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).

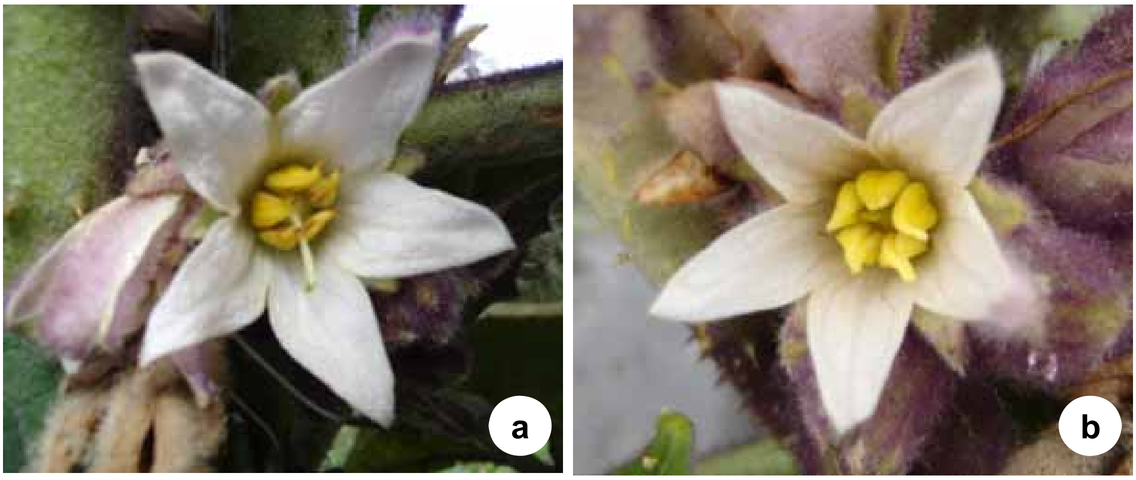

Figure 1.1 Floral morphs of *S. quitoense*. a. Long-style flower (LSF) morph. Note that the style is long exceeding the tip of the anthers with the stigma pointing to one side of the floral axis. b. Short-style flower (SSF) morph. The stigma does not exceed the anthers.

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 (Abrajaminovich 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, Abrajaminovich and Diaz 2002, Abrajaminovich 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 expulsed though 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 must 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).

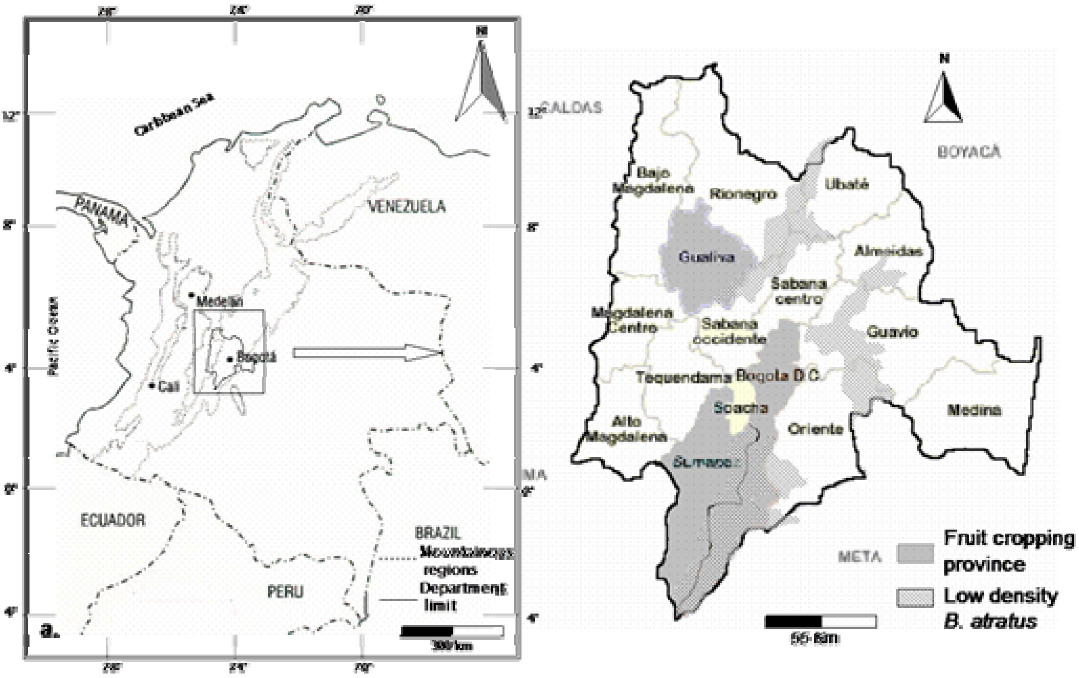

Figure 1.2 a) Department of Cundinamarca in Colombia. b) Location of main fruit production areas Sumapaz, Bogotá D.C. and Gualivá and areas over 3000 m asl where density of *B. atratus* is low.

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