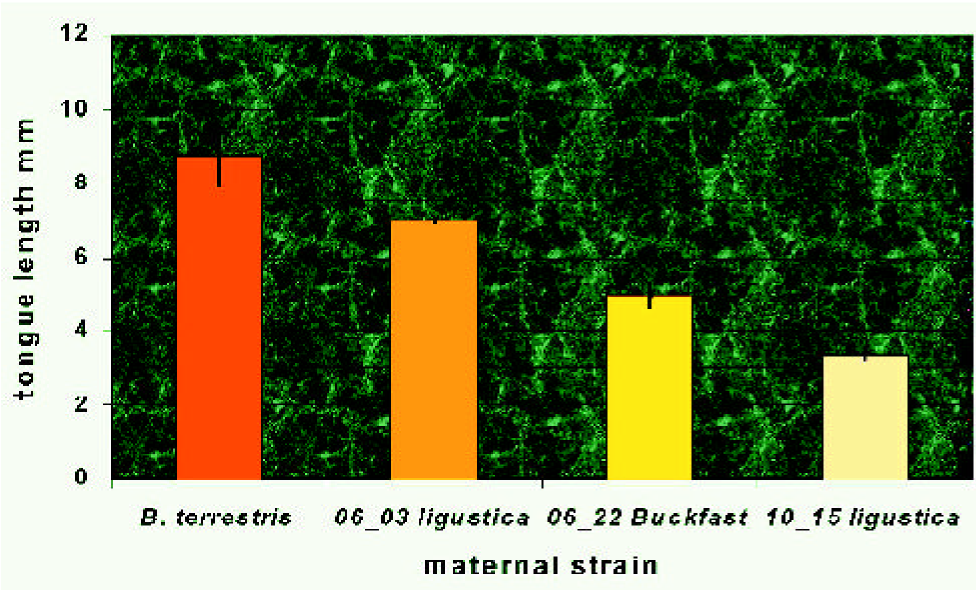


Figure 34. The tongue length in mm (± S.E.M.) of *B. terrestris* and three different honeybee strains placed in cage plots in a red clover field. Tungelængde i mm (± S.E.M.) hos *B. terrestris* og tre forskellige honningbistammer anbragt i bure i en rødkløvermark.

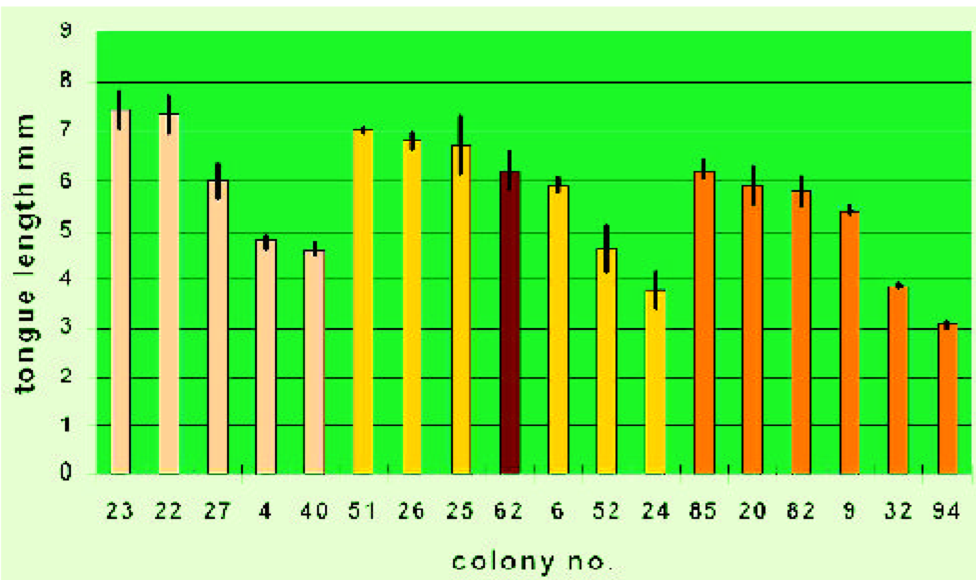


Figure 35. The tongue lengths in mm (± S.E.M.) of 18 of the honeybee colonies placed at the east field. Peach coloured columns are *ligustica* strain 10-15, orange are Buckfast 06-22, tangarine coloured are *ligustica* strain 06-03, and the bordeaux coloured originates from a swarm. Tungelængden i mm (± S.E.M.) hos honningbier i bifamilier i østmarken. De ferskenfarvede søjler er *ligustica* stamme 10-15, lys orange er Buckfast 06-22, mørk orange er *ligustica* stamme 06-03, og den bordeaux søjle er en sværm.

The tongue lengths of the bumblebees and honeybees in the cage plots vary from 3.37±0.18 mm in the *ligustica* 10-15 to 8.77±0.81 mm in *B. terrestris* (figure 34). The tongue of *B. terrestris* is significantly longer than the tongues of the honeybees (Kruskal-Wallace, P<0.001). Also the tongue lengths of the three honeybee strains are significantly different from each other (Kruskal-Wallace, P<0.001).

Figure 35 shows the tongue lengths of the 18 randomly chosen honeybee colonies placed at the east field. The tongue length varies from 3.07±0.07 mm in a *ligustica* 06-03 colony to 7.43±0.38 in a *ligustica* 10-15 colony. The mean tongue length was calculated to 5.65±0.30 mm. There are significant differences among the colonies in the same strains (Kruskal-Wallace, P<0.001) but no significant difference between the strains (Kruskal-Wallace, P>0.05).

There is no significant difference among the red clover seed yield in cage plots with honeybees, with bumblebees feeded or not feeded, and from the uncaged field (figure 36). However, the seed production from the cage plot without bees are significantly lower than the other groups (Kruskal-Wallace, P<0.001).

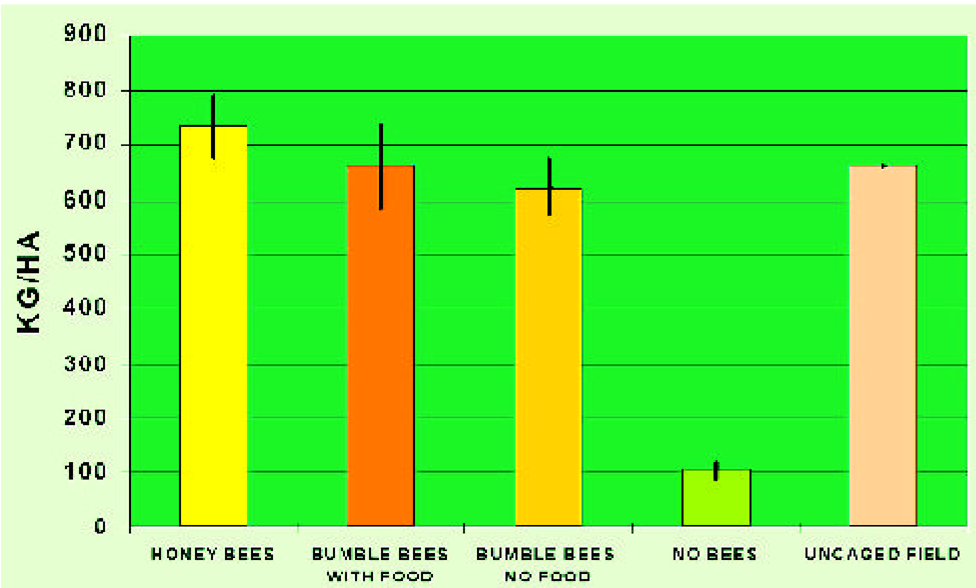


Figure 36. The red clover seed yield in kg cleaned seed/ha (± S.E.M.) in cage plots and in uncaged field. Udbytte af rødkløver i kg renvare/ha **(**± S.E.M.) i burforsøg og i udækket parcel.

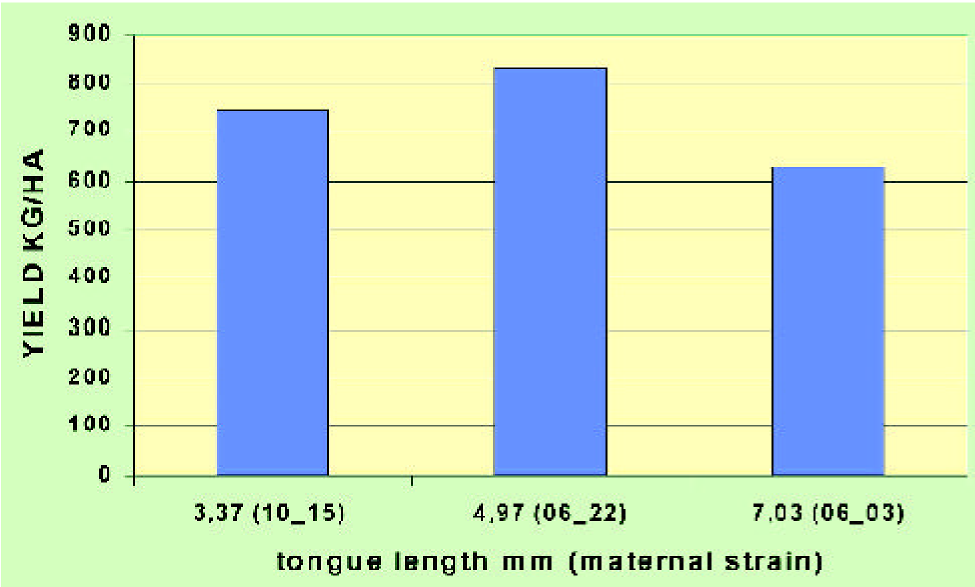


Figure 37. The red clover seed yields in kg cleaned seed/ha in cage plots in relation to the tongue length of different honeybee strains. Udbytte af rødkløver i burforsøg i kg renvare/ ha relateret til tungelængden hos de tre forskellige honningbistammer.

Figure 37 shows the red clover seed yield in cage plots in relation to the three honeybee strains with significantly different tongue lengths. There is no correlation between yield and tongue length.

Discussion

Abundance and distribution of bees in the fields

In 1953, Richards suggested that red clover should be grown in small fields to ensure that a limited bumblebee population would be able to pollinate a sufficient number of flowers throughout the crop. In 1961, Hawkins demonstrated that the number of seeds per red clover head decreased with increasing size of the field. Furthermore, Väre (1960) in Finland has demonstrated that the red clover yield decreases as the distance between the flowers and the nearest bumblebee colony increase from 0 to 200 m.

However, our results from both years show that apparently neither the honeybees nor the bumblebees had difficulties in reaching the middle of the fields (figure 8, 9, 29 and 30). Thus, there are no preferred areas for the bees or limitation in their ability to cover the whole area. These results are in accordance with Calabuig (2000) as she observed a positive correlation between the distance from the edge of an oil seed rape field and the number of *B. terrestris* caught in traps. Furthermore, the yield from the middle of the large scale field (figure 23) - where the distance from the nearest afforestation exceeds 300 m - is not lower than the yield in the vicinity of these possible nesting places (south-southeast of the small forest). The large scale field had a tendency of higher yield (figure 22) than the others in spite of the organic field having a tendency of more bees (figure 10). This of course indicates that beside the abundance of bees soil conditions, pests or fertilization play major roles in successful seed production.

In both years, *A. mellifera*, *B. terrestris*, and *B. lapidarius* are predominant in the fields (figure 10 and 31). Other bumblebees such as *B. agrorum, B. distinguendus, B. hortorum, B. hypnorum, B. muscorum,* and in 1999, *B. soroeënsis* constitute a minor part. In 1999, most honeybees were observed in the west field. This field is located approximately 1 km from the east field where the bee colonies were placed. This indicates that it does not matter whether the honeybee colonies are placed in the field or at a shorter distance from it.

Red clover yield

In 1998, the tretraploid red clover field ('Kvarta') in our experiment had a yield of 328 kg/ha and the diploid large scale field ('Rajah') had a yield of 532 kg/ha (figure 21), which was in accordance with the average yield in Denmark.

In 1999, in Denmark, approximately 40 ha tetraploid red clover ('Kvarta') was harvested for seed production. The mean yield was 247 kg/ha in comparison with diploid ('Rajah') with 576 kg/ha. However, the yield from grower to grower was very variable (A. Larsen, DLF-Trifolium, pers. comm.). Thus, the yield of 'Rajah' fields in our experiment at B. Søgård's farm was above the average yield in Denmark which was 660 kg/ha (figure 36) indicating sufficient pollination was obtained compared to previous years when the grower (B. Søgård) reported lower yields than the national average. In previous years *no* honeybee colonies were placed in the vicinity of these fields (Hansen 1998). Years back, it has been possible to obtain satisfactory yields without the farmers making an active effort to ensure proper pollination because honeybee colonies were placed within the reach of the crop. However, during the last 10 years the number of honeybee colonies, by chance, was placed within reach of the crop. However, during the last 10 years the number of honeybee colonies (figure 38) and especially

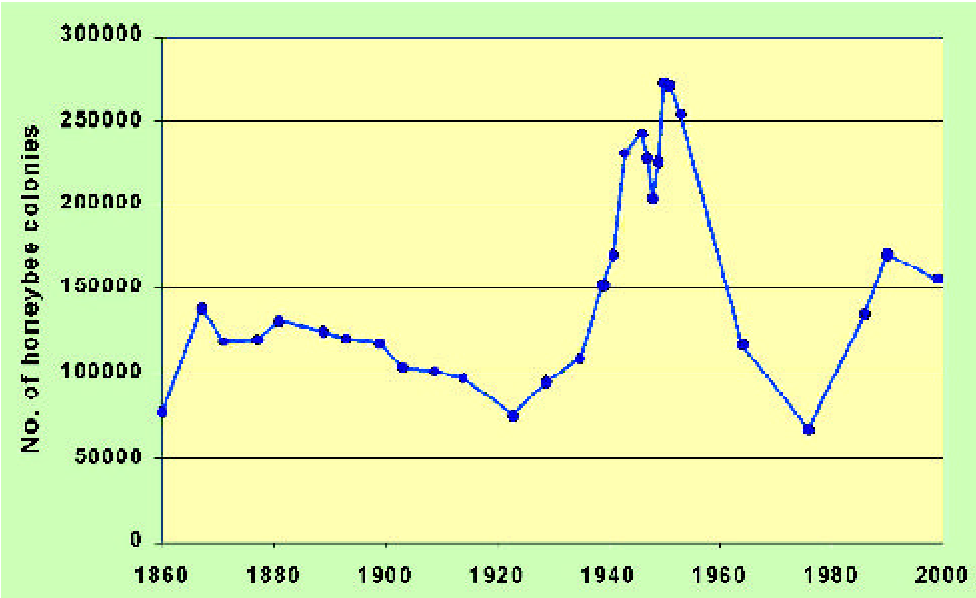


Figure 38. The number of honeybee colonies in Denmark from 1860 to 2000 (data from Hammer 1964, Danmarks Statistik 1965, Hansen 1977, Klug-Andersen 1987, Vejsnæs 1992, Brødsgaard *et al.* 2001). Antallet af honningbifamilier i Danmark fra 1860 til 2000.

the number of honeybee-keepers have decreased (Brødsgaard *et al.* 2001). Therefore, local differences in the concentration of honeybee colonies have become more pronounced. These factors make it more important now than ever for the farmers actively to place honeybee colonies near crops that needs bee pollination. Also fluctuations in the natural bumblebee populations could play a role. Thus, it is important to ensure nesting possibilities for these species, as they are also important pollinators of the natural flora (Calabuig 2000).

In 2000, the yields of ‘Kvarta’ and ‘Rajah’ were 243 kg/ha and 432 kg/ha, respectively. In 2001, the production of seed from tetraploid cultivars in Denmark was given up because of the unsatisfactory seed production (A. Larsen, DLF-Trifolium, pers. comm.). An explanation of the pollination problems of the tetraploid cultivars could be the much larger leaf canopy, which hides the relatively few flowers. Furthermore, this extensive leaf volume might influence the micro-climate in the flowers and reduce the soil temperature and thereby reduce the nectar production. These factors could make the tetraploid cultivar less attractive to bees. However, in the southern part of Sweden, where the climatic conditions are similar to Sealand, the growers seem to have success in pollination of tetraploid cultivars (A. Larsen, DLF-Trifolium, pers. comm.).

Corolla length

According to Free (1993) the most important explanation for the insufficient pollination of tetraploid red clover is its deeper corolla tube. He suggests that the flowers are unattractive to other bees than the long-tongued bumblebees (see figure 2 and 7). Therefore, this lead to experiments with the growth regulatory substance Alar 85 to shorten the corolla (Svendsen

1986). However, our results show no significant differences in the length of the corollas between the current tetraploid and diploid cultivars (figure 18). According to A. Larsen, DLF-Trifolium (pers. comm.) this result is in accordance with the observations of breeders of red clover who, among the current Danish cultivars, find no differences. In the current Danish tetraploid cultivar the corolla length does not seem to play the major role in the seed production problem.

Tongue length

There are different opinions on whether the tongue length of bees plays a role in red clover pollination. E.g. Stapel and Eriksen (1936) found that 33% of the honeybees of an Italian strain (*A. m. ligustica*) visited red clover, whereas only 12% of a Danish strain (*A. m. mellifera*) did. They argued that the longer tongue of the *ligustica* bees (4% longer) was the reason for this difference. Woodrow (1952) on the other hand concluded that the length of the corolla in relation to the honeybee tongue length was an unimportant factor in relation to red clover pollination because the tongue plays no part in the pollination. In Woodrow's opinion the honeybees visited red clover for pollen, and to a lesser extend for nectar.

Though there was a significant difference in the tongue lengths of the three honeybee strains in the caged plots in our experiments (figure 34), this could not be correlated to the difference in red clover seed yield (figure 37). Furthermore, both in 1998 and 1999, a large variation in tongue lengths was seen between and within the different honeybee strains (figure 17 and 35).

Figure 39. *B. agrorum* with the tongue stretched. Agerhumle med strakt tunge.

Our results show that though the *B. terrestris* had significantly longer tongues than the honeybees in the caged plots (figure 34), there were no differences in the yield (figure 36). Furthermore, some of the Buckfast bees placed nearby the fields in the open field trial had as long tongues as *B. terrestris* (figure 35).

Robbing

In the past century, it has been discussed whether the habit of the short-tongued bumblebees (see figure 2) where they gain access to the nectar by biting through the corolla (called robbing, see figure 40) constitutes a problem for the pollination of red clover (Free 1993). One problem could apparently be that when these primary robbers have bitten holes, the honeybees will also collect nectar this way (see figure 41) and, thus, become secondary robbers. These visits are considered negative with respect to pollination. However, Hawkins (1961) found a significant positive correlation between the seed yield and the number of robber bumblebees present. Furthermore, Woodrow (1952) argued that the pollen-collecting honeybees will continue this function regardless of the way other bees collect nectar. Our results support this statement and that this also may be the case for *B. terrestris*. In 1998, data were collected that showed that the corolla biting occurred in all fields (figure 21) but that the large scale field differed from the tetraploid and the organic field, with most biting in the organic crop. However, a direct correlation with the total number of *B. terrestris* present (figure 10) could not be found. On the other hand, the lengths of the corolla in the different fields are positively correlated with the degree of corolla biting as the flowers from the large scale field had significantly shorter corollas than flowers from the organic field (figure 18) and with the number of bitings in the tetraploid crop being intermediate (figure 21). The explanation why the flowers grown

Figure 40. *B. terrestris* bites a hole in the corolla to get access to the nectar. Jordhumlen bider hul i kronrøret for at få fat i nektaren.

Figure 41. A honeybee uses the existing hole in the corolla made by *B. terrestris* resulting in a negative visit. En honningbi bruger det hul, som jordhumlen har bidt, hvilket resulterer i et negativt besøg.

organically have longer corollas than flowers of the same cultivar ('Rajah') grown conventionally could be the differences in fertilization or soil conditions. E.g., if red clover is manured with potassium, the corolla length is increased (Stapel and Götzsche 1941).

In 1999, when the bumblebee breeding company was confronted with the questions concerning negative visits they told us that the problem with robbing was solved by supplying the bumblebee hives with sugar sirup. This procedure should prevent robbing and increase pollination. Because of limited resources, the flowers in the cages were not checked for biting/robbing, but the yield from the cages showed that there were no significant differences in the yield between honeybee (not able to rob) pollinated plot and *B. terrestris* pollinated plot regardless of the food supply to *B. terrestris* (figure 36).

Most suitable pollinators

Our results show that pollen collected from honeybee colonies placed nearby flowering red clover fields is totally dominated by red clover (figure 32). Though there was a large variation among the colonies the result indicates that red clover pollen is attractive to the honeybees, and when this result is related to the seed yield of the caged plots and the uncaged fields it indicates that honeybees are effective as pollinators of red clover.

There were no differences in yield among the different caged plots with bees but all cage plots with bees had significantly higher yield than the control plot (figure 36). This shows that bee pollination clearly increases the yield. Furthermore, the result suggests that the seed production is independent of the bee type (*B. terrestris* or honeybees) providing a sufficient number of bees are present in the flowering period. These observations are not in accordance with the results of Palmer-Jones *et al*. (1966) who found that a caged plot pollinated by short-tongued bumblebees (see figure 2) only produced one third of the yield produced by honeybee pollinated plots. On the other hand van Laere & Martens (1962) argue that *B. terrestris* is a suitable pollinator of red clover but as the population size is unpredictable it cannot be recommended to rely on *B. terrestris* as the only pollinator.

Several authors (e.g. Dennis and Haas, 1967) have reported that relatively more honeybees visit diploid fields whereas relatively more bumblebees visit tetraploid fields. The same tendency is seen in our results from 1998 (figure 10). Furthermore, according to Dennis and Haas (1967) long-tongued bumblebees are relatively more abundant in tetraploid cultivars than short tongued bumblebee species. In our experiments from 1998, this was also seen for *B. lapidarius* whereas *B. hortorum* was more evenly distributed among the fields.

Stapel (1934) recorded that hot dry summers are favourable for nectar secretion in red clover and that in such summers especially many honeybees visit the crop. The summer of 1999, when the red clover was at the peak of bloom, the temperature was high and the precipitation very low (figure 33), and at the same time the pollen collection in the honeybee colonies was at its highest (figure 32). Our results also show that when the temperature dropped to approximately 15°C in the beginning of the bloom the pollen collection also dropped.

Bumblebees are known to visit flowers at lower temperatures than honeybees, thus, they fly earlier in the morning and later in the afternoon (Løken 1949). Furthermore, they work faster than honeybees (Stapel 1933). To be able to rank the pollinating efficiency of the different bee species Stapel (1933) estimated relative values of honeybees, short-tongued, and long-tongued bumblebees (figure 2) based on their working speeds. The value called ‘bee unit’ was defined as 1.0 for honeybees, and relative to that 1.5 for short-tongued and 2.5 for long-tongued bumblebees. Later these results were confirmed for pollination efficiency of tetraploid cultivars (Dennis & Haas 1967).

The recommendation from Landbrugets Rådgivningscenter (1998) for pollination of red clover in Denmark is four to five honeybee colonies per ha. In mid July, a strong Danish honeybee colony consists of 60,000 bees of these approximately 20,000 foragers (Svendsen 1987). This means that with four to five colonies approximately 90,000 foragers is recommended per ha. The short-tongued *B. terrestris* has a ‘bee unit’ of 1.5 which means that approximately 60,000 *B. terrestris* foragers will bee needed per ha to ensure optimal pollination. The medium-size *B. terrestris* colony consists of maximum of 80 foragers which means that approximately 750 hives per ha would be needed to ensure pollination.

However, if Hogborg’s (1966) figures are used the result is somewhat different. He argued that only a 20,000 bee unit per ha was necessary to ensure pollination. This means that only *one* strong honeybee colony is needed and 160 *B. terrestris* colonies. But as mentioned above there are large variations and fluctuations in the collection of pollen by honeybee colonies, therefore, the recommendation from the experienced Danish beekeepers is that as a minimum two strong honeybee colonies per ha are necessary to ensure pollination (P. Kristiansen, pers. comm.).

Furthermore, the price of a medium-size *B. terrestris* colony is approximately 40 EUR (S. Borregaard, pers. comm.) and the rent of a strong honeybee colony is approximately 67 EUR (P. Kristiansen, pers. comm.). With respect to pollination of conventionally or large scale grown diploid red clover the use of honeybees must be recommended.

A nice illustration of honeybees as important and sufficient pollinators of diploid red clover comes from Australia where bumblebees are absent. Here the yield is as good as in areas where bumblebees are present (Hills 1941).

Conclusion

It does not seem to be a problem for neither honeybees nor bumblebees to reach the middle of a large diploid red clover field (16-17 ha). Our results indicate that bee pollination, independent of bee species (*A. mellifera* or *B. terrestris*), clearly increases the yield, and that honeybees are important as pollinators of red clover. But, as the natural *B. terrestris* population size is unpredictable and the cost of *B. terrestris* hives high it is not recommended to rely only on *B. terrestris* as pollinator. In fact, the results indicate that it is more important now than ever to place honeybee colonies near crops that need bee pollination and to ensure nesting possibilities for bumblebees.

The current Danish recommendation for red clover pollination using honeybee colonies (4-5 colonies/ha) is supported. In hot, dry summers honeybee colonies placed up to 1 km from a red clover field are able to provide satisfactory pollination of red clover and even two strong colonies/ha could provide satisfactory pollination under such conditions.

The caged plots study showed that though there was a significant difference in the tongue lengths of the three honeybee groups this could not be correlated to differences in seed yield. Furthermore, though *B. terrestris* had significantly longer tongues than honeybees, and this bumblebee is known to rob flowers, there were no differences in yield between honeybee pollinated plots, *B. terrestris* pollinated plots with or without sugar sirup feeding, or uncaged field whatsoever.

There is a positive correlation between corolla length and the degree of corolla biting of red clover flowers. On the other hand there was no significant difference between the corolla length of the diploid and tetraploid cultivar.

In Denmark, seed production from the tetraploid cultivar ‘Kvarta’ was not satisfactory. Even when honeybee colonies were placed for pollination, the yield was much lower than for the diploid cultivar. The explanation for this phenomenon is not unambiguous and remains a question.

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