

## Stingless bees in applied pollination: practice and perspectives

Ester Judith SLAA<sup>a,b</sup>, Luis Alejandro SÁNCHEZ CHAVES<sup>b</sup>,  
Katia Sampaio MALAGODI-BRAGA<sup>c</sup>, Frouke Elisabeth HOFSTEDÉ<sup>d</sup>

<sup>a</sup> Institute of Integrative and Comparative Biology and Centre for Biodiversity and Conservation,  
University of Leeds, LS2 9JT Leeds, UK

<sup>b</sup> Centro de Investigaciones Apícolas Tropicales, Universidad Nacional, Costa Rica,  
Apartado postal 475-3000 Heredia, Costa Rica

<sup>c</sup> Laboratório de Abelhas, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Travessa 14,  
n.321 CEP 05508-900 São Paulo, Brazil

<sup>d</sup> Tropical Bee Research Unit, Behavioural Biology Department, Utrecht University, PO Box 80.086,  
3508 TB Utrecht, The Netherlands

Received 17 October 2005 – revised 27 February 2006 – accepted 28 February 2006

**Abstract** – At present, numbers of both wild and managed bee colonies are declining rapidly, causing global concern for pollination services. Stingless bees play an important ecological role as pollinators of many wild plant species and seem good candidates for future alternatives in commercial pollination. This paper reviews the effectiveness of stingless bees as crop pollinators. Over the past six years the number of crops reported to be effectively pollinated by stingless bees has doubled, putting the total figure on 18 crops. Eleven stingless bee species across six genera have been found to forage effectively under enclosed conditions, indicating the potential of stingless bees as pollinators of greenhouse crops. The biological features that make stingless bees strong candidates for commercial pollination services are discussed, together with their present limitations. The effects of natural vegetation and wild bees on crop yield are reviewed, and make a strong case for habitat conservation.

**agriculture / alternative pollinators / food crop / greenhouse / Meliponini**

### 1. ECONOMIC IMPORTANCE OF POLLINATION IN COMMERCIALLY GROWN CROPS

Most crop plants depend on pollination for fruit and seed set. For many of these crops, insects are the main pollination vector (with the main exception of grains, which are wind- or self-pollinated). It has been estimated that about 30% of human food is derived from bee-pollinated crops (O’Toole, 1993 cited in Kearns and Inouye, 1997). A wide variety of

bee species are known to be efficient and effective pollinators of many crops (e.g. Freitas and Paxton, 1998; Heard, 1999; Richards, 2001; Kremen et al., 2002). Nevertheless, the European honeybee (*Apis mellifera* L.) is the single most commonly used species in managed pollination services, and the dependency of commercial crop yields on honeybee pollination is enormous everywhere. The economic value of honeybees as agricultural pollinators has been estimated for several countries (e.g. ranging between \$1.6 and \$5.7 billion per year for the United States of America; Southwick and Southwick, 1992) and £137.8 million per year for selected crops in the United Kingdom (Carreck and Williams, 1998)), and far

Corresponding author: E.J. Slaa,  
e.j.slaa@leeds.ac.uk

exceeds their economic value as producers of honey, wax and other hive products (Carreck and Williams, 1998). On a global scale, the total annual value of agricultural pollination has been estimated at \$200 billion (Kearns et al., 1998).

## 2. COMMERCIAL POLLINATORS

### 2.1. Why look for new species?

Recently, numbers of both managed and wild bees are declining rapidly, causing global concern for pollination services (e.g. Watanabe, 1994; Buchmann and Nabhan, 1996; Kearns and Inouye, 1997; Nabhan et al., 1998; Cane and Tepedino, 2001; UNEP, 2002; Villanueva et al., 2005; see Ghazoul (2005a, b) and Steffan-Dewenter et al. (2005) for a discussion on this topic). Threats include habitat destruction or alteration, overuse of pesticides, parasites and diseases, and the introduction of alien species (Buchmann and Nabhan, 1996; Kearns and Inouye, 1997; Kremen et al., 2002). Management of honeybee hives is handicapped worldwide by infectious diseases and parasites such as varroa mites (*Varroa destructor*), American Foul Brood, (*Bacillus larvae*), and Chalk brood (*Ascosphaera apis*) (Watanabe, 1994). In response to the worldwide decline of pollinator populations and pollinator diversity, the “Conference of the Parties to the Convention on Biological Diversity” established an International Initiative for the Conservation and Sustainable use of Pollinators in 2000. One of the main aims of this Initiative is to “promote the [...] sustainable use of pollinator diversity in agriculture and related ecosystems” (UNEP, 2002). Diversification of crop pollinators would help to achieve pollination services when the commonly used pollinator (specifically honeybees for most crops nowadays) is not available in sufficient numbers.

In addition, honeybees are not always the most efficient pollinators due to various factors, e.g. a miss-match in body size and flower size, low nectar production and specialized pollen release mechanisms in some plants, including those with poridical anthers (Kearns

and Inouye, 1997). When honeybees do not efficiently pollinate a given crop, it is probably economically beneficial to search for a better pollinator-plant match. It has been estimated that in the US alone, the commercial value of non-honeybee pollinators to crop yields may be as high as \$6.7 billion per year (see Kearns et al., 1998).

Over the last several decades the management of some other pollinators has been developed which have proven to be much more efficient than the honeybee for certain crops. Examples include *Nomia*, *Osmia*, *Megachile* (for alfalfa), bumble bees (for crops of the Solanaceae family, e.g. tomatoes), flies, and more recently, stingless bees (Torchio, 1987; Free, 1993; Heard, 1999). The best-known example is probably the success-story of the commercial use of bumble bees for the pollination of tomatoes (see Free, 1993). In tomato flowers, as a member of the Solanaceae family, pollen is released through vibration (‘buzzing’) of their poricidal anthers. Bees produce these vibrations by shivering the indirect flight muscles, and anther buzzing has been observed in many bee species, including bumble bees and stingless bees of the genus *Melipona*. Not all bee genera however seem to show this behaviour; e.g. honeybees and stingless bees other than *Melipona* are not buzz-pollinators (Buchmann, 1983). Greenhouse tomato flowers were commonly pollinated by hand using an electrical vibrator, but are now almost all successfully pollinated by commercially bred bumble bees (see Free, 1993). In the UK, the total value of bee pollination was estimated to exceed £200 million per annum, of which bumble bee pollination in green houses accounted for nearly 15% (£29.80 million) (Carreck and Williams, 1998).

A negative side to such a success story is the risk of establishment of exotic pollinators in non-native areas. Both bumble bees and solitary bees used for pollination services have been exported to different parts of the world to enhance crop pollination, and in many cases established successfully. Introduction of exotic bee species causes general concern because of its potentially negative effects on both native pollinators and plants (see Goulson, 2003).

To avoid introduction of exotic flower visitors, some researchers have tested native bumble bee species for pollination services (see Kaftanoglu, 2000 (Turkey); Mah et al., 2000 (Korea)).

Besides physical properties hindering efficient pollination, honeybees may have other drawbacks in pollination services. First, honeybee colonies have seasonal cycles with a long inactive period in temperate regions which makes them less suitable for the pollination of off-season products. Second, honeybees have a functional sting, and although the tendency to sting is quite low in several breeds of European honeybees, it may still cause problems for crop workers who are allergic. In addition, all honeybees present nowadays in the Neotropics are Africanized (e.g. Caron, 2001; Schneider et al., 2004), which requires additional safety measures due to their aggressive nest defense (Winston, 1992). This is especially a problem in enclosed areas such as greenhouses, and in fields close to human or animal establishments.

An economic drawback to the commercial use of bumble bees is that colonies die after reproduction. New hives need to be bought repeatedly, making this pollinator service rather expensive, especially for small-scale farmers in developing countries.

## 2.2. Why stingless bees?

Good candidates for future alternatives in commercial pollination can be found in the diverse group of stingless bees (Meliponini) (Rindfleisch, 1980; Roubik, 1995b; Heard, 1999; Sommeijer and de Ruijter, 2000). Stingless bees comprise a highly diverse and abundant group of eusocial bees that inhabit the tropical and subtropical parts of the world. Stingless bees form perennial colonies from which they forage year-round. Worldwide several hundred species exist, which differ significantly in colony size (from a few dozen to tens of thousands of individuals), body size (from 2 to 14 mm; compare to 12 mm for honeybees), and foraging strategy (some species recruit nestmates to high quality food sources, like honeybees, whereas others forage mainly indi-

vidually, like bumble bees) (e.g. Roubik, 1992; Michener, 2000; Slaa, 2003; Slaa et al., 2003a; Biesmeijer and Slaa, 2004; Nieh, 2004). These inter-specific differences allow for selection of the most appropriate stingless bee for a given crop species and crop breeding system (greenhouse, open field, etc.).

Commercial pollination with stingless bees has hardly been developed yet, and some observed potential problems include domestication, colony reproduction and mass rearing, which are discussed in more detail in the '*general considerations*' section at the end of this paper. Nevertheless, several biological features make stingless bees strong candidates for commercial pollination services, as outlined below.

Stingless bees are true generalists, collecting nectar and pollen from a vast array of plants (Heithaus, 1979a, b; Roubik, 1989; Ramalho et al., 1990; Biesmeijer et al., 2005). A single species can collect floral rewards from up to 100 plant species on a yearly basis (Heithaus, 1979b; Cortopassi-Laurino, 1982). Nevertheless, individuals tend to specialize on a single floral species for a certain amount of time, a behavioural trait commonly referred to as flower constancy (Slaa, 2003; Slaa et al., 2003b). Flower constancy leads to assortative mating of the visited plants and therefore to more efficient pollination (e.g. Darwin, 1876; Thomson, 1983; Campbell and Motten, 1985). Less pollen is wasted due to selective transfer within a species, and less non-specific pollen reaches the stigma, preventing pollen competition and stigma clogging (Waser, 1983). The fact that stingless bees are generalists at the colony level but specialists at the individual level makes them theoretically good pollinators. Indeed, stingless bees are considered important pollinators of the native flora in tropical and subtropical parts of the world, and they have been found to contribute to the pollination of many crops and wild plants (see Heard, 1999 for a review).

Several species of stingless bees have been domesticated for centuries, especially by the Maya people in Latin America (see Weaver and Weaver, 1981; Crane, 1983, 1992; Cortopassi-Laurino et al., 2006, this issue). Nowadays, a number of papers on the use of

rational hive boxes for the keeping of stingless bees is available, and hive management is fairly straightforward for certain species (but see below). Although stingless bees naturally only occur in the tropics and subtropics, they have also been successfully exported and maintained indoors in colder climates, using temperature controlled rooms and/or hives (e.g. Utrecht University, The Netherlands; Japan (Maeta et al., 1992; Amano et al., 2000; Amano, 2004, pers. comm.)).

Besides the fact that many species of stingless bees can be managed in hives, several other features make this group very adequate for pollination services. First, colonies don't die after reproducing, unlike *Bombus*, and colonies are naturally long-lived (Slaa, 2006). This makes it relatively easy to keep individual hives for long periods of time (up to 60 years: Murillo, 1984). Second, they lack a functional sting, which makes them especially suitable for pollination of crops that are cultivated in inhabited areas and in enclosures such as cages and greenhouses. In Costa Rica, for example, many seed producing companies grow ornamental plants in large netted, insect proof, cages. They have a high demand for pollinators, but because all honeybees are Africanised, and hence more defensive, honeybees are hardly used for pollination in such enclosures. In such cases, stingless bees might provide a solution (see Slaa et al., 2000a, b; Sánchez et al., 2002). Third, many stingless bee species have proven to forage well in enclosed areas (see Tabs. I and V), and under adequate climatological conditions they forage year-round. This makes them especially suitable for off-season production of crops in green houses. Most species of stingless bees have a foraging range smaller than that of the honeybee, which may enhance foraging efficiency in confined spaces (Visscher and Seeley, 1982; Seeley, 1985; Katayama, 1987; Kakutani et al., 1993). Fourth, because most stingless bees cannot survive cold winters, there is little risk of invasion when importing stingless bees to temperate climates. Note however that some species do live where it occasionally freezes, and combined with global warming these species might become feral when introduced outside the tropics of Capricorn and Cancer. Furthermore,

they suffer from fewer diseases, pests and parasites than the honeybee (Nogueira-Neto, 1997), which simplifies colony management.

While not all species can be used for commercial pollination (e.g. obligate parasites of other stingless bees, species with restrictive nesting habitats, extremely defensive behavior or destructive use of flowers), several species are good candidates as commercial pollinators because they can easily be kept in hives, have sufficient numbers of workers per hive and are non-aggressive (Roubik, 1995b; Heard, 1999). The diversity of the group indicates that they may be of use to pollinate a wide range of crops and ornamental plants.

### 3. CROPS POLLINATED BY STINGLESS BEES

The first detailed review on the role of stingless bees in crop pollination appeared in 1999 by Tim Heard. Heard (1999) reported that stingless bees are effective and important pollinators of nine crops, and that they contribute to pollination in ~60 other species out of the ~90 crop species they were found visiting. Over the past years, several new studies on stingless bee pollination appeared (Tab. I). After the review by Heard in 1999 there is a clear trend towards a more experimental approach using enclosures such as bags, cages and greenhouses (cases 13–17 Tab. I).

In the sections below a summary of each crop is given, using both published and unpublished data. Only the studies that appeared after 1999 are included. For a review of previous studies see Heard (1999).

#### 3.1. Crops effectively pollinated by stingless bees

##### *Coffea* sp., Rubiaceae

Coffee is one of the most economically important crops, but its pollination requirements are not well understood. The two most important species are *C. arabica* and *C. canephora* (Free, 1993; Roubik, 2002a).

**Table I.** Crops effectively pollinated by stingless bees. Studies 1–12 report on pollination under field conditions, studies 13–18 report on pollination under enclosed conditions. Studies 1–9 are reviewed in Heard (1999).

	Scientific name	Common name	Stingless bee	Reference
Crops reported by Heard (1999)				
1	<i>Bixa orellana</i>	Annato	<i>Melipona melanoventer</i> <i>Melipona fuliginosa</i>	See Heard (1999)
2	<i>Myrciaria dubia</i>	Camu-camu		See Heard (1999)
3	<i>Sechium edule</i>	Chayote	<i>Trigona corvina</i> , <i>Partamona cupira</i>	See Heard (1999)
4	<i>Cocos nucifera</i>	Coconut		See Heard (1999)
5	<i>Averrhoa carambola</i>	Carambola	<i>Trigona thoracica</i>	See Heard (1999)
6	<i>Macadamia integrifolia</i>	Macadamia	<i>Trigona</i> spp.	See Heard (1999)
7	<i>Mangifera indica</i>	Mango	<i>Trigona</i> spp.	See Heard (1999)
8	<i>Poumora cecropiaefolia</i>	Mapati		See Heard (1999)
9	<i>Theobroma grandiflorum</i>	Cupuaçu	<i>Trigona lurida</i>	See Heard (1999)
Studies reported after 1999				
10	<i>Coffea arabica</i>	Coffee	<i>Trigona (Lepidotrigona)</i> <i>terminata</i>	Klein et al. (2003a)
11	<i>Coffea canephora</i>	Coffee	<i>Trigona (Lepidotrigona)</i> <i>terminata</i>	Klein et al. (2003b)
12	<i>Persea americana</i>	Avocado	<i>Trigona nigra</i> , <i>Nannotrigona perilampoides</i> <i>Geotrigona acapulconis</i> , <i>Trigona nigerrima</i> , <i>Partamona bilineata</i> , <i>Nannotrigona perilampoides</i> , <i>Scaptotrigona pectoralis</i> , <i>Trigona nigra</i> , <i>Scaptotrigona mexicana</i> , <i>Trigona fulviventris</i> , <i>Plebeia frontalis</i> ,	Can-Alonso et al. (2005) Ish-Am et al. (1999)
13	<i>Fragaria</i> × <i>ananassa</i>	Strawberry	<i>Plebeia tobagoensis</i> <i>Trigona minangkabau</i> <i>Nannotrigona testaceicornis</i> <i>Tetragonisca angustula</i>	Asiko (2004); Lalama (2001) Kakutani et al. (1993) Maeta et al. (1992) Malagodi-Braga and Kleinert (2004)
14	<i>Nephelium lappaceum</i>	Rambutan	<i>Scaptotrigona mexicana</i> + <i>Tetragonisca angustula</i>	Rabanales et al. (unpubl. data)
15	<i>Capsicum annuum</i>	Sweet pepper	<i>Melipona favosa</i> <i>Melipona subnitida</i> <i>Trigona carbonaria</i> <i>Melipona favosa</i>	Meeuwsen (2000) Cruz et al. (2004) Occhiuzzi (2000) Meeuwsen (2000)
16	<i>Lycopersicon esculentum</i>	Tomato	<i>Melipona quadrifasciata</i>  <i>Nannotrigona perilampoides</i>	Santos et al. (2004a); Sarto et al. (2005) Cauich et al. (2004)
17	<i>Cucumis sativus</i>	Cucumber	<i>Scaptotrigona</i> aff. <i>depilis</i> , <i>Nannotrigona testaceicornis</i>	Santos et al. (2004b)
18	<i>Salvia farinacea</i>		<i>Nannotrigona perilampoides</i> , <i>Tetragonisca angustula</i>	Slaa et al. (2000a, b)

### *Coffea arabica*

*C. arabica* is the most common coffee species and is cultivated throughout the tropics. Coffee flowers are very attractive to a wide array of insects including honeybees and stingless bees (Heard, 1999; Klein et al., 2003a). Although *C. arabica* is largely self-fertile, and a relatively large fruit set may be obtained without any pollinators, several studies indicate that bee pollination increases coffee production (Free, 1993). Recently Roubik (2002a, b) found that in Panama bee pollination resulted in a higher fruit set and heavier mature fruits compared to bagged branches from which pollinators were excluded, and concluded that bees consistently controlled over 36% of the total coffee production. Klein et al. (2003a) found that coffee fruit set in Indonesia was higher in areas with a high bee diversity (approximately 90% fruit set) compared to areas with a low diversity (approximately 60% fruit set), and concluded that bee diversity, not abundance, was important for pollination success. Using bagging experiments, they found that 15 bee species, including four *Trigona* species, contributed to the pollination of this shrub. However, pollination efficiency (fruit set after a single flower visit) varied among the species, with *Trigona (Lepidotrigona) terminata* being the most efficient stingless bee pollinator (80% fruit set). As a group, the less abundant solitary bees were more efficient pollinators than the more abundant social bees (honeybees and stingless bees).

### *Coffea canephora*

*C. canephora* is an important cash crop in many tropical countries (Willmer and Stone, 1989). Flowers are self-sterile and wind was long believed to be the main pollinating vector (see Free, 1993). However, several studies have now indicated that insects do make a considerable contribution to its pollination, with the main visitors being bees (Willmer and Stone, 1989; Klein et al., 2003b, c). In Indonesia, fruit set increased with both abundance and diversity of flower visiting bees (from approximately 70% to 95% fruit set). Honeybees, solitary bees and stingless bees were all effective pollinators of this shrub. As with *C. arabica*, pollination efficiency differed highly

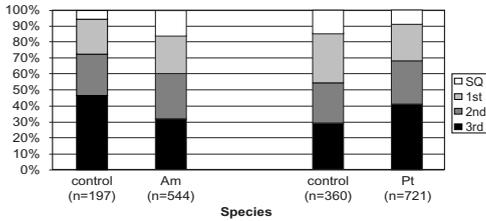
among the species, with *Trigona (Lepidotrigona) terminata* being the most efficient stingless bee pollinator (84% fruit set). As a group, the less abundant solitary bees were more efficient than the more abundant social bees (Klein et al., 2003b).

### **Avocado, *Persea americana* (Lauraceae)**

Avocado originated in Central America, where honeybees are not native. Two recent studies have shown that stingless bees are frequent visitors and efficient pollinators of avocado flowers in Mexico (Ish-Am et al., 1999; Can-Alonso et al., 2005). Ish-Am et al. (1999) conducted their study mainly outside commercial orchards because the application of insecticides highly reduced insect populations in commercial orchards. Based on species abundance on the flowers, foraging behaviour, and number of pollen grains on the insect's body zones that came in contact with the avocado stigma, they concluded that eight to 10 species of stingless bees were effective pollinators of avocado, together with the Mexican honey wasp. Can-Alonso et al. (2005), working in commercial orchards, found that *A. mellifera* and *Trigona nigra* carried comparable amounts of avocado pollen grains on their bodies, but that this number was significantly less on *Nannotrigona perilampoides*. They too concluded that native stingless bees are potentially efficient pollinators of this crop.

### **Strawberry, *Fragaria* × *ananassa* (Rosaceae)**

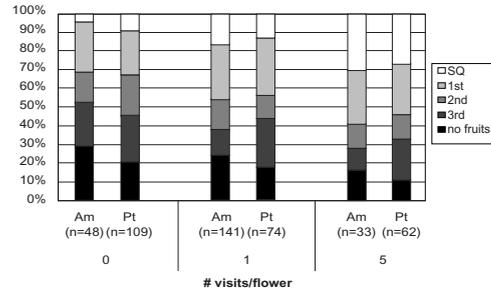
Most strawberry cultivars are hermaphrodite and self-fertile, but cultivars may vary highly in their degree of self-compatibility due to differences in spatial segregation of anthers and stigmata and temporal separation between anther maturation and stigma receptivity (Free, 1993; Zebrowska, 1998; Malagodi-Braga, 2002). Strawberry flowers can be pollinated by a wide range of vectors, such as solitary bees, flies, and even wind (Free, 1993), although these are not (yet) used in commercial strawberry production. Honeybees are often used in greenhouses in Japan and the UK, although they might not be the optimal pollinator for strawberries under greenhouse conditions (Katayama, 1987; Kakutani et al., 1993). McGregor (1976) reports that



**Figure 1.** Effect of bee pollination on strawberry quality. Strawberry fruits were classified into four quality categories (categories derived from The Greenery International qualification for strawberry: SQ: super quality, perfect cone, fruit diameter > 27 mm; 1st: light deformation, fruit diameter > 22 mm; 2nd: some deformation, fruit diameter > 18 mm; 3rd: Industry, deformed, fruit diameter < 17 mm). Control: no bees, Am: *Apis mellifera*, Pt: *Plebeia tobagoensis*. After Lalama (2001).

strawberry plants do not seem to be very attractive to honeybees, and colonies used for strawberry pollination in greenhouses in Japan decreased in population size (Kakutani et al., 1993). However, in commercial strawberry fields in Brazil flowers yielded a lot of pollen and nectar and were abundantly visited by honeybees and stingless bees (Malagodi-Braga, pers. obs.). Since the pollination studies in Japan (Kakutani et al., 1993; Maeta et al., 1992; see Heard, 1999), three more studies on strawberry pollination with stingless bees have appeared.

In The Netherlands imported *Plebeia tobagoensis* from Tobago, West-Indies, and honeybees were tested for their pollination effectiveness and efficiency under greenhouse conditions (each compartment  $9 \times 6 \times 4$  m, one colony for 100 plants; Hofstede, unpubl. data). When the bees were able to forage freely on the strawberry plants (var. Elan), honeybees had a significant positive effect on strawberry quality (Fig. 1, Mann-Whitney test,  $P < 0.005$ ), and fruit quantity was somewhat higher (58% fruit set versus 48% without bees, Student-t test,  $F = 1.84$ ,  $P = 0.063$ ). *P. tobagoensis* had no effect on the number of fruits produced (74% versus 75% without bees, Mann-Whitney test,  $P = 0.8$ ), but had a negative effect on strawberry quality (Fig. 1, Mann-Whitney test,  $P < 0.005$ ). *P. tobagoensis* showed destructive pollen for-



**Figure 2.** Effect of controlled flower visits on strawberry quality. Strawberry fruits were classified into four quality categories (categories derived from The Greenery International qualification for strawberry: SQ: super quality, perfect cone, fruit diameter > 27 mm; 1st: light deformation, fruit diameter > 22 mm; 2nd: some deformation, fruit diameter > 18 mm; 3rd: Industry, deformed, fruit diameter < 17 mm). Bees were allowed controlled visits to 2-day old flowers that were previously bagged to prevent destructive behaviour to the buds. Am: *A. mellifera*, Pt: *P. tobagoensis*. After Asiko, 2004.

aging behaviour by entering closed flower buds (stigma not yet receptive) and biting the anthers (which haven't released pollen yet) with their mandibles (Lalama, 2001). This behaviour could have been caused by the relatively low numbers of flowers available, but more observations are needed to confirm this. When buds were protected from the destructive behaviour through bagging before flower opening, *P. tobagoensis* did have a positive effect on strawberry quality (Fig. 2; Asiko, 2004). Five bee visits resulted in significantly higher quality fruits than no visits for both honeybees and stingless bees (Chi-squared test,  $P < 0.03$ , without significant differences between the two species (Chi-squared test,  $P = 0.7$ ; Fig. 2). Fruit set tended to be higher after 5 bee visits than without visitation, but this was not significant (Chi-squared test,  $P = 0.14$  for *A. mellifera* and  $P = 0.07$  for *P. tobagoensis*; Fig. 2).

In Sao Paulo, Brazil, the only strawberry study site so far where stingless bees are native, five stingless bee species were initially tested for their suitability as strawberry pollinators in greenhouses ( $8 \times 25$  m): *Nannotrigona testaceicornis*, *Tetragonisca angustula*, *Schwarziana quadripunctata*, *Scaptotrigona*

**Table II.** The effect of *T. angustula* pollination on strawberry ('Sweet Charlie' cultivar) production in greenhouses. Given are the mean±SD for various fruit measurements. Each greenhouse contained either one colony of *T. angustula* or no bee colonies (control). In the latter treatment the parcels were covered to prevent flower visitation by bees. After Malagodi-Braga, 2002.

	<i>T. angustula</i>	control	T-test
Fruit number	490 ± 48	519 ± 84	NS
% deformed fruit	6.9 ± 2.2	50.1 ± 12.9	t = 15.1, df = 40, P = 0.0001
Fruit weight (g)	9.6 ± 0.7	8.4 ± 1.2	t = 3.9, df = 40, P = 0.0003

*bipunctata* and *Trigona spinipes* (Malagodi-Braga, 2002). Two species, *S. bipunctata* and *S. quadripunctata*, did not forage under greenhouse conditions, and the other species showed a reduction in their daily foraging activity inside the greenhouse, despite resource availability and favourable values of air temperature and relative humidity. Among all species *T. angustula* was remarkable for its relatively quick adaptation and the ability to keep satisfactory internal colony conditions (continuing cell construction and oviposition, maintaining their honey pots and storing pollen of strawberry flowers), even with frequent removals and introductions in greenhouses (Malagodi-Braga, 2002). *T. angustula* was tested for its pollination effectiveness using two different strawberry cultivars under greenhouse conditions. Despite their small size (about 4.5 mm in length), *T. angustula* was found to be an effective pollinator of both the 'Oso Grande' cultivar (Malagodi-Braga and Kleinert, 2004) and the 'Sweet Charlie' cultivar (Malagodi-Braga, 2002). One colony, allowed to forage freely in a greenhouse with 1350 strawberry plants of the 'Oso Grande' cultivar, resulted in nearly 100% of primary flowers developing into marketable (well-shaped) fruits, compared to 88% for open pollination in the field, and <20% for bagged flowers. Strawberry fresh weight was also markedly higher after pollination by *T. angustula* (41% increase compared to open pollinated plants) (Malagodi-Braga and Kleinert, 2004).

In another pollination experiment using the 'Sweet Charlie' cultivar, a single colony of *T. angustula* was sufficient to pollinate 1350 'Sweet Charlie' plants, reducing significantly and drastically the percentage of misshapen

fruits (by 86%) and yielding a significant increase in strawberry weight (by 14%) compared to the control (Tab. II). The relatively high fruit production in the control was considered a result of selfing (pollen fall caused by gravity and human action during the crop handling, Malagodi-Braga, 2002). This indicates that although fruit set in the 'Sweet Charlie' cultivar does not require additional pollinators, out-crossing through bee pollination greatly enhances fruit quality.

#### **Rambutan, *Nephelium lappaceum* (Sapindaceae)**

Rambutan has separate male and hermaphrodite trees but is considered to be functionally dioecious, with hermaphrodite flowers having non-functional anthers (Free, 1993). This species is native to Southeast Asia, where fruit set under natural conditions was found to be 1% (Free, 1993). Heard (1999) already reported that flowers in Asia are visited by several species of stingless bees, which are potential pollinators. Rambutan is commercially grown in the Neotropics, and Rabanales and co-workers (unpublished document) studied pollination and fruit set in a commercial 'female' rambutan orchard (7 ha, 1000 trees) in Chiapas, Mexico. They recorded flower visitors and compared fruit set under three treatments: (1) open pollination conditions, (2) bagged conditions (panicles bagged, all flower visitors < 1 mm excluded), and (3) 'induced' pollinator conditions, where a cage was placed over a tree. Two stingless bee colonies were placed in the cage; one *Scaptotrigona mexicana* and one *T. angustula* colony. This last treatment resulted in obligate geitonogamy.

Most flowers were hermaphrodite, but only approximately 5% of those actually shed pollen. Flowers were mainly visited by bees, including several species of stingless bees, but were rarely visited by honeybees. The most common flower visitor was *S. mexicana*. Mature fruit set was highest under open pollination conditions and induced pollination conditions (17–23%). Mature fruit set under bagged conditions was only 2–3%, thus the presence of pollinators increased fruit set by nearly 10-fold. Fruit mass was significantly higher under induced conditions (geitonogamy) compared to open pollination where cross-pollination was possible.

*S. mexicana* hives are now used to pollinate rambutan at this orchard, and fruit production has increased significantly (Roubik, pers. comm.).

#### **Sweet pepper, *Capsicum annuum* (Solanaceae)**

Although many Solanaceae species have flowers with poricidal anthers, *C. annuum* does not, and does therefore not require buzz-pollination. Flowers are pollinated through spontaneous selfing, although outcrossing may occur because the stigma is often receptive before anthers dehiscence (Free, 1993). Honeybee pollination can increase fruit size, seed number, and fruit shape (Ruijter et al., 1991 cited in Free, 1993). At the time of the review by Heard (1999) various species of stingless bees were known to visit the flowers, but their pollination potential had not been confirmed. Since then, three species of stingless bees have been reported as effective pollinators.

Occhiuzzi (2000) reported that *Trigona carbonaria* effectively pollinated sweet pepper under glass greenhouse conditions in Australia. Fruit weight had increased by 11% and number of seeds/fruit by 34% compared to crops that were not pollinated by bees. Ongoing research by Greco confirms the effectiveness of *T. carbonaria* as a pollinator of sweet pepper, but also shows that the small *Austroplebeia australis* is not an effective pollinator of this plant (Greco, pers. comm.). Roubik (1995a) already expected that smaller bees would not be effective pollinators of sweet pepper, and this has now also been confirmed for the small stingless bee *Tetragonisca*

*angustula*. In a greenhouse in The Netherlands, *T. angustula* mainly visited the flowers for nectar, during which no contact was made with the stigma and therefore no pollination occurred (Kuyhor, 2001). Using the same settings, Meeuwsen (2000) reports that both *Melipona favosa* and honeybees (both species one colony for 25 potted plants, each compartment 9 × 6 × 4 m) significantly increased the number of fruits/plant compared to the control (no bees present). However, no numbers of flower frequency in the different compartments were given, making it difficult to draw conclusions on pollinator effectiveness. In Brazil, Cruz (2003); Cruz et al. (2004, 2005) studied the pollination effectiveness of *M. subnitida* under greenhouse conditions (83 m<sup>2</sup>), using two colonies for a total of 153 sweet pepper plants. They had four treatments: hand pollination (auto-pollination and cross pollination), pollination by *M. subnitida*, and the control (no pollinators – flowers bagged for 48 h). Although fruit set did not differ among the treatments, *M. subnitida* significantly increased fruit weight (by 30%) and number of seeds per fruit (by 86%) compared to the control. Bee pollination did not significantly differ from hand pollination in terms of fruit weight and seed set. In addition, pollination with *M. subnitida* resulted in significantly less deformed fruits compared to the control (65% decrease). Interestingly, auto-pollination resulted in as much deformed fruits as the control, whereas cross-pollination showed the same reduction in deformed fruits as bee pollination. This clearly shows the value of bees as cross pollinators in selfing crops.

#### **Tomato, *Lycopersicon esculentum* (Solanaceae)**

Tomato flowers are self-compatible but need animal or wind pollination to set fruit (Free, 1993). Nectar production is generally low, and pollen is released from poricidal anthers upon vibration. Tomato is one of the most widely grown vegetable crops in the world, and is commonly produced in greenhouses (Benton Jones, 1998). Although commercially bred *Bombus terrestris* effectively pollinates greenhouse tomatoes, import of this species to areas where it is not native causes general concern. *B. terrestris* already successfully

established in Israel, New Zealand, Japan and Tasmania, and several studies have indicated the negative impact of invasive bumble bees on the native pollinator fauna (Hingston and McQuillan, 1999; Goulson, 2003; Morales and Aizen, 2004). It seems logical to narrow the search for effective tomato pollinators to species that can buzz-pollinate these flowers, and *Melipona* species seem good candidates. Two studies have reported on the pollination effectiveness of *Melipona quadrifasciata* for tomato grown in greenhouses in Brazil. Sarto et al. (2005) found that pollination of tomato (var. Rodas) by *M. quadrifasciata* (six colonies for 700 plants in a 234 m<sup>2</sup> plastic greenhouse, 3 m high) resulted in equal fruit quality (size and shape) compared to hand pollination or bee plus hand pollination. However, bee pollinated fruits contained 11% less seed compared to hand pollination, possibly due to the low temporal overlap in foraging activity and stigma receptivity. Santos et al. (2004a) compared pollination effectiveness of *M. quadrifasciata* and *Apis mellifera* (each species in a 86 m<sup>2</sup> greenhouse), and found that tomatoes were bigger, heavier and had more seeds following pollination by *M. quadrifasciata* compared to *A. mellifera*.

Cauich et al. (2004) reported on the pollination efficiency of *N. perilampoides* on greenhouse tomatoes (var. Maya) in Subtropical México. Although this small stingless bee species is not a buzz-pollinator, it effectively pollinated tomato plants grown in netted cages (4 × 4 × 3.5 m, one colony for 40 plants). Pollination by *N. perilampoides* was as effective as mechanical vibration in terms of percentage fruit set, number of seeds per fruit and fruit weight. However, of tomato flowers that did not receive any pollination treatment more than half did set fruit. Fruits produced without a pollination treatment had significantly less seeds than fruits produced after mechanical vibration or bee pollination, but fruit weight did not significantly differ among the treatments. This finding is in contrast to the study by Sarto et al. (2005), where tomato flowers without vibration did not set fruit. Whether this discrepancy is due to differences in cultivars, differences in housing conditions (plastic house versus netted cage), or differences

in treatments of the non-pollinated plants (inflorescences bagged versus flowers tagged) is unknown.

#### **Cucumber, *Cucumis sativus* (Cucurbitaceae)**

In Yucatán, Mexico, *Partamona bilineata* is a frequent visitor of cucumber (Meléndez-Ramírez et al., 2002). Santos et al. (2004b) reported that *Scaptotrigona* aff. *depilis* and *N. testaceicornis* effectively pollinated greenhouse cucumber in Brazil, resulting in a higher fruit production, higher fruit weight and a higher percentage of perfect fruits compared to the control, where no pollinators were present.

#### ***Salvia farinacea* and *Salvia splendens* (Labiatae)**

So far, *Salvia* is the only ornamental plant for which stingless bee pollination has been studied. In Costa Rica, *Salvia* is an important plant for many seed companies that often grow it in large netted cages to avoid hybridization. At such a company, *T. angustula*, *N. perilampoides* and *A. mellifera* were tested for pollination efficiency and effectiveness of *S. farinacea* in netted cages (6 × 3 × 3 m). Pollination efficiency, expressed as seed set per unit of visitation rate, did not differ among the three species, and all species produced good quality seeds. Because foraging activity was highest in *A. mellifera*, honeybee pollination yielded the highest seed set compared to stingless bee pollination (Slaa et al., 2000a, b). An increase in stingless bee density could potentially increase yields, and stingless bees seem a valuable option as pollinators of *S. farinacea* in enclosures. *S. farinacea* was also commonly visited by *Partamona orizabaensis* and *Trigona fulviventris*, but both species would be difficult to breed commercially as *T. fulviventris* nests in the ground and *P. orizabaensis* is often found in termite nests and defends the nest quite aggressively.

*Salvia splendens* has significantly larger flowers than *S. farinacea*, and was not effectively pollinated by *T. angustula*, due to its small body size in relation to flower size (Sánchez et al., 2002). *Trigona fuscipennis* and *N. perilampoides* did not forage on these flowers when placed in a netted cage (6 × 3 × 2.5 m, Bustamante, 1998). Flowers were visited

by several other species of stingless bees including *Geotrigona* sp. and *Partamona* sp. (Picado, 2000), but those are probably not good candidates for commercial pollination services because of their restrictive nesting habits, and may be too small to be effective pollinators.

### 3.2. Crops visited and occasionally or partially pollinated by stingless bees

#### Calamondin, *Citrus mitis* (Rutaceae)

*Citrus mitis* is a miniature orange, widely grown as an ornamental house pot plant. The flowers are self-fertile and require no cross-pollination (Morton, 1987). Cervancia and Manila (2000) studied the pollination of Calamondin in the Philippines. Bagged inflorescences did not set fruit (0.001% fruit set), whereas fruit set in unbagged inflorescences was 2%. The stingless bee *Trigona biro* was observed to visit the flowers, along with *Apis cerana*, *A. mellifera*, and *Xylocopa* spp. The activity of floral visitors was synchronized with anthesis, which indicates their potential role as pollinators.

#### Cucurbit crops (Cucurbitaceae)

Meléndez-Ramírez et al. (2002) recorded the bee visitors of pumpkin (*Cucurbita moschata*), cucumber (*Cucumis sativus*), melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*), on 14 sites in Yucatan, Mexico. These crops were found to be visited by 58 species of bees. *Partamona bilineata* was the second most dominant species and *Trigona fulviventris* ranked fifth. The other stingless bee visitors were *N. perilampoides*, *Trigona nigra*, *Cephalotrigona zexmeniae*, *Melipona beecheii* and occasionally *Plebeia frontalis* and *Scaptotrigona pectoralis*. The authors conclude that *P. bilineata* is a regional pollinator of watermelon and pumpkin due to its 'high abundance, frequent presence and observed contacts with female and male flower structures'.

#### Radish, *Raphanus sativus* (Cruciferae)

Although radish is mainly known for its succulent root, its propagation is by seed. Most cultivars are self-incompatible, and bees are the main pollinators (McGregor, 1976; Free, 1993). In the Netherlands, fruit set of radish

**Table III.** Relative attractiveness of various vegetable crops to *Nannotrigona perilampoides*, *Tetragonisca angustula*, and *Apis mellifera*. For each bee species the total number of observed visits, the total number of plants present in the cage and the percentage relative preference (Chesson, 1978) is given. After Fonseca and Picado, 2000. \* Cauliflower plants in the cage with *T. angustula* were attacked by *Plutella xilostella*, preventing visitation observations and seed count.

	<i>N. perilampoides</i>	<i>T. angustula</i>	<i>A. mellifera</i>
# visits	523	159	1963
# plants	65	67	59
Broccoli	3	14	18
Rape	2	33	9
Cauliflower*	0		15
Endive	38	0	39
Chicory	12	3	12
Leek	13	49	4
Carrot	33	0	4

was compared in greenhouse compartments (8 × 6 × 3–5 m, 25 potted plants per compartment) using a colony of *Tetragonisca angustula*, a colony of honeybees, or no bees (Meeuwsen, 2000). The number of pods per plant was significantly higher in the compartments with *T. angustula* and honeybees compared to the control (6.6 and 9 time increase respectively), without significant differences between the two bee species. However, no numbers of flower frequency in the different compartments were given, making it difficult to draw conclusions on pollinator effectiveness.

Nevertheless, behavioural observations indicate that although *T. angustula* is able to pollinate radish, it might not be the most effective agent. *T. angustula* only touched the stigma during pollen visits; nectar was extracted from the side of the flower, probably because of its small body size in relation to flower size. In addition, *T. angustula* visited the radish flowers only sporadically for pollen (< 5% of all visits) (Thai, 2001, using the same setting and bee species).

#### Other vegetables

At a seed production company in The Netherlands (Rijk Zwaan), *N. perilampoides*, *T. angustula* and *A. mellifera* were introduced in pollination cages (5 × 5 × 2 m) within a

**Table IV.** Average seed production (number of seeds per flower (head)) in each of the crops when no pollinators were present (control) and after pollination with *N. perilampoides*, *T. angustula* or *A. mellifera*. Different letters in a row indicate significant differences between treatments (Kruskall-Wallis with Multiple Comparisons Test,  $P < 0.05$ ). After Fonseca and Picado, 2000. \* *N. perilampoides* was excluded from the statistical analysis for production of rape seed because original data were lost. \*\* Cauliflower plants in the cage with *T. angustula* were attacked by *Plutella xilostella*, preventing visitation observations and seed count.

Crops	Control	<i>N. perilampoides</i>	<i>T. angustula</i>	<i>A. mellifera</i>
Broccoli (n = 97–155)	3.2 ± 2.7 <sup>a</sup>	5.4 ± 3.6 <sup>b</sup>	5.9 ± 3.5 <sup>b</sup>	6.1 ± 4.4 <sup>b</sup>
Rape* (n = 112–143)	16.3 ± 9.6 <sup>a</sup>	22.6 ± 8.9	17.4 ± 10.9 <sup>a</sup>	29.9 ± 3.8 <sup>b</sup>
Cauliflower** (n = 250)	4.2 ± 2.6 <sup>a</sup>	0.4 ± 1.1 <sup>b</sup>		4.8 ± 3 <sup>a</sup>
Endive (n = 50)	15.2 ± 6.5 <sup>a</sup>	19.4 ± 2.2 <sup>b</sup>	14.2 ± 6.1 <sup>a</sup>	19 ± 2.4 <sup>b</sup>
Chicory (n = 50)	13.6 ± 9.3 <sup>a</sup>	16.1 ± 3.5 <sup>a</sup>	14.6 ± 5.2 <sup>a</sup>	15.2 ± 5.2 <sup>a</sup>
Leek (n = 150–160)	0.9 ± 1.2 <sup>a</sup>	2.2 ± 1.5 <sup>b</sup>	0.88 ± 1.2 <sup>a</sup>	3.6 ± 1.0 <sup>c</sup>
Carrot <sup>1</sup>				
Female line (n = 8–9)		557 ± 312 <sup>a</sup>	0.4 ± 0.7 <sup>b</sup>	1083 ± 561 <sup>a</sup>
Male line (n = 8–10)		2826 ± 1070 <sup>a</sup>	52 ± 69 <sup>b</sup>	3324 ± 1070 <sup>a</sup>

<sup>1</sup> In carrot, seed counts were per umbel. Plants of the female line required cross-pollination, whereas plants of the male line could be self-pollinated.

large greenhouse. Each cage contained several species of vegetables in flower (see Tab. III, IV) and one bee colony or no colony (control). During a pilot experiment, the number of visits to each crop species was recorded, and seed set was recorded in comparison to a control area where no pollinators were present (Tab. IV). The relative attractiveness of each crop to the bees can be expressed as the degree of preference following Chesson (1983) (Tab. III):

$$\frac{r_i/n_i}{\sum_{j=1}^m r_j/n_j}, i=1, \dots, m \text{ (Chesson 1978).}$$

where  $m$  is the number of crop species,  $r_i$  is the number of visits to crop  $i$ , and  $n_i$  is the number of plants of crop  $i$ . During limited observations flower visitation behaviour was recorded, including whether the bee's body contacted the stigma (Fonseca and Picado, 2000).

Foraging behaviour differed significantly between the two stingless bee species, which illustrates the variability in stingless bee preference. *N. perilampoides* visited 6 of the 7 crops present, but preferred endive and carrot flowers (> 30%). Visits to carrot and endive flowers resulted in a good seed set, which

was not significantly lower than seed set after honeybee pollination, but significantly higher than seed set with *T. angustula* and the control (Tab. IV). During limited observations, bees only touched the stigmas consistently in carrot, endive and leek flowers. Bees did not touch the stigmas of broccoli and rape flowers, and in only half of the visits did they touch the stigmas of chicory flowers. *T. angustula* visited only 4 of the 6 crops present, and preferred rape and leek flowers (> 30%). However, during visits to rape and leek flowers the stigma was never touched and those visits did not result in an increased seed set compared to the control (Tab. IV). *T. angustula* only touched the stigma of carrot flowers, but these flowers were hardly visited when other crops were available (Tab. III). *A. mellifera* visited all crops but preferred endive flowers (Tab. III). Bees consistently touched the stigma in all crops, probably because of their larger body size, and increased seed set significantly compared to the control in 5 of the 7 crops.

Overall, *A. mellifera* was the most effective pollinator of the crops tested, followed by *N. perilampoides*. Generally *T. angustula*

was the least effective pollinator, except for broccoli. *N. perilampoides* performed best as a pollinator of carrot and these flowers are relatively attractive to the bees. Although honeybees were at least equally effective pollinators for this crop, carrot flowers were not very attractive to honeybees (Tab. III), which may cause reduced visitation under field conditions when competing plants are nearby (Free, 1993).

## 4. GENERAL CONSIDERATIONS

### 4.1. Domestication

To be able to use stingless bees for commercial pollination purposes, management of colonies in hives is of vital importance. Although many different species have been kept in hives (Cortopassi et al., 2006, this issue), not all species may be easily transferred to hives due to their specific nesting requirements (e.g. *Geotrigona* and *Trigona fulviventris* nest in the ground, *Trigona corvina* builds its own exposed nest, *T. fuscipennis* nests in termite nests). In addition, although they lack a functional sting several species aggressively defend their nest by biting or releasing a caustic substance, which makes them less suitable to manage in hives and for pollination services (e.g. the genus *Oxytrigona* and several species of the genus *Trigona*; see Biesmeijer and Slaa, 2004).

Although several species have been domesticated since ancient times (Cortopassi et al., 2006, this issue), management of stingless bee colonies is not as advanced as management of honeybee colonies (Cortopassi et al., 2006, this issue). In addition, stingless bee management practices have been developed principally for the harvest of hive products, mainly honey. Using colonies for commercial pollination services brings along different management requirements.

Colonies used for pollination services are much more disturbed than colonies used for honey production. Transportation to and from the crop, a limited diet offered by the crop (many crops offer no nectar), and less than optimal foraging conditions in greenhouses all

put stress on the colony, often resulting in a loss of adult bees and a reduced brood production. Some species may be better adapted to these stress factors than others, and some species may not forage at all under confined conditions (see Tab. V).

### 4.2. Mass rearing and colony reproduction

Colonies used for pollination services need to be available in large numbers. Nowadays stingless bee keeping is mainly a non-commercial small-scale business, although a few large-scale beekeepers exist in Mexico, Brazil, and Australia (Murillo, 1984; Heard and Dollin, 2000; Quezada-Euàan et al., 2001; Rosso et al., 2001; Drumond, 2004; Cortopassi et al., 2006, this issue) where it involves the keeping of mainly *Melipona*, *T. angustula*, *Cephalotrigona* and *Scaptotrigona* species. In Australia, several beekeepers sell stingless bee hives (*Trigona* and *Austroplebeia* species), and they are listed on the Australian Native Bee Research Centre website (<http://www.zeta.org.au/~anbr/buy-stingless-bees.html>). Some rent out stingless bee hives for pollination practices, mainly for pollination of macadamia (Heard and Dollin, 2000). In Brazil, many farmers use stingless bees as pollinators of local crops, such as urucum, chuchu, camu-camu, carambola, cocoda-bahia and mango (Drumond, 2004).

Species that show aggressive nest defence also seem to exhibit intra-specific territorial behaviour, which makes them unsuitable for large-scale beekeeping where hives are placed close together (e.g. several *Trigona* species; Hubbell and Johnson, 1977; Wagner and Dollin, 1983).

One of the main problems for cultivating stingless bees at a large scale is that they naturally reproduce at a very low rate. It has been estimated that under natural conditions, colonies of most species reproduce only once every 20–25 years (Slaa, 2006), with the notable exception of a few common species such as the Neotropical *T. angustula* and the Asian *Trigona minangkabau*, that may reproduce up to once a year (Inoue et al., 1993; Slaa, 2006).

**Table V.** Stingless bee species that have been reported to forage under confined conditions and those that have been reported *not* to forage under confined conditions.

Species	Crop	Foraging	Greenhouse size (l × w × h)	Location	Reference
<i>Melipona favosa</i>	Sweet pepper	Yes	9 × 6 × 4 m	The Netherlands	Meeuwssen (2000)
<i>M. quadrifasciata</i>	Tomato	Yes	234 m <sup>2</sup> , 3 m high	Brazil	Santos et al. (2004a); Sarto et al. (2005)
<i>M. subnitida</i>	Sweet pepper	Yes	83 m <sup>2</sup>	Brazil	Cruz et al. (2004)
<i>Nannotrigona perilampoides</i>	Tomato	Yes	4 × 4 × 3.5 m	Mexico	Cauich et al. (2004)
	<i>Salvia farinacea</i>	Yes	6 × 3 × 3 m	Costa Rica	Slaa et al. (2000a, b)
	<i>Salvia splendens</i>	No	6 × 3 × 2.5 m	Costa Rica	Bustamante (1998)
	Broccoli	Yes	5 × 5 × 2 m	The Netherlands	Fonseca and Picado (2000)
	Rape				
	Endive				
	Chicory				
	Leek				
	Carrot				
<i>N. testaceicornis</i>	Strawberry	Yes	4.2 × 8.1 × 2.4 m	Japan	Maeta et al. (1992)
	Cucumber	Yes	86.4 m <sup>2</sup>	Brazil	Santos et al. (2004b)
<i>Plebeia tobagoensis</i>	Strawberry	Yes	9 × 6 × 4 m	The Netherlands	Asiko (2004); Lalama (2001)
<i>Scaptotrigona bipunctata</i>	Strawberry	No	8 × 25 m	Brazil	Malagodi-Braga (2002)
	Cucumber	Yes	10 m high	Japan	Amano (2004)
	Eggplant	Yes	10 m high		
	Paprika	Yes	10 m high		
	Red pepper	Yes	10 m high		
<i>S. aff. depilis</i>	Cucumber	Yes	86.4 m <sup>2</sup>	Brazil	Santos et al. (2004b)
<i>S. mexicana</i>	Rambutan	Yes	16 × 16 × 4 m	Mexico	Roubik pers. comm.
<i>S. quadripunctata</i>	Strawberry	No	8 × 25 m	Brazil	Malagodi-Braga (2002)
<i>Tetragonisca angustula</i>	<i>S. farinacea</i>	Yes	6 × 3 × 3 m	Costa Rica	Slaa et al. (2000a, b); Sánchez et al. (2002)
	Strawberry	Yes	8 × 25 m	Brazil	Malagodi-Braga and Kleinert (2004)
	Rambutan	Yes	16 × 16 × 4 m	Mexico	Roubik pers. comm.
	Broccoli	Yes	5 × 5 × 2 m		Fonseca and Picado (2000)
	Rape	Yes		The Netherlands	
	Chicory	Yes			
	Leek	Yes			
	Radish	Yes			Meeuwssen (2000)
	Sweet pepper	Yes			
<i>Trigona carbonaria</i>	White clover	Yes	0.2 ha	Japan	Amano (2004)
	Tomato	Yes	0.2 ha		
	Cucumber	Yes	10 m high		
	Eggplant	Yes	10 m high		
	Paprika	Yes	10 m high		
	Red pepper	Yes	10 m high		
	Sweet pepper	Yes	3 × 5 × 4 m	Australia	Occhiuzzi (1999)
<i>T. fuscipennis</i>	<i>S. splendens</i>	No	6 × 3 × 2.5 m	Costa Rica	Bustamante (1998)
<i>T. minangkabau</i>	Strawberry	Yes	4.2 × 8.1 m	Japan	Kakutani et al. (1993)

Colony management has been mainly focused on small-scale management practises (Cortopassi et al., 2006, this issue), where low colony reproduction rates have not been a major issue. Colonies can be artificially reproduced by dividing the hive population (adult bees and brood) in two parts, each part resulting in a new colony (e.g. Roubik, 1995b; Cortopassi-Laurino et al., 2006, this issue). In the new colony lacking the mother queen, a virgin queen has to become accepted by the workers and has to mate outside with one or more drones, most likely from another colony. This is no problem in their natural environment, where lots of drones are available, but becomes more problematic when colonies are exported to areas where they are not native. Colonies of *T. carbonaria* have been split successfully in Japan, using a large greenhouse (10 m high) with multiple hives where 'natural' mating can take place (Amano, pers. comm.). Nevertheless, records of successful mating or colony multiplication under enclosed conditions are scarce (but see Camargo, 1972; Cepeda Aponte, 1997; Amano, 2004). So far, artificial insemination has not been developed for stingless bees but may be a valuable alternative to natural mating.

Most people assume that under favourable conditions colonies can be multiplied about once a year. Even for the species that have been kept in hives since ancient times, the limiting factors for colony growth and colony reproduction are still mainly unknown. More research is needed in this area before enough stingless bee colonies can be efficiently managed for commercial pollination purposes.

No major breeding populations exist yet outside the tropics. Further research could solve the current problem of artificial colony multiplication in non-native areas, although it might be economically more beneficial to restrict breeding to the tropical native areas and export existing colonies. Keeping breeding programs in the tropics would also provide the opportunity for local people to benefit from their ecological resources.

### 4.3. Greenhouse pollination

Foraging under confined conditions (e.g. greenhouse, netted cage) brings along its own set of difficulties/complications. One of the most common problems is foragers gathering in the top of the enclosure, especially during the first few days after introduction of the hive. These bees are a loss to the colony; when they manage to escape from the enclosure they do not come back, and if no escape is possible they often die of exhaustion and/or overheating (pers. obs.; Occhiuzzi, 1999; Amano, 2004). These bees are probably experienced foragers in search of a known food source. Pilot experiments have shown that transportation of colonies over rough roads increases the incidence of orientation flights (Lukacs, pers. comm.), and it would be interesting to see whether (gentle) shaking of closed colonies before introduction in the greenhouse could reduce the problem of forager loss. However, shaking may also cause eggs, which float on top of the larval food, to drown, leading to mortality of young brood (Sommeijer, pers. comm.).

Although most stingless bee species that have been tried in pollination studies under confined conditions foraged effectively on the crop, some species were reported to not forage on the crop under confined conditions (Tab. V). This may suggest that some species are not suitable for greenhouse pollination. However, lack of foraging may also reflect suboptimal foraging conditions for the given species, such as a low attractiveness of the crop to the species, rather than a species-specific reluctance to forage under confined conditions. Clearly more studies are needed to get a better understanding of which factors attribute to successful foraging in greenhouses.

### 4.4. Pesticides

Application of pesticides is a common procedure in crop production, especially in the tropics where it still causes major health hazards for both people and animals. Pesticide application to crops often repels insects from the flowers, can kill the pollinators and may

**Table VI.** Mismatches between crop and stingless bee species.

Crop	Bee species	Effective?	Reference
<i>Salvia splendens</i>	<i>T. angustula</i>	No, too small	Sánchez et al. (2002)
Strawberry	<i>P. tobagoensis</i>	No, pollen robbers	Lalama (2001)
	<i>P. tobagoensis</i>	Yes, if buds protected	Asiko (2004)
Tomato	<i>T. carbonaria</i>	No	Amano (2004)
Sweet pepper	<i>T. angustula</i>	No, does not touch stigma during nectar collection	Kuyhor (2001)
Radish	<i>T. angustula</i>	No, reaches nectary from outside corolla	Thai (2001)

kill entire colonies (e.g. Kearns and Inouye, 1997; Ish-Am et al., 1999; pers. obs.). The chemical effect of commonly used pesticides on bees has been documented (see Roubik, 1995a), although most chemicals have only been tested on the honeybee. Smaller-bodied stingless bees are probably even more susceptible than honeybees due to their high surface area-to-volume ratio. During pesticide application managed hives can be removed from the site, but wild colonies may still be exposed to the chemicals. Because colony reproduction rate of stingless bees is very low (see above), colony mortality will have a big impact on natural stingless bee populations. There are several guidelines on pesticide application available to minimize the impact on pollinators, although none make pesticide use completely safe (Kearns and Inouye, 1997). Biological control of pests, as is now offered in conjunction with commercially available bumble bee pollinators (e.g. Koppert BV, The Netherlands), seems an ideal solution. However, special efforts to reduce the use of potent pesticides seem necessary in the tropics, where pesticides are easily available, generally cheap, and where safety risks are commonly unknown to the farmers.

#### 4.5. Further research

From the comparative studies described above it becomes clear that the pollination effectiveness of a specific stingless bee species depends very much on the crop species. Table VI gives some examples of plant-pollinator mismatches from previous studies. A mis-

match may result in visitation without pollination, stealing or robbing pollen and/or nectar. In some instances this can be prevented by evaluation of floral structure and relating the location of the stigma and anthers to bee body size. Literature on techniques and considerations for pollination studies can be found in several other publications (e.g. Kearns and Inouye, 1993; Dafni et al., 2005).

The effects of prolonged enclosed conditions and/or a restricted diet on colony health are largely unknown. Regularly opening the hive to inspect the internal colony status, as often done in honeybees, is very disruptive for stingless bees, and often causes a decline in colony functioning. Monitoring colony weight during pollination services can provide some insight into colony health, but does not provide information on brood status. Recently, X-ray computerized tomography has been successfully used to visualize internal nest structures in a non-invasive way, and this method would provide an excellent research tool when measuring the effect of environmental (greenhouse) conditions on colony health (Greco et al., 2005).

## 5. POLLINATION AND BIODIVERSITY CONSERVATION

Although this manuscript deals mostly with the use of managed stingless bee colonies for pollination services, a fair share of pollination services can come from wild (unmanaged) bees. For several crops it has been shown that growing crops near intact natural habitat (e.g. forest, woodland, chaparral)

increases abundance and diversity of flower-visiting insects, and that these crops have a higher yield than crops growing away from natural vegetation (Wille and Orozco, 1983; Venturieri et al., 1993; Heard and Exley, 1994; Kremen et al., 2002; Klein et al., 2003a, b; Ricketts, 2004; Ricketts et al., 2004; Chacoff and Aizen, 2006). These findings indicate the importance of habitat conservation for pollination purposes. Many wild bees, including stingless bees, depend on trees for nesting, and deforestation significantly reduces their numbers (Slaa, 2003). Even selective logging may severely affect stingless bee populations, especially when the larger trees that are preferred for nesting are harvested (Eltz et al., 2002; Samejima et al., 2004).

In the case of coffee (*C. arabica*, see above), one of the most valuable export commodities from developing countries, yields on a farm in Costa Rica were 20% higher in areas near forest than in areas away from forests. The economic value of the forest in terms of pollination services was estimated to be ca. \$60 000 for one Costa Rican farm, per year. This value is of at least the same order as major competing land uses, which illustrates the economic benefit of forest conservation in agricultural landscapes (Ricketts et al., 2004). Similar results were found in Indonesia where fruit set was negatively correlated with forest distance (Klein et al., 2003a), and in Brazil where coffee plantations near forest fragments had an increase of 15% in production that could be related to pollination services (Marco and Coelho, 2004). Fruit set in the self-sterile lowland coffee species *C. canephora* was found to linearly decrease with distance from the forest (Klein et al., 2003b).

Proper information to farmers about the role of wild bees as pollinators and the pollination services of forests can play a major role in the conservation of wild bees and their natural nesting habitat of tropical forests. Some species of stingless bees, especially from the genus *Trigona*, have dented mandibles and are known to damage fruits, leaves and sometimes even flower buds (Wille, 1961; pers. obs.). Some farmers consider these species as pests and try to eliminate the easily recognizable exposed nests, without knowing that they are

losing valuable pollinators. Wille and Orozco (1983) report that one Costa Rican family with a chayote orchard took one year to eliminate all *Trigona* nests known in their area because they believed these bees ate the tendrils and young leaves. After eliminating all nests, production decreased dramatically from previously high quantities of fruits to no yield at all.

Simple management measures to increase bee abundance and diversity include preservation of natural forests and forest fragments, increasing the availability of nesting sites, and minimizing the use of pesticides including herbicides (Kearns and Inouye, 1997; Klein et al., 2003b).

Conservation of stingless bees may also be affected by the commercial use of stingless bee colonies for pollination services. Provided that colonies for such services are mainly obtained from breeding programs, instead of taken from nature, commercial use of stingless bees does not have to have a negative impact on the feral population, and may actually contribute to their conservation.

## 6. MAIN CONCLUSIONS AND PERSPECTIVES

This manuscript shows that stingless bees are effective pollinators of a wide range of crops. Over the past six years, stingless bees have been confirmed as effective pollinators for nine new crop species, putting the total now on 18 crops. Several species have been domesticated and can be managed in hives. The main limitation to their commercial use as pollinators is lack of mass breeding techniques, which is hampered by low natural colony reproduction rates. Stingless bees may be especially suitable to provide pollination services in greenhouses, as 11 out of the 13 species tested and reported foraged effectively under enclosed conditions. However, more research is needed to find the optimal foraging conditions under enclosed conditions. Although feral colonies are restricted to the tropical and subtropical parts of the world, stingless bees can be kept in cold climates, where they have to be kept indoors in heated hives. Stingless bees have successfully pollinated

several greenhouse crops in regions with temperate climates, such as The Netherlands and Japan. Pesticides may be a severe problem for stingless bees, as they are generally smaller-bodied than the commonly used honeybees and bumble bees, but biological control could provide a good solution.

Although this paper has indicated some potential problems for the use of stingless bees in applied pollination, these can likely be overcome after more research. Stingless bees possess several biological characteristics favourable in applied pollination, and this paper has further strengthened their importance as pollinators of commercially important crops. This indicates that stingless bees are strong candidates in the search for alternative pollinators for our crops.

## ACKNOWLEDGEMENTS

The authors would like to thank Tim Heard and David Roubik for valuable comments on the manuscript. Anne Dollin, Mark Greco, Darci de Oliveira Cruz, Manuel Rincon and David Roubik kindly provided some of the information presented in this paper.

**Résumé – les abeilles sans aiguillon dans la pollinisation appliquée : pratiques et perspectives.** Le nombre de colonies d'abeilles sauvages et élevées connaît actuellement un déclin rapide, provoquant une préoccupation mondiale quant aux services de pollinisation. Les abeilles sans aiguillon (Apidae, Meliponini) sont des abeilles tropicales eusociales qui jouent un rôle écologique important comme pollinisateurs de nombreuses espèces de plantes sauvages. Leur rôle comme pollinisateurs des cultures est actuellement étudié et plusieurs études ont été publiées ces dernières années depuis l'article de synthèse de Heard (1999). Neuf cultures nouvelles ont été mentionnées comme étant efficacement pollinisées par les abeilles sans aiguillon, ce qui monte à 18 le nombre total de cultures en bénéficiant (Tab. I). Cet article passe en revue les informations apparues depuis 1999 et inclut aussi bien des documents publiés que des données non publiées. Les études antérieures sont référencées dans l'article de Heard (1999).

Au cours des six dernières années les abeilles sans aiguillon ont été confirmées comme pollinisateurs du caféier (deux espèces), du fraisier (Figs. 1, 2; Tab. II), de l'avocatier, du rambutan, du paprika doux, de la tomate, du concombre et de la plante

ornementale *Salvia farinacea*. Il a été aussi reporté que les abeilles sans aiguillon visitaient et étaient des pollinisateurs potentiels de l'agrumes calamondin, des cultures de cucurbitacées comme le melon d'eau et la courgette, du radis et de plusieurs autres légumes tels que la carotte et l'endive (Tabs. II, IV). Certaines espèces d'abeilles sans aiguillon diffèrent néanmoins grandement par leur taille et l'efficacité pollinisatrice dépendra de l'adéquation spécifique plante-pollinisateur (voir Tab. VI pour des cas où les deux partenaires sont mal assortis).

La recherche récente s'est concentrée sur l'approche expérimentale à l'aide d'enceintes telles que sachets, cages et serres. On a trouvé que onze espèces d'abeilles sans aiguillon représentant six genres butinaient efficacement sous enceintes (Tab. V), montrant ainsi le potentiel de ces abeilles comme pollinisateurs des cultures protégées. Les colonies peuvent être maintenues à l'intérieur durant des années et, contrairement aux bourdons, ne meurent pas après la période de reproduction. Ceci constitue une incitation économique pour utiliser ces abeilles sans aiguillon comme pollinisateurs commerciaux. Dans les régions tempérées le fait que la plupart des espèces ne survivent pas aux climats froids, ce qui rend donc l'invasion des régions tempérées improbable, constitue une incitation économique. Actuellement la principale limitation à leur usage commercial pour des services de pollinisation réside dans le manque de connaissances pour les élever en masse. Il est nécessaire d'avancer dans la mise au point de la reproduction artificielle des colonies pour que les abeilles sans aiguillon puissent être disponibles comme pollinisateurs commerciaux.

Outre l'utilisation de colonies élevées dans des ruches, les abeilles sauvages de la végétation naturelle environnante peuvent fournir une bonne contribution aux services de pollinisation. Il a été montré un accroissement de la production des cultures dans des plantations situées près de la forêt naturelle pour plusieurs cultures dont le café, qui est partiellement pollinisé par les abeilles sans aiguillon et représente l'une des cultures d'exportations les plus précieuses pour les pays en développement. Ces résultats montrent l'importance économique de la préservation des habitats dans des buts de pollinisation commerciale.

**Apidae / Meliponini / abeille sans aiguillon / pollinisateur / culture protégée / culture alimentaire**

**Zusammenfassung – Stachellose Bienen in der angewandten Pollinisation: Praxis und Perspektiven.** Die Zahlen an wilden und beimkerten Bienenvölkern gehen derzeit rapide zurück, was zu einer weltweiten Bedrohung der Bestäuberdienste führt. Die Diversifizierung der kommerziell verfügbaren Bestäuber kann eine der Antworten sein, um die Lebensmittelproduktion in der Zukunft zu

garantieren. Stachellose Bienen sind tropische eusoziale Bienen, die eine wichtige Rolle als Bestäuber vieler Wildpflanzen spielen. Ihre Rolle als Bestäuber in der Landwirtschaft wird erst seit kurzem untersucht, und seit dem letzten Übersichtsartikel von Heard (1999) wurden viele neue Arbeiten erstellt und publiziert. Neun neue Feldfrüchte konnten durch Stachellose Bienen effektiv bestäubt werden. Dies bringt ihre Gesamtzahl nun auf 18 (Tab. I). Die vorliegende Arbeit stellt die nach 1999 erschienenen Informationen über Stachellose Bienen als Bestäuber in der Landwirtschaft zusammen, und zwar sowohl aus publizierten als auch aus unveröffentlichten Dokumenten. Ältere Studien sind in dem Übersichtsartikel von Heard (1999) berücksichtigt.

Während der letzten sechs Jahre konnten Stachellose Bienen als Bestäuber von Kaffee, Erdbeeren (Abb. 1 und 2, Tab. II), Avocado, Rambutan, Gemüsepaprika, Tomate, Gurke und *Salvia farinacea*, einer Zierpflanze, etabliert werden. Ausserdem werden Stachellose Bienen als Besucher und potentielle Bestäuber von Calamodin, Cucurbitaceen, wie z.B. Wassermelone und Kürbis, für Rettich und verschiedene andere Gemüse wie Karotte und Endivie beschrieben. Stachellose Bienen weisen jedoch erhebliche Artunterschiede hinsichtlich ihrer Körpergrösse auf, und die Bestäubungseffizienz wird davon abhängen wie gut die Bestäuber- und Pflanzenspezies zusammenpassen (siehe Tab. VI für einige Misserfolge).

Neuere Untersuchungen zielen auf experimentelle Ansätze in geschlossenen Systemen, wie Taschen, Käfigen und Gewächshäusern. Elf Arten Stachelloser Bienen aus sechs Gattungen erwiesen sich in solch geschlossenen Systemen als effektive Sammlerinnen (Tab. V), was auf ein beachtliches Potential Stachelloser Bienen als Bestäuber in Gewächshäusern hindeutet. Von wirtschaftlicher Bedeutung für die Nutzung Stachelloser Bienen als Bestäuber dürfte die Tatsache sein, dass ihre Kolonien über Jahre hinweg in geschlossenen Räumen gehalten werden können und dass, im Unterschied zu Hummeln, diese Kolonien nach dem Reproduktionsvorgang nicht eingehen. Von ökologischem Interesse für ihren Einsatz in gemässigten Klimaten ist, dass die meisten Arten nicht kälteresistent sind, was die Gefahr der Invasion und Ausbreitung in diesen Gebieten unwahrscheinlich macht. Das momentan grösste Problem für die kommerzielle Nutzung Stachelloser Bienen für Bestäuberdienste sind die mangelnden Kenntnisse für ihre Massenaufzucht. Die künstliche Reproduktion von Kolonien muss weiterentwickelt werden, bevor sie als kommerzielle Bestäuber in Frage kommen.

Ausser der Nutzung bearbeiteter Völker können auch wilde Kolonien aus der umgebenden natürlichen Vegetation einen wichtigen Anteil an den Bestäuberdiensten erfüllen. Eine gesteigerte Produktivität in nahe an Naturwäldern gelegenen Plantagen konnte für verschiedene Fruchtpflanzen

gezeigt werden, einschliesslich Kaffee, der (teilweise) von Stachellosen Bienen bestäubt wird und der ein wertvolles Exportprodukt in Entwicklungsländern darstellt. Diese Befunde zeigen die Bedeutung der Habitatkonservierung für kommerzielle Bestäubungszwecke.

#### Landwirtschaft / Bestäuberalternativen / Feldfrüchte / Gewächshaus / Apidae / Meliponini

### REFERENCES

- Amano K. (2004) Attempts to introduce stingless bees for the pollination of crops under greenhouse conditions in Japan, Food & Fertilizer Technology Center, [online] <http://www.ffc.agnet.org/library/article/tb167.html> (accessed on 6 March 2006).
- Amano K., Nemoto T., Heard T.A. (2000) What are stingless bees, and why and how to use them as crop pollinators? – a review, JARQ 34, 183–190. [online] <http://ss.jircas.affrc.go.jp/engpage/jarq/34-3/amano/amano.htm> (accessed on 6 March 2006).
- Asiko A.G. (2004) The effect of total visitation time and number of visits by pollinators (*Plebeia* sp. and *Apis mellifera mellifera*) on the strawberry, M.Sc. Thesis, Utrecht University, The Netherlands.
- Benton Jones J. Jr. (1998) Tomato plant culture in the field, greenhouse and home garden, CRC Press, Boca Raton, FL.
- Biesmeijer J.C., Slaa E.J. (2004) Information flow and organization of stingless bee foraging, Apidologie 35, 143–157.
- Biesmeijer J.C., Slaa E.J., Siqueira de Castro M., Viana B.F., Kleinert A., Imperatriz-Fonseca V.L. (2005) Connectance of Brazilian social bee – food plant networks is influenced by habitat, but not by latitude, altitude or network size, Biota Neotrop. 5, 1–9.
- Buchmann S.L. (1983) Buzz pollination in angiosperms, in: Jones C.E., Little R.J. (Eds.), Handbook of experimental pollination biology, Van Nostrand Reinhold Company, N.Y., pp. 73–113.
- Buchmann S.L., Nabhan G.P. (1996) The forgotten pollinators, Island Press, Washington, DC.
- Bustamante M. (1998) Polinización de *Salvia splendens* Sello utilizando cuatro especies de abejas bajo condiciones de invernadero en Cartago, Costa Rica, M.Sc. Thesis, Universidad Nacional, Heredia, Costa Rica.
- Camargo C.A. de (1972) Mating of the social bee *Melipona quadrifasciata* under controlled conditions (Hymenoptera: Apidae), J. Kans. Entomol. Soc. 45, 520–523.

- Campbell D.R., Motten A.F. (1985) The mechanism of competition between two forest herbs, *Ecology* 66, 554–563.
- Can-Alonso C., Quezada-Euán J.J.G., Xiu-Ancona P., Moo-Valle H., Valdovinos-Nunez G.R., Medina-Peralta S. (2005) Pollination of 'criollo' avocados (*Persea americana*) and the behaviour of associated bees in subtropical Mexico, *J. Apic. Res.* 44, 3–8.
- Cane J.H., Tepedino V.J. (2001) Causes and extent of declines among native North American invertebrate pollinators: detection, evidence, and consequences, *Conserv. Ecol.* 5, 1. [online] <http://www.consecol.org/vol5/iss1/art1> (accessed on 6 March 2006).
- Caron D. (2001) Africanized Honey Bees in the Americas, A.I. Root Company, Ohio, USA.
- Carreck N., Williams I. (1998) The economic value of bees in the UK, *Bee World* 79, 115–123.
- Cauich O., Quezada-Euán J.J.G., Macias-Macias J.O., Reyes-Oregel V., Medina-Peralta S., Parra-Tabla V. (2004) Behavior and pollination efficiency of *Nannotrigona perilampoides* (Hymenoptera: Meliponini) on greenhouse tomatoes (*Lycopersicon esculentum*) in Subtropical México, *Hortic. Entomol.* 97, 475–481.
- Cepeda Aponte O.I. (1997) Mating of *Melipona favosa*: behavioral description and some regulating factors (Apidae: Meliponinae), *Proc. Exp. Appl. Entomol.*, N.E.V. 8, 75–78.
- Cervancia C.R., Manila A.C. (2000) Pollination of calamondin (*Citrus mitis* Blanco), *Abstr. 7th IBRA Conf. Trop. Bees: Managem. Div. and 5th Asian Apic. Ass. Conf. Thailand*, p. 136.
- Chacoff N.P., Aizen M.A. (2006) Edge effects on flower-visiting insects in grapefruit plantations bordering premontane subtropical forest, *J. Appl. Ecol.*, doi: 10.1111/j.1365-2664.2005.01116.x.
- Chesson J. (1978) Measuring preference in selective predation, *Ecology* 59, 211–215.
- Chesson J. (1983) The estimation and analysis of preference and its relationship to foraging models, *Ecology* 64, 1297–1304.
- Cortopassi-Laurino M. (1982) Divisão de recursos tróficos entre abelhas sociais, principalmente em *Apis mellifera* Linné e *Trigona (Trigona) spinipes* Fabricius (Apidae, Hymenoptera), M.Sc. dissertation, Instituto de Biociências, Universidade de São Paulo, Brazil.
- Cortopassi-Laurino M., Imperatriz-Fonseca V.L., Roubik D.W., Dollin A., Heard T., Aguilar I.B., Eardley C., Nogueira-Neto P. (2006) Global meliponiculture: challenges and opportunities, *Apidologie* 37, 275–292.
- Crane E. (1983) *The archeology of beekeeping*, Duckworth, London.
- Crane E. (1992) The past and present status of beekeeping with stingless bees, *Bee World* 73, 29–42.
- Cruz D. de O. (2003) Uso e eficiência da abelha jandaíra (*Melipona subnitida* Ducke) na polinização do pimentão (*Capsicum annuum* L.) sob cultivo protegido, M.Sc. dissertation, Universidade Federal do Ceará, Brazil.
- Cruz D. de O., Freitas B.M., Silva L.A. da, Silva S.E.M. da, Bomfim I.G.A. (2004) Use of the stingless bee *Melipona subnitida* to pollinate sweet pepper (*Capsicum annuum* L.) flowers in greenhouse, *Proc. 8th IBRA Int. Conf. Trop. Bees and VI Encontro sobre Abelhas*, p. 661.
- Cruz D. de O., Freitas B.M., Silva L.A. da, Silva S.E.M. da, Bomfim I.G.A. (2005) Pollination efficiency of the stingless bee *Melipona subnitida* on greenhouse sweet pepper, *Pesq. Agropec. Bras.*, Brasília 40, 1197–1201.
- Dafni A., Kevan P.G., Husband B.C. (2005) *Practical Pollination Biology*, Enviroquest, Cambridge, Ontario, Canada.
- Darwin (1876) *The effects of cross and self fertilization in the vegetable kingdom*, Murray, London, UK.
- Drumond P. (2004) Abelhas indígenas sem ferrão, Embrapa/CPAFAC: Acre. [online] <http://www.cpfac.embrapa.br/chebias/cna/artigos/abelhas.htm> (accessed on 6 March 2006).
- Eltz T., Brühl C.A., van der Kaars S., Linsenmair K.E. (2002) Determinants of stingless bee nest density in lowland dipterocarp forests of Sabah, Malaysia, *Oecologia* 131, 27–34.
- Fonseca A., Picado A. (2000) The use of the stingless bees for pollination in production of vegetables seeds, Report, Faculty of Biology, Utrecht University, The Netherlands.
- Free J.B. (1993) *Insect pollination of crops*, 2nd ed., Academic Press, London, UK.
- Freitas B.M., Paxton R.J. (1998) A comparison of two pollinators: the introduced honey bee (*Apis mellifera*) and an indigenous bee (*Centris tarsata*) on cashew (*Anacardium occidentale*) in its native range of NE Brazil, *J. Appl. Ecol.* 35, 109–121.
- Ghazoul J. (2005a) Buzziness as usual? Questioning the global pollination crisis, *Trends Ecol. Evol.* 20, 367–373.
- Ghazoul J. (2005b) Response to Steffan-Dewenter et al.: Questioning the global pollination crisis, *Trends Ecol. Evol.* 20, 652–653.
- Goulson D. (2003) Effects of introduced bees on native ecosystems, *Annu. Rev. Ecol. Syst.* 34, 1–26.
- Greco M., Spooner-Hart R., Holford P. (2005) A new technique for monitoring *Trigona carbonaria* nest contents, brood and activity using X-ray computerized tomography, *J. Apic. Res.* 44, 97–100.
- Heard T.A. (1999) The role of stingless bees in crop pollination, *Annu. Rev. Entomol.* 44, 183–206.

- Heard T.A., Exley E.M. (1994) Diversity, abundance, and distribution of insect visitors to macadamia flowers, *Environ. Entomol.* 23, 91–100.
- Heard T.A., Dollin A. (2000) Stingless beekeeping in Australia, snapshot of an infant industry, *Bee World* 82, 116–125.
- Heithaus E.R. (1979a) Flower-feeding specialization in wild bee and wasp communities in seasonal neotropical habitats, *Oecologia* 42, 179–194.
- Heithaus E.R. (1979b) flower visitation records and resource overlap of bees and wasps in northwest Costa Rica, *Brenesia* 16, 9–52.
- Hingston A.B., McQuillan P.B. (1999) Displacement of Tasmanian native Megachilid bees by the recently introduced bumblebee *Bombus terrestris* (Linnaeus, 1758) (Hymenoptera: Apidea), *Aust. J. Zool.* 47, 59–65.
- Hubbell S.P., Johnson L.K. (1977) Competition and nest spacing in a tropical stingless bee community, *Ecology* 58, 949–963.
- Inoue T., Nakamura K., Salmah S., Abbas I. (1993) Population dynamics of animals in unpredictably-changing tropical environments, *J. Biosci.* 18, 425–455.
- Ish-Am G., Barrientos-Priego F., Castañeda-Vildozola A., Gazit S. (1999) Avocado (*Persea americana* Mill.) pollinators in its region of origin, *Rev. Chapingo Serie Hortic.* 5, 137–143.
- Kaftanoglu O. (2000) The diversity and faunistics of bumblebees for pollination in greenhouses, in: Sommeijer M.J., Ruijter A. de (Eds.), *Insect Pollination in Greenhouses: proceedings of the specialists' meeting held in Soesterberg, The Netherlands*, pp. 73–81.
- Kakutani T., Inoue T., Tezuka T., Maeta Y. (1993) Pollination of strawberry by the stingless bee, *Trigona minangkabau*, and the honey bee, *Apis mellifera*: an experimental study of fertilization efficiency, *Res. Popul. Ecol.* 35, 95–111.
- Katayama E. (1987) Utilization of honeybees as pollinators for strawberries in plastic greenhouses, *Honeybee Sci.* 8, 147–150 (in Japanese).
- Kearns C.A., Inouye D.W. (1993) Pollinators, flowering plants, and conservation biology, *BioScience* 47, 297–307.
- Kearns C.A., Inouye D.W. (1997) *Techniques for pollination biologists*, University Press of Colorado, Colorado.
- Kearns C.A., Inouye D.W., Waser N.M. (1998) Endangered mutualisms: The conservation biology of plant-pollinator interactions, *Annu. Rev. Ecol. Syst.* 29, 83–112.
- Klein A.M., Steffan-Dewenter I., Tscharntke T. (2003a) Fruit set of highland coffee increases with the diversity of pollinating bees, *Proc. R. Soc. Lond. B* 270, 955–961.
- Klein A.M., Steffan-Dewenter I., Tscharntke T. (2003b) Pollination of *Coffea canephora* in relation to local and regional agroforestry management, *J. Appl. Ecol.* 40, 837–845.
- Klein A.M., Steffan-Dewenter I., Tscharntke T. (2003c) Bee pollination and fruit set of *Coffea arabica* and *C. canephora* (Rubiaceae), *Am. J. Bot.* 90, 153–157.
- Kremen C., Williams N.M., Thorp R.W. (2002) Crop pollination from native bees at risk from agricultural intensification, *Proc. Natl Acad. Sci. (USA)* 99, 16812–16816.
- Kuyhor T. (2001) Foraging and pollination efficiency of *Tetragonisca angustula* on sweet pepper in a greenhouse, M.Sc. Thesis, Utrecht University, the Netherlands.
- Lalama K. (2001) Pollination effectiveness and efficiency of the stingless bee *Plebeia sp.* and the honeybee *Apis mellifera* on strawberry *Fragaria* × *ananassa* in a greenhouse, M.Sc. Thesis, Utrecht University, The Netherlands.
- Maeta Y., Tezuka T., Nadano H., Suzuki K. (1992) Utilization of the Brazilian stingless bee, *Nannotrigona testaceicornis*, as a pollinator of strawberries, *Honeybee Sci.* 13, 71–78 (in Japanese).
- Mah Y.I., Man-Youn L., Bilinski M. (2000) Some characteristics of Korean indigenous bumblebee species (Hymenoptera; *Bombus* spp.) in the laboratory conditions, in: Sommeijer M.J., Ruijter A. de (Eds.), *Insect Pollination in Greenhouses: proceedings of the specialists' meeting held in Soesterberg, The Netherlands*, pp. 107–115.
- Malagodi-Braga K.S. (2002) *Tetragonisca angustula* as strawberry pollinator in greenhouses, Ph.D. Thesis, Brazil.
- Malagodi-Braga K.S., Kleinert A.M.P. (2004) Could *Tetragonisca angustula* Latreille (Apinae, Meliponini) be used as strawberry pollinator in greenhouses? *Aust. J. Agric. Res.* 55, 771–773.
- Marco P. de, Coelho F.M. (2004) Services performed by the ecosystem: forest remnants influence agricultural cultures, pollination and production, *Biodivers. Conserv.* 13, 1245–1255.
- McGregor S.E. (1976) *Insect pollination of cultivated crop plants*, Agriculture Handbook No. 496, Washington, DC: US Dep. Agric. [online] [http://www.beeculture.com/content/pollination\\_handbook/index.html](http://www.beeculture.com/content/pollination_handbook/index.html) (accessed on 6 March 2006).
- Meléndez-Ramírez V., Magaña-Rueda S., Parra-Tabla V., Ayala R., Navarro J. (2002) Diversity of native bee visitors of cucurbit crops (Cucurbitaceae) in Yucatán, México, *J. Insect Conserv.* 6, 135–147.
- Meeuwsen F.J.A.J. (2000) Stingless bees for pollination purposes in greenhouses, in: Sommeijer M.J., Ruijter A. de (Eds.), *Insect Pollination in Greenhouses: Proc. specialists' meeting held in Soesterberg, The Netherlands*, pp. 143–147.
- Michener C.D. (2000) *The bees of the world*, Johns Hopkins University Press, Baltimore, Maryland.

- Morales C.L., Aizen M.A. (2004) Potential displacement of the native bumblebee *Bombus dahlbomii* by the invasive *Bombus ruderatus* in NW Patagonia, Argentina, Proc. 8th IBRA Int. Conf. Trop. Bees and VI Encontro sobre Abelhas, pp. 70–76.
- Morton J. (1987) Calamondin, in: Morton J.F. (Ed.), Fruits of warm climates, Miami, USA, pp. 176–178, [online] <http://www.hort.purdue.edu/newcrop/morton/calamondin.html#Propagation> (accessed on 6 March 2006).
- Murillo M. (1984) Uso y manejo actual de las colonias de *Melipona beecheii* Bennett (Apidae: Meliponini) en el estado de Tabasco, Mexico, Biotica 9, 423–428.
- Nabhan G.P., Allen-Wardell G., Bernhardt P., Bitner R. (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields, Conserv. Biol. 12, 8–17.
- Nieh J.C. (2004) Recruitment and communication in stingless bees (Hymenoptera, Apidae, Meliponini), Apidologie 35, 159–182.
- Nogueira-Neto P. (1997) Vida e criação de abelhas indígenas sem ferrão, Nogueirapis, São Paulo.
- Occhiuzzi P. (1999) Stingless bees thrive in glasshouse trial, Aussie Bee 12, 8–11, Published by Australian Nature Bee Research Centre, North Richmond NSW Australia.
- Occhiuzzi P. (2000) Stingless bees pollinate greenhouse Capsicum, Aussie Bee 13, 15. Published by Australian Nature Bee Research Centre, North Richmond NSW Australia.
- Picado A. (2000) Sistema reproductivo, de *Salvia splendens* Sello y el efecto de las abejas *Tetragonisca angustula* y *Apis mellifera* en su polinización, M.Sc. thesis, Universidad Nacional de Costa Rica, Heredia.
- Quezada-Euàan J., May-Itzá W., González-Acereto J. (2001) Meliponiculture in Mexico: problems and perspective for development, Bee World 82, 160–167.
- Ramalho M., Kleinert-Giovannini A., Imperatriz-Fonseca V.L. (1990) Important bee plants for stingless bees (*Melipona* and Trigonini) and Africanised honeybees (*Apis mellifera*) in neotropical habitats: a review, Apidologie 21, 469–488.
- Richards A.J. (2001) Does low biodiversity resulting from modern agricultural practice affect crop pollination and yield? Ann. Bot. 88, 165–172.
- Ricketts T.H. (2004) Tropical forest fragments enhance pollinator activity in nearby coffee crops, Conserv. Biol. 18, 1262–1271.
- Ricketts T.H., Daily G.C., Ehrlich P.R., Michener C.D. (2004) Economic value of tropical forest to coffee production, Proc. Natl Acad. Sci. (USA) 101, 12579–12582.
- Rindfleisch (1980) A case for meliponiculture in pollination, Am. Bee J. 120, 468–470.
- Rosso J.M., Imperatriz-Fonseca V.L., Cortopassi-Laurino M. (2001) Meliponicultura en Brasil II: técnicas de manejo, II Seminario Mexicano sobre abejas sin aguijón, Memorias, Mérida, Yucatán, Mexico, p. 132.
- Roubik D.W. (1989) Ecology and natural history of tropical bees, Cambridge Univ. Press, Cambridge, UK.
- Roubik D.W. (1992) Stingless bees: a guide to Panamanian and Mesoamerican species and their nests (Hymenoptera: Apidae: Meliponinae), in: Quintero D., Aiello A. (Eds.), Insects of Panamá and Mesoamerica, Oxford University Press, Oxford, UK, pp. 495–524.
- Roubik D.W. (1995a) Pollination of cultivated plants in the tropics, FAO Agric. Serv. Bull. 118, Rome.
- Roubik D.W. (1995b) Stingless bee colonies for pollination, in: Roubik D.W. (Ed.), Pollination of cultivated plants in the tropics, FAO Agric. Serv. Bull. 118, Rome, pp. 150–154.
- Roubik D.W. (2002a) The value of bees to the coffee harvest, Nature 417, 708.
- Roubik D.W. (2002b) Feral African Bees augment Neotropical coffee yield, in: Pollinating Bees: The Conservation Link Between Agriculture and Nature, Ministério do Meio Ambiente, Brasília, Brazil [online] [http://striweb.si.edu/publications/PDFs/Roubik\\_coffee97.pdf](http://striweb.si.edu/publications/PDFs/Roubik_coffee97.pdf) (accessed on 6 March 2006).
- Samejima H., Marzuki M., Nagamitsu T., Nakasizauka T. (2004) The effects of human disturbance on a stingless bee community in a tropical rainforest, Biol. Conserv. 120, 577–587.
- Sánchez L.A., Picado A., Sommeijer M.J., Slaa E.J. (2002) Floral Biology, pollination ecology and seed production of the ornamental plant *Salvia splendens* Sello, J. Hortic. Sci. Biotech. 77, 498–501.
- Santos S.A.B. dos, Bego L.R., Roselino A.C. (2004a) Pollination in tomatoes, *Lycopersicon esculentum*, by *Melipona quadrifasciata anthidioides* and *Apis mellifera* (Hymenoptera, Apinae), Proc. 8th IBRA Int. Conf. Trop. Bees and VI Encontro sobre Abelhas, p. 688.
- Santos S.A.B. dos (2004b) Pollination of cucumber – *Cucumis sativus* – by stingless bees (Hymenoptera, Meliponini), Proc. 8th IBRA Int. Conf. Trop. Bees and VI Encontro sobre Abelhas, p. 689.
- Sarto M.C.L. del, Peruquetti R.C., Campos L.A.O. (2005) Evaluation of the Neotropical stingless bee *Melipona quadrifasciata* (Hymenoptera: Apidae) as pollinator of greenhouse tomatoes, J. Econ. Entomol. 98, 260–266.

- Schneider S.S., DeGrande-Hoffman G., Smith D.R. (2004) The African honeybee: factors contributing to a successful biological invasion, *Annu. Rev. Entomol.* 49, 351–376.
- Seeley T.D. (1985) *Honeybee Ecology*, Princeton University Press, Princeton, New Jersey.
- Slaa E.J. (2003) Foraging ecology of stingless bees: from individual behaviour to community ecology, Ph.D. dissertation, Utrecht University, The Netherlands.
- Slaa E.J. (2006) Population dynamics of a stingless bee community in the seasonal dry lowlands of Costa Rica, *Insectes Soc.* 53, 70–79.
- Slaa E.J., Sánchez L.A., Sandí M., Salzar W. (2000a) A scientific note on the use of stingless bees for commercial pollination in enclosures, *Apidologie* 31, 141–142.
- Slaa E.J., Sánchez L.A., Sandí M., Salzar W. (2000b) Pollination of an ornamental plant (*Salvia farinacea*: Labiatae) by two species of stingless bees and Africanised honey bees (Hymenoptera: Apidae), in: Sommeijer M.J., Ruijter A. de (Eds.), *Insect Pollination in Greenhouses: Proc. specialists' meeting held in Soesterberg, The Netherlands*, pp. 209–215.
- Slaa E.J., Wassenberg J., Biesmeijer J.C. (2003a) The use of field-based social information in eusocial foragers: local enhancement among nestmates and heterospecifics in stingless bees, *Ecol. Entomol.* 28, 369–379.
- Slaa E.J., Tack A.J.M., Sommeijer M.J. (2003b) The effect of intrinsic and extrinsic factors on flower constancy in stingless bees, *Apidologie* 34, 457–468.
- Sommeijer M.J., Ruijter A. de (2000) Insect pollination in greenhouses, *Proc. specialists' meeting held in Soesterberg, The Netherlands*.
- Southwick L., Southwick E.E. (1992) Estimating the economic value of honey bees as agricultural pollinators in the United States, *J. Econ. Entomol.* 85, 621–633.
- Steffan-Dewenter I., Potts S.G., Packer L. (2005) Pollinator diversity and crop pollination services are at risk, *Trends Ecol. Evol.* 20, 651–652.
- Thai P.H. (2001) Foraging and pollination efficiency of the stingless bee, *Tetragonisca angustula*, on radish, *Raphanus sativus*, in a greenhouse, M.Sc. thesis Utrecht University, The Netherlands.
- Thomson J.D. (1983) Component analysis of community-level interactions in pollination systems, in: Jones C.E., Little R.J. (Eds.), *Handbook of Experimental Pollination Biology*, Van Nostrand Reinhold Company Inc., New York, pp. 451–460.
- Torchio P.F. (1987) Use of non-honeybee species as pollinators of crops, *Proc. Entomol. Soc. Ont.* 118, 111–124.
- UNEP (2002) Action for a sustainable future, Decisions from the Sixth meeting of the conference of the parties to the convention on Biological diversity, United Nations, The Hague, The Netherlands, [online] <http://www.biodiv.org/doc/meetings/cop/cop-06/official/cop-06-20-en.pdf> (accessed on 6 March 2006).
- Venturieri G.A., Pickkersgill B., Overal W.L. (1993) Floral biology of the Amazonian fruit tree “cupuassu” (*Theobroma grandiflorum*), in: *Biodiversity and Environment – Brazilian Themes for the Future*, London, Linn. Soc. London, R. Bot. Gard. P. 23.
- Villanueva-G.R., Roubik D.W., Colli-Ucán (2005) Extinction of *Melipona beecheii* and traditional beekeeping in the Yucatán peninsula, *Bee World* 86, 35–41.
- Visscher P.K., Seeley T.D. (1982) Foraging strategy of honeybee colonies in a temperate deciduous forest, *Ecology* 63, 1790–1801.
- Wagner A., Dollin L. (1983) Swarming in Australian native bees – help solve the mystery! *Australas. Beekeeper* 84, 34–37.
- Waser N.M. (1983) Competition for pollination and floral character differences among sympatric plant species: a review of evidence, in: Jones C.E., Little R.J. (Eds.), *Handbook of Experimental Pollination Biology*, Van Nostrand Reinhold Company Inc., New York, pp. 277–293.
- Watanabe M. (1994) Pollination worries rise as honey bees decline, *Science* 265, 1170.
- Weaver E.C., Weaver N. (1981) Beekeeping with the stingless bee (*Melipona beecheii*) by the Yucatecan Maya, *Bee World* 62, 7–19.
- Wille A. (1961) Las abejas jicotes de Costa Rica, *Rev. Univ. de Costa Rica* 22, 1–30.
- Wille A., Orozco E. (1983) Polinización del chayote *Sechium edule* (Jacq.) Swartz en Costa Rica, *Rev. Biol. Trop.* 31, 145–154.
- Willmer P.G., Stone G.N. (1989) Incidence of entomophilous pollination of lowland coffee (*Coffea canephora*); the role of leaf cutter bees in Papua New Guinea, *Entomol. Exp. Appl.* 50, 113–124.
- Winston M.L. (1992) The Africanized honey bee, *Annu. Rev. Entomol.* 37, 173–193.
- Zebrowska J. (1998) Influence of pollination modes on yield components in strawberry (*Fragaria x ananassa* Duch.), *Plant Breeding* 17, 255–260.