Universidad Autónoma de Querétaro

Facultad de Ciencias Naturales

Posgrado en Recursos Bióticos

"Abejorros nativos de México como polinizadores manejados"

T E S I S

Que como parte de los requisitos para obtener el grado de:

DOCTOR EN RECURSOS BIÓTICOS

P r e s e n t a:

M. en C. ALFONSO TORRES RUIZ

Director de Tesis: Dr. Robert Wallace Jones

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Presenta:
M. en C. Alfonso Torres Ruiz

Dirigido por:
Dr. Robert Wallace Jones

Dr. Robert Wallace Jones
Presidente — firma

Dra. Guadalupe Malda Barrera
Secretario — firma

Dr. Humberto Suzan Azpiri
Vocal — firma

Dr. Ricardo Ayala Barajas
Suplente — firma

Dr. Remy Vandame
Suplente — firma

Dra. Margarita Teresa de Jesús García Gasca
Director de la Facultad

Dr. Irineo Torres Pacheco
Director de Investigación y Posgrado

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DEDICATORIA

A quienes iluminan mi vida: Héctor, Mateo, Alfonso y Tamara

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INDICE

RESUMEN

El uso de polinizadores manejados –producidos masivamente y comercializados- es una práctica común en la agricultura actual. México cuenta con una alta diversidad de especies vegetales cultivadas, donde cerca del 85% de esos cultivos dependen de los polinizadores para una producción eficiente. En este trabajo se selecciona y evalúa la efectividad de *Bombus ephippiatus* Say (Hymenoptera: Apidae: Bombini), una especie de abejorro nativo de México, como polinizador manejado en cultivo de Tomate (*Solanum lycopersicum* Mill.), con el fin de determinar su factibilidad de uso como polinizador de tomate bajo invernadero. Se presenta una revisión de las especies de abejas comercialmente disponibles en México como polinizadores manejados, analizando en particular: 1) disponibilidad comercial, 2) cultivos donde se utilizan, 3) perspectivas de su uso sustentable y conservación. Así mismo, se evaluó de forma experimental el esfuerzo de forrajeo y eficiencia de polinización de dos especies de abejorros en un invernadero comercial de tomate. Las especies comparadas fueron: *Bombus impatiens* Cresson, nativo del noreste de Norteamérica, comercialmente disponible para polinización en México; y *Bombus ephippiatus*, nativo de gran parte de México y Centroamérica. A pesar de la importancia de los polinizadores manejados, sólo se cuenta con dos especies de polinizadores apoideos comercialmente disponibles en México: la abeja melífera *Apis mellifera* L. y el abejorro *B. impatiens.* Las especies *B. ephippiatus* y *B. impatiens* presentaron eficiencias de polinización similares. Las variables de calidad del fruto, como peso fresco, número de semillas, y diámetro máximo, no fueron diferentes de forma significativa. No se encontró mas actividad de *B. ephippiatus* durante los periodos de mayor temperatura en el invernadero. Ambas especies de abejorros lograron porcentajes de polinización cercanos al 100% durante el periodo de evaluación. Este estudio establece que *B. ephippiatus* es un polinizador eficiente de tomates bajo invernadero, comparable a la eficiencia de *B. impatiens*, por lo que se puede considerar con potencial para su uso como polinizador manejado. Sin embargo, métodos de crianza masiva de dicha especie no están aún disponibles y son necesarios, para asegurar la calidad y sanidad de los nidos y disminuir la posible presión para la colecta no regulada de esta especie.

(Palabras Clave: Apidae, Abejorros, polinización, polinizadores manejados)

ABSTRACT

The use of managed pollinators –both, marketed and mass produced- is a common practice in today's agriculture. Mexico has a high diversity of cultivated plants, 85% of these crops depend on pollinators for efficient production. The selection and effectiveness of one Mexican bumblebee as a managed pollinator in tomato (*Solanum lycospersicum* Mill), is evaluated on this work. A review of commercially available bee species in Mexico as managed pollinators was done, analyzing in particular: 1) commercial availability, 2) where crops are used, 3) prospects for sustainable use and conservation. Also, the foraging effort and efficiency of pollination of two bumble bees species were experimentally evaluated in a commercial greenhouse tomato. The compared species were: *Bombus impatiens* Cresson, native from northeastern North America, commercially available for pollination in Mexico and *Bombus ephippiatus* Say, native from Mexico to Central America. Despite the importance of managed pollinators, there are only two Apoideae pollinator's species commercially available in Mexico: the honeybee *Apis mellifera* L. and the bumblebee *B. impatiens*. Both species presented similar pollination efficiency. Fruit quality variables such as fresh weight, number of seeds, and maximum diameter were not significantly different. There was no increased activity of *B. ephippiatus* during periods of elevated temperatures in the greenhouse. Both species reached nearly 100% pollination rates through the evaluation period. This study establishes that *B. ephippiatus* is an efficient pollinator for greenhouse tomatoes, comparable to the efficiency of *B. impatiens*; thus *B. ephippiatus* is considered with potential for use as managed pollinator. However, methods of mass rearing of this species are not available yet and still needed to ensure nest quality and health, as well as to reduce the potential of unregulated collection of this species.

(Key works: Apidae, Bumblebees, Pollination, Managed Pollinators)

INTRODUCCIÓN GENERAL

La polinización es una interacción mutualista fundamental para el mantenimiento de los ecosistemas naturales y los agroecosistemas (Nabhan and Buchmann, 1997). De hecho, esta interacción es uno de los más importantes servicios ecosistémicos a la agricultura a nivel mundial. Se ha señalado que la reproducción sexual de más del 80% de las plantas terrestres vasculares, incluyendo la mayoría de las plantas cultivadas, es llevada a cabo por vectores animales (Nabhan and Buchmann, 1997). De acuerdo a Prescott-Allen y Prescott-Allen (1990), el 90% del insumo *per capita* en el mundo proviene de únicamente 100 especies de plantas cultivadas; de dichos cultivos el 72% son polinizados por abejas. Lo anterior da cuenta de la gran importancia ecológica y económica de los apoideos como polinizadores. Por ejemplo, en los Estados Unidos se ha estimado el valor de la polinización para las abejas melíferas en 9.7 billones de dólares por año (Losey y Vaughan, 2006) y para otro tipo de polinizadores de 3 a 6.7 billones de dólares al año (Nabhan y Buchmann, 1997).

La introducción de polinizadores manejados para aumentar la tasa de visita a las flores y por lo tanto mejorar la producción de frutos es una práctica común en muchos cultivos. El polinizador más comúnmente manejado en diversos cultivos es la abeja melífera (*Apis mellifera* Linnaeus). Lo anterior es debido a que forman colonias muy grandes las cuales pueden polinizar amplias áreas, aunado al desarrollo de las técnicas de su manejo, disponibilidad comercial, y finalmente a la producción de miel (Gouldson, 2003). Sin embargo, la eficiencia de polinización de la abeja melífera disminuye drásticamente en cultivos en cuyas flores requieren polinización por vibración como el arándano y el tomate (Stubbs y Drummond, 2001; Javorek *et al*., 2002; Torres *et al.*, 2007). Aunado a esto, el gran tamaño de las colonias de la abeja melífera, la necesidad de especialización técnica para su manejo y la susceptibilidad de africanización que aumenta la posibilidad de ataques masivos como respuesta a un disturbio, limita su utilización, sobre todo en ambientes restringidos como los invernaderos. Como una alternativa a las abejas melíferas, se han utilizado como polinizadores manejados en cultivos protegidos, diferentes especies de abejas tanto solitarias y sociales, como las abejas sin aguijón y los abejorros agrupados todos en la superfamilia Apoidea (O'Toole y Raw, 1991). Las abejas apoideos (Anthophila), dependen casi exclusivamente de recursos florales (polen y néctar) para la obtención de nutrientes y energía, lo que las hace mantener una alta tasa de visitas (comparado con mariposas, por ejemplo) para proveer el alimento a las larvas en desarrollo, características que los vuelve excelentes polinizadores de las flores de plantas silvestres y de cultivos (O'Toole y Raw, 1991; Gouldson, 2003).

Los abejorros (Apidae: *Bombus*) son capaces de polinizar por vibración (Buchmann, 1985). Este tipo de polinización es requerida para la fertilización de plantas de la familia Solanaceae, como el tomate (*Solanum lycopersicum* L.), y Ericaceae, como los Arándanos (*Vaccinium* spp.) y el Kiwi (*Actinidia deliciosa* Chev.) (Cane y Payne, 1988; Gouldson, 2003). Actualmente, se ha documentado ampliamente el efecto del uso de abejorros como polinizadores de tomate (Morandin *et al*., 2001b; Pressman *et al*., 1999). El efecto positivo en la producción de frutos y semillas, se traduce para el agricultor en frutos de mayor calidad y mejores cosechas. Un efecto colateral de la introducción de abejorros en invernaderos, ha sido la substitución de pesticidas que los pueden dañar, por el control biológico (Kearns y Thomson, 2001). Los efectos directos e indirectos, aunados a su menor costo con respecto a métodos mecánicos, la facilidad de monitoreo de su actividad de polinización en cultivo de tomate, y el desarrollo de alternativas de solución al control de plagas y enfermedades compatibles con los abejorros han contribuido al rápido incremento en su uso como polinizadores manejados de tomates bajo invernadero en el mundo (Morandin *et al*., 2001a; Morandin *et al*., 2001b; Kearns y Thomson, 2001).

La utilización comercial de abejorros como polinizadores manejados inició en la década de los ochenta en Bélgica y Holanda, en cultivos de tomate bajo invernadero, utilizando la especie europea, *Bombus terrestris* L. (Velthuis, 2002). Esta práctica revolucionó la polinización comercial, pues a solo cinco años de estar disponibles por varias compañías, ya el 100% de los invernaderos en Holanda los utilizaba. El uso de abejorros rápidamente se convirtió en algo habitual en los cultivos de jitomate en Europa. En Norteamérica, fue hasta principios de 1990s que se desarrolló la producción comercial de una especie de abejorro nativa del Oeste de Estados Unidos y Canadá, *Bombus impatiens* Cresson y posteriormente del abejorro del éste *Bombus occidentalis* Greene (Velthuis y Van Doorn, 2006).

Actualmente solo seis especies de abejorros son criadas comercialmente en el mundo, siendo las más utilizadas; *Bombus terrestris* para Europa, Asia, Nueva Zelanda y Sudamérica y *B. impatiens* para Norte América (Velthuis y Van Doorn, 2006). Se han utilizado estas especies de abejorros como polinizadores en países donde no son nativos (Gouldson, 2003 y Velthuis y Van Doorn, 2006). Sin embargo, diversos trabajos han señalado el riesgo de la introducción de abejorros no nativos (Freitas *et al*., 2009; Morales, 2007; Gouldson, 2003; Gouldson, 2010). Los posibles riesgos se pueden resumir en cuatro potencialidades: establecimiento, competencia, transmisión de enfermedades e hibridación. Sin embargo, la gran mayoría de la evidencia científica sobre este tema se ha generado en estudio de una sola especie: *B. terrestris,* lo que puntualiza la necesidad de mayores estudios en otras especies de abejorros.

En México, el abejorro *Bombus impatiens* es utilizado desde 1994 para polinización principalmente de tomate bajo invernadero. El rango natural de distribución de esta especie es el este de los Estados Unidos y Canadá (Velthuis y Van Doorn, 2006; Williams, 2009). La superficie actual de cultivos bajo cubierta en el País es de aproximadamente 12,000 ha (INEGI, 2007) de las cuales 8569 ha corresponden a tomate (AMHPAC, 2009). El crecimiento de la superficie de invernaderos dedicados al cultivo de Jitomate ha sido constante durante los últimos 10 años y se espera que esta tendencia continúe en el mediano y largo plazo. De tal forma, los abejorros constituyen un insumo altamente valorado y que provee de ventajas competitivas a los agricultores nacionales, por lo que resulta importante el estudio de estos polinizadores para asegurar la sustentabilidad de los agro-ecosistemas y la industria nacional de producción de tomate, sin riesgo para la conservación de los sistemas naturales.

En este trabajo se selecciona y evalúa la efectividad como polinizador manejado en el cultivo de Tomate (*S. lycopersicum*) a una especie de abejorro nativo de México, con el fin de determinar su factibilidad de uso como polinizador de tomate dentro de invernaderos, desde el punto de vista de su aplicación en campo, sin abarcar el estudio de métodos de crianza comercial.

La tesis se estructura en tres capítulos planteados para resolver los objetivos particulares de la presente investigación. Los capítulos fueron preparados como manuscritos independientes, por lo que en cada uno de estos se incluye la metodología, resultados, conclusiones y bibliografía particulares. Los capítulos son los siguientes:

Capítulo I. Present and Potential use of Bees as Managed Pollinators in Mexico.
En este capítulo se revisa las especies de abejas comercialmente disponibles en México como polinizadores manejados, analizando en particular: 1) disponibilidad comercial, 2) cultivos donde se utilizan, 3) perspectivas de su uso sustentable y conservación. Este escrito fue publicado por la revista *Southwestern Entomologist* en Marzo del 2013.

Capítulo II. Abejorros del Estado de Querétaro.

En este capítulo, se reportan nuevos registros de especies de abejorros, en particular se identifica el área del Cerro del Zamorano, en el Estado de Querétaro, como de alta importancia para la conservación de este grupo. Este manuscrito está aceptado para su publicación en el libro “Historia Natural de Querétaro” Editado por Robert Wallace Jones. Facultad de Ciencias Naturales, Licenciatura en Biología (UAQ). Septiembre 2012.

Capítulo III. Comparison of the efficiency of the bumblebees *Bombus impatiens* and *Bombus ephippiatus* (Hymenoptera: Apidae) as pollinators of tomato in greenhouses.

Finalmente, en el tercer capítulo se evaluó el esfuerzo de forrajeo y eficiencia de la polinización en un invernadero comercial de tomate *Solanum lycopersicum* L. (Solanaceae), de dos especies de abejorros: *Bombus impatiens* Cresson, nativo del noreste de Norteamérica y comercialmente disponible para polinización en México; y *Bombus ephippiatus* Say, nativo de México y Centroamérica. Este manuscrito fue publicado en la revista *Journal of Economic Entomology* en Diciembre del 2012.

OBJETIVOS

OBJETIVO GENERAL

- Evaluar la factibilidad de utilización de abejorros nativos de México como polinizadores manejados en cultivo de jitomate (*S. licopersicum*) bajo invernadero.

OBJETIVOS PARTICULARES

- Elaborar una revisión sobre la utilización de polinizadores manejados en cultivos protegidos en México. (Capítulo I)
- Colecta de abejorros en campo para establecer las colonias experimentales utilizadas en otros objetivos de la presente investigación. (Capitulo II)
- Evaluar la eficiencia de polinización del cultivo de tomate en invernadero de una especie de abejorro nativa en comparación con la especie *B. impatiens*, comercialmente disponible. (Capitulo III)

CAPITULO I

PRESENT AND POTENTIAL USE OF BEES AS MANAGED POLLINATORS IN MEXICO[1]

Alfonso Torres-Ruiz, [2,5], Robert Wallace Jones[3], and Ricardo Ayala Barajas[4]

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[1]Hymenoptera : Apidae

[2] Departamento de Investigación y Desarrollo (I+D), Koppert México SA de CV, Calle Circuito El Marqués Norte No. 82. Parque Ind. El Marqués, C.P. 76246, Querétaro, México.

[3] Universidad Autónoma de Querétaro, Licenciatura en Biología, Facultad de Ciencias Naturales, Laboratorio de Ecología y Diversidad Faunística. Avenida de las Ciencias s/n, Juriquilla, C.P. 76230 Querétaro, Querétaro, México.

[4] Estación de Biología Chamela, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). Apartado Postal 21, C.P. 48980, San Patricio, Jalisco, México.

[5] Corresponding author: atorres@koppert.com.mx

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Present and Potential Use of Bees as Managed Pollinators in Mexico[1]

Alfonso Torres-Ruiz[2,5], Robert Wallace Jones[3], and Ricardo Ayala Barajas[4]

Abstract. A diversity of crops is grown in Mexico. Of the plants that produce fruits and/or seeds for human consumption, 85% depend to some degree on pollinators for efficient production. Mexico also has a great diversity of native bees, some of which have been recognized as having potential for use as managed pollinators; defined as a semi-domesticated species, produced in large quantities, and bought and sold commercially. The objective of the present work was to review the species of bees presently commercially available in Mexico as managed pollinators and to analyze in particular: 1) their commercial availability, 2) crops for which they are used, and 3) the perspectives for their sustainable use and conservation. Presently, only two species of bees are used commercially in Mexico: the honey bee, *Apis mellifera* L., and the common eastern bumble bee, *Bombus impatiens* Cresson. Mexican native bee species have been used experimentally, but at this time, none is commercially available. The principal threats to the conservation of native bees in Mexico are the elevated rate of deforestation combined with intense use of agrochemicals and particularly insecticides for control of pests in extensive monocultures. Continued research and conservation programs considering native bee species are needed to ensure the survival of as many species as possible to provide a wide array of biological characteristics with potential for use as managed pollinators in Mexico. This is critical to ensure the future necessities of the wide diversity of Mexican crops with unique agronomic characteristics within the diversity of habitats where they are cultivated.

Resumen. México es un país con alta diversidad de especies vegetales cultivadas. El 85% de las especies de plantas que producen frutas y/o semillas para consumo humano, dependen en algún grado de los polinizadores para una producción eficiente. Alternativamente, México tiene gran diversidad de abejas nativas y algunas de ellas se han señalado con potencial para ser usadas como polinizadores manejados. El objetivo de este trabajo es revisar las especies de abejas que están comercialmente disponibles en México como polinizadores manejados, analizando en particular: 1) disponibilidad comercial, 2) cultivos donde se utilizan, y 3) las perspectivas de su uso sustentable y conservación. Actualmente, sólo se cuenta con dos especies de abejas polinizadoras comercialmente

[1]Hymenoptera: Apidae

[2]Departamento de Investigación y Desarrollo (I+D), Koppert México SA de CV, Calle Circuito El Marqués Norte No. 82. Parque Ind. El Marqués, C.P. 76246, Querétaro, México.
[3]Universidad Autónoma de Querétaro, Licenciatura en Biología, Facultad de Ciencias Naturales, Laboratorio de Ecología y Diversidad Faunística. Avenida de las Ciencias s/n, Juriquilla, C.P. 76230 Querétaro, Querétaro, México.
[4]Estación de Biología Chamela, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). Apartado Postal 21, C.P. 48980, San Patricio, Jalisco, México.
[5]Corresponding author: atorres@koppert.com.mx

disponibles en México: la abeja melífera *Apis mellifera* L. y el abejorro *Bombus impatiens* Cresson. Otras especies de abejas nativas han sido utilizadas experimentalmente, pero sin que haya disponibilidad comercial hasta el momento. La elevada tasa de deforestación, aunado al uso intensivo de agroquímicos y pesticidas para el control de plagas en monocultivos extensivos, son las principales amenazas para la conservación de las abejas polinizadoras y silvestres. Es necesario que se continúe realizando investigaciones que permitan tener mayor número de especies de abejas que puedan ser usadas como polinizadores manejados en México, con el fin de cubrir las necesidades futuras de la gran diversidad de los cultivos mexicanos, con sus características agronómicas únicas.

Introduction

Pollination is a critical ecosystem service in agriculture. Many food crops in the world depend to some degree on visits by animal pollinators for production of seeds or fruit (Nabhan and Buchmann 1997). It has been estimated that 75% of human food crops require pollination by insects for adequate production (Klein et al. 2007). Of these pollinators, bees (Hymenoptera: Apoidea) are the most important and most used in managed pollination programs. In recent decades, a decline in natural populations of pollinators, principally Apoidea, has been documented throughout the world (National Research Council 2007, Potts et al. 2010, UNEP 2010). The causes of the decline are complex and multifaceted, but include habitat alteration and loss due, in part, to the expansion of areas dedicated to agriculture combined with the use of agrochemicals leading to reduction in diversity and abundance of native pollinators (Kremen et al. 2002, Freitas et al. 2009).

A further complication in crop pollination is the increase in cultivars grown in protected environments, such as greenhouses and shade houses, which restrict the access of pollinators to crops. Protected agriculture either reduces the role of insect pollinators in these crops or requires the use of managed pollinators to achieve acceptable amounts of production. According to the National Research Council (2007), managed pollinators are those that are semi-domesticated, produced in large quantities, and bought and sold commercially. The managed pollinator of greatest importance globally and used in the widest diversity of crops is the honey bee, *Apis mellifera* L. (Delaplane and Mayer 2000). In various countries, such as the United States and Canada, several other bee species, including both native and introduced, are used as managed pollinators in open fields and greenhouses. Commercially available species in the United States and Canada include: *Megachile rotundata* F., *Osmia lignaria* Say, *Osmia cornifrons* Radoszkowski, *Nomia melanderi* Cockerell, and *Bombus impatiens* Cresson (National Research Council 2007). Other managed pollinators of the Americas include several species of carpenter bees, *Xylocopa frontalis* Olivier, *Xylocopa suspecta* Moure and Camargo, and *Xylocopa grisescens* Lepeletier, used to pollinate distinct species of passion flowers in Brazil (Freitas and Filho 2001).

Mexico has the need and potential to develop management programs for underexploited pollinators considering the wide diversity of both pollinators and species of cultivated plants in the country. More than half of the cultivated plants of Mexico are cultivars with fruits or seeds consumed by humans, of which 85% depend to some degree on insect pollinators for efficient production (Ashworth et al. 2009). Mexico is a principal world supplier of fresh fruits and vegetables. For example, México is the major exporter of tomatoes, *Solanum lycopersicum* L., for the world, with slightly more than a million tons of exports annually (FAO 2007). The country is also the principal exporter of avocadoes, *Persea americana* Mill, with annual exports exceeding 300,000 tons, and ranks third in exportation of green peppers, *Capsicum annuum* L. For these crops, visits by pollinators have a positive effect on production (Meisels and Chiason 1997, Morandin et al. 2001b, Gazit and Ish 2007), emphasizing the need for conservation of native pollinators, as well as the maintenance and development of new managed pollinator systems for the production of these and other fruits and vegetables in Mexico.

The current rate of loss of biodiversity in Mexico due to habitat destruction and degradation combined with accelerated climate change will almost certainly result in a decrease in pollination services by native bees and necessitates an increase in use of managed pollinators to ensure agricultural production. The objective of the present work was to review the species of bees presently commercially available in Mexico and analyze specifically: 1) their commercial availability, 2) crops where they are used as pollinators, and 3) perspectives on their sustainable use and conservation.

Availability of Managed Pollinators in Mexico

The honey bee is the most common managed pollinator in Mexico and the most valued for honey production, wax, and pollination services (Fig. 1B). *Apis mellifera* was probably introduced into Mexico from Cuba, through the states of Veracruz and Tabasco, in the late 1760's or early 1770's (Labougle and Zozaya 1986). Honey bees are found throughout Mexico. Approximately 1.8 million commercial honey bee hives were in Mexico in 2010 (SIAP 2010).

The technology of apiculture in Mexico is heterogeneous (Labougle 1990). Apiculture enterprises range from small-scale operations (10-60 hives) using traditional methods, to large-scale operations implementing advanced technology (Cajero-Avelar 1999). The practice of moving hives from one location to another for crop pollination is only reported for large-scale operations and from the northern states and state of Veracruz. An average of 131,000 hives was annually used for this purpose from 2000 to 2007, with a total of 135,586 hives in 2008 (SAGARPA 2010).

Before the introduction of honey bees, other bee species had been domesticated for many centuries in Mexico. The Maya of Mexico and Central America managed various bee species for hundreds of years, and many of the practices are used by the present-day Mayan people (Quezada-Euàn et al. 2001). The most important of the native domesticated species are stingless bees, managed principally for the harvest of honey (Slaa et al. 2006), cerumen ("cera de Campeche"), propolis, and pollen. Stingless bees have been reported to be effective pollinators of 16 crops (Slaa et al. 2006, Quezada-Euàn 2009), of which coffee, *Coffea arabica* L., may be most benefited. However, at present there are no species of stingless bees available on a commercial scale for use as managed pollinators in Mexico.

The common eastern bumble bee, *Bombus impatiens* Cresson (Fig. 1a), has been used since 1994 in México, principally for pollination of tomatoes in greenhouses (Winter et al. 2006). The natural distribution of this species is the eastern US and southeastern Canada (Velthuis and Van Doorn 2006, Williams 2009). This species is used as a managed pollinator in protected and open field crops in the US and Canada, within the natural distribution of the bumble bee in

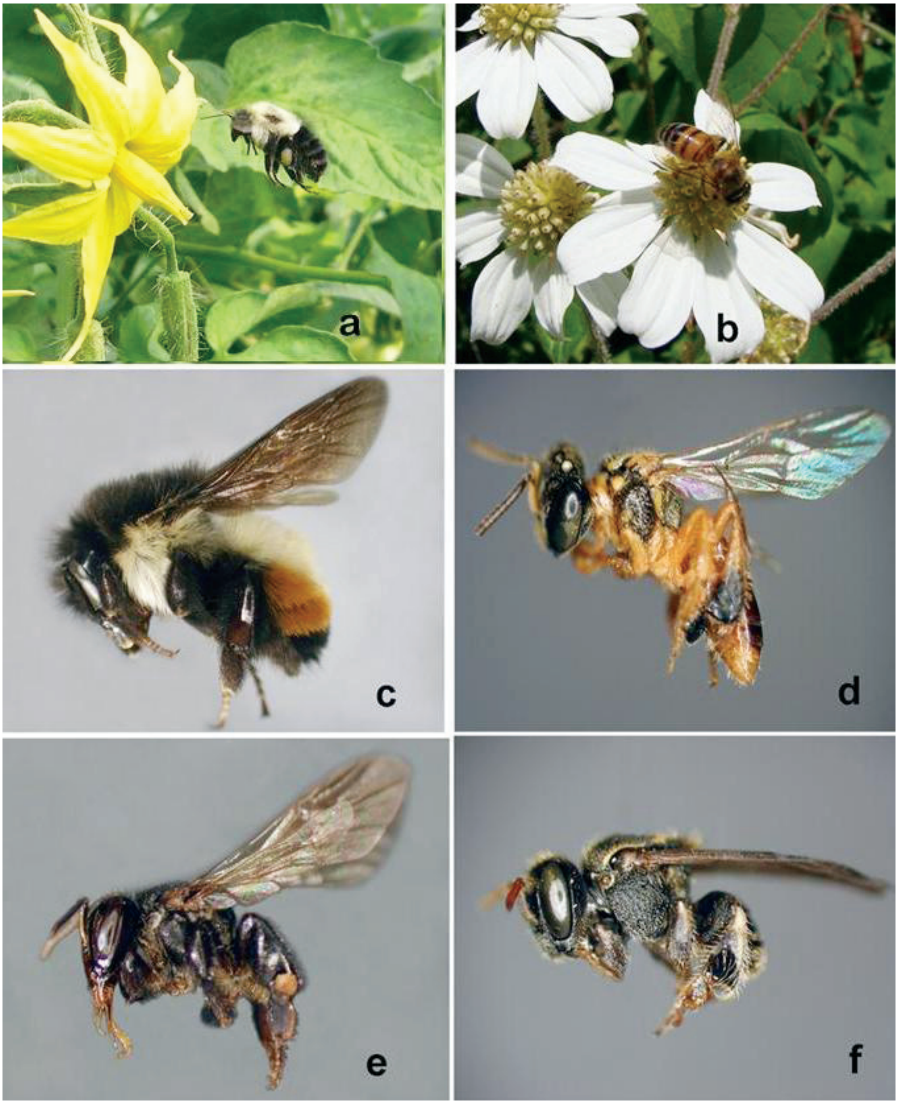

Fig.1. Bee fauna used as commercial and experimental pollinators in Mexico. a) *Bombus impatiens* Cresson b) *Apis mellifera* L., c) *Bombus ephippiatus* Say, d) *Tetragonisca agustula* Latreille, e) *Scaptotrigona mexicana* Guérin-Méneville, f) *Nannotrigona perilampoides* Cresson.

both countries. It is also used outside of its distribution in the western US but only within protected agriculture and with some restrictions (Winter et al. 2006). In Mexico, the Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA), through the National Center of Health, Safety and Agricultural Food Quality (SENASICA), regulates the importation of agricultural food products and is in charge of regulating the importation of species of bumble bees (*Bombus*) (SAGARPA 2010). Presently, two companies in Mexico produce and commercially market the common eastern bumble bee for use as a managed pollinator and have production plants with a complete production cycle: Koppert México in the state of Querétaro and Biobest in the state of Jalisco (Díaz 2011, Torres-Ruiz et al. 2011).

A native bumble bee, *Bombus ephippiatus* Say (Fig. 1c), has a widespread distribution throughout Mexico, with the exception of Baja California and the Yucatan Peninsula, and is found south through Central America to Panama (Labougle 1990, Ascher and Pickering 2012). This species is found in pine and oak forests above 1,500 m. *Bombus ephippiatus* has been considered a potential managed pollinator in previous studies (Fuentes and Madrid 2004, Hines et al. 2006) and used experimentally as a managed pollinator in tomatoes in greenhouses in the states of San Luis Potosí, Coahuila (Cuadrillero and Salinas-Navarrete 2006), Querétaro (Torres-Ruiz and Jones 2012), Jalisco (Cuadrillero 2011), and Chihuahua (Romo and Acosta 2010). Currently however, this species is not commercially available because it has not been successfully reared for mass production in a complete production cycle.

In conclusion, only two commercially produced species of Apoidea are available for use as managed pollinators in México: *Apis mellifera* and *Bombus impatiens.* This is few in comparison with the six species commercially available in the US and Canada (Table 1).

Table 1. Bee Species Commercially Available as Managed Pollinators in North America (National Research Council 2007)

Bee species	Countries of North America where commercially available		
Apis mellifera	Canada	United States	Mexico
Bombus impatiens	Canada	United States	Mexico
Nomia melanderi	Canada	United States	
Osmia cornifrons	Canada	United States	
Osmia lignaria	Canada	United States	

Crops for which Managed Bee Pollinators are Used

Honey Bees. The practice of renting honey bees for pollination is not as prevalent in Mexico as in other countries. A total of 135,000 hives was rented in Mexico for pollination in 2008 which represented only 7.5% of the honey bee hives present that year (SAGARPA 2010). Estimates from SAGARPA (2010) indicated that 70% of the rental of hives for pollination occurred within the northern states, of which the states of Sinaloa, Sonora, and Chihuahua account for 26, 18, and 15% of hive rentals, respectively. In contrast, the western zone annually rents approximately 22% of the hives. Hives are rented for a diverse number of crops in Mexico, principally for those destined for fruits and vegetables for exportation.

These crops include eggplant (*Solanum melongena* L.), squash (*Cucurbita* spp. L.), safflower (*Chartamus tinctorius* L.), strawberries (*Fragaria* spp. L.), apple (*Malus* spp. L.), melon (*Cucumis melo* L.), cucumbers (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* [Thunb.] Matsum and Nakai), and tomatoes (*Solanum lycopersicum* Mill), primarily in open fields (Table 2).

Although the honey bee is a generalist pollinator and used in diverse crops, it is clearly not the most effective pollinator for all crops or the most practical. The honey bee is especially deficient as a pollinator for crops such as cranberries (*Vaccinium* sp.) and tomatoes that require pollination by vibration or "buzz pollination" (Stubbs and Drummond 2001, Javorek et al. 2002, Torres et al. 2007), because this kind of pollination is not characteristic of honey bees (Buchmann 1985). Another problem is the large size of honey bee hives that require special techniques and experience to manage. Africanization of honey bees also augments the possibility of massive attacks on workers within the vicinity of hives (Guzmán-Novoa et al. 2011). These problems limit the use of honey bees in restricted spaces such as greenhouses. For these reasons, the use of honey bees in greenhouses in Mexico is limited and only reported from greenhouses for pollination of cucurbit crops and strawberry in Baja California (Roberto Altamirano, Koppert México, personal communication).

Apple orchards of Mexico often require anti-hail nets because of frequent hail storms in the mountainous regions where apples are grown. In large orchards (>5 hectares) covered with anti-hail nets, honey bees often fail to pollinate the central portion of the orchard which results in reduced production (A. Torres and V. Gómez, unpublished data). In addition, honey bees are not active at temperatures colder than 15°C, which becomes a limiting factor where the flowering period is very short and environmental conditions are not optimal. Although the climate in regions where apples are grown in Mexico is considered to be temperate, with freezing temperatures in winter months, the annual oscillation in temperature is often less than 10°C which contrasts to greater oscillations and warmer spring and summer temperatures in more northern regions (Hernández-Cerda and Carrasco-Anaya 2004). These relatively constant but cool temperatures can limit pollination and production in Mexican orchards of apples and peaches (Torres, unpublished data) and in blueberries (Torres et al. 2007).

Bumble Bees. All commercial bumble bee colonies presently in use in Mexico are *B. impatiens.* This *Bombus* species is primarily utilized for pollination of protected crops (greenhouses, shade houses, and macro tunnels). The estimated area of protected crop in Mexico is 12,000 hectares (INEGI 2007). Approximately 40% of this area is devoted to tomatoes, of which most are destined for exportation (SAGARPA 2010). Of the crops pollinated by *B. impatiens*, 90% are tomatoes. The remaining crops include other Solanaceae such as bell and chili peppers, as well as various cucurbits such as cucumbers and squash in greenhouses. Strawberries and raspberries (*Rubus* sp.) in shade tunnels are also pollinated by *B. impatiens*. It is estimated that 61% of the commercial colonies of bumble bees in Mexico are used in the northern states, of which three states are most important: Sinaloa (21% of the total colonies), Baja California (16%), and Sonora (9%) (Koppert México 2011, unpublished data). In contrast to *A. mellifera*, bumble bee colonies are not perennial, and no maintenance is required by the producer, which facilitates its implementation in the crop (Castañeda 2011).

Other Bee Species Used Experimentally as Managed Pollinators. Three Mexican species of stingless bees (meliponines) have been evaluated to date as

Table 2. Bee Species Used as Managed Commercially Available or Experimentally Managed Pollinators in Mexico

Bee species	Crop pollinated: Common name	Crop pollinated: Species name	Reference
Commercially available bee pollinators:			
Apis mellifera	Tomato	*Solanum lycopersicum*	
	Bell pepper	*Capsicum annuum*	
	Habanero pepper	*Capsicum chinense*	
	Squash	*Cucurbita* spp.	
	Strawberry	*Fragaria* spp.	
	Watermelon	*Citrullus lanatus*	
	Melon	*Cucumis melo*	Reyes and Cano (2006)
	Avocado	*Persea americana* Mill.	SAGARPA (2010)
	Apple	*Malus sylvestris*	
	Cucumber	*Cucumis sativus*	
	Safflower	*Chartamus tinctorius*	
	Mango	*Mangifera indica* L.	
	Citrus	*Citrus* spp. L.	
Bombus impatiens	Tomato	*Solanum lycopersicum*	Torres-Ruiz and Jones (2012)
	Bell pepper	*Capsicum annun*	Garcia and Gastelum (2012)
	Habanero pepper	*Capsicum chinense*	Palma et al. (2008a)
	Squash	*Cucurbita* spp.	Rogelio Castañeda Godoy personal communication
	Strawberry	*Fragaria* spp.	Rogelio Castañeda Godoy personal communication
Bee pollinators tested experimentally:			
Scaptotrigona mexicana	Rambutan	*Nephelium lappaceum*	Guzmán-Díaz et al. (2005), Esponda-Muñóz et al. (2005)
Tetragonisca angustula	Rambutan	*Nephelium lappaceum*	Slaa et al. (2006)
Nannotrigona perilampoides	Tomato	*Solanum lycopersicum*	Palma et al. (2008b), Cauich et al. (2004)
	Habanero pepper	*Capsicum chinense* Jacq.	Palma et al. (2008a), Cauich et al. (2006)
Bombus ephippiatus	Tomato	*Solanum lycopersicum*	Torres-Ruiz and Jones (2012), Romo Chacón and Acosta Muñiz (2010), Vergara and Fonseca-Buendia (2012)

managed pollinators: *Scaptotrigona mexicana* (Fig. 1e) (Arzaluz Gutiérrez et al. 2002, Esponda-Muñoz et al. 2005, Guzmán-Díaz et al. 2005), *Tetragonisca angustula* (Fig. 1d) (Slaa et al. 2006), and *Nannotrigona perilampoides* (Fig. 1f) (Cauich et al. 2004, 2006; Palma et al. 2008a,b; Quezada-Euán 2009). In Mexico, *S. mexicana* has been successfully used as a managed pollinator of rambutan, *Nephelium lappaceum* L. (Esponda-Munoz et al. 2005, Guzmán et al. 2005), and in small family-owned orchards in Chiapas (Remy Van Dame, personal communication). This species is also managed using traditional methods in coffee-growing and has been reported to significantly increase fruit production (Slaa et al. 2006).
The stingless bee *Nannotrigona perilampoides* has notable potential for commercial production in Mexico for several reasons. First, it is the stingless bee with the greatest distribution in Mexico. It is present the length of both coasts, from the Yucatán Peninsula to San Luis Potosí to the east and from Chiapas north to Sinaloa in the west, with populations found inland in the Rio Balsas depression (Ayala 1999). This distribution suggests that the species is most apt as a managed pollinator in tropical climates (Cauchi et al. 2004, 2006; Quezada-Euán 2009). Second, the species is relatively resistant to the warm temperatures common in greenhouses of Mexico (Cauich et al. 2004). Third, the species has been confirmed to be an effective pollinator of several crops. In habanero peppers (*Capsicum chinense* Jacq.) and tomato in greenhouses in the Yucatan Peninsula, the percentage of fruits and number of seeds produced, and fruit weight in treatments using *N. perilampoides* were similar in both crops to treatments pollinated by mechanical vibration (Cauchi et al. 2004, 2006; Palma et al. 2008a). However, *N. perilampoides* is not a buzz pollinator (Slaa et al. 2006), which is preferred for solanaceous crops such as tomato and peppers and for cranberry. Pollination by this species needs to be compared with that of bumble bees for solanaceous crops.

Perspectives on Sustainable Use and Conservation of Bees in Mexico

On a global perspective, the factors that impact the conservation of bees are habitat loss, invasive species, parasites, diseases, pesticides, overexploitation, extinction cascades, and climate change (Brown and Paxton 2009). For the neotropics, the conservation of native bees is threatened especially by human activities including deforestation, intensification of agriculture, and introduction of exotic species (Freitas et al. 2009). The deforestation rate for Mexico is approximately 347,000 hectares annually (FAO 2003), which is equivalent to 0.5% of the country's total forest surface area. This deforestation rate combined with the intensive use of agrochemicals, especially neocotinoid insecticides used in extensive monocultures, are the principal threats to bee pollinators in Mexico (Freitas et al. 2009, Brittain and Potts 2011, Whitehorn et al. 2012).

The presence of the widely reported Colony Collapse Disorder, a syndrome characterized by a marked decrease in managed honey bees reported in the United States and Europe (van Engelsdorp et al. 2008, Neumann and Carreck 2010, Potts et al. 2010) has not been detected in Mexico (Vandame and Palacios 2010). The number of honey bee hives reported for Mexico has decreased recently from 1.9 million in 1999 to 1.8 million in 2010 (SIAP 2010). Given the threat of Colony Collapse Disorder, various authors have advocated for improved apicultural practices in Mexico, with standardized methods for disease and pest prevention, in conjunction with improved training of apiculturists (Reyes and Cano 2006, SAGARPA 2010).

A total of 21 bumble bee species has been registered in Mexico (Labougle and Zozaya 1986). Three Mexican endemic species are considered most threatened: *Bombus haueri* Handlirsch, *Bombus macgregori* Labougle & Ayala, and *Bombus trinominatus* Dalla Torre (Williams and Osborne 2009). These three species have limited distributions and have been rarely collected in recent decades, a decline possibly caused by human activities. Given that *B. haueri*, *B. macgregori*, and *B. steindachneri* Handlirsch are Mexican endemics with reduced populations, we recommend that actions should be taken to categorize these species in the risk category of threatened (=”amenazada”) in accordance with Mexican law Nom-59-ECOL-2010 (SEMARNAT 2010).

Although these species are especially vulnerable, several biological characteristics of bumble bees in general make them susceptible to population declines including: climatic specialization, competition for food resources, and asynchrony of annual patterns of foraging activities with the presence of flowers (Williams et al. 2008). Climatic specialization limits bumble bees to temperate habitats generally higher than 1,500 m (Labougle 1990, Ascher and Pickering 2012). These temperate habitats, and especially cloud forests, have been greatly reduced in size in Mexico (Challenger 1998) and are one of the habitats most threatened by global warming (Ponce-Reyes et al. 2012).

The use of native bumble bee species as managed pollinators is recommended to reduce the risk associated with introduced species and possible displacement of native pollinators (Velthuis and Van Doorn 2006, Gouldson et al. 2008, Madjiiian et al. 2008). However, the development of the necessary technology for the production of multiple generations of bumble bee colonies under artificial conditions requires both basic and applied research during many years. In Mexico, two species of native bumble bees have been evaluated as managed pollinators: *Bombus ephippiatus* and *B. sonorus* Say (Vergara 2012). Most of these studies have evaluated efficiency of pollination in the field, but some studies have involved rearing methods. To date, all studies have used wild queens collected in the field without sustained reproduction, and no methods are yet available for rearing of these species. Such methods are necessary to ensure the quality of colonies for effective managed pollination. In addition, commercial rearing of native bumble bees would reduce the necessity for massive collection of bumble bee queens in natural habitats for single-generation colonies for pollination of crops in greenhouses, a practice presently utilized in the region of Autlan in Jalisco, Mexico (Cuadrillero 2011). More research is required to define the critical characteristics of successful managed pollinators. Recent information regarding the biological characteristics that favor the dispersion and successful establishment of the bumble bee, *Bombus terrestris* L. in non-native areas in Europe, offer guidelines for the selection of native species focusing on decreasing this environmental risk (Dafni et al. 2010). Finally, more effort is needed to develop rearing methods to ensure reliable procedures and rigorous hygienic standards.

Stingless bees have been considered to have potential for development as commercially managed pollinators based on the diversity of species and a long history of management (Slaa et al. 2006). Although some methods for maintenance of this species in hives with good development and colony viability have been developed (Arzaluz Gutiérrez et al. 2002), the limiting factor in the commercial use of this group of bees as managed pollinators is still the lack of technique for mass rearing. A total of 46 species of stingless bees (Meliponinae) is reported from Mexico (Ayala 1999). The conservation status of these bees has not been studied in detail, but evidence from other regions suggests the bees are dependent on tropical forests and vice versa (Slaa et al. 2006). Bees use forest resources for food (floral resources) and nesting sites and may have specific requirements for each (Reyes-Novelo et al. 2009). Thus, the relatively high deforestation rates in Mexico threaten populations of stingless bees, although no species is presently classified with a threatened status under Mexican law Nom-59-ECOL-2010 (SEMARNAT 2010).

The promotion of traditional use of native stingless bees combined with development of new technologies for the use of these species as managed pollinators offer potential for their conservation in Mexico. In addition, because wild bees are good indicator groups of biodiversity and fragmentation (Reyes-Novelo et al. 2009), supporting conservation and use of stingless bees in general, would likewise encourage conservation of tropical habitats. Research to improve and support programs promoting stingless bee management would also offer sustainable alternatives for use of natural resources in rural communities, as well as provide insights into methods for commercial use of these pollinators.

Conclusions

Mexico has a rich diversity and heritage of cultivated plants, more than half of which produce seeds or fruits consumed by humans, and approximately 85% of which depend to some degree on pollinators for efficient production. Mexico also has a great diversity of species of bees, some of which have potential for use as managed pollinators. Managed pollinators are crucial for intensive agricultural practices, especially considering the accelerated loss of biodiversity and recent problems with honey bee decline. Despite the importance of managed pollinators, only two species are commercially available in Mexico: the honey bee, *Apis mellifera,* and common eastern bumble bee, *Bombus impatiens.* The honey bee is rented for pollination of fruit, horticultural, and industrial crops in open fields principally in the north of Mexico, whereas *B. impatiens* is used principally for pollination of tomatoes in protected cultivation. Deforestation combined with the intensive use of agrochemicals in extensive monocultures areas are the principal threats to conservation of wild and managed bees in Mexico. Improved monitoring and sanitary practices are necessary for honey bee hives in Mexico, and could prevent the marked decline in hives observed elsewhere. There has been concern about use of the common eastern bumble bee in Mexico given that the species in not native. Several other native bumble bees show potential as managed pollinators, and methods could be developed for their commercial production. Research is needed to provide a greater diversity of bee species that can be used for commercial, managed pollination services that could benefit the preservation of the grand diversity of crops and the habitats where they are grown in Mexico.

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CAPITULO II

ABEJORROS DEL ESTADO DE QUERÉTARO

Alfonso Torres Ruiz.

Koppert México SA de CV. Circuito El Marqués Norte No. 82. Parque Industrial El Marqués. El Marqués, Querétaro. atorres@koppert.com.mx

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ABEJORROS DEL ESTADO DE QUERÉTARO

Los abejorros son un grupo de insectos himenópteros pertenecientes al género *Bombus*, agrupados en la tribu monotípica Bombini, que es una de las 19 tribus de la subfamilia Apinae. Los abejorros se caracterizan por ser insectos sociales y organizados en castas: reina, obreras y machos. Son insectos de tamaño grande, cubiertos con muchas vellosidades, principalmente de color negro y amarillo y algunas veces con colores blanco, rojo o naranja. Las hembras presentan corbícula para la colecta de polen; el cual constituye su única fuente de obtención de proteínas (Kearns & Thomson, 2001). Los abejorros juegan un papel muy importante en la polinización de una amplia variedad de plantas cultivadas y silvestres y son utilizados como polinizadores manejados principalmente para el cultivo de jitomate (*Solanum lycopersicum* L.) en agricultura protegida (Veltuis & Van Door, 2006), por lo que sus hábitos de forrajeo y comportamiento social han sido ampliamente estudiados (Gouldson, 2003).

Los abejorros, se distribuyen principalmente en las regiones frías del hemisferio norte, aunque algunas especies están presentes también en regiones tropicales, principalmente en el continente Americano (Labougle 1990). Los abejorros son mucho más comunes y diversos en regiones templadas que en tropicales. Pocos estudios se han hecho sobre ellos en áreas al sur de los Estados Unidos (Labougle, 1990); en general, se ha señalado la escasez de trabajos sobre diversidad, taxonomía y distribución de las abejas en México (Ayala, *et al*., 1993). De acuerdo a Williams *et al*., 2008, se han descrito alrededor de 250 especies de abejorros en el mundo, mientras que en México se han documentado hasta el momento 21 especies (Labougle, 1990). El género *Bombus* es relativamente de baja diversidad en México comparado con otros géneros de la familia Apidae como *Exomalopsis* (74 especies); *Centris* (54), *Xylocopa* (36) y *Anthophora* (31) (Ayala *et al*., 1996). En particular para el estado de Querétaro sólo se han registrado tres especies de abejorros: *Bombus ephippiatus, B. pensilvanicus sonorus* y *B. pensilvanicus pensilvanicus*, todas ellas colectadas en localidades de zonas áridas como Vizarron, Peñamiller y Peña Blanca (Labougle, 1990); sin embargo, se ha señalado en general que las zonas boscosas de clima frío, con presencia de Pinos, Encinos y Abetos son el lugar donde se encuentra la mayor diversidad de abejorros. Dada la alta diversidad de tipos de vegetación que incluyen zonas altas y boscosas en el Estado de Querétaro (Zamudio *et al* 1992), es de esperar una mayor riqueza de especies de abejorros para el Estado que las reportadas hasta el momento. En colectas recientes, se han encontrado tres especies más de abejorros, que constituyen nuevos registros para el Estado de Querétaro: *Bombus diligens, B. huntii* y *B. weisi*. Dichas especies fueron colectadas en la localidad el Cerro del Zamorano, donde están presentes al menos cuatro especies de abejorros de forma simpátrica, por lo que esta zona resulta importante para el estudio y conservación de estos polinizadores. Dichos ejemplares están depositados en la colección del laboratorio de entomología de la Facultad de Ciencias Naturales de la UAQ.

Desde principios de 1990 los abejorros han sido utilizados como polinizadores manejados en cultivos protegidos, actualmente su uso es ya generalizado en México principalmente para cultivos de hortalizas bajo invernadero. Dos de las especies presentes en Querétaro: *B. ephippiatus* y *B. huntii* poseen las características necesarias para ser consideradas especies con potencial para su uso como polinizadores manejados (Veltuis & Van Door, 2006). En particular, *Bombus ephipiatus* Say ya ha sido utilizado de forma experimental y semicomercial como polinizador manejado en tomate bajo invernadero en varios estados de México [San Luis Potosí, Jalisco, Coahuila, Puebla (Cuadriello & Salinas 2006; Vergara & Fonseca 2012) y Querétaro (Torres & Jones, 2012)]. Sin embargo, actualmente no hay disponibilidad de esta especie de forma comercial por lo que se deberá tener especial atención en evitar colectas masivas de reinas en la naturaleza con el fin de hacer crecer la colonia y utilizarlas para la polinización, más bien, se deben hacer esfuerzos en documentar el efecto de esta especie en el tomate en invernadero para definir su potencial como polinizador y finalmente enfocarse a la investigación para lograr la crianza masiva de forma confiable a fin de evitar desabastos en los invernaderos y también el dispersar plagas y enfermedades. El laboratorio de investigación y desarrollo (I+D) en Koppert México SA de CV, ha desarrollado esta línea de investigación, con el objetivo de evaluar la efectividad de *B. ephippiatus* y otras especies de abejorros nativas de México como polinizadores manejados; enfocados también en el desarrollo del método de crianza confiable y probado para una producción estable y de calidad que garantice el insumo de este importante polinizador manejado para los cultivos protegidos en México.

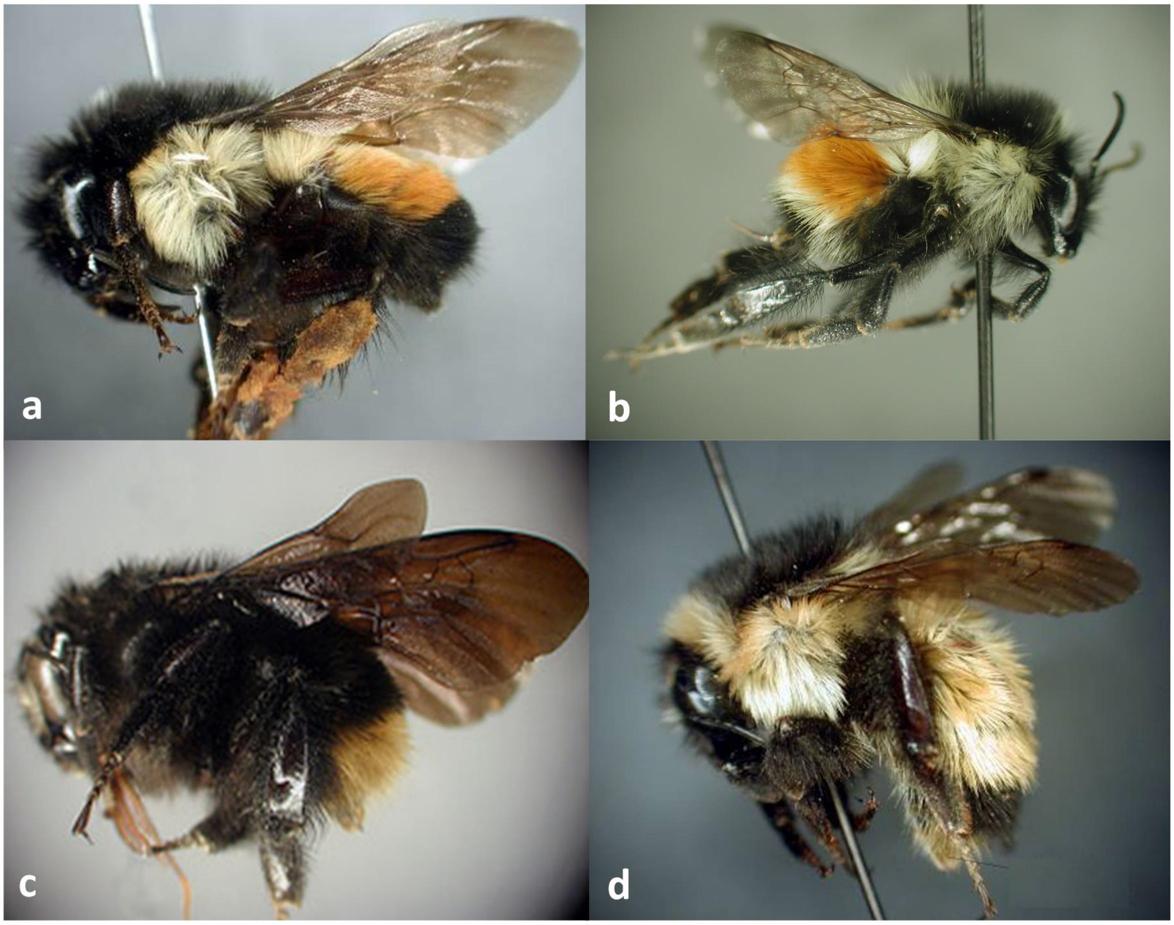

Figura 1. Especies de abejorros del género *Bombus* presentes en el Cerro del Zamorano. a) *Bombus ephippiatus*; b) *Bombus huntii*; c) *Bombus diligens*; d) *Bombus weisi*. (Fotos por Ricardo Ayala Barajas).

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CAPITULO III

Comparison of the efficiency of the bumble bees *Bombus impatiens* and *Bombus ephippiatus* (Hymenoptera: Apidae) as pollinators of tomato in greenhouses.

Torres-Ruiz, Alfonso[1*,] and Jones, Robert W.[2]

[1]R & D Department, Koppert Mexico SA de CV, Calle Circuito El Marqués Norte No. 82, Parque Ind. El Marqués, Querétaro, México.
[2]Universidad Autónoma de Querétaro, Facultad de Ciencias Naturales, Laboratorio de Ecología y Diversidad Faunística; Querétaro, México.

[*]Corresponding author. E-mail: atorres@koppert.com.mx
Running head: Torres and Jones: Efficiency of two bumble bees as tomato pollinators

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Comparison of the Efficiency of the Bumble Bees *Bombus impatiens* and *Bombus ephippiatus* (Hymenoptera: Apidae) as Pollinators of Tomato in Greenhouses

ALFONSO TORRES-RUIZ[1] and ROBERT W. JONES[2]

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ABSTRACT Experiments were conducted in a commercial tomato, *Solanum lycopersicum* L. (Solanaceae) greenhouse to compare the relative foraging effort and efficiency of two bumble bee species: *Bombus impatiens* Cresson, a species from northeastern North America, commercially reared and used for pollination in Mexico; and *B. ephippiatus* Say, a native species of Mexico and central America. *B. ephippiatus* was as efficient in pollination of tomatoes as *B. impatiens*, as indicated by all variables of fruit quality: fruit weight, number of seed per fruit, and maximum fruit diameter. The two species had similar levels of hourly and daily foraging activity. They had the same response to temperature fluctuation. Pollination rates by both species were similar and close to 100% throughout the sample period. However, *B. impatiens* showed greater foraging activity during the first half of the 27-d sample period, whereas *B. ephipiatus* had greater relative activity during the last half. This study establish that *B. ephippiatus* is as efficient as *B. impatiens* as a pollinator of tomatoes in greenhouses and thus a candidate as a managed pollinator. However, standard reliable methods for mass rearing of *B. ephippiatus* are not yet available. Such methods are necessary to ensure healthy colonies and optimum pollination for producers and will reduce the pressure for the unregulated collection of queens in the field and the subsequent reduction of populations of this species.

RESUMEN Se evaluó el esfuerzo de forrajeo y eficiencia de polinización de dos especies de abejorros en un invernadero comercial de tomate *Solanum lycopersicum* L. (Solanaceae). Las especies comparadas fueron: *Bombus impatiens* Cresson, nativo del noreste de Norteamérica, comercialmente disponible para polinización en México; y *Bombus ephippiatus* Say, una especie de abejorro nativo de México y Centroamérica. Ambas especies presentaron eficiencias de polinización similares, pues todas las variables de calidad del fruto, como peso fresco, número de semillas, y diámetro máximo, no fueron diferentes de forma significativa. Ambas especies no difirieron en la actividad de forrajeo en función de la temperatura. No se encontró mayor actividad de *B. ephippiatus* durante los periodos de mayor temperatura en el invernadero. Ambas especies de abejorros lograron porcentajes de polinización similares, cercanos al 100% durante todo el periodo de evaluación. Este estudio establece que *Bombus ephippiatus* es un polinizador eficiente de tomates bajo invernadero, comparable a la eficiencia de *Bombus impatiens*, por lo que se puede considerar con potencial para su uso como polinizador manejado. Sin embargo, métodos de crianza masiva de dicha especie no están aún disponibles y son necesarios para asegurar la calidad y sanidad de los nidos y al mismo tiempo disminuir la posible presión para la colecta no regulada de esta especie.

KEY WORDS bumble bee, pollination, tomato, greenhouse, *Bombus ephippiatus*

Protected agriculture in Mexico is expanding. In 2005, the country had a total of 3,214 ha of protected crops, which increased to 12,000 ha in 2007 (Instituto Nacional de Estadística Geografía e Informática [INEGI] 2007). Approximately 40% of this area is devoted to tomatoes (*Solanum lycopersicum* L.), of which the majority is destined for exportation (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y alimentación [SAGARPA] 2009). This total, combined with open field cultivation, makes México the largest exporter of tomatoes in the world with more than a million tons shipped annually (Food and Agriculture Organization of the United Nations [FAO] 2007). Tomatoes grown in protected agriculture will constitute an increasing percentage of this market.

One of the advantages of protected agriculture is that colonization of crops by insect pests is greatly restricted, especially for larger species. However, this

[1] Corresponding author: R & D Department, Koppert Mexico SA de CV, Calle Circuito El Marqués Norte No. 82, Parque Ind. El Marqués, Querétaro, México (e-mail: atorres@koppert.com.mx).

[2] Universidad Autónoma de Querétaro, Facultad de Ciencias Naturales, Laboratorio de Ecología y Diversidad Faunística; Querétaro, México.

restriction also limits access of floral visitors, which for entomophilous crops requires artificial pollination practices or managed pollinators. In the case of tomato although the flower is autocompatible, the anthers are poricidal and require vibrations to liberate pollen and to fertilize ovaries (Harder and Barclay 1994). Bumble bees (Apidae: *Bombus*) are specialized for pollination through vibration, that is, "buzz pollination" (Buchmann 1985), for which they have been widely used for pollination of tomatoes in greenhouses. The superior pollination efficiency of bumble bees has been demonstrated by significant increases in the number of seeds and fruit weight when compared with manual pollination (Banda and Paxton 1991, Pressman et al. 1999, Morandin et al. 2001b).

The bumble bee, *Bombus impatiens* Cresson, has been used in Mexico since 1994 for managed pollination of food crops, particularly in greenhouse tomatoes (Winter et al. 2006). The natural range of this species is restricted to the eastern United States and Canada (Velthuis and van Doorn 2006, Williams 2010). Although this species has been a very successful pollinator in greenhouses in Mexico, it has been suggested that native pollinators from tropical regions may be better adapted to the higher temperatures and climatic regimes of greenhouses of Mexico. In addition, the concerns over the potential environmental and sanitary risks posed by non-native species (Goulson 2003a,b; Goulson 2010; Otterstater and Thompson 2008), could limit the use of *B. impatiens* and necessitate the development of technology for the use of a native bumble bee species.

Bombus ephippiatus Say is a native bumble bee species of Mexico with a wide distribution in the country (except for the Baja and Yucatan peninsulas) that extends south to Central America, Colombia, and Venezuela (Labougle 1990, Williams 2010). The potential of this species as a managed pollinator has been previously noted (Fuentes and Madrid 2004, Hines et al. 2006; Vergara et al. 2006). However, the efficiency of this species as a pollinator of tomato under commercially managed greenhouse conditions has not been compared with *B. impatiens.*

In the current study, the pollination efficiency of *B. impatiens* and *B. ephippiatus* in tomatoes grown in greenhouses is compared to evaluate the potential of the native species for use as a managed pollinator. The variables measured for these comparisons were the quality of fruit (number seeds, weight, and diameter of fruit), the percentage of flowers pollinated by bumble bees, and foraging activities (number of foraging flights from the colony, and the length of time of visits per flower).

Materials and Methods

Study Area. The study was carried in one hydroponic greenhouse managed by the Center for Technological Transfer of Protected Agriculture (CEICKOR) in Ezequiel Montes, State of Querétaro, Mexico. The greenhouse had an area of 4,950 m^2 and a height of 5 m. The tomato variety used was 'Tricia,' although some rows in the native bee treatment had some plants (<1.5%) of the varieties of 'Cherry,' 'Cocktail,' and 'Saladette.' The planting density was 2.94 plants/m^2. The plants were transplanted to the greenhouse on 19 May 2008.

The temperature of the greenhouse was regulated using lateral vents for cooling and natural gas heaters for heating. The mean, maximum, and minimum temperatures during the experiment were 20.9, 34.5, and 13.5°C, respectively. The operation of the vents and heaters was controlled by computer. All windows and walls were covered with antiaphid screen. The agronomic management of the tomato crop (nutrition, cultural management, and harvest) and the pest and disease control (using Integrated Pest Management [IPM] based on biological control), were carried out according to established practices of CEICKOR and were the same in both treatments.

Bumble Bee Colonies. The colonies of *B. impatiens* were obtained from established colonies maintained by Koppert México (El Marqués, Querétaro) under artificial rearing conditions. The colonies of the native species, *B. ephippiatus,* were obtained from queens captured in the field and reared in the laboratory to the phase of worker production, following the general techniques reported for other bumble bee species (Roseler 1985, Kearns and Thomson, 2001). Before introduction of the colonies into the greenhouse, the numbers of workers of each colony was counted and varied between 115 and 144 individuals. Additionally, the presence of the founder queen was verified. The colonies of both species were established and moved within the standard commercial packaging system, Natupol, which consists of plastic containers covered with a 35 × 30 × 25 cm cardboard container. This packaging permitted the normal maintenance practices for *B. impatiens;* these practices were used for both species. Each container held a plastic tank in the base that stored a sugar solution as a substitute for nectar, as tomato flowers do not produce nectar.

Pollination Treatments. Three treatments were implemented: 1) *B. ephippiatus* pollination (BE), 2) *B. impatiens* pollination (BI), and 3) nonpollinator (control). The greenhouse was separated by a mesh antiaphid screen into two areas of approximately equal surface area (2,500 m^2). The screen prevented movement of bumble bees between separated areas (treatments 1 and 2). Racemes with open flowers were marked in both treatments using a marking tape labeled as to treatment and raceme number. The control treatment was established by covering racemes with a 20 × 15 cm cloth bag that prevented the entrance of bees, but did not reduce the ventilation and entrance of light. The bag was removed after flowers dried or small fruits appeared to permit unimpeded fruit growth. We collected 45 and 51 fruits for the pollinator treatments, *B. impatiens* and *B. ephippiatus,* respectively, and 67 fruits for the control treatment.

In total, four colonies of each species were used for each pollination treatment. Initially, three colonies were introduced on 19 June 2008, with an additional colony introduced for each species 3 wk later.

Fig. 1. Bee activity recorder in place on a bumble bee hive. (Online figure in color.)

Fruit Quality and Harvest. The development of the truss for each treatment was monitored for 8 wk until fruits were ripe (uniform red coloration) and separated 1 d before the programmed commercial harvest. Fruits were weighed using a balance (model AR5120, OHAUS, Parsippany, NJ) and minimum and maximum diameters were measured with a digital caliper. For each fruit, seeds were manually separated and counted.

Percentage and Levels of Pollination. The percentage of flowers visited by bumble bees was determined by collecting 100 flowers per week in each treatment. A flower was considered pollinated if it displayed a characteristic necrotic mark in the cone of the anthers that is produced during the process of buzz pollination by both species of bumble bees. A flower with this mark was considered pollinated, and the percentage of pollinated flowers was determined weekly and compared between species. In addition, necrotic marks were recorded into five categories based on methodologies of Morandin et al. (2001b) to determine the pollination level, a standard measurement on greenhouse tomato pollination.

Foraging Activity. Foraging activity was measured by the numbers of bees leaving the hives per minute. A digital counter with an infrared optical reader, developed by R&D Koppert B.V. (Berkel en Rodenrijs, The Netherlands) was fixed to each colony and connected to a data logger (HOBOPRO, Cape Cod, MA). The optical reader was fixed on the exit hole of the hive to record the number of bees leaving for each date and hour for a selected time period (Fig. 1). Before placement of the colony in the greenhouse, the number of bees (workers and drones), per hive were determined visually using red light. The counter did not record for the first 2 d after placement, for bees to become accustomed to the presence of the apparatus. The sensor recorded the departure of each worker bee during the recording period, but also the activity of guard bees and others that left and entered the nest for reasons other than foraging. To discard the activity of bees other than foraging, we calibrated the sensors through direct observations. For this we used a total 6 h of direct observations and constructed a linear regression that was used to transform the data ($y = 0.9524x - 0.0437$).

Data were stored in the data logger and downloaded weekly using a software program (HOBOPRO). In total, the digital counters were placed on the hives and recorded data for 27 d of the experiment.

Duration of Flower Visits. The time spent by individual worker bees per flower was determined by direct observation. Observations were made on four different days during the peak period of foraging between 11:00 and 13:00 hours for both bee species. A digital stopwatch was used to record the time from when a bumble bee landed on the flower until the point when it left. In total, 50 observations were made for *B. impatiens* and 77 for *B. ephippiatus.*

Statistical Analysis. To determine the effect of treatments (*B. impatiens, B. ephippiatus,* and control) on the parameters of fruit quality, a one way analysis of variance (ANOVA) was used to compare fruit weight, number of seeds, and maximum fruit diameter, with mean separation using Tukey's test. Differences in foraging activity (mean number of bees leaving the hive per hour) for each bumble bee species were analyzed with two-way ANOVA. To determine if there was difference between bumble bee species in foraging activity per day of sampling, we used a two-way ANOVA with multiple comparisons using a Tukey analysis with $\alpha = 0.05$. For this analysis, we used the data of total number of foraging trips for the first nine sampling dates compared with the last nine sampling dates. Additionally, during the time of the experiment the average ambient temperature and humidity were recorded at 30-min intervals with a data logger (HOBO). Pearson's correlations were used to analyze the effect of these environmental variables on the number of bees leaving the hives. A *t*-test was used to

Table 1. Comparison of fruit quality variables of tomatoes under different pollination treatments

Treatment	Fruits (n)	Weight (g)[a]	No. of Seeds[a]	Maximum diameter (cm)[a]
B. ephippiatus	45	135.60 ± 3.12a	178.24 ± 3.70a	6.51 ± 0.06a
B. impatiens	51	143.03 ± 4.2a	170.94 ± 6.33a	6.59 ± 0.08a
Control	67	69.17 ± 4.7b	68.05 ± 8.36b	4.87 ± 0.13b

[a] Values within a column with the same letter not significantly different based on a one way analysis of variance and Tukey test ($\alpha = 0.05$).

determine if there were differences in the average pollination percentage during the experiment. A one way ANOVA was used to determine if there were differences in the time spent on flowers for the two bumble bee species. A test of homogeneity of the variance was used to test normality in all ANOVA analysis. As a consequence of these test we found it necessary to use a $\log_{10}$ transformation of all the data. All statistical analysis was performed using SPSS version 18.0 (Chicago, IL).

Results

Fruit Quality and Harvest. There was a significant effect of pollination treatment on fruit weight ($F = 95.2$; df = 2,162; $P < 0.001$), number of seed per fruit ($F = 81.5$; df = 2,162; $P < 0.001$), and maximum fruit diameter ($F = 88.6$; df = 2,162; $P < 0.001$). However, these significant differences were because of differences between bumble bee pollinated treatment groups and nonpollinated (control) groups. We found no difference in fruit weight, the number of seeds, and maximum diameter of fruit between plants pollinated by either *B. impatiens* or *B. ephippiatus* (Table 1).

Percentage and Levels of Pollination. Pollination efficiency was similar for the two bumble bee species. Both species pollinated 100% of the sample flowers during 16 of the 20 sample week's (Fig. 2). The mean percentage of pollination over the 20-wk sample period was 98 and 97% (t-test; $P = 0.709$), respectively, for *B. impatiens* and *B. ephippiatus.* The only notable difference in pollination percentages occurred during the first 3 wk after hive introduction when *B. ephippiatus* had slightly lower efficiency levels for the first 2 wk and for week 19 after colony placement, compared with 100% levels for *B. impatiens* after week 1 (Fig. 2).

Foraging Activity. The two species had similar foraging patterns. The mean time per flower visit by *B. ephippiatus* and *B. impatiens* was very similar (11.08 ± 1.08 ($n = 50$) and 11.64 ± 1.16, ($n = 77$), respectively) and not significantly different ($F = 0.113$; df = 1, 126; $P = 0.717$). The daily fluctuation in the number of bees leaving the hive also followed similar patterns. There were significant differences in foraging activity between hours of the day for the two species taken together ($F = 16.924$; df = 23; $P < 0.0001$), but not between species ($F = 2.457$; df = 1; $P = 0.117$), and the interaction of species and hours was not significant ($F = 1.371$; df = 23; $P = 0.115$). The foraging activity began for both species at daybreak (0600–0700 hours) and increased to peak activity at mid-day (1100 and 1200 hours). This activity remained high throughout the afternoon and early evening hours then decreased after 1700 and 1800 hours and ended at 2200 hours (Fig. 3). Sensors detected low numbers of exits from the hive during night hours for both species, although no artificial lights illuminated greenhouses during these hours. There was a positive correlation between temperature and the number of foraging flights for

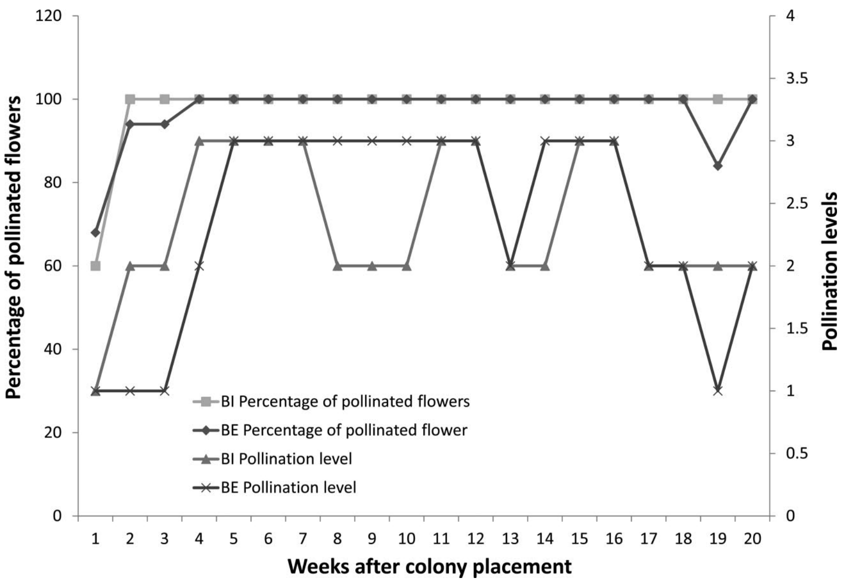

Fig. 2. Percentage of pollinated flowers and levels of pollination (index based on Morandin et al. 2001) of tomatoes grown in greenhouses using the bumble bee species, *B. impatiens* (BI) and *B. ephippiatus* (BE).

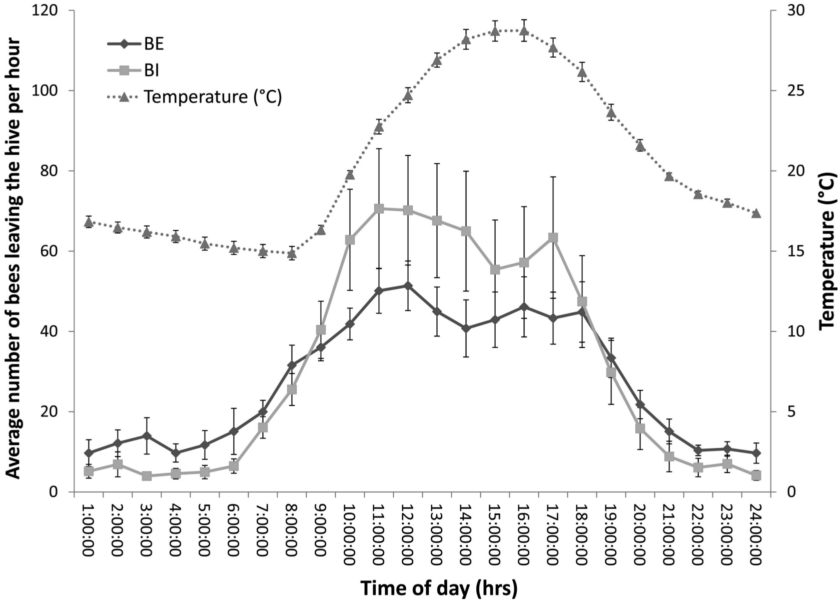

Fig. 3. Hourly average temperature (dotted line) and activity of foraging workers of *B. ephippiatus* (BE) and *B. impatiens* (BI) as measured using an optical counter of bees leaving the hive. All points represent the mean ± SE of bees/h the hive from three nests per species over 26 sample days in commercial production of tomatoes in greenhouses.

both species (Table 2). In contrast, humidity did not have a significant correlation with the number of foraging flights (Table 2).

The patterns in foraging activity over the 20-wk sampling period, grouped in two periods of nine sampling dates, revealed significant differences between species ($F = 189.030$; df = 1; $P = 0.046$) (Fig. 4). Both species were active during the length of the sample period, but the number of foraging trips by workers of *B. impatiens* bee workers was significantly greater ($F = 19.6$; df = 1; $P < 0.001$) in the first 9 d after hive introduction (1,221.86 ± 96.92) than the last 9 d of sampling (294.81 ± 80.64). This was in contrast to workers of *B. ephippiatus* for which the number of foraging flights were similar during all of the 20-d sampling period (first nine sampling days, 696.83 ± 91.95; second nine sampling days, 608.14 ± 76.36).

Discussion

The bumble bee species, *B. ephippiatus,* was equally efficient as *B. impatiens* in pollinating tomatoes grown in greenhouses based on measures of the production-related variables (fruit weight, number of seeds, and maximum diameter). Both species also spent similar amounts of time per flower, have similar daily pattern of foraging activity, and also showed a comparable percentage and level of pollination on tomato crop in greenhouses. The parity of the two species in their capacity for tomato pollination was not expected given that the colonies of the native species were first generation colonies, founded in the laboratory by a wild queen. In contrast, *B. impatiens* has been domesticated, and reared in captivity, in the artificial commercial packaging system for >14 yr.

One of the incentives for evaluating native bumble bees for pollination services in protected environments is the expectation that these species would be more tolerant of the higher temperatures characteristic of greenhouses in some regions of Mexico. For example, Palma et al. (2008) report important behavioral differences in the foraging activity between the native stingless bee, *Nanotrigona perilampoides* (Cresson) when compared with *B. impatiens,* suggesting that the native species is better adapted to the high temperatures characteristic of greenhouses in Mexico. However, our results indicate that the bumble bee species had similar daily fluctuations in the number of workers leaving the hive during the day and that temperature was positively correlated with foraging activity of both species. There was no evidence of greater or more efficient foraging activity of the native bumble bee species *B. ephippiatus* during the hottest periods of the day. However, in the current study the temperatures did not reach the extreme levels that can occur in low technology greenhouses of Mexico, as reported in Palma et al. (2008), where temperatures were >30°C at 0800 hours with a maximum of 38°C at 1300 hours. Further research is needed to compare the for-

Table 2. Coefficient of correlation between temp and number of foraging flights during 26 sample days in commercial production of tomatoes in greenhouses

Variables	*B. ephippiatus*		*B. impatiens*	
	(r)	P value	(r)	P value
Temperature	0.63	0.004	0.659	0.003
Humidity	−0.346	0.94	−0.347	0.94

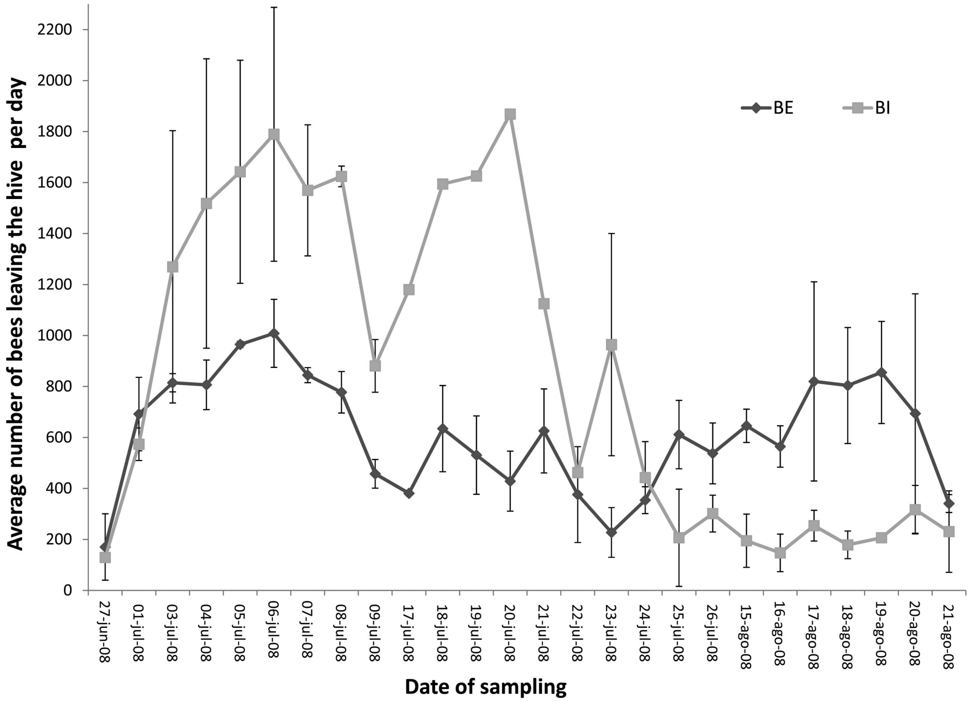

Fig. 4. Mean number of bees leaving the hive (±SE) of *B. ephippiatus* (BE) and *B. impatiens* (BI) per day over 26 sample days in commercial production of tomatoes in greenhouses.

aging behavior of both species under these extreme conditions.

It has been estimated that a minimum of 2,000 bumble bees foraging trips per day are required to ensure adequate pollination of 1 ha of greenhouse tomatoes (Morandin et al. 2001a). In the current study, the number of foraging trips (foraging trips = average number of bees leaving the hive/two) was greater than this minimum for both species when calculated on a hectare basis (2,887 and 2,885, per hectare, respectively, for *B. ephippiatus* and *B. impatiens* using the data from 26 sampling days). However, there was significant variation over the course of the sample period in foraging activity between species. *B. ephippiatus* had fewer numbers of foraging flights when compared with *B. impatiens,* but more uniform activity during the length of the sample period. When using lower colony densities, this pattern in foraging activity may result in more superior pollination rates of the first flowers for *B. impatiens,* although more information is needed to confirm this assumption.

Nocturnal activity was unexpectedly recorded at the nest entrance in both species. This activity was a common occurrence and was recorded during 95% of the sampled nights. This behavior has previously been documented for *B. impatiens* by Kevan et al. (2008) who postulated that it is the result of unnatural, nocturnal disturbances within greenhouses. However, there were no artificial lights and disturbances were minimal during the experimental period of the current study. This low-level nocturnal activity may be the result of movement of guard bees at the nest entrance being registered by the counter. Further study is needed to confirm and observe this activity to evaluate its possible importance in nest function.

The current study is the first to establish that *B. ephippiatus* is as efficient as *B. impatiens* as a pollinator of tomatoes in greenhouses. Because *B. impatiens* has been widely used in the United States and other countries and compared in efficiency to other bumble bee species (Whittington and Winston 2004) it also can be concluded that *B. ephippiatus* is comparable to other species used in greenhouse pollination. Although this species has been used experimentally for pollination of tomatoes in greenhouses on a small scale in several states of Mexico [San Luis Potosí, Jalisco, Coahuila, Puebla (Cuadriello–Aguilar and Salinas–Navarrete 2006, Vergara and Fonseca–Buendía 2012) and Querétaro (A.T.-R., unpublished data)], there was no comparison with other pollinators to evaluate foraging activity and efficiency of pollination. All studies to date have used field collected queens to establish colonies and, as yet, production of multiple generations of colonies under artificial conditions have not been reported. Standard reliable methods for mass rearing of *B. ephippiatus* are not available. Such methods will ensure that colonies used in greenhouses are uniform and healthy and provide optimum pollination for producers. Equally important, the availability of commercially produced colonies will reduce the pressure for the unregulated collection of queens in the field and the subsequent reduction of populations of this species. Given the present crisis in loss of important pollinators (i.e., Poots et al. 2010, Kremen et al.

2002, Freitas et al. 2009), further research is needed to evaluate other species of native pollinators in Mexico for their potential as managed pollinators and to develop new methods for mass rearing.

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CONCLUSIONES

En este trabajo se seleccionó y evaluó al abejorro *Bombus ephippiatus* Say (Hymenoptera: Apidae: Bombini), una especie de abejorro nativo de México, como polinizador manejado en cultivo de Tomate (*Solanum lycopersicum* Mill.), con el fin de determinar su factibilidad de uso como polinizador de tomate bajo invernadero.

Como resultado de la revisión del uso presente y potencial de abejas como polinizadores manejados en México se concluye que a pesar de la importancia de los polinizadores manejados en la producción agrícola intensiva, sólo se cuenta con dos especies de polinizadores Apoideos comercialmente disponibles en México: la abeja melífera *Apis mellifera* L. y el abejorro *B. impatiens.* Las abejas melíferas son rentadas para la polinización de frutales, hortalizas y cultivos industriales en campo abierto, principalmente en el norte del País. Los abejorros, en cambio, son utilizados para la polinización de hortalizas, principalmente de tomate bajo invernadero. Aunque en México existe una gran diversidad de abejas nativas, solo se ha reportado la evaluación a nivel experimental como polinizadores manejados de tres especies de abejas meliponas. Sin embargo, ninguna de ellas se encuentra en la actualidad comercialmente disponible, situación similar a lo ocurrido con otras especies de Apoideos como los abejorros. La deforestación en combinación con el uso de agroquímicos en la agricultura extensiva han sido señalados como los principales factores que amenazan la conservación de las abejas silvestres.

Se reportan nuevos registros de 3 especies de abejorros para Querétaro: *Bombus diligens*, *B. huntii* y *B. weisi*. Dichas especies fueron colectadas en la localidad el Cerro del Zamorano, donde están presentes al menos cuatro especies de abejorros de forma simpátrica, por lo que esta zona resulta importante para el estudio y conservación de estos polinizadores.

La evaluación experimental del esfuerzo de forrajeo y eficiencia de polinización de tomate (*S. lycopersicum*) en un invernadero comercial utilizando al abejorro *Bombus* *impatiens*, nativo del noreste de Norteamérica y *Bombus ephippiatus*, nativo de gran parte de México y Centroamérica, mostró que ambas especies presentaron eficiencias de polinización similares. Ya que las variables de calidad del fruto, como peso fresco, número de semillas, y diámetro máximo, no fueron diferentes de forma significativa.

Los resultados muestran que ambas especies de abejorro presentan una fluctuación similar en el número de obreras promedio en actividad de forrajeo por día y que la temperatura está correlacionada positivamente con la actividad de forrajeo de ambas especies. Al contrario de lo esperado, no se encontró evidencia de mayor o más eficiente actividad de forrajeo por parte de *B. ephippiatus* durante el periodo del día con mayor temperatura ambiental.

Este estudio establece que *B. ephippiatus* es un polinizador eficiente de tomates bajo invernadero, comparable a la eficiencia de *B. impatiens*, por lo que se puede considerar con potencial para su uso como polinizador manejado. Sin embargo, métodos de crianza masiva de dicha especie no están aún disponibles y son necesarios, para asegurar la calidad y sanidad de los nidos y disminuir la posible presión para la colecta no regulada de esta especie.

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ANEXOS

Universidad Autónoma de Querétaro Facultad de Ciencias Naturales

Licenciatura en Biología

20 de agosto de 2012

A QUIEN CORRESPONDA:

Por medio de la presente hago constar que el capítulo de libro,

ABEJORROS DEL ESTADO DE QUERÉTARO

escrito por el **Pas. De Dr. Alfonso Torres** Ruiz ha sido aceptado para publicación en el libro *HISTORIA NATURAL DE QUERETARO, MEXICO.*

Sin mas por el momento y agradeciendo de antemano a la presente me despido a usted enviándole un cordial saludo.

A T E N T A M E N T E
"Naturalza, Vida y Ciencia"

Dr. Robert W. Jones
Editor del Libro
HISTORIA NATURAL DE QUERETARO, MEXICO

Licenciatura en Biología, Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Avenida de las Ciencias s/n, Juriquilla, C.P. 76230, Querétaro, QRO, MEXICO, Tel.: (442) 192-1327, Ext: 5333, Fax: (442) 192-1328, Email : rjones@uaq.mx