

Linking pollinator behavior to selfing rate for three distinct pollinators of alfalfa

Emmanuel Santa-Martinez¹ and Johanne Brunet^{1,2}

¹Department of Entomology, University of Wisconsin-Madison, ²United States Department of Agriculture, ARS

Abstract

Insect pollinators are important to seed production but little is known about their impact on pollen dispersal and the amount of selfed seeds produced on a plant. Selfing can decrease the level of heterozygosity within populations and increase genetic differences among populations. High selfing can reduce fitness and yield, especially when inbreeding depression is high. Pollinator movements on a plant can affect selfing. We examined how the differences among pollinators in foraging behavior and pollen deposition patterns can affect a plant's selfing rate. Selfing rate was measured on an alfalfa field pollinated with distinct pollinators. Field-wide selfing rate was estimated at 27%. To compare the impact of these pollinators on selfing rate in alfalfa, we conducted individual greenhouse experiments with honey bees, common eastern bumble bees, and alfalfa leafcutting bees. We measured the number of open flowers per raceme, and number of racemes per plant to assess the effect of different floral displays on bee behavior and the impact on selfing rate. The results showed that large amount of flowers available on a plant can increase attractiveness and visitation rates of pollinators. Visiting more flowers in succession on a plant increases the selfing rate. An increase in the total number of open flowers per raceme and in the number of racemes per plant increase selfing rates with the three bee species studied. This study will shed light on how distinct pollinators affect plant mating systems and the genetic structure of plant populations.

Introduction

Selfing

Decrease in heterozygosity

- Deleterious alleles in homozygous plants

Negatively impact plant fitness

- Inbreeding depression = reduction in seed yield

Selfing rate

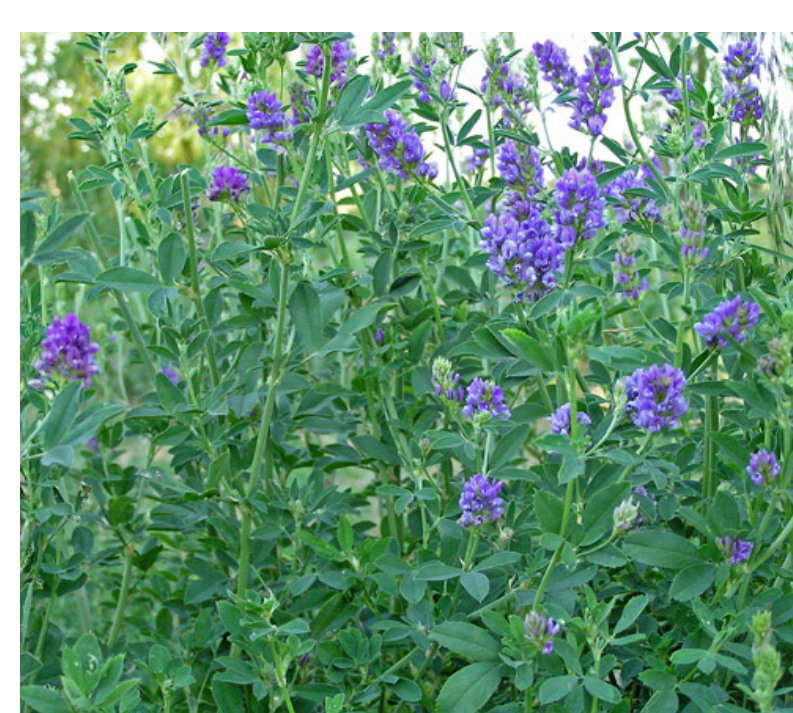
Selfing rate: the proportion of the seeds on the plant that result from self pollination.

High selfing rate = reduce gene flow.

Selfing in agricultural crops is affected by how pollinators forage on plants and plants floral display size.

Pollinators visiting more flowers in succession on a plant can increase the level of geitonogamous (within plant) selfing.

Study species



Alfalfa, *Medicago sativa*

- Outcrossed perennial legume
- Tripping mechanism
- Seed and hay production
- Relies on insect pollinators for seeds
- 28 million kg of seeds per year in USA



Bombus impatiens
Bumblebee



Apis mellifera
Honeybee



Megachile rotundata
Alfalfa Leafcutting bee

Methodology

1) Before bee behavior experiments:

Selected thirty alfalfa plants with unique genotypes

- 3 clones per plant (one per bee species)
- 10'x10'x6' cages in a large greenhouse

Each plant had a different floral display:

- Count the number of racemes per plant.
- Count the number of flowers in marked racemes

2) Bee behavior experiments:

For each bee species foraging bout (n=20) we measured:

- The number of plants visited
- The number of racemes visited per plant
- The number of flowers visited per raceme

3) After bee behavior experiment:

Counted fruits and seeds developed in marked racemes:

- 24 seeds selected to genotype per plant (30 plants)



Greenhouse setting for each bee type



Thirty unique alfalfa plants with different genotypes and floral display size.

Estimating Selfing Rate in Field and Bee Greenhouse Experiments

Used **Microsatellites (SSR)** for paternity analysis

DNA extracted from leaf tissue (mother) and seeds (offspring)

Genotypes of DNA samples were scored with **12 SSRs**

Multilocus genotypes of mothers and offspring analyzed using an Exclusion-based paternity

- SAS code developed by Heathcliffe Riday

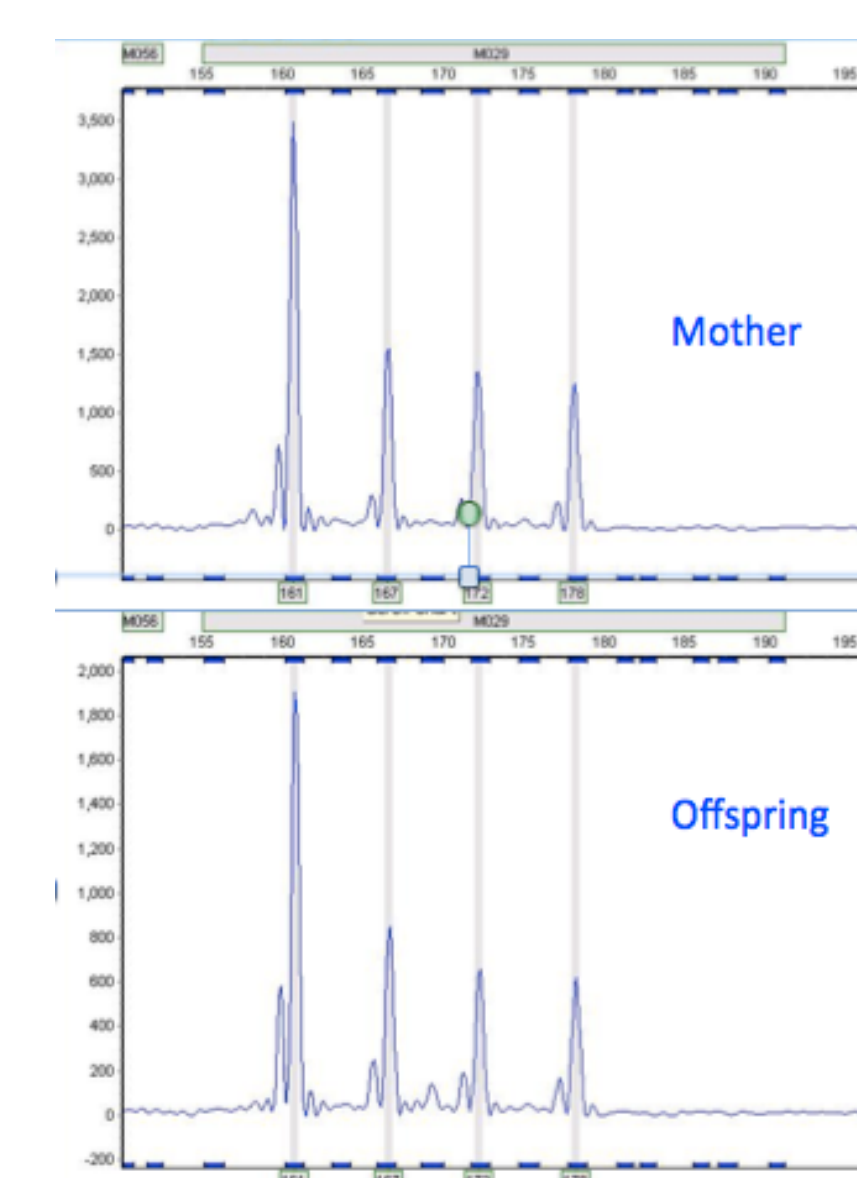


Fig 1. Example of comparing mother and offspring genotypes. If alleles observed in progeny = alleles observed in maternal plants, self-fertilization may occur. Multiple loci are needed for a better estimation of selfing rate.

Results

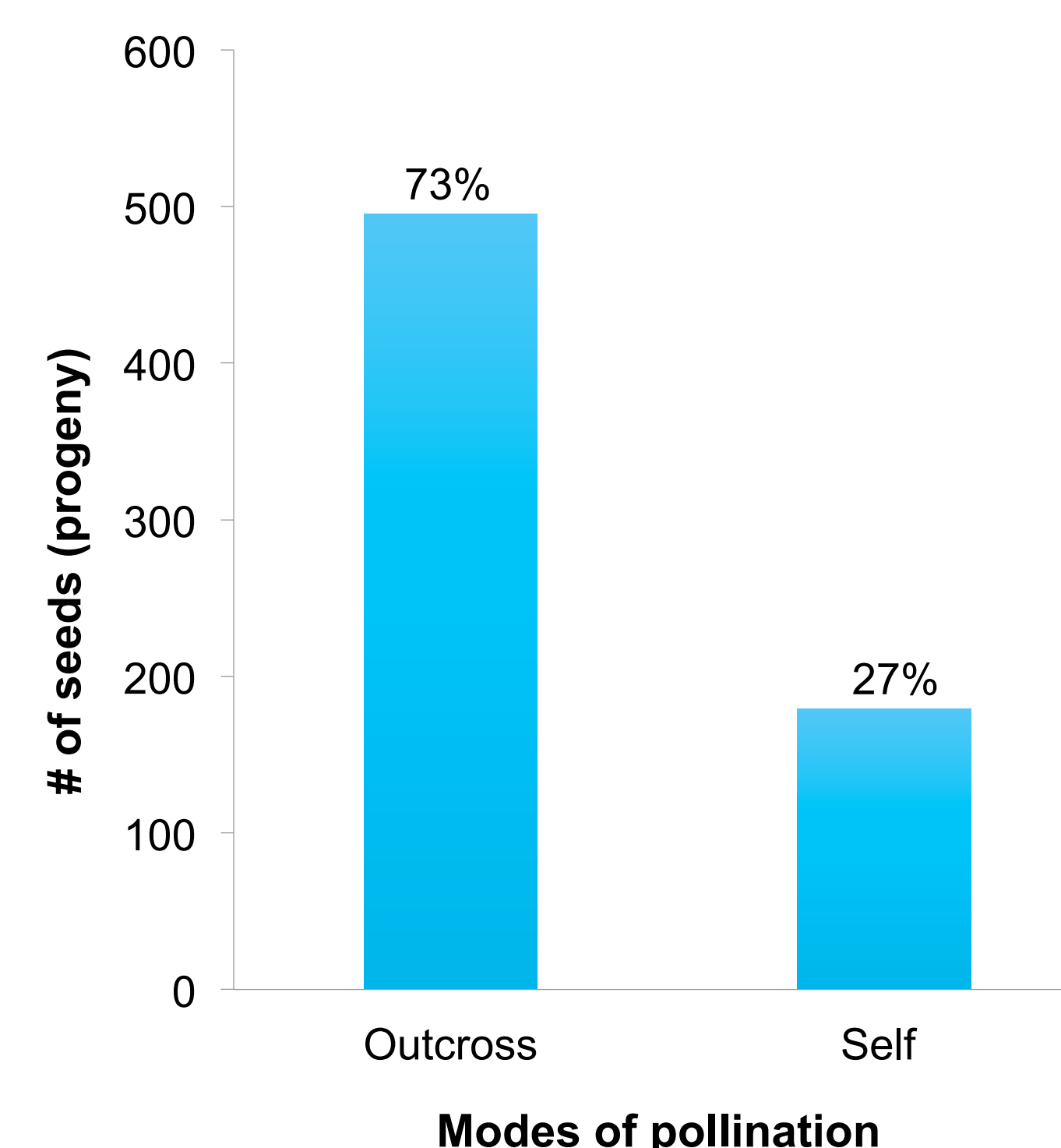


Fig 2. Selfing rate in an alfalfa field setting pollinated by the three distinct pollinators

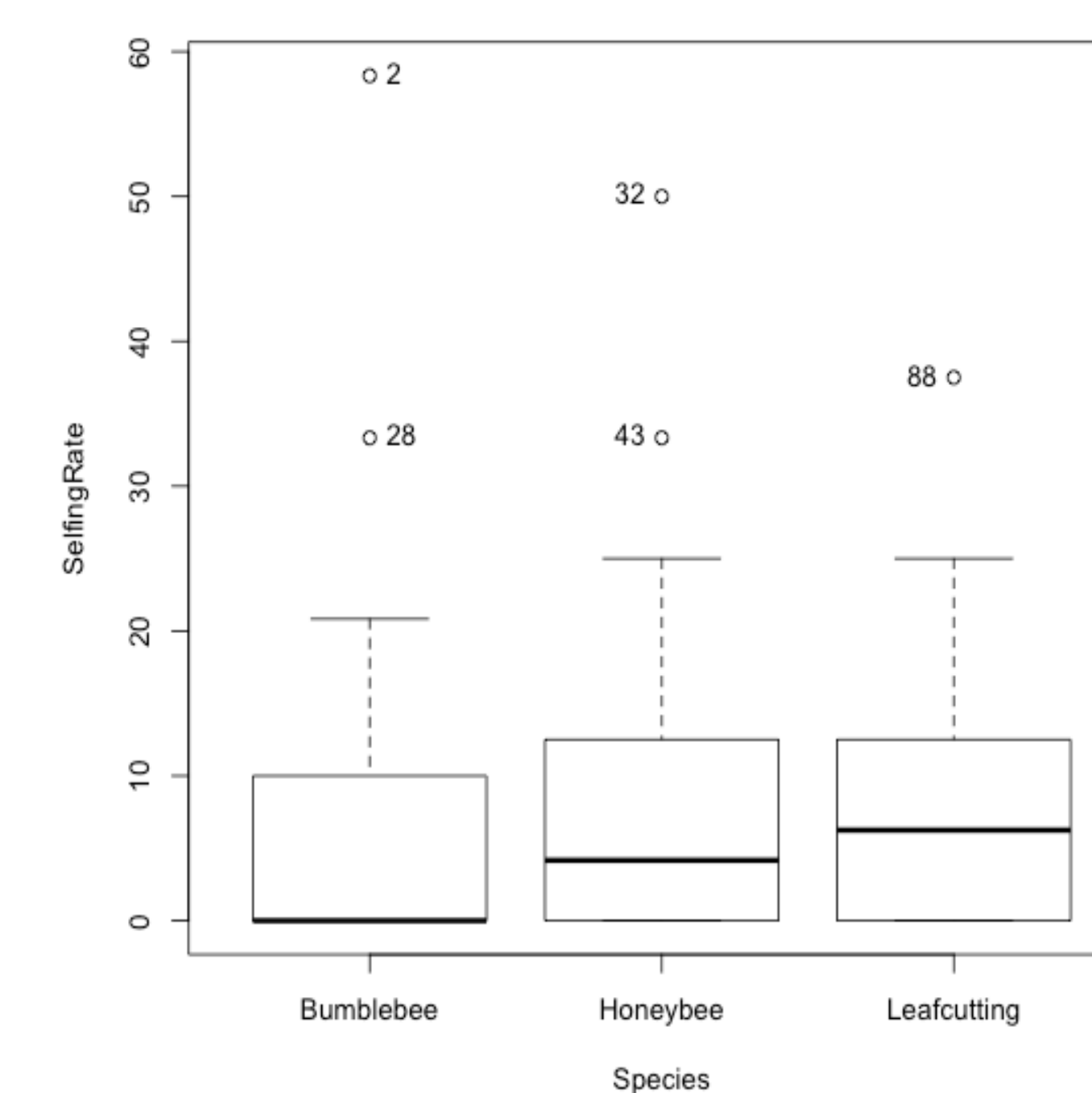
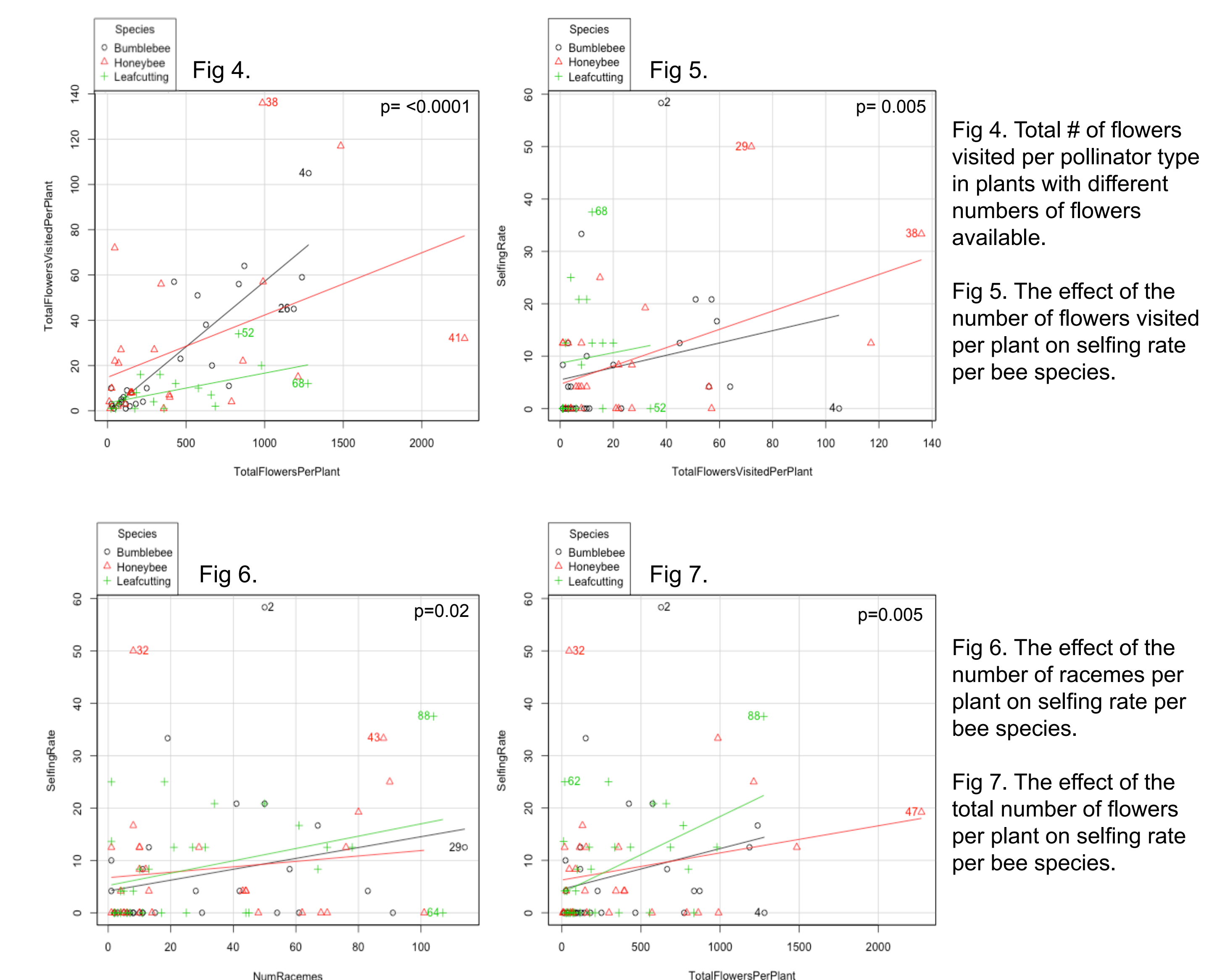


Fig 3. The impact of three bee species on selfing rate in alfalfa (bee greenhouse experiments).

Results



Discussion

Selfing rate in the field was 27%, which is high for a plant that relies mainly on outcross pollination.

Pollinator type does not influence selfing. Fields using different managed pollinators may not exhibit differences in selfing rate.

Large amount of flowers available on a plant can increase attractiveness and the visitation rates of pollinators.

Visiting more flowers in succession on a plant increase selfing rate (geitonogamous selfing).

Higher selfing rates in plants with larger floral displays:

- Selfing rate increases with the number of racemes per plant.
- Selfing rate increases with the number of flowers per plant

In well-managed alfalfa seed production fields we expect geitonogamous selfing in plants with larger floral displays.

Large floral displays > increase visitation rates > higher selfing rate = inbreeding depression = decrease in seed yield

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Contact information: E. Santa: santamartinez@wisc.edu, J. Brunet: jbrunet@wisc.edu