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Bee (Hymenoptera: Apidae) pollination of *Vanilla planifolia* in Florida and their potential in commercial production

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Abstract

Male orchid bees are known pollinators of some *Vanilla* species (Orchidaceae), but there is little direct evidence for the pollination of *V. planifolia* L., the most important source of commercial vanilla. This study was undertaken to determine if the naturalized orchid bee*, Euglossa dilemma* Bembé & Eltz (Hymenoptera: Apidae), is a pollinator of *V. planifolia* in Florida*,* where the orchid is currently being considered as a potential crop. The flowers of *V. planifolia* were determined to have no nectar. Fragrance from in situ flowers was analyzed and found to contain principally 1-8 cineole and β-ocimene, compounds known to attract some male orchid bees. Timed watches documented pollen removal by a single female orchid bee, but no male orchid bees visited the flowers. Separate applications of 1-8 cineole, that we found in the flowers, and eugenol, which does not occur in the flowers, were made to flowers and baits, but the male orchid bees came only to flowers and baits with eugenol. Eugenol is a known attractant and principal component of many of the perfumes of the orchid mutualists of male *E. dilemma*. The rapid and intensive visitation and pollination that male *E. dilemma* bees exhibit towards their mutualist orchids did not occur, indicating *V. planifolia* is not an orchid mutualist of *E. dilemma*. During a timed watch, a female worker honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), pollinated a flower by entering it, backing out with pollen on its thorax, then re-entering the flower and exiting without the pollen. Dissection of the flower found pollen on the stigma and the anther cap disarticulated. A female *E. dilemma* removed pollen from a flower, indicating that she was also a probable pollinator. Morphometric measurements determined there was a good match between the interior dimensions of the tubular lip of the flowers and the thickness of the bodies of both the female orchid bee and honey bee, enabling good contact between the bees and the flowers sexual organs. Season-long fruit set was 0.655%, similar to the 1% or less reported for *V. planifolia* in its native Mexico range. Although the visitation rates were low, the potential of both the female honey bee and *E. dilemma* as pollinators of *V. planifolia* cultivated in screen houses is considered. Managed honey bees for pollination of vanilla appears to have more potential and it could reduce the need for expensive hand pollination, if sufficient pollination rates could be achieved.

Key Words: agriculture; *Apis mellifera*; *Euglossa dilemma*; orchid

Resumen

Las abejas macho de las orquídeas son polinizadores conocidos de algunas especies de *Vanilla* (Orchidaceae), pero hay poca evidencia directa de la polinización de *V. planifolia* L., la fuente más importante de vainilla comercial. Se realizó este estudio para determinar si la abeja de las orquídeas naturalizada, *Euglossa dilemma* Bembé & Eltz (Hymenoptera: Apidae), es un polinizador de *V. planifolia* en la Florida, donde actualmente se considera a la orquídea como un cultivo potencial. Se determinó que las flores de *V. planifolia* no tenían néctar. Se analizó la fragancia de las flores in situ y se encontró que contenía principalmente de 1 a 8 cineol y β-ocimeno, compuestos que se sabe atraen a algunas abejas orquídeas macho. Relojes cronometrados documentaron la eliminación de polen por parte de una sola abeja orquídea hembra, pero ningún abeja orquídea macho visitó las flores. Se hicieron aplicaciones separadas a las flores y los cebos de 1-8 cineol, que encontramos en las flores, y eugenol, que no se encuentra en las flores, pero las abejas orquídeas macho solo llegaron a flores y cebos con eugenol. El eugenol es un conocido atrayente y componente principal de muchos de los perfumes de las orquídeas mutualistas del macho *E. dilemma*. La ausencia de visita y polinización rápida e intensiva que las abejas macho de *E. dilemma* exhiben hacia sus orquídeas mutualistas no ocurrió, lo que indica que *V. planifolia* no es una orquídea mutualista de *E. dilemma*. Durante una vigilancia cronometrada, una abeja melífera, *Apis mellifera* L. (Hymenoptera: Apidae), polinizó una flor entrando en ella, saliendo con polen en su tórax, luego volvió a entrar en la flor y salió sin polen. La disección de la flor encontró polen en el estigma y el casquete de las anteras desarticulado. Una hembra de *E. dilemma* eliminó el polen de una flor, lo que indica que ella también era una probable polinizadora. Las mediciones morfométricas determinaron que había una buena coincidencia entre las dimensiones interiores del labio tubular de las flores y el grosor de los cuerpos tanto de la abeja orquídea hembra como de la abeja melífera, lo que permitió un buen contacto entre las abejas y los órganos sexuales de las flores. El cuajado de frutos durante toda la temporada fue del 0,655 %, similar al 1 % o menos reportado para *V. planifolia* en su área de distribución nativa de México. Aunque las tasas de visitas fueron bajas, se considera el potencial de la abeja melífera y de la hembra de *E. dilemma* como polinizadores de *V. planifolia* cultivada en invernaderos. Las abejas melíferas manejadas para la polinización de la vainilla parecen tener más potencial y podrían reducir la necesidad de una costosa polinización manual, si se pudieran lograr tasas de polinización suficientes.

Palabras Clave: agricultura; *Apis mellifera*; *Euglossa dilemma*; orquídea

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Natural and synthetic vanilla is the most popular and most widely used flavor and fragrance with a broad array of uses in food and beverages, household products, pet products, perfumes, pharmaceuticals, and is even used to coat toys to make them smell better (Havkin-Frenkel et al. 2019). Natural vanilla is the highly valued cured fruit capsules of 3 tropical orchids (Orchidaceae; *V. planifolia* Andrews, *V. tahitensis* Moore, and *V. pompona* Shiede), of which *V. planifolia* is by far the most important due to its high vanillin content (Ranadive 2019). Vanilla is an exceptionally labor-intensive crop due to the need for hand pollination of the flowers and the complex treatment of the mature fruit to cure them, in order to fully develop their fragrance and desired market value (Hernandez-Hernandez 2019). Due to the high labor costs, vanilla is largely cultivated in countries where labor is inexpensive, most importantly in Madagascar, but also in Tahiti, Indonesia, India, Papua New Guinea, Reunion, Mexico, and Uganda (Korthou & Verpoorte 2007, Ranadive 2019). *Vanilla planifolia,* which is native to Mexico and Central America, was first used as a wild-gathered flavor plant and appears to have come into cultivation in Mexico during the 1760s for export to Europe (Lubinsky et al. 2019). The plant is vine propagated by stem cuttings, resulting in a clonal crop with very low diversity. Although it is cultivated, it is essentially undomesticated due to the lack of agricultural selection and breeding (Chambers 2019). Research is underway in southern Florida, USA, to develop a vanilla breeding program and to investigate the potential of the plant as a specialty crop (Chambers 2019). Chambers et al. (2019) in a description of this effort, suggested that *Euglossa dilemma* Bembé & Eltz, a non-native orchid bee, could be a potential pollinator of *V. planifolia* in Florida.

The natural pollination of *V*. *planifolia* in its native region (Mexico and Central America) has been the subject of speculation and some observation, but little direct evidence for it exists (Lubinsky et al. 2006). Natural pollination in Mexico is uncommon, accounting for only about 1% of the flowers setting fruit (Hernandez-Hernandez 2019). Dressler (1981) indicated that the stingless bee *Melipona beecheii* Bennet, although popularly believed to be the pollinator of vanilla, is unlikely to be an effective pollinator because it is too small to contact the sexual organs when it visits the flower. He proposed that orchid bees (Euglossini) may be the pollinators of *V. planifolia*. Ackerman (1983) found pollinia of *Vanilla planifolia* (as *V. fragrans* Andrews) on the scutellum of a male *Euglossa* orchid bee in Panama. Soto-Arenas (1999, 2003) wrote that *Euglossa viridissima* Friese visits the flowers of *V. planifolia* in Mexico and that this results in fruit set in less than 1/100 or 1/1000 of the flowers but provided no evidence. *Euglossa dilemma* in Florida was identified as *E. viridissima* before it was determined to be an undescribed cryptic species also native to Mexico and Central America (Eltz et al. 2011). Roubik and Hanson (2004) listed *V. planifolia* as one of the orchids associated with male *Euglossa tridendata* Moure in Panama. In an effort to document the pollination of *V. planifolia*, Lubinsky et al. (2006), spent 2 wks conducting intensive field observations of the orchid in Oaxaca, Mexico during the spring of 2004. They saw flower visits by ants, hummingbirds, stingless bees (*Melipona*), and orchid bees (*Euglossa* and *Exeretes*), but no pollination events occurred.

Male orchid bees have been reported to be the pollinators of many other *Vanilla* species including *V. cribbiana* Soto Arenas, *V. dressleri* Soto Arenas, *V. hameri* Soto Arenas (Soto Arenas 1999)*, V. pompona* Schiede (Pridgeon et al. 2003), *V. grandiflora* (Lindl.) Soto Arenas (Lubinsky et al. 2006), *V. trigonocarpa* Hoehne (Soto Arenas & Dressler 2010), and *V. dubia* Hoehne (Pansarin and Pansarin 2014).

Euglossa dilemma was first detected in southeastern Florida in 2003 (Skov & Wiley 2005; Pemberton & Wheeler 2006). This orchid bee now occurs widely in the southern two thirds of Florida (Pemberton & Escalona 2023), but it has a very patchy occurrence, being abundant in some areas but uncommon or absent in others. For instance, the bee is absent from Fairchild Botanical Garden in Miami-Dade County and currently occurs at low levels in Homestead, also in Miami-Dade County, the area where the potential of *V*. *planifolia* as a commercial crop is being studied.

The primary goal of the present study was to determine if the naturalized orchid bee, particularly male *E. dilemma*, pollinates *V. planifolia* in subtropical Florida. A secondary goal was to detect other pollinators of the orchid that might also be useful in commercial vanilla production. An effective pollinator could reduce the need for expensive hand pollination, which can be a ‘make or break’ cost in vanilla production. A shortage of labor needed to hand pollinate the flowers was one of the factors that led to the end of commercial vanilla production in Puerto Rico (Bayman 2019). “The study of *Vanilla* pollination is extremely important because the need for manual pollination, added to the ripening and curing time, causes vanilla to be one of the most expensive spices in the world” (Pansarin 2021, p 1).

Materials and Methods

THE STUDY SYSTEM

The research was carried out in a commercial nursery (26.0583021 °N, 80.2399487 °W) in Davie (Broward county) in southeastern subtropical Florida, with a single 14 m long vine. The nursery usually hand pollinates the flowers of this vine to obtain fruit capsules but did not do so during the year when this study was done. The plant was growing in an open shade structure with a screened ceiling about 3 m high, but otherwise was open on all sides. The plant had lost its connection to the ground and was growing as an epiphyte on the shade house’s vertical and horizontal wooden support posts and beams. It bore 8 inflorescences, each bearing numerous flower buds. Each day, 4 to 8 new flowers would open. The orchid bee is abundant in this nursery, which has both commercial growing and sales areas that are mostly in the sun, and a partly shaded, diversely planted wooded landscape comprising the owner/plant collector’s garden. The nursery contains many flowers that *E*. *dilemma* uses to obtain nectar, especially members of the Acanthaceae and Bignoniaceae, and pollen sources such as *Solanum* and *Senna* species, as well as many *Salvia* species that provide both nectar and pollen (Pemberton & Wheeler 2006; Pemberton unpublished observations). Importantly, the nursery does not apply insecticides to control pest insects. The research was conducted for 2 wks during Apr of 2022 from the midpoint of the plant’s flowering almost to its end.

TIMED WATCHES

Our key approach was to conduct timed watches of the flowers when the bees were active. It was determined on early morning visits, that new flowers usually open every morning between 6 AM and 7 AM and wilt around noon, or as early as 11 AM, when the temperature approached 28 °C. When wilting, the lateral petals of the flowers collapse against the lip until they narrow then block the entrance to the lip. The orchid bees were not active until well after sunrise, usually when conditions became sunny and warm or hot after 8 AM. For these reasons the watches were carried out in the morning between 8 AM and 12 PM. The anther cap of each flower was examined before, periodically during, and after each timed watch session, to see if it had been moved. Flower visitors large enough to deliver or remove pollen would disturb the hinged anther cap, so examining the anther cap was a means of verifying that no undetected visits had occurred prior to or during the watches. During the watches, the observer sat quietly in a chair 1.5 to 2.5 m from the flowers, except when capturing flower visitors and checking the anther caps in the flowers, the distance was dependent on the location of the open flowers on the vine. Bees that visited flowers were captured when they backed out of the flowers into 15 dram snap top vials placed open end against the entrance to the flower's lip. The tubes containing captured bees were then put on ice and the bees were later pinned.

NECTAR DETERMINATION

To determine if nectar was present in the flowers, 3 flowers were cut from the vine at 9:30 AM one morning and placed in uncapped 50 ml vials with distilled water and uncooked rice to hold the flowers erect. The vials with the flowers were carefully placed in a small cooler between ice packs to prevent the flowers from wilting or being damaged, and then taken quickly to a nearby laboratory for examination. The fresh flowers' trumpet shaped lips bearing the column with the single stamen and stigma were dissected under a dissecting microscope to look for liquid secretions and/or shiny areas. The narrow linear hair bed on the inner dorsal roof of the tubular lip posterior to the column, was examined for nectar but was difficult to assess visually. To determine if nectar was present, the hair bed was lightly brushed with moistened fibers from Diastix regent strips (Ascensia Diabetes Care US Inc., Parsippany, New Jersey, USA) used to detect glucose in urine. Additionally, the inaccessible interior base of the tubular lip was gently probed with Drummond Microcaps (Drummond Scientific Co., Brommal, Pennsylvania, USA) capillary tubes to collect liquids.

FLOWER AND BEE MORPHOMETRICS

The morphometrics of the interior of *Vanilla* flowers and their pollinating bees has been shown to be important for pollination success because it determines whether the visiting bees contact the column bearing the anther and stigma (Pansarin & Pansarin 2014). To access the interior of the tubular lip to take measurements, a longitudinal lateral window was cut out of 2 flowers, to expose the interior of the lip with the column dorsally and the penicillate callus on the floor beneath the column (Fig. 1). Other studies of bee pollination of *Vanilla* orchids (Rasmussen 1985; Pansarin & Pansarin 2014) showed that the penicillate callus lifts the body of the visiting bee as it backs out of the flower to facilitate its contact with the rostellum and the anther on the column. The rostellum is a flange of tissue between the stigma and the anther on the column, which prevents automatic self-pollination. In bee pollination of *Vanilla* species, the lower distal edge of the rostellum scrapes pollen from the thorax of the visiting bee as it backs out of the flower and puts it on the stigma. The callus lifting the body of the exiting bee also enables the bee to contact and displace the hinged anther cap, causing pollen to be applied to bee's scutum or scutellum more readily. Photographs were taken of these windowed lips and an adjacent aligned ruler to enable measurements of the critical vertical distance between the penicillate callus and the rostellum and between the callus and the anther. Digital calipers were used to measure the vertical thickness of the thoraxes of the specimens of the bees that visited the flowers.

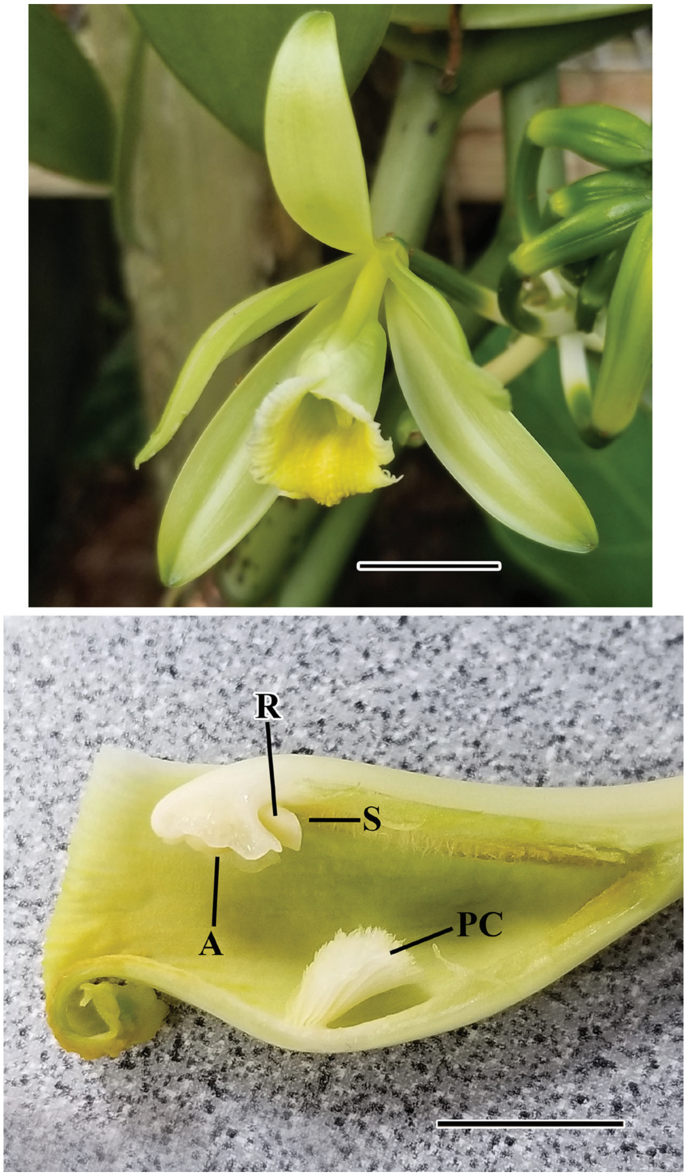

Fig. 1. *Vanilla planifolia* flower and the pollination mechanism. Top - intact flower, scale bar = 2 cm. Bottom - lateral view of bisected tubular lip showing the interior, scale bar = 4 mm. The column is appressed to the top with the single anther (A) not fully visible in this view because it is hidden behind a flange of tissue. Adjacent and to the right the anther and this flange is the rostellum (R), which separates the anther and the stigma S, which is on the right posterior interior surface of the rostellum (R). The ovary is within the flower stalk so not visible in this photo. These parts are more easily seen and understood in Figure 2. The penicillate callus (PC) lifts the exiting bee's body up causing it to better contact the rostellum (R) and the anther (A). The vertical thickness of the pollinating bees thoraxes was found to be similar to the distance between the penicillate callus (PC) and the rostellum (R), and the penicillate callus (PC) and the anther (A). When a pollinating bee exits the flower, it backs out and the dorsal surface of its thorax first contacts the rostellum (R), which scrapes any existing pollen on the top of its thorax off and onto to the stigma (S). Then as the bee continues to back out, its thorax contacts the anther and dislodges its hinged anther cap, which deposits pollen on the top of its thorax.

FLORAL FRAGRANCE

Sample collections

Volatiles were collected from 3 *V. planifolia* flowers over 2 consecutive days during Apr 2022 between 9 AM and 11 AM. To collect volatiles, in situ flowers on the vine were enclosed in polytetrafluoroethylene gas sampling bags (Tedlar® 10L; 231-10, SKC, Eighty Four, Pennsylvania, USA) fitted with a septum. The open side of each bag was secured over the flower base with a zip tie that was constricted over a foam stopper (46–65 mm) (W.W. Grainger Corp., Lake Forest, Illinois, USA) to the flower base. Flower volatiles were collected with solid phase microextraction (SPME) fibers (100 uM PDMS; Sigma-Aldrich, Inc., St Louis, Missouri, USA). Collections were made by piercing the bag septum with the SPME collector and exposing the fibers to the sample volatiles for 30 min. At the beginning of each day the fibers were conditioned by exposing them to 280 °C for 30 min within a gas chromatograph inlet (Agilent 6890, Wilmington, Delaware, USA). The inlet was equipped with a direct SPME type liner that was 0.75 mm with a single tapered end (Sigma-Aldrich, Inc., St Louis, Missouri, USA).

Volatile analysis

Analysis of volatiles was conducted by gas chromatography-mass spectroscopy (GC-MS) according to standard methods (Wheeler et al. 2014). The identities of constituents were determined with an Agilent 8890 instrument fitted with a HP-5ms FSOT (fused silica open tubular) column (30 m x 0.25 mm, 0.25 micron film thickness) with helium at 36 cm/s as a carrier gas, injector port (split 1:20) at 250 °C, mass selective detector (HP 5977B) at 250 °C (source) and 150 °C (quad) with transfer line 280 °C and ion source filament voltage of 70 eV. Volatiles were thermally desorbed by exposing the SPME fibers to the GC injection port (250 °C) operated in splitless mode. Injections were programed to be in split (20:1) mode after 2.5 min. We identified individual chemical constituents based on mass spectral fragmentation and retention indices calculated from injections of n-paraffins under identical conditions. The identity of the components was confirmed by searching their mass spectra against NIST20 using the internal library search algorithm for Agilent MassHunter (ver. 10.2). Calculated retention indices were compared with those published in NIST20.

FRAGRANCE TREATMENTS AND BAITS

Because 1-8 cineole was the most abundant compound identified in the floral fragrances of *V. planifolia*, we decided to add the chemical to the flowers to see if this enhanced the attractiveness of flowers to male *E. dilemma*. Ten µL of 1-8 cineole was carefully pipetted deeply into the tubular lips of all 6 flowers open early one morning, followed by a 4 h watch from 8 AM to 12 PM to see if the bees responded. Another treatment of 10 µL of eugenol, a known attractant for *E. dilemma* and a principal fragrance component of many of its orchid mutualists (Pemberton & Wheeler 2006), was added to all 4 open flowers another morning, followed by a 3 h timed watch from 8 AM to 11 AM to see if the bees would respond. Another day, after timed watches of the flowers and fragrance treatments of the flowers were completed, baits with these chemicals were created and exposed. Four 10 cm squares of blotter paper were saturated with 5 cm wide areas, 2 with 1-8 cineole and 2 with eugenol and hung 1.5 m above the ground in a wire fence near the studied vanilla plant and watched for 3 h from 9 AM to 12 PM for response by the bees.

POLLINATION AND FRUIT SET

At the end of the timed watches in Apr, only 1 flower on the vine appeared to have set fruit, exhibiting an elongate ovary about twice as long as the largest flower buds. At this time the vine had 11 flower buds with few if any opening each day. In Jun 2022, the fruit set was determined by counting the number of fruits forming on the plant and dividing this by the total number of flowers. The total flowers were the number of fruits plus the number of unpollinated flowers that was indicated by the distinctive scars on the inflorescences left by fallen unpollinated flowers.

Results

TIMED WATCHES

Timed watches were conducted for 7 consecutive days from Apr 20 to 26, during which the weather was favorable for both the flowers and the bees, warm to hot (with 25 to 29 °C highs) rainless and light wind. Each day, between 4 to 8 flowers opened and a total of 43 unmanipulated flowers were watched during 20 hrs of total observation time. During this period 2 bees visited 2 flowers. On the first bee visit on the first day of observations at 9:22 AM, a female worker honey bee landed on an inflorescence, walked around on the sides and back of flowers and then entered a flower, staying about 30 s. The honey bee then backed out of the flower with pollen on its scutellum. The honey bee then walked back into the flower and stayed for about 15 s, then backed out without the pollen. The honey bee was captured in a snap top vial when it backed out of the flower. Examination of the flower from the outside found that the anther cap had been dislodged. Dissection of the flower found the anther cap dislodged exposing pollen, the rostellum pushed forward and pollen deposited on the stigma. Figure 2 shows the column of this honey bee pollinated flower and the column of an unpollinated flower for comparison. A few other honey bees were observed in the area around the vanilla plant on most days, but none approached or entered the flowers.

The second bee visit was a female orchid bee entering a flower on day 3 at 9:16 AM. This bee arrived and briefly hovered around an inflorescence then flew directly into a flower. The orchid bee remained in the flower approximately 2–3 s and then backed out with pollen on her

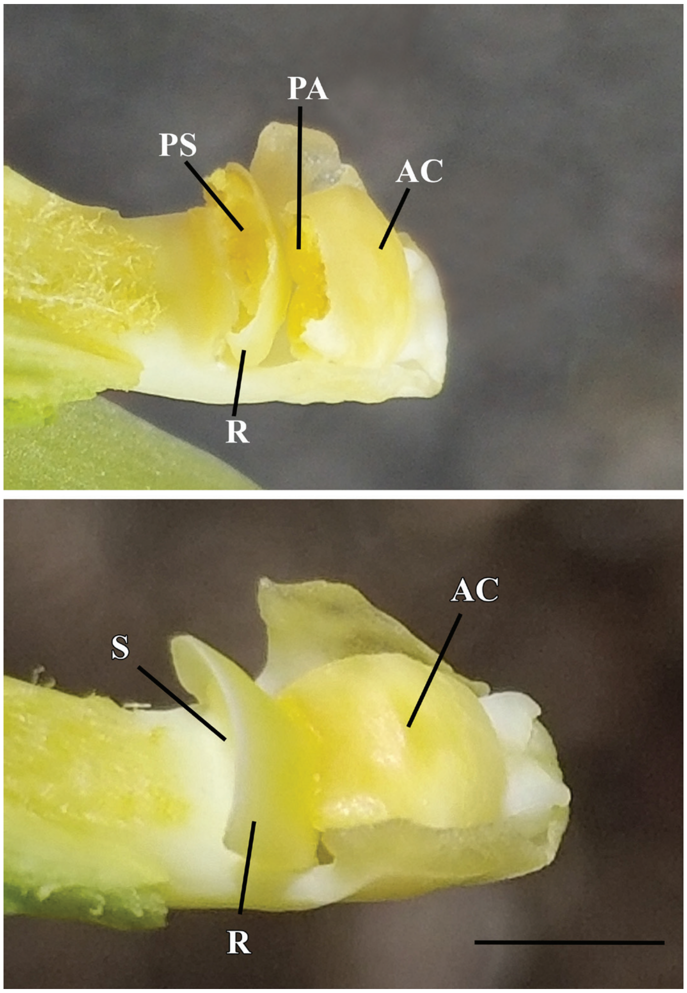

Fig. 2. Dissected columns of *Vanilla planifolia*. Top - flower visited by a female worker honey bee showing the anther cap (AC) disengaged revealing pollen within the anther (PA), the broken rostellum (R), and pollen on the stigma (PS). Bottom - flower unvisited showing for comparison the intact anther cap (AC), the unbroken rostellum (R) and stigma (S) without pollen. Scale bar 4 mm.

scutum, the dorsal surface of her thorax. The orchid bee was captured in a snap top vial as she backed out the flower. The anther cap of the visited flower was dislodged. The visited flower was left in place to allow for more potential visits. Three other orchid bees appeared briefly near the vine during the timed watches. The closest bee flew within approximately 8 cm of the entrance of the floral lip for 1 s before flying off. One of the orchid bees was a female but the others flew too rapidly and too briefly for their sex to be determined. No male orchid bees (when sex could be determined) visited the flowers or were observed near the plant during the timed watches of unmanipulated flowers. Anther cap examinations before and after the timed watches found that none had been displaced, indicating that no unobserved flower visits had occurred.

NECTAR DETERMINATION

No nectar was detected in the flowers either by visual inspection with the dissecting microscope, by micro pipette probing, or by color change in the moistened glucose test strips used to brush the interior of the flower.

MORPHOMETRIC MEASUREMENTS

The morphometric measurement of the interior of the floral tube of the labellum (Fig. 1) indicated that the distance between the penicillate callus on the floor of the tube beneath the column and the rostellum was 4 mm and 5 mm (n = 2). The distance between the penicillate callus and the anther cap was 4 mm and 4.5 mm (n = 2). The penicillate callus is a fleshy projection that is not rigid so some slight variation in its position occurs. The thickness of the thorax of the specimen of the female worker honey bee observed to pollinate a flower was 3.5 mm, and the vertical thickness of the thorax of the orchid bee that removed pollen from a flower was 4 mm. There was a good morphometric match between the vertical thickness of the bees and the interior distances between the callus and the rostellum and the callus and the anther cap, even without considering that the legs of the bees lift their bodies as they walk, which increases the functional thickness of the bees. The removal of pollen by both bees and the deposition of pollen by the honey bee confirms that both bees are of appropriate sizes to be pollinators of *V. planifolia*.

FRAGRANCE ANALYSIS

Flower fragrance analysis identified 9 volatile constituents from the *V. planifolia* flowers (Table 1). Nearly half of the percent total volatiles collected consisted of 1-8 cineole (48.9%) whereas nearly a third consisted of β-ocimene (31.8%).

Table 1. Volatile constituents from 3 *Vanilla planifolia* flowers from 1 plant (mean percentage ± SE). Constituents arranged by their order of elution from the GC. (E)-β-Ocimene was found in only 1 of the 3 samples (NP=not present).

Constituent	Mean %	SE
Benzaldehyde	4.7	1.6
β-Myrcene	0.8	0.0
1-8-Cineole	48.9	4.2
(E)-β-Ocimene	1.0	NP
β-Ocimene	31.8	4.6
Linalool	4.7	0.3
α-Terpineol	1.2	0.1
Caryophyllene	3.1	0.3
Z-Nerolidol	4.7	0.3

FRAGRANCE TREATMENTS AND BAITS

No male orchid bees or other bees investigated or visited the 6 flowers to which 1-8 cineole had been added during the 4 h timed watch. Five male *E. dilemma* bees came to the 4 flowers to which eugenol had been added during the 3 h timed watch. One male *E. dilemma* bee investigated multiple flowers and came within 2.5 cm of a flower but did not touch a flower. The other 4 bees touched multiple flowers and exhibited typical fragrance collection behavior in which they brushed the surfaces of the lips of the flowers with their front tibia, hovered to transfer collected oils into the storage tanks on their hind legs, then landed again to repeat the process. However, the 5 male bees spent 4, 3, 5, 8 and 16 min, collecting or attempting to collect from the eugenol-treated flowers. The added eugenol caused the distal tips of the lips of the flowers to wilt, and after 2 h to block the entrance, which prevented the bees from entering the flowers. During the fragrance baiting, no male orchid bees came to the 1-8 cineole baits, but 6 bees came to the eugenol baits and they either hovered near the baits or exhibited fragrance collecting behavior.

FRUIT SET

Fruit set was 1 fruit out of 160 flowers or 0.655%. If the honey bee pollinated flower had not been picked and dissected to document pollination, it probably would have developed into a fruit, which would have increased the fruit set to 1.25%.

Discussion

Vanilla planifolia, as a commercial plant and crop, is entirely dependent on hand pollination to produce fruits. In hand pollination, the flower is skillfully manipulated so that the rostellum, the flap of tissue between the anther and the stigma, is lifted away with a small stick or toothpick and then the anther is pushed onto the stigma (Hernandez-Hernandez 2019). The lack of fruit, or almost no fruit, without hand pollination indicates that the flowers are not apomictic or automatically self-pollinating to any extent. Fruit set following hand pollination of single flowers demonstrates that flowers are self-compatible but need a pollinator, human or other agent to produce fruit. For these reasons, no pollinator exclusion treatments or research to define the breeding system were necessary to demonstrate the need for pollinators.

Male *E. dilemma* bees did not visit the unmanipulated flowers of *V. planifolia* in this study. In addition, they did not visit the flowers to which 1-8 cineole, the flowers' principal fragrance component, was added, nor did they come to 1-8 cineole baits on the single day when this was done. Orchid flowers pollinated by male orchid bees usually have large amounts of terpenes in their fragrances that are readily detectable by human noses. We found that the fragrance of the studied *V. planifolia* flowers was barely detectable to our noses, so we thought that the amount of 1-8 cineole present in the flowers may have been suboptimal to attract male orchid bees and that adding more might stimulate the bees to visit the flowers. Male *E. dilemma* bees only came to the flowers when eugenol, a known attractant for male *E. dilemma* bees (Pemberton & Wheeler 2006), was added, and they also came to eugenol baits. The fact that male *E. dilemma* bees came to the flowers to which eugenol was added and to eugenol bait, demonstrates that the bees were present and could have visited the flowers of *V. planifolia* if they were attracted to them, which they were not.

Male orchid bee pollination of its orchid mutualists is a very apparent and even a dramatic affair, with male bees quickly visiting and even mobbing the flowers to collect the volatiles fragrance oils for use in their courtship (Zimmermann et al. 2006). Male bees come quickly to these flowers as soon as they begin to produce fragrance. A previous study (Pemberton RW, unpublished) exposed flowering plants of some of the orchid mutualists of *E. dilemma* in Broward county, Florida, where the present pollination study was done. The orchids were species of *Gongora*, *Lycaste*, and *Stanhopea*, and the bees exhibited this stereotypic male orchid bee behavior, quickly and intensively visited the flowers (Pemberton RW, unpublished data). If *V. planifolia* was one of this orchid bee's orchid mutualists, it would have been apparent by a rapid response and intensive visitation of the flowers by males, but this did not occur.

Although male *E. dilemma* bees were not attracted to *V. planifolia* in this study, male *Euglossa* species (Ackerman 1983), including *E. viridissima* (Soto-Arenas 1999, 2003), have been reported as apparent pollinators of *V. planifolia* in tropical America. No evidence was provided by Soto-Arenas (1999, 2003) to support his claim that *E. viridissima* pollinated *V. planifolia*. There are numerous similar sized green *Euglossa* species in most of tropical America (Roubik & Hanson 2004), which can make identifying active orchid bees difficult.

The segregation of *E. dilemma* from *E. viridissima* (Eltz et al. 2011) may explain this possible anomaly, the bees are different species, which relate differently to *Vanilla* orchids. A more likely pollinator of *V. planifolia* in tropical America is *E. tridentata*, an associate of the orchid in Panama (Roubik & Hanson 2004). The male bees of *E. tridentata* come to baits with 1-8 cineole, which we found to be one of the main compounds of the fragrance of this orchid, and the bees are 11 to 12 mm in body length (Roubik & Hanson 2004). The body length of *E. tridentata* is the same as that of *E. dilemma,* so like *E. dilemma* it is a good morphometrical match for *V. planifolia* flowers. *Euglossa tridentata* occurs from Mexico to Colombia (Roubik & Hanson 2004).

The flower visit and pollen removal by the female *E. dilemma* in this study is significant because pollination of vanilla has not been previously documented. Flower visitors that remove pollen from orchids are also their pollinators (Dressler 1981; Ackerman 1983), so by this criterion this bee is a pollinator of *V. planifolia*, a rare documentation of pollination of this important plant. However, because only 1 fruit was set on the studied plant, the orchid bee may not have pollinated the flower because it is not known what bee pollinated the flower that set fruit. If the single female observed to back out of a *V. planifolia* flower with pollen of the flower on her thorax had not been captured, she may have visited (and potentially pollinated) additional flowers. The low visitation observed is probably because the flowers have no nectar, and that male *E. dilemma* were not interested in the flowers. Pollination by this orchid bee would probably depend on naïve bees because the bees probably would not visit vanilla flowers after learning that they have no nectar. Although orchid bees are important pollinators of many plant species in nature (Dressler 1982), they have not been used as pollinators in agriculture (S. Ramirez, personal communication). Also, unlike some commercialized solitary bees, such as the alkali bee *Nomia melanderi* (Cockerell) (Mayer & Milicsky 1998), orchid bees do not nest in aggregations. *Euglossa dilemma*, previously thought to be a solitary bee, has been found to be semi social with nests comprised of usually 1 to 2 adult female bees and around 12 potential brood cells (Saleh & Ramirez 2019). Ramirez and Saleh have successfully used trap boxes in Florida to induce this bee to nest in them and have learned not only that they are semi social but that they can be exceptionally long-lived, with females living up to 9 mo. Orchid bees have not been reared in captivity, but Ramirez and Saleh are having some success (S. Ramirez, personal communication). Rearing this bee is quite complicated because females mate only with males displaying with a particular species-specific blend of collected aromatic compounds (Brandt et al. 2021). In addition, the small nest size means that the number of new naïve bees produced may be too few, even with multiple nests, to pollinate a screenhouse full of short-lived vanilla flowers.

The pollination of a single *V. planifolia* flower by a female worker honey bee is a novel documentation of vanilla pollination and is significant because it suggests the potential use of the honey bee as a pollinator of this potential crop in Florida. Although honey bees may have potential as vanilla pollinators, they have not been used in commercial crop pollination in glass or screen houses, but they have pollinated flowers when contained experimentally in large screen cages (K. Delaplane, personal communication). A hive placed in screenhouse with a vanilla crop might result in pollination of the flowers and fruit set because the flowers may appear to be rewarding to the bees even though they have no nectar. Individual honey bees visiting the flowers would quickly learn that the flowers are rewardless and stop visiting them. This means that the pollination of vanilla flowers would depend on naïve honey bees. With a colony, there would be continuous production of naïve bees that could visit and pollinate the flowers, either through delivering pollen from different flowers or repeat visits of the same flower as seen in this study. The number of bees in a hive varies considerably, ranging from 17,000 to 24,000 or more (Dyer & Seeley 1987), but increases during the spring, when vanilla plants flower in Florida. The number of new naïve bee foragers produced each day during the spring is estimated to be a thousand or more. Because new short-lived flowers open every morning and last only for a morning, naïve bees would be needed every morning. The number of bees needed would depend on the level of fruit production desired. Because the *V. planifolia* flowers offer no food, the bees would need to have access to rewarding flowers or other feedstocks. Even though honey bees are inferior pollinators of crops such as squashes, blueberries, and others compared with other bees (Delaplane & Mayer 2000), the large number of honey bees that can be employed make them highly valued pollinators in agriculture.

The potential use of honey bees as pollinators of vanilla would be a significant departure from what hand pollination achieves. In hand pollination only about half of the flowers in an inflorescence are usually pollinated because over pollination of the vines can stress them and eventually cause their failure (Exley 2019). In addition, only the flowers on the lower part of the inflorescence are hand pollinated, so that the developing fruit hang down to produce straight beans, an essential trait in the premium market (Exley 2019). Flowers lower on the inflorescence bloom first and then the bloom progresses upward. It may be possible to employ honey bees during the early to middle bloom period so that over pollination does not occur, and so that only flowers on the more proximal parts of the inflorescence are pollinated. Research to evaluate the potential for honey bee pollination should reveal its feasibility.

The level of season-long fruit set of 0.655% or 1.25%, if the honey bee pollinated flower is included, is similar to the 1% natural fruit set observed in the plant's native range in Mexico (Hernandez-Hernandez 2019). *Vanilla planifolia* is a member of the so-called *V. planifolia* group, which includes *V. dubia* Hoehne, *V. insignis* Ames as well as *V. planifolia* (Pridgeon et al. 2003). These orchids have greenish flowers, a penicillate callus on the interior floor of their tubular lips and appear to be pollinated by food deceit (Ackerman 1986; Pansarin & Pansarin 2014). Our study, which found a lack of floral nectar, the presence of fragrance, and low-level visitation by bees, supports the suggestion that *V. planifolia* is pollinated by food deceit mimicry. Our finding of 1-8 cineole as a principal fragrance component of the flowers suggests that pollination by male *E. tridendata*, reported to be associated with the orchid (Roubik & Hanson 2004), may occur in the orchid's native region. About one third of orchid species lack floral nectar (van der

Pijl & Dodson 1966; Ackerman 1986), including most *Vanilla* species (Pansarin 2021). These nectarless orchids are pollinated by deceit, so consequently have lower visitation and fruit set than nectar-producing rewarding orchids (Neiland & Wilcock 1998). Nectarless orchids are thought to have evolved from nectar producing ancestors and producing no nectar is thought to be adaptive because these orchids have higher outcrossing rates (Neiland & Wilcock 1998). This occurs because insect pollinators leave plants with nectarless flowers more quickly and transfer pollen more frequently to flowers of other plants rather than to flowers on the same plant. Although being nectarless may be adaptive to wild *V. planifolia,* it is not normally a positive trait in the flower of a crop plant, especially one without an easily gathered pollen reward. The potential use of large numbers of honey bees to pollinate the flowers of commercial vanilla could overcome this limitation. The possibility of using intensively managed honey bees as pollinators may reduce or eliminate the need for expensive hand pollination. This could make the production of vanilla more economically viable, especially in Florida, where labor costs are higher than in other areas of the world where vanilla is cultivated.

The present study had limitations. It proved challenging to locate flowering *V. planifolia* plants where the orchid bee is abundant. The plants require trees or structures to climb on, a significant amount of shade, and 3 yrs are needed for them to begin to flower after planting (Hernandez-Hernandez 2019). Flowering *V. planifolia* vines are uncommon in private or public gardens or other landscapes in southern Florida. Due to the incipient development of commercial cultivation of *V. planifolia* in southern Florida, plants were not available for purchase, nor were we able to borrow any flowering plants to expose to *E. dilemma*. We were able to examine the pollination of only 1 large vanilla vine where the naturalized orchid bee was abundant in an insecticide free environment. Because vanilla is clonal, there may be little difference in pollinator attraction to flowers on a single vanilla vine versus flowers on pieces of vine growing as separate plants. We observed only 1 female worker honey bee pollinator and 1 female *E. dilemma* as a probable pollinator. The primary goal of the study, however, was met because clearly *V. planifolia* is not an orchid mutualist of male *E. dilemma,* which means that these male bees will be of little use in the pollination of commercial vanilla in Florida. The occurrence of a honey bee pollinator and a probable female orchid bee pollinator are the first direct evidence of the pollination of this important plant anywhere. These novel observations allowed us to speculate about their potential utility as pollinators of commercial vanilla in Florida, but more research is needed to better understand these possibilities.

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