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THE BEHAVIOUR OF *BOMBUS IMPATIENS* (APIDAE, BOMBINI) ON TOMATO (*LYCOPERSICON ESCULENTUM* MILL., SOLANACEAE) FLOWERS: POLLINATION AND REWARD PERCEPTION

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Abstract—The foraging behaviour of pollinators can influence their efficiency in pollinating certain plant species. Improving our understanding of this behaviour can contribute to an improvement of management techniques to avoid pollination deficits. We investigated the relationship between the number of visits of bumble bees (*Bombus impatiens*) to tomato flowers (*Lycopersicon esculentum*) and two variables related to the quality of the resulting fruits (weight, number of seeds), as well as the relationship between foragers' thoracic weights, physical characteristics of thoracic vibrations (main frequency, velocity amplitude), amount of pollen removed from flowers, and the quality-related variables. In addition, we studied the capability of foragers to assess the availability of pollen in flowers. Tomato weight and seed number did not increase with the number of bee visits, neither were they correlated with the foragers' thorax weight. Thorax weight also did not correlate with the amount of pollen removed from the flowers nor with the physical characteristics of vibration. Vibration characteristics did not change in response to the amount of pollen available on tomato flowers. Instead, foragers adjusted the time spent visiting the flowers, spending fewer time on flowers from which some pollen had already been removed on previous visits. The quantity and the production-related variables of tomatoes are not dependent on the number of bee visits (usually one visit suffices for full pollination); bigger foragers are not more efficient in pollinating tomato flowers than smaller ones; and *B. impatiens* foragers are capable of evaluating the amount of pollen on a flower while foraging and during pollination.

Keywords: *bumblebee, pollination, tomato, vibration*

INTRODUCTION

Bumble bees (*Bombus* spp.) are highly efficient pollinators of tomato (*Lycopersicon esculentum* Miller) flowers and, for commercial purposes, yield far better results than honeybees, manual vibration, or self-pollination (Banda & Paxton 1991; Kevan et al. 1991; Dogterom et al. 1998; Morandin et al. 2001a, 2001b; Palma et al. 2008; Choi et al. 2009; Torres-Ruiz & Jones 2012). Today, approximately 95% of all bumble bee sales worldwide are destined for tomato production, with the estimated value of the bumble bee-pollinated crops reaching 12 billion Euros per year (Velthuis & van Doorn 2006).

Although tomato plants are self-compatible, the anthers need to be shaken to allow effective pollen release (Buchmann 1983). Many bee species, among them the bumble bees, generate thoracic vibrations when visiting tomato flowers therewith facilitating the release pollen from the anthers ("buzz-pollination"; Buchmann & Hurley 1978, Buchmann 1983). However, whether and to which extent the physical characteristics of thoracic vibrations are correlated with fruit characteristics (e.g. weight, size, seed number) remains unknown.

Tomato fruit size depends, to a certain extent, on the amount of pollen transferred to the stigma (Morandin et al. 2001a). Even so, it has been suggested that the quality of tomatoes (weight, size, seed number) does not increase any further at flower visitation rates above one or two bumble bee visits (*Bombus impatiens*; Morandin et al. 2001a). In case, however, pollen is transferred inadequately to the stigma, seed production is impaired, therewith resulting in sub-optimal crop yields ("pollination deficit"; Vaissière et al. 2011).

The adequacy, efficiency, and quality of bee pollination are affected by many factors, such as the floral characteristics of a plant species that influence the behaviour of flower visitors (Lefebvre & Pierre 2006). Tomato flowers, for instance, produce certain chemicals (β-phellandrene and 2-carene) as part of their scent bouquet that reduce the visitation frequency of *B. impatiens* to the flowers, thus impeding bee pollination (Morse *et al.* 2012). The amount

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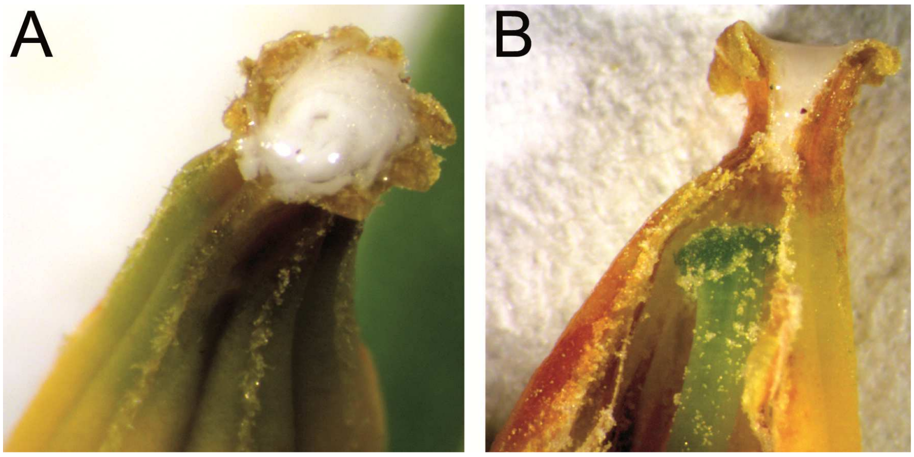

FIG. 1. (A) The pore of the anthers' cone of tomato (*Lycopersicon esculentum*) flowers after being blocked with silicon. (B) By opening the cone it was possible to verify that the silicon was not removed by *Bombus impatiens* (Apidae) visitation and that pollen was deposited on stigma.

of these chemicals can be altered by different cultivation practices: vegetative plants produced less β-phellandrene and 2-carene and received more visits than generative plants (Morse 2009). Another important factor influencing pollinator visits is the presence of floral rewards.

In the case of tomato flowers, pollen is the only resource collected by bees, and its availability may affect the behaviour of these pollinators, yet only if the bees are capable of assessing the amount of pollen in flowers. Few studies have tackled this subject so far. But most of these investigations point to the ability of bees to evaluate the amount of pollen in flowers (Buchmann & Cane 1989, Harder 1990, Shelly et al. 2000, see however Hodges & Miller 1981).

Given the importance of bumble bees in tomato pollination and considering the putative relationship between the bees' behaviour and their efficiency as pollinators, we investigated the following questions: (1) Do fruit set and production-related parameters (weight and seed number) depend on the number of bumble bee (*Bombus impatiens*) visits? (2) Are big foragers more efficient in pollinating tomato flowers than small individuals? (3) Are *B. impatiens* foragers capable of evaluating the amount of pollen available in a flower during pollination?

MATERIALS AND METHODS

Study site and bee species

The present study was performed at the Greenhouse and Processing Crops Research Center of Agriculture and Agri-Food Canada (Harrow, Ontario, Canada) between April and June of 2010. The tomato plants (*Lycopersicon esculentum* Mill var. Clarance: Solanaceae) to be used in the experiments were grown and maintained in one compartment of the experimental greenhouse complex (plant compartment). A second compartment (bee compartment) contained two screened cages (2.5m x 5.3m x 2.3m), each of which contained one bumble bee colony (*Bombus impatiens* Cresson, Apidae) and 20 tomato plants, even during the experiments. The colonies were provided by Biobest Canada (Leamington, Ontario, Canada) and consisted of one queen and initially 15 workers. Due to the fact that tomato flowers do not produce nectar, the hives had their own compartment containing a sugar solution as carbohydrate substitute.

Experiment 1

This first set of experiments was designed to evaluate whether and to which extent the number and duration of bee visits to tomato flowers influence posterior fruit set, weight and seed number. Additionally, it allowed us to investigate whether or not *B. impatiens* foragers are capable of assessing pollen reward.

We transferred tomato plants from the plant compartment to the bee compartment. Prior to the transfer, we covered the completely opened flowers to be used in the experiments with mesh bags to prevent bee visitation. In the bee compartment, the mesh bag of one flower was removed and only a single forager was released from the colony. The bumble bee nest was kept closed for the remainder of experiment. After the forager had visited an experimental flower for the desired number of times (see flower visit treatments), we covered the flower again with a mesh bag. Each forager was used for one to four subsequent flower visit treatments and then collected and killed by freezing. Immediately after death, the thorax was separated form head, abdomen, legs and wings and afterwards weighed on a precision scale (10^{-4} g).

The flower visit treatments were: C: control, no visit (n=18 flowers); 1V: one bee visit (n=16); 2V: two visits (n=16); 3V: three visits (n=17); 4V: four visits (n=18); SV: several visits, plants from the plant compartment that had virgin flowers were kept for 8 hours in the bee compartment where the hive was opened and bees could forage freely (n=20); C2: control 2, a drop of silicon placed on the surface of the anther cone (n=16); and BP: anther pores blocked, one visit to flowers which had the pore of the anther cone blocked with silicon to stop pollen release (n=16, Fig. 1). All visits were video-taped (JVC Everio GZ-MS 100V camcorder) for later analysis. After the visitation treatments, the plants were returned to the plant compartment, where they were kept until fruit ripening. Each flower and subsequent fruit was tagged for individual identification. After ripening, fruits were weighted (10^{-4} g) and their seeds counted.

FIG. 2. Bumble bee (*Bombus impatiens*) vibration recording. (A) The setup showing the Laser Doppler Vibrometer mounted on a small four-wheeled cart (1) and the flower fixed to a tripod pan handle with adhesive tape (2) being visited by a forager. (B) The red dot on the scutum of the bee is the laser beam of the vibrometer.

Bee visits were analysed by observing the video recordings concerning the total visit duration (visit duration = time between first landing and leaving for the colony or another flower). The number of buzzes made by the foragers could not be analysed from the videos because the noise of the ventilation system of the greenhouse interfered with detection of the bee sounds, thus compromising the accuracy of data. For evaluating the reward perception by bumble bee foragers, we compared the visit duration among the different treatments. As described by Buchmann & Cane (1989) it was expected that foragers spend more time visiting flowers with higher pollen reward.

Experiment 2

In a second set of experiments, we evaluated whether the amount of pollen removed from tomato flowers is related to the physical aspects of the thoracic vibrations generated by the bumble bee foragers during flower visits.

Individual virgin flowers were transferred to the bee compartment and fixed to a tripod pan handle with adhesive tape (Fig. 2A). Single bumble bee foragers were allowed to visit the flowers as described above. The thoracic vibrations generated by the foragers were recorded using a portable Laser Doppler Vibrometer (PDV-100, Polytec, Waldbronn, Germany; Fig. 2A), mounted on a small four-wheeled cart (for details see Hrncir *et al.* 2004) and positioned on a table right beneath the flower (Fig. 2A). The laser beam of the vibrometer was directed upwards via a diagonal mirror, and oriented perpendicular to the surface of the thorax as the bee hung inverted from the anther cone of the flower. Movements of the foragers could be followed by moving the cart. Thus, the laser beam aimed at the scutum of a forager during the entire flower visit (Fig. 2B). The output of the vibrometer was fed into a notebook using the software Soundforge 7.0 (Sony Pictures Digital Inc., Madison, WI, USA). Vibration analyses were performed using the software SpectraPro 3.32 (Sound Technology Inc., Campbell, CA, USA). For each forager, we calculated the average main frequency (Hz) and the average velocity amplitude (mm/s) of its thoracic vibrations (average of 3 to 108 pulses). Statistical tests were performed using these individual averages.

The visitation treatments (1V: n=15; 4V: n=15; C2: n=14; BP: n=15) were the same as in experiment 1. In treatment 4V, bee vibrations were recorded only during the first and the last visit, based on our observations in the first experiment that revealed a great difference in behaviour between the first and the fourth visit (see results).

After the respective treatment, the anther cones were carefully removed from flowers and stored individually in tubes containing 1 ml of alcohol 70%. Afterwards, the anthers (C: n=13; 1V: n=15; 4V: n=15) were dissected and the pollen grains removed. The pollen grains were diluted in 15 ml of a saline solution for numerical enumeration using a particle counter (MultisizerT 3 COULTER COUNTER®). The total number of pollen grains in a sample was estimated from three subsamples of 0.5 ml each. The amount of pollen removed from anthers by bees was determined by subtracting the mean amount of pollen left inside anthers after the visits (treatments 1V and 4V) from the mean amount of pollen found in virgin flowers (treatment C).

Data analysis

Statistical analyses were performed using the software packages BioEstat, Statistica, and Sigma Plot. Because data were not normally distributed (Shapiro-Wilk test; P<0.05), we performed non-parametric statistical tests only. The respective tests are given in the results section. The α-level of significance was $P \leq 0.05$. Throughout the text, data are presented as mean values ± standard deviation.

RESULTS

Are fruit set and weight and seed number of tomatoes related to the number of bee visits?

Fruit set was similar in most visitation treatments. In the treatment groups C (n=18), 2V (n=16), 4V (n=18), and C2 (n=16), fruit set was 100%. In groups 1V (n=16) and 3V (n=17), one flower (1V) and two flowers (3V) were aborted after one day of lack of water caused by a failure in the irrigation system, resulting in reduced fruit sets of 93.8% (1V) and; 88.2% (3V). Fruit set in treatment group SV (n=20) was 90%. The only group with clearly reduced success was BP (anther pores blocked, n=16), where fruit set was 75%.

Fruit weight was significantly lower in the control group C compared to the treatment groups 1V, 2V, 3V, 4V, SV and C2 (Kruskall-Wallis test, X^2=40.9, P<0.05; Dunn's pairwise comparison, P<0.05). There were no statistically significant differences in fruit weight between C and BP, neither among treatment groups 1V, 2V, 3V, 4V, SV and C2 (Dunn's pairwise comparison, P>0.05) (Fig. 3A). Fruits of control group C produced significantly fewer seeds than fruits of the treatment groups 1V, 2V, 3V, 4V, SV and BP (Kruskall-Wallis test, X^2=26.2, P<0.05; Dunn's pairwise comparison, P<0.05). There were no statistically significant differences in seed number between C and C2, neither among treatment groups 1V, 2V, 3V, 4V, SV, C2, and BP (Dunn's pairwise comparison, P>0.05) (Fig. 3B).

Are bigger foragers more efficient in pollinating tomato flowers than smaller ones?

The mean thoracic weight of foragers was 48.3±11.4 mg (n=20; maximum: 68.8 mg; minimum: 26.1 mg). The investigated physical parameters of the thoracic vibrations, main frequency and velocity amplitude, did not correlate with the thoracic weight of the forager generating them (Tab. 1). Neither of these vibration parameters nor the thoracic weight correlated with the amount of pollen removed after one and after four flower visits (Tab. 1). Also concerning fruit quality, we found no significant correlations between the thoracic weight of the forager and fruit weight (Spearman Rank Correlation: r=0.00, P>0.05, n=88), or seed number (Spearman Rank Correlation: r=0.10, P>0.05, n=88) of the tomatoes that resulted from the respective bee's visit.

Are foragers capable of assessing the amount of available pollen during a flower visit?

The mean number of pollen grains in the anthers of virgin tomato flowers (control group C) was 96,561 ± 28,220 (n=13). After a single bumble bee visit, the number of pollen grains dropped to an average of 40,768 ± 32,701 (n=15) and after four visits further to 30,595 ± 36,794 (n=15). Thus, a forager removed, on average, 57.8% of a flower's pollen during the first visit, and 68.3% within four visits. The number of pollen grains in virgin flowers was significantly larger than the number of pollen grains after one and after four visits (Kruskall-Wallis test: X^2=21.6, P<0.05; Dunn's pairwise comparison: C *vs* 1V: P<0.05; C *vs* 4V: P<0.05; Fig. 5); however, there was no statistically significant difference concerning the number of pollen grains after the first visit or after four visits (Dunn's pairwise comparison: 1V *vs* 4V: P>0.05; Fig. 4).

During the first visit, foragers remained significantly longer on a flower than during all the following visits (Tab. 2), as expected. When the pores of the anthers were blocked (treatment BP, no access to pollen), the foragers' visits were

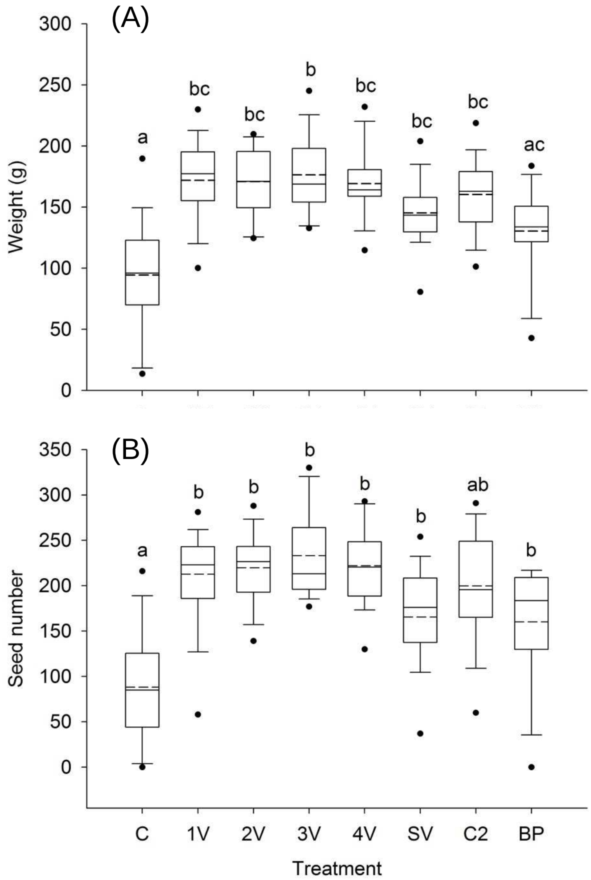

FIG. 3. (A) Mean weight and (B) mean seed number of tomato fruits produced by different treatments (C = control, 1V = one visit, 2V = two visits, 3V = three visits, 4V = four visits, SV = several visits: virgin flowers from plants kept for 8 hours in the bee compartment, C2 = control 2: a drop of silicon on the surface of the anther cone, BP = blocked pore: one visit flowers which had the pore of the anther cone blocked with silicon to stop pollen release. All visits were performed by *Bombus impatiens* (Apidae). Different letters (a, b) indicate statistical differences at P<0.05. *Box plots:* box indicates the distribution of 50% of the values, horizontal full line indicates median, horizontal dashed line indicates mean, whiskers indicate standard error (above 90% and below 10%) and spheres indicate outliers.

TABLE 1. Spearman correlation coefficients among main frequency (Hz), velocity amplitude (mm/s), amount of pollen remaining on tomato flowers after one (1V) and four visits (4V) and thoracic weight of *Bombus impatiens* foragers (mg).

Treatment		Main frequency (Hz)	Velocity amplitude (mm/s)	Amount of pollen left
1V	Amount of pollen left	0.30ns	0.19ns	—
	Thoracic weight of forager (mg)	-0.23ns	-0.10ns	-0.45ns
4V	Amount of pollen left	-0.32ns	-0.51ns	—
	Thoracic weight of forager (mg)	0.36ns	0.23ns	-0.47ns

ns: not significant at *P*<0.05

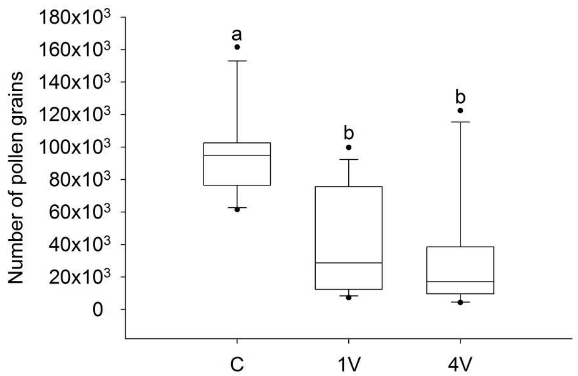

FIGURE 4. Mean number of pollen grains of tomato virgin flowers (C), of flowers visited once (1V) and four times (4V) by a forager of *Bombus impatiens*. Different letters (a, b) indicate statistical differences at *P*<0.05 (Kruskall-Wallis, pair comparison: Dunn's method). *Box plots:* box indicates the distribution of 50% of the values, horizontal full line indicates median, horizontal dashed line indicates mean, whiskers indicate standard error (above 90% and below 10%) and spheres indicate outliers.

significantly shorter compared to visits to flowers with pollen access (Tab. 2).

Although visit duration changed significantly, the physical parameters of thoracic vibrations generated during the visits did not. Both mean main frequency (1V = 334.3 ± 11.4 and 4V = 344.7 ± 20.4) and velocity amplitude of the foragers' vibrations (1V = 195.0 ± 16.4 and 4V = 223.6 ± 64.4) did not differ between the first and the fourth visit (Wilcoxon Signed Rank-Test: for frequency - T=24.0, Z=1.5, *P*>0.05; for velocity - T=19.0, Z=1.8, *P*>0.05). The vibration parameters did also not differ between the first visit to untreated flowers (1V: mean main frequency = 332.8 ± 19.3, velocity amplitude= 194.7 ± 20.5), flowers of control treatment 2 (C2: mean main frequency = 337.9 ± 18.0, velocity amplitude=188.8 ± 27.5) and flowers with no access to pollen (BP: mean main frequency = 344.0 ± 18.5, velocity amplitude= 188.8 ± 27.5) (Kruskal-Wallis test: for frequency - X^2=2.5, *P*>0.05; for velocity - X^2=2.8, *P*>0.05).

DISCUSSION

Bumble bees are important pollinators for tomato crops. With the present study, we wanted to contribute to a more profound understanding of the biological background of the efficiency of *Bombus impatiens* in pollinating tomato flowers. Our results indicate that (1) foragers are capable of assessing the pollen reward of the flowers, (2) the first flower visit is the most effective concerning pollen removal and, consequently, pollination, and (3) small foragers are as efficient in pollinating tomato flowers as are big ones. From these results, we can draw important conclusions concerning both the foraging biology of this bee species and their use and management for tomato crop pollination.

Foraging biology of Bombus impatiens

Foragers of *B. impatiens* spent less time visiting flowers with low amounts of pollen than they spent on virgin flowers (Tab. 2). This points to the bees' capability of evaluating the resource value during their visit. The perceptual mechanism by which bees assess the amount of pollen available while removing it from a flower is still far from understood. Hodges & Miller (1981) proposed that bees are not capable of evaluating the amount of pollen obtained during a single visit. Also, assessing the quantity of pollen loaded may be difficult for bees because, in contrast to nectar, pollen is deposited on the body, not ingested (Hodges & Miller 1981).

Our results, however, in line with some earlier studies (Buchmann & Cane 1989, Harder 1990, Shelly et al. 2000) provide clear evidence that bees are indeed capable of perceiving the amount of pollen obtained while visiting a flower. In accordance with Buchmann & Cane (1989), we observed that bees groomed several times during a visit, thereby transferring pollen to their corbiculae. Thus, one possibility is that bees evaluate the amount of pollen during grooming. A second possibility is that bees directly register the pollen that falls on their body (head, thorax and abdomen) through mechano-sensitive hair, the sensilla trichodea, which are highly sensitive to tactile stimuli (McIver 1975). A third possibility is that bumble bee foragers make use of scent marks, deposited by previous flower visitors, to evaluate whether or not the flower still provides pollen (e.g. Stout et al. 1998; Goulson et al. 2000; Stout & Goulson 2001). Although these possibilities are not mutually exclusive, and bees may use more than one information for reward evaluation, our finding that foragers spent significantly less time on flowers without pollen reward (visitation treatment BP, pollen release blocked) that on virgin flowers (Tab. 2) corroborate the direct perception-mechanism. Just like the virgin flowers, flowers with blocked

TABLE 2. Mean, minimum (Min) and maximum (Max) visit duration of *Bombus impatiens* foragers to tomato flowers and respective standard deviations (SD) and sample sizes (N). Treatments: (1V) one visit; (2V) two visits; (3V) three visits; (4V) four visits; (C2) control 2: a drop of silicon on the surface of the anther cone; and (BP) blocked pore. Different letters (a, b) indicate statistical differences at P<0.05

		Visit duration (s)				Statistics	
		Mean ± SD	Min	Max	N	Test	*P*
1V		89 ± 71a	11	242	17		
C2		89 ± 70a	10	292	16	Kruskal-Wallis	X^2=17.4, *P* <0.05
BP		12 ± 8b	1	29	16		
2V	1st visit	78 ± 61a	24	269	16	Wilcoxon Matched Pairs	*T*=27.0, *Z*=2.1, *P* <0.05
	2ndvisit	32 ± 42b	3	147			
3V	1st visit	107 ± 83a	1	323	17	Friedman ANOVA	X^2= 10.5, *P* <0.05
	2ndvisit	24 ± 29ab	1	105			
	3rdvisit	14 ± 16b	1	66			
4V	1st visit	112 ± 71a	2	276	17	Friedman ANOVA	X^2= 19.9, *P* <0.05
	2ndvisit	15 ± 18b	2	66			
	3rdvisit	30 ± 60b	1	247			
	4thvisit	11 ± 9b	1	30			

pores had not been visited before, thus they carried no scent marks.

Although visit duration changed significantly with pollen reward (Tab. 2), the mechanical characteristics of the thoracic vibrations (main frequency and velocity amplitude) generated by the forager during the flower visits did not. Also, the thoracic vibrations did not differ between bees of different size (Tab. 1). This result seems surprising on first sight, given that the force of the thoracic vibrations is determined, in parts, by the mass of the flight muscles and, thus, depends on thorax size (Buchmann et al. 1977; Buchmann & Hurley 1978; Morse 1981; King & Buchmann 1995, 1996; Hrncir et al. 2008). Furthermore, in a recent study, De Luca et al. (2012) found a significant correlation between bumble bee (*B. terrestris*) forager mass and peak amplitude of the vibrations of *Solanum rostratum* flowers caused by the foragers. Probably, these differences between our findings and those by De Luca et al. (2012) stem from methodological differences. Whereas de Luca et al. (2012) measured vibrations on the petals of flowers, we picked up the vibrations directly from the thoraces of the bees. From this, we can assume that small bees, even when generating thoracic vibrations of similar amplitudes as big bees (Tab. 1), vibrate the flowers with reduced force due to reduced body mass compared to big bees (force = mass × acceleration, where acceleration is proportional to amplitude × frequency). Consequently, flower vibrations caused by small bees are of smaller amplitude than those caused by big bees. Here, future investigations on the vibration transfer between bees and flowers shall test our assumption.

The use and management of Bombus impatiens *for tomato crop pollination*

Bumble bee pollination increases tomato production, weight and seed number, which guarantees a better market price (Kevan et al. 1991; Velthuis & van Doorn 2006). In addition to weight as important factor for the value of a crop, a recent study indicates that the number of seeds is important for the sensory characteristics of tomatoes, resulting in the preference of bee-pollinated tomatoes over wand-pollinated ones by consumers (Hogendoorn et al, 2010).

Our results show that a single bee visit is enough to guarantee heavier fruits with elevated seed number (Fig. 3). Interestingly, both these fruit characteristics did not increase significantly when flowers were visited more than once by bumble bees. Our finding corroborates the results by Morandin et al. (2001a), who examined the relation between tomato weight and seed number and the bruising (caused by the bees biting the anther cones) on the anthers, which indicate the approximate number of bee visits. In compliance with our findings, these authors observed that tomato weight did not increase with bruising levels above one (one visit) and seed number did not increase with bruising levels above two (one or two visits). A possible explanation for this observation that a single bee visit is sufficient to promote high-quality tomatoes is that bees remove a significantly larger amount of pollen from flowers during the first visit than on subsequent visits (Fig. 4). This elevated pollen removal may result in the deposition of enough pollen grains to fertilize most ovules during the first visit.

Controlling the intensity of bumble bee visits is important for tomato production because a high level of visitation damages the reproductive organs of flowers, causing abortion (Morandin *et al.* 2001b; Morse 2009). In our study, the plants in the bee compartments were intensely visited, causing flower destruction and abortion (Fig. 5). Tomato growers need to consider this potential damage through bumble bees when planning the pollination management of their crop. Morandin *et al.* (2001b) suggest that 7 to 15 colonies of *B. impatiens* per hectare are

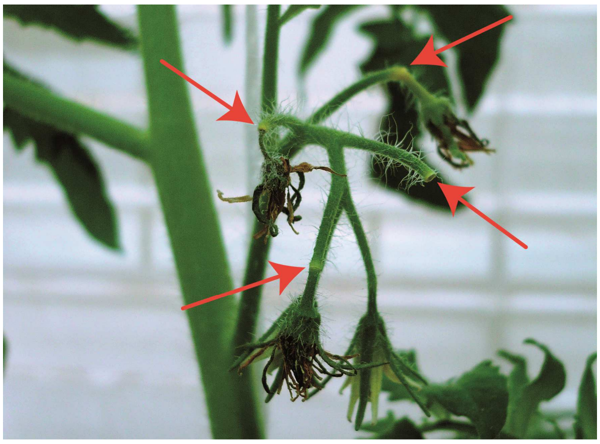

FIGURE 5. Tomato flowers damaged from intense visitation by *Bombus impatiens* and showing signs of abortion (red arrows).

sufficient to guarantee adequate pollination (one visit). In fact, a single bumble bee visit considerably increases the economic value of tomato crops. From our results on fruit set and fruit quality (weight), the estimated base-value of 100 tomatoes (control group C, no visits) would be C$14.62 (estimated value = mean fruit weight: 94.34g × fruit set: 100% × price/kg tomato $_{\text{in 2010, 2011}}$: C$1.55, Shalin Khosla, personal communication). For the visitation treatments 1V, 2V, 3V, 4V and SV the estimated values were C$25.00, C$26.48, C$24.11, C$26.23, and C$20.26, respectively. Thus the estimated yield due to bumble bee visits was 71.0% (1V), 81.1% (2V), 64.9% (3V), 79.4% (4V), and 38.5% (BP) higher than that for the control group C.

The second important result from our study concerning the commercial use and management of *B. impatiens* as tomato pollinators was that fruit quality (weight, number of seeds) was not related to forager size (thorax weight). This independence of bee size may be due to tomato floral morphology: tomato flowers have a cone of anthers surrounding the pistil, thus forming a chamber (Rick & Robinson 1951; McGregor 1976; Fig. 1B). This structure allows for self-pollination because the pollen, when released from the anthers, falls directly onto the stigma inside the chamber (Rick & Robinson 1951; Rick & Dempsey 1969). This self-pollination mechanism and the fact that the first bee visit causes the release of sufficient pollen to fertilize most ovules of a flower, independently of forager size (Tab. 1), could explain why fruit quality is not related to the size of the pollinator.

This finding may have an important impact for the management of bumble bees as pollinators for tomato crops. First, the size of *B. impatiens* workers decreases with increasing age of the colony (Couvillon et al. 2010). Hence, if forager size affected pollination efficiency, tomato growers would have to substitute their colonies frequently in order to assure the availability of big foragers. Our results now suggest that there is no real need for this practice. Second, the independence between bee size and pollination efficiency indicates that other bumble bee species may have potential for commercial tomato pollination. Recently, Torrez-Ruiz and Jones (2012) showed that *B. ephippiatus* is as efficient in pollinating tomatoes as is *B. impatiens*, despite differences in foraging pattern. Colony foraging pattern, however, may have an important contribution to pollination efficiency. Whittington and Winston (2004) compared the behaviour of *B. occidentalis* and *B. impatiens* in tomato greenhouses. The observed differences in foraging pattern lead these authors to the conclusion that *B. impatiens* is a better pollinator for tomatoes than *B. occidentalis*. Therefore, to explore the possibility of using different bumble bee species for tomato pollination, more comparative studies are needed. We suggest that these studies explore not only colony foraging pattern and yield, but also the bees' efficiency in pollen removal. Although our results indicate that tomato pollination efficiency is not related to bee size, this relationship should be further investigated.

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