São Paulo Declaration on Pollinators plus 5 Forum

# Group 1. Discussing the surveying and monitoring of pollinators at natural landscapes and cultivated fields

Group 1

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## SUMMARY

### THE POLLINATION CRISIS AND THE NEED FOR SURVEYS AND MONITORING PROGRAMS

The impact of deforestation, habitat fragmentation, introduction of exotic species and unfriendly agricultural practices is believed to be causing a decrease in wild pollinator populations. This, on turn, is suspected to be the cause of low fruit and seed productivity in many crop plants with economic consequences in many parts of the world. Also the productivity of wild plants may be affected and this can lead to local extinction of populations of those plants, as well as of those animals depending on them.

Since this “pollination crisis” was recognized, much effort has been put on initiatives to conserve and sustainable use wild pollinators. However, it is widely recognized that we lack much of the knowledge we need to propose effective actions to achieve

conservation and management practices. Even are not even certain about the geographic extension and intensity of pollinator population decreases. We also lack basic information on how different factors affect wild populations of flower visiting organisms.

Two basic questions stand as of surmount importance for any conservation or sustainable management initiative to succeed: 1) which pollinator species exist in any given place? And 2) how are their populations fluctuating along the time?

For these questions to be answered, we need to invest on pollinator faunistic surveys and on monitoring programs.

**AIM:**

The establishment of standard methodologies for bee surveys and monitoring at natural landscapes and crops.

**EXPECTED PRODUCT:**

The production of a manual of standard methodologies for bee surveys and monitoring at natural landscapes and cultivated fields.

**RESULTS:**

The group has discussed the general structure of standard procedures to survey and monitor bees in cultivated fields and natural areas.

**Rationale**:

- Data to be obtained by the suggested guidelines will be used in the context of the Brazilian Pollinator Initiative and should be useful for other initiatives around the world.
- As there are countries and regions with practically no information about pollinator fauna, three strategies for pollinator investigation were addressed: 1) rapid assessments; 2) surveys and 3) monitoring programs.

**Recommendations**

· *Basic principles*

The group agreed that suggested actions should:

- Assure data quality, i.e., data must be reliable and adequate for statistical analyses.
- Be realistic, considering time, personnel and costs constraints.
- Be flexible enough to be applied in different environments.
- Be question oriented.

· *Difficulties*

Members of the group suggested and discussed various methods in use across the world. Different people had different experiences with different methods. For example, some had very good results in using pan traps for collecting bees, while other obtained meager data from their use. Such differences could be due to different designs, different environment conditions, etc. It became obvious that no rigid protocol could be built that could be recommended for all situations across the World or, even, Brazil and that the group would not be able to decide on specific methods to be employed, due to the varying opinions on their efficiency.

Thus, it was decided that only general guidelines would be built, so that data obtained from surveys and monitoring programs, using any combination of the suggested methods, would meet the basic principles listed above. It was hoped that further comparison of different methodology would enable sound choice of methods in the future.

Considering the difficulties exposed above the following recommendations should be accepted as guidelines, not protocols, in order to assure their applicability.

## 1) Rapid Assessments

| | |
|---|---|
| **Aims** | |
| To best describe given local faunas.<br>To maximize number of species recorded. | |
| **Type of data** | |
| Species records | Through collection and deposit as vouchers in public collections. |
| Species Abundance | Not necessary. Priority should be given to increase the number of new species detected |
| Habitat description | Follow a basic protocol** that describe the collection site in many scales. Geographical coordinates must be taken for species distribution analysis. When GPS is not available, geographical clues should be used*. (T. Griswold from CA, USA, will send a model to be adapted to Brazil.) |
| Association with plants | *Whenever possible, plants visited by pollinators should be recorded, in order to give clues on possible target plants for next surveys.* |
| **Sampling design** | |
| Plan a pilot study to verify the adequacy of techniques | |
| Plan data to be useful in the future as meta data | |
| Sampling effort must be measurable and recorded. | |
| **Sampling techniques** | |
| A combination of methods may be used, but sampling effort for each method should always be recorded. Whenever possible hand netting should be applied. Other recommended methods are: trap nesting; aspirators, malaise traps, pan traps. | |
| **Statistical analysis** | |
| Use recommended statistical analysis. Statistical techniques should be known in advance. A guide of statistical procedures or references should be part of the manual. | |
| **Observations** | |
| Species identification should be made by trained people, with the aid of taxonomic keys and reference collections. Responsible for identifications should be contacted in advance. Manual should bring information on national collections and taxonomy services. | |

## 2) Surveys

| **Aims** | |
|---|---|
| Best estimate of local fauna and bee plant relationships.<br>To allow for comparison among areas. | |
| **Type of data** | |
| Species records | Through collection and deposit as vouchers in public collections. |
| Species Abundance. | Number of individuals must be recorded allowing post collection analysis based on numbers per plant, per hour, per species, and any other relevant unit |
| Habitat description | Follow a basic protocol** that describe the collection site in many scales. Geographical coordinates must be taken for species distribution analysis. When GPS is not available, geographical clues should be used*. (T. Griswold from CA, USA, will send a model to be adapted to Brazil.) |
| Association with plants | Plants should be collected and deposited as vouchers in public collections for identification. Record the resource used by plant visitor. Weather conditions and time of the day must be recorded for resource availability analysis |
| **Sampling design** | |
| Use previous data to plan collection and build a list of species of expected occurrence | |
| Plan a pilot study to verify the adequacy of techniques | |
| Plan data to be useful in the future as meta data | |
| Sampling effort must be measurable and recorded. | |
| Adequate number of replications should be employed. Environment patchiness and plot design should be used to define the number of replicates. | |
| The sampling area should be visited before sampling, and plant collection and individual plant labeling should be done whenever possible to facilitate plant identification. | |
| Consider time to be spent in obtaining information on habitat and surroundings that may be useful in the future | |
| Identify data that should be collected and collect only data that will be useful in future analyses | |

| **Sampling techniques** |
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| A combination of methods may be used, but sampling effort for each method should always be recorded. Whenever possible hand netting should be applied. Other recommended methods are: trap nesting; aspirators, malaise traps, pan traps. |
| **Statistical analysis** |
| Use recommended statistical analysis. Statistical techniques should be known in advance. A guide of statistical procedures or references will be part of the manual. |

*Observation:* Species identification should be made by trained people, with the aid of taxonomic keys and reference collections. Responsible for identifications should be contacted in advance. Manual should bring information on national collection and taxonomy services.

### 3) Monitoring

| **Aims** | |
|---|---|
| To identify and describe patterns and variations through time and changing conditions of selected variables.<br>To evaluate population fluctuations<br>To guide decision in conservation actions;<br>To guide decisions in management actions;<br>To generate Basic data for selecting potential pollinators for further studies | |
| **Type of data** | |
| Species records | Species can be counted or collected, depending on the facility of identification and objective of the program |
| Species Abundance | Number of individuals must be recorded allowing post collection analysis based on numbers per plant, per hour, per species, and any other relevant unit |
| Habitat description | Follow a basic protocol** that describe the collection site in many scales. Geographical coordinates must be taken for species distribution analysis. When GPS is not available, geographical clues should be used*. (T. Griswold from CA, USA, will send a model to be adapted to Brazil.) |
| Association with plants | Plants should be collected and deposited as vouchers in public collections for identification. Record the resource used by plant visitor. Weather conditions and time of the day must be recorded for resource availability analysis |
| **Sampling design** | |
| Use previous data to plan collection and build a list of species with expected occurrence | |
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| Consider time to be spent in obtaining information on habitat and surroundings that may be useful in the future |
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| **Statistical analysis** |
| Use recommended statistical analysis. Statistical techniques should be known in advance. A guide of statistical procedures or references will be part of the manual. |

**Observations**
Species identification should be made by trained people, with the aid of taxonomic keys and reference collections. Responsible for identifications should be contacted in advance. Manual should bring information on national collection and taxonomy services.

**FINAL REMARKS**

It was obvious that no fixed protocol could be provided for all situations. Moreover, different people had different experiences with different sampling methods. Since no comparative data exists on those methodologies, no consensus was reached on which methods to recommend for given situations. Thus, such comparative data should be sought for before any strict recommendations can be built in the context of the Brazilian Pollinator Initiative.

## CASE STUDIES – AN EXERCISE

**Aim:** To provide examples of how the recommendations above could be used in the construction of survey and monitoring protocols.

The group was divided in three subgroups, each of which worked on one case study. The resulting protocols presented below were constructed upon literature information and the expertise of group members, for surveying and monitoring bees at specific crops and different kinds of environments taken as examples.

### 1) Monitoring flower-visiting bees at cotton fields

**Background**

According to Barroso & Freire (2003), three species of cotton are found in Brazil, *Gossypium hirsutum* (L.), *G. barbadense* (L.) and *G. mustelinum* (Mier). Of these, only herbaceous cultivars of the introduced *G. hirsutum* are currently

cultivated in commercial scale in Brazil. However, cultivation systems are not homogeneous across the large cotton-producing regions of Brazil. An evident contrast exists, for example, between the small-scale production found in the small family-held farms in the northeastern region of Brazil, which employ a low technology crop system, and the large scale production system employed in the huge commercial farms in central Brazil.

The cotton flower may produce nectar through five different kinds of nectaries distributed inside and outside the flower. However, not all of these nectaries occur in every cultivar (Free, 1970; McGregor, 1976). Many different organisms are attracted to the cotton flower by the nectar and pollen it produces. Among these, insects and especially bees are the most abundant. These flower visiting species may contribute for increasing fiber production and/or quality (Free, 1970; McGregor, 1976).

The suggestions below were constructed considering a small-scale system. Considerations on how to expand this protocol for a large-scale, high-technology system are presented at the end of this exercise.

**Survey**

The survey of cotton-flower visiting species is proposed for 1 ha fields, considered here as sampling units. This is an average size field for cotton in Northeastern Brazil. In each such sampling unit, two sampling procedures would be executed in parallel:

*A) Arbitrary sampling.* This aims to maximize the number of flower visiting species recorded on cotton plants. The field is slowly inspected and all bees found on the cotton flowers or flying above them are collected. It is important to call attention to the fact that the deep corolla of the cotton-flower makes the use of hand nets much inefficient, protecting the visiting insects. For this reason, a complement of capture methods was suggested: forceps, insect aspirators and hand nets, according to the size and position of the bees.

*B) Systematic sampling.* This aims to quantify the relative density and abundance of flower-visiting species. Sampling is to be done weekly in 10 plots, each including 80 cotton plants, 20 in each of four neighbor rows. Those plots should be homogeneous distributed across the field, including areas close to its border and center. Areas close to patches of natural vegetation and other special environments around the field also should be considered. Each plot is sampled for 10 min by slowly walking between the rows. Any bees found inside the flowers and on extra floral nectarines will be collected.

Both sampling procedures should be executed weekly, between 8:00 and 12:00, along the flowering season. Sampling should be done preferably during

sunny days, when bees are most active at flowers. Any cultivation practice proceeded between and on sampling days should be recorded.

### Monitoring

Monitoring can be done by repeating yearly the systematic sampling procedure described above. In this way, average abundance of the whole flower-visiting assemblage and of target species can be compared between years and along longer periods. Those numbers can also be associated to factors such as climatic parameters and amount of pesticide application.

### Adapting the protocols for large-scale, high-technology systems

The same 1 ha sampling units could be used, each with 10 sampling plots, as explained above. Such sampling units should be homogeneous distributed inside the cotton fields, the number of such units being proportional to the size of the fields, with some of them close to the borders and others at the interior of the fields.

## 2) Bee survey and monitoring at a fragmented landscape in the Atlantic Rain Forest Biome

### Background

The Atlantic Tropic Rain Forest is a recognized biodiversity spot. Its original vegetation cover has been reduced to 8%, and it is threatened by human presence. Population growth has led to destruction of the forest through uncontrolled urban expansion, industrialization and migration of people from other areas (Galindo –Leal & Câmara 2003). About 100 million people live in the mega cities located in the Atlantic Forest Region, along with the largest industrial and silvicultural centers. In the other hand the biodiversity harbored by the Atlantic forest is one of the greatest of the world. We believe that 60 percent of the terrestrial species of the planet live on the remaining areas of this forest. This is probably a result of the large range of latitude it covers, its variation in altitude, the diverse climatic regimes, and the availability of water and energy to the system (Pinto & Brito 2003). The forests is highly stratified, with a canopy as high as 35 meters.

### Survey

Pollinator survey should be done along transects. The determination of size, placement and number of transects will depend on the heterogeneity of the forest community, which should be understood beforehand. The size of the transects should be such as to be covered in one day. They should be 100 m long.

The sampling units will be flowering plants, with all bees found being collected at all flowering plants visited by them. Hand nets will be the principal collection method and will be complemented by euglossine baits. Additional methods such

as pan, malaise and light traps and also new baits like salt/ammonia and “anti-freeze” should be tried.

Sampling should be proceeded 3 to 5 times a month for 8 to 12 months per year, depending on flowering phenology and flower density.

**Monitoring**

The goal of the suggested monitoring program is to detect differences in bee diversity in disturbed and undisturbed forests over time. It could also be use to compare different degrees of disturbance.

Sampling units are similar to those used in the survey. Monitoring subjects may be selected, based on survey results. For example, euglossine or *Melipona.* Such subjects *a)* include species sensitive to deforestation; *b)* occur in large numbers and *c)* be easily identified. Still other subjects could be considered, such as *Apis mellifera,* trap-nesting *Centris* or specialist taxa.

The monitoring design should include site in or adjacent to undisturbed area (control), disturbed area (treatment 1) and intermediate area (treatment 2).

A minimum of five years of sampling is needed for conclusions to be drawn.

## 3) Survey of potential pollinators in Brazilian savanna, and a monitoring program to evaluate the impact of grazing on flower visitants´ richness

## Background

The biome of the Cerrado is a gradient of vegetation physiognomies, including open fields, savannas and open-canopy forests. It covers about 25% of the Brazilian territory and was included among the world's hotspots (Myers et al 2000), for combining high biodiversity and high rates of disturbance. Until 40 years ago the Cerrado was primarily used for extensive cattle raising and it is estimated that 35% of the natural cover has already been removed (Klink & Moreira 2002) According to Buschbacher (2000) about 60% of the Cerrado Biome area is pasture, what comprises 73,000 hectares. Agriculture occupies 6% of the total area, but this figure is increasing stimulated by present agricultural national policy. Large plantations, especially of soybean and cotton, are an important threat to the native Cerrado and its fauna (Fearnside 2001). Major impacts on pollinators are supposed to be caused by the intense use of chemicals, aerial spraying, habitat removal, including reduced nesting opportunities and food availability.

According to Silberbauer-Gottesberger and Eiten (1987) the plant species richness of the Cerrado open areas is among the highest known for non-forests vegetation. Seasons are very well defined. The dry season lasts from 3 to 5 months, during winter, and the wet season peaks at December-January. Flower resource availability varies through the seasons (Oliveira & Gibbs 2002), but flowers are found throughout the year (Batalha, 1997). Bee surveys in Cerrado area were carried in the Central Brazil, around Brasília, in Minas Gerais State, in the Northeast and in some peripheral areas in Sao Paulo state (Pinheiro-Machado et all. 2002), allowing for a baseline data set for native fauna.

## Survey

The Cerrado vegetation is a natural mosaic, with many vegetation types, varying from open grass fields to dry forests. Therefore a previous analysis of the sampling area has to be done before designing the survey. The following steps guide sampling procedure

1. The very first of all activities must be the definition of the question that the survey is aiming to ask. All the steps ahead depend on a very clear and objective question posed;
2. a good view of the large area, using satellite image or local driving around to picture the heterogeneity of the area to be sampled;
3. accessing previous studies in the area, or similar areas, to create estimates of diversity and sampling effort necessary to best describe the focal fauna. Some calculations involving sampling curves in a standardized way with previous data may be necessary. The studies should indicate period of activity, both seasonally and daily;
4. visits to collections produce a better species list and expected richness numbers, because collections are believed to have many unpublished data;
5. local evaluation prior to work provides familiarization with flora and allows identification of potential plant species for a target survey. It may be of

interest, especially in case of very short budgets. Simple things that might turn into difficulties in the field can be observed, like the height of trees to be sampled. Previous studies may also provide information about plant species intensively visited by bees;

6. although Brazilian researchers use hand netting as the main technique for sampling bees, evaluation of other method's results from literature indicate what part of the community is not being sampled if hand netting is the only technique.
7. Plan the type of analyses that will be carried out and assure that design will provide proper data.
8. Sampling area should be marked at the field.

***Site selection*** must be guided by the question proposed for the survey. The chosen site must be representative of the environmental situation to be investigated. If a unique type of vegetation or physiognomy is aimed, evaluate if the chosen site is representing well the situation to be surveyed. It also applies when gradients or mosaics are the case. Replication and control areas are important parts of some surveys and have to be considered during site selection. Another very important point is the accessibility of the site and all the permits that should be obtained, both from private land owners and governmental agencies.

**Sampling design and sampling techniques.**

Sampling design involves choices of sampling unit's format, size, number and spatial distribution. For instance, if an overview of diversity in the area is aimed, sampling units will be randomly distributed, if diversity information is to be linked to habitat, sampling units should be placed in order to represent all desired situations (Alonso & Agosti 2000). Sampling design has to consider areas greater than 2 hectares because of the typical spatial distribution of plants in the cerrado. At least 10 transects of 2 meters wide per 1 km long or 5 quadrates of 40x100m are to be established. Calibrate method against known area by follow up procedures using the species accumulation curves (Soberón & Llorente 1993) to inform about the efficiency of the chosen design and techniques. Species accumulation curves are produced from species-by-sample matrix in a spread sheet, and can be carried out by the EstimateS program (Colwell 1997).

Sampling techniques that minimize the collectors' interference are preferred, but best results in Brazil were, so far, achieved using hand nets. To minimize differences among collectors a previous training is mandatory. Different species have different behavior at flowers, and some of them can be very fast and sensitive to movement. Collectors must be advised to collect any bees and all wasps and small flies, because some bee species looks like wasps or are too small to be differentiated from another insect with a bare eye in the field.

Recommended techniques are hand netting transect, sweep netting transect. Although pan traps have not a good acceptance among Brazilian researchers,

good results and new fluorescent colors reported from other countries speak for its use. Pan traps tend to be selective for certain groups, so it should not be used as the only technique for inventories. The best method may vary according to site and logistics, but best results in species numbers are usually achieve when multiple methods are applied.

The length of the transect is to be standardized, but it will depend on the aim of the survey, as discussed above. If the transect is divided in sub samples, they should be kept separated for posterior analyses. The starting point of the transect should be picked at random, in order to avoid collecting during peak activity period always at the same part of the transect. Attention should be given that the peak activity period is always covered by sampling effort. Randomize also collectors along sites to minimize bias from collecting ability.

Complementary data is very important for data analysis, so a protocol should be applied to every survey. Recommended data to be gathered are the following:

- Site location with coordinates. Use a GPS, if not available report local geographic references like roads, bridges, or equivalent.
- Date, including month/day/year
- Time of the day, indicating sampling hours
- A clear vegetation classification, informing not only details about the vegetation found in the sampling areas, but also the characteristic if the landscape in which is it situated.
- The size of the total area from which samples are taken must be indicated, because results are expected to vary if collecting sites are a part of a 1ha, a 100ha or larger area of cerrado.
- General climate description and classification is very useful and must be complemented with local weather condition during the collection days and information about averages temperature and rainfall whenever possible.

Floral associations are important complementary information, but time can be saved if collectors add flowers to a bag, identify it, and leave a numbered tag on the plant to proceed plant identification later on.

Field equipment must include spare hand nets, and vials enough to avoid insect jam in each vial. All material is to be labeled in advance allowing quick field information to be taken promptly.

After field work some procedures are recommended, which can make data easy to work for anyone interested on it:

- Standardize the format of data presentation and codes to facilitate understanding at all instances of the study.
- Present results by simple summary descriptors, like species richness, and common and popular diversity indices.
- Feed data bases that provide access to the general pubic. Indicate the collection where voucher material was deposited.

**Monitoring**

The proposed exercise for this group was to give directions for monitoring the impact of cattle raising activities on the biodiversity of pollinators.

The group started with the statement of the leading question:

What is the impact of introducing cattle “on pollinators”?

To address this question, previous data about bees and their relationship with flowers of open areas will be gathered. This information is used to choose focal groups for monitoring activities. Preference will be given to bees sensitive to gradients that are this case is understood a range from original non grazed areas to heavily grazed areas. If preliminary data fails to point out the taxa to be monitored, the first part of monitoring is used to establish a baseline for further comparisons. The preliminary data will be tested for correlation with desired variables, like bee diversity. Once a strong and significant statistical relationship is found, collections will be repeated through time. The sampling design is the same one proposed for the survey, but will be repeated through time.

<u>How data from monitoring will be used in the context of BPI?</u>

Monitoring depends on the choice of sound and easy to measure variables. Variable selection can be made upon previous data. It is recommended that a selected taxa or a guild is used for monitoring, but the relationship between the measured variable and the object of monitoring should be understood and supported by significant strong positive correlation. Direct relationships are preferred. If the relation between the measured variable and the object of monitoring is not already known, assumptions must be stated clearly at the beginning of the monitoring program. The selection of a specific taxa or a guild must take into consideration that the focal organisms are abundant and easy to identify. A previous survey is mandatory for areas where data is lacking, to improve the chance of choosing good taxa to be monitored.

- Evaluating population fluctuations.
- Guide decisions in conservation actions.
- Guide decisions in management actions.

**Important considerations:**

Adequate replication.

Controls.

How to test differences?

## GENERAL RECOMMENDATIONS

Some general recommendations could be made for all situations involving pollinator surveys and monitoring:

**Training.** One problem raised about the use of "manual" collection methods, such as hand-netting is the effect of different abilities of different people to find and capture bees. This problem can be reduced by properly training collectors before actual sampling is begun.

**Replication.** An appropriate number of replicates should be set, according to environment heterogeneity and/or other important factors. Pseudo-replication should be avoided. For instance, 12 monthly samples collected at one site cannot be considered as replications of different disturbance grades or environment types. In these cases, on needs different areas should be sampled as replications of each treatment.

**Identification and voucher specimens.** Specimens (both of target (pollinators) and associate *(e.g.* food sources) taxa should be properly collected, preserved and labeled to facilitate proper identification. Such identification should be done preferably by experienced personnel. Moreover, voucher specimens of all taxa involved should be deposited in public collections that should be indicated in reports and publications, so that their identification can be checked at anytime.

## FINAL REMARK

The group was composed by a number of researches with experience in bee survey and monitoring. However, different people had different thoughts about different methods. In part, this may be consequence of the effect of different environment (including composition of regional bee fauna) on sampling methods. In this way, methods that were reported by some as very efficient, did not produced good results in other places, when run by other people. It was suggested that experiments (like those going on under the auspices of the European Pollinator Initiative) should be proceeded in regional scale, so that a final choice of methods could be done for each region.

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