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Management of *Bombus atratus* bumblebees to pollinate
Lulo (*Solanum quitoense* L), a native fruit from the Andes
of Colombia

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*Dedicated to the memory of my mother, Maria Teresa, who guides and
accompanies me always*

ABSTRACT

The purpose of this study was to set the base for a management program for the bumblebee species *Bombus atratus* to pollinate Lulo crops (*Solanum quitoense*). Lulo is a fruit of the northern Andes. It has a high export potential due to its attractive taste. However, increasing the production yields is hampered in part by lack of appropriate pollination. To solve this problem, it is proposed to manage pollination using bumblebees native of Colombia reared in captivity. The advantage of such a pollination management system is that the import of alien bumblebee species is avoided.

The reproductive system of *S. quitoense* is characterized by andromonoecy, where each plant bears a variable proportion of hermaphroditic long-style flowers and functionally male short-style flowers. The flowers have poricidal anthers that enclose the pollen that can only be liberated through vibrations. *Bombus atratus* workers are adapted to perform this so-called buzz-pollination.

The results show that hermaphrodite flowers have simultaneously both male and female phases. Hand-pollination experiments revealed that cross-pollination results in significantly higher seed production over self-pollinated flowers. Bumblebees visited in sequence 7 to 32 flowers of Lulo on different plants during their foraging flights. Their visits to flowers promoted cross-pollination and with it, fruit set was doubled in comparison to spontaneous self-pollinated flowers. Those fruits had a better quality as they were significantly bigger and bore more seeds.

A model was used to simulate colony development to identify the effect of the main parameters: rate of oviposition and worker production, mortality, pollen and nectar consumption. The development of four reared colonies was compared with the simulation. The simulation showed that oviposition determines the beginning of colony development in the solitary phase. In the eusocial phase the capacity of adult workers to feed larvae becomes a limiting factor. Workers can acquire more pollen if they can forage on flowers in the nature over those that only rely on the experimental feeding scheme where they are fed with a mass of pollen in complete captivity. For this reason, a combined scheme of semi-captivity and complete captivity is suggested to improve the rearing method. The establishment of these methods can be applied in the region of Cundinamarca.

Zucht einheimischer Hummeln (*Bombus atratus*) und deren Einsatz zur Bestäubung in Lulo-Kulturen (*Solanum quitoense* L), einer lokalen Frucht der kolumbianischen Andenregion

Ziel der Arbeit war, die Basis für ein Management-Programm für die Zucht und den Einsatz von Hummeln (*Bombus atratus*) zur Bestäubung von Lulo-Kulturen (*Solanum quitoense*) zu entwickeln. Lulo ist im Gebiet der nördlichen Anden beheimatet. Die Früchte haben aufgrund ihres guten Geschmacks ein hohes Exportpotential. Jedoch ist eine Produktionssteigerung u.a. durch mangelhafte natürliche Bestäubung begrenzt. Durch die Zucht und den Einsatz von einheimischen Hummeln soll die Bestäubung der Kulturen optimiert werden. Dadurch soll auch der Import und Einsatz nicht-einheimischer Hummeln vermieden werden.

Das Fortpflanzungssystem von *S. quitoense* wird als andromonoëzisch bezeichnet, d.h. die Pflanzen produzieren sowohl zwittrische langröhrliche Blüten als auch funktionell männliche kurzröhrliche Blüten. Der Pollen muss aus den poriziden Antheren durch Vibration freigesetzt werden. Zu dieser sogenannten Buzz-Bestäubung sind die Arbeiterinnen von *B. atratus* bestens geeignet.

Meine Untersuchungen zeigen, dass die zwittrischen Blüten die männliche als auch die weibliche Phase synchron durchlaufen. Handbestäubung zeigte, dass bei Fremdbestäubung grundsätzlich mehr Samen ausgebildet wurden als bei Selbstbestäubung. Es wurde beobachtet, dass die Hummeln auf ihren Sammelflügen 7- bis 32-mal Blüten von verschiedenen Lulo-Pflanzen besuchen. Durch eine häufige Fremdbestäubung mit Hummeln konnten doppelt so viele Früchte geerntet werden wie bei spontaner Selbstbestäubung. Diese Früchte hatten auch ein signifikant höheres Gewicht, und einen höheren Anteil an Samen.

In einer Simulation wurde die Auswirkung der Parameter Eiablage, Produktionsrate von Arbeiterinnen, Mortalität, Pollen- und Nektarverbrauch auf die Entwicklung von 4 Völkern untersucht. Es zeigte sich, dass die Eiablage am Beginn der Solitärphase die Entwicklung eines Volkes determiniert. Limitierender Faktor in der eusozialen Phase des Volkes ist die Kapazität der Arbeiterinnen, die Larven zu ernähren. Wesentlich ist auch der Befund, dass Arbeiterinnen die Gelegenheit zur natürlichen Nahrungssuche haben, mehr Pollen herbeischaffen und damit eine bessere Nahrungsgrundlage schaffen, als dies bei einem gänzlich künstlich aufgezogenen Volk der Fall ist. Daher wird zur Optimierung der Aufzucht von *B. atratus* eine Kombination von halbkünstlicher und künstlicher Anzucht empfohlen. Dieser Zuchtansatz kann im agrarischen Sektor der Region Cundinamarca umgesetzt werden.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
asl	above sea level
ASOHOFrucOL	Asociación Hortifrutícola de Colombia
CBD	Convention on Biological Diversity
COP 3	Third Conference of the Parties
DANE	Departamento Administrativo Nacional de Estadística
DC	Distrito Capital
df	degrees of freedom
FAO	Food and Agriculture Organization of the United Nations
IAvH	Instituto de Investigación de Recursos Biológicos Alexander von Humboldt
IBM	International Business Machines
ICA	Instituto Colombiano Agropecuario
ICONTEC	Instituto Colombiano de Normas Técnicas y Certificación
IPI	International Pollination Initiative
LSF	Long style flower
MinAmbiente	Ministerio de Ambiente, Vivienda y Desarrollo Social
OTS-USAP	Organization for Tropical Studies-Undergraduate Semester Abroad Program
p	probability
PASCAL	Programing Language
S/D	Supply/Demand
SPSS	Statistical Product and Service Solution
SSF	Short style flower
UNEP	United Nations Environmental Program
var.	variety

1 GENERAL INTRODUCTION

1.1 Importance of pollination in crops

At a worldwide level, the trend in agriculture is to become environmentally sustainable and at the same time to respond to human population needs in terms of food security and livelihood opportunities for farmers, especially in developing countries. Consequently, international efforts are directed towards the identification of species that play an important role in agriculture and towards practices that are friendly to the environment (Article 10 of the Convention on Biological Diversity CBD) (UNEP 1996).

In the biodiversity arena, pollinators have gained key importance because of the value that represents the service of pollination in many crops. A recent review on the importance of pollination in crops worldwide shows that 87 out of 124 leading food crops are dependent on animal pollination (Klein et al. 2006). In particular, tropical crop species seem to rely on pollen vectors even more, as 70% of the species have at least one variety where production is improved with animal pollination (Roubik 1995). The estimated value that pollination by bees brings to production exceeds millions of dollars and euros every year (Williams et al. 1991, Buchmann and Nabhan 1997, Velthuis and van Doorn 2006). Furthermore, losses due to poor pollination range from 26% to 50% for tropical tree crops (Free 1993), which are figures not to be ignored.

The recognition that pollinators have gained came after the identification of a global “pollinators crisis” (Buchmann and Nabhan 1997). In regions where the domesticated honey bee is managed for pollination of apple and cherry there has been a remarkable decrease of hives due to diseases and a decline of beekeeping (Williams 1991, Kremen et al. 2002)

In addition, there are many reports on local extinctions and decrease of natural populations of wild bees (Williams 1989, Williams 1991). As a result, crops face productivity gaps because of insufficient pollination.

Within the goals of the Convention of Biological Diversity (CBD), the scientific community set up measures to protect pollinator species and to use them in a sustainable way (International Pollinators Initiative (IPI) 2000) (Brazilian Ministry of Environment 1999).

The present study bears in mind two goals of the IPI: 1) to quantify the value of pollination service and 2) to promote the conservation, restoration and sustainable use of pollinator diversity in agriculture and related ecosystems.

1.2 Fruit producers' profile

In Colombia, fruit production is one of the most important sources of income in rural areas. After coffee and flower production, exotic fruits are one of the best known products of Colombia in the international market. Examples are tamarillo (*Cyphomandra betacea*), passion fruit (*Passiflora edulis*), granadilla or golden passion fruit (*P. lingularis*), and cape gooseberry (*Physalis peruviana*) among others. However, most of the national production is assigned to the demand of local markets. In contrast to banana, another traditional Colombian export product, those fruit crops are produced by small farmers who mostly practice traditional agriculture in small monoculture plantations of 0.6 ha. (Table 1.1).

Table 1.1 Fruit producers' profile. General characteristics of farms and agriculture practices. Data extracted from DANE 2004.

Farm land type	Average scheme	Fruit crop agriculture management	Percentage of farmers that apply
Average cropped area	0.6 ha	Chemical pesticides	70%
Land tenure scheme	> 50% private	Chemical fertilization	70%
	< 50% leasing and sharecropping	Technical assessment	13%
Vegetation cover management	13% fruit crops	Management of pollination	None or not reported
	50 % pastures and rest land	Harvest classification before marketing	70%
	16% transitory crops	Financing with own resources	> 90%
	20% non-cleared native vegetation		

This management scheme has as consequence that the presence of natural beneficial organisms is not encouraged, as clearing of native vegetation diminishes

pollen and nectar sources, and the frequent use of pesticides as a phytosanitary measure affects insect species.

Farmers additionally face the problem of fluctuating local prices. The possibility of serving export markets is very attractive and promising, but it is not possible for many producers because of the requirements of quality and constant production. Consequently, the adoption of technologies that improve production is a priority factor for reaching international markets, but this means not only that yields must be secured but also that quality standards must be reached.

1.3 *Solanum quitoense*

Lulo or Naranjilla is the common name of *S. quitoense* fruits. It originated in the high mountain regions of the northern Andes. It is on the list of the 10 most promising agro-industrial fruits of the Colombian ministry of agriculture due to its highly appreciated taste and nutrition content. Although *S. quitoense* fruits are named “the fruit of the Gods” by many authors, the plant is only locally commercialized, but its market has a high potential in wider international trade (National Research Council 1989, Nee 1991). Furthermore, the studies for pest risk analysis (PRA) (FAO 2003) have already been done for this species and it is listed as a product allowed to enter big markets such as the American market (ICA et al. 2004). Unfortunately, farm production is insufficient because of inefficient agricultural techniques (Angulo 2003; Bernal et al. 1996; Franco et al. 2002; National Research Council 1989).

Solanum quitoense is cropped at altitudes between 1600 and 2600 m asl depending on variety. Plantations are sowed with either one of the two natural varieties (*Solanum quitoense quitoense* and *Solanum quitoense septentrionale*) or the hybrid *S. quitoense* var. La Selva (Eraso 1991a). The optimum altitude has been determined at around 1800 m asl for the *quitoense* and 2200 m asl for the *septentrionale* variety. Optimal temperatures range between 15°C and 22°C but the species is very susceptible to frost. As an under-story growing species, it needs short days and shadow of 25-50%. Optimal soils are characterized by high organic matter, rather acid pH values and good drainage (Estrada 1992; Franco et al. 2002). The plant is susceptible to several pests and fungal diseases but, their control is strongly related with crop management, and some can be avoided with the control of abiotic conditions (Eraso 1991b). On the other hand,

it is necessary to study the improvement of genetic material used to propagate and produce seeds. Until now, only low attempts have been made for genetic selection because the species itself is still in the process of domestication and has a too low genetic variability to allow development of resistant varieties (Estrada 1992).

The plant is a bush of up to 3 m height with a thorny stem and branches. The oblong-oval leaves are 25 to 30 cm long and also have thorns and abundant hairs on the dark green blade. Each plant produces inflorescences with up to 15 flowers of two morphs: long-style flowers (LSF) and short-style flowers (SSF) (Whalen and Costich 1986, Miller and Diggle 2003). In the inflorescence of *S. quitoense*, not more than one mature flower is open at the same time. This reproductive system is called Andromonoecious in which only the LSF flowers are functionally female and produce a fruit.

The flower of *S. quitoense* has typical *Solanum*-type morphology (*sensu* Vogel 1978). The sepals and corolla of LSF and SSF flowers are identical, but the principal morphological difference between them is the length of the style (Whalen and Costich 1986). The sepals are green violet covered with hairs and the corolla has five conspicuous white petals. Each flower bears five dark yellow anthers, not fused at the base in form of elongated sacs around 11 mm long with two apical rostrate pores. In the bud, the anthers have a yellow-cream color. They gain their dark-yellow coloration during dehiscence (opening of the pores) and become brown yellow in the senescent flower. Because pollen is enclosed in the poricidal anthers and can only be liberated through vibrations, the flower of *S. quitoense* is classified as buzz pollinated.

Moreover, the gynoecium of both flower morphs differs mainly on the longitude of the style, so that the position of the stigma is within the androecium of SSF flowers while in LSF flowers the stigma exceeds the anther length, opposite to their apical tips due to a deflection of the style (Figure 1.1).



Figure 1.1 Floral morphs of *S. quitoense*. a. Long-style flower (LSF) morph. Note that the style is long exceeding the tip of the anthers with the stigma pointing to one side of the floral axis. b. Short-style flower (SSF) morph. The stigma does not exceed the anthers.

Although SSF flowers are complete, they do not set fruit.

The ripe fruit is a gold-orange berry with green-yellow flesh and more than 900 seeds. Fruits have a diameter of 5 cm and a weight of 40 g. They are juicy with a high content of vitamin C (30 mg/100 ml) and calcium (34,2 mg/100ml) (Franco et al. 2002).

1.4 Andromonoecy in *Solanum*

The andromonoecy reproductive system is commonly present in species of the *Leptostemonum* subgenus of *Solanum*. The sex-expression pattern varies from weak andromonoecy species that bear inflorescences with few staminate flowers at the tip to strongly andromonoecious species that bear just one hermaphrodite flower at the base (Whalen and Costish 1986). No species with purely female flowers have so far been found (Symon 1979).

The evolutionary meaning of this reproductive system is not clear, and several hypotheses have been stated to explain its origin (for a list of references see Whalen and Costish 1986). One hypothesis states that this reproductive system is a halfway to dioecy so that cross-pollination is promoted. A second one explains the excess of staminate flowers as attraction and maintenance for specific pollinators (Symon 1979). Although studies of some species such as *S. hirtum* (Diggle 1991) and *S. carolinense*

(Solomon 1987) support the second hypothesis as the role of andromonoecy, for *S. quitoense* it remains unknown.

1.5 Bumblebees of Colombia

Colombia is a mega-diverse country that lodges a high number of species, ecosystems and endemic organisms within biodiversity hot-spot territories (Didier et al. 2000). Unfortunately, due to a lack of bee surveys, there is no clear overview of the fauna of native bees. Until now 564 bee species have been described but a much higher diversity is expected (Nates and Gonzalez 2000). There are still taxonomic problems in many groups, and for most of them the natural history and ecology are unknown. Therefore, bee fauna in ecosystems in Colombia might be endangered because of a lack of information that would help to set environmental measures to protect them.

In the Andean high mountains there are more than 70 bee species from 25 genera (Gonzalez et al. 2005), which evolve under the particular abiotic and biotic conditions at altitudes higher than 2500 m asl. Most of them are endemic species that depend on specific plant resources to maintain and develop their populations. The fauna of large bees (body size larger than 15 mm) is relatively poor in the high Andes with two established genera *Centris* spp (*C. festiva*) and *Bombus* spp and individual bees from two genera from low lands that are occasional visitors of altitude ecosystems (*Xylocopa* spp and *Eulaema* spp) (Gonzalez et al. 2005).

In Colombia, the genus *Bombus* (bumblebee) is composed of 9 to 12 species (Abrajinovich and Diaz 2002, Liévano et al. 1991) but just one occurs at low tropical altitudes (*B. transversalis*). The other species are typical of the Andean mountain fauna. Bumblebee species are distributed up to 4750 m asl (Osorno and Osorno 1938, Liévano et al. 1991, Abrajinovich and Diaz 2002, Abrajinovich et al. 2004). Although the genus *Bombus* has been studied worldwide, the biology of Colombian bumblebees is not known. For example, the nest architecture of the *Robustobombus* species of the Neotropics has not yet been described and the only available report is of a single nest of *B. malaleucus* (Hoffmann et al. 2004). Furthermore, the taxonomy of *B. hortulanus* and *B. robustus*, species of the same group, is still confusing (Williams 1998).

Bumblebees of the *Bombus* genus are known as good pollinators of many wild plants in regions where other pollinators are often absent due to low temperatures at

high altitudes. This is the case for the plants of the Asteracea family which covers a high range of altitude. Its flowers are frequently visited by workers and queens of the *Bombus* species (Cruz et al. 2005, Cuervo 2002), principally in search of nectar. Additionally, bumblebee workers collect pollen from flowers of Solanaceae and Melastomataceae, species that need buzz pollination. As explained above, this type of pollination consists of the extraction of pollen through the vibration of the poricidal anthers that enclose it. The vibrations are caused by the bumblebees when they visit the flower. The bee grasps the anther and buzzes its thorax muscles so that the movement is transferred to the anthers and the pollen is expelled through the pores of the anther (Buchmann 1983). For example, in tomato, the intensity of the bruises left by foragers on the stamen are interpreted as a sign of the amount of vibrations that the forager performed during the visit. These bruises are related with fruit and seed set and also to a certain extent with fruit quality (Morandin et al. 2001).

The neotropical bumblebee *B. atratus* (*Fervidobombus*) belongs to the group of pocket-maker species (*sensu* Sladen 1912). They offer food to their larvae in a wax pocket built on the side of the larva cell. The distribution of *B. atratus* is very wide in latitude as well as in altitude. The species occurs from the northern regions of the Colombian Andes to the south of Brazil (Williams 1998). Liévano et al. (1991) reported that the altitudinal distribution of this species in the oriental cordillera of Colombia ranges from 150 to 3500 m asl. However, populations are denser at the low montane range (1800 to 2800 m asl) and density rapidly decreases at 3000 m asl where the species is rarely found in comparison to other *Bombus* species.

Nests of *B. atratus* are perennial as are most species of the neotropics. Colonies of *B. atratus* can reach very large sizes (1000 workers) through the replacement of the founder queen by young queens, descendants of the same nest, during a polygynous phase that alternates with a monogynous phase (Cameron and Jost 1998). Nevertheless, in the central region of Colombia, queens cannot be seen flying all year round, and the phenology of queen production is related with the periods after the rainy seasons of the year (Gonzalez et al. 2004, personal observations). In contrast, workers are common all year round. When colonies reach a high number of workers, they are especially aggressive, which makes them unpopular among farmers. The queens frequently build a nest in open grass lands on the ground which is covered by a

layer of dry vegetation. This is an interesting characteristic of *B. atratus*, because it might be the only species that has not been displaced by agriculture and cattle pastures. The workers have a long tongue that allows them to forage on flowers with a deep corolla such as red clover (*Trifolium pratense*) and white clover (*T. repens*), as well as in the disc flowers of Asteraceae. Foragers collect pollen for the immatures from a wide range of plant species (Cortopassi-Laurino et al. 2003, Cruz et al. 2005).

First results on rearing native species in captivity have been achieved with *B. atratus* (Almanza et al. 2003, 2006). Following the methods from Gretenkord (1997) and Plowright and Jay (1966), wild queens were captured and reared in wooden nest boxes at 26°C and 70% relative humidity in a dark isolated rearing room. They were fed with a mixture of fresh pollen and a sugar solution together with an additional sugar solution (50% concentration) in unlimited quantities. When the colonies reached a size of 30 to 40 workers, they were transferred to greenhouses for pollination. Workers in small colonies are less aggressive and thus do not disturb farmers.

1.6 Study area

The study was performed in the department Cundinamarca, a central political division on the eastern cordillera of Colombia where the capital district Bogotá is located. It is a mountainous region with highly different climatic zones that range from low lands at 300 m asl to high mountain “Páramos” that reach 3500 m asl. The region was already formed by human activity even before colonial times; therefore a high proportion of the vegetation has been transformed to culture landscape and pastures. The area is divided into 15 provinces, where economical development is mainly based on agriculture and cattle, while industry is concentrated in the capital district, Bogotá, and its surrounding municipalities. The variety of soils, climatic zones and availability of water make three of the provinces of the Cundinamarca main fruit production areas: Sumapaz, Bogotá D.C. and Gualivá (ASOHOFrucol 2006, MinAgricultura et al. 2004). Particularly, most of *S. quitoense* producers are concentrated in Sumapaz province (MinAgricultura 2004, DANE 2004) where the bee fauna is especially diverse (Nates and Parra 2006) and where *B. atratus* is one of the most common species in the regions below 3000 m asl. *S. quitoense* can also be frequently found growing wild in natural forests in shadow

and humid conditions. Therefore, *S. quitoense* and *B. atratus* have altitudinal overlapping distribution areas and share common biotopes (Figure 1.2).

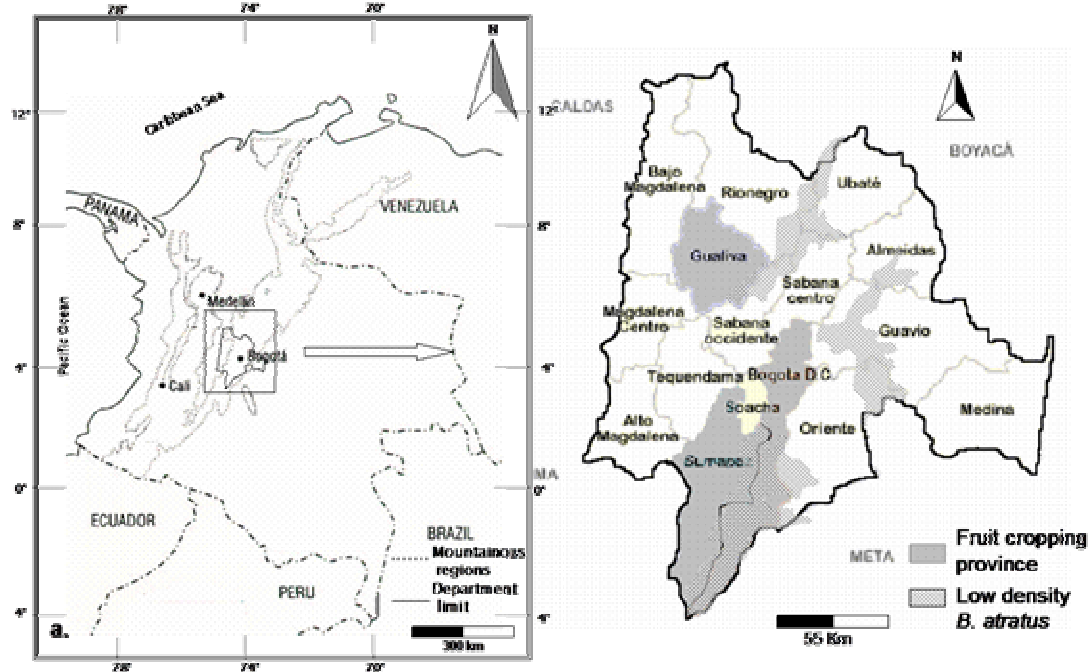


Figure 1.2 a) Department of Cundinamarca in Colombia. b) Location of main fruit production areas Sumapaz, Bogotá D.C. and Gualivá and areas over 3000 m asl where density of *B. atratus* is low.

One of the aims of this study was to develop a management program for a wild bumblebee species for the pollination of *S. quitoense* crops. It avoids introduction of alien *Bombus* species to biotopes where locally adapted species could be endangered.

1.7 Modeling colony dynamics of *Bombus atratus*

In agriculture, models have become a common tool especially for phytosanitary management, but so far little attention has been paid to the use of this tool for pollination purposes (Harder and Wilson 1997). Although pollination has other ecological complexities than predation and parasitism (Thomson 1983), and experimentally it is difficult to track pollen, it is possible to integrate pollination in a model to analyze how plant and pollinator affect each other.

The purpose of developing a model to simulate the development of a colony of *B. atratus* bumblebees was to set up an analytic tool. First, the rearing of bumblebee

colonies can only be improved when the main variables influencing the development of the colony are identified and their effect understood. For example, it is important to quantify the effect of temperature on thermoregulation costs and nectar consumption and its effect on immature development duration and pollen consumption. With the help of a model, the almost impossible task of experimentally testing all variables and their interactions is facilitated through the identification of key parameters. Second, if bumblebee colonies are to be used in commercial scale plantations, the potential effects of new abiotic conditions and resources availability on the development of the colonies need to be foreseen. Here again, a model helps to simulate the dynamics of the colony and identify potentially limiting factors in the field.

The modeling approach starts with the development of the bumblebee colony, which is affected by the plants and species present in the lower trophic level (bottom-up effects) as well as by the species on the upper trophic level (bottom-down effects) (Figure 1.3). The model was initially set up to simulate the development of the colony under controlled conditions, i.e., in captivity.

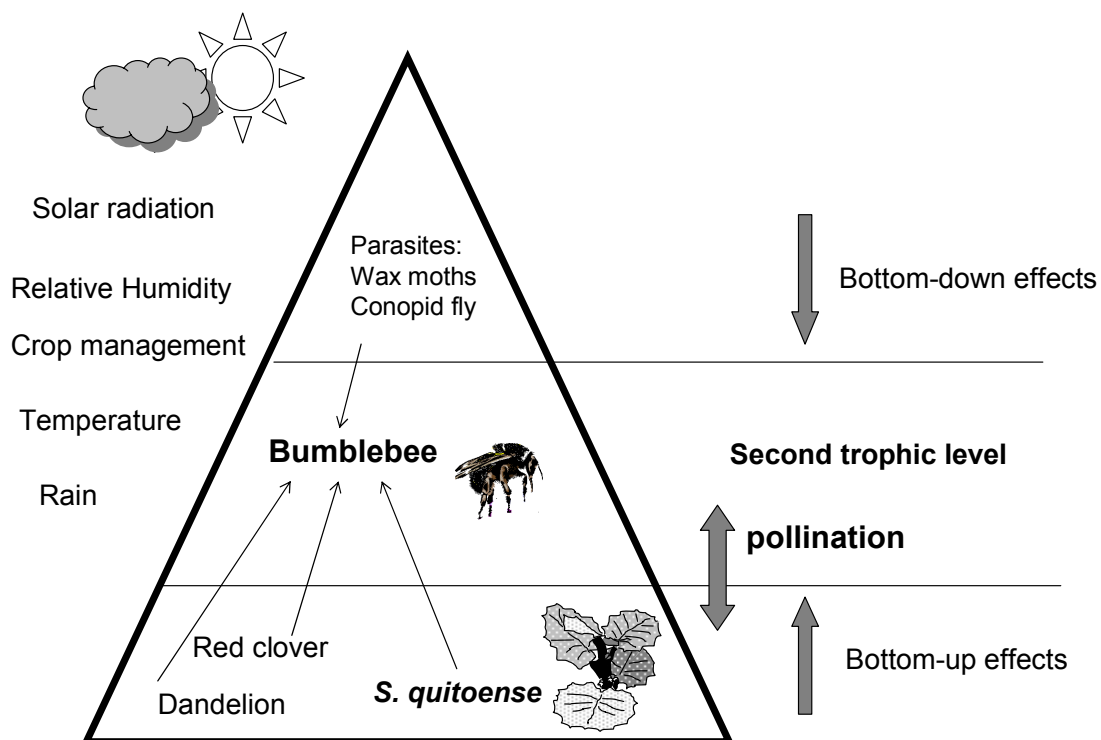


Figure 1.3 Ecological trophic scheme relating bumblebee species with plant's lower trophic level and parasite's higher trophic level. Abiotic conditions affect all levels.

1.8 Proposal for a bumblebee pollination management program

The overall goal of this work was to develop an agriculture practice that can help to overcome the decrease of natural pollination services caused by ecosystem degradation. It includes pollination practices as part of integral production programs (de Oliveira 1997, Westercamp and Gottesberg 2001).

One option to overcome insufficient pollination is to restore natural populations of bumblebees with native colonies reared *ex-situ* (Bohart 1972). Bumblebee rearing world-wide has had already a century of development but the commercial production scale was reached just two decades ago for tomato crops of Europe and America. However, it is still not available for farmers in South America. This is the approach adopted in this study: management of a native bumblebee species within a program that includes rearing of colonies in captivity and setting them in crops for pollination. As in integrated pest management programs (IPM), the base of the practice is the natural biology and behavior of the species. The approach also integrates the ecology of plant-pollinator relationships (de Oliveira 1997, Potts et al. 2001). The approach follows a scheme of integrated elements (Figure 1.4).

In Chapter 2 of this study, the reproductive systems of *S. quitoense* and the effect of bumblebee pollination on fruit yield and the quality of fruits are described. Pollination success was quantified through experiments that compared spontaneous self-pollination with forced self-pollination, geitonogamy and cross-pollination, all three performed by the bumblebee workers when visiting the flowers.

Chapter 3 deals with the behavior of the foragers and with the floral morphology to determine the ability of *B. atratus* to perform as a pollinator of *S. quitoense*. Additionally, the development of a *B. atratus* colony in a *S. quitoense* crop was tracked to evaluate it pollen as resource.

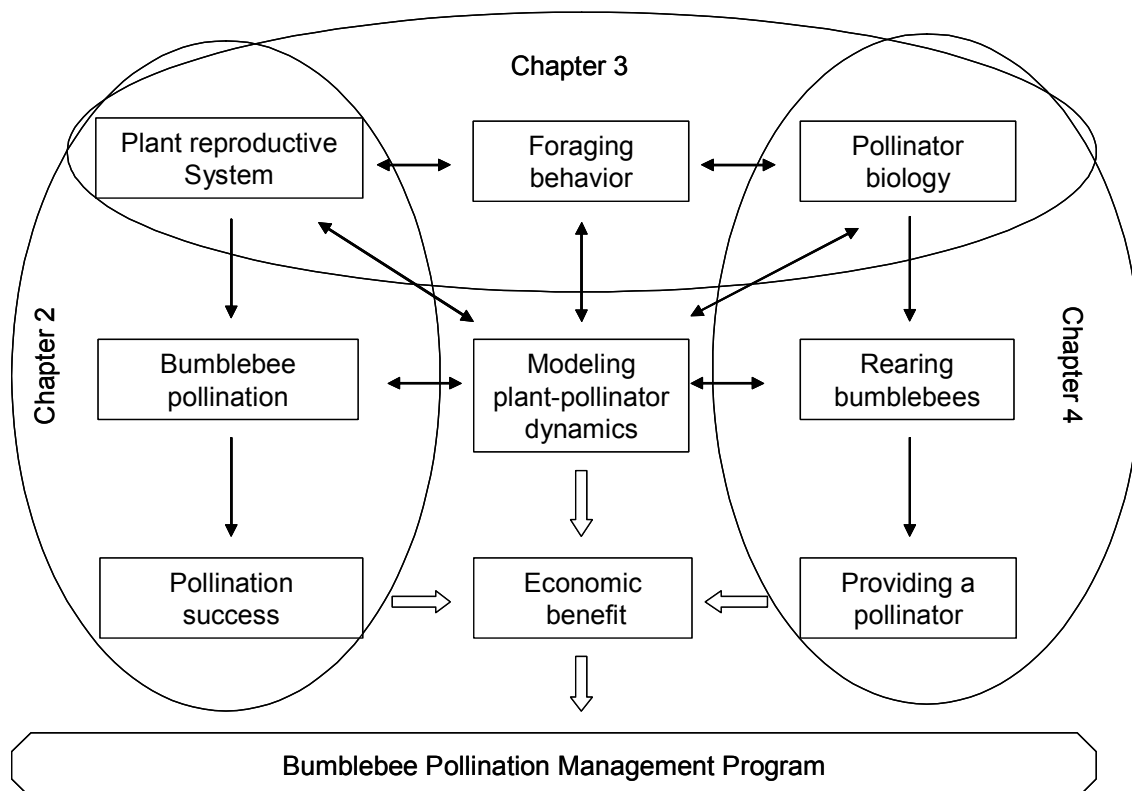


Figure 1.4 Elements and their relationships in an integrated approach to bumblebee pollination management

Chapter 4 describes the dynamics of a colony in captivity under rearing conditions with the help of a model that simulates the growth of the colony depending on the trophic status under two different scenarios: complete captivity and semi-captivity. The model helps to understand the biology of the species so that rearing techniques can be improved.

1.8.1 Objectives and questions

General objectives

- To describe the reproductive system of *S. quitoense*.
- To quantify the effect of pollination by bumblebees on the production of *Solanum quitoense*.
- To describe the behavior of *B. atratus* foragers on *S. quitoense* flowers.
- To simulate the growth of a colony of *B. atratus*.

Questions to be answered

In each chapter, the following questions were answered to reach the above objectives:

Chapter 2

- Why do short-style flowers do not set fruit?
- Does fertilization depend on the floral morph from which donor pollen comes?
- Which pollination cross produces higher fertilization of ovules?
- Is there different fertilization efficiency among spontaneous self-pollination and forced self-pollination?

Chapter 3

- How do *B. atratus* workers handle floral cues of *S. quitoense*?
- Do *B. atratus* workers deposit pollen on the stigma?
- Are sequences of visitation among flowers of different plants more frequent than among flowers of the same plant?
- Is foraging activity synchronized with receptivity of flowers?
- Which resource (pollen or nectar) is collected the most by the colony?
- Do foragers remain constant to *S. quitoense* flowers?
- Can a *B. atratus* colony exist on a diet of mainly *S. quitoense* pollen?

Chapter 4

- To what extent does oviposition rate determine workers' production rate?
- Is immature development restricted by the capacity of the worker to supply their demands despite available unlimited resources?
- In which development phase of the colony is the demand for pollen and nectar highest?

2 **REPRODUCTION OF *SOLANUM QUITOENSE* AND ITS POLLINATION BY THE NEOTROPICAL BUMBLEBEE *BOMBUS* *ATRATUS***

2.1 **Introduction**

Despite the importance of *Solanum quitoense* (lulo) as crop plant in Colombia, there is a general gap in the knowledge on the biology of its reproductive system and its pollination. The information about reproduction and pollination of *S. quitoense* given in cropping guides is scarce, contradictory in scientific publications (see Franco et al. 2002, Hernandez and Martinez 1993 vs. Miller and Diggle 2003, Whalen and Costish 1986) or sometimes even absent (Bernal et al. 1996). Consequently, farmers neglect its importance and employ no pollination management although there is low percentage of fruit set (16%) in commercial plantations (Hernandez and Martinez 1993, Bernal et al. 1996, Franco et al. 2002, Angulo 2003).

So far, it is well known that plants with poricidal anthers, like *S. quitoense*, that enclose pollen need buzz pollination such that performed by bumblebees (Buchmann 1983). It is also known that *S. quitoense* bears a variable proportion of flowers with a short style (SSF) that are female-sterile, and flowers with a long style (LSF) that are female-functional in an andromonoecy reproductive system (Whalen and Costish 1986). However, it is necessary to clarify whether flowers of *S. quitoense* are self-compatible or if, on the contrary, there are mechanisms to avoid it. The possible mechanisms involved are dichogamy (asynchrony of male and female phases of the flower), genetic incompatibility and/or herkogamy (spatial separation of male and female organs within a flower) (Heß 1983). Additionally, because there is a wide range of variation in the expression of andromonoecy (Whalen and Costish 1986) and it is not yet known how gender expression in *S. quitoense* is regulated, the physiology of androecium and gynoecium were tested to reveal the function of the floral morphs (SSF and LSF flowers).

Gender expression *S. quitoense* has been described as a species with a 60% level of andromonoecy (Miller and Diggle 2003). This means that most of the flowers produced by a plant are female-sterile. This characteristic has low phenotypic plasticity

to be expressed, and it varies slightly under different abiotic conditions and fruiting regimes (Miller and Diggle 2003).

The purpose of this study is to reveal particular aspects of the reproductive system and to quantify the effect of bumblebee pollination on yield and fruit quality.

2.2 Question statement

The following questions are answered in this chapter:

- Why do short-style flowers not set fruit?
- Does fertilization depend on the floral morphs from which donor pollen comes?
- Which pollination cross produces higher fertilization of ovules?
- Is there different fertilization efficiency among spontaneous self-pollination and forced self-pollination?

2.3 Materials and methods

Plant material for experimentation was obtained from an experimental crop in Cajicá, a central municipality in Colombia. The region is located at 2558 m asl. Annual mean temperature is 14°C, although extreme temperatures can range from frost to 35°C. External temperatures in the plantation were registered with a data logger (Hobbo®). In July 2004, the culture was set into an area of 312 m² that contained 78 lulo plants (*S. quitoense*) homogeneously arranged in 6 rows of 13 plants each. Agricultural practices for production management of the plantation followed Angulo (2003).

The *S. quitoense* pollination problem was first approached by studying the reproductive system of the plants under the conditions described above. Additionally, two experimental treatments were used to compare the effect of bumblebee visits to flowers on fruit yield and quality.

2.3.1 Reproductive system

Development and reproductive phases of the flower

The continuous process of development of 90 flowers that had not yet opened was tracked every two days. Each day of observation, the flowers were registered and classified into one of five defined stages. The classification of the stages was defined on

the base of observable morphological changes in one of the floral elements (Figure 2.1): 1) Closed corolla bud: the sepals opened and the petals exceeded the length of these but remained closed at the tip. 2) Young flower: corolla opened but whitish-yellow anthers had no anthesis as pores remained closed. 3) Mature flower: corolla reached maximal aperture and anthers had dehiscence. 4) Senescent flower: petals and anthers have a brownish color. 5) Fruit set: petals and anthers dropped, ovary grew. The average duration of each stage was quantified in days.

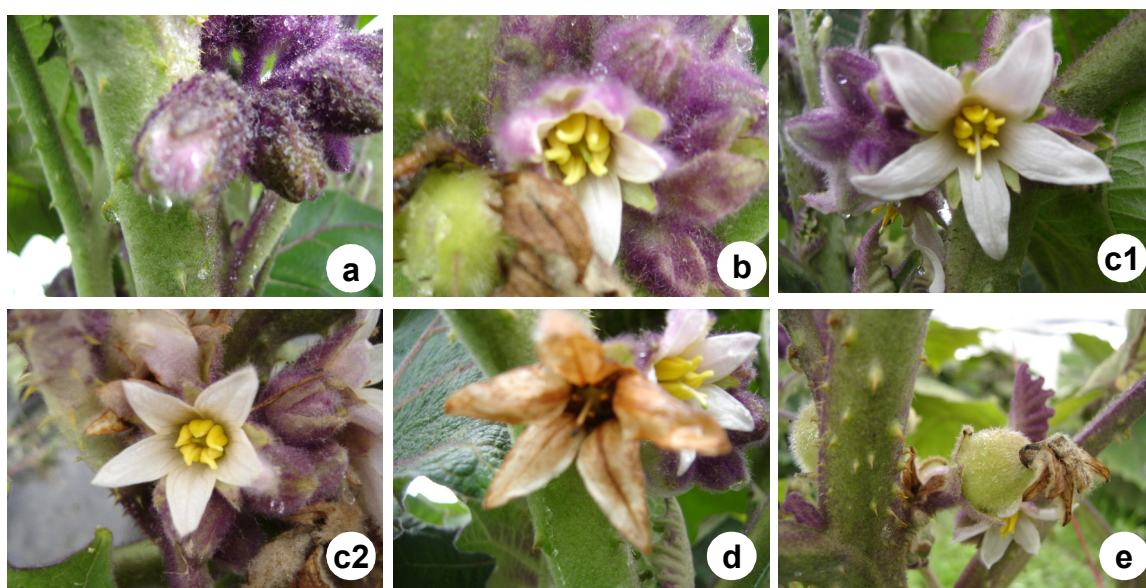


Figure 2.1 Sequence for classification of development stages of flowers. a) closed corolla bud, b) SSF young flower, c1) LSF mature flower, c2) SSF mature flower d) senescent flower, and e) fruit set.

The receptivity of the stigma at the stages when the corolla was open (young, mature and senescent flower) was measured in 31 flowers to determine the duration of the female phase. The receptivity of the stigma was measured with the hydrogen peroxide method ($[H_2O_2] = 3\%$) described by Kearns and Inouye (1993). The presence of bubbling reaction reflects the receptivity of the stigma because of the oxidation process of the peroxidase present on the stigma surface. Buds were bagged to avoid contamination of the stigmas. For each stage, a sample of flowers was cut from the plant and immediately taken to the laboratory where the assay was performed (10 minutes later at the most). In the laboratory, the stigmas were separated from the style taking care that there was no damage to the stigma to avoid scars where the reaction of the peroxidase would be positive (Kearns and Inouye 1993). Each stigma was deposited on

a Petri glass and 0.5 ml of H₂O₂ were dropped onto it. In the following 5 minutes, under a stereoscope the presence of reaction and its intensity was observed.

First, the intensity was measured with a scale of four stages depending on the amount of bubbles seen on the stigma: I. No reaction; II. Low; III. Middle; IV. High. Statistical differences among the categories of intensity were tested using a t-test.

To measure the male phase of flowers, the viability of pollen from bud to senescent flower was measured with the pollen germination test in the Brewbaker-Kwack medium with a 20% sucrose concentration (Kearns and Inouye 1993). A sample of 35 flowers from the 5 defined development stages was cut from the plants and within 15 minutes taken to the laboratory to perform the measurements. At the laboratory, pollen grains of a single anther were collected and deposited on a microscope glass slide (sample unit), and then 0.5 ml of the sucrose medium was dropped onto the pollen. Samples were incubated in a humid chamber (18°C temperature and > 80% relative humidity) and then examined after 24 hours. The percentage of pollen grains that exhibited a pollen tube (out of 100) was determined with a hemacytometer under a microscope. To detect statistical differences between germination of pollen from the two flower morphs, a t-test was done.

Differences among floral morphs

In order to measure the functional differences between the two floral morphs, the receptivity of the stigma and the viability of pollen grains were tested with the same experimental test described above.

The stigmas of 30 open flowers (14 SSF flowers and 16 LSF flowers) were used to compare the receptivity among floral morphs. Two additional variables were measured: 1) the duration of a positive reaction was timed with a chronometer and 2) the percentage of change in color from green to dark-brown on the stigma surface as an indirect sign of esterase enzyme presence and thus receptivity. The differences between the variables were analyzed with a t-test.

In a separate experiment, the duration of stigma receptivity during the day and the highest receptivity moment was measured in a new sample of 31 stigmas from LSF flowers and 19 stigmas from SSF flowers taken at 7:30, 12:00 and 18:30 hours to

perform the peroxidase test. Stigma receptivity was measured again following the same criteria explained above.

The evolution of auto-incompatibility systems in andromonoecious species includes in some cases the evolution of pollen dimorphism, which may be detected as differences in viability of pollen grains (Buchmann 1983). For that reason, the viability of pollen grains coming from anthers of different flower morphs was measured with the germination test in the same medium used in the above described assays. A sample of 70 flowers of both morphs was taken, their pollen germination measured and the differences between flower morphs analyzed with a t-test.

A second approach to determine differences among floral morphs was to compare the weight of androecium and gynoecium. The set of 5 anthers and the ovary with the style were dissected from 18 young open flowers. Each fresh organ was separately weighed on a precision balance (Ohaus). The style was then separated from the ovaries and each was weighed separately. The analysis for significant differences among weights of organs from floral morphs was done with a t-test.

Plant population phenology

Because the sexual expression of SSF staminate and LSF hermaphrodite flowers is important to establish the effect of pollination on fruit set, the amount of flowers produced by 31 plants was measured during 11 months in the research plantation. A census of the total opened flowers was done once a month. During the last month of measurements (July 2005), counting was done more frequently every three days to detect week fluctuations in the relative occurrence of the flower morphs.

2.3.2 Self-incompatibility experiments

Most of the *Solanum* species are self-compatible (Whalen and Costich 1986) but the presence of andromonoecy reproduction like the one present in *S. quitoense* could suggest that out-crossing is promoted (Heithaus et al. 1974). Consequently, a hand-pollination experiment was done to test the hypothesis that cross-pollination (C) has an advantage over self-pollination (S) and geitonogamy (G) (Table 2.1). Additionally, hand-cross- pollination can be used to confirm the need of bumblebee pollination to set fruit (Corbet, et al. 1991).

Table 2.1 Five fertilization crosses between receptor LSF (♀) flowers and SSF (♂) or LSF (♀) flowers as pollen donors. Three possible pollination types derive from the fertilization crosses.

Fertilization cross	Receptor	Donor	Pollination
1. Autogamy	♀	♀	Self-pollination
2. Geitonogamy	♀	♀	Geitonogamy
3. Geitonogamy	♀	♂	Geitonogamy
4. Allogamy	♀	♀	Cross-pollination
5. Allogamy	♀	♂	Cross-pollination

Hand-pollination was done with a 7mm deep plastic vial adapted for that purpose (Figure 2.2). For each trial, the pollen of a donor flower was extracted from dissected anthers and collected in the vial. Then the stigma of the receptor flower (on the plant) was dipped in the donor's pollen. Spontaneous self-pollination was avoided by emasculation of the flowers before anthesis, i.e., the pores were still closed. To maximize pollen viability, fresh pollen from recently opened anthers was used (Stone et al. 1995).

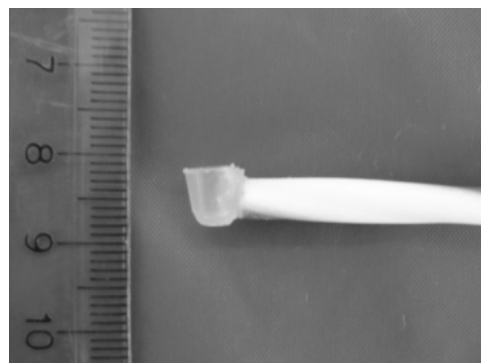


Figure 2.2: Plastic vial used for the collection of pollen and for dipping the stigma of the receptor flower.

Each individual cross trial was tracked until the flower dropped or a fruit developed. Those fruits were harvested after 10 weeks, measured and weighed and their

seeds counted. A comparison between pollination treatments was done with an ANOVA analysis.

The above-described method used was chosen because of the difficulty of hand-pollination of buzz-pollinated flowers (Buchmann 1983, Dafni 1992, Kearns and Inouye 1993). It was assumed that pollen deposition on the stigma was maximized through this method.

2.3.3 Pollination experiments

The effect of single visits of bumblebees to flowers was experimentally tested. A colony of *B. atratus* was placed in the plantation on 27 January 2005 when the *S. quitoense* plants started to flower. The colony contained 30 workers, which were very active at the moment of introduction. The plantation area was enclosed with a shadow mesh above and on all sides so that no other pollinators had access to the flowers.

The experimental treatments were applied simultaneously to avoid the effect of changing climatic conditions on plant development that could affect fruit set. Furthermore, the spatial distribution of the plants in the crop area was homogenous, forming a grid of 6 rows by 13 columns. The treatments were assigned at random to different plants in the grid so that any uncontrolled spatial effect was minimized. In the morning hours of 9 to 15 March 2005, in the crop area with the plants in the first treatment, i.e., pollination by bumblebees, an observer waited until a LSF flower of one of the assigned plants was visited by a bumblebee. The flower was then tagged to record the visit and the date. This procedure was repeated for 7 days until 50 flowers had been marked. The second treatment, i.e., bagged flowers, was applied to closed corolla buds. By bagging the buds with a fine-mesh bag, visits of bumblebees to the open flower were avoided, but wind action was allowed (Corbet et al. 1991, Kearns and Inouye 1993). In the following days, it was checked whether the bagged buds had opened to confirm if they were LSF flowers. When SSF flowers had been bagged, these were discarded and a new bud was bagged to replace them until 50 LSF flowers were bagged. During the next two weeks, tagged and bagged flowers were daily checked to register fruit set or flower drop. Fruits were collected when ripe; weight and size were measured and seeds per fruit counted. Fruit set in treatments was compared using Chi-square, and the mean

value for each fruit variable was compared among treatments with a t-test (Lozán and Kausch 2004).

2.4 Results

2.4.1 Development and reproductive phases of *Solanum quitoense* flowers

The closed corolla bud was the shortest transitory stage and lasted 2.8 days. Then the corolla opened, but the young flower stage when the anthers remained closed was not observed. In all cases, the style had already reached its final length, the anthers had a dark-yellow color and the pores on the tip were opened. This stage lasted for 9.2 days (mature flower) until petals and anthers lost turgidity and gained a brownish color. The senescent flower lasted 14.6 days until it dropped off or alternatively, if pollinated, it lasted 18.5 days when the development of a fruit could be observed (Table 2.2).

Table 2.2 Average duration in days of development stages of *S. quitoense* flower.

	a. Closed corolla bud	b. Young flower	c. Mature flower	d. Senescent flower to drop	d. Senescent flower to fruit set
Average duration of stage (days)	2.8 ± 1.5	Not observed	9.2 ± 3.6	12.6 ± 8.9	18.0 ± 10.7
Maximum (days)	8		18	33	35
Minimum (days)	1		3	2	2
n	87		80	34	37

The young flower stage was not observed; the duration was shorter than the period of observation (two days). The variation of the duration of the mature flower depends on the event of pollination, which shortens the duration. In the senescent flower, the standard deviation is higher than half the average duration. This variation is due to the fact that the non-pollinated flower drops from the plant sooner in windy conditions. On the other hand, when the flower was pollinated it was difficult to see the expansion of the ovary to detect fruit set.

The results of the esterase activity show that LSF flowers are receptive at the moment of corolla opening, as stigmas from young flowers exhibited a positive reaction with H₂O₂ (Figure 2.3). Therefore, the female phase lasted on average 6 days until

senescence when receptivity decreased. The stigmas of SSF flowers also showed positive reactions to the H_2O_2 but the intensities of the reaction were commonly low (Figure 2.3).

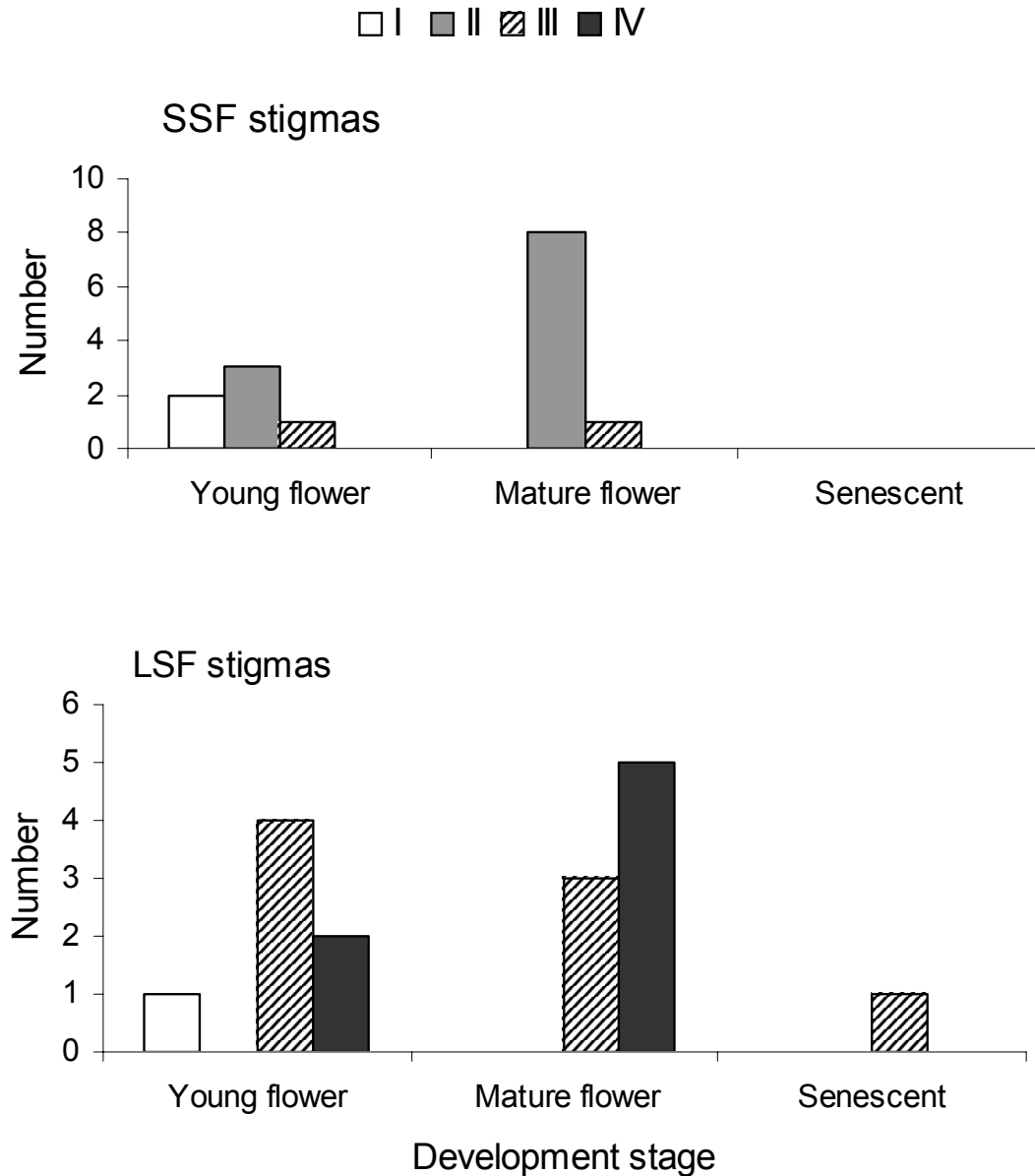


Figure 2.3 Reaction intensities when H_2O_2 was applied to stigmas of two floral morphs at different development stages. SSF stigmas $n=15$, LSF stigmas $n= 16$. Categories: I. No reaction; II. Low, III. Middle; IV. High.

The results of pollen germination in flowers at different stages show that pollen grains of both floral morphs reached maturity before anther pores opened, as anthers from young flowers lodged pollen that germinated (Figure 2.4).

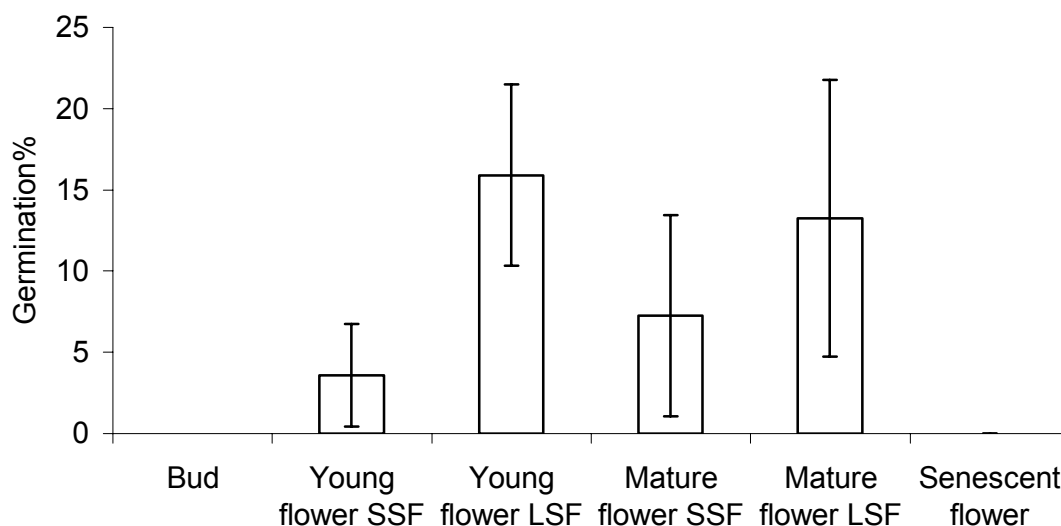


Figure 2.4 Percentage of pollen germination in flowers at different development stages. Bud n= 4; Young flower n= 12; Mature flower n=12; Senescent flower n= 15.

The beginning of the male phase is therefore determined by anthesis in the mature flower and finishes after 7 to 9 days when the flower becomes senescent.

Consequently, reproductive phases are synchronic in LSF flowers with exception of a maximum period of two days between opening of the corolla (young flower) and dehiscence (mature flower).

2.4.2 Physiology of reproductive organs

Stigma receptivity

The positive reactions of the hydrogen peroxide test showed that stigmas of LSF flowers have higher receptivity than those of SSF flowers. Furthermore, stigmas of LSF flowers rarely exhibited reactions of categories I and II but frequently categories III and IV (Figures 2.5). On the other hand, SSF stigmas showed no (category I) or a weak reaction (category II).

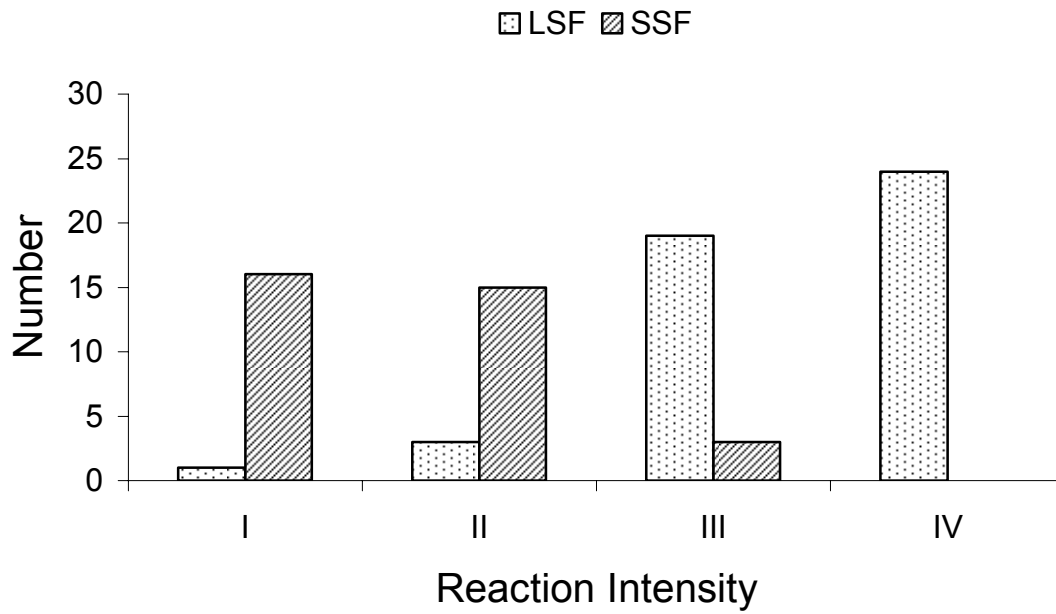


Figure 2.5 Reaction intensities observed when H₂O₂ was applied to stigmas of two floral morphs. LSF stigmas n=47, SSF stigmas n=34. Categories: I. No reaction; II. Low; III. Middle; IV. High.

The second measured indicator of viability, i.e., percentage of stigma surface oxidation, supports these results (Figure 2.6). The percentage of stigma surface that changed color indicating receptivity was larger in LSF than in SSF, as more than 80% of the LSF stigmas turned brown while less than 50% of the surface of SSF stigmas exhibited this change.

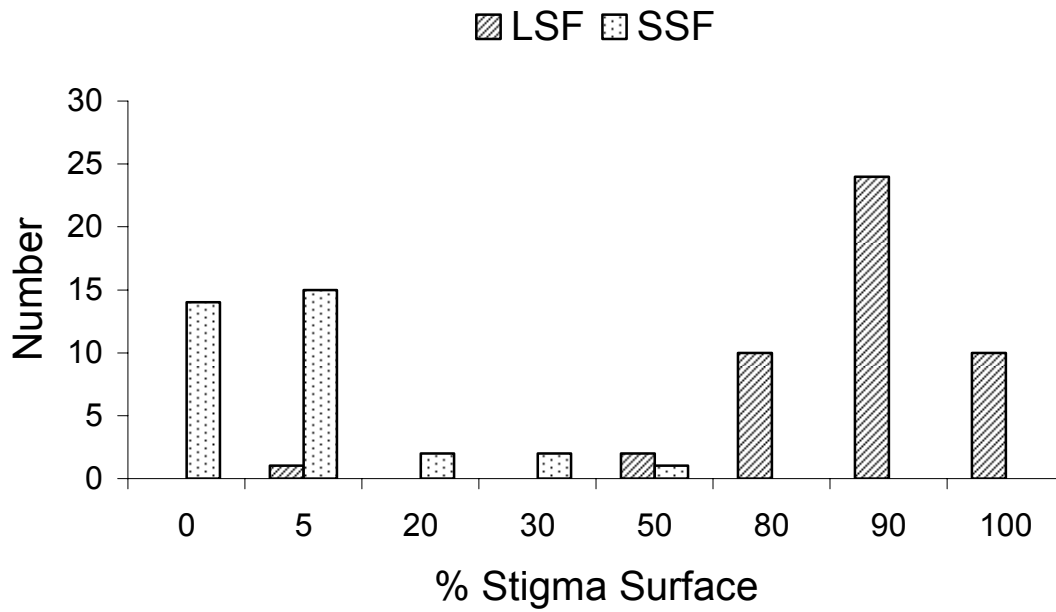


Figure 2.6 Distribution of the change of surface coloration on stigmas after application of H_2O_2 in LSF flowers (LSF stigmas $n=46$) and SSF flowers (SSF stigmas $n=34$).

The mean duration of the reaction was also significantly different between floral morphs ($t=2.12$, $df=21.86$, $p=0.02$), as the stigmas of the hermaphrodite flowers reacted for 193 ± 94 seconds and those of the staminate flowers reacted for 141 ± 43 seconds.

The tests of stigma receptivity at different hours of the day revealed that the reaction of LSF stigmas with H_2O_2 was stronger in the morning and around midday (Figure 2.7)

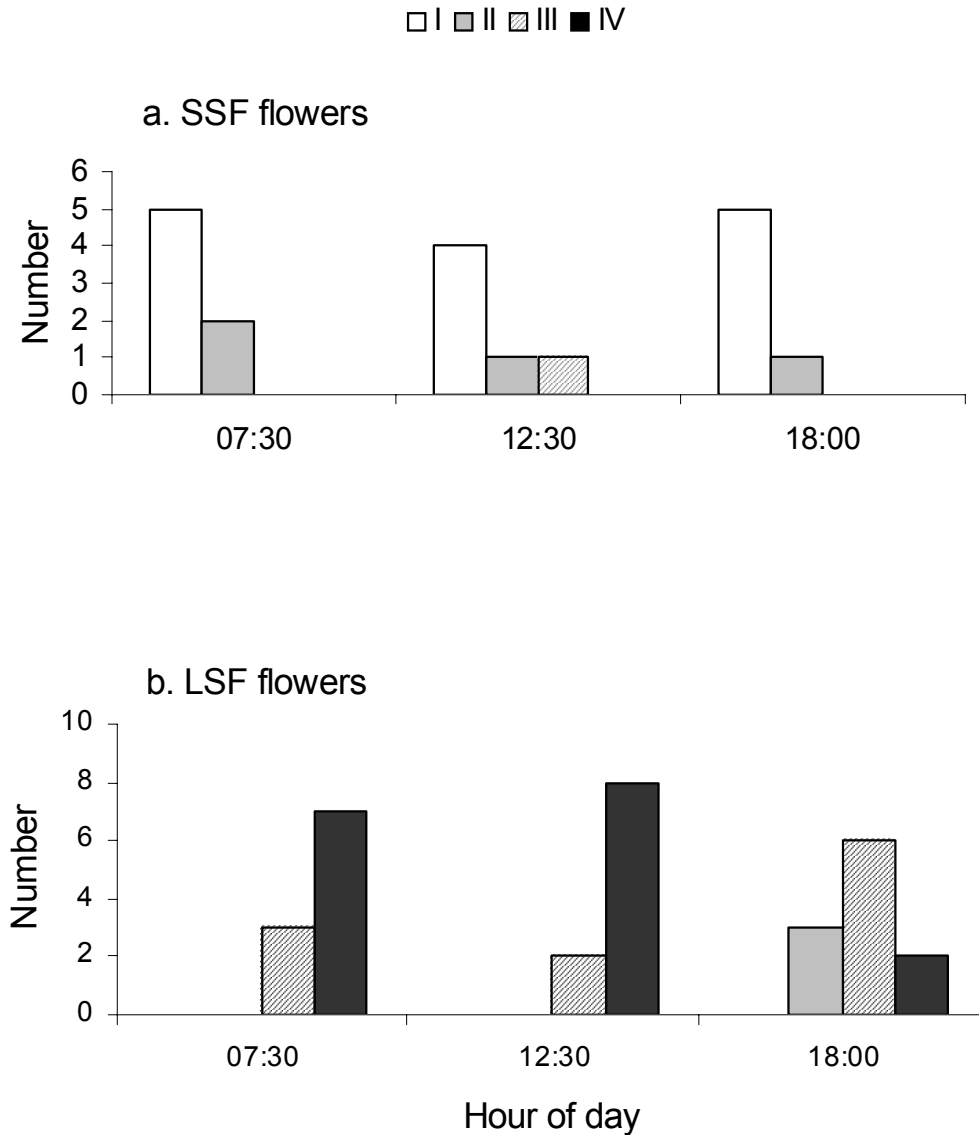


Figure 2.7 Distribution of reaction intensities observed when H_2O_2 was applied to stigmas of SSF flowers (n=19) (a) and to stigmas LSF flowers (n=31) (b) at different times of the day. Categories: I. No reaction; II. Low; III. Middle; IV. High.

The percentage of stigma surface that changes of color is no clear indicator of daily timing of receptivity (Figure 2.8), on the contrary, the total duration of the reaction supports the observation that stigmas of LSF flowers are more receptive around midday (Figure 2.9).

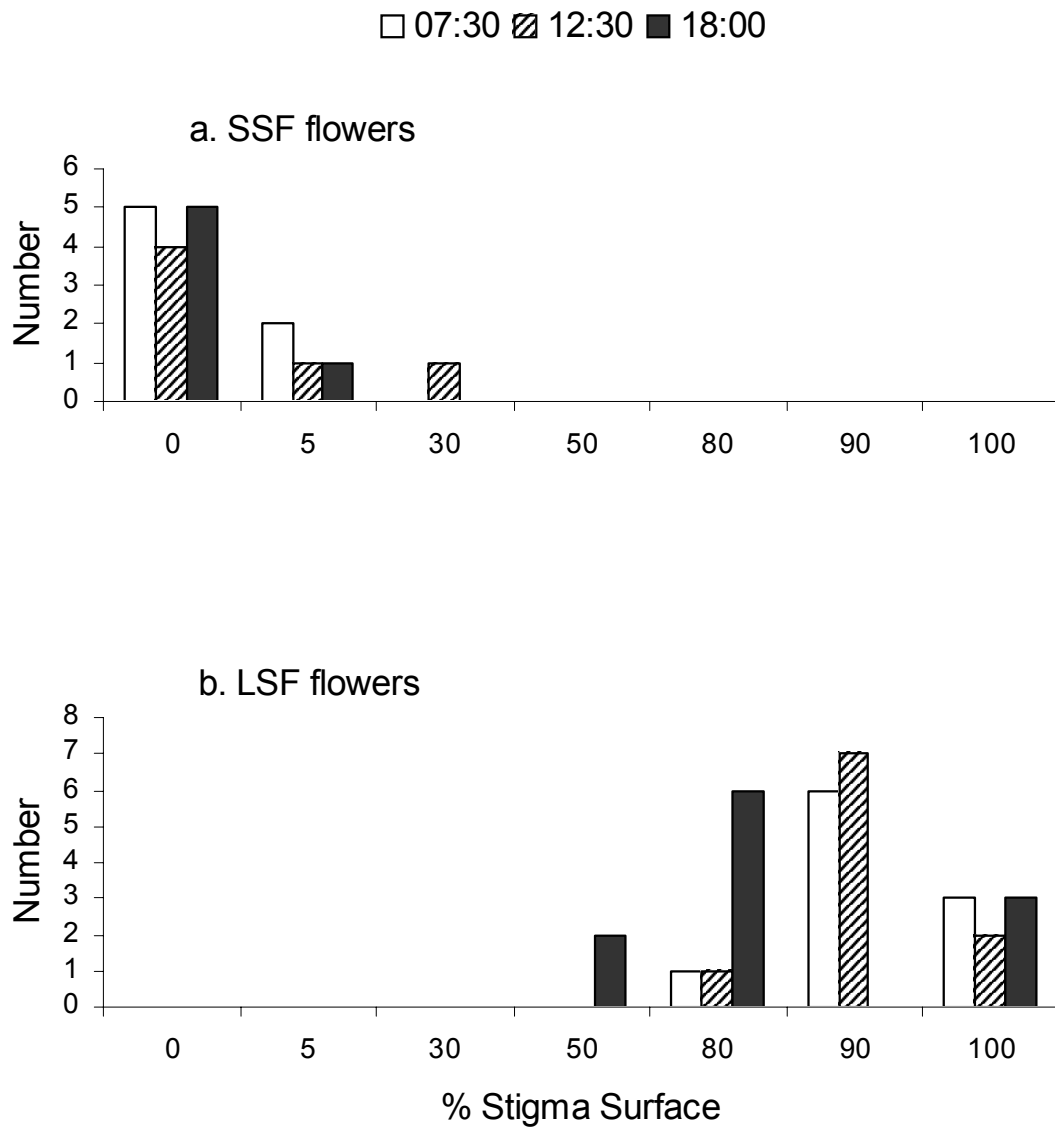


Figure 2.8 Distribution the changes in surface coloration of stigmas after test with H_2O_2 with SSF and LSF flowers at different times of the day. SSF stigmas $n=19$, LSF stigmas $n=31$.

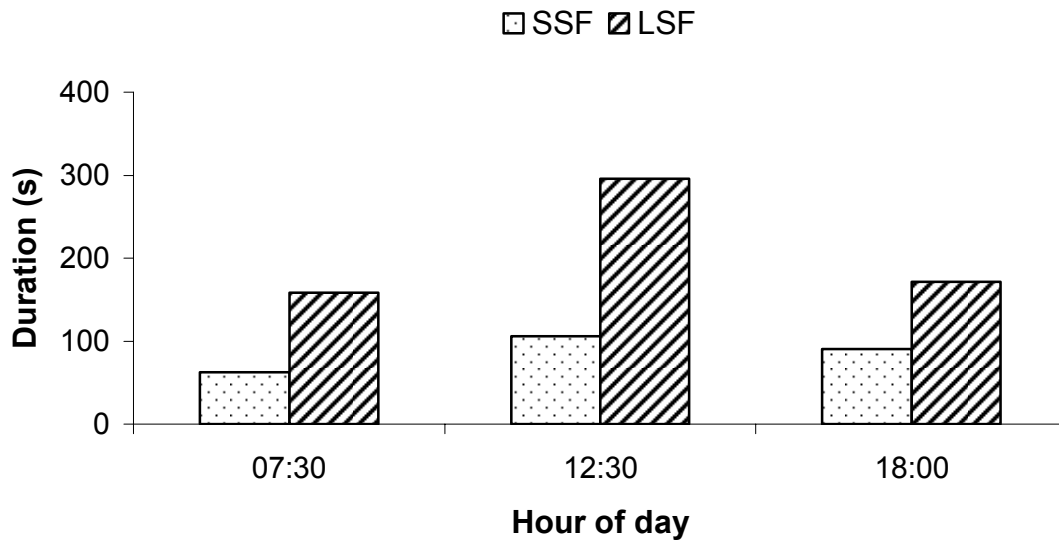


Figure 2.9 Total duration of H_2O_2 reaction on stigmas of SSF and LSF flowers at different hours of the day. SSF stigmas $n=19$, LSF stigmas $n=31$.

Pollen viability

The results show that the proportion of pollen grains from LSF flowers that germinated was higher than that of the SSF flowers ($t = -3.673$, $df = 22$, $p = 0.001$), indicating that anthers of both floral morphs have different pollen viability (Figure 2.10). Percentages of germination in both cases were low (5.42 % and 14.58 %).

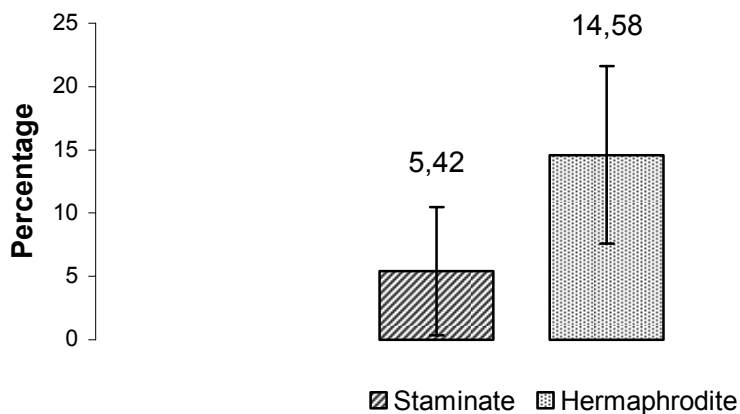


Figure 2.10 Mean percentage germination of pollen grains from anthers of both floral morphs (Glass slides with SSF pollen $n = 12$; glass slides with LSF pollen $n = 12$).

The comparison of the weight of the reproductive organs among floral morphs shows that the androecium of SSF flowers is lighter than that of LSF flowers (Table 2.3) ($t = 4.43$, $df = 14.02$, $p = 0.0003$). Similarly, the gynoecium of LSF flowers is significantly heavier than that of SSF flowers (Table 2.3.). This is due to the weight of the longer style but also because the ovary alone is significantly heavier (Table 2.3) ($t = 6.28$, $df = 16$, $p = 5.436e-06$).

Table 2.3 Weight of reproductive structures of *S. quitoense* flowers.

Floral morph	Mean weight of androecium (g)	Mean weight of gynoecium (g)	Mean weight of ovary (g)	n
LSF	0.23 ± 0.05	0.11 ± 0.03	0.10 ± 0.03	9
SSF	0.17 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	9

Floral phenology

The floral phenology of the plants in the experimental crop showed a degree of andromonoecy lower than 60% at all times of the study. It fluctuated around 29% and never exceeded 45% (Figure 2.11).

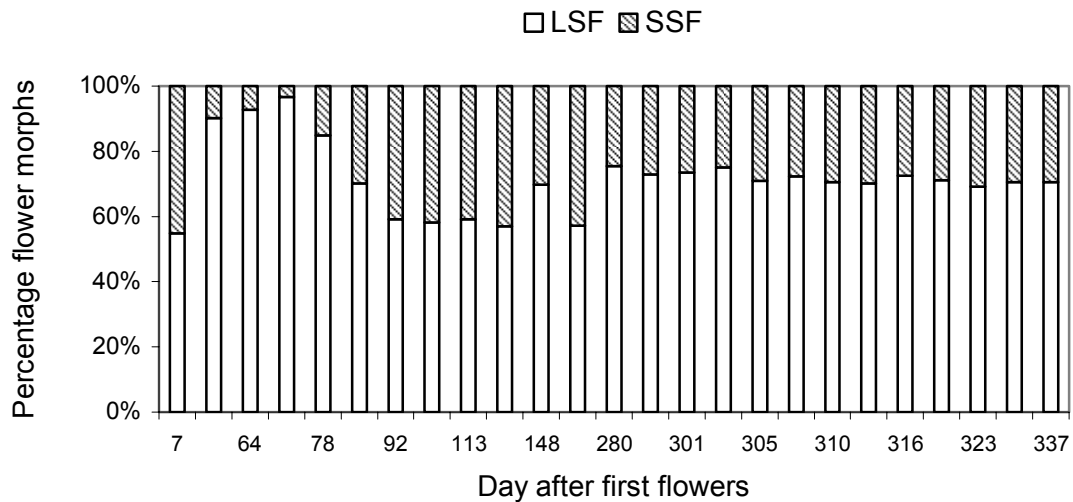


Figure 2.11 Distribution of andromonoecy percentage during a period of 337 days starting at the moment when the first open flowers appeared in the crop (day 1).

However, a sudden increase in staminate flowers was detected around day 213th that coincided with a strong infection of the plants with the *Sclerotinia*

sclerotiorum disease. This observation shows that the expression of gender is labile and that plant condition can affect it.

Hand-pollination experiments

First, by comparing the mean number of seeds per fruit between the three types of possible crosses (self-pollination, geitonogamy and cross-pollination) it can be seen that the fruits obtained through cross-pollination have a significant higher number of seeds (Figure 2.12) (ANOVA $F=9.479$, $df=2$, $p=0.000$)



Figure 2.12 Mean numbers of seeds per fruit for each cross type (self-pollination $n=17$; geitonogamy $n=20$; cross-pollination $n=20$).

Mean fruit weight with cross-pollination was heavier, and consequently the fruits were larger in this treatment (Figure 2.13).

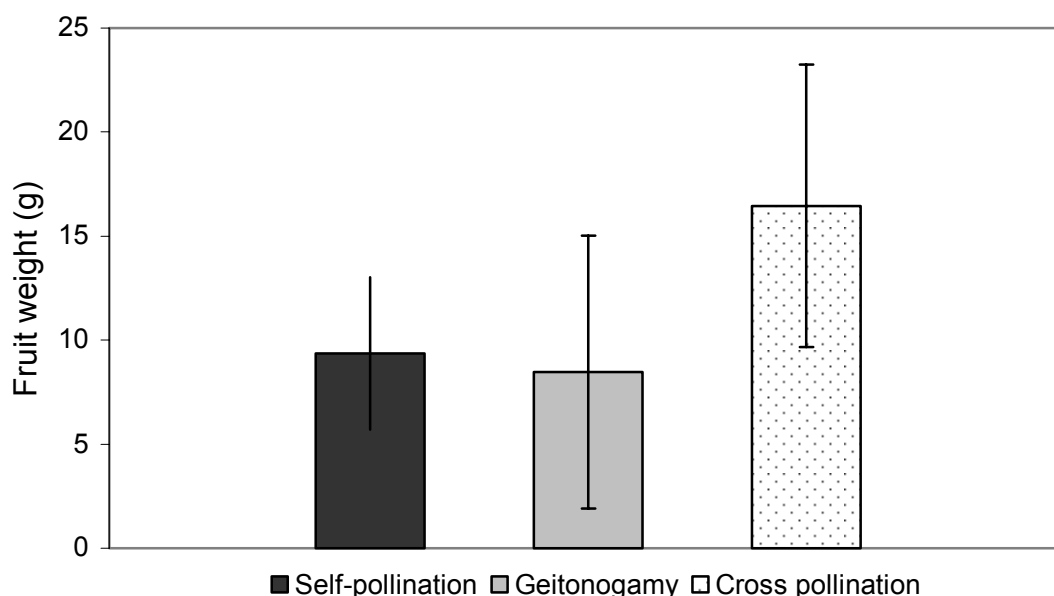


Figure 2.13 Mean fruit weight for three different cross types (self-pollination $n=17$; geitonogamy $n=20$; cross-pollination $n=20$).

Differences were also significant (ANOVA $F=10.65$; $df=2$; $p=0.000$) although the obtained weights are from fruits cropped after 10 weeks when they were not completely ripe and therefore the sizes had not reached commercial standards.

Furthermore, the mean number of seeds obtained with the hand-pollination method was lower than that obtained in the pollination experiments with bumblebees, showing that buzz pollination is more suitable for reaching a higher fecundation proportion and seed formation. As will be explained with more detail in the next chapter, the behavior of the bumblebee worker during flower visit explains the high pollen deposition.

Second, to confirm differences among fecundation efficiency between pollen from the two floral morphs, the same variables (seed number per fruit and fruit weight) were compared within cross classes taking into account the floral morph that donated the pollen for the cross. No significant difference was found in any of the groups (geitonogamy and cross-pollination) for any of the variables ($t > -1.835$ and $p > 0.083$ in all cases). Therefore, the finding that pollen from different flower morphs has an equal fecundation proportion is not consistent with the results of pollen germination.

2.4.3 Pollination experiments with bumblebees

The results of bumblebee pollination compared with the bagging treatment show that fruit set was highly improved when bumblebees visited the flowers and pollinated them. A significantly higher proportion of fruits was obtained when flowers were visited by bumblebees (76%) against a lower amount of fruits (38%) with bagged flowers that allowed only wind pollination ($\chi^2 = 13.823$; $df = 1$; $p < 0.0001$). In this experiment, bumblebee pollination doubled the fruit yield (Figure 2.14).

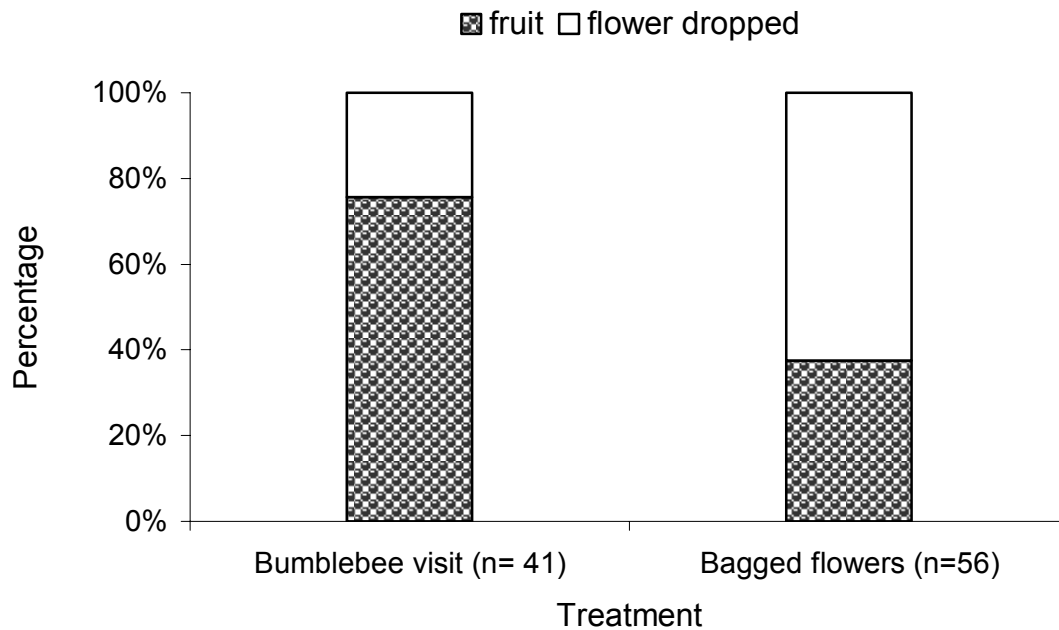


Figure 2.14 Proportion of fruit obtained and floral drop with bumblebee pollination and pollination of bagged flowers by the wind.

Nevertheless, the most important improvement was in the quality of fruits. All fruits obtained through bumblebee pollination fulfilled the commercialization class 1 requirements (ICONTEC, 2002), i.e., the diameter of the fruits was > 5 cm (Table 2.4). For those fruits obtained without pollination, mean size was smaller (< 5 cm) and only 20 % of them reached class 1 quality.

Table 2.4 Comparison of fruit quality variables among two treatments: bumblebee pollination and bagged flowers. (Fruits from bagged flowers n= 5, Fruits from bumblebee visit= 8).

Fruit variable	Treatment		t-test		
	Bumblebee visit	Bagged flowers	t	df	p
Weight (g)	109.4 ± 27.9	26.6 ± 17.8	5.33	10	0.000
Diameter (cm)	5.8 ± 0.7	3.8 ± 0.4	5.27	10	0.000
Seeds per fruit	942.0 ± 367.8	52.8 ± 69.1	4.68	10	0.001

The mean number of seeds per fruit was significantly higher in the bumblebee pollination treatment (Table 2.4). Therefore there is a positive relation between the three variables. The relation between fruit diameter and weight was to be expected, as a bigger fruit contains more fruit flesh. In the same way, the variables fruit weight and number of seeds vary positively and they are highly correlated (Figure 2.15).

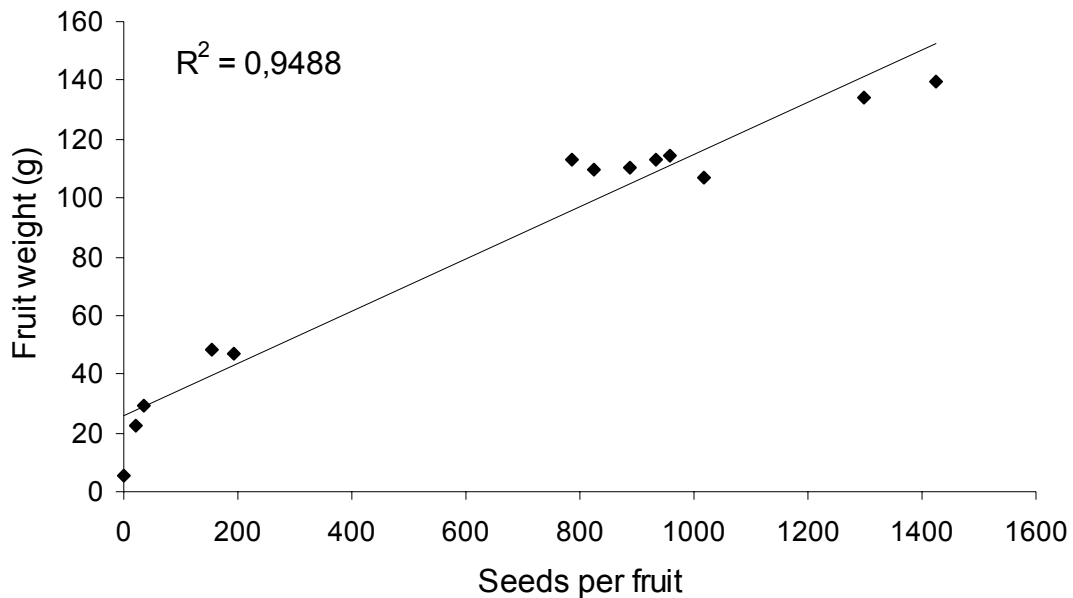


Figure 2.15 Relation among number of seeds per fruit and fruit weight. In the sample, fruits produced by the two treatments were pooled.

2.5 Discussion

2.5.1 Used methods

Pollen germination

The germination method used for pollen viability determination showed that SSF flowers had pollen with significantly lower viability than LSF flowers. This is not consistent with the results obtained in the comparison of seed set in crosses from SSF anthers and LSF anthers in hand-pollination. Seed set parameter was independent from the floral morph that was used as a pollen donor for the cross, so that pollen from SSF flowers rendered the same fecundation proportion as LSF pollen. This contradiction suggests that the germination method to test pollen viability might not be the suitable for *S. quitoense*.

Additionally, the pollen viability found in both floral morphs is extremely low, similar to that reported by Buchmann, who found an extreme case of 95% and 96% inviability in *Casia quiedondilla* (unpublished, cited in Buchmann 1983). One possible explanation is the influence of an external factor affecting pollen viability (Chauhan 1986). However, it is not to be expected that the effect of external factors such as soil quality could differently affect pollen viability among floral morphs. The second possibility is that 20% sucrose medium is not appropriate for *S. quitoense*. Furthermore, other *Solanum* species like *S. elaeagnifolium* exhibit 36% pollen viability when using the method of staining pollen grains with aniline blue in lactophenol (Linsley and Cazier 1963).

2.5.2 Reproductive system of *S. quitoense*

The evolution of andromonoecy has been explained as the result of a selective pressure to promote cross-pollination. It is proposed that the species evolved into a system with functional staminate flowers and hermaphrodite flowers under a selective pressure against self-pollination because of the presence of self-incompatible systems. Andromonoecious is understood as a middle point on the way to dioecy evolution. Consequently, cross-pollination by bumblebees would be advantageous. However, the results of this study do not support this hypothesis.

First, flower development revealed that there is no asynchronous mechanism between the female and male phase to avoid self-pollination in hermaphrodite flowers.

In andromonoecy species of *Solanum*, a weak protandry has been reported but at inflorescence level (Steven et al. 1999). The inflorescence of *S. quitoense* bears up to 15 flowers but they open sequentially so that no more than one mature flower is open at the same time. Therefore, protandry is probably absent in *S. quitoense*.

Second, hand-pollination assays show that *S. quitoense* is self-compatible. This is a common trait shared by species from the *Leptostemonum* subgenus to which *S. quitoense* belongs (Whalen and Costich 1986). However, fruits obtained with cross-pollination treatment show significantly more seeds than with geitonogamy and self-pollination, thus showing that exogamy is advantageous even though self-compatibility is possible.

If andromonoecy in *S. quitoense* is not a mechanism to avoid self-pollination, then the next possibility to explore is the function of the floral morphs and their relation with their potential pollinators. The role of andromonoecious systems has been related to the interaction of the plants and their pollen vectors in many studies done on the reproduction of poricidal Solanaceae and to the high variation of mating systems within the genus *Solanum* (Bertrin 1982, Whalen and Costich 1986, Solomon 1987, Anderson 1989).

2.5.3 Function of floral morphs

The results of the esterase reaction on stigmas of LSF and SSF stigmas confirm one of the main differences between floral morphs. LSF flowers are female functional while SSF flowers are male functional; the fact that their stigmas have low receptivity explains in part the female un-functionality of this floral morph. Additionally, the weight of the ovaries of LSF flowers suggest that they contain functional ovules while gynoecium of SSF flowers might be lighter, because they bear undeveloped ovaries as is the case with other *Solanum* species (Whalen and Costich 1986).

Long-style Flowers

LSF flowers have a clear role in the reproduction of the species, as fruit is exclusively formed out of hermaphrodite flowers. The assays comparing self-spontaneous pollination with self-forced pollination by bumblebees show the need of a vector to increase pollen deposition on the receptive stigma. It was indirectly shown that the visit

of a bumblebee causes high fecundation of ovules that lead to the formation of seeds (Dafni 1992, Kearns and Innouye 1993). Because the fruit starts to grow after fecundation of ovules is achieved due to the activation effect of hormones secreted by the seed on the surrounding cellular layers of the mesocarp (Lobo 1988, Hernandez and Martinez 1993, Raven et al. 2000), fruits with a higher number of seeds are bigger and heavier. The results show the positive effect of bumblebee pollination on fruit growth and quality and the important implications for fruit marketing.

The pollen from LSF anthers is viable as it exhibited the highest germination percentage among the floral morphs (14%). Additionally, in hand-pollination crosses done with LSF pollen as a donor, fruit-bearing seeds were produced. Based on these two facts, it can be stated that LSF flowers of *S. quitoense* retain their male functionality and are therefore hermaphrodite.

Short-style Flowers: pollen flowers

It is evident that SSF flowers of *S. quitoense* are “pollen-flowers” with typical “*Solanum*-type” characteristics (*sensu* Vogel 1978); the flower has radial or zygomorphic symmetry, offers no nectar as reward for pollinators, and has enlarged turgid anthers with showy a yellow color that strongly contrasts with the whitish corolla. The morphology of anthers among floral morphs shows no evident heteranthery, but the comparison of pollen viability shows that the pollen grains from the SSF flowers had a lower mean value of germination. These differences can be interpreted as an intraspecific dimorphism in pollen viability commonly found in poricidal plants (Buchmann 1983). Additionally, the difference in the weight of anthers from the two floral morphs could be explained by a larger amount of pollen in the anthers of LSF flowers or alternatively by a pollen dimorphism with SSF flowers having smaller pollen grains. This observation needs to be tested, but it is consistent with the significantly low pollen viability of SSF flowers. Nevertheless, as the method to test pollen viability is not congruent with the results of hand-pollination crosses, further experiments to test the existence of a pollen dimorphism *S. quitoense* should be performed.

If the fact that pollen of SSF flowers is less viable, it could be interpreted that SSF flowers are a production surplus on which the plant invests as “bee food” to attract

and maintain pollinators (Symon 1979). The SSF flowers would promote visitation but do not necessarily increase cross-pollination (Anderson 1989, Symon 1979, Whalen and Costich 1986). Nevertheless, it is necessary to test if a large number of SSF flowers in a plant is related with high visitation rates of bumblebees.

Other authors have suggested that production of staminate flowers enhances male reproduction of the plant (Wilson 1979, Bawa and Beach 1981, Bertrin 1982) as this also occurs in other andromonoecious plants (Podolsky 1993). However, the results showing a low germination percentage for pollen grains of SSF flowers might exclude this possibility. A high proportion of staminate flowers (degree of andromonoecy) has been also interpreted as a sign of sexual investment of the plant in male gender as a response to the availability of resources. Solomon (1987) studied the investment of resources in hermaphrodite and staminate flowers in *S. carolinense* and found that male flower production increases when the fruit set is limited. In the present study, the strength of andromonoecy was never higher than 45% but it slightly increased after an event of plant infestation by a fungus. The latter suggests that availability of resources can affect sex gender expression of *S. quitoense*. This is also congruent with the labile sex expression found in *S. hirtum* by Diggle (1991), who found that the ratio of staminate flowers to the total of flower changes under the influence of fruiting regimes and external factors. However, the results of the present study strongly contrasts with a second study by Miller and Diggle (2003) done with four species of *Solanum*. They found that the sexual expression of *S. quitoense* is the least plastic and is not influenced by external factors or fruiting regimes.

2.6 Conclusions

- Although completely compatible, cross-pollination has an advantage in *Solanum quitoense* reproduction, thus favoring pollen transport by *Bombus* bumblebee workers.
- Pollen deposition on the stigma is increased by bumblebee visit.
- Yield and quality of fruits was significantly improved through pollination by *B. atratus*.

3 FORAGING BEHAVIOUR OF THE NEOTROPICAL BUMBLEBEE *BOMBUS ATRATUS* (FRANKLIN) IN A LULO (*SOLANUM QUITOENSE*) CULTURE

3.1 Introduction

In South America, a wide range of native and non native crops could be potentially pollinated by native *Bombus* species such as *Bombus atratus*, a common species that is distributed over a wide latitude range from Colombia to Brazil (Moure and Sakagami 1962, Lievano et al. 1991, Abrahamovich et al. 2004).

In the wild, workers of *B. atratus* forage mostly on flowers of Asteraceae, Fabaceae, Leguminosae, Melastomataceae, Verbenaceae and Solanaceae (Abrahamovich et al. 2001, Cortopassi-Laurino et al. 2003, Cruz et al. 2005). Their pollen loads show that their diet is composed of at least 45 plant species in Brazil (Cortopassi-Laurino et al. 2003) and 14 plant species in 3 localities in Colombia (Cruz et al. 2005). Solanaceae species are always present. However *B. atratus* is a polylectic species, and individual foragers have an aggregated foraging behavior and concentrate on fewer plant species locally (Cortopassi-Laurino et al. 2003).

Due to their body size and the length of their tongue, workers can collect nectar and pollen from flowers with different morphologies. They can reach the nectaries in the bottom of the deep corolla of the red clover (*Trifolium pratense*) or the foxglove (*Digitalis purpurea*) (Cortopassi-Laurino et al. 2003). *Bombus atratus* is a species that is an important pollinator of plants, whereas other *Bombus* species act as nectar robbers (Riveros et al. 2006).

The visit of *B. atratus* workers had good effect on the fruit and seed set of lulo (*Solanum quitoense*) but a description of their foraging behavior was necessary to link this result to the bumblebee's role as a pollinator. For this purpose, parameters of the behavior of the bumblebee were selected after a model adapted from the "horizontal pollination model" of Harder and Wilson (1998). It explains the effect of foraging sequence on pollen deposition.

For pollination to be effective, three main steps must be accomplished: 1) pollen removal from the pollen pool (anther removal), 2) transport of pollen to the next flower of the same species and, 3) deposition of the pollen grains on a recipient stigma to reach the pool of ovules (Figure 3.1). During the process, loss of pollen for pollination purposes are distributed among: losses by falling after being extracted

from the anthers, losses by packing in the corbiculae (the proportion that the pollinator takes) and losses when pollen is not deposited on the stigma because no contact with the stigma takes place. Each of the three steps acts like a filter that diminishes the amount of pollen reaching the ovule pool. The viability of the pollen grains then affects the fecundation rate.

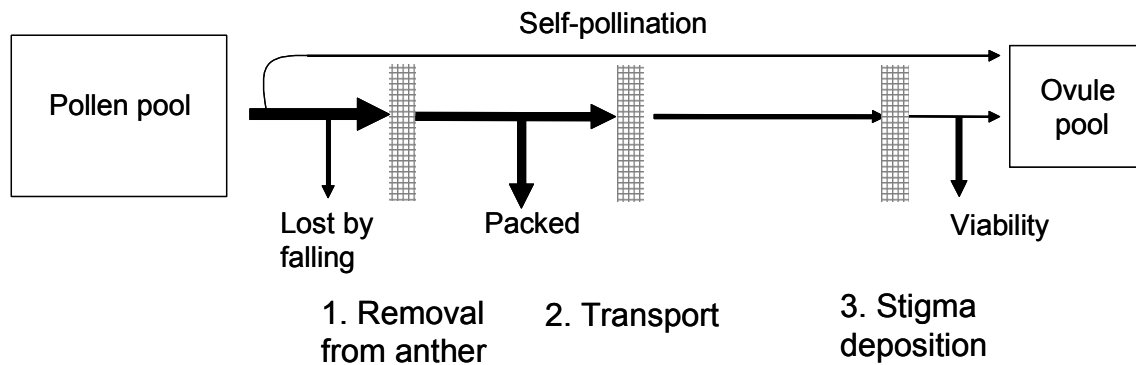


Figure 3.1 Pollination model. The transport of pollen from pollen pool to ovule pool is in three main steps in cross-pollination during which there are losses. In self-pollination, there is direct deposition of pollen on the stigma of the visited flower; consequently it brings pollen directly to ovules pool.

This sequence is that of buzz-pollinated species, such as *S. quitoense*, where an effective pollination can only occur if pollen liberation through vibration of the anthers occurs. Furthermore, studies of the reproductive system of *S. quitoense* revealed (Chapter 2) that forced pollen deposition on the stigma is necessary because spontaneous pollination leads to low fecundation of seeds.

However, it is difficult to track the path of pollen grains transported by the bumblebees to test if the sequence to pollination is achieved. For that reason, other indirect characteristics of the behavior of the bumblebee were registered. For instance, handling time, visitation rate, stigma contact, visitation sequence and pollen load composition are related with high efficiency of pollination (Engel and Irwin 2003, Heinrich 1984, Lavery 1980). Handling time is the duration between landing on the flower until the moment when the bee leaves the flower with pollen. This characteristic shows if foragers correctly manipulate poricidal anthers by buzzing. It also shows how fast the bumblebee can collect the pollen, since time is a critical matter in terms of foraging energetics (Heinrich 1983).

Visitation rate is here defined as the number of flowers that a forager visits per minute. Stigma contact is the contact of body parts of the bumblebee that carry pollen with the stigma. This characteristic is a direct parameter that indicates forced pollen deposition on the stigma. Visitation sequence is the order of visits to flowers during a foraging bout. One foraging bout is defined as the time that a bumblebee spends between leaving the nest and flying back to it after it has foraged several flowers (Waddington 1983). Additionally, the traffic of foragers in and out of the nest quantifies the amount of resources that are brought to the nest (Heinrich 1979, Plowright 1979) and the overlap of foraging activity with the receptivity time of the flowers.

3.2 Questions

- How do workers of *B. atratus* handle floral cues of *S. quitoense*?
- Do workers of *B. atratus* deposit pollen on the stigma?
- Is there a visible mark left after the visit of the worker to the flower that indicates pollination?
- Are sequences of visitation among flowers of different plants more frequent than among flowers of the same plant?
- Is foraging activity synchronized with receptivity of flowers?
- Which resource (pollen or nectar) is collected the most by the colony?
- Do foragers remain constant to *S. quitoense* flowers?
- Does pollen provided by *S. quitoense* allow the development of a colony?

3.3 Methods

3.3.1 Foraging behaviour on *S. quitoense* flowers

A colony of *B. atratus* was introduced to the crop at the end of January 2005. The colony had initially 35 workers among which one developed as a false queen and assumed the reproductive role in the colony. A false queen is a worker of generally large size that dominates the workers after the mother queen of the colony has died. After she mates, its ovaries develop and she starts to lay eggs in the mother colony. However, the fecundity of such false queens is notably inferior to that of a queen (da Silva-Matos and Garófalo 1995).

Before the colony was brought to the crop, all workers were individually marked with numbered plastic discs. The crop was completely enclosed with a 30% shadow mesh to protect the plants from direct sunlight. However, this did not present an obstacle for the bumblebees, as some of them were seen flying outside the crop and returning to the nest. The colony grew during 4 months until the end of April when the activity of the foragers in the crop decreased almost to zero. A feeder with sugar solution (50% concentration) was located at the entrance of the nest because the flowers of *S. quitoense* offer no nectar.

Foraging behavior of individual *B. atratus* workers

The individual behavior of foragers on the flower was qualitatively and quantitatively described by tracking 315 visits of the foragers to the flowers of the crop. A trained observer followed individual workers during 64 foraging bouts. All observations were done at different times of the day during the 4 months by the same observer.

During 26 of the bouts, the following time measurements were done with a professional chronometer (to 0, 1 seconds precision): 1) total bout time as the time between leaving the nest and flying back to it after visiting several flowers, 2) handling time as the duration between landing on the flower and leaving it to collect pollen by vibrating the anthers, and 3) time within flower flight. Additionally, to understand the spatial distribution of foraging behavior and the fidelity to the *S. quitoense* flowers (Waddington 1983), in 38 bouts the sequence of visits to flowers was registered in the following way: S) for a sequential visit to the same flower; G) for a sequential visit to another flower within the same plant and C) for a sequential visit to another flower of another plant. The classes reflected the possible type of pollination that occurred, i.e., S for self-pollination, G for geitonogamy, and C for cross-pollination. If visits to flowers of different plants within the crop area such as dandelion or red clover were seen, they were also recorded.

Foraging activity of *B. atratus* colony

To determine activity of the colony, a second observer stood 5 m away from the nest entrance and counted the number of workers leaving and entering the nest for periods of one to two hours at different times of the day. These observations were done during 47 days between 30 January and 30 April 2005. It was possible to observe the resource that each worker brought to the colony. For each entry, resources were

recorded as 1) nectar if the worker had no pollen load 2) nectar and pollen if the worker had a pollen load and was also observed drinking from the sugar solution in the feeder at the entrance of nest before it entered or 3) pollen if the worker had only pollen loads. In the latter case, it was possible to discriminate by color if the pollen load had more than one type of pollen (white pollen of *S. quitoense*, orange pollen of dandelion and brownish pollen of red clover). If this was the case, then the entry was registered as 4) other pollen. The presence of the observer did not disturb bumblebee activity.

A pollen trap was attached to the nest entrance at three different days (18 and 25 February, and 3 March 2005) from 10:00 h to 13:00 h to take a sample of pollen loads. The trap consisted of a wooden disk with a hole of 6 mm diameter that allowed the average size workers to get into the nest with some effort. When they came with pollen loads, the size of the hole allowed just the worker to go in but removed the pollen loads. The pollen pellets fell into a collecting tray (Figure 3.2). Every hour the tray was checked to collect the loads and take them to the laboratory for weight determination. This was done to avoid fresh weight changes. Unfortunately, the pollen trap notably affected the traffic of workers; sampling was therefore abandoned and the sample size of pollen loads was small (n=13).

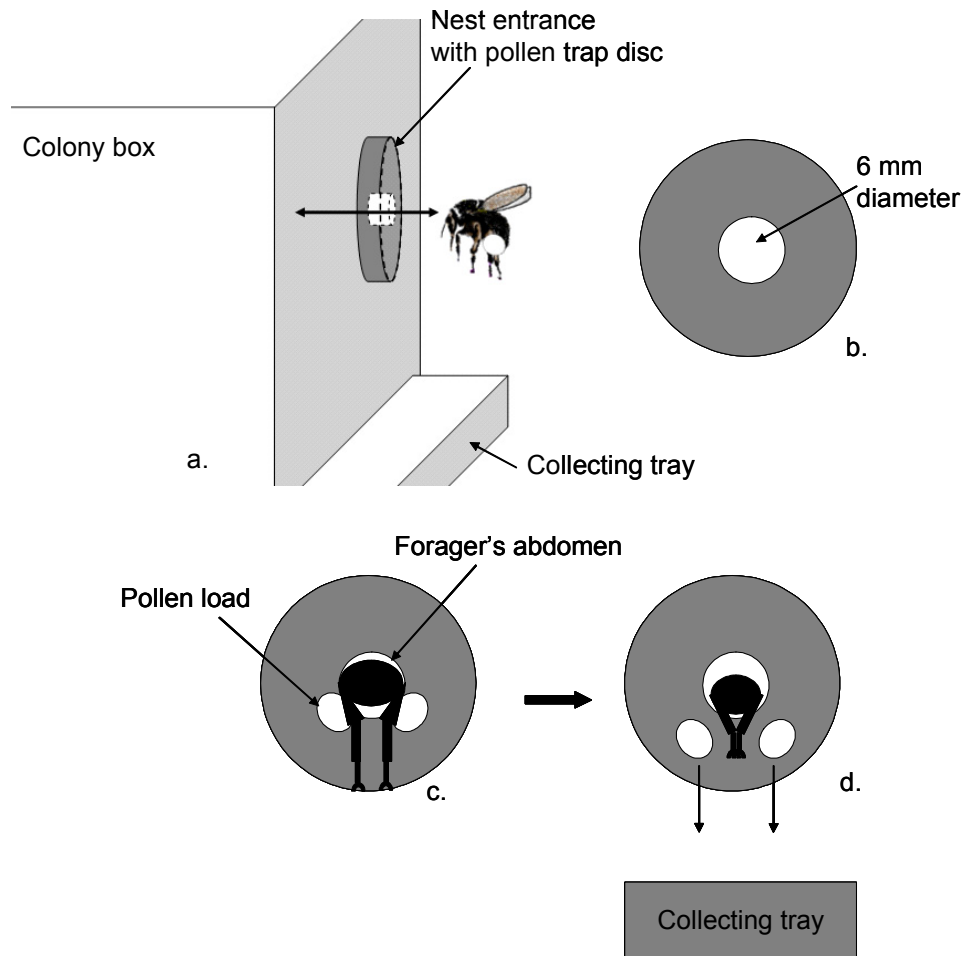


Figure 3.2. Scheme of pollen trap on the nest entrance a. Lateral view of the pollen trap disc attached to the nest entrance. b. Frontal view of the pollen trap disc with hole of 6 mm diameter through which foragers entered and left the nest. c. Frontal view of forager going into the nest. d. Forager goes into the nest but loses pollen loads, which fall into a collecting tray.

Colony development

The development of the colony was tracked once per month (four times) by moving the nest to the laboratory during the early morning hours when most of the workers were still in the nest. The nest was opened and the adults and immatures were censused (when possible). The new workers were tagged with a plastic number on the thorax.

3.4 Results

3.4.1 Behaviour of individual foragers

The behavior of foraging workers on flowers of *S. quitoense* was tracked for 64 foraging bouts. The variables measured during the visit of 315 flowers show that individual behavior varies quantitatively (Table 3.1).

Table 3.1 Foraging behavior on flowers of *S. quitoense*

Behavior variable	mean value	maximum	minimum	n (sample unit)
Handling time	18 ± 23 s	03:22 min	1 s	315 visits
Duration of vibration activity on the flower	1.7 ± 1.1 s	5.0 s	0.6 s	20 buzz
Total bout time	7:18 ± 5:57 min	21:24 min	0:51 min	23 bouts
% visits with more than one round of vibrations	41%			53 visits
% visits with pollen packing in corbiculae	29%			40 visits
% visits with stigma contact	97%			47 visits on LSF flowers

However, the behavior is homogenous between foragers when described qualitatively, so that the foraging pattern of an experienced forager can be described as follows: First, a it landed directly on one of the five anthers. As the corolla diameter of the *S. quitoense* flower is in average 4 cm and the anthers are also large, the forager grasped a single anther at the external side of the base with its mandibles and vibrated it strongly for periods between 0.6 and 5.0 s. This vibration caused a spray of pollen out of the anther through the pores on the apical part. A large amount of pollen grains fell on the body of the forager. After buzzing the first anther, the forager rotated in an anticlockwise direction to the next anther and performed the same action. Each anther was buzzed at least once during a visit, but in 41% of the cases the bee buzzed the anthers up to five times during one visit. In 29% of the visits, the worker combed the pollen on her body and packet it in the corbiculae. Visible pollen grains remained on the body of the bumblebee, especially on the areas on the ventral middle part of the metasoma and the anterior ventral region of the thorax it could not reach. Finally, the bee left the flower. Total duration of the visit changed

considerably between 1 s and 3:22 min depending on the number of times that the bee vibrated the set of anthers and if the worker combed her body and packed the pollen in the corbiculae or not.

On SSF flowers, workers could not contact the stigma because it is hidden under the set of anthers. The bee did not try to reach the interior bottom of the anthers where there are no nectaries. In contrast, stigma contacts were registered on 97% of the visits to LSF flowers. *Bombus atratus* foragers are generally more than 12 mm long. As a result, the body of the forager touched the stigma either while turning around to change from one anther to the other or when combing its body. The bumblebee hung with its fore legs from the anthers and moved the middle and hind legs so that the body contacted the stigma several times. As the bee always turned around the flower, the stigma on the long style was touched at least once. The contact was ventrally with the abdomen or the pronotum of the worker (Figure 3.3).



Figure 3.3 Behavior during the visit to a flower of *S. quitoense*. a. Landing on the flower. b. Vibration of first anther. c. Rotation to vibrate second anther. d. Visit to a short-style flower (arrow signals hidden stigma). e. Pollen combing and packing in corbiculae. Stigma contact. f. Stigma contact during rotating. Note the pollen loads.

After the visit, no visual mark could be detected such as the presence of bruises on the anthers or change in size or turgidity of the anther. It was also observed that flowers were visited more than once by other foragers.

Foraging behaviour of workers

The bumblebees visited preferentially mature flowers of *S. quitoense*. Of the 315 recorded visits, none was observed on a senescent flower and no preferential choice for any of the flower morphs was detected.

Bumblebees visited in average 14.1 flowers per bout but this variable was significantly different at different times of the day (Kruskal-Wallis test: $\chi^2 = 22.16$, $df = 6$, $P = 0.001$) (Figure 3.4). The number of visits varied between 2 and 32 flowers per bout (Appendix.1).

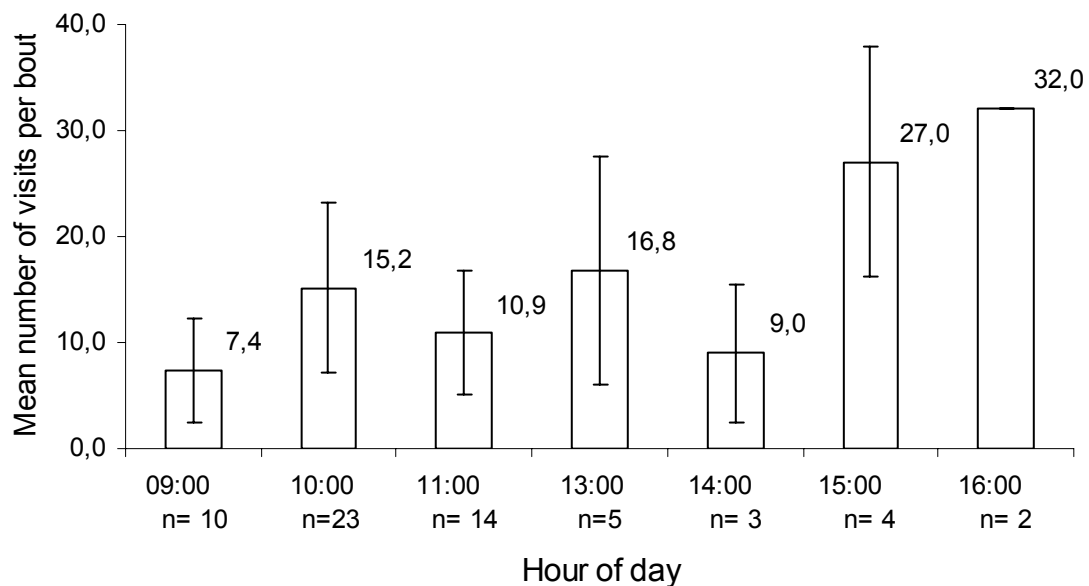


Figure 3.4 Average numbers of flowers visited by foragers during one bout at different hours of the day. One foraging bout of an individual forager is the sampling unit. n indicates the number of foraging bouts tracked at each observation hour.

As foragers returned to the nest as soon as their pollen loads were full, the variation of flower visits per bout shows that they had to visit more flowers in the late afternoon to obtain the same amount of pollen as in the morning.

The duration of the foraging bout varied consequently with the number of flowers visited. Its total duration varied highly between 51 seconds and 21:24 minutes (Figure 3.5). There is a co-relation between the number of flowers visited and the

duration of the total foraging bout ($R^2 = 0.6526$). Therefore it can be inferred that the visitation rate (number of visited flowers per minute) is almost constant at all times of the day.

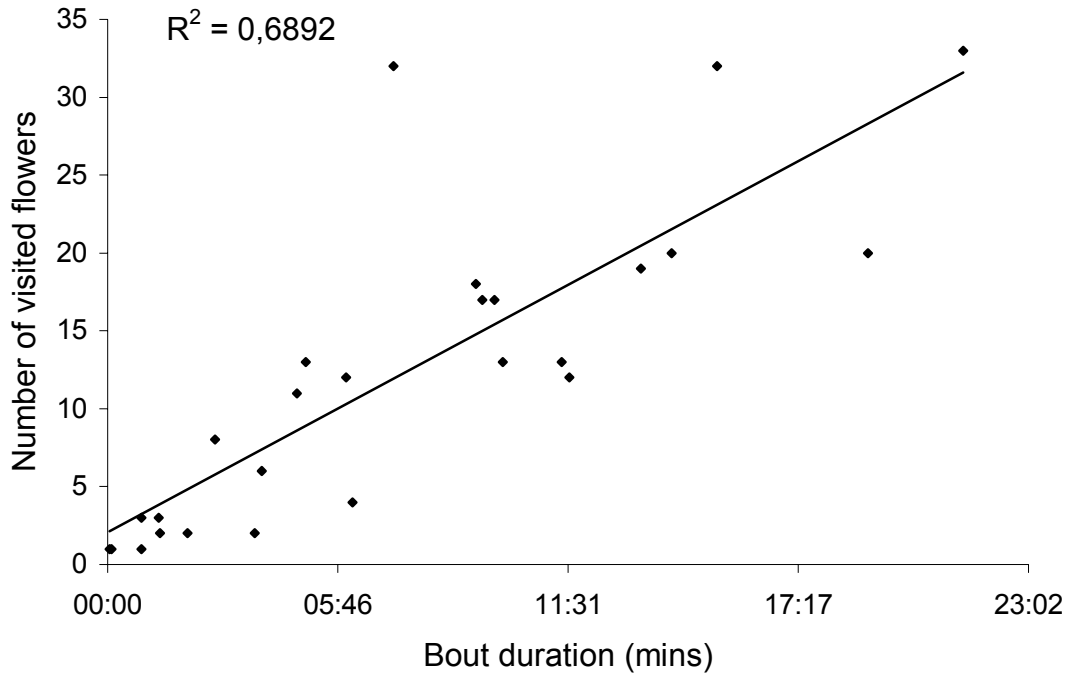


Figure 3.5 Relationship between foraging bout duration and number of flowers a single worker visited per bout.

Visitation sequence

Subsequent visits to flowers of different plants were more frequent than visits to flowers of the same plant ($\chi^2 = 218.09$ $p < 0,001$, $df = 2$, $n = 488$). Interesting is the fact that the visit to flowers within the same plant increased at the end of the day when foragers visited the largest number of flowers per bout (Figure 3.6).

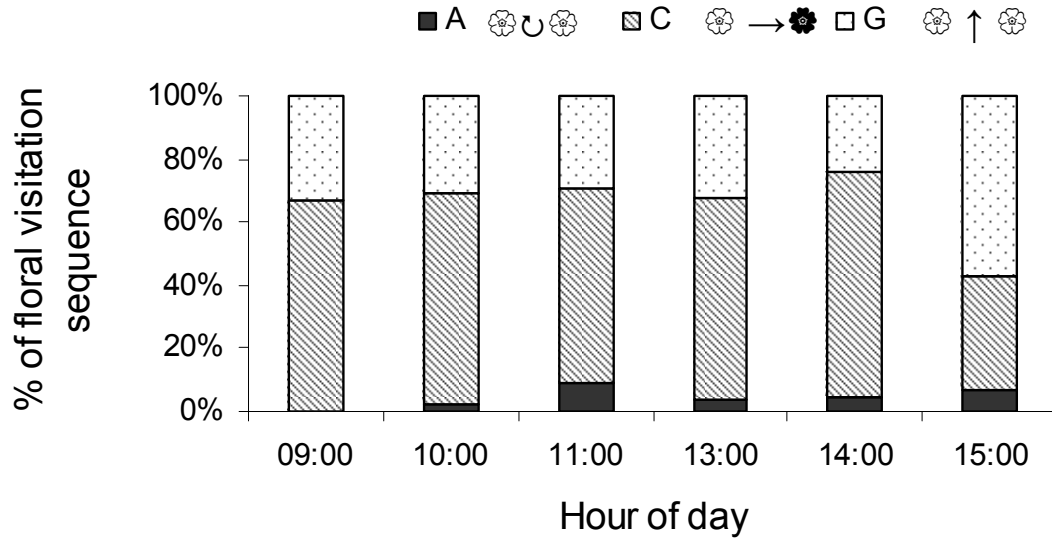


Figure 3.6 Proportion of floral visitation sequences at different hours of the day. 488 interfloral flights are represented as A) sequential visit to the same flower (solid color); C) sequential visit to a flower on a different plant (banded color) or G) sequential visit to a flower on the same plant (dotted color).

During one visit, it is highly probable that self-pollination occurs. However, a proportion of the deposited pollen must come from previously visited flowers as non-combed pollen grains remain on the bumblebee body.

Pollen loads

The sample of pollen loads was limited because of the method used. During the first sampling date on 18 February 2005 no pollen load was collected. The foragers that returned after collecting pollen either did not recognize the entrance of the nest or waited on one side near the nest but never entered it. On the two other sampling days (25 February and 4 March 2005), the foragers actually entered the nest, but the activity of the colony diminished notably as foragers stopped leaving the nest. For these reasons, it was decided to stop the sampling of pollen loads.

The mean weight of pollen loads was 0.012 ± 0.004 g. The content of the pollen loads was not pure and most of them were a mixture of *S. quitoense* pollen with dandelion or red clover pollen (Table 3.2).

Table 3.2 Pollen loads from *B. atratus* workers captured at nest entrance. L: *S. quitoense* pollen, RC: red clover pollen, DL: dandelion pollen

Sampling date	Pollen load	weight (g)	Pollen content
25/02/2005	1	0.012	L + DL
25/02/2005	2	0.014	L + RC
25/02/2005	3	0.010	DL
25/02/2005	4	0.024	L + DL
25/02/2005	5	0.010	DL
25/02/2005	6	0.014	L + DL
25/02/2005	7	0.010	L + DL
25/02/2005	8	0.009	L + DL
25/02/2005	9	0.007	L
25/02/2005	10	0.009	not determined
04/03/2005	11	0.007	not determined
04/03/2005	12	0.005	not determined
04/03/2005	13	0.006	not determined

3.4.2 Colony foraging activity

Daily foraging activity

Bumblebees foraged between 8:00 and 17:00 hours when the activity stopped completely until next morning (Figure 3.7). Maximal foraging activity occurred in the morning, so that it overlapped with the period of receptivity from *S. quitoense* LSF flowers.

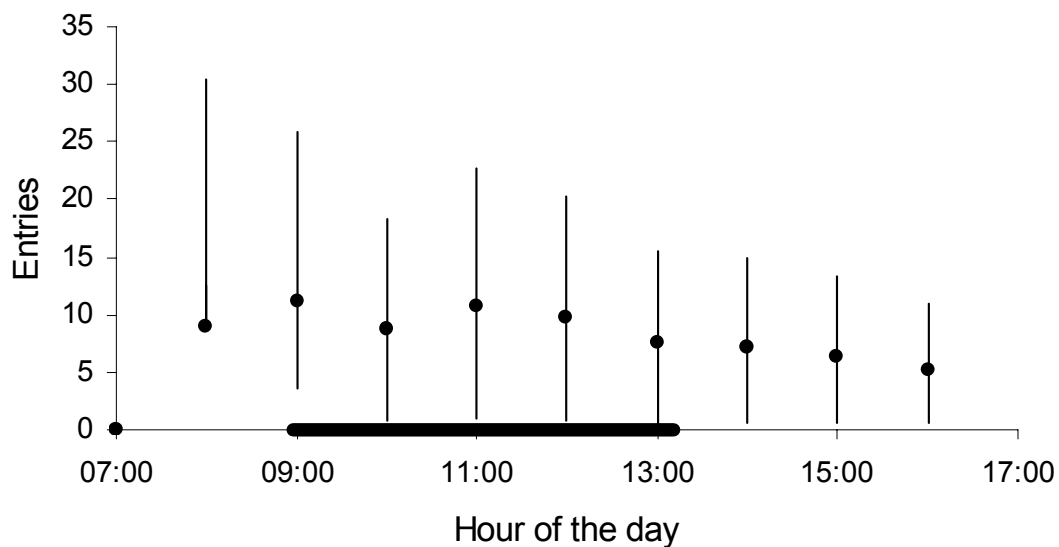


Figure 3.7 Mean entries to the nest at different times of the day. 95% confidence interval is indicated for each value (n= 1290 events of entry). Heavy line on x axis shows highest stigma receptivity period

Collected resources

The bumblebees concentrated their foraging activity on different resources during the day. In this way, in their first bouts at 8:00 hours they collected only nectar and started to collect pollen one hour later (Figure 3.8). Most of the workers that collected pollen did it in *S. quitoense* flowers, although other pollen such as red clover (*Trifolium pratense*) and dandelion (*Taraxacum officinale*) was also present in the plantation area.

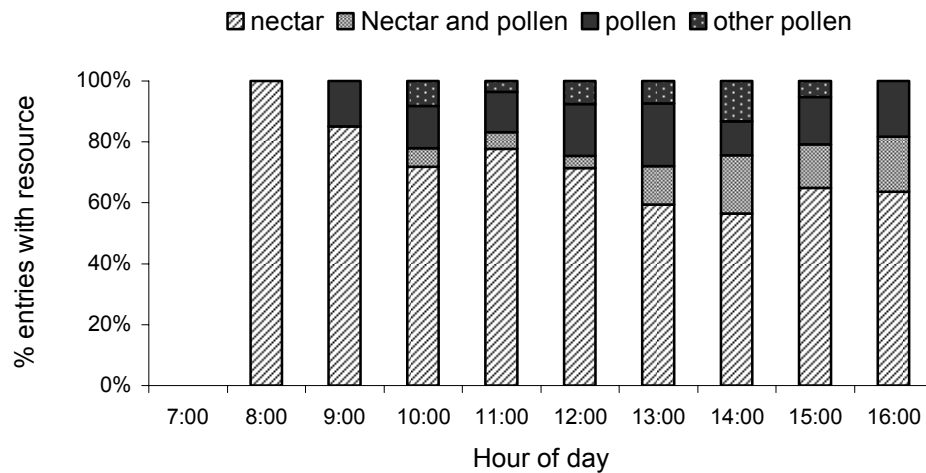


Figure 3.8 Proportion of resources brought to the nest at different times of the day.

Foraging constancy

The different colors of pollen loads brought by foragers to the nest were used to determine flower fidelity of the workers. Although these were able to leave the crop, flower fidelity was high (Figure 3.9). Out of 1290 entries, 1% pollen was collected from sources other than *S. quitoense*, and 20 % came from the crop. However, the resource that the bumblebees collected the most was nectar from the bird feeders provided on the side of the nest. Almost 80% of the workers came without pollen loads and were therefore interpreted as nectar foragers.

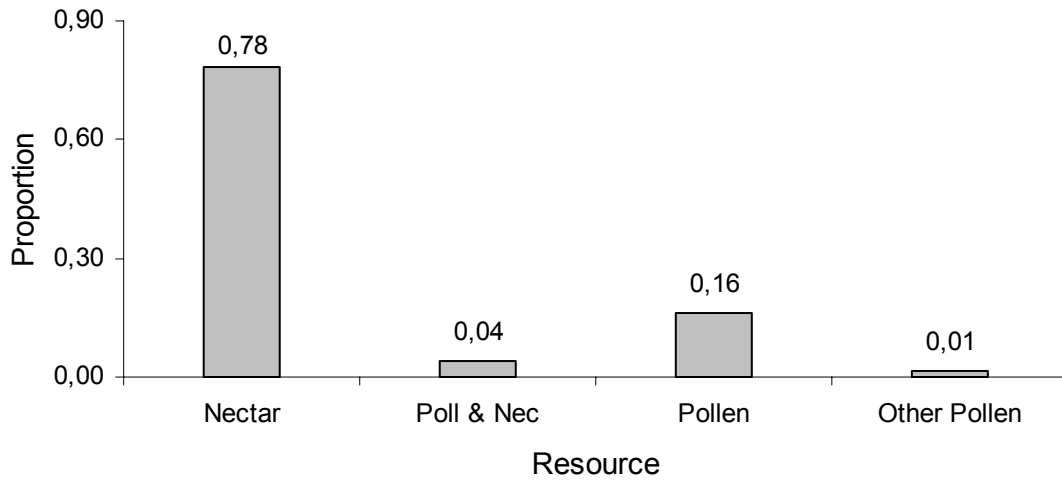


Figure 3.9 Proportion of resources brought to the nest by workers (n=1290 entries)

3.4.3 Use of *S. quitoense* pollen by the colony

The nest started to forage in *S. quitoense* flowers five days after it was placed in the crop area. After four months, the colony produced a total of 60 workers, 4 males and 2 queens, when it totally decayed and foraging activity was no longer detected. The censuses of the colony show that the average colony size was 20 workers (Table 3.3). The amount of immatures was not possible to quantify because of the presence of a wax involucre that covered the brood so that it could not be observed. The involucre was not taken away since a destructive sampling was not desired.

Table 3.3 Census of immatures, workers and reproductive individuals in the experimental colony during four months. Quantification of immatures was especially difficult because of the formation of a wax involucre that covered the nest.

Date	Cells of immatures	Workers	Drones	New queens
26 January-05	11	20	0	0
28 February-05		15 new	0	0
30 March-05		27 new	4	2
28 April-05		5	0	0

Although the colony remained small, new workers and even reproductive individuals were produced. The growth of the colony and its increased foraging activity show that the colony developed further under the conditions of the crop.

3.5 Discussion

3.5.1 Methods

Pollen load sampling

The wooden disc used in this study as a pollen trap at the entrance of the nest followed the same principle used very successfully with honey bees (*Apis mellifera*). However, the pollen trap completely disturbed the foraging activity of the bumblebees, since some of the workers were bigger than the hole in the disc. Some bumblebees were smaller and could easily get into the nest without losing the pollen loads. There is a design problem with this method, since the size variation of bumblebee workers is higher than that among honey bee workers. A new design for pollen traps in bumblebee nests is necessary, as the method of analyzing pollen loads can be very accurate. In this study, the results of pollen loads are inconsistent with the methods on the observation of traffic of foragers coming into the nest. This second method can have a higher sampling error because it relies on the observer's accuracy. Unfortunately, the sample of pollen loads cannot be representative, as collection was carried out during 8 hours on two days, while the activity of incoming foragers was registered during 160 hours along four months. In this study, the second method rendered better results.

3.5.2 Individual behavior

The general foraging behavior of *B. atratus* on *S. quitoense* flowers follows the description done for buzzing bees on *Solanum* flowers (Buchmann 1983, Faegri and van der Piel 1971). This stereotype behavior could be expected due to the conspicuous *Solanum*-type morphology from the flower (Vogel 1978). The arrangement of anthers around the style and the yellow color are one of the floral cues that elicit the behavior of foragers to vibrate them (Buchmann 1983). Consequently, foragers visited mature flowers more frequently than senescent ones with a brown color and lack of contrast. The flower of *S. quitoense* might have other attractants such as odor.

The fact that during the visit the body of the forager touches the stigma several times confirms that it actually plays a role as a pollinator and not as a robber

of pollen. This is similar to the case of *S. rostratum* pollination, to which only buzzing bees with large bodies play a role as pollen vector (Bowers 1975). Therefore, other bee species that potentially could act as pollinators of *S. quitoense* must have not only the vibration behavior but also a large body size so that pollen is effectively deposited on the stigma. For example, other buzzing species have been proposed as potential pollinators of fruits in Colombia (*Partamona peckolti*, *Tetragonisca angustula*, *Nannotrigona testaceicornis*, *Scaptotrigona limae*), but the average body size of those species is 5-7 mm. Alternatively, *Tetragonistica* spp. and *Nannotrigona* spp. are not elements of the high mountain bee fauna (Gonzalez et al. 2005). In Brazil, *Melipona* spp. has been proposed to pollinate tomato in greenhouses thanks to its buzzing behavior (Del Sarto et al. 2005), but *Melipona* species generally have a small to moderate size body (Michener et al. 1994) and they might not pollinate effectively in the case of *S. quitoense*.

Bombus atratus foragers visited almost exclusively *S. quitoense* flowers although flowers of two other plants were present in the experimental crop area. This result contrasts with the description of their behavior as inconstant foragers by Heinrich (1979). Furthermore, the content of sampled pollen loads of workers was rarely pure as also is the case in the wild (Cruz et al. 2005). One explanation for the fidelity observed to *S. quitoense* might be that *B. atratus* workers forage near the nest (Osorno and Osorno 1938). This behavior is called doorstep foraging (Goulson and Stout 2001), and in the wild it is easy to locate nests when workers and young queen are seen foraging on flowers within a range of a few meters from the nest entrance (personal observation). The foragers kept to a single pollen source due to the spatial arrangement and to the relative proportions of the plants around the nest. Thus, the resulting constancy can be then classified as a “passive one” (Thomson 1983).

A second reason for the fidelity of the colony might be that the reward collected from *S. quitoense* flowers was so good that the foragers returned to the same foraging patch (Buchmann and Cane 1989). Bumblebees can assess the reward that they get from one plant and use it as decision criteria to direct their next foraging choices (Heinrich 1979, Hodges and Miller 1981, Dukas and Real 1993). The long handling time of *S. quitoense* flowers and the low number of visited flowers per bout in comparison to other reported values for buzz-pollinated flowers (Buchmann 1983, Lavery 1980) can be interpreted as a high return of pollen during a visit.

It was also observed that individual workers specialized their foraging effort in just one resource, either pollen or nectar. Those foragers that collected both were rare and probably they were pollen-collecting foragers that took nectar for their own flight activity demand. This might also be the case for workers that collected pollen in *T. pratorum* and *T. officinale*. They changed from *S. quitoense* to take nectar in those species and collected pollen as a side-by activity when collecting nectar in those plants, as the main resource offered by these is nectar (Corbet et al. 1994).

An interesting result is the pattern of the floral visitation sequence of *B. atratus* foragers because of its consequences for promoting cross-pollination. Each plant has more than one inflorescence simultaneously with one mature open flower, but bumblebees did not explore all inflorescences of a plant, on the contrary, they visited just one flower per plant and then changed to a flower of a different plant. This behavior follows the model for the horizontal pollination sequence proposed by Harder and Wilson (1998). In this case, however, the variation of flower morphs also influences the efficiency of pollination since visits to a SSF leads to pollen deposition and thus no pollination.

The following short theoretical analysis shows that the probability of efficient pollination of LSF flowers increases with the number of visits per bout.

If the bumblebees forage without any preference for a floral morph, then the probability that a forager pollinates a LSF flower depends on the frequency of LSF flowers in the crop. Therefore, if LSF flower frequency is 0.7, then a forager will find this floral morph with a probability equal to 0.7. As the forager flies in sequence among several flowers (in average 14 flowers), then the probability of distribution follows the polynomial equation:

$$V = (p + q)^b$$

where V is the probability distribution, p the frequency of LSF flowers, q the frequency of SSF flowers and b the number of visits per bout by a forager does. The higher the number of flowers visited during a bout, the higher will be the probability to “find” a LSF flower at observed frequencies of LSF flowers. The event that 50% of the visited flowers are LSF in an average bout is higher than 0.8 at LSF frequency of 0.6 (Appendix A1). This hypothesis works for the plant purposes only if the forager is motivated to visit further flowers. Then the return of pollen might be restricted by mechanisms of the plant in a way that foragers have good pollen returns but not

enough to complete their collecting capacity (Harder and Wilson 1997, Shelly et al. 1997). If SSF flowers render less pollen than LSF flowers, gender variation could be a mechanism to modulate the behavior of the pollinator to its own benefit. In that sense, the SSF flowers would attract and maintain constancy of the pollinator to secure pollination. To test this hypothesis it would be necessary know the mechanism of pollen maturation in the anther and the amount of pollen produced by each floral morph.

Furthermore, foragers in the late afternoon more often explored a whole plant and visited more than one flower before changing to a different plant. If pollen in flowers of *S. quitoense* is partially depleted in the afternoon when the amount of pollen decreases in already visited flowers, it is possible that foragers change their behavior and aggregate to plants where they find better pollen returns. However, this foraging pattern requires further experimentation on the presentation cues of the plant and the response to them by the bumblebee. The effect of multiple visits on fruit quality and the buzzing intensity (number of vibrations per visit) should also be further assessed to detect possible damages that foragers can do to the receptacle of flowers with their mandibles by trying to extract pollen from depleted ones during repeated visits as happens in tomato flowers (Velthuis and Doorn 2006).

3.5.3 Colony development

A colony of *B. atratus* can reach as many as 1000 workers and persist for more than one year (Zucchi 1967), but the colony used for this study showed a development pattern far from this description. The experimental condition could be regarded as if the colony had developed under an enforced monolectic diet that would seem inappropriate for a polylectic species. However, in this case the slow growth rate of the colony was probably due to the low oviposition rate of the false queen. The reported false-queen oviposition rate is 1,07 egg/day (da Silva-Matos and Garófalo 1995), i.e., lower than that of a queen.

In addition, *Solanum* plants offer a high amount of pollen and its pollen grains also have a high protein content (Buchmann 1983, Buchmann and Cane 1989), consequently the colony was probably not limited by a nutritional deficiency despite a diet based mainly on one pollen type.

3.6 Conclusions

- *Bombus atratus* workers vibrate the flower of *S. quitoense* to extract pollen from the anthers. During the visit, they contact the stigma of hermaphrodite flowers with the parts of their body that carry pollen grains.
- Workers that collected pollen did it mostly from flowers of *S. quitoense*. Their foraging behavior was highly constant to these flowers.
- *Bombus atratus* workers foraged on flowers of different plants more frequently than on flowers of the same plant so that they promoted cross-pollination.
- The foraging activity of *B. atratus* workers during the morning overlapped with the receptivity of the LSF flowers of *S. quitoense*.
- A colony of *B. atratus* can develop with the pollen rewards from *S. quitoense* flowers.

4 MODELLING COLONY DYNAMICS OF *BOMBUS ATRATUS*: FACTORS AFFECTING REARING IN CAPTIVITY AND POLLINATION MANAGEMENT

4.1 Introduction

Bombus atratus is a neotropical bumblebee species that lives in social colonies composed of a founder queen, females of the worker's caste and the brood of immatures. The colony goes through a cycle that starts with a solitary gyne. It grounds a colony by building an egg cell with wax in a protected place just under the ground surface. During the subsocial phase before the first workers emerge, the queen must take care of the brood and forage to collect pollen and nectar. After the first batch of workers emerges, the queen only leaves the nest on very seldom occasions. It lays eggs and also incubates, while the search of food is done by the forager workers who bring it into the nest. Pollen is deposited in the larvae pockets and nectar is stored in pots. The house workers remain in the nest feeding and incubating the brood. To attain reproduction, the colony must reach the conditions that allow it to rear new drones and gynes. Unfortunately, these conditions are so far not completely understood for most species of *Bombus* and it is not possible to predict the moment when reproduction is triggered. The cycle finishes with the death of the queen and the emergence of new reproductive individuals that leave the nest. However, as new gynes do not enter in the diapause there are cases when the nests of *B. atratus* can be perennial if the cycle continues by the replacement of the founder queen by one or more new mated young queens. This polygynous cycle allows the nests to reach a large size and to last for more than one year (Cameron and Jost 1998, Sakami and Zucchi 1965). In that sense, development of colonies from this neotropical bumblebee species is similar to most of the bumblebee species with exception of the possibility of being perennial and the presence of a polygynous phase.

4.2 Observation and question statement

The management of a species under artificial conditions requires profound knowledge of the organism's life cycle (Velthuis and van Doorn 2006). From literature sources (Corbet et al. 1993, Beekman and van Stratum 2000) and from own observations

(Almanza et al. 2006), have been identified the following factors that affect the development and behavior of a *Bombus* colony:

- **Poor development of the colony and early death of the queen:** A high percentage (90%) of queens from the wild start to lay eggs and take care of the brood up to the beginning of the eusocial phase when the first workers emerge. However, during the phase in which the colony should rear at least 30 individuals, the workers discard the brood and subsequently the colonies decay and collapse after the queen dies. This problem causes a decrease of 40% of reared colonies.

- **No production of reproductive individuals:** Of those colonies that develop to a larger size (up to 200 workers), it is expected that colonies reach the switch point where new drones and gynes are produced. It has been observed that just 20% of the colonies reared males and 10% new gynes.

The many possible factors that cause this fate of colonies can hardly be tested experimentally in the laboratory or the field. Therefore, it was decided to simulate colony development with a model in order to answer the following questions:

- Does oviposition rate determine worker's production rate?
- In which development stage is mortality higher?
- Is immature development restricted by the number of workers that supply the demands of the larvae despite available unlimited resources?
- In which development phase of the colony (solitary, eusocial, and competence) is the demand for pollen and nectar highest?

4.2.1 Methods

4.2.2 Superorganism concept to approach system modelling

A bumblebee colony can be interpreted as a superorganism because it accomplishes at least four main constraints of an organism (Moritz and Southwick, 1992). First, the colony remains sessile in a physiographic space, i.e., the nest, where most of the individuals remain during their life time. Within this space, nectar is additionally stored and a reproductive brood is reared. Second, the constant regulation of the nest temperature shows an intraorganismic homeostasis and implies the storage of nectar that allows maintenance of this steady-state during unfavorable conditions. Third, the aggressive behavior of bumblebees serves as a defense of the stored resources, and

fourth, members of mature colonies are numerous and work as a cooperative unit. This concept suits the approach that is adopted in this model of a bumblebee colony, especially because nest thermoregulation and storage of nectar are characteristics that influence the flow of energy at the colony level and make the colony the center for regulation.

Bumblebees regulate their temperature so that the thorax has on average 30°C. To produce heat, the thorax muscles rapidly shiver with low amplitude vibrations without moving the wings. This adaptation enables them to forage at times and places with lower temperatures than other bee species. In addition, the ability to elevate their body temperature allows them to incubate the brood in the nest so that development is speeded despite low external temperatures (Heinrich 1979). Nevertheless, the mechanism demands high amounts of energy acquired from nectar. Consequently, energy flow will direct the growth of a colony. A lack of nectar brings adult workers rapidly into a torpor stage and they abandon incubation. In the same way, growth of larvae is negatively affected at temperatures below 30°C (Heinrich 1979).

A bumblebee colony functions like a module system composed of specialized subunits to transform masses of pollen and nectar into masses of bumblebees. In that sense, the approaches of Heinrich (1979) and Wilson (1974) are adopted for the model. Within each subunit age-structured cohorts of eggs develop to produce workers, drones or gynes. The colony starts with a founder queen (first subunit) whose final goal is to reach reproduction. This subunit is composed of a single individual (the queen) that does not grow but that controls colony development through oviposition. Consequently, it generates a demand for resources to produce eggs. The second subunit has a somatic function, i.e., rearing of workers for the growth of the colony. In this period, batches of fecundated eggs develop into larvae, pupae and adult workers. This subunit also generates a demand for pollen and nectar. In the subsocial phase, the demands are supplied by the founder queen and afterwards by the workers.

The third subunit is composed of the reproductive individuals: new gynes and drones. Their production start with the switch point of the colony (the founder queen lays its first haploid egg) and they are the final output from the colony. The demands of this subunit are assumed to be higher per individual because gynes and drones generally have a larger body than workers. During the social phase and reproductive phases of the

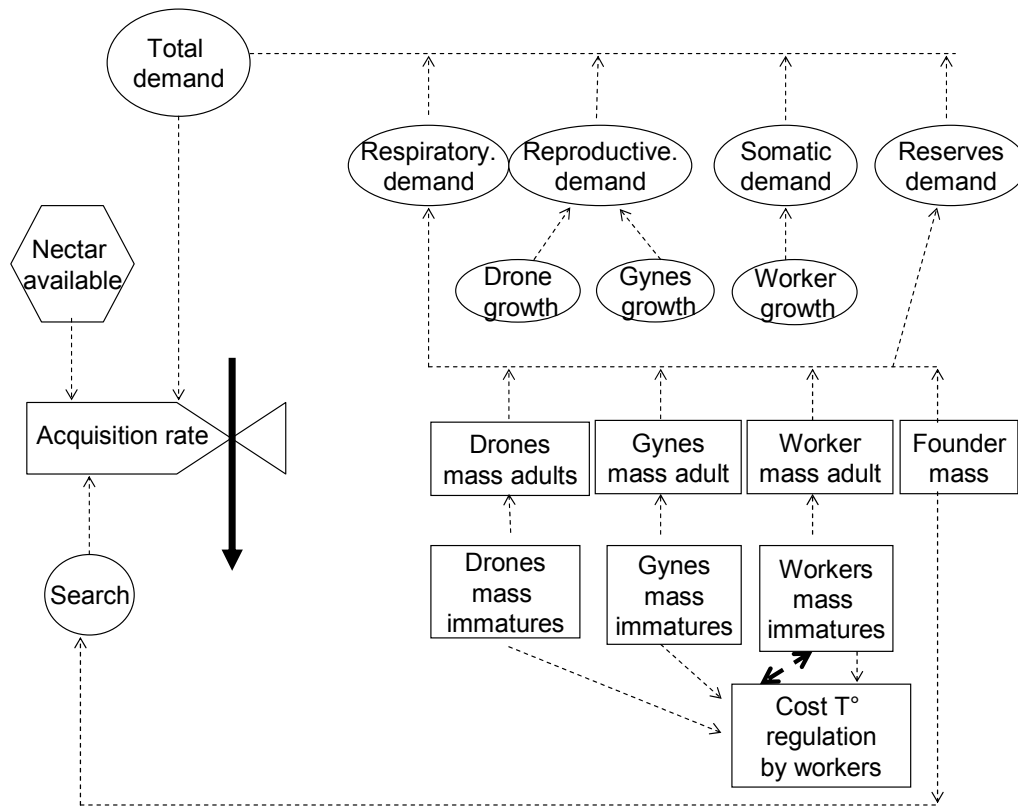
Rate of resource acquisition

The biological goal of the colony to produce reproductive individuals out of pollen and nectar resources is attained through search, collection, assimilation and transformation of these resources. The colony as a superorganism develops a strategy for that purpose that takes into account the colony phase, the temperature conditions and the amount of resources present in the environment. The model integrates these factors with the rate of resource acquisition defined by a non-linear function. The use of a non-linear function avoids the false assumption that the organism is insatiable. The function follows the equation (from Gutierrez-Baumgärtner 1984):

$$S = bN * (1 - e^{-aN/b})$$

where S is the rate of resource acquisition in grams of sugar per day, b is the total physiological demand of the colony in grams of sugar per gram of bee per day (g/g/day). The function introduces into the strategy the physiological motivation and the temperature as an abiotic condition. N is the mass of sugar (g) actually collected by the workers and quantifies the amount of resources that is available for consumption in the nectar pool, and a is the efficiency of nectar collection which considers the probability that the forager cannot fill its honey stomach due to competition through high densities of foragers.

Three main factors (total demand, nectar availability and resource acquisition) affect the consumption of energy in the colony (Figure 4.2).



Metabolic pool model

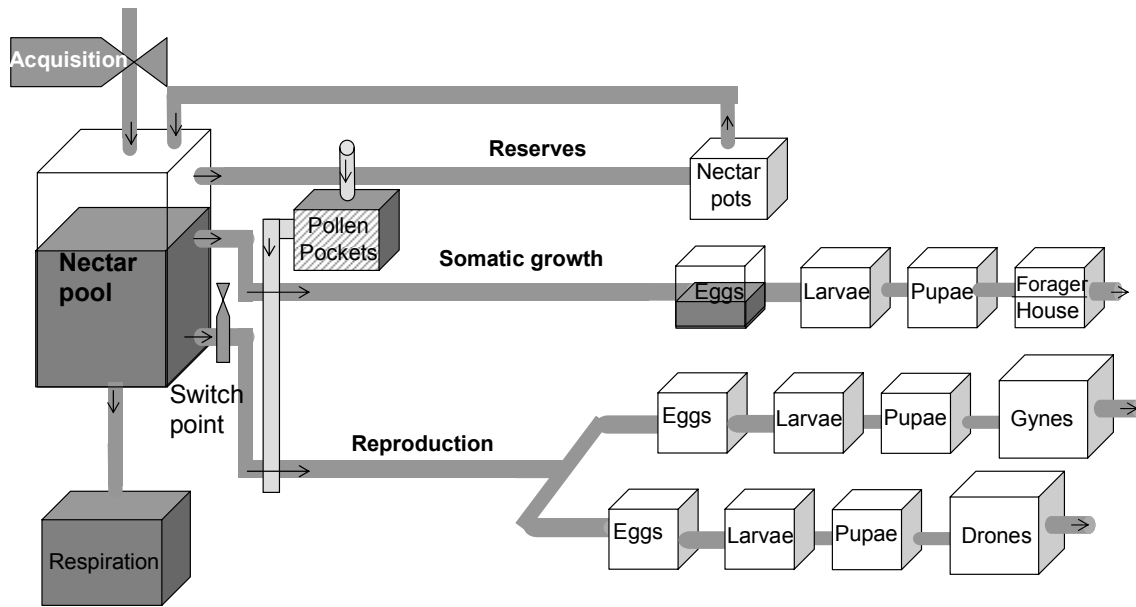


Figure 4.3: Metabolic pool model of nectar combined with metabolic pool of pollen for a colony of bumblebees. The resources are allocated between respiration, reproduction, somatic growth and reserves.

In the model, the metabolic pool of nectar is combined with the metabolic pool of pollen. The metabolic pool of pollen is included in the model as it comprises 40% of the food that immatures get for development. The percentage was assumed on the base that adults mix pollen and nectar to feed the larvae and regurgitate the mixture without adding secretions (Alford 1974, Pereboom 2000). Additionally, it is assumed that the consumption of pollen by adults can be neglected, and that the costs of respiration and temperature regulation are covered by the energy provided by nectar.

The priority for distribution is respiration followed by reproduction. The production of workers can be achieved as long as the key for the switch point remains closed. While the colony grows, every stage has a certain mortality (not indicated in the Figure 4.3) due to lack of resources and age, but mortality during foraging is not included. The individuals that finally reach the adult stage are classified as house workers or forager workers depending on their size. With bumblebees, distribution of

labor depends on the size of workers and not on age as with honeybees (*Apis* spp.). When the difference between acquisition and costs is positive, nectar can be stored as the last priority. A proportion of the nectar reserves can be reintegrated to the nectar pool when they attain maximum. This concept follows the assumption that bumblebees do not forage unnecessarily, and that reserves correspond to a demand for resources. This assumption has been proved for other *Bombus* species (Pelletier and McNeil 2004).

However, when the colony reaches the reproduction stage (switch point key opens), resources are exclusively allocated to drones and gynes. The sex ratio in *Bombus* colonies varies, and it is not yet clear which factors define it. For the model, it is assumed that haploid eggs are laid by the queen in a ratio of 6:1 diploid eggs (Cameron and Jost 1998, Garófalo 1979). It is assumed that gynes and drones do not forage and that they leave the colony after reaching sexual maturity. The competition phase and the replacement of the founder queen by a new queen are not included in the model, but the plasticity of the model will allow this in the future.

The ratio among supply and demand (S/D) shows the interplay between the rates of resource acquisition and physiological demands. It varies between 0 and ≤ 1 , because in theory the demands will be always higher than the supply of resources even when they are non-limited due to the inefficiency of foraging. Consequently, the rate of production of workers, gynes and drones is regulated by this ratio (Figure 4.4).

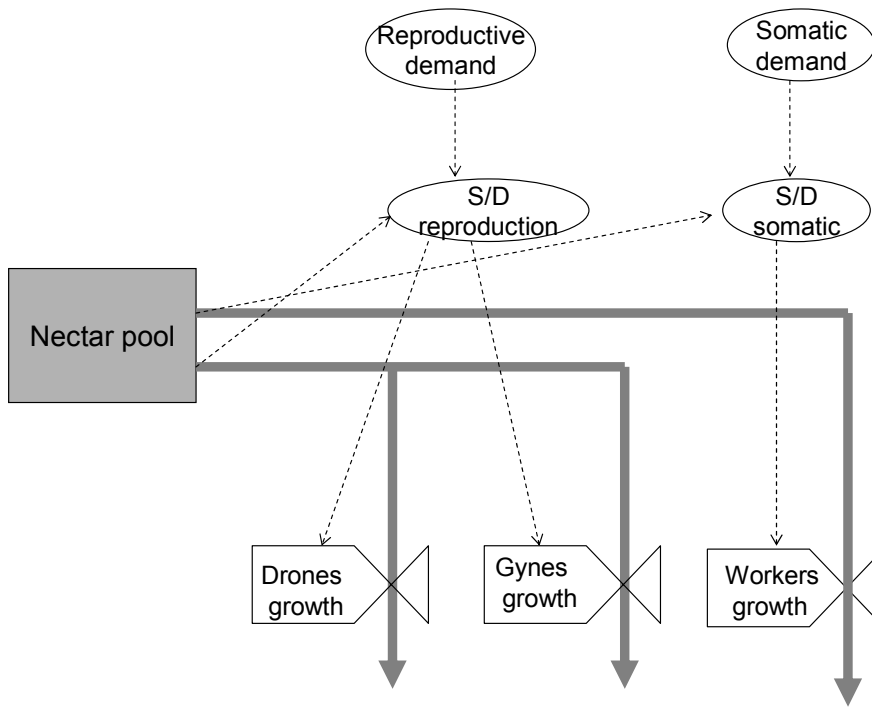


Figure 4.4: Effects of the supply/demand ratio of reproduction and somatic growth on the allocation of nectar.

4.2.4 Physiological time

In the model, the effect of temperature on larval development is included as physiological time (measured in degrees/day). The larvae grow at temperatures higher than 23°C, which is their physiological threshold. The effect of temperature differs for immatures and adults so, and the threshold defined for adults is 16°C. Threshold temperatures are estimated with brood temperature data from *B. vosnesenskii* (Heinrich 1974) and the average temperature of *B. atratus* nests in the wild (Orozco et al. 2006). The costs for growth and incubation at lower temperatures demand higher amounts of energy in the form of sugar. If sugar is not available, incubation is not achieved, and growth of immatures stops. Temperatures lower than 16°C will affect adult longevity.

This approach allows simulating colony development under different temperatures.

4.2.5 Laboratory rearing methods

In order to set up a life table of *B. atratus*, four colonies were reared in the laboratory (Figure 4.5). Four wild young queens were captured in Ubaté, a rural region in Cundinamarca, 100 km north of Bogotá at 2800 m asl. The queens were captured on different dates; one was captured in December 2004 and the other three in May 2005. They were transported to the laboratory in Bogotá within a time span of 5 hours. Each queen was weighed and then transferred to the rearing room at 26 to 28 °C, 70% relative humidity and without illumination. Each queen was kept in a wooden initiation box (Gretenkord 1997). Without any stimulation, the queens started to lay eggs within a week (Gretenkord 1997). Every three days, the queens were fed with 5 g of a mixture of fresh pollen collected by honey bees and a sugar solution (50% concentration). The mixture was prepared with 40 g of pollen and 10 ml of the sugar solution so that it had a soft and humid texture (Gretenkord 1997).

An additional 50 ml of sugar solution were provided to the queen in bird feeders on one side of the initiation box. Both resources were replaced every three days by fresh ones. After the first 8 workers had emerged, the colony was moved to a bigger box and sufficient amounts of pollen mix and sugar solution were provided.

One of the boxes (colony 4) had an entrance that allowed the foragers through the wall of the rearing room to leave the nest and forage outside in the gardens of the experimental center. It was opened when the colony had 15 workers and from this moment on, no additional food was offered to this colony. Three events of queen replacement occurred, i.e., a foreign queen invaded the colony and killed the established queen and took control of the colony. Finally, a dominant worker killed the last queen and controlled the nest, since it was not fecundated; the colony stopped growing and decayed. In this colony, data were registered until the last queen died.

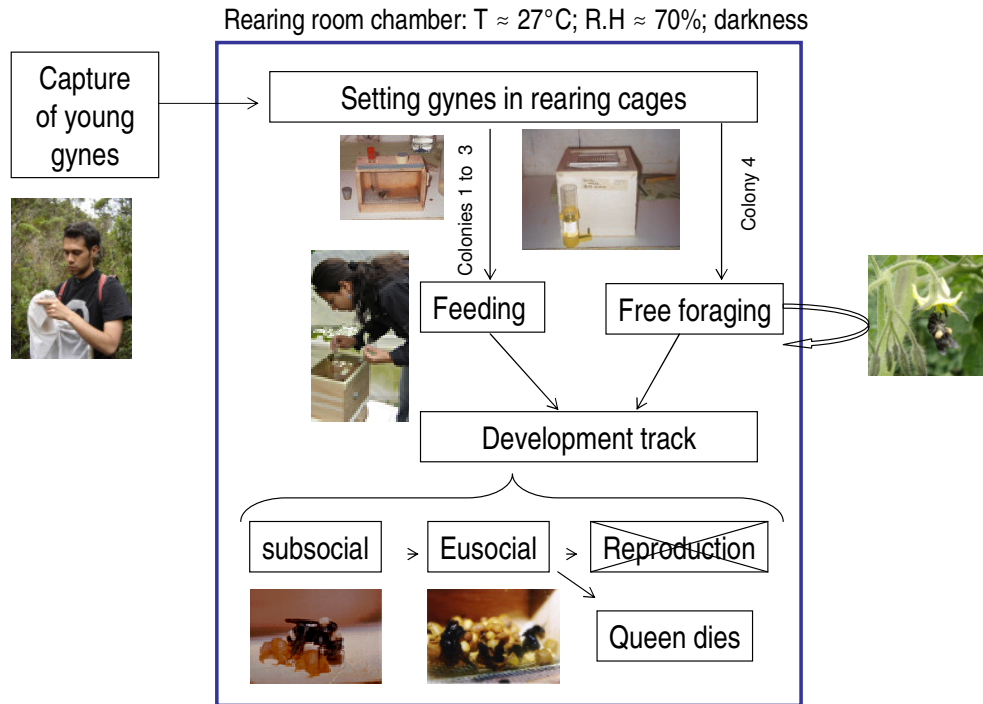


Figure 4.5: Steps for rearing bumblebees in captivity.

4.2.6 Parameter estimation though life table

The parameters for modeling the dynamics of a colony were obtained from the life table of the four colonies reared in the laboratory. Because the colonies were reared under constant conditions and with unlimited resources, it was assumed that the obtained values represent an optimum. The rearing methods in the laboratory were the same as for rearing colonies for pollination experiments described in previous chapters.

A trained observer daily recorded the number of cells of each development stage: egg cell, larva cells, pupa cells and new emerged adults. Each type of cell can be distinguished by size, color and form. Egg cells contain six to eight eggs covered by wax. Larvae cells can be recognized by a pocket of wax on the side of the cell, which means that the larvae have hatched and are fed through the pockets. As the larvae develop and increase in size, each is separated into an individual cell until time of pupation. These bigger larva cells are easier to determine so that individual larva can be quantified. The next development stage, the pupa is the easiest to recognize, as it changes in color, i.e., light yellow and is oval. Finally, just emerged adults can be

recognized by their body hairs that are not yet black and the soft wings, bent on the abdomen and not fully extended.

Each emerged adult worker was tagged with a plastic disc number in order to track the longevity of the individuals.

The life table was developed following the methods of Gutierrez (1996) and Silva-Matos and Garófalo (2000). It provided the following demographic parameters for each stage: duration of worker development, oviposition rate, worker production rate, and mortality at each immature stage.

The amount of pollen and nectar consumed was measured in one of the colonies by taking the difference of the amount initially given and the amount left after three days. The temperature and humidity conditions of the rearing room had a desiccation effect on the pollen mass; the ensuing lost of weight was measured in a separate sample under the same conditions but without the presence of bumblebees. In the same way, nectar consumption was measured taking the evaporation factor into account.

Those parameters that could not be estimated from the data were obtained from studies done for *B. atratus* colonies in Brazil (Cameron and Jost 1998, da Silva-Matos and Garófalo 1995, 2000, Garófalo 1979, Garófalo et al. 1986, Sakagami and Zucchi 1965, Sakagami et al. 1967) and general data from other *Bombus* species (see Appendix 3). The energetics studies performed by Heinrich (1979) were used to obtain the parameters of incubation costs and foraging by adjusting the units of calories to grams of sugar. Appendix 3 contains a list with the initial parameters, a description and the source of the obtained value for each parameter.

4.2.7 Hardware and software

The model was developed using Borland Object PASCAL™ as the programming language. Files of observed data were created with Dbase IV format, so that the program runs independently of other software programs. It runs on Microsoft system hardware, IBM™ compatible.

4.3 Results

4.3.1 Development rate of reared colonies

Four colonies were reared with the methods above described and their development tracked until the last worker produced by the queen died. None of them reached the switch point before the competition phase started and then the queen died. Therefore, no reproductive individual was produced by the founder and the reproduction phase of the colonies was not attained. Total life time of nests showed variations because of different longevities between the queens. In the table 4.1, the characteristics attained by each colony are given.

Table 4.1 Characteristics of four colonies of *B. atratus* reared in captivity and semi-captivity conditions. Foragers from colony 4* were left to forage freely after the size of the colony reached 15 workers.

Colony N°	Total life time of (days)		Total number of			Number of workers emerged from		Rate of worker production (workers/day)
	Founder queen	Colony	Worker	♀	♂	First batch	Second batch	
1	166	173	145	0	0	2	12	1.88
2	150	164	103	0	0	7	0	1.27
3	Not known	108	40	0	0	5	4	0.68
4*	Not known	97	91	0	0	8	4	1.93

It can be seen that colonies 1 and 4 had a faster and better development than colonies 2 and 3 despite the total number of workers produced. Their rate of worker production was faster than that of colonies 2 and 3. These differences of growth can also be detected when the accumulated number of egg batches and adults are plotted against time (Figures 4.6 to 4.9).

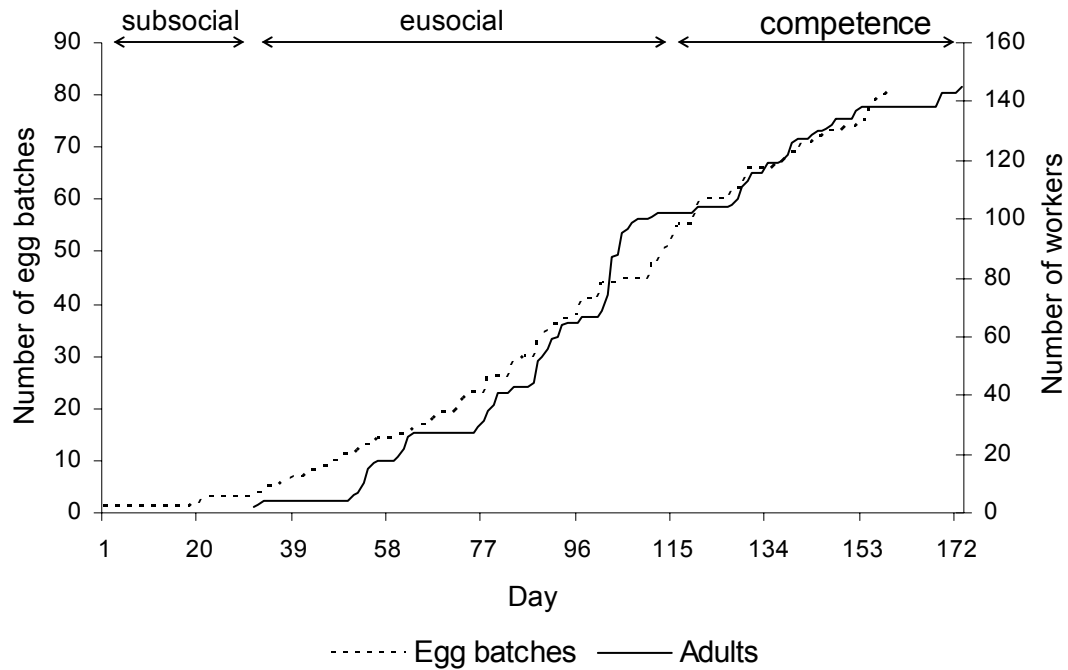


Figure 4.6 Accumulation of individual egg batches and adult workers in colony 1. Note that colony phases have different rate of worker production.

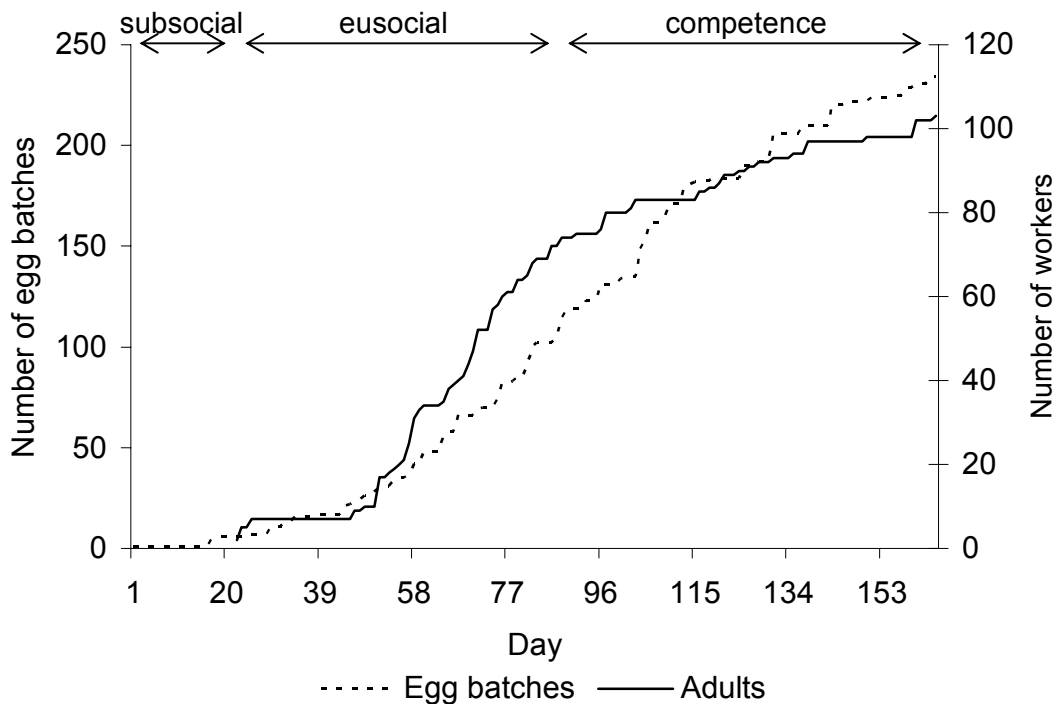


Figure 4.7 Accumulation of individual egg batches and adult workers in colony 2. Note that colony phases have different rate of worker production.

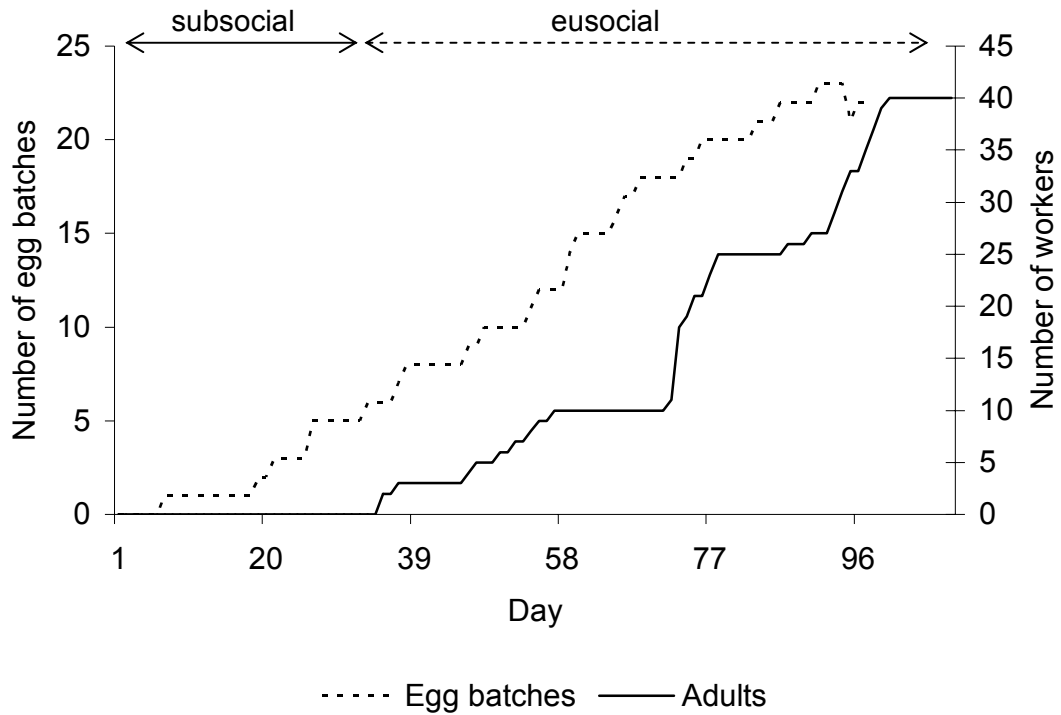


Figure 4.8 Accumulation of individual egg batches and adult workers in colony 3. Note the rate of worker production was not continuous (stepwise growth).

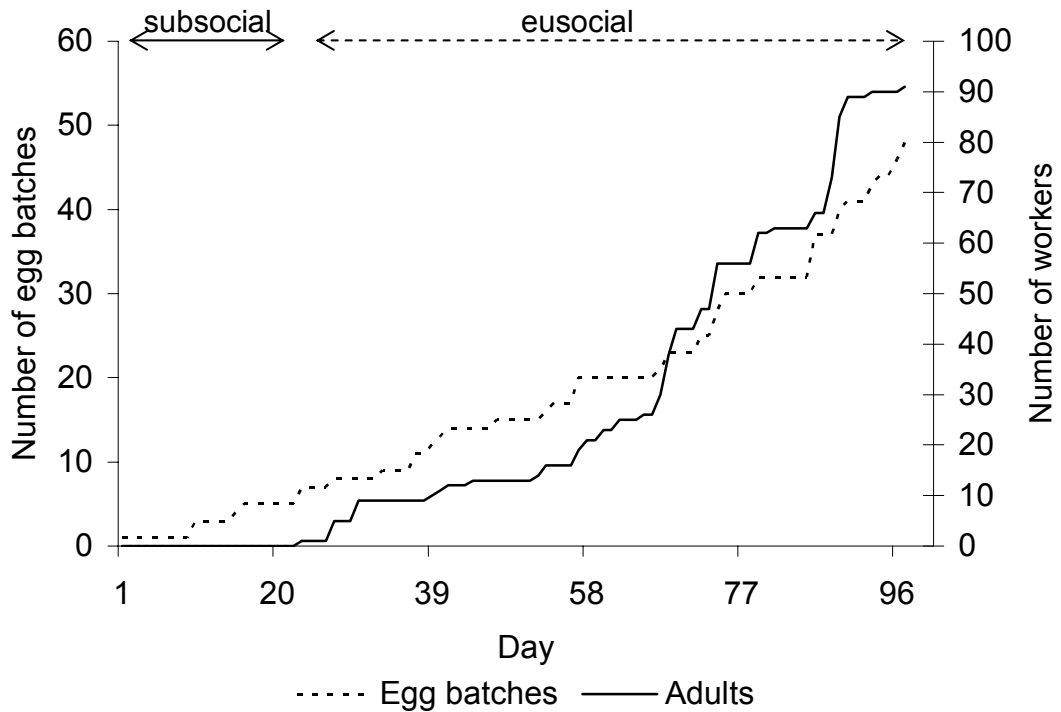


Figure 4.9 Accumulation of individual egg batches and adult workers in colony 4. Note that the rate of worker production was high from day 58 on.

The development up to the emergence of the first workers (subsocal phase) was different among colonies. This can be explained by different rates of oviposition. Colonies 1 and 2 exhibited an oviposition pattern typical of *Bombus* species for the subsocial phase, since the queens did not lay additional batches of eggs until the first batch pupated. In contrast, queens of colonies 3 and 4 laid 2 additional egg batches during the subsocial phase. Nevertheless, queens of all colonies laid eggs in the time between first pupa and their emergence as adults, so that all colonies accumulated in average 7.75 batches to the beginning of eusocial phase.

Once the first workers emerged, every colony went through a stepwise growth phase and workers accumulated faster because the frequency of oviposition increased and workers helped the queen with the brood care. The number of adults emerged from the first batches was higher for colonies 1 and 4, as 3 to 5 additional workers emerged from the first and second batches (Table 4.1). The subsequent linear growth phase was faster in colonies that were larger from the start. In sum, colonies 1 and 4 reached a larger size faster than colonies 2 and 3 during the eusocial phase (Figures 4.6 to 4.9).

These differences in the development during the subsocial phase of the colony can be due to queens with a high capacity, able to take care of more brood simultaneously. In contrast, colony 2 exhibited an extremely high oviposition rate, but the rate of worker production was not consequently higher (Table 4.1).

For modeling purposes the development observed from colony 4 was taken as reference to calculate the demographic parameters included in growth, because the colony went through subsocial-eusocial phases faster than colonies 1, 2 and 3.

The reproductive phase was never reached in any of the colonies. Instead of large immatures that indicate rearing of new drones and gynes, signs of the competition phase (oophagy, laying workers) were detected in colonies 1 and 2. They had different sizes at the moment of the competition point (67 workers for colony 1; 83 for colony 2). Some workers started to lay eggs; therefore, the oviposition rate observed after that moment was the result simultaneous laying activity of queen and workers. However, there was a notable decrease in the rate of pupae development, as workers do not attend the brood, and mortality of the larva increased (Table 4.2). This last phase could not be identified for colony 4 because of the events of queen replacement.

4.3.2 Life table of reared colonies

The second factor that could cause differences between the developments of colonies is the duration of immature stages. The life table was developed for immature workers in each colony (Table 4.2). The total duration of immature development lasted between 32.7 and 38.4 days. Immatures of colonies 2 and 3 developed slower than those from colonies 1 and 4, but the differences in the average duration of immature stages between colonies are not significant (ANOVA larvae $F = 0.279$ $df = 3$ $p = 0.76$; ANOVA pupae $F = 2.034$ $df = 3$ $p = 0.141$) with the exception of the egg stage (Kruskal-Wallis $\chi^2 = 15.53$ $df = 3$, $p = 0.000$). The egg stage exhibited the highest variation due to the difficulty in observing the change between egg cell and larva cell.

Table 4.2 Life table for immatures of four *B. atratus* colonies reared in captivity and semi-captivity conditions. Foragers from colony 4* were left to forage freely after the size of the colony reached 15 workers.

Colony	Stage	Age (days at 27°C)	Proportion dying in phase		
			subsocial	eusocial	competence
1	egg	6.8 ± 1.8	0.27	0.50	0.80
	larva	12.9 ± 3.3	0.34	0.40	0.77
	pupa	12.9 ± 3.7	0	0.02	0.52
2	egg	7.5 ± 2.3	0.60	0.50	0.75
	larva	14.1 ± 4.3	0.44	0.68	1.00
	pupa	14.2 ± 5.2	0	0.12	
3	egg	11.0 ± 4.6	0.57	0.67	1.00
	larva	15.0 ± 2.9	0.13	0.50	
	pupa	12.5 ± 3.4	0	0.24	
4*	egg	7.4 ± 2.6	0.23	0.56	not determined
	larva	14.7 ± 3.6	0.45	0.25	not determined
	pupa	11.5 ± 3.2	0.18	0.23	not determined

Mortality of immatures is a third factor that affects growth of colonies. Each development stage has a different mortality rate (Table 4.2). Egg and larva have higher mortalities than pupa. Furthermore, each stage has a different mortality rate in each phase of the colony, so that egg and larva mortality increases in the competence phase (Table 4.2). Eggs are eaten by the workers and larvae are not fed and starve to death. As a result, the somatic growth of a colony stops when the competition phase starts. Colonies 2 and 3 had always high mortalities of eggs, but mainly in the subsocial phase.

4.3.3 Larva/worker ratio

A parameter of the colony that helps to understand the mortality of immatures is the ratio among number of larva and number of workers. When the observed data are used to calculate this ratio for each colony, it can be seen that the four studied colonies show a similar pattern in the eusocial phase (Figure 4.10) despite strong differences among their developments. Initially, the ratio has a value over 2 larvae per adult worker (approximately until day 40), but from that point on a stable ration of around 2 larva per worker is a common element among colonies independent of size, queen age or feeding regime.

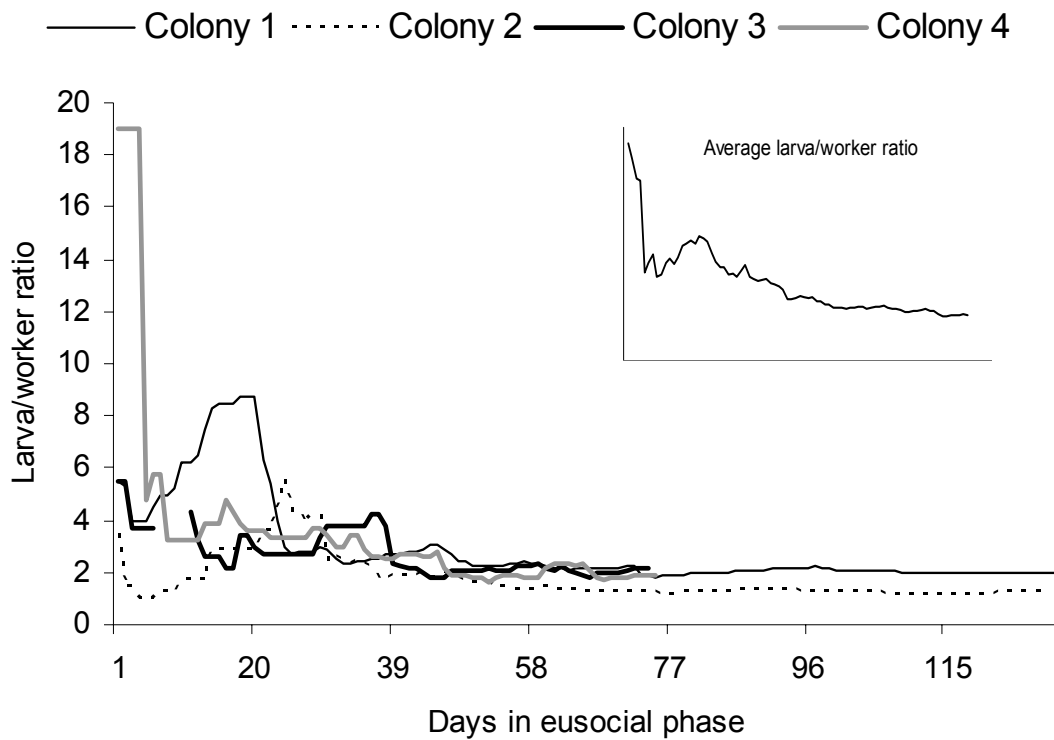


Figure 4.10 Pattern of the larva/worker ratio in 4 colonies of *B. atratus*. The upper graphic box shows the average pattern.

Only colony 2 deviates from that pattern during the first period, as it exhibits an initial distribution with values lower than 2 larvae/worker ratio.

4.3.4 Colony growth simulation

The above description of colony characteristics shows that variations in the development are a combination of factors not easy to explain from the observed data set. With simulation, it is possible to compare the observed data set with a common expected pattern (Figures 4.11 and 4.12). The simulation follows the trend of oviposition but predicts a lower number of eggs. The correlation among the observed data of batches and the simulated data is high ($R^2 = 0.9865$). This difference indicates that the number of eggs laid in each batch can vary and that the assumption of eight eggs per batch might be an underestimation. The more extreme case is represented by colony 2. The simulation of accumulated larva also correlates with the observed data of colony 4 ($R^2 = 0.9649$), but colonies 2 and 3 exhibit data very different to that of the simulation for larvae. Mortality of eggs and larvae was extremely high in these two colonies. The model only includes mortality due to senesce or lack of resources, but in these cases there must be additional causes for mortality, so that the simulation is an overestimation. Finally, pupae and adult simulations also correlate with the observed data of colony 4 ($R^2 > 0.9704$ in both cases). In the other colonies, the simulation is an overestimation of pupae and adults, but it diminishes if the larvae simulation adjusted. In other words, mortality and production rates fit the data but are dependent on the input of the previous stage. The simulation also permits predicting an average size for colonies of *B. atratus* of around 60 individuals.

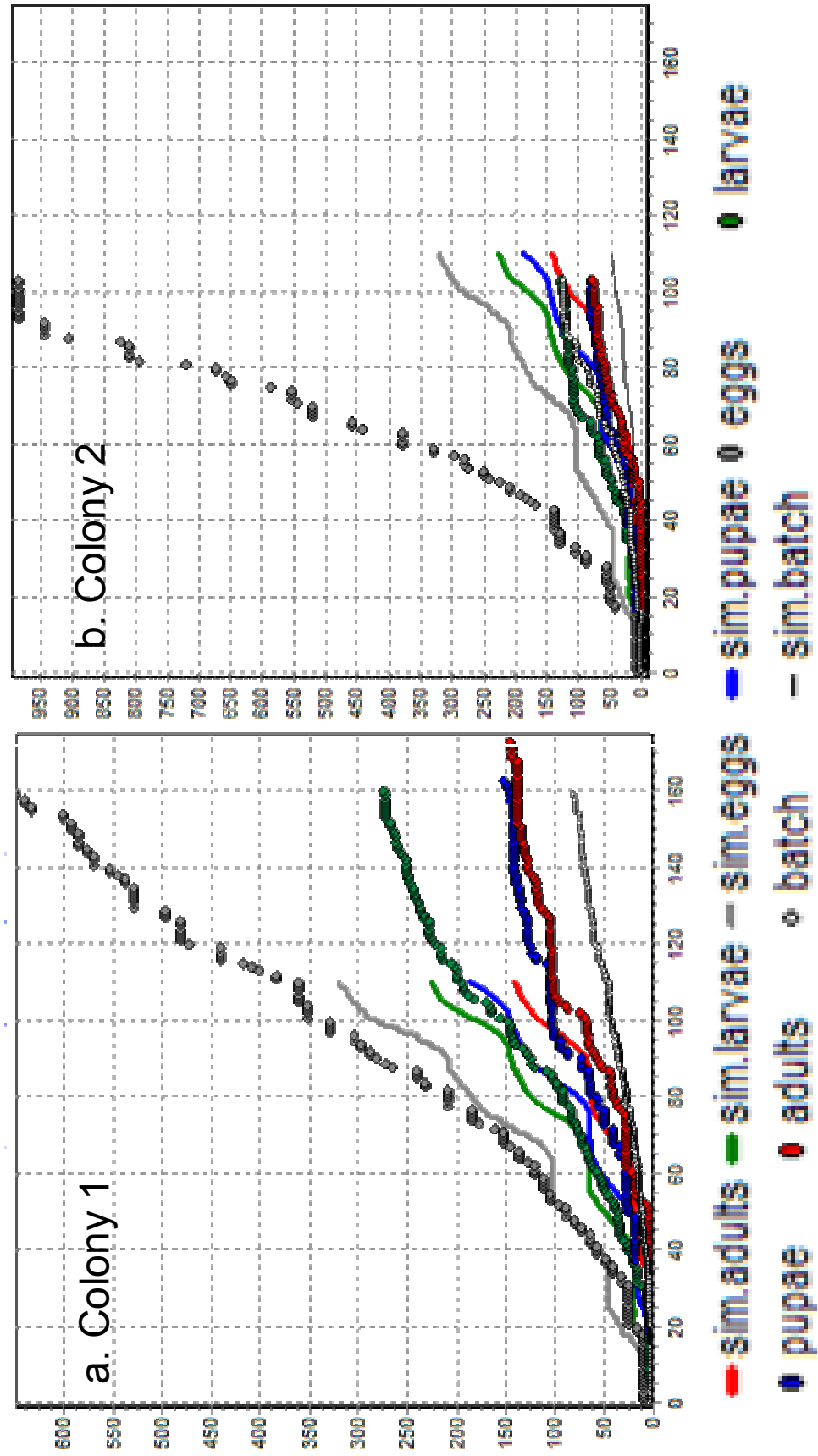


Figure 4.11 Simulated and observed accumulated data from four *B. atratus* colonies. (a) Colony 1, (b) Colony 2;

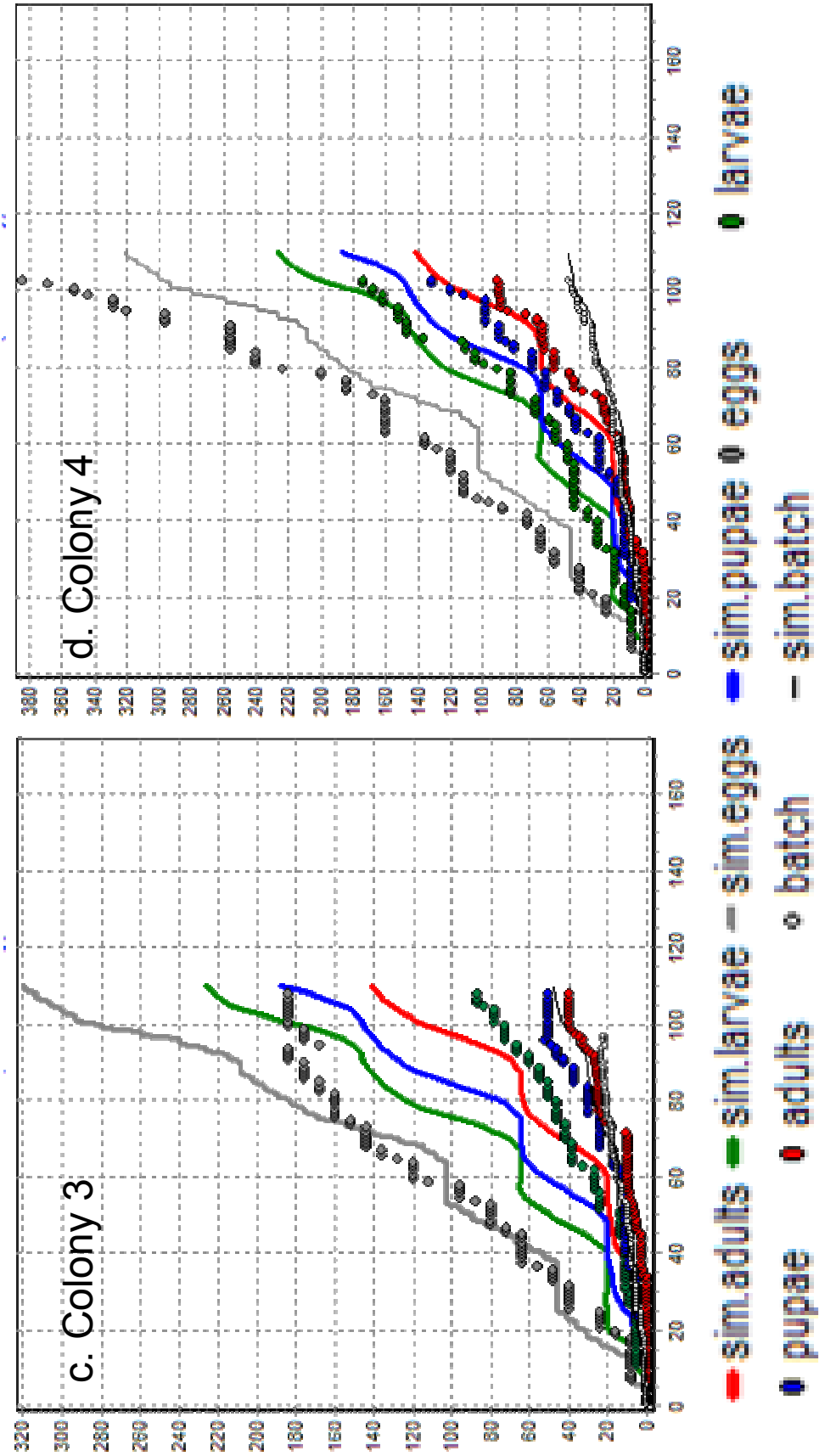


Figure 4.12 Simulated and observed accumulated data from four *B. atratus* colonies. (c) Colony 3 and (d) Colony 4.

4.3.5 Supply/Demand ratio and colony growth

The distribution of the supply/demand (S/D) parameter for the simulation was plotted against the time (Figure 4. 13). It has a value of 1 during the subsocial phase (until day 18). Once the eusocial phase starts, the relationship between supply and demands oscillates at values < 1 , because demands increase so that they are not completely fulfilled. In Figure 4.13 it also can be seen that eggs are laid in four main periodical pulses. The curve of workers appears more continuous despite the formation of the same four peaks but with a lower magnitude. The reason is the longevity of the adult stage, which is longer than that of immatures and causes an overlap of adult workers from different egg batches. The comparison of the non-cumulative data simulation with the distribution of S/D suggests that an increase in the amount of workers leads to an increase in the S/D ratio towards its maximum value.

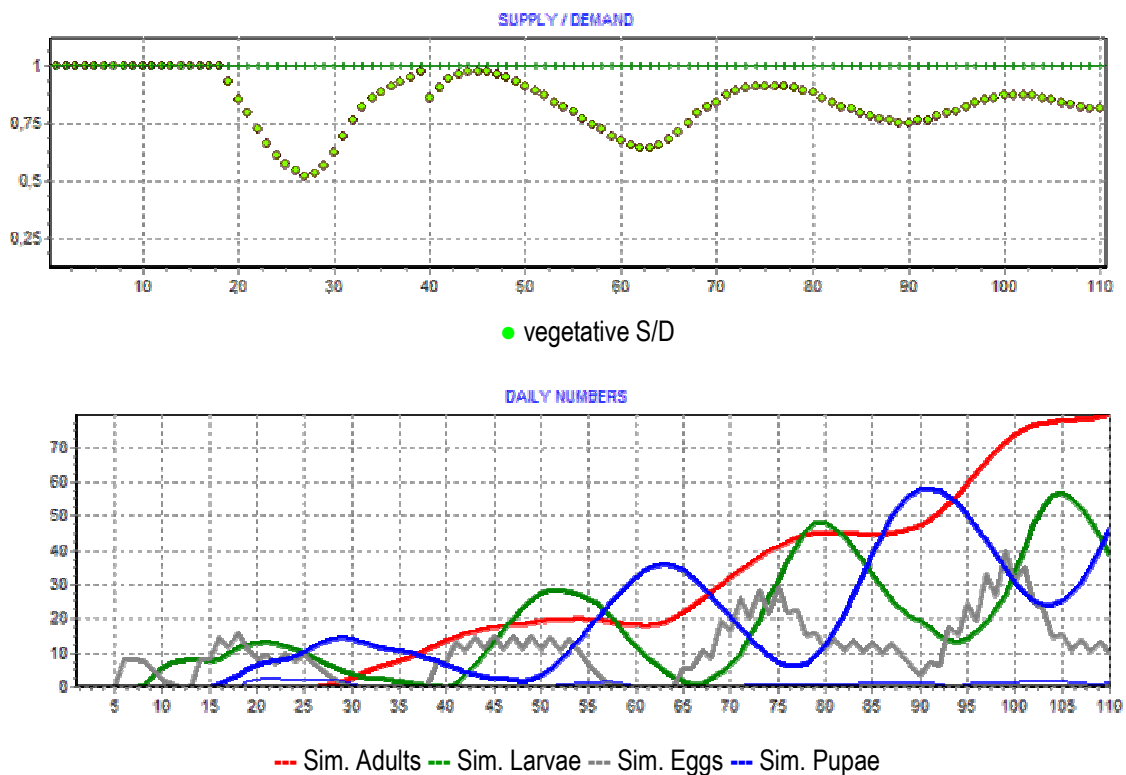


Figure 4.13 Daily simulation of supply/demand ratio for the somatic growth and simulation of not cumulative number of individuals at subunits eggs, larvae, pupae and adult workers from colony 4. Mortality of adult workers is calculated as a physiological senescence and not as the effect of foraging.

The S/D ratio shows that demands were higher at around days 37 and 60 when the colony had an average size of 10 and 20 workers, respectively, and oviposition of the next brood was about to take place. It indicated critical moments for the colony, which make it susceptible to food shortage or low temperature periods.

4.4 Discussion

The differences in the development during the subsocial phase of the colonies can be attributed to the fact that some queens are better able to take care of larger brood than others. During this phase, the queen is responsible for all rearing activities (oviposition, foraging, feeding and incubating); therefore, it has limited means to rear a large number of individuals (Alford, 1974). In the wild, mortality of eggs during this phase can be due to low temperatures when the queen is absent for incubation. In the reared colonies, the mortality of eggs was higher than 23% despite the fact that the queen did not need to leave the nest to find nectar, and temperatures were regulated to 27°C in the rearing room. Larva mortality depends on the amount and quality of the pollen provided as well as the temperatures at which the larvae are reared. No difference in the quality of food between colonies could be attributed to this difference, since all four colonies were confined in captivity and maintained under the same conditions during the subsocial phase.

In the eusocial phase, the factors that lead to immature mortality depend mainly on the house workers that attend the brood. In the same way as in the previous phase, pollen is a critical element and the lack of it causes starvation of larvae. The temperature in the nest becomes more stable as the colony grows and more adult individuals contribute to incubation and collection of nectar (Heinrich 1974). Nevertheless, the observed mortalities were higher than in previous phase. The relationship among the amount of workers and larvae has been proposed as an important characteristic of the colony that influences its development (Duchateau and Velthuis 1988, 1991). The average larva/worker ratio of observed data has the same pattern as that of *B. terrestris* of 2 larvae per worker in the last part of the distribution (Duchateau and Velthuis 1988). This suggests that the number of workers determined the capacity of the colony to grow, as it influenced the supply/demand ratio. Even though pollen and nectar were provided *ad-libitum*, not all available resources could be acquired to supply the demands of the immatures because of collection was inefficient when the density of

workers increased. This inefficiency was possibly higher in complete captivity than in semi-captivity. Additionally, the quality of pollen among the colonies fed in captivity was probably different from that in colony 4 that foraged in the gardens. As this variable was not controlled, it is possible that this factor could be a source of variation in larvae mortality. The effect of different pollen qualities must be included in the model.

The reported sizes of nests with 800 individuals (Sakagami and Zucchi 1965) contrasts strongly with this model, as such dimensions would only be possible with queens whose production life span would be eight times longer than that of the queens in this study (longevity of more than two years). Therefore, it can be stated that the model correctly simulates the dimensions of monogyny colonies, but that nesting behavior in the wild must follow dynamics different to those in this study.

Although the biology of temperate species is greatly different to that of *B. atratus*, the pattern of development between them is similar. As was mentioned above, growth phases (steps and linear) and larva/workers ratio are a common element in both species. The main difference is the rate of growth. The production of workers during the linear phase of *B. terrestris* colonies is higher than 2.5 workers/day (estimated from the data of Duchateau and Velthuis 1988) and can even reach 5.65 workers/day (Beekman and van Straum 2000). The differences in speed of development can be understood as the adaptation to seasons and the great shortage of resources that temperate species suffer from after the summer. The particular difference regarding how many workers emerge from the two first broods might be a key element that explains differences among colonies.

Under conditions of optimal temperature, feeding behavior might be a principal factor during the somatic growth of the colony. No reproduction was attained, probably because there was no oviposition of haploid eggs with the consequent triggering of gyne feeding behavior of the workers. This last process is still to be studied.

4.5 Conclusions

- The simulation of the colony development adjusts to the general pattern of colony development described for *Bombus* species in general but the main difference lies in the rate of development.
- With the methods used to rear colonies in captivity, the simulation shows that colonies can grow until a medium size similar to those of commercial *B. terrestris* colonies.
- There are two critical moments in the development cycle of a colony shortly before the end of the subsocial phase and shortly before linear growth begins that make it susceptible to shortage of food and/or exposition to low temperatures.

5 GENERAL DISCUSSION

5.1 Pollination

The results reveal that *S. quitoense* depends on pollination on a vector such as *B. atratus*. Flower bagging showed that wind pollination is not adequate, whereas hand cross-pollination improved seed yield. In practice, hand-pollination is not applicable, since it is extremely time consuming. Pollination through bumblebee was the method with the highest seed yields. Additionally, it was revealed that *B. atratus* is an important pollinator of *S. quitoense*, and not so much other bee species like the honey bee and smaller buzzing bees due to their behavior cues or distribution range.

According to Miller and Diggle (2003) *S. quitoense* has low plasticity of sex expression. However, the results of the present study show a different trend. The detected differences can be due to the kind of plant material used for the experimental crop. The 78 plants were probably genetically quite similar as they came from a single seed provider. However in general, *S. quitoense* has been under low genetic selection and consequently the variation for the plasticity of sex expression can be wider as Miller und Diggle (2000) reported. Therefore, the physiology of gender investment of the plant among male and hermaphrodite flowers should be further studied. Pollen addition not only influences fruit set but can also affect sex expression of andromonoecious plants. In *S. carolinense* plants, the production of male flowers is higher in plants with a higher fruit set (Steven et al. 1999). Therefore pollination could cause an increase of non-functional female flowers (SSF) and thus a decrease in potential fruit set.

5.2 Bumblebee behavior

Bombus atratus bumblebees foraged in the *S. quitoense* crop in search of pollen. They buzzed the anthers of the flowers in sequence and packed the released pollen grains in their corbiculae. They thus pollinated the hermaphrodite flowers, as they contacted the stigma with the parts of their body that bore pollen grains. Additionally, they foraged preferentially around the nest, so that they remained constant to the flowers of the crop and in this way promoted pollination.

The colonies of *B. atratus* can continue their development even in the absence of a queen, as was the case with the colony used in the pollination experiments. The

potential of this particularity of the specie's biology is that colonies display the same behavioral patterns as a queen-right colony where workers forage and take care of the brood (da Silva-Matos and Garófalo 1995). Moreover, for rearing purposes colonies could also be reproduced additionally by laying-workers that mate and become false queens. Nevertheless, the size of false-queen colonies is smaller, thus its foraging force is not as strong as that of a queen-right colony.

Temperature conditions are not limiting for *B. atratus* adults because of their individual thermoregulation, but the results show that thermoregulation determines most of the foraging activity of the colony as most of the foragers collected nectar. Wild nests of *B. atratus* are built covered by thin grass vegetation and a wax involucre (Gonzales et al. 2004, Morales et al. 2006) to isolate them. Consequently, colonies set up in a crop must be also isolated to minimize foraging activity for nectar and promote pollen collection thus *S. quitoense* pollination.

5.2.1 Rearing

Larvae feeding behavior has been a critical characteristic for the choice of species for rearing purposes, as pollen storer workers accept well the pollen provided in captivity and use it to feed immature individuals by regurgitation. By comparison, pocket-maker species do not so efficiently use pollen provided in mixture placed next to the brood when in captivity (Griffin et al. 1991). Although *B. atratus* is classified as a pocket-maker species (Sladen 1912) it does not follow the rigid behavior distinction of that group. Larval feeding is also done like by pollen storer species by regurgitating into individual larval cells and by reaching the larvae through the wax pocket (Rojas et al. 2003). Therefore, although Velthuis and van Doorn (2006) mention this as a problem when rearing pocket-maker species, the results of this study show that this is not a limitation in the case of *B. atratus*. However, wild *B. atratus* foragers deposit collected pollen from their corbiculae into the lateral wax pockets of larvae cells. For instance, the observation that few colonies reach the switch point can be related with this rearing procedure, because the capacity of feeding might be nevertheless affected by captivity limitations. As in the case of other *Bombus* species, gynes and drones are reared with larger amounts of food than workers (Pereboom 2000, Ribeiro 1994), consequently it is possible that acquisition limitations results in low queen rearing. To compare the results

of reproductive individuals rearing, a system of free-foraging colonies under semi-captivity conditions could be adopted to test this hypothesis.

5.2.2 Colony modeling

The modeling approach for population dynamics of Gutierrez (1996) was adapted to the dynamics of a bumblebee colony through the use of the colony energetics as the basis. This is a new application for this modeling approach, which has been used to model plant production (Gutierrez et al. 1994), and insect predators and parasitoids in programs of integrated pest management (Gutierrez et al. 1988,). The present model on bumblebee colonies allowed the analysis of a data set of four colonies. First, it was identified that the number of workers is the factor that determines further growth or decay of the colony during the eusocial phase of the colony. The implication for pollination management is the measures that have to taken to avoid initial loss of workers at the moment of introduction to the crop and the design of an isolating box that diminishes energy expenditure via thermoregulation. Second, the model also helped to understand that the growth of colonies in complete captivity has no numerical disadvantage, but that pollen acquisition by the larva when feed via the pocket in nos-captivity conditions is higher. With it, the effect is detected at a mass level. It is important to test if this has implications on the triggering of reproduction in the colony.

5.3 Management of *B. atratus* as pollinator: outlook

5.3.1 Cost/benefit analysis of pollination program with *B. atratus* colonies in *Solanum quitoense* crops

The following analysis is done taking into account the impact and profitability at the producer's level during a production cycle of one year. The time span is selected because it is estimated that the impact of pollination on fruit set and quality is significantly highest during the first production phases of the plant. A higher level analysis is not carried out, because implementation of the proposed technique the first of all, the producer's decision. The analysis considers the feasibility of pollination strategies following the profile of farmers and the local conditions in the Cundinamarca region.

Benefits of *B. atratus* colonies for pollination

The experimental results on the effect of bumblebee pollination show that crop plants such as *S. quitoense* need pollination management to fill production gaps. According to the data, lack of a vector for pollen transport could be responsible for a fruit production decrease of around 50% consequently the first foreseeable benefit for the farmer is a higher fruit yield.

The growth phases of *S. quitoense* plants play a role with regard to the quality of fruits seen in plants of indeterminate growth like tomato (Heuvelink et al. 2004). The initial harvest of the first fruit clusters has a medium quality, whereas the second to fourth or fifth fruit cluster has potentially the highest quality (extra). Therefore, pollination management must be also analyzed under production purposes. The effect of bumblebee pollination will be higher in markets where quality is taken into account so that an additional profit can be included in the income. Alternatively, the effect of pollination could have a greater effect on producers that sell their harvest for exportation purposes. Here, quality standards are higher, and fruits must have a homogeneous size around 5 cm diameter with no sign of damage.

An additional indirect benefit of bumblebee pollination is that it indicates the use of cleaner practices without or only low amounts of chemical pesticides. This benefit might not be reflected in the cash flow, but it is important for certification processes and could in future organic markets represent an added value.

Costs of *Bombus atratus* colonies

The international pollination initiative has identified that the lack of information on the costs for crop pollination is one of the main problems to overcome in the coming years. The viability of a program for pollination is affected by additional management costs that have to be quantified to predict the economical viability of the project in individual and local situations (Corbet et al. 1993).

The costs of pollination depend on the number of colonies needed per cultivated area. A single colony can exist for four two to four months; therefore in one year cycle, colonies would be set up twice for pollination. Additional costs of colony management arise from the supply of the sugar solution that the colony must receive because *S. quitoense* flowers do not produce nectar. Alternatively, landscapes can be

managed such that other sources of nectar such as red clover plants within *S. quitoense* crops can be offered.

5.3.2 Actors a pollination program

The conditions here discussed include to begin with the local producers of *S. quitoense* in Cundinamarca, who would be the beneficiaries if the technology of pollination is practiced. Their participation can only be achieved by an active diffusion of the beneficial results that bring pollination. There are two challenges to overcome in order to obtain their acceptance. First, as explained above, farmers underestimate the impact of insufficient pollination on production. Second, is bumblebees unfortunately are not well accepted by the farmers because of their aggressive behavior towards field workers and grazing cattle, which is frequently affected by wild bumblebee nests. Skepticism regarding the feasibility of managing colonies in the crop area are higher even when family members are active participants in crop management. In order to overcome these two factors, a participatory diffusion of the present results at a community level is proposed, preferably with the help of demonstrative units.

Second, the colony production has so far been achieved by members of the scientific research group on native bees and pollination ecology of the Universidad Nueva Granada in Bogotá in the academic research area of bumblebee rearing for pollination of crops.

The next step to implement the program of bumblebee pollination management is to increase production further to a commercial scale. This is more than for academic purposes and requires additional legal and financial requisites.

First, the use and commercial exploitation of native species is regulated by the Colombia legislation through the Law 611 of 2000 (for the normalization on sustainable use of wild and aquatic fauna), and the Decree 1608 of 1978 (for the regulation of the national code on renewable natural resources and the protection of natural environments). Consequently, a legal process to set up the bumblebee rearing must follow the legal animal husbandry framework.

Second, to reach extensive levels of colony production, it would be necessary to attract private industry investment. At this point, the strategy to follow is critical and depends on the kind of development that is foreseen for bumblebee domestication in

Colombia. The proposal is to follow the scheme of programs on the use and valuation of biodiversity, such as that of the Colombian governmental environmental system, Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH). IAvH is a governmental research organization for coordinating, promoting and studying biodiversity, its conservation and sustainable use. Under the program “Use and valuation of biodiversity” there is a scheme with which it would be possible to develop a company that responds to environmental concerns as well as to social distribution equilibrium through the products developed from the use of biodiversity.

5.3.3 Which other fruit crops could *B. atratus* potentially pollinate?

The range of potential crops that could be pollinated by *B. atratus* is as wide as that of bumblebee-pollinated crops in Europe and North America: strawberries, tomatoes, sweet pepper, egg plants, cucumber, among others. Even without knowing the particularities of the breeding systems of native species, the range of crops can be easily broadened. For example, Tamarillo (*Cyphomandra betacea*) also has a buzz-pollinated flower, and the cropping range of this species overlaps with that of *S. quitoense*. In the fruit producing region of Sumapaz, blackberry is also frequently cropped. The morphology of the flower is totally different, therefore, an additional study needs to be carried out to quantify the effects of pollination on this species. The advantage in these cases is that the adoption of the technique could be very quickly implemented.

Additionally, *S. quitoense* has at least 4 related species within the section *Lasiocarpa* of the *Solanum* genus that have not been explored as intensively as *S. quitoense* for economic purposes (Bernal et al. 1996). Generally, these species (*S. hyporhodium*, *S. pectinatum*, *S. sessiliflorum* and *S. stramonifolium*) grow wild in areas of lower altitudes and their pollination is by wild bee species. *Bombus atratus* is very probably among the wild pollinators of these species but a further survey is necessary to confirm this statement.

5.3.4 Limitations of the proposed program for pollination management

Application of chemical insecticides is the main limitation identified for the management of bumblebee colonies in *S. quitoense* crops. The plant is susceptible to various pests, and most of the farmers control these chemically. Unfortunately, there is

no available study on the effect of active components of insecticides on bumblebees. Biological control is applied in only 25% of the cases. To produce guides for pollination management in the field, specific information is necessary. The costs of bumblebee colonies might be not so significant, but alternative pest control is expensive as well as harvest management and post-harvest management with higher technical input. This is the case in other continents where the use of bumblebees is generally in combination with biological control or practices that tend to reduce the amount and frequency of insecticides (Velthuis and van Doorn 2006).

Furthermore, rearing of species *ex-situ* can be part of conservation strategies but it must be followed by landscape management to provide suitable habitats for introduced individuals (Owen and Chiras 1995). Crops and agro-ecosystems where colonies of bumblebees are introduced must be managed such that colonies can develop and reach reproduction with diverse pollen sources and areas for nesting places. Again changes in traditional pest control practices are necessary.

5.3.5 Recommendations

It would be desirable for government institutions to increase their efforts on the protection of beneficial organisms for agriculture, especially pollinators. Environment policies with legislation for direct protection of pollinators and the adoption of pollinator friendly practices in agriculture are necessary.

Fortunately, in Colombia there is already a national strategy on biodiversity related to agriculture development headed by the IAvH with support from UNEP. The project goal is to evaluate the effects of agriculture market liberation on Colombian biodiversity. Among the priorities of this strategy, food security and biodiversity are included but not pollinators. As food production is affected by pollination, it is therefore desirable for management of pollinators to be included.

Finally, the experiences of other countries have shown that research is an essential element for the development of sustainable pollination (Velthuis and Doorn 2006). Moreover, the results of the present study also show that scientific research can bring concrete alternatives for pollination management, so that the efforts of the Colombian government should be directed towards research on the native bee fauna.

6 SUMMARY

6.1 General introduction

Solanum quitoense is a fruit from the northern Andes. As many others, this is a good example for the diversity of the local fruits found in Colombia with a potential for export due to the attractiveness of its taste. However, an increase in production is hampered in part by lack of appropriate pollination. One of the reasons is that populations of wild pollinators are scarce. To solve this problem, we proposed to manage pollination with colonies of the native bumblebee *Bombus atratus* reared in captivity. The study was done in three parts that integrate the biology of the plant and the pollinator: 1) description of the *S. quitoense* breeding system with experiments that measured the effect of bumblebee pollination on quality and fruit set, 2) description of the behavior of bumblebees while they visited *S. quitoense* flowers, 3) development of a bumblebee colony was simulated with a trophic model.

6.2 Breeding system of *S. quitoense* and its pollination with bumblebees

Flowers of *S. quitoense* have poricidal anthers that enclose the pollen, which can only be liberated through vibrations. The breeding system is called andromonoecy in which each plant bears a variable proportion of long-style flowers (LSF) and short-style flowers (SSF). Since it is not yet clear which consequences this breeding system and floral morphology have on pollination requirements, the function of floral morphs was studied. Pollination through bumblebees was compared with spontaneous pollination by wind.

Questions

- Why do short-style flowers not set fruit?
- Does fertilization depend on the floral morphs from which donor pollen comes?
- Which pollination cross produces higher fertilization of ovules?
- Is fertilization efficiency different between spontaneous self-pollination and forced self-pollination?

Methods

In July 2004, an experimental crop was placed in an area of 312 m² that contained 78 *S. quitoense* plants evenly arranged in 6 rows of 13 plants each. The crop area was located in Cajicá, a central municipality in Colombia. The region is located at 2558 m asl and the mean annual temperature is 14°C, although extreme temperatures can range from frost to 35 °C. Plant material for the experiments was obtained from the experimental crop.

The maturity of stigma and pollen grains was tested in different stages of flower development to determine the overlap of male and female phases. The maturity of the stigma was tested with the reaction of the stigma enzyme esterase to hydrogen peroxide (H₂O₂). Pollen maturity was tested with a pollen germination test in a 20% sugar medium. Additionally, the moment of anther dehiscence was determined in the development of the open flower.

The esterase reaction on stigmas of LSF and SSF flowers was compared to test female functional differences among flower morphs. Additionally, pollen grain germination of pollen from anthers of LSF and SSF flowers was compared to detect differences between the male function of both floral morphs.

The phenology of flower production was tracked in 31 plants. The number of LSF and SSF flowers was counted in each plant during 11 months.

The self-compatibility of LSF flowers was tested with hand-pollination experiments. Pollen from different donors (flower from a different plant, different flower of the same plant and same flower) was deposited on the stigma of LSF flowers. The proportion of fruit set, fruit weight and number of seeds per fruit were compared among crosses.

A. B. atratus colony was placed in the crop area and the effect of pollination by the bumblebees was quantified by comparing fruit set and fruit characteristics with those of spontaneous self-pollination by wind in bagged flowers.

Results

The results show that LSF flowers of *S. quitoense* have simultaneously male and female phases, as pollen grains are mature and stigma are receptive when the flower opens. There is a short period of asynchrony of one to two days while the pores of the anthers

remain closed. The comparison among floral morphs of stigma receptivity and pollen germination confirmed that LSF flowers retain both reproductive functions while SSF flowers are female sterile as the activity of the esterase on stigmas is minor. The germination of pollen grains showed that LSF flowers have more viable pollen than SSF flowers.

Hand-pollination experiments revealed that although completely self-compatible, cross-pollination resulted in significantly higher seed production. The visit of bumblebees to flowers doubled fruit set and produced significantly bigger fruits that bore more seeds.

Discussion

There is no asynchronous mechanism to avoid self-pollination. However, there might be a degree of self incompatibility in flowers of *S. quitoense* so that cross-pollination produces more seeds, but the species is not allogamous. The finding that pollination of bumblebees rendered more fruits that bore more seeds shows that pollen deposition is high when flowers are visited by bumblebees. The andromonoecious system of *S. quitoense* might be a mechanism to attract pollinators like *B. atratus* and to a lesser degree a mechanism to avoid self-pollination.

Conclusions

- Although completely compatible, cross-pollination has an advantage in *Solanum quitoense* reproduction, thus favoring pollen transport by the workers of *Bombus*.
- Pollen deposition on the stigma is increased by bumblebee visits.
- Yield and quality of fruits were significantly improved through pollination by *B. atratus*.

6.3 Foraging behavior of *B. atratus* workers

Introduction

Bombus atratus visit a wide range of plant species in the wild and they thus feed their brood with different pollen. They collect pollen from flowers with poricidal anthers by vibrating the anthers with their typical buzzing behavior. However, they are inconstant foragers in a foraging bout and change from flowers of one species to flowers of other

species. The goal of the experiment was to relate the behavior of the foragers with the beneficial effects of pollination obtained in the previous chapter.

Questions

- How do workers of *B. atratus* handle floral cues of *S. quitoense*?
- Do workers of *B. atratus* deposit pollen on the stigma?
- Are sequences of visitation among flowers of different plants more frequent than among flowers of the same plant?
- Is foraging activity synchronized with receptivity of flowers?
- Which resource (pollen or nectar) is collected the most by the colony?
- Do foragers remain constant to *S. quitoense* flowers?
- Is pollen provided by *S. quitoense* is used by the colony?

Methods

The foraging activity of bumblebees of a reared colony was tracked in *S. quitoense* crop. Before the colony was brought to the crop, all workers were individually marked with numbered plastic discs. A trained observer followed individual foragers during their foraging bouts. On each visit of a bumblebee to a flower, the observer timed the handling time and registered if the bumblebee buzzed the anthers and contacted the stigma with its body. The sequence of visited flowers was tracked and each visit classified as visit to: 1) the same flower, 2) a flower of the same plant or 3) a flower of a different plant.

The resources that the bumblebees collected were quantified by registering the resource that foragers carried when coming into the nest. A sample of pollen loads was taken with a pollen trap but the device affected the activity of the colony.

The development of the colony was tracked. Every month, the number of adults and immatures were counted in the colony in the laboratory. Newly emerged workers were tagged.

Results

During the visits to the flower, bumblebee workers extracted pollen from poricidal anthers by buzzing them and contacted several times the stigma of LSF flowers with parts of the body that bore pollen grains. They showed no preference for any flower morph, but they never visited senescent flowers. During one foraging trip, a worker visited in average 14 flowers, most of them on different plants.

Most of foragers collected nectar from the feeders that were provided near the colony. Those workers that specialized in collecting pollen, mostly visited *S. quitoense* flowers. The foraging activity of the colony overlapped with the receptivity period of the flowers during the morning.

The colony produced 30 new workers and 4 reproductive individuals after four months of activity in the crop.

Discussion

The results show that *S. quitoense* flowers are attractive to *B. atratus* workers. They act as pollinators because they extract the pollen from the anthers and deposited it on the stigma. The pattern of flight among flowers of different plants also promoted cross-pollination. Despite the variation of the proportion of LSF flowers, the probability that the bumblebee pollinated them increased with the number of flowers that each bumblebee visited during one single foraging trip.

Although *B. atratus* are generalists and feed their brood with pollen from more than one family of plants, the brood in the colony was able to develop with a diet of mainly *S. quitoense* pollen. Therefore, the conditions of the crop allowed further development of the colony during four months until the queen died.

Conclusions

- *Bombus atratus* workers vibrated the flower of *S. quitoense* to extract pollen from the anthers. During the visit, they contacted the stigma of hermaphrodite flowers with the parts of their body that bore pollen grains.
- *Bombus atratus* workers that collected pollen did it mostly from flowers of *S. quitoense*. Their foraging behavior was highly constant to these flowers.

- *Bombus atratus* workers foraged on flowers of different plants more frequently than on flowers of the same plant, so that they promoted cross-pollination.
- The foraging activity of *B. atratus* workers during the morning overlapped with the receptivity of the LSF flowers of *S. quitoense*.
- The colony of *B. atratus* was able to develop with the pollen rewards from *S. quitoense* flowers.

6.4 Modeling colony dynamics of *B. atratus*

Introduction

Rearing of *B. atratus* colonies requires good knowledge of its biology. The first steps of the domestication of this species have been reached, but problems with the development of the colonies still exist. A model was used to simulate colony development to identify the effect of the main parameters: rate of oviposition and worker production, mortality, and pollen and nectar consumption. The modeling approach was with a trophic model for acquisition of nectar and pollen that is regulated by supply and demand.

Questions

- Does oviposition rate determine worker production rate?
- In which development stage is mortality highest?
- Is immature development restricted by the number of workers that supply demands of the larvae despite unlimited resources?
- In which development phase of the colony (solitary, eusocial, and competence) is the demand for pollen and nectar highest?

Methods

The model was built using the concept of superorganism to describe the colony. It functions like a module system composed of specialized subunits to transform masses of pollen and nectar into masses of bumblebees. The first subunit is the founder queen; the second subunit is the group of workers; the third subunit is the gynes and drones. The theory of resource acquisition and resource allocation of Gutierrez (1996) was adapted to the energetics of the colony. The acquisition of nectar and pollen by the workers of

the colony and its effects on colony development were quantified. Resource acquisition followed a non linear distribution. It was determined by total demand, availability nectar and foraging efficiency of workers. The allocation of resources was determined by the relationship between the supply and the demand of resources (S/D - supply/demand ratio); when close to 1, the rate of worker production was faster, and when the S/D was zero, demands were not fulfilled and the colony stopped growing.

Four colonies were reared from wild captured queens. They developed in captivity under constant conditions of temperature and relative humidity. Three colonies were in complete captivity. One of the boxes (colony 4) had an entrance that allowed the foragers to leave the nest through the wall of the rearing room and forage outside in the gardens of the experimental center. The parameters for the model were collected from the life table of colony 4, which showed a better development than the other colonies. Their development was compared by simulation with a linear regression of the observed data and the simulated data.

Results

The development of the colonies was different, and colonies 1 and 4 had higher rates of worker production than colonies 2 and 3. However, none of them reached reproduction. The oviposition rate during subsocial phase regulated the amount of emerged workers in the first two broods. In the eusocial phase the mortality of larvae was the main factor that determined colony growth. This pattern was common for all four colonies. The relation among the number of larvae and number of workers also followed a common pattern, with the exception of colony 2, which showed the slowest development.

The simulation of the colony is highly correlated with the observed data ($R^2 > 0.96$ in all cases), but the model sub estimates the production of eggs while it overestimates the production of larvae, pupae and adults.

The S/D ratio detected that at the beginning of the eusocial phase the colony is susceptible to workers loss.

Discussion

The differences in the development during the subsocial phase of the colony can be attributed to the queens, which have highest capacity to take care of a larger brood. In

the eusocial phase, the factors that lead to immature mortality depended mainly on the house workers that attended the brood. In the same way as in the previous phase, pollen was a critical element and the lack of it caused starvation of larvae. Even though pollen and nectar were provided *ad-libitum*, not all available resources could be acquired to supply the demands of immatures because of the collection inefficiency when the density of the workers increased. This inefficiency was probably higher in complete captivity than in semi-captivity, because workers were able to acquire more pollen if they were able to fly freely and forage flowers in comparison to those who collected a mass of pollen in the rearing room. Additionally, the quality of pollen among the colonies fed in captivity was probably different to that in colony 4 that foraged in the gardens. As this variable was not controlled, it is possible that this factor could be a source of variation in the mortality of larvae. The effect of different pollen qualities must be included in the model.

Conclusions

- The simulation of the colony development adjusts to the general pattern of colony development described for *Bombus* species in general, but the main difference lies in the rate of development.
- With the methods used to rear colonies in captivity, the simulation shows that colonies can grow until a medium size similar to that of commercial *B. terrestris* colonies.
- There are two critical moments in the development cycle of a colony shortly before the end of the subsocial phase and short before linear growth begins that make it susceptible to shortage of food and/or exposition to low temperatures.

6.5 General discussion

Based on these results, it can be confirmed that pollination of *S. quitoense* requires buzz pollination. Furthermore, the visit of a large-body bee is necessary to reach the stigma of LSF flowers to deposit pollen. As fruit set increases significantly when bumblebees pollinate the flowers, further research on the effect of fruit set is necessary on gender expression in *S. quitoense* that could affect fruit production.

To improve the rearing methods of *B. atratus* colonies, it is recommended to combine semi-captivity and total captivity conditions so that worker intake of pollen increases.

The use of colony energetics to simulate the colony allowed adoption of the model to the initial development of a bumblebee colony. In the model it is necessary to include the quality of the pollen as a variable to simulate more accurately its effect on assimilation and weight of reared workers.

6.6 Outlook

The benefits of managing reared colonies of bumblebees to pollinate are the increase in profit for farmers, the use of cleaner practices, and the sustainable use of a wild species as pollinator. However, the adoption of this technique implies the use of more expensive practices in agriculture such as biological control over insecticides and better landscape management that favour the re-establishment of wild pollinators. The foreseen costs are the cost of the colonies and their feeding with nectar in cultures that does not offer nectar in their flowers.

The feasibility of this technique depends on the participation of the community of farmers in the region of Cundinamarca and the support of local environmental organizations that regulate the use of wild species.

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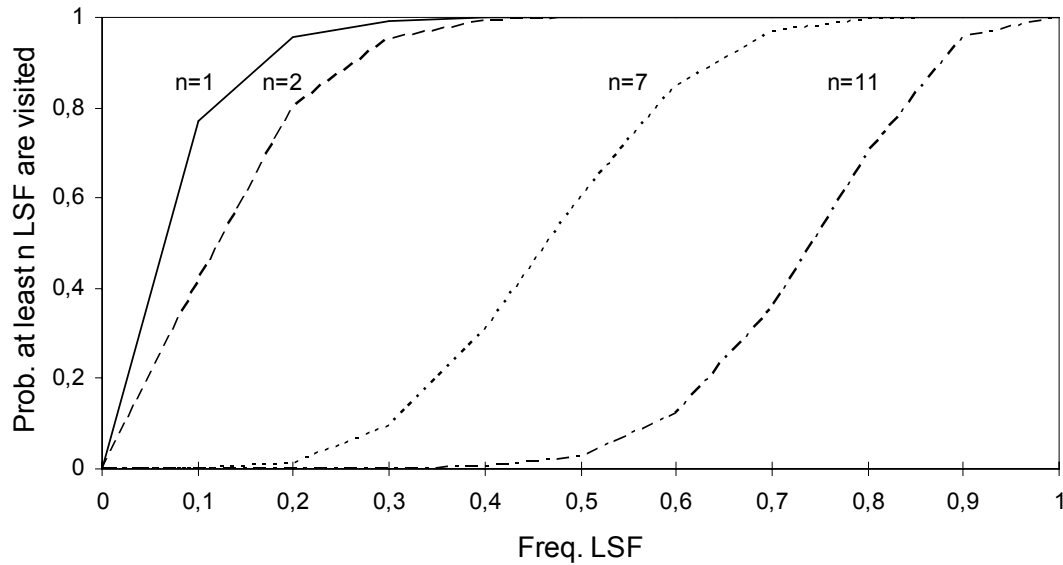
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8 APPENDICES

Appendix A1: List of bouts and the number of flowers that the forager visited. The time when the data were registered appears in the first column.

Bout	Hour	Visited flowers	Bout	Hour	Visited flowers
1	09:00	11	43	11:00	17
2	09:00	2	44	11:00	24
3	09:00	8	45	11:00	4
4	09:00	12	46	11:00	14
5	09:00	3	47	11:00	11
6	09:00	6	48	13:00	17
7	09:00	17	49	13:00	33
8	09:00	9	50	13:00	15
9	09:00	2	51	13:00	3
10	09:00	4	52	13:00	16
11	10:00	20	53	14:00	8
12	10:00	13	54	14:00	16
13	10:00	18	55	14:00	3
14	10:00	2	56	15:00	15
15	10:00	13	57	15:00	40
16	10:00	2	58	15:00	31
17	10:00	20	59	15:00	22
18	10:00	19	60	16:00	32
19	10:00	12	61	16:00	32
20	10:00	29		Mean	14.08
21	10:00	15			
22	10:00	16			
23	10:00	14			
24	10:00	21			
25	10:00	20			
26	10:00	21			
27	10:00	16			
28	10:00	35			
29	10:00	4			
30	10:00	15			
31	10:00	9			
32	10:00	5			
33	10:00	10			
34	11:00	4			
35	11:00	3			
36	11:00	13			
37	11:00	12			
38	11:00	10			
39	11:00	4			
40	11:00	11			
41	11:00	12			
42	11:00	14			

Appendix A2: Probability distribution of the event that a worker visits at least n LFS flowers during one foraging bout at different LSF flowers frequencies. It is assumed that the forager visits 14 flowers during a bout and that it has no preference for none of the floral types.



Appendix A3: Estimation of pollination capacity of colonies from different sizes. It is assumed that a forager bumblebee visits 14 flowers during one bout. Because receptivity of LSF flowers is limited to the hours of the morning, total value of visited flowers is calculated for the hours when foraging activity overlaps with receptivity (4 hours per day). Additionally it is assumed that pollen demand increases linearly with the size of the colony and that there is no effect from density of foragers on the efficiency of pollination. Colony marked with * would be the case of the colony used for pollination experiments (chapter 2 and 3).

Colony size (number of workers)	Potential foragers collecting pollen	Potential visited flowers	Number of plants	Crop area (m ²) with 3x3 sowing density	Colonies/ha
15	3	672	96	288	35
20*	4	896	128	384	26
50	10	2240	320	960	10
100	20	4480	640	1920	5
200	40	8960	1280	3840	3

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Appendix A4: Parameters of initial values for founder queen and workers. Timing for each parameter is also given.

Factor	Parameter	description	units	Calculation logic	Source
Founder Queen	Founder mass	mass of the founder queen= 0,96 g	g	average weight= 0,9632 +/- 0,1876 n=11	Own data queens captured in the wild and weighted short after capture and before they started to build a nest in captivity
	Weight adult W Weight adult Wf	mass of adult worker mass of forager adult worker	g g	average weight= 0,24 +/- 0,068 n=30 average weight is > 0,19 g the threshold of 0,19 was assumed by comparing the mean weight of workers of a foraging nest producing "bigger" workers and two nest in captivity that produced mean small workers, mean weight of "big" workers 0,24	Own data of <i>B. atratus</i> wild nest in lulo Own data of <i>B. atratus</i> colonies C159- C157 and foraging nest in lulo crop
Workers	Weight adult Wh	mass of house adult worker	g	average weight is < 0,19 g the threshold of 0,19 was assumed by comparing the mean weight of workers of a foraging nest producing "bigger" workers and two nest in captivity that produced mean small workers, mean 0,16 g and 0,2 g 0,3994	Own data of <i>B. atratus</i> colonies C159- C157 and foraging nest in lulo crop
	Weight pupa W	mass of one pupa of worker	g		Own data of <i>B. atratus</i> colony C159
	Weight egg W	weight of an egg of worker	g	the value is 0,001 n=8 eggs	own data of <i>B. atratus</i> colony C159
	Eggs per batch	number of eggs per batch		Each batch contains 5 to 8 eggs. We took 8 as the value for every egg cell	
	K worker			K worker=30	
Timing	Base foraging	Time for first workers to start foraging	dd	48	

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Factor	Parameter	description	units	Calculation logic	Source
	Base immature	is this the threshold	dd	23	The value is calculated through the difference of the ambient temperature and the temperature threshold value. 27°C is the rearing room temperature and 23°C is the assumed value for the threshold. Reasons: 1) Heinrich states that under 30 °C the larval development stops.2) For honey bees the value is 34,5°C (source Wittmann) and 3) the mean nest temperature of a wild nest is 22°C (Riaño, Velosa and Orozco, 2005).
	tsp	time switch point (=tffb time first fruiting branch) it is the moment when the first haploid egg is laid by the queen	dd	84	
	Start queens		dd	84	
	Start eggs W	time when first diploid eggs are laid	dd	0 this moment is assumed to be the same as start cells W	
	Start larvae W	time when eggs hatch as larva of a worker	dd	value= 27dd because development duration of egg is around 7 days in rearing chamber at 27°C	Own data from <i>B. atratus</i> C159
	Start pupae W	time when larvae of a worker pupate	dd	value= 79dd because development of larva is around 13 days in rearing chamber at 27°C	Own data from <i>B. atratus</i> C159
	Del W	time when adult workers emerge	dd	value=131dd because duration of pupa development is around 13 days in rearing chamber at 27°C	Own data from <i>B. atratus</i> C159
	Begin growth W	time when mass accumulation actually starts	dd	value= 44dd because larva starts growing at day 11 of development (first instars is assumed as no mass accumulation) just after day 4 as larva (7+4)	mass accumulation with age from worker's larvae in work by Ribeiro, M.F. 1994 Growth in the bumblebee larvae: relation between development time, mass, and amount of pollen ingested
	Stop growth W	time when accumulation of mass stops	dd	value= startpupaeW because after pupa the individual grow no longer	

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Factor	Parameter	description	units	Calculation logic	Source
	Start adults Wf	time when an adult worker that has been rated as forager when it emerged and is considered as young an do not leave the nest	dd	value=0 dd when the worker just emerged from the pupa	
	Age Ad1 Wf	time when an adult worker that has been rated as forager is considered as mature and is able to leave the nest to forage	dd	value=20 dd when the worker is as adult 5 days old and then might be ready to leave the nest	E. Da Silva-Matos and C.A. Garófalo 2000 Workers life tables, survivorship, and longevity in colonies of <i>Bombus (Fervidobombus) atratus</i> (Hymenoptera: Apidae). Rev. Biol. Trop. 48 (2/3): 657-664 Own data from <i>B. atratus</i> C159
	Del Ad W	duration of the adult life of the worker (for foragers and house workers)	dd	value=168 calculated with a longevity of 42 days under captivity conditions when no foraging is done	
	Start adults Wh	time when an adult worker that has been rated as house worker is still considered as young	dd	value=0 dd when the worker just emerged from the pupa	
	Age Ad1 Wh	time when as adult worker that has been rated as house worker remains in the nest	dd	value=0 dd when the worker just emerged from the pupa	
	Start eggs D	time when first haploid eggs are laid it starts the switch point (tsp)	dd	value= 0dd from the start of the tsp	
	Start larvae D	time when eggs hatch as larva of a drone	dd	value= 27 it s assumed the same duration as worker's eggs	
	Start pupae D	time when larvae of a drone pupate	dd	value= 95dd because males have a longer development	
	Del D	time when adult drone emerges	dd	value= 163	
	Begin growth D	time when mass accumulation actually starts in larvae of drones	dd	value= 44dd because larva starts growing at day 11 of development (first instar is assumed as no mass accumulation) just after day 4 as larva (7+4)	mass accumulation with age from worker's larvae in work by Ribeiro, M.F. 1994 Growth in the bumblebee larvae: relation between development time, mass, and amount of pollen ingested
	Stop growth D	time when accumulation of mass stops	dd	value=95 startpupaeD because after pupa the individual grow no longer	

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Factor	Parameter	description	units	Calculation logic	Source
	Start adults D	time when male still remains in the nest after it emerges. It probably feeds and in some occasions it can even help by incubating. But this help will be neglected in the present work.	dd	value=0 when drone just emerged	
	Age Ad1 D	time when male is to leave the nest in search for Gynes to mate. This might be the reproductive mature time when they are able to mate	dd	Value =16 because males are seen to stay for around 4 days in the nest.	C.A. Garòfalo, R. Zucchi and G. Muccillo 1986 Reproductive studies of a neotropical bumblebee <i>Bombus atratus</i> (Hymenoptera:Apidae) REV. Brasil. Genet. IX,2: 231-243
	De1 Ad D	duration of the adult life of the drone	dd	value=168 assumed from a longevity of 42 days under captivity conditions when no foraging is done similar as workers	Own data from <i>B. atratus</i> C159
	tst	It is assumed that diploid eggs are as well laid by the queen and fed to produce gynes. Simultaneously with time when first haploid eggs are laid it starts the switch point (tsp).	dd	value= 0 form the start of the tsp. The proportion of eggs laid simultaneously is assumed to be 6 males to 1 gyne	S.A. Camero and M.C. Jost 1998 Mediators of dominance and reproductive success among queens in the cyclically polygynous Neotropical bumblebee <i>Bombus atratus</i> Franklin. Insectes Sociaux 45: 135-149. Other reports for other <i>Bombus</i> species are the same 6:1 sex ratio.
	Start larvae G	time when eggs hatch as larva of a female larva that will be fed as gyne	dd	value= 27 it s assumed the same duration as worker's eggs	
	Start pupae G	time when larvae of a gyne pupate	dd	value=95 if the duration of gyne development is longer from the one of workers then it is assumed that the larva stage is the stage that makes the bigger difference in comparison to the one of the workers (17 days)	Ribeiro, M.F. 1994 Growth in the bumblebee larvae: relation between development time, mass, and amount of pollen ingested. Among other references Plowright and Duchateau

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Factor	Parameter	description	units	Calculation logic	Source
	De1 G	time when adult gyne emerges end of immature phase of development	dd	value=179 dd (26 days) it is assumed that queen have a total development time of 44,5 days (14 days longer as <i>B. terrestris</i>) as workers of <i>B. atratus</i> have this difference in development duration with <i>B. terrestris</i> workers (33 vs. 22 days respectively)	M.J. Duchateau and H.W. Velthuis 1988 Development and reproductive strategies in <i>Bombus terrestris</i> colonies Behavior 107: 186-207.
	Begin growth G	time when mass accumulation actually starts in larvae of gynes	dd	value= 44dd because larva starts growing at day 11 of development (first instars is assumed as no mass accumulation) just after day 4 as larva (7+4)	
	Stop growth G	time when accumulation of mass stops	dd	value= 95startpupaeG because after pupa the individual grow no longer	
	Start adult G	time when gynes emerged and remain in the nest and do not leave it yet	dd	value=0 dd when the gyne just emerged from the pupa	
	Age Ad1 G= mating maturity	time when gynes are ready to leave the nest to mate and start a new colony. They are potential queens	dd	Value =40dd Gynes stay in the nest around 10 days taking pollen and nectar from the nest while their corpora allata develops. In captivity gynes of 6 days old are ready to mate (<i>B. terrestris</i>)	C.Gretenkord1996 Laborzucht der Dunklen Erdhummel <i>Bombus terrestris</i> L. und toxikologische Untersuchungen unter labor- undHalbfreilandbedingungen Dissertation Uni-Bonn
	Start cells W	time when the new gynes mated and can start to build a nest (first cell or the nectar pot)	dd	value=100 it is the theoretical begin of the next nest development calculating that after the mating queens of <i>B. atratus</i> started to lay eggs in average in the next 15 days, i.e 60 dd	C.A. Garòfalo, 1979 Observacoes preliminares a fundacao solitaria de colonias de <i>Bombus atratus</i> Franklin (Hymenoptera: Apidae) Bolm.Zool.Univ.S.Paulo 4:53-64
	De1 Ad G	duration of the adult life of the gyne (queen)	dd	value=720 assuming that a queen can last 6 months. The queen of C159 colony died after 166 days in captivity and had an unknown time of solitary phase in the wild before the capture	Own data from <i>B. atratus</i> C159

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