Pollen diversity collected by honey bees in the vicinity of *Vaccinium* spp. crops and its importance for colony development

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Abstract

Access to a rich diversity of flowering plants is very important for the development of honey bee colonies introduced in crops for pollination. The aim of this observational study was to determine the impact of surrounding pollen diversity on the health of honey bee colonies introduced in lowbush blueberries (*Vaccinium angustifolium)* in June and cranberries (*V. macrocarpon*) in July. The results suggest that monocultures of lowbush blueberries are not suitable for optimal brood rearing. In the blueberry environments we studied, the dominant pollen collected by honey bees were *Alnus* spp. and *Taraxacum officinale*, which are deficient in some essential amino acids. Significant reduction of brood rearing during bees' stay in blueberry monocultures in June may therefore be explained by nutritional deficiencies. In July, the polliniferous flora in the vicinity of cranberry monocultures was poorer, but of better nutritional quality. Pollen analysis allowed the identification of Brassicaceae, *Trifolium* spp. and *V. macrocarpon* as the three dominant taxa collected by bees during this period. The complete lists of plant taxa foraged by honey bees for pollen during the pollination of lowbush blueberries and cranberries are provided.

Keywords:

Honey bees / pollination / pollen / *Vaccinium* / floral diversity / nutritional deficiencies

Résumé

L'accessibilité à une flore diversifiée est très importante pour le développement des colonies d'abeilles domestiques introduites dans une culture pour le service de pollinisation. Notre étude observationnelle visait à déterminer l'impact de la diversité florale environnante sur la santé des colonies d'abeilles introduites dans les cultures de bleuets nains (*Vaccinium augustifolium*) en juin et de canneberges (*V. macrocarpon*) en juillet au Québec (Canada). Les résultats suggèrent que les monocultures de bleuets nains n'offrent pas une flore adéquate pour l'élevage optimal du couvain. Les plantes qui furent le plus butinées pour le pollen dans cet environnement, *Alnus* spp. et *Taraxacum officinale*, sont déficientes en certains acides animés essentiels à l'abeille. La diminution significative de la surface de couvain dans les monocultures de bleuets en juin pourrait donc être expliquée par des carences alimentaires. Dans l'environnement de la canneberge, les grains de pollen récoltés par les abeilles furent moins taxonomiquement variés que dans l'environnement du bleuet mais étaient de meilleures qualités nutritionnelles. Les taxons dominants récoltés par les abeilles furent les Brassicacées, *Trifolium* spp. et *V. macrocarpon.* Les listes complètes des taxons des plantes butinées pour le pollen pendant la pollinisation du bleuet nain et de la canneberge sont fournies.

Mots clés :

Abeilles domestiques/ pollinisation/ pollen/ Vaccinium/ diversité florale/ carences nutritionnelles

65

Introduction

A combination of multiple factors has been causing a decline in the world's honey bee stock. Although the *Varroa* mite has been identified by some researchers as the main parasite responsible for colony losses (Neumann and Carreck 2010), many other pathogens, such as *Nosema ceranae* (Currie et al. 2010), as well as a few viruses (Bromenshenk et al. 2010; Ratnieks and Carreck 2010), are known to affect honey bee health. Pesticides (Charrière et al. 2006; Desneux et al. 2007) and commercial beekeeping practices, e.g., long-distance transportation of beehives for pollination services (Kevan et al. 2007), are also important factors known to cause lethal and sublethal stresses to honeybee colonies.

Over the last few decades, the intensification of agriculture combined with the use of herbicides over vast areas have profoundly changed the agricultural landscape, depleting the natural floral diversity (Brodschneider and Crailsheim 2010). The cultivation of pollinator-dependent crops has escalated simultaneously, increasing the demand for commercial pollination services (Klein et al. 2007; Aizen and Harder 2009). The crops in which honey bee colonies are introduced for pollination thus tend to offer low floral diversity, forcing the foraging bees to visit a single plant species extensively (Brodschneider and Crailsheim 2010). Furthermore, in order to optimize pollination, the fields are often overcrowded with honey bees (Johnson 2010), causing a scarcity of nectar and pollen resources to feed the disproportionately high honey bee population. In addition, the pollen of some cultivated plants cannot satisfy the nutritional needs of the developing honey bee colonies (Brodschneider and Crailsheim 2010; de Groot 1953; Westerkamp and Gottsberger 2000). For this reason, indigenous flora established in the area surrounding the crops are still essential for pollinators, serving as a complementary source of pollen and

89 nectar (Haubruge et al. 2006). As a result of the actual loss in floral diversity, colonies used
90 for crop pollination are subjected to an additional stress factor: nutritional deficiencies
91 (Glare and O'Callaghan 2008; Kevan and Ebert 2005; Oldroyd 2007; Westerkamp and
92 Gottsberger 2002).

93 To limit the consequences of nutritional deficiencies, pollen substitutes and/or sugar
94 syrup must often be provided to colonies (Decourtye et al. 2010). These supplements do not
95 supply the same nutritional elements as natural pollen and nectar, but when essential
96 nutrients are lacking, some supplemental feeding is recommended (Brodschneider and
97 Crailsheim 2010). In addition to protein deficiencies, nectar can also be scarce in some
98 environments, e.g., cranberry monocultures (Cane and Schiffhauer 1997; Kevan et al. 1983;
99 Ramsay 1987). According to Decourtye et al. (2010), growing a diversity of plants is the
100 best way to provide a natural and complete source of proteins and essential nutrients.
101 Finally, Brodschneider and Crailsheim (2010) have demonstrated that bees and colonies
102 with natural diets exhibit superior performance compared to those fed on artificial diets.

103 In the province of Quebec, Canada, honey bee pollination services are mainly
104 requested for two berry crops, lowbush blueberries (*Vaccinium angustifolium* Ait.,
105 Ericaceae) and cranberries (*Vaccinium macrocarpon* Ait., Ericaceae), which are both
106 highly dependent on insect pollinators for optimal fruit set (Delaplane and Mayer 2000).
107 Over the last five years, the managed areas devoted to these two crops has increased by
108 24% and 35% respectively (Association des Producteurs de Canneberges du Québec 2009;
109 Lavaute 2009). The demand for honey bee hives for pollination has risen proportionately.
110 Unfortunately, *Vaccinium* crops often offer environments with poor floral diversity. To
111 further compound this problem, the same honey bee colonies are regularly used to pollinate

112 both crops consecutively during the same season, increasing bees' exposure to nutritional
113 stress.

114 This observational study had two main objectives. The first was to compare the
115 floral diversity found in landscape surrounding of lowbush blueberries to that found around
116 cranberry crops by sampling the pollen brought into hives by forager honey bees. The
117 second was to compare the health and development of honey bee colonies introduced in
118 *Vaccinium* crops for pollination to that of colonies which were not exposed to *Vaccinium*
119 crops. To our knowledge, no study has yet investigated the effects of the pollen diversity of
120 a crop's surroundings on the health of honey bee colonies introduced for commercial
121 pollination services in Canada, or anywhere else. The results of our study will provide a
122 better understanding of the natural floral diversity available to honey bees in the vicinity of
123 blueberry and cranberry fields, thereby providing guidelines for further improvement of the
124 landscape adjacent to these crops, and ultimately, improvement of honey bee health and
125 increased crop yields.

126

127 **Materials and methods**

128 *Honey bee supplies*

129 The honey bee colonies came from the Centre de recherche en sciences animales de
130 Deschambault (CRSAD), Quebec, Canada. Colonies were *Apis mellifera ligustica*
131 hybridized with *Apis mellifera primorsky*. Thirty-six colonies were used for each year of
132 the study. Their brood frame surfaces were equalized in late spring with an average of
133 30 400 ± 1709 cells of brood (sealed and unsealed brood) per colony to obtain colonies of a
134 similar strength before their exposure to different site categories. Surplus frames of sealed

brood with adhering bees were taken from a strong colony and given to a weak colony. Each colony was comprised of a double brood chamber Langstroth hive.

Study regions

The study was conducted in different regions of the province of Quebec, in 2008 and 2009. The experimental blueberry fields were located in the Côte-Nord region (2008; 48° 44′ 00″ North; 69° 06′ 00″ West) and in the Lac St-Jean region (2009; 48° 53′ 00″ North; 72° 14′ 00″ West). The cranberry fields studied in both years were in the Bois-Francs region (46° 30′ 00″ North; 75° 59′ 00″ West). These three regions are the most important production areas for their respective crops in Quebec.

Study sites

Three categories of sites were compared: 1) monocultures; 2) mixed; and 3) non-*Vaccinium* fields. Sites labelled as «monocultures» were fields of *Vaccinium* crops larger than 15 hectares on which weeds were intensively managed. Sites labelled as «mixed» were fields of *Vaccinium* crops smaller than 3 hectares on which weeds were left unmanaged. *Vaccinium* crops were lowbush blueberries in June and cranberries in July, which corresponds to the blooming period when pollination services are required. Sites labelled as «non-*Vaccinium* fields» were fields on which fodder plants attractive to honey bees were grown (e.g., rapeseed, clover). Each category of sites was repeated four times, for a total of 12 study sites, and three experimental hives were placed at each site, for a total of 36 hives. All sites were situated at least five kilometres apart to minimize any overlapping of honey

157 bee foraging areas. Distances between sites were confirmed using a GPS and ArcGIS
158 software.
159 In early June, the hives were transported from Deschambault to the blueberry fields
160 for a three-week stay. At the end of June, the hives were brought back to Deschambault for
161 brood evaluation prior to being transported to the cranberry fields at the beginning of July.
162 After being left in place for two to three weeks for cranberry pollination, the hives were
163 brought back to Deschambault for the remainder of the beekeeping season.
164 Pollen sampling and identification were carried out for both years of the study while
165 brood surface data were collected for 2009 only.
166
167 *Pollen sampling*
168 Pollen pellets harvested by honey bee foragers were collected using two methods.
169 First, a pollen trap (Shaparew model) was installed randomly on one of the three hives at
170 each of the 12 sites. Drawers were emptied every other day, and the pollen pellets were
171 frozen until they could be analyzed in the laboratory during the fall. Second, foragers
172 returning to the hives were captured (N = 40) every other day using an insect net. Captured
173 bees were put on dry ice and kept frozen until laboratory analysis was performed in the fall.
174
175 *Pollen identification*
176 The entire sample from each bag of pollen harvested in traps was mixed, and two
177 one-gram subsamples were removed (Louveaux 1958). The subsamples were sorted into
178 lots according to the different colours of the pellets under normal and UV light. For each
179 colour lot, 1/8 mm^3 of pollen (equivalent to the tip of a needle) was mounted on a

180 microscope slide using glycerin-gelatin dyed with fuchsine (Louveaux et al. 1970). Pollen
181 grains were identified under the microscope (x1000) to the plant family, genus or species
182 level, when possible, using a collection of references and a taxonomic key (Moore and
183 Webb 1983).

184 Pollen pellets on the foragers' hind legs (N=40 foragers for each sample date) were
185 removed using forceps and mixed together with distilled water (2 mL) to form a
186 homogeneous mixture. A small drop of this mixture, together with the glycerin-gelatin, was
187 placed on two different glass slides and covered with a glass cover. One hundred grains
188 were identified randomly on each slide.

189

190 *Brood surface evaluation*

191 In the context of pollen studies, brood surface is the most meaningful indicator of
192 colony health because pollen provides the protein source needed by the colony to ensure
193 sufficient egg laying by the queen and proper larval development (Brodschneider and
194 Crailsheim 2010). Thus, healthy colonies tend to show greater brood surface area than
195 unhealthy ones. The evaluation method used here was the same as the one described by
196 Giovenazzo and Dubreuil (2011). The surface area occupied by total brood (eggs + larva +
197 capped brood) was evaluated by measuring width and length of the brood surface on each
198 side of every brood frame. The rectangular surface obtained was multiplied by 0.8 to
199 compensate for the elliptical form of the brood pattern. These values were then added in
200 order to calculate the total brood surface in each colony. A factor of 25 worker cells per
201 6.25 cm^2 (i.e., a square inch) was used to convert the area to obtain a number of brood cells.

202 The evaluation was performed four times for each hive during the study: 1) prior to
203 introduction in the blueberry fields (late May); 2) between stays in the blueberry and
204 cranberry fields (late June); 3) after their stay in the cranberry fields for pollination (late
205 July); and 4) before wintering (late August).
206
207 *Statistical analyses*
208 Brood surface
209 Repeated measures ANOVA was done to compare brood surface variations over
210 time. Following a significant effect, some contrasts were calculated to better understand
211 the effect and some Fisher's protected least significant difference (LSD) multiple
212 comparisons were also used to compare, two by two, the levels of the effect of interest.
213 The variable was transformed ($[brood/1000]^2$) to satisfy the assumptions of the model,
214 where brood represents the total number of brood cells (sealed + unsealed cells) obtained as
215 described in the section entitled «Brood surface evaluation». This transformation was
216 chosen among the Box-Cox family (Montgomery 2009) and best fitted the data. The
217 dependency structure that best described the data was the autoregressive covariance
218 structure. The assumption of normality was verified using the Shapiro-Wilk test, while the
219 homogeneity of variances was verified using traditional residual plots. All statistical
220 analyses were performed using SAS-STAT proc MIXED (SAS Institute Inc. 2008) at the α
221 = 0.05 level of significance.
222
223 Pollen analyses

Diversity at each site was calculated using Simpson's index (Peet 1974). The analyses were done using MVSP 3.1 (MVSP 1998). A Wilcoxon test for paired samples was used to find significant differences between the site categories (SIMSTAT 1996). Jaccard community indices were measured between crop sites and their statistical significances were established using the statistical table provided by Real (1999). This statistical table shows the lower (J-(P)) and upper (J+(P)) critical values of Jaccard's index with the probability levels 0.05, 0.01 and 0.001, when any possible distribution for the N elements in the two OTUs (Operational Taxonomic Units) is considered. In this case, the probabilities associated with Jaccard's index depend only on the total number (N) of plant taxa identified via pollen analysis present in either crop sites (Real 1999). Finally, rarefaction curves were generated using R software (The R Foundation for Statistical Computing 2009).

Results

Pollen taxa abundance and richness

Examination of microscope slides of mounted pollen grains revealed 61 720 taxon occurrences over both years combined. In 2008, pollen grains included 43 and 32 different taxa from the blueberry and cranberry crop environments, respectively. In 2009, pollen grains included 54 and 34 different taxa for the same crops. During these two crops' pollination periods, the co-flowering plants in the study sites included trees, shrubs, herbaceous plants and grasses (Table I). Jaccard's community indices revealed that the plant taxa identified by pollen analysis differed greatly between these two crops and their

246 respective flowering periods in both 2008 (J-=0.154; N=33; P=0,0317) and 2009 (J-=0.110;
247 N=53; P<0,001).

248 In 2008, the dominant pollen taxa foraged by the bees were *Acer spicatum*
249 (Aceraceae) and *Nemopanthus* sp. (Aquifoliaceae) during the blueberry blooming period,
250 and *Trifolium* sp. (Fabaceae) and *V. macrocarpon* during the cranberry blooming period
251 (Table I). Overall, the 10 most dominant pollen taxa foraged by bees accounted for up to
252 89.9% and 90.8% of the total number of identified pollen pellets for June and July,
253 respectively (Table I).

254 Data analysis of the pollen harvested during the 2008 blueberry pollination period
255 showed that the Simpson's diversity indices were comparable for all site categories,
256 monocultures (0.925), mixed (0.934) and fields (0.942) (Table II A); and no significant
257 differences were found between sites using a Wilcoxon matched-pairs signed-rank test (P =
258 0.8741). For the cranberry crops, as in the blueberry crops, the Simpson's diversity indices
259 were comparable for all sites. However, the mixed sites (0.874) had a slightly lower
260 diversity index than the non-*Vaccinium* field (0.906) and monoculture (0.905) sites (Table
261 II A). No significant differences were found between sites using a Wilcoxon matched-pairs
262 signed-rank test.

263 In 2009, the most abundant pollen taxa recorded were *Taraxacum officinale*
264 (Asteraceae) and *Salix* sp. (Salicaceae) in June (blueberry) and plants from the Brassicaceae
265 family followed by *V. macrocarpon* in July (cranberry). The 10 most dominant taxa
266 collected by bees accounted for up to 74.9% and 96.3% of the total taxa occurrences in
267 June and July, respectively (Table I).

The Simpson's diversity indices were comparable for all site categories during the blueberry pollination period: monocultures (0.968), mixed (0.972) and non-*Vaccinium* fields (0.966). These indices were higher than those found during the cranberry pollination period. For this period, the monoculture sites had a lower Simpson's diversity index (0.845) compared to the non-*Vaccinium* field sites (0.912), with the latter being more comparable to the mixed sites (0.896) (Table II B). During the blueberry blooming period, floral diversity had a tendency to be poorer in the monoculture sites than in the non-*Vaccinium* field sites, but these differences were not statistically significant. However, in July (cranberry blooming period) a significant difference in the pollen diversity foraged by bees between the monoculture and non-*Vaccinium* field sites was confirmed by the Wilcoxon signed-rank test ($z = -2.82$; $P = 0.0047$). The non-*Vaccinium* field sites also differed significantly from the mixed sites ($z = -1.97$; $P = 0.0048$).

Species rank abundance curves comparing the blueberry and cranberry pollination periods and years (2008 and 2009) showed that the abundance of pollen species most foraged by bees declined much faster in the cranberry crops than in the blueberry crops for both years (Figure 1). This graph also illustrates the high number of taxa with very few occurrences.

Species accumulation curves, excluding rare species, showed that the expected number of pollen taxa collected by bees from the blueberry and cranberry environments were 19 and 10 in 2008 (Figure 2 A) and 25 and 9 in 2009, respectively (Figure 2 B). Both curves quickly reached a threshold, with that of the combined samples from the cranberry crop sites below that of the combined samples from the blueberry crop sites, both in 2008 and 2009. In 2008, pollen from twice as many taxa was collected by bees in blueberry sites

as compared to cranberry sites. In 2009, bees collected almost three-fold as many pollen species in blueberry sites. The plateau phase of the curves indicates that sampling effort was sufficient during both years for the two cropping environments. These results also demonstrate that by collecting as few as 10 samples, one could obtain around 80% of potential bee-collected pollen in blueberry, while an effort of 20 samples would probably capture around 95% of pollen species collected by honey bees in this crop. In cranberry, as few as 10 samples from bees and pollen traps would capture close to 100% of the pollen taxa foraged by honey bees.

The complete lists of pollen taxa collected by bees and their relative abundance are provided for each year of the study (Tables III and IV).

Brood surface

The repeated measures ANOVA table illustrated in Table V shows a significant interaction Site*Date ($F=2.36$, $df=6, 82$, $p=0.0371$). The contrasts revealed that colonies from all category sites were similar at the beginning of the experiment ($F=0.05$, $df=2, 82$, $p=0.9525$) with an average of $30\,252 \pm 1090$ brood cells. The contrasts revealed a significant effect only at the second sampling date, June 25 ($F=4.08$, $df=2,82$, $p=0.0205$). The LSD multiple comparisons showed that the significant difference involved non-*Vaccinium* fields and monoculture sites, with a higher number of brood cells in colonies from non-*Vaccinium* fields ($t = -2.78$, $df=82$, $p=0.0067$). This result indicates that colonies in monocultures suffered low brood development during blueberry pollination (Figure 3). Finally, the contrasts found no difference for the third and the fourth sampling dates, i.e.

July 22 and August 26 ($F=1.17$, $df=2,82$, $p=0.3151$ and $F=0.83$, $df=2,82$, $p=0.4413$ respectively), suggesting that after the cranberry pollination period, colonies in monocultures were able to recover from the low brood development they momentarily underwent during blueberry pollination period (Figure 3).

Discussion

Identification of pollen pellets collected in traps and removed from honeybees' hind legs allowed us to characterize the flora visited by pollen foragers in the most important regions known for blueberry and cranberry crop production in Quebec. The listing of harvested pollen taxa showed the high number of plants foraged by honeybees in both regions. However, many plant taxa were represented by a very small number of pellets, suggesting the tendency of honey bee colonies to optimize their foraging activities. In many ways, the results also revealed access to a richer flora during the blueberry pollination period in June than during the cranberry pollination period in July. There was a higher relative abundance of the combined 10 most dominant pollen taxa collected by bees during the blueberry flowering period compared to the cranberry flowering period. This is confirmed by examining the species rank abundance curves, which also showed that the richness of the most abundant pollen species collected by bees was lower during the cranberry pollination period. Moreover, the low Jaccard's index measure comparing the cranberry and blueberry crops demonstrated that the pollen collected by bees differed greatly between the two crop regions, but this difference could also be explained by the succession of plants during the season.

336 The species accumulation curves showed that the honey bees had access to a greater
337 number of plants with pollen resources during the blueberry blooming period than during
338 the cranberry blooming period. These curves rapidly reached a threshold, revealing that the
339 number of pollen samples was high enough to characterize the richness of the flora
340 (Thompson and Withers 2003). The combined sampling methods, i.e., the collection of
341 pollen via the traps and captures of foragers, thus allowed an exhaustive sampling of plant
342 taxa foraged for pollen. However, the data that enabled us to evaluate the relative
343 abundance of each taxon are not as accurate as the estimate of their richness. For instance,
344 the size of the pellets formed by the foragers differed among plant taxa and can change
345 according to climatic conditions. Therefore, the biggest pellets were easily removed from
346 the honey bee's hind legs by the pollen trap meshing while the smaller pellets were not.
347 This may explain the low proportion of blueberry pollen harvested in the pollen traps, for
348 the foragers tend to form half-load pellets (i.e., very small pellets) with *V. angustifolium*
349 pollen because the grains of this species do not hold together well (Hodges 1974).
350 Another bias in the way we assessed floral diversity is the fact that the visited plant
351 taxa identified using the second method (captures of foragers) only represent a specific time
352 of day, since the foragers were captured within a five- to ten-minute period during each of
353 our visits to the sites. It is known that honeybee colonies from a single apiary placed within
354 the same habitat do not forage exactly the same flora (Louveaux 1954; Synge 1947).
355 Nonetheless, since we could only install a single pollen trap at each site, the capture of
356 foragers still complements the data we gathered using the traps. We believe that installing
357 one or more additional pollen traps at each site would have yielded negligible new

358 information, since the species accumulation curves show that the sampling effort was
359 already exhaustive.

360 Evaluation of changes in total brood surface (sealed and unsealed brood) was used
361 to evaluate the development and health of honey bee colonies. In June, when the hives were
362 placed in blueberry fields, results showed that the colonies in the monoculture sites reduced
363 their brood rearing, probably due to nutritional deficiencies. However, comparison of the
364 diversity and richness of pollen entries by bees does not suggest that the colonies
365 introduced in blueberry monocultures had access to a much poorer flora than those
366 introduced in the mixed sites. It is important to remember that pollen data alone do not
367 reveal the effort (e.g., flying range, number of trips) the foragers had to make to gather the
368 high diversity of pollen observed in the traps. Therefore, we speculate that in the
369 monoculture sites, the foragers most likely had to fly much farther to accumulate a
370 comparable abundance and richness of pollen species than those from colonies placed in
371 the mixed and field sites, where the floral resources were probably more easily available.
372 Moreover, the dominant pollen taxa foraged by bees in each site category were different,
373 with a different pollen quality (Ramsay 1987). In the blueberry monoculture sites, the four
374 main pollen taxa collected by the bees were *Alnus* sp. (14.8%), *Taraxacum officinale*
375 (11.9%), *Vaccinium angustifolium* (10.4%) and *Picea* sp. (10.3%). *Alnus* pollen is of poor
376 quality for brood rearing and *Picea* has a very poor protein content (Ramsay 1987). In
377 addition, according to Loper and Cohen (1987), dandelion pollen is low in tryptophan,
378 phenylalanine and arginine and does not foster brood rearing. Finally, the third most
379 important pollen collected by the bees whose hives were placed in blueberry monocultures
380 was from the lowbush blueberry itself, *V. angustifolium.* This plant genus has a very low

protein content (13.9% crude protein content; Somerville and Nicol 2006)). In the mixed sites, the three main pollen taxa were *Salix* spp. (19.1%), *T. officinale* (14.9%) and *Rubus* type (8.7%). Finally, the colonies introduced in the non-*Vaccinium* field site category foraged mainly on *Salix* spp. (18.3%), *T. officinale* (16.0%) and Brassicaceae (15.4%). *Salix* spp*., Rubus* spp. and Brassicaceae pollen have an excellent protein quality and content (very poor protein content = 10% vs excellent = 30% according to Ramsay, 1987), thus allowing normal brood rearing in colonies placed in the mixed and non-*Vaccinium* field site categories.

In July, the colonies in each site category had access to a better quality of pollen in terms of protein percentage and amino acid content, with plants such as the Brassicaceae*, Trifolium* spp*., V. macrocarpon, Oenothera biennis* and *Rubus* type (Loper and Cohen 1987; Ramsay 1987). This may explain why colonies that had suffered brood rearing problems in blueberry monocultures recovered and started rearing broods as fast as the colonies introduced in the mixed sites for the cranberry flowering period. The brood surface of the colonies introduced in the non-*Vaccinium* field sites declined slightly in July because at least two out of 12 colonies swarmed during this period. No swarm was detected in the colonies that had been introduced in the two other site categories.

Conclusion

The results of this study suggest that colonies may suffer from a nutritional deficiency during their stay in large-scale blueberry crop in which weeds are intensively managed. This can affect honey bee colony brood development quite rapidly, although the effects are only observable a few weeks later by beekeepers in the field. Our finding

404 reflects results from a recent study by Odoux et al. (2012) in which the authors
405 demonstrated that weeds are a critical source of pollen for honey bees in agrarian
406 environment.

407 In the context of commercial pollination services, competition between the crop and the
408 surrounding flora is of concern for berry growers. Competition can occur when one plant
409 produces more pollen and/or nectar than another (Feinsinger 1987), but in monocultures,
410 flowers from the crop are always higher in number than field margin flora. According to the
411 optimal diet theory described in Wells and Wells (1983), pollinators will search for the
412 closest food source to obtain the greatest reward, i.e., the crop they are introduced in. If the
413 pollinated crop does not produce enough nectar (e.g., cranberry), alternative flora is needed
414 to meet the colonies' nutritional needs. Although interactions between flowers of different
415 species are often thought to be competitive, facilitation can occur. Moeller (2004) and
416 Rands and Whitney (2009) explained that different plant species in an area can act together
417 to attract and maintain a population of pollinators and thus contribute to enhancing the
418 pollination of a monoculture. Further experiments would be needed following our system to
419 determine whether such facilitation occurs with with lowbush blueberry and cranberry.

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Table I. Ten most dominant pollen taxa (%) collected by honey bees during A) the blueberry pollination period (June 2008 and 2009) and B) the cranberry pollination period (July 2008 and 2009); average of the three site categories combined.

A)

2008		2009	
Taxa	%	Taxa	%
Acer spicatum	31.1	*Taraxacum officinale*	14.2
Nemopanthus sp.	10.9	*Salix* sp.	13.4
Taraxacum officinale	8.8	Type *Rubus* sp.	9.4
Sambucus pubens	8.3	*Alnus* sp.	7.5
Rosaceae (shrubby)	7.7	*Sambucus pubens*	5.7
Type* *Rubus* sp.	7.0	Type *Malus* sp.	5.5
Cornus canadensis	4.8	*Carex* sp.	5.2
Cornus stolonifera	4.3	Brassicaceae family	5.2
Prunus pensylvanica	3.5	*Picea* sp.	4.5
Picea sp.	3.4	*V. angustifolium*	4.3
Total	89.9	Total	74.9

B)

2008		2009	
Taxa	%	Taxa	%
Trifolium sp.	57.8	Brassicaceae	29.7
V. macrocarpon	13.5	*V. macrocarpon*	23.5
Thalictrum pubenscens	3.3	*Trifolium* sp.	20.9
Type *Rubus* sp.	2.9	Type *Rubus* sp.	10.9
Ilex sp.	2.6	*Oenothera biennis*	5.0
Rhus typhina	2.4	*R. typhina*	2.4
Brassicaceae family	2.3	Type *Spiraea latifolia*	1.3
Hypericum perforatum	2.1	*Lotus corniculatus*	1.1
Lathyrus Latifolius c.f.	2.0	*Polygonatum fagopyrum*	0.8
Sambucus canadensis	1.9	*Thalictrum pubenscens*	0.7
Total	90.8	Total	96.3

*Type = Used to indicate the genders and species represented by the same morphological type.

Table II. Simpson's diversity indices for each site category during the blueberry and cranberry pollination periods both in A) 2008 and B) 2009.

A)

Site category*	Simpson's diversity indices	
	Blueberry (June)	Cranberry (July)
Monocultures	0.925	0.905
Mixed	0.934	0.874
Non-*Vaccinium* fields	0.942	0.906

B)

Site category*	Simpson's diversity indices	
	Blueberry (June)	Cranberry (July)
Monocultures	0.968	0.845
Mixed	0.972	0.896
Non-*Vaccinium* fields	0.966	0.912

472 **Table III.** Bee harvested pollen taxa and their relative abundance (%) for each crop
473 (geographic region) and each site category in 2008.

Blueberry (Côte-Nord)				Cranberry (Bois-Francs)			
Taxa	M	MX	F*	Taxa	M	MX	F*
Acer spicatum	38,2	33,8	19,1	*Apocynum androsaemifolium*	0,3	1,6	0,6
Alnus sp.	5,4	0,0	0,0	Brassicaceae	11,5	0,1	2,4
Aralia nudicaulis	0,0	0,0	0,0	Compositae	0,0	0,0	0,0
Carex sp.	0,6	0,5	0,0	*Epilobium angustifolium*	0,0	3,0	1,1
Clintonia borealis	0,0	0,4	0,6	*Hypericum perforatum*	4,1	2,0	2,5
*Clintonia borealis c.f.***	0,0	0,0	0,8	*Ilex verticillata*	0,5	2,9	2,8
Compositae	0,0	0,3	0,2	*Lathyrus latifolius c.f.*	1,3	1,9	3,1
Cornus canadensis	8,3	5,1	0,3	Liliaceae	0,8	0,5	0,5
Cornus stolonifera	8,2	2,4	2,2	*Lotus corniculatus*	0,3	3,6	1,8
Ericaceae	0,2	0,4	0,3	*Melilotus alba*	0,0	0,0	0,3
Fragaria sp.	0,0	0,9	0,8	Poaceae	0,0	0,1	0,0
Iris sp.	0,4	0,6	0,3	*Polygonum fagopyrum*	3,5	0,2	0,5
Ledum groenlandicum	0,2	0,0	0,5	*Ranunculus acris*	0,0	0,0	0,1
Liliaceae	0,4	0,6	0,3	*Rhus radicans. c.f.*	0,0	0,0	0,3
Lonicera tatarica	0,0	0,0	1,2	*Rhus typhina*	0,0	3,9	6,5
Nemopanthus sp.	18,2	7,2	6,7	Rosaceae arbustive	0,4	0,0	0,1
Picea sp.	2,4	1,1	7,1	*Rubus* sp.	1,0	0,3	1,2
Pinus sp.	0,3	0,3	0,0	*Sambucus canadensis*	5,3	0,5	1,9
Potentilla norvegica	0,1	0,5	0,0	*Sambucus pubens*	0,0	0,0	0,1
Prunus pensylvanica	1,4	2,3	7,9	*Sorbus americana*	0,2	0,0	0,0
Prunus virginiana	0,4	0,0	0,0	*Taraxacum officinale*	0,0	0,0	0,1
Ranunculus acris	0,4	0,0	0,6	*Thalictrun pubescens*	0,8	3,4	5,2
Rosaceae arborescente	0,9	2,1	1,0	*Trifolium*	27,7	65,9	62,6
Rosaceae arbustive	3,8	5,4	14,8	Type *Rubus*	5,0	3,4	3,1
Rumex acetosella	0,3	0,0	0,0	Type *Thalictrum*	0,0	0,0	0,0
Salix sp.	0,0	0,0	0,6	Type *Vicia*	0,0	0,0	0,1
Sambucus pubens	0,0	10,1	16,9	*Typha latifolia*	0,9	0,0	0,2
Sisyrinchium bermudiana	0,0	0,1	0,0	*Vaccinium macrocarpon*	35,9	6,4	2,8
Sisyrinchium bermudiana c.f.	0,5	0,5	0,2	*Vicia cracca*	0,3	0,0	0,1
Sorbus americana	1,3	0,0	0,0				
Spores	0,0	0,0	1,6				
Taraxacum officinale	0,2	21,2	3,2				
Trifolium repens	0,1	1,1	0,9				
Type *Rubus****	7,4	3,0	11,5				
Type *Syringa*	0,0	0,0	0,4				
Vaccinium angustifolium	0,3	0,1	0,0				

474

475 **Table IV.** Bee harvested pollen taxa and their relative abundance (%) of taxa for each crop
476 (geographic region) and each site category in 2009.

Blueberry (Lac Saint-Jean)				Cranberry (Bois-Francs)			
Taxons	M	MX	F	Taxons	M	MX	F
Acer spicatum	0,0	0,1	0,1	*Apocynum androsaemifolium*	0,0	0,2	0,1
Alnus sp.	14,8	7,6	0,0	*Asclepias syriaca*	0,1	0,0	0,3
Aralia nudicaulis	0,0	0,0	0,2	Brassicaceae	17,3	23,3	48,4
B****	0,0	0,0	0,1	*Cirsium arvense*	0,0	0,0	0,1
Brassicaceae	0,1	0,1	15,4	Compositae	0,0	0,0	0,1
Carex sp.	9,2	5,6	0,9	*Epilobium angustifolium*	0,1	0,2	0,0
Cassandra calyculata	0,0	1,3	0,0	*Hypericum perforatum*	0,0	0,0	0,0
Clintonia borealis	0,0	0,0	0,0	*Ilex verticillata*	0,0	0,2	0,0
Cornus canadensis	3,7	0,0	0,0	J	0,0	0,1	0,2
Cornus stolonifera	2,3	2,1	2,5	*Linaria vulgaris*	0,0	0,0	0,8
F	0,0	0,4	0,1	*Lotus corniculatus*	0,0	2,8	0,4
Fragaria sp.	0,0	0,4	0,2	*Melilotus alba*	0,1	0,2	1,0
G	0,0	0,0	0,1	Mixtes	0,8	1,3	0,0
Ledum groenlandicum	0,2	0,3	0,0	*Oenothera biennis*	0,0	2,5	12,6
Liliaceae	0,6	3,7	0,0	P	0,0	0,0	0,2
Lonicera canadensis	0,0	0,7	0,0	*Polygonum fagopyrum*	2,3	0,0	0,1
Lonicera tatarica	1,2	0,0	0,0	*Ranunculus acris*	0,1	0,0	0,0
Menyanthes trifoliata	2,1	0,0	0,0	*Rhus typhina*	0,0	3,3	4,1
Mixtes	0,3	0,0	0,0	Rosaceae	0,2	0,3	0,2
Myrica gale	4,4	0,0	0,0	*Rudbeckia hirta*	0,0	0,1	0,0
Nemopanthus mucronatus	3,3	3,7	0,4	*Sambucus canadensis*	0,3	0,3	0,1
Picea sp.	10,3	2,9	0,2	*Sonchus arvensis*	0,0	0,1	0,0
Pinus divaricata	0,5	0,7	0,0	*Spiraea latifolia*	0,0	0,2	0,3
Potentilla argentea	0,5	0,1	0,0	*Taraxacum officinale*	0,0	0,2	0,0
Prunella vulgaris	0,3	0,0	0,2	*Thalictrum pubescens*	1,2	0,2	0,5
Prunus pensylvanica	0,4	3,3	1,2	*Trifolium hybridum*	9,9	39,1	13,8
Prunus virginiana	1,8	0,9	3,4	Type *Calystegia*	0,0	0,0	0,1
Q	0,0	0,0	0,3	Type *Carum c.*	0,2	0,0	0,1
Ranunculus acris	0,1	0,0	0,5	Type *Rhus t.*	0,0	0,0	1,4
Rhododendron sp.	1,4	0,0	0,0	Type *Rubus*	13,1	8,0	11,5
Rosaceae	0,0	0,0	0,3	Type *Spiraea l.*	0,1	0,9	2,8
Rumex acetosella	0,0	3,7	3,5	*Typha latifolia*	0,0	0,1	0,1
Salix sp.	2,8	19,1	18,3	*Vaccinium macrocarpon*	54,0	16,5	0,1
Sambucus pubens	5,4	5,9	5,8	*Vicia cracca*	0,0	0,0	0,3
Similacina stellata	0,1	0,5	0,0				
Spores	0,7	2,0	0,0				
Taraxacum officinale	11,9	14,9	16,0				

Trifolium hybridum	0,0	0,1	5,3
Type *Barbarea v.*	0,0	0,0	0,0
Type *Betula*	0,2	0,0	0,0
Type *Carum c.*	0,0	0,0	0,2
Type *Crateagus*	0,0	0,0	0,4
Type *Malus*	2,6	5,5	8,3
Type *Nemopanthus*	0,0	0,6	0,0
Type *Prunus v.*	0,0	0,1	0,1
Type *Rubus*	7,0	8,6	12,4
Type *Rumex a.*	0,0	0,0	0,1
Type *Salix*	0,0	0,5	0,0
Type *Sambucus p.*	0,1	0,5	0,0
Type *Sorbus a.*	0,0	0,6	0,0
Type *Sorbus/Amelanchier*	0,1	0,0	3,1
Type *Spiraea l.*	0,0	0,6	0,0
Type *Syringa*	1,0	0,0	0,3
Vaccinium angustifolium	10,4	2,6	0,0

480 **Table V.** Repeated measures ANOVA table for brood surface evaluation over time.

Source	df	F value	P value
Site	2	0.79	0.4659
Trap	1	0.12	0.7276
Site x Trap	2	0.36	0.7010
Error1= Hives(Site*Trap)	28		
Date	3	11.12	<0.0001
Site*Date	6	2.36	0.0371
Trap*Date	3	0.17	0.9191
Site*Trap*Date	6	1.03	0.4119
Error2 = Date*Hives(Site*Trap)	82		
Corrected Total	133		

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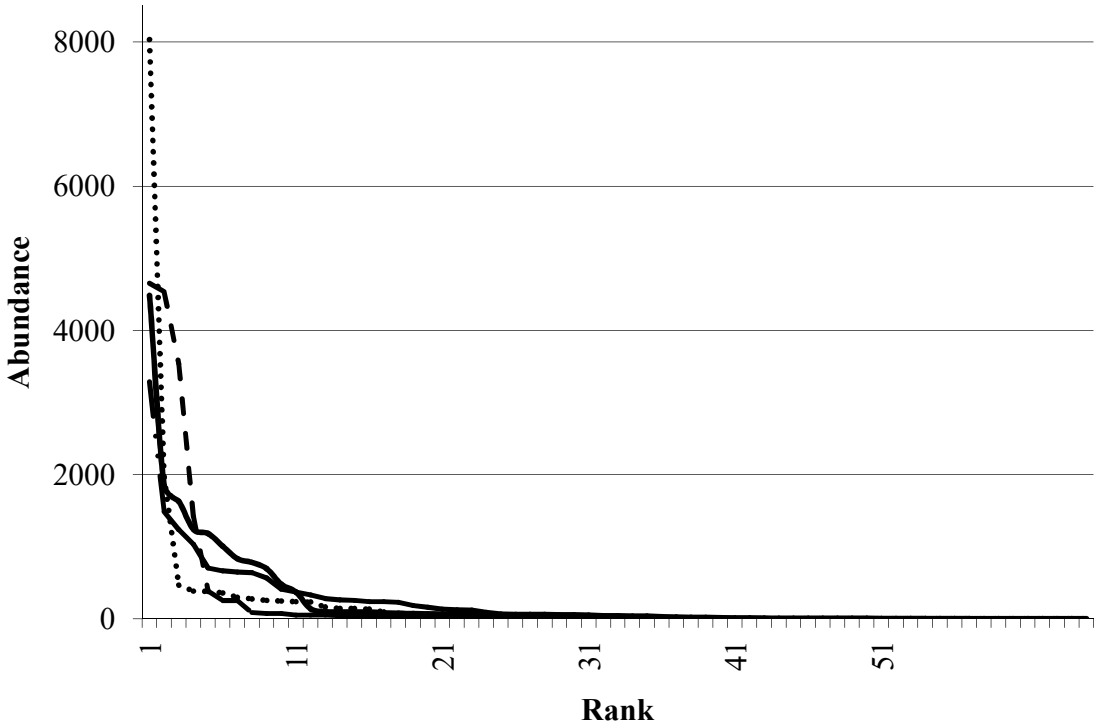
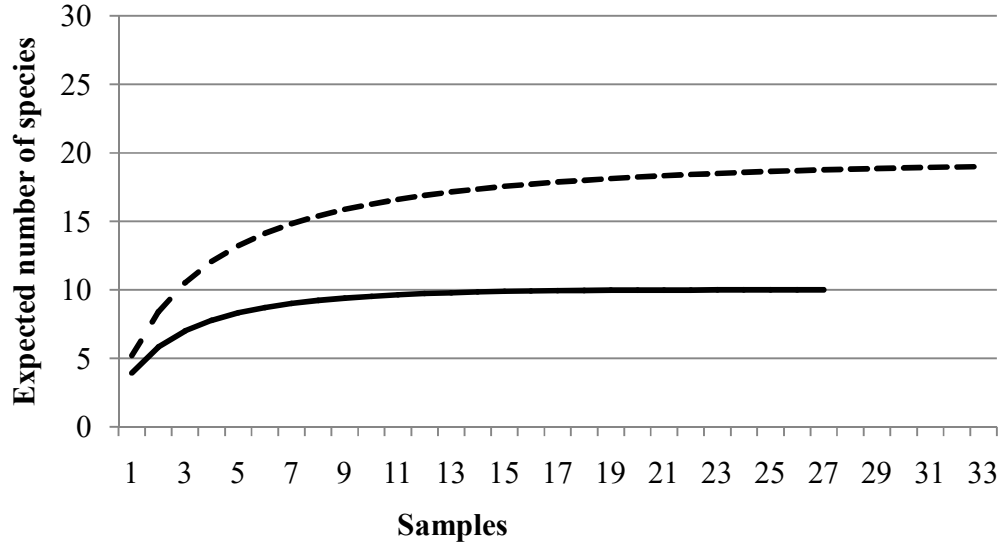


Figure 1. Species rank abundance curves for blueberry and cranberry crops for 2008 and 2009. The y-axis represents the number of pollen pellets (abundance) from a plant species in relation to its rank (x-axis).

504 **A)**



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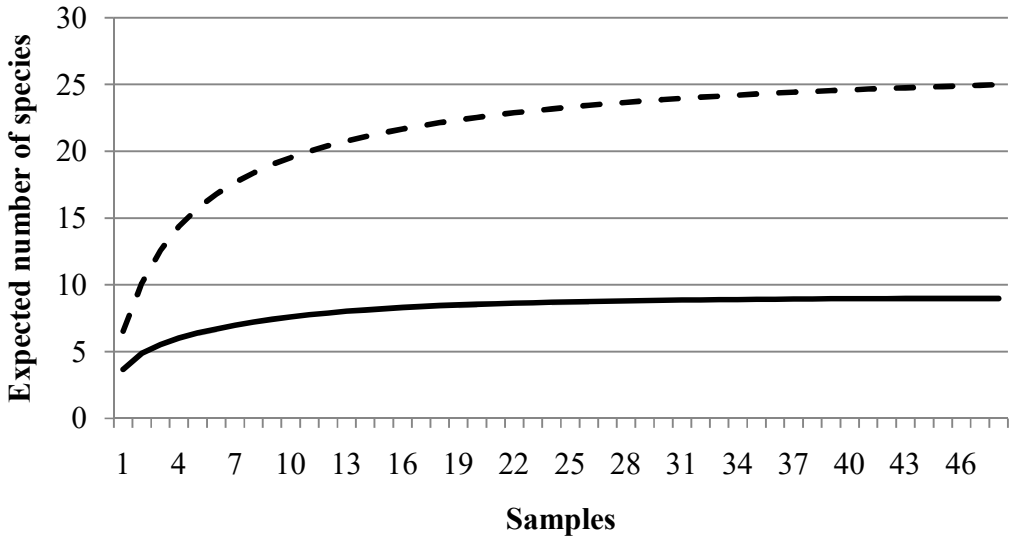
507 **B)**



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509 **Figure 2.** Species accumulation curves for blueberry and cranberry crops in A) 2008 and
510 B) 2009.

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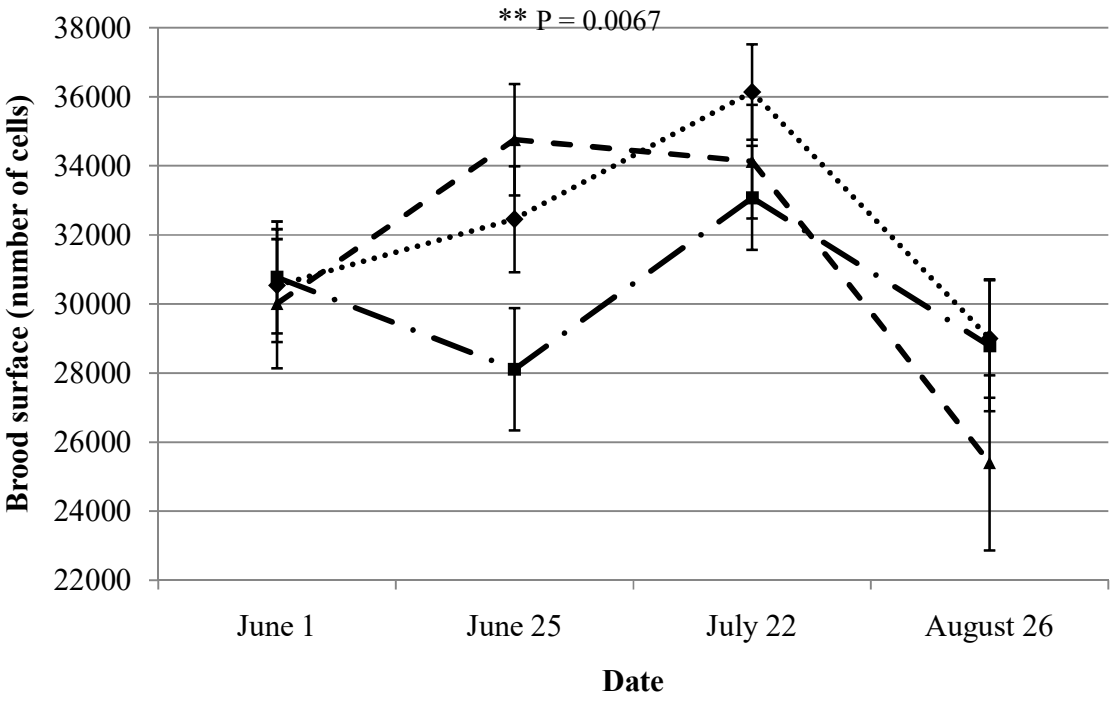

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516 **Figure 3.** Evolution of the total brood surface (# cells sealed + unsealed) before (June 1,
517 2009) and after (June 25, 2009) blueberry pollination, after cranberry pollination (July 22,
518 2009) and before overwintering (August 26, 2009).

519 Each dot represents the average brood surface of the 12 colonies introduced in one site
520 category. The symbol (**) indicates that the total brood surface from colonies introduced
521 in monocultures was significantly lower than the one from colonies in field sites at the end
522 of the blueberry pollination period (June 25, 2009).
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Legends

Table I

None

Table II

Monocultures: *Vaccinium* fields whose size were >15 ha and on which weeds were intensively managed.

Mixed: *Vaccinium* fields whose size were < 3 ha and on which weeds were left unmanaged.

Fields: non-*Vaccinium* fields.

Tables III and IV

* M (monocultures): *Vaccinium* fields whose size were > 15 ha and on which weeds were intensively managed.

MX (mixed): *Vaccinium* fields whose size were < 3 ha and on which weeds were left unmanaged.

F (non-*Vaccinium* fields): fields on which fodder plants are grown.

** c.f.*: confer* in Latin signifies close to.

*** Type is use to designate the taxa having very similar morphological characteristics.

**** The taxa named by a letter are pollen grains which could not be identified.

Table V

Two (one in Monoculture and one in non –*Vaccinium* sites) out of the 36 study hives had to be discarded for brood surface evaluation because all brood cells were found empty on the last sampling date (August 26). Queen failure was most likely the cause.

Figure 1

Crops and year Blueberry 2008 _____

Cranberry 2008 .....

Blueberry 2009 _.._.._.._

Cranberry 2009 _ _ _ _ _

Figure 2

Crops Cranberry _____

Blueberry _ _ _ _ _

Figure 3

Site categories Non-*Vaccinium* fields _ _ _ _ _

Mixed .....

Monocultures __.__.__._

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