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INSECT POLLINATORS

Diversity of Floral Visitors in Apple Orchards: Influence on Fruit Characteristics Depends on Apple Cultivar

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Abstract

Most cultivars of apple trees are highly dependent on insects for successful pollination and fruit production. In this study, we evaluated the insect diversity in apple orchards of southern Brazil and verified whether or not there is a relationship between the diversity of insect visitors and the characteristics (weight, seed number, and symmetry) of the fruits of 'Fuji' and 'Gala' apples produced by the orchards. We also evaluated the diversity of insects on flowering weeds within apple orchards and compared it with the apple flowers. Diversity of anthophilous insects was low, in general, and differed between the regions. Furthermore, regarding insect diversity, orchards were grouped by management system: organic orchards were more similar to each other than to conventional orchards. The insect diversity of weed flowers was higher than apple flowers, but insect abundance was greater on apple flowers, suggesting that weeds may increase insect diversity within apple orchards and may sustain pollinators. We found a positive effect of insect diversity on the number of seeds of 'Fuji' apples and of honeybee abundance on their weight, suggesting that honeybee management is important in the studied areas. In contrast, we found no significant effect of insect diversity and abundance on 'Gala' apple characteristics. Despite this, the analyses of the seeds of 'Gala' apples indicate that the orchards may suffer a pollination deficit, which could be overcome by improving insect pollination. These results reinforce previous findings that insect diversity is important for apple yield, but its influence varies with cultivar.

Introduction

Biodiversity influences the functioning and provision of ecosystem services (Allen-Wardell *et al* 1998, Díaz *et al* 2006, Brittain *et al* 2013). One of these services is the pollination provided by animals, which impacts both wild (Ollerton *et al* 2011) and agricultural plants (Klein *et al* 2007, Giannini *et al* 2015). For agriculture, around 70% of the crops are dependent on animal pollination and they are responsible for 35% of the volume of the agricultural production (Klein *et al* 2007). Estimates on the loss of all agricultural production without animal pollinators vary but range from 5 to 8% (Aizen *et al* 2009) to 9.5% and the insect pollination value has been estimated in €153 billion (Gallai *et al* 2009).

Beyond the economic value, pollination has also a nutrition value. Despite the greatest amount of food and calories come from crops that do not require animal pollination (Prescott-Allen & Prescott-Allen 1990, Klein *et al* 2007), most lipids, vitamins A, C, and E, and a great part of the minerals calcium, fluoride, and iron, an important part of human diet, are provided by animal pollinated plants (Eilers *et al* 2011, Chaplin-Kramer *et al* 2014). Although effects can vary among human populations and nutrients (Chaplin-Kramer *et al* 2014, Ellis *et al* 2015), food security could be endangered by the reduction of the yield of these crops due to pollinators' decline, leading to micronutrient deficiency (Eilers *et al* 2011, Chaplin-Kramer *et al* 2014, Smith *et al* 2015).

Fruits are important sources of vitamins and micronutrients (Eilers *et al* 2011) and several fruit crops depend on animal pollination (Klein *et al* 2007, Giannini *et al* 2015), which even enhance the nutrient content of fruits, e.g., apple (Volz *et al* 1996, Buccheri & Di Vaio 2005, Garratt *et al* 2014a), almonds (Brittain *et al* 2014), and strawberries (Klatt *et al* 2014). Indeed, fruit is one of the crop categories for which insect pollination has a high value compared to the total value of the crop and, in the case of pollinator loss, production would be below the worldwide consumption (Gallai *et al* 2009).

Apple is a fruit crop which most cultivars require animal pollination (Delaplane & Mayer 2000) because insect pollinators are indispensable for pollen movement between cultivars in apple orchards that grow self-incompatible ones (Thomson & Goodell 2001, Jahed & Hirst 2017). These services have been provided by both wild and managed bees (McGregor 1976, Ortolan & Laroca 1996, Delaplane and Mayer 2000, Garratt *et al* 2014a, Viana *et al* 2014), but the last ones are represented by few species, e.g., *Apis mellifera* Linnaeus (honeybees) (Delaplane & Mayer 2000), *Bombus* spp. (bumble bees) (Thomson & Goodell 2001, Velthuis & Van Doorn 2006), and *Osmia* spp. (mason bees) (Delaplane & Mayer 2000, Vicens & Bosch 2000, Bosch & Kemp 2002). Honeybees are the sole managed pollinator available and/or used in many places and the most important worldwide (Morse 1991, Rucker *et al* 2012).

Although honeybees are effective apple pollinators (Stern *et al* 2001, Park *et al* 2016), they are less effective collecting nectar from the side of the flower (side-working) instead of from the top (Thomson & Goodell 2001, Schneider *et al* 2002) and when they collect nectar instead of pollen (Vicens & Bosch 2000). Furthermore, honeybees tend to forage for resources outside the orchards (Balfour & Ratnieks 2017). Honeybees are also less effective than other bee species, e.g., *Osmia cornuta* Latreille (Vicens & Bosch 2000) and *Bombus* spp. (Thomson & Goodell 2001), even though their abundance can compensate per-visit performance (Park *et al* 2016).

Wild bees, such as solitary bees, contributes much more to apple pollination and yield than it was often believed (Garratt *et al* 2014b, Mallinger & Gratton 2015, Földesi *et al* 2016); hence, the diversity of pollinators becomes important, because different species can be complementary (Tscharntke *et al* 2005, Sapir *et al* 2017) and also provide pollination stability (Allen-Wardell *et al* 1998, Díaz *et al* 2006). Indeed, insect diversity in general, not only bee diversity, influences apple yield and quality (Garratt *et al* 2014a, 2014b, Garibaldi *et al* 2016, Rader *et al* 2016). Honeybees, bumblebees (*Bombus terrestris* Linnaeus), solitary bees (*O. cornuta*), and syrphid flies (*Episyrphus balteatus* De Geer) produces apples with similar width and weight (Garratt *et al* 2016). Even though biotic and abiotic environmental factors acting on plant metabolism are important for apple yield and quality (e.g., Nava *et al* 2007, Neilsen *et al* 2008, Sawicki *et al* 2015), pollinators play an important role on these because apple weight and shape are influenced by seed number (Sheffield 2014, Sapir *et al* 2017). Seeds stimulate the growth of the ovaries, so fruits with more seeds are heavier (Sheffield 2014, Sapir *et al* 2017). Furthermore, if one or more sides of the ovary grows bigger than others, apples may become asymmetric (Sheffield 2014).

Apple orchards can sustain a large number of arthropod species, including insect pollinators (Cross *et al* 2015). Although bees have been the focus of most of the research on the topic (e.g., Russo *et al* 2015), other groups of insects are also crop pollinators (Delaplane & Mayer 2000, Garibaldi *et al* 2013, Garratt *et al* 2014a). This is the case of Diptera, especially Syrphidae, which are visitors of several crops and pollinate some (Inouye *et al*, 2015), including apple (Boyle & Philogène 1983, Garratt *et al* 2016). Although their pollinator role is not defined for apple, other insects, such as Coleoptera, are present on agricultural areas and contribute to crop production (Rader *et al* 2016).

Conservation of the insect diversity in agricultural areas depends, among other factors, on the maintenance of other plants within these areas (Nicholls & Altieri 2013, Kremen & M'Gonigle 2015, Campbell *et al* 2017). One source of food is the ground vegetation in orchards, but it is a common practice to mow this vegetation, because these plants would compete for pollinators, reducing their visitation on the target species. However, the few studies that have tested this assumption have shown that, in fact, the ground vegetation enhances the diversity of visitors on crop species (Carvalheiro *et al* 2011, Samnegård *et al* 2018) or has no effect on it (Holzschuh *et al* 2012). In the case of apple orchards, growers commonly mow the ground vegetation, which is a recommended management practice for the flowering and fruiting period (September to April in Brazil) (Hoffmann *et al* 2004).

For improving insect pollination on orchards and to conserve insects on agricultural areas, it is necessary to know their insect diversity (Russo *et al* 2015) and how this diversity affects production. The aim of this study was to evaluate the diversity of anthophilous insects in apple orchards of southern Brazil and to verify whether there is a relationship between their diversity and the characteristics (weight, seed number, and symmetry) of the fruits of "Fuji" and "Gala" cultivars produced by the orchards. "Fuji" and "Gala" cultivars represent around 30% and 60% of the apple production in Brazil, respectively (Petri *et al* 2011). Furthermore, we evaluated the diversity of insects on flowering weeds within apple orchards to compare it with the diversity of insects present on apple flowers.

Material and Methods

Study sites

The study was done in eight commercial apple orchards (Table 1), four of them in Rio Grande do Sul State (hereafter called area 1—A1) and four (Table 1) in Santa Catarina State (hereafter called area 2—A2), the two main apple production areas in Brazil. The distance between the two areas is approximately 138 km. The climate of both areas is humid subtropical, oceanic, without a dry season; however, while A1 presents a hot summer (Köppen's climate classification Cfa), A2 presents a temperate one (Cfb). In A1, the air temperature is between − 3and 18°C in the coldest month and ≥ 22°C in the hottest, and the rainfall in the driest month is > 40 mm. In A2, the air temperature of the hottest month < 22°C and on 4 months of the year the temperature is above 10°C, with an average annual temperature of 13°C (Alvares *et al* 2013).

The vegetation of the farms of area 1 was composed mainly by crops (e.g., other apple orchards, strawberry, kiwi, grape) with few fragments of forests (Atlantic Forests mixed with Araucaria). On area 2, it was composed of a mixture of grasslands and forested areas (fragments of Atlantic Forests mixed with Araucaria) and other apple orchards.

The agricultural practices on conventional orchards, for soil fertilization dolomitic limestone and conventional formulations containing NPK, were used, but also calcium chloride and leaf fertilizers containing NPK and micronutrients were used according to soil analysis recommendations. Insecticides and fungicides applied were the ones used for conventional production according to the pesticide grid recommended by the technical board of the Integrated Apple Production (PIM—"Produção Integrada de Maçãs"). On organic orchards consisted on using poultry manure as fertilizer, as well as rock dust, oxides and carbonates of Ca and Mg. Silicones and foliar fertilizers containing NPK and micronutrients, all certified for the use in this production system, were also used. Bordeaux mixture and lime sulfur were used and as insecticides *Bacillus thuringiensis*, neem oil, and sexual pheromones for mating interruption. On both agricultural practices and areas (1 and 2), there was no artificial irrigation; the water was from rain only.

Insect collection

In each orchard, two 50-m transects were determined side by side and each one was limited by two rows of apple trees, being one row common for the two transects (Fig 1). There were 50 trees in each row. Transect 1 was delimited by two rows of "Gala" trees (total: 100 trees) and transect 2 by one row of "Gala" trees (50 trees) and one row of "Fuji" trees (50 trees, Fig 1). Simultaneously, two people, one in each transect, while slowly walking the 50 m for 15 min, collected all flower visitors found in one of the rows of trees. The capture was done using a net and the collectors never collected on the same row of trees at the same time. When they reached the end of the transect, they came back collecting the insects in the other row of trees, also for 15 min (Fig 1). In total, each collector walked 100 m for 30 min per sampling, which was repeated at 10:00, 12:00 14:00 in the orchards of A1 and

Table 1 Location, size and cropping systems of the orchards and number of sampling days for each orchard in 2013 and 2014.

Area	Orchard	Location	Size (ha)	Cropping system	Number of sampling days	
					2013	2014
A1	1	S29°07′58.7″, W051°24′31.3″	6	Conventional	3	3
	2	S29°12′17.1″, W050°58′11.0″	125	Conventional	1	3
	3	S29°10′37.4″, W050°53′59.8	380	Conventional	1	4
	4	S28°53′38.5″, W051°22′56.6″	4	Organic	2	3
A2	5	S 28°15′31.2″, W49°53′38.0″	15	Conventional	0	4
	6	S 28°15′0.5″, W49°53′27.0″	6.5	Organic	0	4
	7	S 28°17′37.2″, W49°52′54.0″	4.1	Organic	0	4
	8	S 28°17′58.0″, W49°53′41.1″	2.3	Conventional	0	4

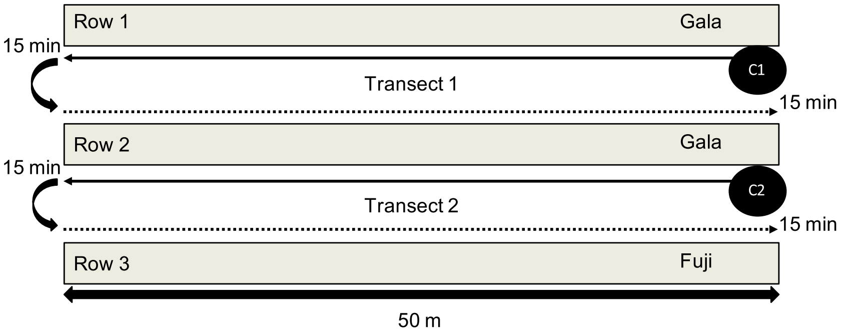

Fig 1 Insect collection method. The 50-m transects were determined side by side and each one was limited by two rows of apple trees, being one row common for the two transects. Transect one was delimited by two rows of “Gala” trees and transect two by one row of “Gala” trees and one row of “Fuji” trees. Two collectors (C1 and C2), one in each transect, slowly walked simultaneously the 50 m for 15 min and collected all flower visitors found in one of the rows of trees using a net. When they reached the end of the transect, they collected the insects in the other row of trees, also for 15 min. The collectors never collected at the same time on the same row of trees.

10:00, 13:00 16:00 in the orchards of A2. The difference in insect sampling time is due to the activity of the insects in the two areas; in A1, the activity of insects on flowers dropped after 15:00, while this was not observed in A2.

The sampling was done at least on 1 and at maximum on 4 days (Table 1) during the flowering season (October) of 2013 and of 2014 in A1 and on four days in A2 (Table 1) during the flowering season of 2014. In total, the sampling effort was 30 h for A1 and 24 h for A2.

For evaluating the insect diversity on the flowering weeds within apple orchards, the same procedure was done after the end of the insect sampling on apple trees, at 10:30, 12:30, and 14:30 in the orchards of A1. Collected insects were killed in jars containing ethyl acetate, mounted and identified later under a stereoscopic microscope, using dichotomic keys for each family. Voucher specimens were deposited at insect collection of the PUCRS Science and Technology Museum.

Apple fruit analysis

In A1, during the harvest period (2014 and 2015), we collected “Fuji” and “Gala” apples of trees located in the transects for evaluating their weight and number of seeds. The number of “Fuji” apples analyzed in 2014 was 149, 150, and 146 for orchards 2, 3, and 4, and in 2015, 151, 143, and 141 for orchards 1, 2, and 3. For “Gala,” the number of fruits analyzed in 2014 was 138, 142, and 137 for orchards 1, 2, and 3, respectively; and in 2015, 105, 151, 144, and 106 for orchards 1, 2, 3, and 4, respectively. We analyzed the fruits in relation to weight, maximum and minimum diameter, maximum and minimum height, and number of seeds.

The diameter and height of the fruits were used to calculate symmetry indexes A and B, respectively. This was done dividing the minimum diameter or height by its maximum, which means that values closer to 1 meant more symmetric (the minimum and the maximum measure are more similar). Number of seeds was used to classify fruits into eight categories, according to the presence (P) or absence (0) of seeds in the five carpels (Sheffield 2014): (a) all carpels contain seed(s); (b) P, P, P, P, 0; (c) P, P, P, 0, 0; (d) P, P, 0, 0, 0; (e) P, 0, 0, 0,0; (f) P, 0, P, 0, P; (g) P, 0, P, 0, 0; and (h) no carpels contain seed.

Statistical analysis

We analyzed the sample completeness of A1 (apple and weeds) and A2 using iNEXT Online (Chao *et al* 2016). Because sample completeness was high and similar for both areas (A1 apple: 97.2%; A2: 99.2%; see Fig S1), we compared areas, even though the number of sampling days was not equal for all orchards and areas. Information on individual orchards can be found on supplementary material (supplementary material Fig S1).

We used two approaches for comparing the insect diversity and richness of the two areas. One was the rarefaction and extrapolation with Hill numbers, using sample-size-based and coverage-based rarefaction and extrapolation curves (Chao *et al* 2014). The curves were constructed in iNEXT Online (Chao *et al* 2016). The other was a neighbor joining analyses (Bray-Curtis similarity index) using Past 3.12 (Hammer *et al* 2001), for verifying whether the two analyzed regions were similar regarding insect diversity and native bee diversity. We also analyzed whether the orchards would be grouped by management (conventional and organic) using the same analyses.

We calculated Shannon diversity indexes for each orchard using Past 3.12 (Hammer *et al* 2001). The Shannon diversity indexes for all insect species and exclusively for bee species visiting apple flowers were compared to the Shannon diversity indexes of weed flowers using the diversity *t* test also using the Past software.

For assessing whether “Gala” and “Fuji” apple quality (fruit weight, number of seeds, number of category A apples and symmetry) could be explained by insect diversity (Shannon index) and honeybee abundance, we constructed generalized linear mixed model (with Poisson distribution). This test was performed in R software (R Core Team 2016) with the *glm* function from "lmer" package (Bates *et al* 2015).

Results

Insect diversity—apple flowers

In area 1 (A1), we collected 301, 380, 392, and 324 insects at orchards 1, 2, 3, and 4, respectively. Hymenoptera was the most abundant order (Table 2), because honeybees were the most abundant insect in the orchards (Fig 2a). Other hymenopterans were mainly native bees, except in orchard 3 (Fig 2a). The second most abundant order was Diptera (Table 2), which was mainly composed by syrphid flies (Fig 2b), followed by Coleoptera (Table 2). Hemiptera and Lepidoptera were found in very low numbers (Table 2). In relation to species richness, 62 insect species visited apple flowers (a list of the species is given on supplementary material Table S1). Diptera was the richness (23) order, followed by Hymenoptera (20), Coleoptera (15), Lepidoptera (3), and Hemiptera (2). Native bee abundance and richness on apple flowers was low: five species of Apidae and four of Halictidae.

In area 2 (A2), we collected 426, 408, 534, and 533 insects at orchards 5, 6, 7, and 8, respectively. Hymenoptera was also the most abundant order (Table 2), because honeybees were the most abundant insect in the orchards (Fig 2a). Other hymenopterans were mainly native bees, except in orchard 6 (Fig 2a). The second most abundant order was Diptera (Table 2), which was mainly composed by syrphid flies in orchards 6 and 8, but not in orchards 5 and 7 (Fig 2b). Coleoptera, Lepidoptera, and Hemiptera vary in numbers and presence in the orchards of A2 (Table 2). In relation to species richness, 44 insect species visited apple flowers in area 2. Hymenoptera was the richness (33) order, followed by Diptera (4), Coleoptera (5), Lepidoptera (1), and Hemiptera (1). Native bee abundance on apple flowers was also low (a list of the species is given on supplementary material Table S1); however, richness was high (54.5% of the hymenopteran species) compared to A1: 12 species of Apidae and 6 of Halictidae.

Table 2 Percentage of each insect order on the flowers of apple (areas 1 and 2) and weeds (area 1) on commercial apple orchards from southern Brazil. *Hym*: Hymenoptera. *Dip*: Diptera. *Col*: Coleoptera. *Hem*: Hemiptera. *Lep*: Lepidoptera.

	Apple					Weed				
Orchard	Hym	Dip	Col	Hem	Lep	Hym	Dip	Col	Hem	Lep
1	93	6	0.7	0	0.3	66.8	30.6	2.1	0	0.5
2	90.8	3.2	6.1	0	0	57.9	33.9	8.2	0	0
3	89.5	8.2	1.8	0.3	0.3	84.1	15	0.3	0.6	0
4	38.3	34.6	26.9	0.3	0	31.6	36	32	0.3	0
5	95.5	4.2	0	0	0.2	–	–	–	–	–
6	90	5.1	4.7	0.3	0	–	–	–	–	–
7	82.8	11.8	4.1	0.3	1.1	–	–	–	–	–
8	94.6	4.7	0.4	0	0.4	–	–	–	–	–

The orchards of A1 and A2 presented 62 and 44 insect species respectively (supplementary material Table S1). Of those species, only 10 occurred in both areas, 52 occurred only in A1 and 34 in A2. In respect to native bees, A1 presented 9 species while A2 presented 18. Four species occurred in both areas, five only in A1 and 14 only in A2. Sample-size-based and sample-coverage-based rarefaction and extrapolation curves for species richness ($q = 0$; Fig 3(A, B)) indicated that A1 and A2 differed in species richness.

There was a consistent pattern in the sample-size-based (Fig 3(C, E)) and coverage-based (Fig 3(D, F)) rarefaction and extrapolation curves for Shannon ($q = 1$; Fig 3(C, D)) and Simpson ($q = 2$; Fig 3(E, F)) diversities. The 95% confidence intervals for A1 and A2 in the curves are disjoint, which implies a significant difference in diversity (Chao *et al* 2014). The diversity curves for A1 were always lying under the curves of A2, indicating that A2 is more diverse than A1.

The neighbor joining analysis of all insects clustered the orchards by type of management, resulting in two main clusters, one formed by the conventional orchards and one by the organic orchards (Fig 4). The cluster formed by the conventional orchards was divided into two groups, one formed by the orchards located at area 1 and the other by the ones of area 2 (Fig 4). For native bee species (all species excluding honeybees), the neighbor joining analysis clustered the orchards into two groups; within the bigger one, the orchards from area 1 were more similar to each other than to the ones from area 2 (Fig 5). The groups were clustered according to geographic position (Fig 5).

Insect diversity—weeds

Eleven weed species were flowering on the ground vegetation of the orchards of A1: *Hypochaeris* sp. (Asteraceae); *Trifolium* sp. 1 and *Trifolium* cf. *polymorphum* Poir. (Leguminosae; white and pink clover, respectively); *Oxalis* sp. 1, sp. 2, sp. 3, and sp. 4 (Oxalidaceae); *Lysimachia arvensis* (L.) U. Manns & Anderb. (Primulaceae); Gratiolaceae sp. 1; *Vicia angustifolia* L. ex Reichard (Fabaceae); and *Galega officinalis* L. (Fabaceae).

The number of collected insects was 193, 171, 346, and 297 for orchards 1, 2, 3, and 4, respectively. Hymenoptera was

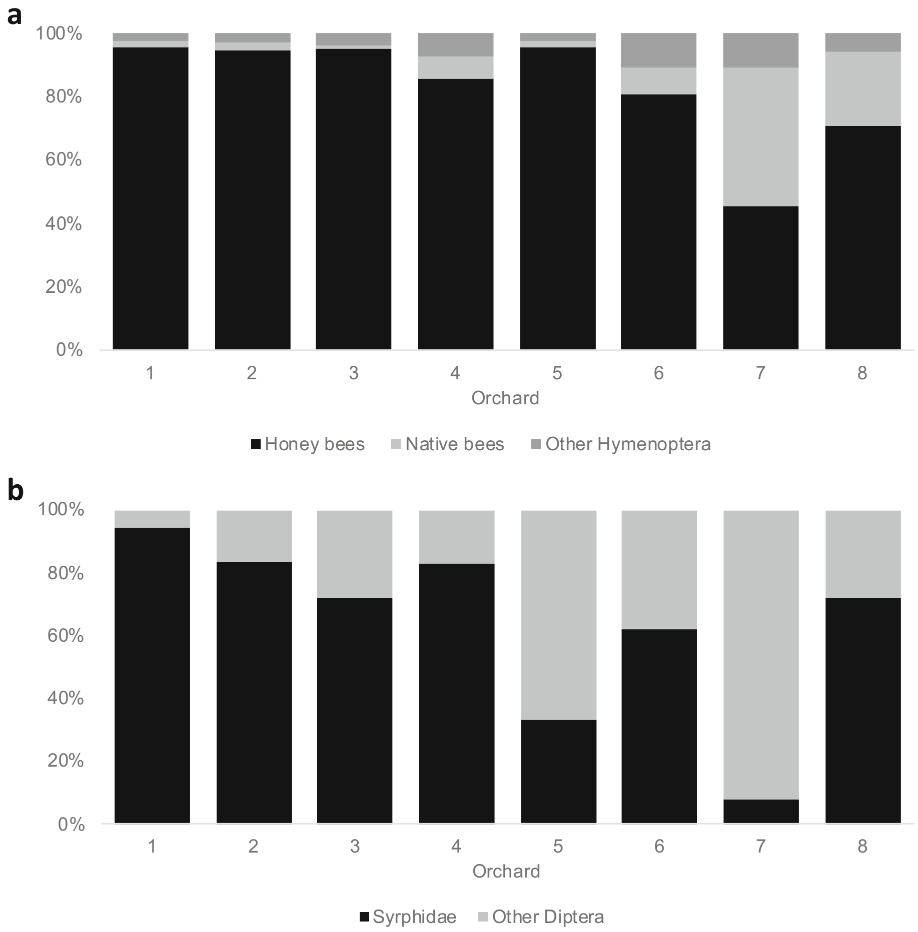

Fig 2 Percentages of Hymenoptera (**a**) comprised by honeybees, native bees, and other hymenopterans and of Diptera (**b**) comprised by syrphid flies and other dipterans collected on apple flowers.

the most abundant order in orchards 1, 2, and 3, but not in orchard 4, where Diptera was most abundant (Table 2). The dominance of Hymenoptera in orchards 1, 2, and 3 reflects the abundance of honeybees (Fig 6a), which was also the most abundant Hymenoptera in orchard 4 (Fig 6a). Other hymenopterans were mainly native bees (Fig 6a). For orchards 1, 2, and 3, the second most abundant order was Diptera (Table 2), which was mainly composed by syrphid flies, as it was in orchard 4 (Fig 6b). Coleoptera occurred at low abundance in all orchards (Table 2). Hemipterans were found in very low numbers in orchards 3 and 4 and not found in orchards 1 and 2 (Table 2). This is also the case of Lepidoptera, which was found in orchards 1, but not in orchards 2, 3, and 4 (Table 2).

In relation to species richness, 58 insect species visited the flowering weeds. Hymenoptera (24) was the richness order, followed by Diptera (20), Coleoptera (11), Hemiptera (2), and Lepidoptera (1). Native bee abundance and richness was low: five species of Andrenidae, five of Apidae, and eight of Halictidae (supplementary material Table S1).

Apple trees vs. weeds

Shannon diversity of weed visitors ($H = 0.678$) was higher than of the apple trees ($H = 0.159$) (t test, $t = -8.03$; $P < 0.05$). This is also indicated by the sample-size-based and sample-coverage-based rarefaction and extrapolation curves (Fig 3(C, D)). These curves for Simpson diversity (Fig 3(E, F)) also indicates that weed insect visitors are more diverse than apple visitors. However, the abundance of insects was greater on apple flowers than on weeds (see supplementary material Table S1).

In relation to insect species richness, apple trees were richer than weeds (Table 3), as indicated by the sample-size-based and sample-coverage-based rarefaction and extrapolation curves for species richness ($q = 0$; Fig 3(A, B)). However, regarding each insect order separately, while Diptera and Coleoptera were richer on apple trees, Hymenoptera was richer on weeds (Table 3). The two orders left, Lepidoptera and Hemiptera, presented no difference in species richness between apple trees and weeds (Table 3).

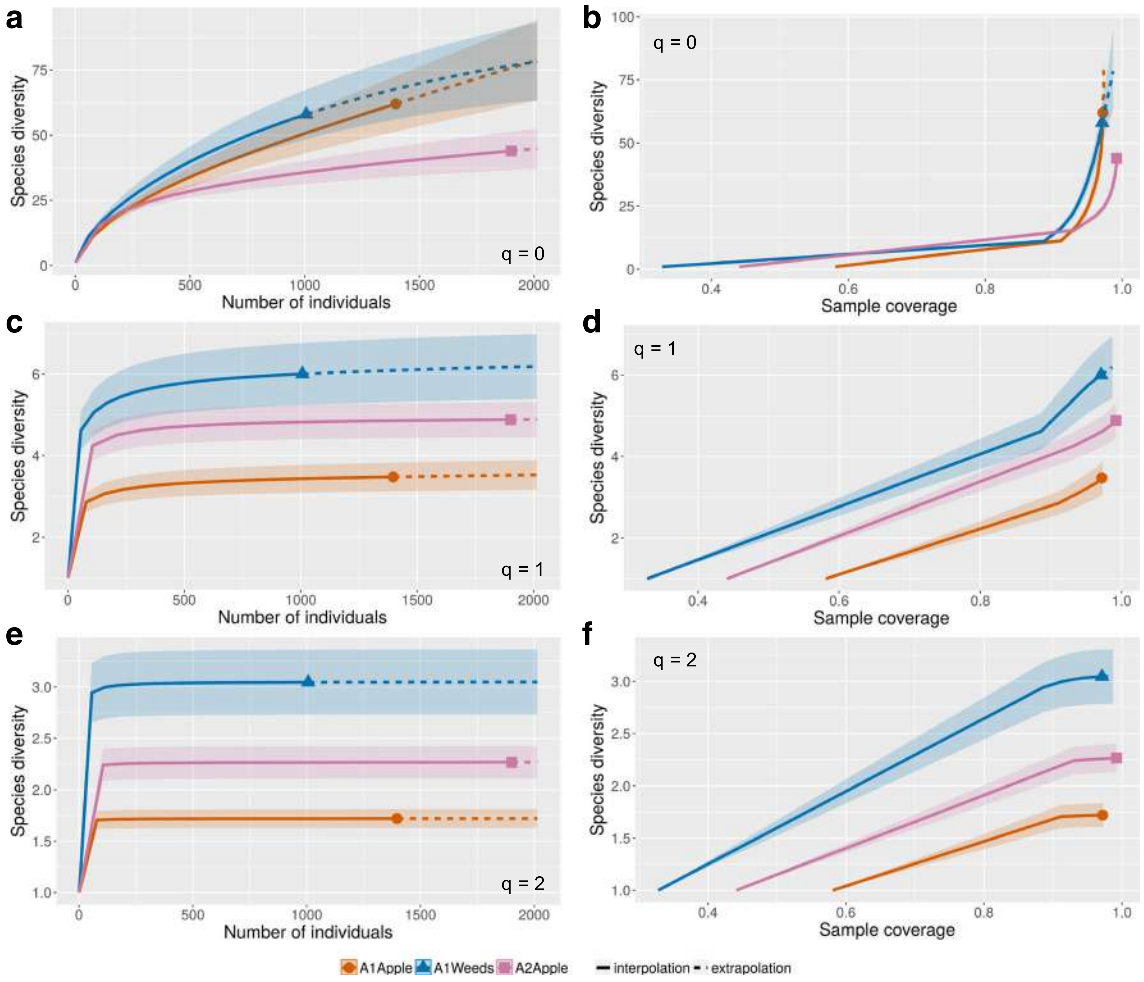

Fig 3 Sample-size-based (a, c, e) and sample-coverage-based (b, d, f) rarefaction (solid) and extrapolation (broken) curves for species richness (a, b; $q = 0$), Shannon diversity (c, d; $q = 1$), and Simpson diversity (e, f; $q = 2$) of insects visiting apple flowers in areas 1 (A1Apple) and 2 (A2Apple) and weed flowers (A1Weeds). The 95% confidence intervals (shaded areas) were obtained by a bootstrap method based on 50 replications (Chao *et al* 2016).

Ninety-six insect species were found in the orchards; however, only 25 were common apple and weed flowers (supplementary material Table S1). Thirty-seven and 33 insect species visited only apple and weed flowers, respectively

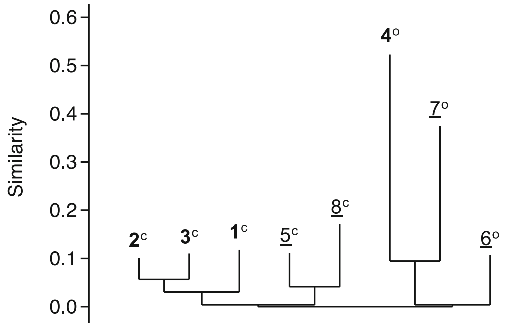

Fig 4 Dendrogram of insect species visiting apple flowers in eight orchards. The tree was obtained through neighbor joining analyses, using the Bray-Curtis similarity index. Bold numbers indicate that the orchard is located in area 1 and underlined numbers area 2. The letters beside orchard numbers indicates the management type of the orchard, which can be conventional (c) or organic (o).

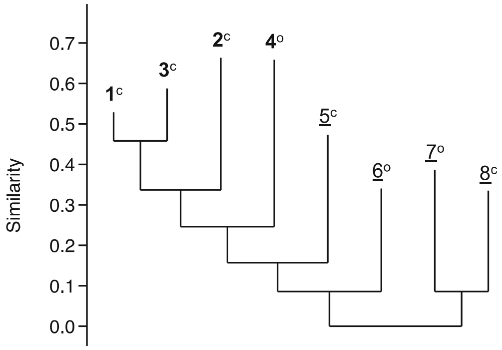

Fig 5 Dendrogram of native bee species visiting apple flowers in eight orchards. The tree was obtained through neighbor joining analyses, using the Bray-Curtis similarity index. Bold numbers indicate that the orchard is located in area 1 and underlined numbers area 2. The letters beside orchard numbers indicates the management type of the orchard, which can be conventional (c) or organic (o).

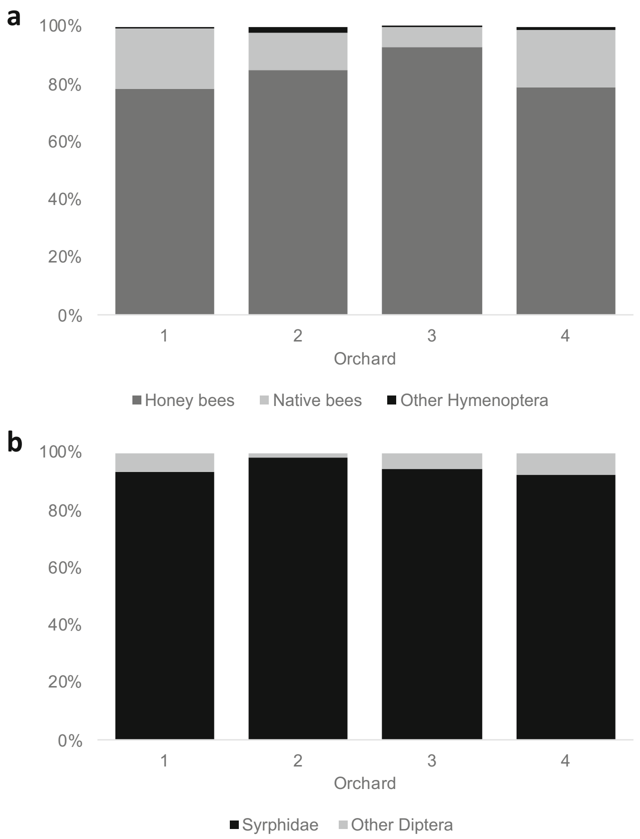

Fig 6 Percentages of Hymenoptera (**a**) comprised by honeybees, native bees and other hymenopterans and of Diptera (**b**) comprised by syrphid flies and other dipterans collected on weed flowers.

(supplementary material Table S1). When considering only the species of bees, of the 24 species found in the orchards, only five species were common to apple and weed flowers (Fig 7). Furthermore, 14 species occurred only on weed flowers and five exclusively on apple flowers (Fig 7).

Fruit characteristics vs. insect diversity

There was no significant effect ($P > 0.05$) of the Shannon index on "Fuji" fruit weight, symmetry index A (diameter) and B (height) and on the percentage of fruits of category A, the amount of fruits that contained seeds in all carpels in relation to the number of fruits analyzed, except for the number of seeds on fruits ($t = -2.90$, $P = 0.044$). Honeybee abundance did not affect the number of seeds on fruits, symmetry index A and B, and the percentage of fruits of category A ($P > 0.05$) but affected fruit weight ($t = 3.88$, $P = 0.018$).

For "Gala," there was no significant effect of the Shannon index on fruit weight, number of seeds, symmetry index A and B, and the percentage of fruits of category A ($P > 0.05$). Honeybee abundance did not affect fruit weight, the number of seeds on fruits, symmetry index A and B, and the percentage of fruits of category A ($P > 0.05$).

Table 3 Insect species richness on the flowers of apple and weeds on commercial apple orchards from southern Brazil (area 1).

	Number of species		Wilcoxon test
	Apple	Weeds	*p* value
Diptera	23	20	< 0.001
Hymenoptera	20	24	< 0.001
Coleoptera	15	11	< 0.001
Lepidoptera	3	2	0.095
Hemiptera	2	1	0.479
Total	62	58	< 0.001

Discussion

In general, orchards presented a low diversity of insects (supplementary material Table S1), as indicated by the Shannon indexes, even though total species richness (area 1 + area 2) was 96 (supplementary material Table S1). In both areas, the most abundant order was Hymenoptera with *A. mellifera* being the most dominant species (Table 2; Fig 2a), followed by native bees (supplementary material Table S1). The Shannon index accounts for both species richness and evenness, thus the dominance of honeybees results in overall low insect diversity.

We found nine species of native bees on apple flowers in area 1 and 18 in area 2 all in low abundance (supplementary material Table S1). In the 1980s, sampling two orchards, Ortolan and Laroca (1996), found 26 species of native bees visiting apple flowers in Santa Catarina State in an area less than 100 km from area 2 of our study. One possible cause for the low number of bee species on apple orchards may include the time of the year when flowering occurs. Flowering begins very early in spring and lasts only for the first month of this season (Petri 2006), when some solitary bee species may not be active yet. Another factor could be the weather, because this period often presents low temperature (under 15°C) and windy rainy days, which negatively influence bee foraging.

The landscape of the region where the orchards are located may also be a cause for the low diversity of insects, at least for the bees. The landscape around apple orchards, in special when composed of forested areas, influences the number of species and abundance of wild bees present on them (Watson *et al* 2011). The heterogeneity of the landscape also influences positively the species richness of wild bees within orchards (Földesi *et al* 2016). We did not analyze the landscape around the orchards; however, the regions are dominated by agricultural properties and are under anthropogenic disturbances, which are threats to the populations of pollinators (Potts *et al* 2016).

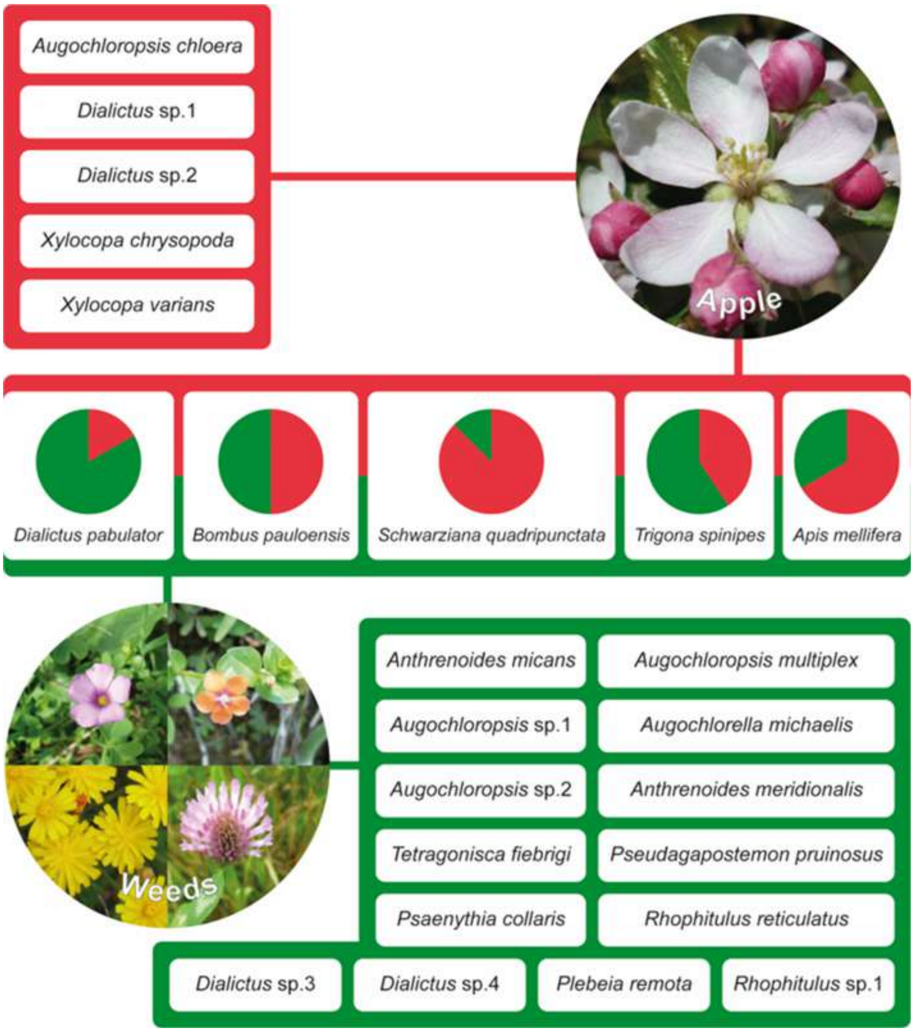

Fig 7 Bee species found on apple (indicated by red) and weed (indicated by green) flowers. The pie graphics indicate the proportion of the bee species abundance on apple and weeds for the species that were found on both.

The rarefaction and extrapolation diversity curves (Fig 3) showed that the diversity of the two regions is different: A2 is more diverse than A1 but presented lower species richness. This was also indicated by the neighbor joining analysis of insects and native bees (Fig 4). The orchards were clustered by management type; that is, independently of the region, organic orchards were more similar to each other than to conventional ones regarding insect diversity. Type of management influences insect diversity and abundance in agriculture areas (Kennedy *et al* 2013, Tuck *et al* 2014, Power *et al* 2016, Schon *et al* 2017). The secondary grouping by region may be explained by the landscape where the orchards are, because it affects pollinator communities in apple orchards (Watson *et al* 2011, Marini *et al* 2012, Joshi *et al* 2016, Power *et al* 2016). Furthermore, different regions may have different communities of wild insects.

However, when we analyzed the native bee species, the geographic region (area 1 versus area 2) explains better the grouping than orchard management type (Fig 5). A study investigated the influence of management type on pollination success and concluded that organic management may not improve pollination services on apple orchards (Porcel *et al* 2018). This was attributed to the fact that apple orchards are composed of time-stable habitats that attract pollinators to the orchards (Porcel *et al* 2018). In addition, pollinators are less prone to the effects of orchard management because of their mobility and short visitation time periods (only during flowering) in the orchards, when the use of pesticides is discouraged (Porcel *et al* 2018). On the other hand, the richness and abundance of native bees in apple orchards are positively influenced by the presence of forested areas surrounding orchards (Watson *et al* 2011). Even though we made no landscape analysis, orchards on area 2 presented forested areas, while on area 1, except for orchard 4, there was no forested areas surrounding the orchards.

We also evaluated insect diversity of flower visitors of weeds. Sown flower strips and ground vegetation usually enhance the abundance of wild insects in agriculture areas (Carvalheiro *et al* 2011, Campbell *et al* 2017). In our study, the insect richness of weeds was higher than of apple trees (Fig 3; Table 3; supplementary material Table S1) and few insect species (26.3%) visited both (supplementary material

Table S1), 39% of the insect species were exclusive to apple flowers and 34.7% were exclusive to weed flowers. Furthermore, only 20% of the collected bees visited both weeds and apple flowers (Fig 7) and 58.3% were collected only on weed flowers. Our results suggest, in agreement with previous studies (Carvalheiro *et al* 2011), that weeds increase general insect diversity on agricultural areas, and in our case, in apple orchards. However, it is not possible to hypothesize about the effect of presence of weeds on apple flower visitation. Despite that, weeds are a food resource for insects on agricultural areas and as recommended by other studies (Carvalheiro *et al* 2011, Holzschuh *et al* 2012, Nicholls and Altieri 2013, Kremen and M'Gonigle 2015, Campbell *et al* 2017) should be kept in those areas.

Remarkably, in our study, andrenid bees were captured exclusively on weed flowers, even though it has been frequently recorded on apple flowers (Watson *et al* 2011, Marini *et al* 2012, Russo *et al* 2015) and sometimes being more abundant than honeybees on those flowers (Campbell *et al* 2017). However, another study in Brazil also did not record andrenid bees on apple flowers (Ortolan and Laroca 1996), indicating that the species occurring in the country may not be attracted to them. Andrenid bees visit other plant species within apple orchards, including weeds like dandelions (*Taraxacum*) (Campbell *et al* 2017, Russo and Danforth 2017). In cider apple orchards, andrenid bees (*Andrena*) visited several plant species on flowers of strips but were one of the main apple flower visitors (Campbell *et al* 2017). It is important to acknowledge though that the andrenid species on the studies cited here are not the same from ours and are even from different genera.

Besides *Dialictus pabulator* Schrottky, the bee species (*Bombus pauloensis* Friese, *Schwarziana quadripunctata* Lepeletier, *Trigona spinipes* Fabricius, and *A. mellifera*; Fig 7) that visited both weeds and apple flowers are generalist social bees. Interestingly, two other generalist social bee species, the stingless bees *Plebeia remota* Holmberg and *Tetragonisca angustula* Latreille (Fig 7), were observed only on weed flowers, but not on apple. However, due to their generalist habit, we do not discard the possibility of them being apple flower visitors. In the case of bees that we collected only on apple flowers (Fig 7), other species of their genera (*Augochloropsis*, *Dialictus*, and *Xylocopa*) are food generalists, but there is no information on the diet of those species. One species of *Augochloropsis* was recorded by Ortolan and Laroca (1996) visiting only apple flowers and other species visiting other plant species besides apple flowers. The same was observed by them for *Dialictus* species, but they recorded *D. pabulator* only on apple flowers, what differed from our study.

Although it is known that insect diversity influences fruit production and quality (Garratt *et al* 2014b, a, Garibaldi *et al* 2016, Rader *et al* 2016) and the number of wild bees species influences pollination success (Földesi *et al* 2016, Campbell *et al* 2017), we found no relation between insect diversity and "Gala" fruit characteristics. However, there are some factors to consider: (a) apples analyzed on the orchards of A1 had half of the potential seeds (4.7 ± 0.7, $n = 7$) possible (10); (b) seed number influences fruit weight (Webb *et al* 1980, Matsumoto *et al* 2012, Sheffield 2014); (c) few apples had seeds in all carpels (category A = 23.2% ± 7.1%, $n = 7$); and (d) we found low insect diversity in apple orchards, where honeybees were the most abundant insects on flowers. In this scenario, we suggest that, regarding "Gala," orchards from southern Brazil possibly present a pollination deficit that could be overcome by improving insect pollination. In Brazil, managed apple pollination relies solely on honeybees. Adding more species to the orchards may improve cross-pollination and fruit size; for example, bumble bees changed the behavior of honeybees, making them move more between rows of pollinizer and the target apple cultivar, which may result in improved fruit quality (Sapir *et al* 2017). Among the stingless bee species that visited apple flowers in our study, only *Plebeia saiqui* Friese could be managed for pollination because techniques for keeping them in hives exist. However, their efficiency and the feasibility of using them as managed pollinators must be tested, as well as their effect on other bee species. For the other stingless bees observed, *Mourella caerulea* Friese, *S. quadripunctata*, and *T. spinipes*, as well as all other native bee species, there is no current technique for breeding colonies in hives or managing the nests (in the case of solitary bees). This is unfortunate, because Ortolan & Laroca (1996) have also recorded the three species visiting apple flowers.

For "Fuji," the results indicate that insect diversity influences the number of seeds within fruits. Although the relationship varies with cultivar, apple weight, diameter, shape/symmetry, and quality (calcium content, flesh firmness, pulp acidity) increase with seed number (Brault and de Oliveira 1995, Buccheri & Di Vaio 2005, Sheffield 2014, Sapir *et al* 2017), indicating that insect diversity is important for apple yield. In fact, seed number varies with pollinator identity, because pollinators vary in effectiveness (Garratt *et al* 2016). Nevertheless, even though syrphid flies contribute less to apple seed number than bees, they result in increased economic output (Garratt *et al* 2016); consequently, their presence benefit apple production from an economic point of view. In our study, we found 13 species of syrphids visiting apple flowers (supplementary material Table S1) and it is possible that they are contributing to apple pollination because they walk over the reproductive parts during feeding, suggesting that they are pollinators. Beyond that, pollinators have complementary and synergistic effects on apple pollination, increasing fruit quality, as explained above (Sapir *et al* 2017). Other visitors found, as the coleopterans *Diabrotica speciosa* Germar and *Astylus quadrilineatus* Germar, *Polybia* wasps and *Campsomeris* spp. (Scoliidae wasps), feed on nectar and/or pollen and may contribute to apple pollination (Nunes-Silva *et al* 2016), which must be investigated to explain why our results indicated that insect diversity influences seed number in apple.

Furthermore, honeybee abundance influenced the weight of "Fuji" apples, what was also observed for other cultivars. Honeybee (*A. mellifera* and *Apis cerana* Fabricius) visits increased fruit quality (weight, length, breadth, and number of seeds) when compared to the control (no visits) in "Golden Delicious" and "Red Gold," and to open pollination in "Golden Delicious," "Red Gold," "Royal Delicious," and "Red Delicious" (Bhagat & Mattu 2015). In line with Bhagat & Mattu (2015), our results varied between cultivars, confirming that different cultivars have distinct pollination requirements (Garratt *et al* 2014a, 2016).

Honeybees were the dominant insect visitor, which was expected because there were hives in the study areas. Other studies have also observed a dominance of honeybees on apple orchards (Russo *et al* 2015, Földesi *et al* 2016). They are, in fact, efficient pollinators of apple (Stern *et al* 2001), at least when they collected pollen and nectar from the top of the flower (Vicens & Bosch 2000, Thomson & Goodell 2001, Schneider *et al* 2002). Our results indicate that honeybees are the main pollinator of "Fuji" apples and may compensate the lack of other pollinators, because even though we found low insect diversity in apple orchards, apples had almost all the seeds (7.4 ± 0.3, $n = 6$) possible (10) and most fruits had seeds in all carpels (70.4% ± 9.1%, $n = 6$). Therefore, the current management of honeybee hives becomes essential, possibly combined with stingless bee hives like *M. quadrifasciata* (Viana *et al* 2014) or *P. emerina* (Ortolan & Laroca 1996). Furthermore, the conservation of other pollinators should be promoted for improving and securing the pollination services.

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Author Contribution Statement PNS, SW, MB, and BB planned and designed the experimental work; PNS, SW, JMR, CJA, LMS, and JD executed the experimental work; RH and LMS worked on insect identification; PNS and RH conducted the data analyses; PNS wrote the manuscript; and all authors revised it.

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References

Aizen MA, Garibaldi LA, Cunningham SA, Klein AM (2009) How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. Ann Bot 103:1579–1588. https://doi.org/10.1093/aob/mcp076

Allen-Wardell G, Bernhardt P, Bitner R, Burquez A, Buchmann S, Cane J, Cox PA, Dalton V, Feinsinger P, Ingram M, Inouye D, Jones CE, Kennedy K, Kevan P, Koopowitz H, Medellin R, Medellin-Morales S, Nabhan GP (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of fruit crop yields. Conserv Biol 12:8–17

Alvares CA, Stape JL, Sentelhas PC, De Moraes Gonçalves JL, Sparovek G (2013) Köppen's climate classification map for Brazil. Meteorol Z 22: 711–728. https://doi.org/10.1127/0941-2948/2013/0507

Balfour NJ, Ratnieks FLW (2017) Using the waggle dance to determine the spatial ecology of honey bees during commercial crop pollination. Agric For Entomol 19:210–216. https://doi.org/10.1111/afe.12204

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67. doi: https://doi.org/10.18637/jss.v067.i01

Bhagat T, Mattu VK (2015) Effect of honeybee pollination on quantity and quality of apple crop in Kullu Hills of Himachal. Int J Sci Res 4:624–629

Bosch J, Kemp WP (2002) Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. Bull Entomol Res 92:3–16. https://doi.org/10.1079/BER2001139

Boyle RMD, Philogène BJR (1983) The native pollinators of an apple orchard: variations and significance. J Hortic Sci 58:355–363. https://doi.org/10.1080/00221589.1983.11515130

Brittain C, Kremen C, Garber A, Klein A-M (2014) Pollination and plant resources change the nutritional quality of almonds for human health. PLoS One 9:e90082. https://doi.org/10.1371/journal.pone.0090082

Brittain C, Kremen C, Klein AM (2013) Biodiversity buffers pollination from changes in environmental conditions. Glob Chang Biol 19:540–547. https://doi.org/10.1111/gcb.12043

Brault AM, de Oliveira D (1995) Seed number and an asymmetry index of 'McIntosh' apples. HortScience 30:44–46

Buccheri M, Di Vaio C (2005) Relationship among seed number, quality, and calcium content in apple fruits. J Plant Nutr 27:1735–1746. https://doi.org/10.1081/pln-200026409

Campbell AJ, Wilby A, Sutton P, Wäckers FL (2017) Do sown flower strips boost wild pollinator abundance and pollination services in a spring-flowering crop? A case study from UK cider apple orchards. Agric Ecosyst Environ 239:20–29. https://doi.org/10.1016/j.agee.2017.01.005

Carvalheiro LG, Veldtman R, Shenkute AG, Tesfay GB, Pirk CW, Donaldson JS, Nicolson SW (2011) Natural and within-farmland biodiversity enhances crop productivity. Ecol Lett 14:251–259. https://doi.org/10.1111/j.1461-0248.2010.01579.x

Chao A, Gotelli NJ, Hsieh TC *et al* (2014) Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecol Monogr 84:45–67. https://doi.org/10.1890/13-0133.1

Chao A, Ma KH, Hsieh TC (2016) iNEXT (iNterpolation and EXTrapolation) Online. https://chao.shinyapps.io/iNEXTOnline/. Accessed 20 Feb 2019

Chaplin-Kramer R, Dombeck E, Gerber J, Knuth KA, Mueller ND, Mueller M, Ziv G, Klein AM (2014) Global malnutrition overlaps with pollinator-dependent micronutrient production. Proc R Soc B 281: 20141799. https://doi.org/10.1098/rspb.2014.1799

Cross J, Fountain M, Markó V, Nagy C (2015) Arthropod ecosystem services in apple orchards and their economic benefits. Ecol Entomol 40:82–96. https://doi.org/10.1111/een.12234

Delaplane KS, Mayer DF (2000) Crop pollination by bees. CABI, Wallingford, p 352

Díaz S, Tilman D, Fargione J, Chapin FS III, Dirzo R, Kitzberger T, Gemmill B, Zobel M, Vilà M, Mitchell C, Wilby A, Daily GC, Galetti M, Laurance WF, Pretty J, Naylor R, Power A, Harvell A, Potts S, Kremen C, Griswold T, Eardley C (2006) Biodiversity regulation of ecosystem services. In: Hassan H, Scholes R, Ash N (eds) Ecosystems and human well-being: current state and trends. Island Press, Washington, pp 297–329

Eilers EJ, Kremen C, Smith Greenleaf S, Garber AK, Klein AM (2011) Contribution of pollinator-mediated crops to nutrients in the human food supply. PLoS One 6:e21363. https://doi.org/10.1371/journal.pone.0021363

Ellis AM, Myers SS, Ricketts TH (2015) Do pollinators contribute to nutritional health? PLoS One 10:1–17. https://doi.org/10.1371/journal.pone.0114805

Földesi R, Kovács-Hostyánszki A, Kőrösi Á, Somay L, Elek Z, Markó V, Sárospataki M, Bakos R, Varga Á, Nyisztor K, Báldi A (2016) Relationships between wild bees, hoverflies and pollination success in apple orchards with different landscape contexts. Agric For Entomol 18:68–75. https://doi.org/10.1111/afe.12135

Gallai N, Salles JM, Settele J, Vaissière BE (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol Econ 68:810–821. https://doi.org/10.1016/j.ecolecon.2008.06.014

Garibaldi LA, Carvalheiro LG, Vaissiere BE, Gemmill-Herren B, Hipolito J, Freitas BM, Ngo HT, Azzu N, Saez A, Astrom J, An J, Blochtein B, Buchori D, Chamorro Garcia FJ, Oliveira da Silva F, Devkota K, Ribeiro Mde F, Freitas L, Gaglianone MC, Goss M, Irshad M, Kasina M, Pacheco Filho AJ, Kiill LH, Kwapong P, Parra GN, Pires C, Pires V, Rawal RS, Rizali A, Saraiva AM, Veldtman R, Viana BF, Witter S, Zhang H (2016) Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. Science (80) 351:388–391. https://doi.org/10.1126/science.aac7287

Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Harder LD, Afik O, Bartomeus I, Benjamin F, Boreux V, Cariveau D, Chacoff NP, Dudenhoffer JH, Freitas BM, Ghazoul J, Greenleaf S, Hipolito J, Holzschuh A, Howlett B, Isaacs R, Javorek SK, Kennedy CM, Krewenka KM, Krishnan S, Mandelik Y, Mayfield MM, Motzke I, Munyuli T, Nault BA, Otieno M, Petersen J, Pisanty G, Potts SG, Rader R, Ricketts TH, Rundlof M, Seymour CL, Schuepp C, Szentgyorgyi H, Taki H, Tscharntke T, Vergara CH, Viana BF, Wanger TC, Westphal C, Williams N, Klein AM, Dudenhöffer JH, Freitas BM, Ghazoul J, Greenleaf S, Hipólito J, Holzschuh A, Howlett B, Isaacs R, Javorek SK, Kennedy CM, Krewenka KM, Krishnan S, Mandelik Y, Mayfield MM, Motzke I, Munyuli T, Nault BA, Otieno M, Petersen J, Pisanty G, Potts SG, Rader R, Ricketts TH, Rundlöf M, Seymour CL, Schüepp C, Szentgyörgyi H, Taki H, Tscharntke T, Vergara CH, Viana BF, Wanger TC, Westphal C, Williams N, Klein AM (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science (80-) 339:1608–1611. https://doi.org/10.1126/science.1230200

Garratt MPD, Breeze TD, Boreux V, Fountain MT, McKerchar M, Webber SM, Coston DJ, Jenner N, Dean R, Westbury DB, Biesmeijer JC, Potts SG (2016) Apple pollination: demand depends on variety and supply depends on pollinator identity. PLoS One 11:e0153889. https://doi.org/10.1371/journal.pone.0153889

Garratt MPD, Breeze TD, Jenner N, Polce C, Biesmeijer JC, Potts SG (2014a) Avoiding a bad apple: insect pollination enhances fruit quality and economic value. Agric Ecosyst Environ 184:34–40. https://doi.org/10.1016/j.agee.2013.10.032

Garratt MPD, Truslove CL, Coston DJ, Evans RL, Moss ED, Dodson C, Jenner N, Biesmeijer JC, Potts SG (2014b) Pollination deficits in UK apple orchards. J Pollinat Ecol 12:9–14

Giannini TC, Cordeiro GD, Freitas BM, Saraiva AM, Imperatriz-Fonseca VL (2015) The dependence of crops for pollinators and the economic value of pollination in Brazil. J Econ Entomol 108:1–9. https://doi.org/10.1093/jee/tov093

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4(1):1–9. https://doi.org/10.1016/j.bcp.2008.05.025

Hoffmann A, Petri JL, Leite GB *et al* (2004) Tratos culturais. In: Nachtigall GR (ed) Maçã - Produção. Embrapa Uva e Vinho, Bento Gonçalves, pp 78–102

Holzschuh A, Dudenhöffer JH, Tscharntke T (2012) Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. Biol Conserv 153:101–107. https://doi.org/10.1016/j.biocon.2012.04.032

Inouye DW, Larson BMH, Ssymank A, Kevan PG (2015) Flies and Flowers Iii : Ecology of Foraging and Pollination. J Pollinat Ecol 16:115–133

Jahed KR, Hirst PM (2017) Pollen tube growth and fruit set in apple. HortScience 52:1054–1059. https://doi.org/10.21273/HORTSCI11511-16

Joshi NK, Otieno M, Rajotte EG, Fleischer SJ, Biddinger DJ (2016) Proximity to woodland and landscape structure drives pollinator visitation in apple orchard ecosystem. Front Ecol Evol 4:1–9. https://doi.org/10.3389/fevo.2016.00038

Kennedy CM, Lonsdorf E, Neel MC, Williams NM, Ricketts TH, Winfree R, Bommarco R, Brittain C, Burley AL, Cariveau D, Carvalheiro LG, Chacoff NP, Cunningham SA, Danforth BN, Dudenhöffer JH, Elle E, Gaines HR, Garibaldi LA, Gratton C, Holzschuh A, Isaacs R, Javorek SK, Jha S, Klein AM, Krewenka K, Mandelik Y, Mayfield MM, Morandin L, Neame LA, Otieno M, Park M, Potts SG, Rundlöf M, Saez A, Steffan-Dewenter I, Taki H, Viana BF, Westphal C, Wilson JK, Greenleaf SS, Kremen C (2013) A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. Ecol Lett 16:584–599. https://doi.org/10.1111/ele.12082

Klatt BK, Holzschuh A, Westphal C, Clough Y, Smit I, Pawelzik E, Tscharntke T (2014) Bee pollination improves crop quality, shelf life and commercial value. Proc Biol Sci 281:20132440. https://doi.org/10.1098/rspb.2013.2440

Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. Proc R Soc B 274:303–313. https://doi.org/10.1098/rspb.2006.3721

Kremen C, M'Gonigle LK (2015) Small-scale restoration in intensive agricultural landscapes supports more specialized and less mobile pollinator species. J Appl Ecol 52:602–610. https://doi.org/10.1111/1365-2664.12418

Mallinger RE, Gratton C (2015) Species richness of wild bees, but not the use of managed honeybees, increases fruit set of a pollinator-dependent crop. J Appl Ecol 52:323–330. https://doi.org/10.1111/1365-2664.12377

Marini L, Quaranta M, Fontana P, Biesmeijer JC, Bommarco R (2012) Landscape context and elevation affect pollinator communities in intensive apple orchards. Basic Appl Ecol 13:681–689. https://doi.org/10.1016/j.baae.2012.09.003

Matsumoto S, Soejima J, Maejima T (2012) Influence of repeated pollination on seed number and fruit shape of 'Fuji' apples. Sci Hortic (Amsterdam) 137:131–137. https://doi.org/10.1016/j.scienta.2012.01.033

McGregor SE (1976) Insect pollination of cultivated crop plants. Agriculture Handbook No. 496. United States Department of Agriculture, Washington, p 411

Morse RA (1991) Honeybees forever. Trends Ecol Evol 6:337–338. https://doi.org/10.1016/0169-5347(91)90043-W

Nava G, Dechen AR, Nachtigall GR (2007) Nitrogen and potassium fertilization affect apple fruit quality in southern Brazil. Commun Soil Sci

Plant Anal 39(1–2):96–107. https://doi.org/10.1080/00103620701759038

Neilsen GH, Neilsen D, Toivonen P, Herbert L (2008) Annual bloom-time phosphorus fertigation affects soil phosphorus, apple tree phosphorus nutrition, yield, and fruit quality. HortScience Horts 43:885–890. https://doi.org/10.21273/HORTSCI.43.3.885

Nicholls CI, Altieri MA (2013) Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. Agron Sustain Dev 33: 257–274. https://doi.org/10.1007/s13593-012-0092-y

Nunes-Silva P, Witter S, Schlemmer L, Halinski R, Ramos J, Arioli C, Botton M (2016) Visitantes florais e potenciais polinizadores da cultura da macieira. Embrapa Uva e Vinho - Comunicado Técnico 184 (INFOTECA-E) http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/1047639

Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? Oikos 120:321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Ortolan S, Laroca SMLS (1996) Melissocenótica em área de cultivo de macieira (*Pyrus malus* L.) em Lages (Santa Catarina, sul do Brasil), com notas comparativas e experimento de polinização com *Plebeia emerina*. Acta Biológica Paranaense 25:1–113. https://doi.org/10.5380/abpr.v25i0.696

Park MG, Raguso RA, Losey JE, Danforth BN (2016) Per-visit pollinator performance and regional importance of wild *Bombus* and *Andrena* (*Melandrena*) compared to the managed honey bee in New York apple orchards. Apidologie 47:145–160. https://doi.org/10.1007/s13592-015-0383-9

Petri JL (2006) Formação de flores, polinização e fertilização. In: A cultura da macieira, 1st edn. EPAGRI, Florianópolis, pp 229–260

Petri JL, Leite GB, Couto M, Francescatto P (2011) Avanços na cultura da macieira no Brasil. Rev Bras Frutic 33(spe1):48–56

Porcel M, Andersson GKS, Pålsson J, Tasin M (2018) Organic management in apple orchards: higher impacts on biological control than on pollination. J Appl Ecol 55:2779–2789. https://doi.org/10.1111/1365-2664.13247

Potts SG, Imperatriz-Fonseca V, Ngo HT, Aizen MA, Biesmeijer JC, Breeze TD, Dicks LV, Garibaldi LA, Hill R, Settele J, Vanbergen AJ (2016) Safeguarding pollinators and their values to human well-being. Nature 540:220–229. https://doi.org/10.1038/nature20588

Power EF, Jackson Z, Stout JC (2016) Organic farming and landscape factors affect abundance and richness of hoverflies (Diptera, Syrphidae) in grasslands. Insect Conserv Divers 9:244–253. https://doi.org/10.1111/icad.12163

Prescott-Allen R, Prescott-Allen C (1990) How many plants feed the world? Conserv Biol 4:365–374. https://doi.org/10.1111/j.1523-1739.1990.tb00310.x

R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna https://www.R-project.org/

Rader R, Bartomeus I, Garibaldi LA, Garratt MPD, Howlett BG, Winfree R, Cunningham SA, Mayfield MM, Arthur AD, Andersson GKS, Bommarco R, Brittain C, Carvalheiro LG, Chacoff NP, Entling MH, Foully B, Freitas BM, Gemmill-Herren B, Ghazoul J, Griffin SR, Gross CL, Herbertsson L, Herzog F, Hipólito J, Jaggar S, Jauker F, Klein AM, Kleijn D, Krishnan S, Lemos CQ, Lindström SAM, Mandelik Y, Monteiro VM, Nelson W, Nilsson L, Pattemore DE, Pereira NO, Pisanty G, Potts SG, Reemer M, Rundlöf M, Sheffield CS, Scheper J, Schüepp C, Smith HG, Stanley DA, Stout JC, Szentgyörgyi H, Taki H, Vergara CH, Viana BF, Woyciechowski M (2016) Non-bee insects are important contributors to global crop pollination. Proc Natl Acad Sci 113:146–151. https://doi.org/10.1073/pnas.1517092112

Rucker RR, Thurman WN, Burgett M (2012) Honey bee pollination markets and the internalization of reciprocal benefits. Am J Agric Econ 94: 956–977. https://doi.org/10.1093/ajae/aas031

Russo L, Danforth B (2017) Pollen preferences among the bee species visiting apple (*Malus pumila*) in New York. Apidologie 48:806–820. https://doi.org/10.1007/s13592-017-0525-3

Russo L, Park M, Gibbs J, Danforth B (2015) The challenge of accurately documenting bee species richness in agroecosystems: bee diversity in eastern apple orchards. Ecol Evol 5:3531–3540. https://doi.org/10.1002/ece3.1582

Samnegård U, Alins G, Boreux V, Bosch J, García D, Happe A, Klein A, Miñarro M, Mody K, Porcel M, Rodrigo A, Roquer-Beni L, Tasin M, Hambäck PA (2018) Management trade-offs on ecosystem services in apple orchards across Europe: direct and indirect effects of organic production. J Appl Ecol 00:1–10. https://doi.org/10.1111/1365-2664.13292

Sapir G, Baras Z, Azmon G, Goldway M, Shafir S, Allouche A, Stern E, Stern RA (2017) Synergistic effects between bumblebees and honey bees in apple orchards increase cross pollination, seed number and fruit size. Sci Hortic (Amsterdam) 219:107–117. https://doi.org/10.1016/j.scienta.2017.03.010

Sawicki M, Barka EA, Clément C, Vaillant-Gaveau N, Jacquard C (2015) Cross-talk between environmental stresses and plant metabolism during reproductive organ abscission. J Exp Bot:661707–661719. https://doi.org/10.1093/jxb/eru533

Schneider D, Stern RA, Eisikowitch D, Goldway M (2002) The relationship between floral structure and honeybee pollination efficiency in “Jonathan” and “Topred” apple cultivars. J Hortic Sci Biotechnol 77: 48–51. https://doi.org/10.1080/14620316.2002.11511455

Schon NL, Poveda K, Freitas BM, Kennedy CM, Winfree R, Clough Y, Berendse F, Morandin L, Wilson JK, Kremen C, Jonason D, Bommarco R, Klatt BK, Crowder DW, Klein AM, Gratton C, Bosque-Pérez NA, Williams NM, Holzschuh A, Sidhu CS, Isaacs R, Brittain C, Danforth B, Jha S, Pocock MJO, Chaplin-Kramer R, Ramos M, Carvalheiro LG, Elle E, Macfadyen S, Rundlöf M, Lichtenberg EM, Gaines-Day HR, Eigenbrode SD, Benjamin F, Åström S, Tscharntke T, Batáry P, Potts SG, Saunders ME, Ponce C, Memmott J, Isaia M, Jones VP, Pfiffner L, Weisser WW, Ekroos J, Neame L, Martinez E, Fukuda Y, Otieno M, Snyder WE, Martin EA, Mallinger RE, Steffan-Dewenter I, Sardiñas H, Letourneau DK, Park MG, Veselý M, Grab H, Diekötter T, Sciligo AR, Krauss J, Rosenheim JA (2017) A global synthesis of the effects of diversified farming systems on arthropod diversity within fields and across agricultural landscapes. Glob Chang Biol 23:4946–4957. https://doi.org/10.1111/gcb.13714

Sheffield CS (2014) Pollination, seed set and fruit quality in apple: studies with *Osmia lignaria* (Hymenoptera: Megachilidae) in the Annapolis Valley, Nova Scotia, Canada. J Pollinat Ecol 12:120–128

Smith MR, Singh GM, Mozaffarian D, Myers SS (2015) Effects of decreases of animal pollinators on human nutrition and global health: a modelling analysis. Lancet 386:1964–1972. https://doi.org/10.1016/S0140-6736(15)61085-6

Stern RA, Eisikowitch D, Dag A (2001) Sequential introduction of honeybee colonies and doubling their density increases cross-pollination, fruit-set and yield in “Red Delicious” apple. J Hortic Sci Biotechnol 76: 17–23. https://doi.org/10.1080/14620316.2001.11511320

Thomson JD, Goodell K (2001) Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. J Appl Ecol 38:1032–1044. https://doi.org/10.1017/CBO9781107415324.004

Tscharntke T, Klein AM, Kruess A, Steffan-Dewenter I, Thies C (2005) Landscape perspectives on agricultural intensification and biodiversity - ecosystem service management. Ecol Lett 8:857–874

Tuck SL, Winqvist C, Mota F, Ahnström J, Turnbull LA, Bengtsson J (2014) Land-use intensity and the effects of organic farming on biodiversity: a hierarchical meta-analysis. J Appl Ecol 51:746–755. https://doi.org/10.1111/1365-2664.12219

Velthuis HHW, Van Doorn A (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. Apidologie 37:421–451. https://doi.org/10.1051/apido

Viana BF, Gabriel J, Coutinho DE, Garibaldi LA, Laercio G, Gastagnino B, Gramacho KP, Silva FO (2014) Stingless bees further improve apple pollination and production. J Pollinat Ecol 14:261–269

Vicens N, Bosch J (2000) Pollinating efficacy of *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae, Apidae) on 'Red Delicious' apple. Environ Entomol 29:235–240. https://doi.org/10.1093/ee/29.2.235

Volz RK, Tusk DS, Ferguson IB (1996) Pollination effects on fruit mineral composition, seeds and cropping characteristics of 'Braebum' apple trees. Sci Hortic 66:169–180

Watson JC, Wolf AT, Ascher JS (2011) Forested landscapes promote richness and abundance of native bees (Hymenoptera: Apoidea: Anthophila) in Wisconsin apple orchards. Environ Entomol 40:621–632. https://doi.org/10.1603/EN10231

Webb RA, Purves JV, Beech MG (1980) Size factors in apple fruit. Sci Hortic 13:205–212. https://doi.org/10.1016/0304-4238(80)90057-6