Supplementary material

Pollination deficits and their relation with insect pollinator visitation are cultivar-dependent in an entomophilous crop

Maxime Eeraerts*, Stan Chabert, Lisa W. DeVetter, Péter Batáry, John J. Ternest, Kris Verheyen, Kyle Bobiwash, Kayla Brouwer, Daniel García, G. Arjen de Groot, Jason Gibbs, Lauren Goldstein, David Kleijn, Andony Melathopoulos, Sharron Z. Miller, Marcos Miñarro, Ana Montero-Castaño, Charlie C. Nicholson, Jacquelyn A. Perkins, Nigel Raine, Sujaya Rao, James R. Reilly, Taylor H. Ricketts, Emma Rogers, Rufus Isaacs
*Contact: maxime.eeraerts@gmail.com

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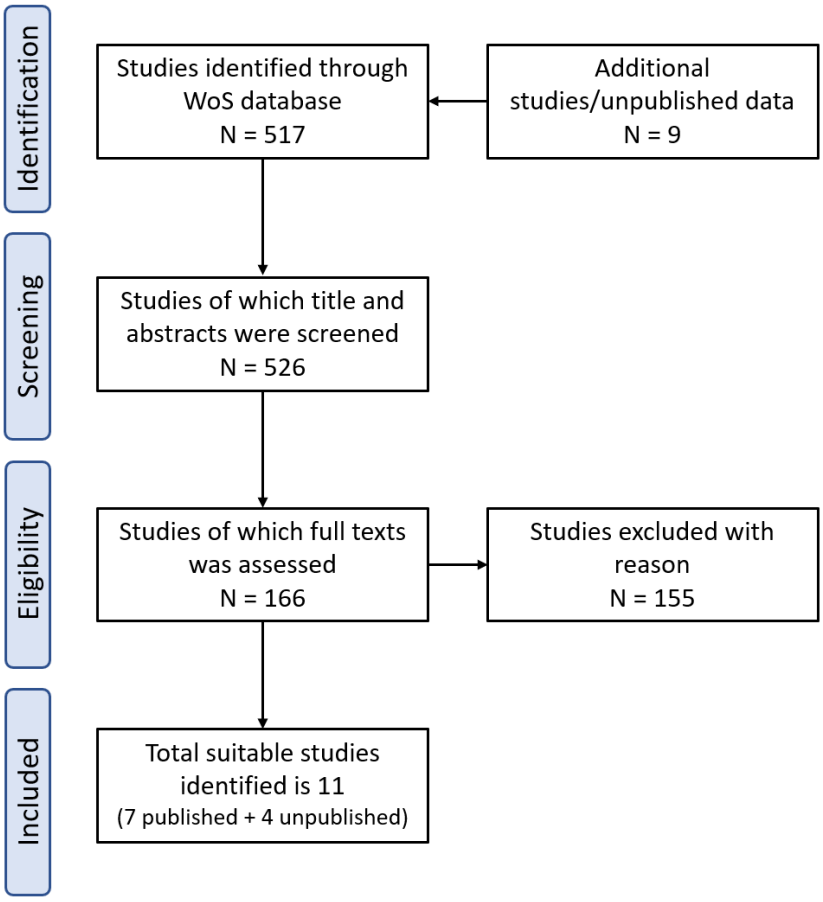

Fig. S1: PRISMA diagram showing the process of study selection.

Table S1: Overview of each study with the year(s), region(s), cultivar(s) and number of sites that were included in the study (states in the USA: FL = Florida, MI = Michigan, NJ = New Jersey, OR = Oregon, VT = Vermont, WA = Washington; provinces in Canada: BC = British Columbia, ON = Ontario). The inclusion of each study for each pollination metric is given (i.e., fruit set, berry weight and seed set), together with the origin of the pollen for the hand pollination treatment and level over bee identification (S = species or M = morphospecies).

Study	Year	Regions	Cultivar	Sites	Fruit set	Berry weight	Seed set	Pollen origin	Richness
Benjamin and Winfree 2014	2011	NJ, USA	Bluecrop	5		x		Same cultivar	NA
	2012	NJ, USA	Bluecrop	8		x		Same cultivar	NA
	2011	NJ, USA	Duke	6		x		Same cultivar	NA
	2012	NJ, USA	Duke	4		x		Same cultivar	NA
de Groot et al. 2015	2013	The Netherlands	Duke	15	x	x		Same cultivar	S
Gibbs et al. 2016	2013	BC, Canada	Bluecrop	17	x	x	x	Same cultivar	S
	2013	MI, USA	Bluecrop	17	x	x	x	Same cultivar	S
Nicholson and Ricketts 2019	2014	VT, USA	Bluecrop	9	x	x	x	Other cultivar	S
	2015	VT, USA	Bluecrop	8	x	x	x	Other cultivar	S
Reilly et al. 2020	2014	BC, Canada	Bluecrop	17	x	x		Same cultivar	S
	2015	BC, Canada	Bluecrop	12	x	x		Same cultivar	S
	2014	MI, USA	Bluecrop	16	x	x		Same cultivar	S
	2015	MI, USA	Bluecrop	17	x	x		Same cultivar	S
	2014	OR, USA	Bluecrop	6	x	x		Same cultivar	M
	2015	OR, USA	Bluecrop	6	x	x		Same cultivar	M
Eeraerts et al. 2023	2021	WA, USA	Duke	14	x	x	x	Same cultivar	S
Miñarro et al. 2023	2019	Spain	Duke	20	x	x		Cultivar mix	S
	2021	Spain	Duke	20	x	x		Cultivar mix	S
Montero-Castaño et al. unpub	2018	ON, Canada	Bluecrop	9	x	x	x	Cultivar mix	S
Melathopoulos et al. unpub	2021	OR, USA	Duke	6	x	x	x	Same cultivar	M
	2022	OR, USA	Duke	10	x	x	x	Same cultivar	M
Isaacs et al. unpub	2021	MI, USA	Bluecrop	16	x	x	x	Same cultivar	M
	2022	MI, USA	Bluecrop	16	x	x	x	Same cultivar	M
DeVetter et al. unpub	2022	WA, USA	Duke	12	x	x	x	Same cultivar	M
			Bluecrop	**179**	**165**	**179**	**91**		
			Duke	**107**	**91**	**107**	**42**		
			Total	**286**	**256**	**286**	**133**		

Table S2: Description of the materials and methods of the unpublished studies.

Study	Material and methods
DeVetter et al.	The study was performed in 2022 in Skagit and Whatcom counties in Washington, USA. A total of 12 conventionally managed 'Duke' fields were selected. In the centre of every field four transects of 100 m were constructed for data collection. Honey bees and wild bees were sampled in all fields in April and May 2022 (mid-bloom of 'Duke'), via transect walks performed for 30 minutes per transect. Transect walks were performed between 11:00 and 17:30 with temperatures ranging from 14°C to 20°C. Using insect nests, all honey bees and wild bees foraging on blooming blueberry flowers were sampled. Sampled specimens were kept in individual conical tubes until after the survey. Wild bees were identified to genus and morphospecies. Blueberry pollination was measured on 20 bushes per field (five bushes in each 100 m transect). From each bush, three branches were selected and assigned to one of the following treatments: 1) open, 2) bagged, and 3) hand pollination. For supplemental hand pollination, pollen was collected from adjacent bushes of the same cultivar and applied on the stigma of each open flowers with a fine paintbrush twice during the bloom period. All flowers from each branch were counted for fruit set estimates. After bloom, all branches were covered with a mesh bag to protect the berries. Ripe fruits were harvested by hand over two to three subsequent visits per field and visits were timed to precede commercial harvest except for the last harvest where all berries were collected to include in the analyses. For each branch, total berry number and berry weight were determined, and these data were pooled for the harvest rounds. Berry number was used to calculate percent fruit set and berry weight (gram/berry). Three berries per branch were then randomly selected from the pooled berry sample per branch. One by one, these berries were macerated in a clear plastic bag to extract and quantify seed set per berry. Only dark-plump seeds were counted and considered viable.
Isaacs et al.	The study was performed in 2021 and 2022 in Ottowa, Allegan, Van Buren and Muskegon counties in Michigan State, USA. A total of 16 conventionally managed 'Bluecrop' fields were selected. In the centre of every field four transects of 100 m were constructed for data collection. Honey bees and wild bees were sampled in all fields in April and May in both 2021 and 2022 (mid-bloom of 'Bluecrop'), via transect walks performed for 30 minutes per transect. Transect walks were performed between 11:00 and 17:30 with temperatures ranging from 14°C to 20°C. Using insect nests, all honey bees and wild bees foraging on blooming blueberry flowers were sampled. Sampled specimens were kept in individual conical tubes until after the survey. Wild bees were identified to species, genus and morphospecies. Blueberry pollination was measured on 20 bushes per field (five bushes in each 100 m transect). From each bush, three branches were selected and assigned to one of the following treatments: 1) open, 2) bagged, and 3) hand pollination. For supplemental hand pollination, pollen was collected from adjacent bushes of the same cultivar and applied on the stigma of each open flowers with a fine paintbrush. All flowers from each branch were counted for fruit set estimates. Hand pollination was performed three to five times per field during the bloom period. After bloom, all branches were covered with a mesh bag to protect the berries. Ripe fruit was harvested during three subsequent visits per field, with each visit organized in sync with the growers so that the berries were harvested before the field was subject to a harvest round of the farmer (during the last harvest round all berries were picked to include them in the data). For each branch, total berry number and berry weight were determined, and these data were pooled for the two harvest rounds. Berry number was used to calculate percent fruit set and berry weight (gram/berry). Three berries per branch were then randomly selected from the pooled berry sample per branch. One by one, these berries were macerated in a clear plastic bag, and seeds were extracted and counted to determine viable seed number.
Melathopoulos et al.	The study was performed in 2021 and 2022 in Washington, Polk and Linn counties in Oregon State, USA. A total of 6 and 8 conventionally managed 'Duke' fields were selected in 2021 and 2022, respectively. In the centre of the field four transects of 100 m were constructed for data collection. Honey bees and wild bees were sampled in all fields

	in April and May 2022 (mid-bloom of 'Duke'), via transect walks performed for 30 minutes per transect. Transect walks were performed between 11:00 and 17:30 with temperatures ranging from 14°C to 20°C. Using insect nests, all honey bees and wild bees foraging on blooming blueberry flowers were sampled. Sampled specimens were kept in individual conical tubes until after the survey. Wild bees were identified to genus and morphospecies. Blueberry pollination was measured on 20 bushes per field (five bushes in each 100 m transect). From each bush, three branches were selected and assigned to one of the following treatments: 1) open, 2) bagged, and 3) hand pollination. For supplemental hand pollination, pollen was collected from adjacent bushes of the same cultivar and applied on the stigma of each open flowers with a fine paintbrush. All flowers from each branch were counted for fruit set estimates. Hand pollination was performed twice during the bloom period. After bloom, all branches were covered with a mesh bag to protect the berries. When approximately 60% of the fruits were ripe, fruits were harvested at the branch level. Fruits were only counted a second time but not harvested a second time as the historic heatwave of 2021 damaged fruit quality of the remaining fruits. For each branch, total berry number and berry weight were determined, and these data were pooled for the two harvest rounds. Berry number was used to calculate percent fruit set and berry weight (gram/berry). Three berries per branch were then randomly selected from the pooled berry sample per branch. One by one, these berries were macerated in a clear plastic bag, and seeds were extracted and counted to determine viable seed number.
Montero-Castaño and Raine	The study was conducted in Southern Ontario, Canada, from mid-May to early June 2018. Within an area of c.a. 69 km2, 9 managed blueberry fields were selected. Nine had a conventional management, while two were organic. Average distance between study fields was 40.1 ± 3.2 km, being 3.3 km the minimum distance between pairs. Though different varieties are usually grown in a single field, our sampling was conducted only on the mid-season varieties bluecrop and blueray in fields where either one or both varieties were grown. Each field was visited twice at different time slots to conduct pollinator surveys. Surveys consisted on focal observations of 15 min during which all the flowers under observation were counted and all the flower visitors conducting a flower visits were recorded. Observations were standardized per flower and per time unit. Surveys were conducted from 10 am to 4 pm during sunny and no windy days with temperature ranging from 15 to 31 ºC. After the two visits, a total of 3 to 4 hours of focal censuses were conducted in each study field. Identifications were done at the species level. Honeybees were identified in the field. Bumblebees were captured and if not identified in the field, photos were taken for later identification before releasing them. All other flower visitors were collected in individual clean plastic vials for identification in the lab. These were then identified based on their morphology or sent (only some tissue sample, usually legs) for molecular identification to the Canadian Centre for DNA barcoding. In nine of the study fields, pollination experiments were also conducted. In May 2018, 20 individuals per field and variety (in six study fields only the 'Bluecrop' variety was studied while in the other three, the experiments were conducted on a mix of 'Bluecrop' and 'Blueray' varieties) were randomly selected and three treatments were applied in randomly selected flowers of bugs: 1) open pollination, 2) bagged, and 3) pollen addition. From mid-July and mid-August all marked flowers were checked to estimate fruit set (%) and ripped fruits were collected to be weighted and measured in the lab, where seed set was also calculated.

Table S3: Model ranking for the influence of total bee visitation on blueberry pollination deficits for fruit set, berry weight and seed set. For each response a null model, linear model and negative exponential model was tested per cultivar. Values in bold indicate models within the 2 ΔAICc unit range of the best fitting model.

Response	Cultivar	Model	AICc	ΔAICc
Fruit set deficit	Bluecrop	**Null**	**-160.26**	**0**
		Linear	-157.02	3.24
		Exponential	-157.31	2.95
	Duke	**Null**	**-40.47**	**0**
		Linear	-35.04	5.43
		Exponential	-35.98	4.49
Berry weight deficit	Bluecrop	Null	-110.48	19.08
		Linear	-123.62	5.95
		Exponential	**-129.57**	**0**
	Duke	**Null**	**-91.97**	**0**
		Linear	-88.45	3.51
		Exponential	-89.81	2.15
Seed set deficit	Bluecrop	Null	-31.66	4.03
		Linear	-33.80	1.88
		Exponential	**-35.69**	**0**
	Duke	**Null**	**-39.87**	**0**
		Linear	-38.11	1.75
		Exponential	-38.43	1.43

Table S4: Model ranking for the influence of bee richness on blueberry pollination deficits for fruit set, berry weight and seed set. For each response a null model, linear model and negative exponential model was tested per cultivar. Values in bold indicate models within the 2 ΔAICc unit range of the best fitting model.

Response	Cultivar	Model	AICc	dAICc
Fruit set deficit	Bluecrop	**Null**	**-60.06**	**0**
		Linear	-55.05	5.01
		Exponential	-54.15	5.89
	Duke	**Null**	**-40.47**	**0**
		Linear	-35.29	5.18
		Exponential	-34.45	6.02
Berry weight deficit	Bluecrop	**Null**	**-46.16**	**0**
		Linear	-40.39	5.77
		Exponential	-41.49	4.68
	Duke	**Null**	**-82.99**	**0**
		Linear	-76.76	6.23
		Exponential	-77.16	5.38
Seed set deficit	Bluecrop	**Null**	**-31.66**	**0**
		Linear	-28.03	3.63
		Exponential	-27.21	4.45
	Duke	**Null**	**-39.87**	**0**
		Linear	-34.39	5.48
		Exponential	-33.81	6.05

Table S5: Model assessing blueberry pollination deficits between cultivars. Models are tested only for data from the studies that used pollen from the same cultivar for the hand pollination treatment. Reported are the model estimates, standard error (SE), t-values and p-values are given.

Response	Cultivar	estimate	SE	t	p
Fruit set deficit	Bluecrop	0.061	0.037	1.67	0.16
	Duke	0.074	0.037	2.01	0.10
Berry weight deficit	Bluecrop	0.086	0.033	2.63	< 0.01
	Duke	0.046	0.033	1.38	0.17
Seed set deficit	Bluecrop	0.13	0.032	3.94	0.023
	Duke	0.069	0.031	2.23	0.11

Table S6: Model ranking for the influence of total bee visitation on blueberry pollination deficits for fruit set, berry weight and seed set. Models are tested only for data from the studies that used pollen from the same cultivar for the hand pollination treatment. For each response a null model, linear model and negative exponential model was tested per cultivar. Values in bold indicate models within the 2 ΔAICc unit range of the best fitting model.

Response	Cultivar	Model	AICc	dAICc
Fruit set deficit	Bluecrop	**Null**	**-162.97**	**0**
		Linear	-160.58	2.39
		Exponential	-161.11	1.86
	Duke	**Null**	**5.40**	**0**
		Linear	8.72	3.31
		Exponential	9.22	3.81
Berry weight deficit	Bluecrop	Null	-107.10	24.83
		Linear	-123.52	8.41
		Exponential	**-131.93**	**0**
	Duke	**Null**	**-34.79**	**0**
		Linear	-31.53	3.26
		Exponential	-33.24	1.55
Seed set deficit	Bluecrop	Null	-52.02	5.95
		Linear	-55.21	2.76
		Exponential	**-57.97**	**0**
	Duke	**Null**	**-39.87**	**0**
		Linear	-38.11	1.75
		Exponential	-38.43	1.43

Table S7: Linear mixed effect models assessing the effect of total bee visitation on blueberry pollination deficits for fruit set, berry weight and seed set. Models are tested only for data from the studies that used pollen from the same cultivar for the hand pollination treatment. For each response a null model, linear model and negative exponential model was tested per cultivar. Only best models are reported and model estimates, standard error (SE), t-values, p-values and model selection statistics ΔAIC_c (difference between the AIC_c of the two best models based on all possible models constructed based on the full model) are given.

Response	Cultivar	Best model	ΔAICc	Fixed factor	estimate	SE	t	p
Fruit set deficit	Bluecrop	Null	1.86					
	Duke	Null	3.31					
Berry weight deficit	Bluecrop	Exponential	8.41	exp(-bees)	0.37	0.062	5.91	< 0.001
	Duke	Null	1.55					
Seed set deficit	Bluecrop	Exponential	2.76	exp(-bees)	0.31	0.076	3.5	< 0.01
	Duke	Null	1.43					

Table S8: Model ranking for the influence of bee richness on blueberry pollination deficits for fruit set, berry weight and seed set. Models are tested only for data from the studies that used pollen from the same cultivar for the hand pollination treatment. For each response a null model, linear model and negative exponential model was tested per cultivar. Values in bold indicate models within the 2 ΔAICc unit range of the best fitting model.

Response	Cultivar	Model	AICc	dAICc
Fruit set deficit	Bluecrop	**Null**	**-53.87**	**0**
		Linear	-49.11	4.77
		Exponential	-48.24	5.63
	Duke	**Null**	**5.40**	**0**
		Linear	9.57	4.17
		Exponential	11.18	5.78
Berry weight deficit	Bluecrop	**Null**	**-44.02**	**0**
		Linear	-42.85	1.16
		Exponential	-43.63	0.38
	Duke	**Null**	**-25.77**	**0**
		Linear	-20.01	5.76
		Exponential	-20.84	4.93
Seed set deficit	Bluecrop	**Null**	**-52.02**	**0**
		Linear	-48.70	5.48
		Exponential	-48.54	3.32
	Duke	**Null**	**-39.87**	**3.48**
		Linear	-34.39	5.48
		Exponential	-33.81	6.05

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