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PROTOCOL TO DETECT AND ASSESS POLLINATION DEFICITS IN CROPS: A HANDBOOK FOR ITS USE

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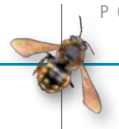

PROTOCOL TO DETECT AND ASSESS POLLINATION DEFICITS IN CROPS: A HANDBOOK FOR ITS USE

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, ROME 2011

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00153 Rome
Italy

The field testing and adaptation of the protocol described in this handbook, and the production of the book, was made possible through the support of the International Fund for Agricultural Development (IFAD), Rome, Italy.

This publication provides guidance, as part of the GEF supported Project "Conservation and Management of Pollinators for Sustainable Agriculture, through an Ecosystem Approach" implemented in seven countries - Brazil, Ghana, India, Kenya, Nepal, Pakistan, and South Africa. The project is coordinated by the Food and Agriculture Organization of the United Nations (FAO) with implementation support from the United Nations Environment Programme (UNEP).

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PREFACE

In agro-ecosystems, pollinators are essential for orchard, oilseed crop, horticultural and forage production, as well as the production of seed for many root and fibre crops. Pollinators such as bees, birds and bats affect 35 percent of the world's crop production, increasing outputs of 87 of the leading food crops worldwide, plus many plant-derived medicines in the world's pharmacies.

Just as the agricultural community is taking stock of the contribution of pollination to crop production, populations of managed pollinators (the Western honey bee *Apis mellifera*, the Eastern honey bee *Apis cerana*, and their Asian relatives) are experiencing new and poorly understood threats. Wild pollinators in agricultural landscapes can provide important pollination services and serve also as a critical form of insurance against the risks of pests and diseases amongst managed pollinators.

Within the context of its lead role in the implementation of the Initiative for the Conservation and Sustainable Use of Pollinators (also known as the International Pollinators Initiative-IPI) of the United Nations Convention on Biological Diversity adopted in 2000 (COP decision V/5, Section II), FAO has established a "Global Action on Pollination Services for Sustainable Agriculture". FAO has also developed a global project, supported by the Global Environment Facility (GEF) through the United Nations Environment Programme (UNEP) entitled "Conservation and management of pollinators for sustainable agriculture, through an ecosystem approach". Seven countries (Brazil, Ghana, India, Kenya, Nepal, Pakistan and South Africa) have worked together with FAO to identify and carry out targeted activities that can address threats to pollinators in agricultural landscapes. The outcomes of the global project are expected to expand global understanding, capacity and awareness of the conservation and sustainable use of pollinators for agriculture.

As a contribution to the IPI, FAO and its partners have collaborated with INRA (Institut National de la Recherche Agronomique, a public research body of the French government) to develop a protocol for assessing and detecting if a crop production system is suffering a pollination deficit. Field testing and adaptation of the protocol for the variable cropping systems in different countries was made possible through a grant from the International Fund for Agricultural Development (IFAD) on the "Development of Tools and Methods for Conservation and Management of Pollination Services for Sustainable Agriculture", in 2009 and 2010. This document thus presents a handbook for the application of the protocol, outlining the underlying concepts, the hypothesis to be tested, and the modification and application of the protocol to a variety of circumstances in developing countries, such as small fields, home gardens, and high environmental variability. As the protocol is applied, FAO and its partners will be able to provide information on the results of detecting and assessing levels of pollination deficit in crops important for nutrition and food security around the world.

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INTRODUCTION

The following describes a protocol to be applied to focal crops at the farm scale level to (i) detect and assess pollination deficits in field situations in a standard and statistically testable way; and (ii) draw management conclusions from the proposed experiment for possible action to eliminate or at least reduce these deficits. It can also be used simply to assess pollinator density and diversity on a focal crop for comparison purposes among different sites.

Pollination is the transfer of pollen from the producing anthers to the receptive stigma and it is an essential preliminary step for the sexual reproduction of flowering plants. Pollination level can be precisely measured as the number of compatible and viable pollen grains that reach a stigma during the effective pollination period, and it is therefore directly related to yield for all crops in which the output is a product of sexual reproduction. Indeed, pollination management should be regarded as a production factor in its own right for all these crops as it can affect the agronomic yield and its many components such as fruit set and seed set, fruit quality (e.g. size, aspect, sugar content, flavor and nutritional content), seed quality (e.g. germination rate, oil content), and other characteristics such as earliness and uniformity of output (e.g. rape *Brassica napus* L.: Lerin 1982, Sabbahi *et al*. 2006), market value and profitability, and finally the environmental and societal impacts of a crop (McGregor 1976; Free 1993).

FAO facilitates and coordinates the International Initiative for the Conservation and Sustainable Use of Pollinators (IPI: http://www.internationalpollinatorsinitiative.org/), which was established in 2000 by the Fifth Conference of Parties of the Convention on Biological Diversity. One of the objectives of the IPI is to promote the conservation and the restoration and sustainable use of pollinator diversity in agriculture and related ecosystems based upon the four elements of the IPI Plan of Action: assessment, adaptive management, capacity building, and mainstreaming. It is in this context that FAO commissioned in 2008 a literature review on

the topic of detecting and assessing pollination deficit in crops. This review study then served as background for an expert workshop to identify methods for detection and assessment of pollination deficit in crops and develop a practical yet efficient protocol to assess such deficits. This FAO-sponsored workshop was held on 3-5 April 2008 nearby Avignon, France, under the auspices of INRA (Institut National de la Recherche Agronomique) with 13 participants from around the world (Figure 1.1).

The workshop considered two perspectives that establish the context for a focus on pollination deficits and human livelihoods: (i) from a pollinator perspective, pollination crises appear increasingly likely, as evidence of pollinator declines become more and more apparent in numerous locations; and (ii) from a plant perspective, there are many potential drivers of increasing pollination deficits such as lack of compatible pollen for self-incompatible and dioecious species, and reduced pollen production and/or poor pollen quality due to genotype and its interaction with nutrient status, water deficits or other aspects of growing conditions. Climate change may be contributing to pollination deficit by affecting the phenology of both the plant and its pollinators in different ways so as to lead to asynchrony, or reducing the durations of pollinator activity and plant flowering.

The workshop then examined the definitions, concepts and theory of pollination deficits and pollen limitation in broad terms. The context of 'optimal pollination' from a plant perspective (fitness) is clearly different from that of a farmer's perspective (agronomic or economic yield), and also from the perspective of sustainable development (which may be more oriented toward long-term sustainability and reliability depending on the area ; Figure 1.2). With this background, the workshop participants agreed on the following definition: **Crop pollination deficit refers to inadequate pollen receipt that limits agricultural output**. The review of the methods used to assess pollination deficit in crops was based on 67 papers. The synthesis of this large array of case studies was conducted along 3 axes: (i) the dependent variable(s) used to assess pollination deficit (e.g. number of pollen tubes per style or pollen grains per stigma, fruit set, seed set, fruit characteristics, or seeds characteristics); (ii) the experimental unit used in the assay (a sample of flowers, of branches, a whole plant, a plot or a whole field or larger area); and (iii) the demand of the crop, that is the intrinsic pollination need for optimal field productivity based upon the sexual reproductive biology and physiology of the crop, the temporal scale of the demand (duration of flowering: determinate versus undeterminate species), the spatial scale of the demand (field size and landscape pattern), and the production strategy (e.g. off-season production of covered crops). The main methodological problems and possible improvements

Figure 1.1

PARTICIPANTS IN THE FAO-SPONSORED EXPERT WORKSHOP ON *ASSESSING POLLINATION DEFICITS IN CROPS*

From left to right: Jim Cane (USDA, Logan, Utah, USA), Resham Bahadur Thapa (Institute for Agriculture and Animal Sciences, Chitwan, Nepal), Paulo Eugênio Oliveira (Universidade Federal de Uberlândia, Brazil), Jérôme Vandame (INRA Avignon, France), Wanja Kinuthia (National Museums of Kenya, Nairobi, Kenya), Barbara Gemmill-Herren (FAO Rome, Italy), Simon Potts (University of Reading, UK), Bernard Vaissière (INRA Avignon, France), Linda Collette (FAO Rome, Italy), Ruan Veldtman (South African Biodiversity Institute, Cape Town, South Africa), Breno Freitas (Universidade Federal do Ceará, Fortaleza, Brazil), Natacha Chacoff (Centro Regional de Investigaciones Cientificas y Tecnológicas, Mendoza, Argentina).

Figure 1.2

OPTIMAL POLLINATION LEVELS - WITHIN THE RESOURCE ALLOCATION PATTERNS OF THE CROP

Cocoa (*Theobroma cacao* L.) flowers, and the subsequent pods, are borne on the trunk of the cocoa tree. On average, only about 5 percent of flowers on a cocoa tree will give rise to a mature pod (Free 1994). In a study where all the flowers on a cocoa tree were hand-pollinated, the yield of the tree exceeded the yields of all other cocoa trees; but the tree died the next year (Falque *et al.* 1996)! It is most often the case that optimal yields are considerably less than 100 percent fruit or seed set, and a certain percentage of flowers abort.

in assessing pollination deficits were then reviewed with the clear goal to develop a practical ready-to-use protocol that could be readily implemented to detect and assess pollination deficits for the major crops in the seven countries that are taking part in the GEF/UNEP/FAO project on the "Conservation and Management of Pollinators for Sustainable Agriculture through an Ecosystem Approach" (Brazil, Ghana, India, Kenya, Nepal, Pakistan and South Africa). It is this protocol that has been refined, detailed and improved in concert with stakeholders and end-users that is presented here.

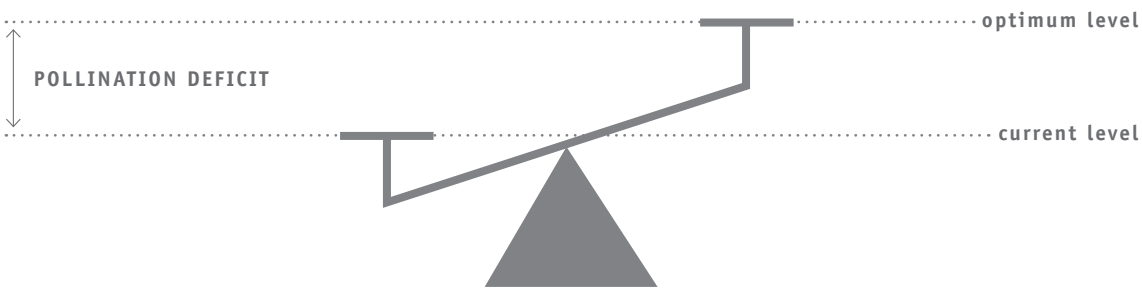
SECTION 1
DEFINITIONS AND CONCEPTUAL FRAMEWORK

The following conceptual framework underlies the protocol; the definitions of terms often lead to the need for further definitions, in a logical sequence. The terms defined are underlined.

Optimum pollination: Pollination that leads to maximum sexual reproductive output given the current available resources over the lifetime of the plant. In the case of crops, this refers to the agricultural output that depends upon pollination, and it takes into account the production objectives in relation to the market and the sustainability of the crop management. To define pollination deficits, it is necessary to define (and understand) how to attain optimum pollination levels (Figure 1.3).

Pollination deficit: Quantitative or qualitative inadequate pollen receipt which decreases the sexual reproductive output of plants (from Wilcock and Neiland (2002) who defined the concept of pollination failure).

Figure 1.3
POLLINATION DEFICIT IN RELATION WITH OPTIMUM POLLINATION LEVEL



Crop pollination deficit: Quantitative or qualitative inadequate pollen receipt that limits agricultural output in yield or economic terms (Figure 1.4).

Figure 1.4

OPTIMUM POLLINATION OF RUNNER BEANS IN KENYA

© Bernard Vaissière

Flowers of runner beans (*Phaseolus coccineus* L.) that do not receive sufficient pollen form distorted, sickle-shaped pods, instead of long, straight pods. Distorted pods are rejected by the export market. A producer nearby Nanyuki, Kenya, estimated that mishapen pods made about one-fifth of his crop despite the colonies of honey bees located nearby his production fields.

Further defining this concept:

The **inadequate pollen receipt** may be quantitative/qualitative due to a deficient quality of the pollen grains deposited, or inadequate with respect to timing, that is occurring outside the period of effective pollination based on stigmatic receptivity and ovule senescence.

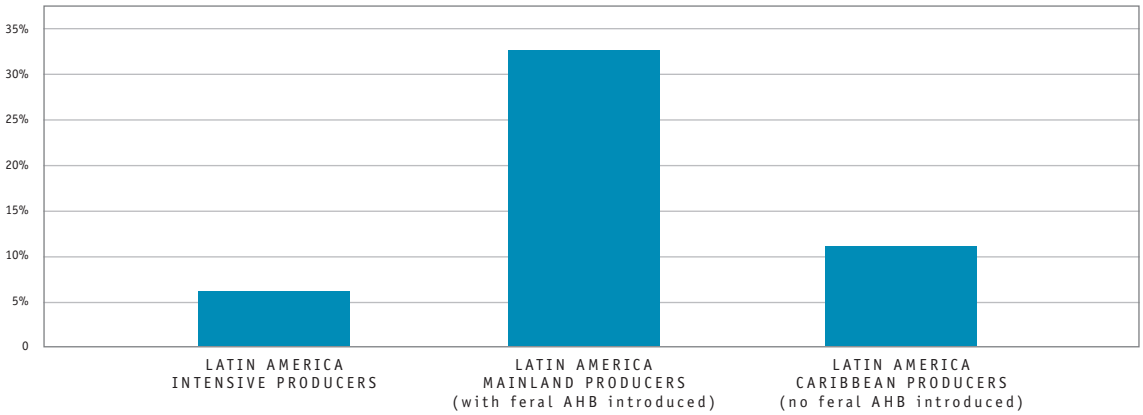
A **quantitative pollination deficit** is an insufficient number of conspecific pollen grains deposited onto the stigma during the **effective pollination period** (see below). It is often the result of an insufficient number of visits by pollinators (Figure 1.5).

A quantitative pollination deficit could be an outcome of conditions such as:

- Ineffective/insufficient transport and deposition of pollen onto the stigmas;
- Insufficient pollen production (Figure 1.6);
- Lack of male flowers relative to female ones in dioecious crop species, such in orchards of kiwifruit (*Actinidia deliciosa* (A. Chev.) C. F. Liang & A.R.Ferguson);
- Lack of staminate flowers relative to pistillate ones in monoecious crops, as can occur at the onset of flowering in very early plantings of zucchini (*Cucurbita pepo* L); and
- Lack of male-fertile flowers relative to male-sterile ones in hybrid seed production.

Figure 1.5

IMPACT OF A SIGNIFICANT INCREASE IN THE NUMBER OF INSECT VISITORS TO COFFEE CROPS IN LATIN AMERICA



PERCENT CHANGE IN COFFEE PRODUCTION FROM 1961-1980 (BEFORE AHB) TO 1981- 2001 (AFTER AHB)

A vast, continent-wide "experiment" showing the value of increased pollination levels took place in Latin and Central America between 1980 (before the arrival of feral Africanized honey bees (AHB) and after that date. A substantial increase in coffee (*Coffea arabica* L.) yield coincided with the establishment of Africanized honey bees in those countries it invaded, an increase that did not occur amongst African nor Asian producers. It also did not occur amongst intensive producers in Latin America who leave little habitat for bees to nest, nor among Carribean producers untouched by feral AHB. These findings are by no means presented to advocate the introduction of alien pollinators, but solely to illustrate the levels of increase in production possible when levels of pollination services are increased and habitat is available to permit sufficient nesting resources for increased pollinator density.

Source: Roubik (2002)

Figure 1.6

LACK OF POLLEN PRODUCTION IN STRAWBERRY

Primary flower of a strawberry *Fragaria* x *ananassa Duch*. plant grown in greenhouse for out-of-season production at anthesis in February. A single anther is well formed while all others are aborted. Often many flowers at the onset of flowering are totally male-sterile resulting in a severe shortage of pollen to enable adequate pollination.

A **qualitative pollination** deficit is when sufficient conspecific pollen is deposited onto the stigma, but this pollen is not effective for fertilization. This reduced pollen quality may result from a low intrinsic viability and/or the genetic origin of the pollen in self-incompatible species for which the pollen must come from a plant genetically different from that of the receptive stigma for fertilization to occur.

A qualitative pollination deficit could be an outcome of conditions such as:

- Poor pollen viability, as in some fruit varieties and crops such as strawberry when grown under low light conditions early on under greenhouses; or
- Lack of pollenizer flowers in self-incompatible crops (Figure 1.7).

The **effective pollination period** is the period during which the pollen deposited onto the stigma can result in fertilization. Pollen that is deposited either before or after this period will not be effective for fertilization and therefore for production (Sanzol and Herrero 2001).

Figure 1.7

BOUQUET OF POLLENIZER FLOWERS IN PEAR ORCHARD

Bouquet of flowers from a cross-compatible variety installed at the onset of flowering to mitigate the qualitative pollen deficit in a pear orchard planted with a single self-incompatible variety. Effective pollination will require that pollinators transfer the pollen from these bouquets of pollenizer flowers to the flowers of the orchard.

The **limitation of agricultural output** may be quantitative (that is, with respect to yields), or qualitative (with respect to fruit or seed characteristics; Figures 1.4, 1.8 and 1.9), or inadequate output with respect to timing (e.g. because of delayed or extended fruiting). Limitation of agricultural output may impact a farmer on an annual basis, but it may also have longer term impacts when a useful component of a sustainable farming system, such as a valuable entomophilous crop, is dropped because of poor pollination (e.g. yield of lowbush blueberry *Vaccinium angustifolium* Aiton in southern in New Brunswick because of pesticide applications, Kevan 1977; see also Figure 1.10).

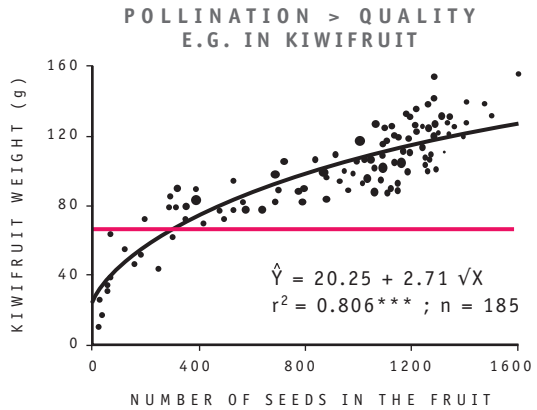
Figure 1.8

CROP POLLINATION DEFICIT: STRAWBERRIES IN KENYA

Two strawberries (*Fragaria x ananassa* Duch.) grown near Nanyuki, Kenya: the strawberry on the left is well shaped and it developed from a flower that received sufficient pollination on most of its stigmas, while the one on the right shows evidence that only the side stigmas, those that usually touched the anthers, received suffient pollination while all the central stigmas did not get pollinated and so the central part of the strawberry did not develop. In many markets, the strawberry on the right would be discarded.

Figure 1.9

CROP POLLINATION DEFICIT AS DEFINED BY MARKET STANDARDS



The weight of a kiwifruit (*Actinidia deliciosa* (A.Chev.) C.F.Liang & A.R.Ferguson)) is well correlated with its number of seeds, which directly depends upon the level of pollination service of the flower it came from as there is neither parthenocarpy nor apomixy in kiwifruit. Within the European Union, it is unlawful to sell kiwifruits below the weight of 65 g (http://www.unece.org/trade/agr/standard/fresh/FFV-Std/English/46kiwifruit.pdf), illustrating how in some markets, quality considerations can translate directly into marketability.

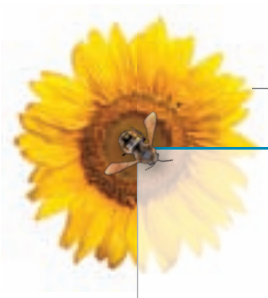
adapted from Vaissière *et al.*, 1992

Figure 1.10

CROPS CULTIVATED LESS BECAUSE OF POOR POLLINATION

Farmers in northern India and in the Chitwan district of Nepal are choosing to grow less of their traditional crops, such as mustard (*Brassica rapa* L.), because yields have declined. The crop is important for both food security and animal feed. In the Chitwan region, farmers recognise that the bee pollinators of mustard have been negatively impacted by the high levels of pesticides applied to crops.

This protocol has been developed to address pollination in a way that is realistic for farmers, and so the yield is the primary focus. The fact that crop plants can compensate for pollen limitation with longer flowering periods and more flowers means that the whole plant, rather than individual flowers or even a sample of flowers, needs to be considered. Along the same line, fruit set and/or seed set can be resource-limited, and thereby the results obtained by increasing pollination levels on a subset of flowers on a plant may result in a larger fruit from those flowers, but not greater overall production on a plant basis (Knight *et al*. 2005). Agricultural output should therefore always be based on a whole plant or larger scale (plot, field), and pollination treatments must be carried out on a similar scale, that is with the whole plant as the smallest experimental unit.



SECTION 2
PROTOCOL OBJECTIVE AND STRUCTURE

The protocol aims at applying methods following a standard experimental design to assess the degree to which pollination is a limiting factor in the production of a focal crop at the field scale. Comparing crop responses under pollination levels resulting from current practices with those from enhanced pollinator abundance or diversity will indicate the presence, and degree, of a pollination deficit.

The protocol is structured as a hypothesis that there is a relationship between the pollination level X, the independent variable, and a part or the whole of crop yield Y, the dependent variable, as reflected in the following equation and overview of parameters.

$$Y = F(X) + A$$

where:

- Y is the total crop yield measured in agronomic or economic units;
- F(X) is the yield resulting from the level of pollination service X, and is measured in the same unit as Y; and A is the yield resulting from autonomous self-pollination and wind pollination measured in the same unit as Y (Figure 2.1).

The pollination level is critical for the yield for all crops in which the output is a product of sexual reproduction. But, unless the precise relationship between the yield and the number and genetic diversity of pollen grains that reach the stigma during the effective pollination period is known, it is not possible to quantify directly the optimum level of pollination service needed to achieve maximum sustainable output. It then becomes necessary to use alternate variables as proxies to assess this level of pollination. Assuming that the main pollinating species are known among the floral visitors, such proxies include **pollinator density (number of pollinators/floral unit) and pollinator diversity**.

Figure 2.1

RELATIONSHIP BETWEEN POLLINATION LEVEL AND CROP YIELD

The protocol hypothesises a relationship between the pollination level X, and a part or the whole of crop yield Y, as reflected in the following equation and overview of parameters.

$$Y = F(X) + A$$

where Y is the total crop yield measured in agronomic or economic units;
F(X) is the yield resulting from the pollination service measured in the same unit as Y;
and A is the yield resulting from autonomous self-pollination and wind pollination measured in the same unit as Y. The possible application of this equation to wheat (*Triticum aestivum* L.- left) and apples (*Malus domestica* Borkh - right) is illustrated.

Based upon the above, the protocol will now be described in 6 sections as follows:

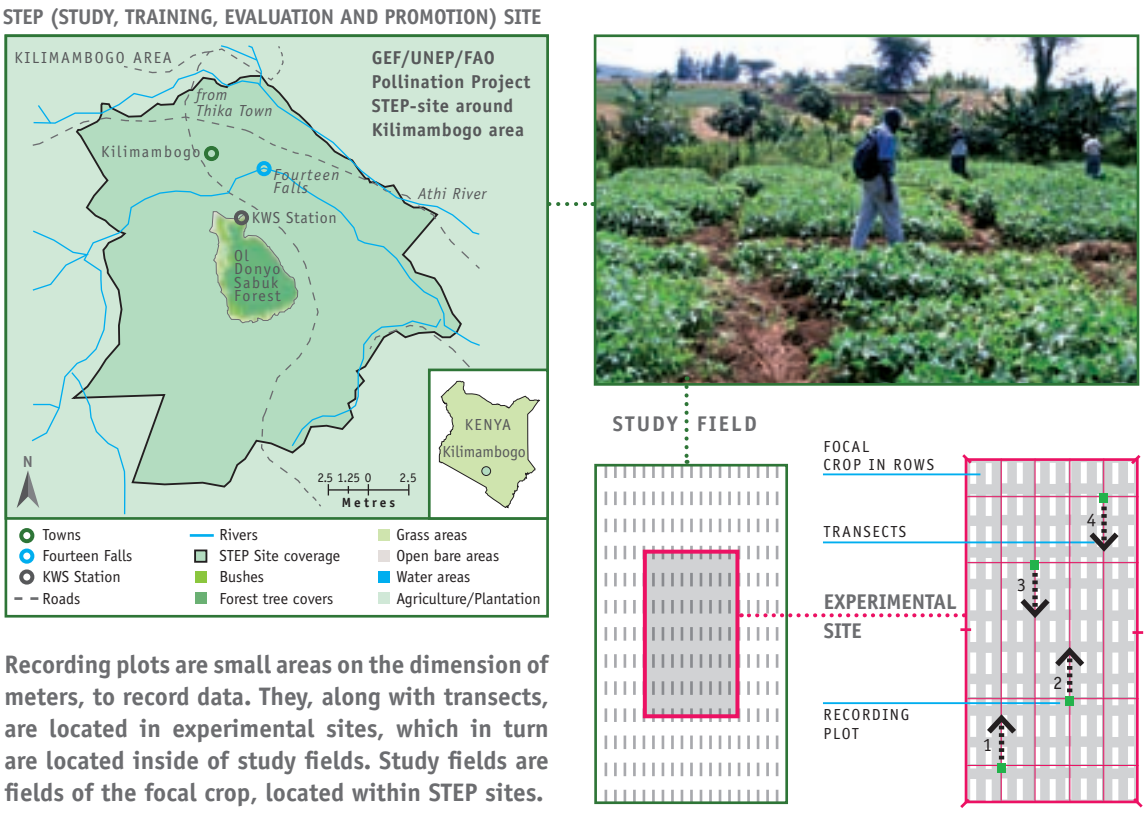
- General considerations for experimental design and study field selection (see Section 3)
- Treatments to modulate the pollination level and independent variables (see Section 4)
  - Local pollinator supplementation
  - Landscape context / field location in relation to natural habitats
- Layout of experimental sites (see Section 5)
  - Establishing the experimental site
  - Locating the experimental site within a study field
- Pollinator dependent variables and data collection (see Section 6)
  - Pollinator density
  - Pollinator diversity
  - Covariables
- Production dependent variables and sampling units (see Section 7)
  - Agronomic yield
  - Economic yield
- Statistical analyses (see Section 8)
- General conclusions (see Section 9)

SECTION 3
GENERAL CONSIDERATIONS FOR EXPERIMENTAL DESIGN & STUDY FIELD SELECTION

Within the GEF/UNEP/FAO project on the "Conservation and Management of Pollinators for Sustainable Agriculture through an Ecosystem Approach", demonstration sites have been selected, termed "STEP" sites, where STEP stands for Study, Training, Evaluation and Promotion Sites (Figure 3.1). In this project, and similarly in other efforts to identify and assess pollination deficits, sites should be

Figure 3.1

HIERARCHY OF LOCATIONAL TERMINOLOGY USED IN THIS HANDBOOK

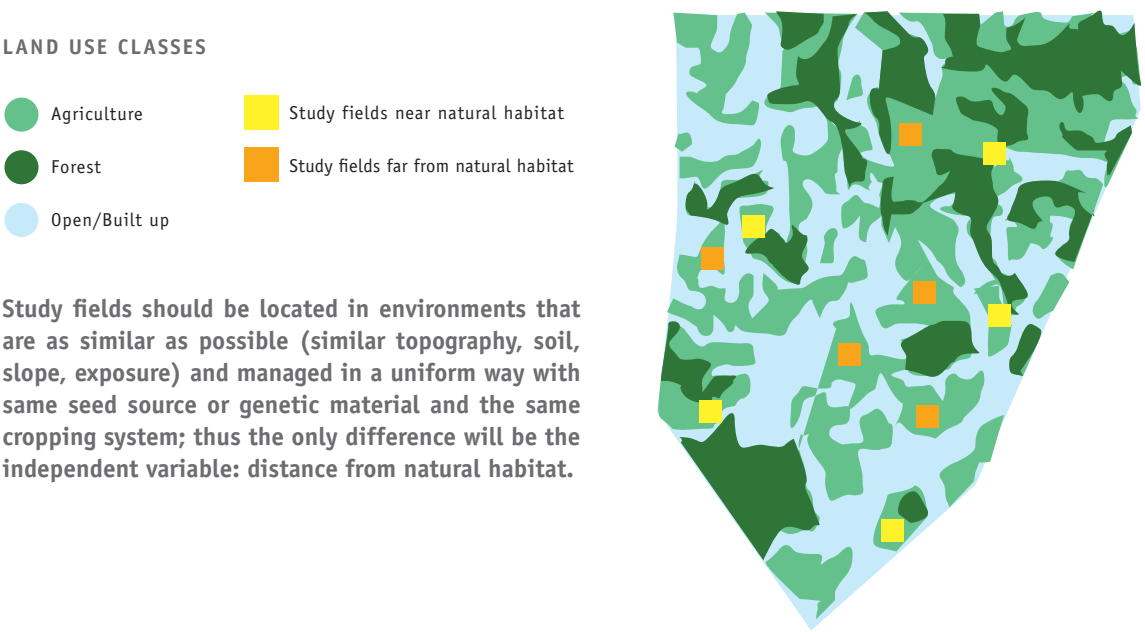


Recording plots are small areas on the dimension of meters, to record data. They, along with transects, are located in experimental sites, which in turn are located inside of study fields. Study fields are fields of the focal crop, located within STEP sites.

identified where farmers are growing pollinator-dependent crops under a range of conditions that lend themselves to making comparisons. Such sites can be used to implement a protocol to detect and assess pollination deficits with the goal that farmers can be involved in the study, and the results can be useful to raise the awareness about the significance of pollinators in farming communities and also promote the use of pollinator-friendly practices. Thus the protocol has to be straightforward and address pollination in a way that is realistic to farmers. To this end, the use of dependent variables such as the number of pollen grains per stigma for self-compatible species or the number of pollen tubes per style for self-incompatible ones was not considered. Rather yield, whether the agronomic yield or the economic yield, is the primary focus so that, as indicated above, the whole plant is the smallest experimental unit possible to avoid the confounding effects of plant response and resource allocation. However, such an experimental unit has its drawbacks and it prevents the use of hand pollination as a way to achieve maximum pollination because it is practically impossible to hand pollinate all the flowers of a plant. The pollination treatment to assess deficits will therefore have to be done indirectly by manipulating the pollinator fauna. The use of screen cages or enclosures in general is a common way to easily control the number of pollinators onto one or several plants at once with several replicates possible per treatment (e.g. Steffan-Dewenter 2003). The use of enclosures, however, was not considered here either because of their cost and the fact that they modify the microclimatic conditions, such as humidity, air flow and solar radiation, and therefore photosynthesis which can lead to the reduction of assimilate availability and lower seed set (Bouwmeester and Smig

Figure 3.2

HYPOTHETICAL PLACEMENT OF STUDY FIELDS WITH A COMPLETELY RANDOMIZED DESIGN USING TWO DISTANCES TO NATURAL HABITAT AS TREATMENT



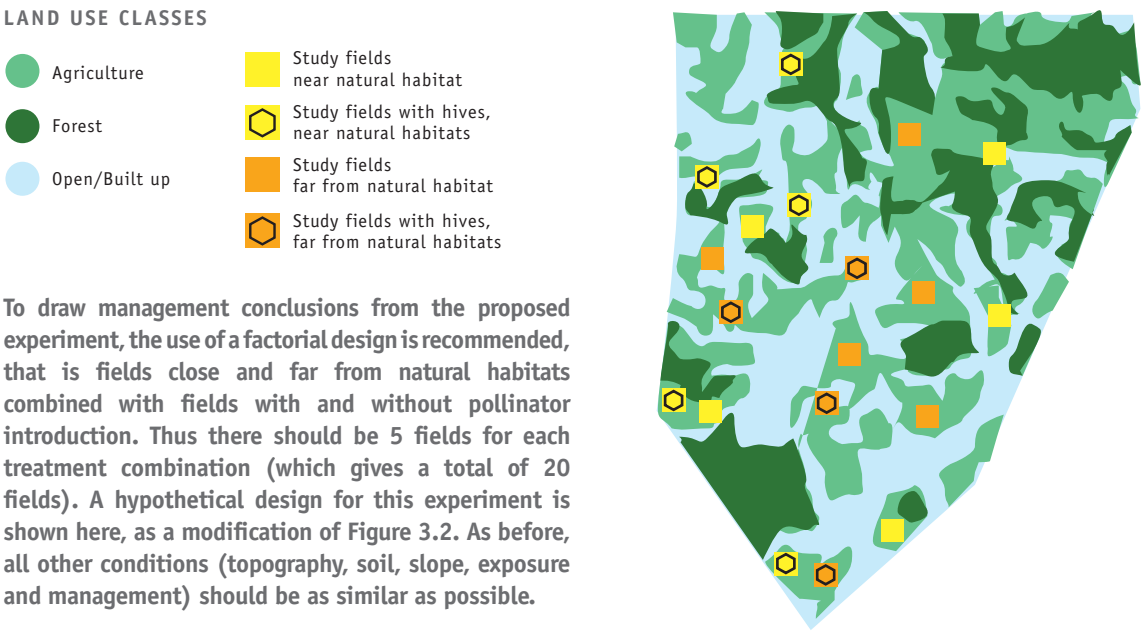
Study fields should be located in environments that are as similar as possible (similar topography, soil, slope, exposure) and managed in a uniform way with same seed source or genetic material and the same cropping system; thus the only difference will be the independent variable: distance from natural habitat.

1995). In addition, they also eliminate access to alternate floral sources so that pollinator behavior is considerably altered compared to their foraging in the open (e.g. honey bees will visit and pollinate tomato flowers under closed greenhouses, which hardly ever takes place in the open; Banda and Paxton 1991). For this reason, the protocol as presented here is designed to be used in fields in the open. It relies on free flying pollinating species with the constraint that pollinator treatment will act at the level of the foraging area of these species, which may commonly extend over at least 1 to 2 km radius, though pollinator density will clearly not be uniform over this range. For this reason, individual study fields should always be separated from each other by a distance at least equal to 2 km and if possible greater than the maximum modal foraging distance of the managed pollinator species used (2 to 3 km for social bees such as honey bees and bumble bees – Buchmann and Shipman 1991; Steffan-Dewenter and Tscharntke 2000; Osborne *et al*. 2008). In the case of solitary bees, the maximum foraging distance can range from 1.2 km for small bees (Beil *et al*. 2008) up to 6 km for large carpenter bees such as *Xylocopa flavorufa* (Pasquet *et al*. 2008).

For randomized designs where comparisons will be made between study fields, these should be located in environments that are as similar as possible (similar topography, soil, slope, exposure), and also managed in a uniform way (same seed source or same genetic material, same cropping system) with the exception of the one factor being manipulated between sites, such as the introduction of pollinators to complement the local fauna or the distance to natural habitat (Figure 3.2). If two factors are being manipulated, a factorial design is required (Figure 3.3).

Figure 3.3

HYPOTHETICAL PLACEMENT OF STUDY FIELDS IN A FACTORIAL DESIGN WITH TWO LEVELS OF TWO TREATMENTS



To draw management conclusions from the proposed experiment, the use of a factorial design is recommended, that is fields close and far from natural habitats combined with fields with and without pollinator introduction. Thus there should be 5 fields for each treatment combination (which gives a total of 20 fields). A hypothetical design for this experiment is shown here, as a modification of Figure 3.2. As before, all other conditions (topography, soil, slope, exposure and management) should be as similar as possible.

For long fields (> 450 m in length), comparisons can be made along a gradient between different areas within the field if it is possible to locate a "pollinator front" – either colonies, nesting sites, or natural area on one side only (Aras *et al*. 1996; Figure 3.4). It is the uniformity within a field that will be especially important in both the environment (uniform topography, soil, slope, exposure) and management (same seed source or same genetic material, same cropping system). In this case, there can be important differences in the environment and management between the different fields since each field will be considered as a block for the statistical analyses.

Figure 3.4
POLLINATOR FRONTS

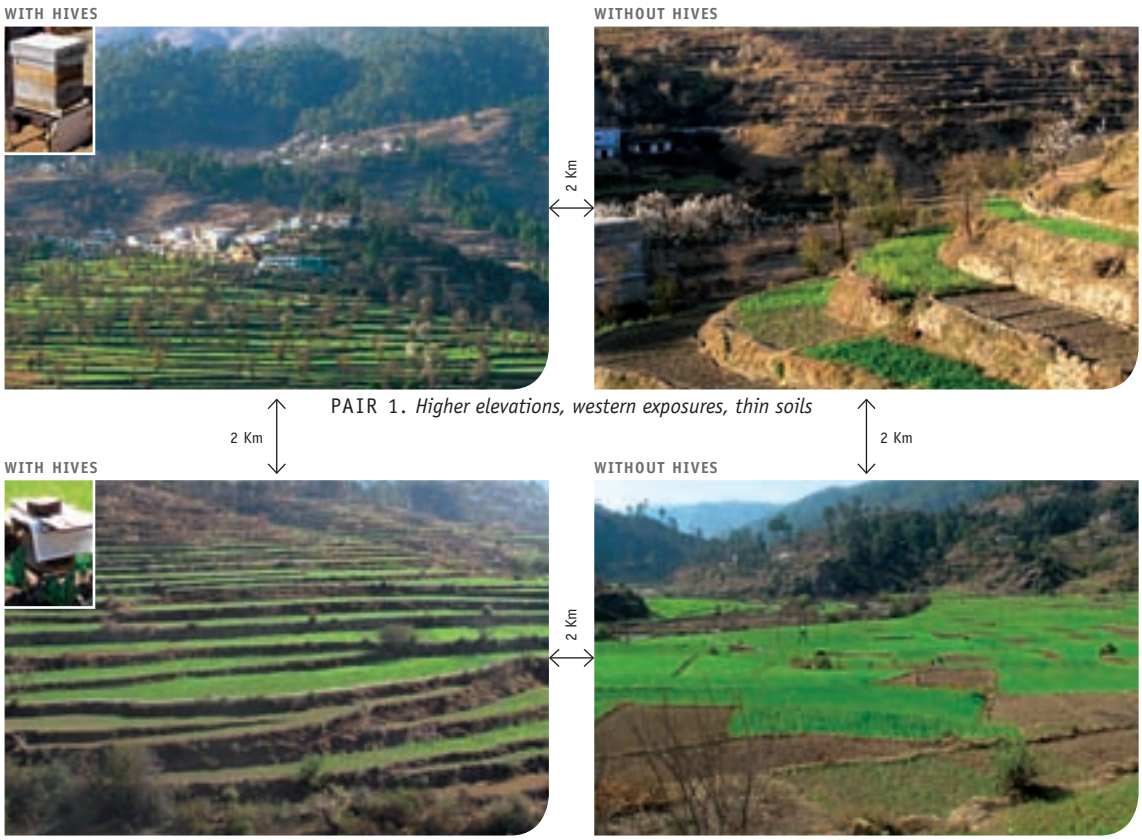
Remnants of semi-natural habitat along one edge of intensive grapefruit (Mach) plantation in the Northwest of Argentina.

If fields are long, that is, more than 450 m in length, comparisons can be made along a gradient between different areas within the field if it is possible to locate a "pollinator front" – either hives, or a natural area – on one side. It is the uniformity within a field that will be especially important in both the environment (uniform topography, soil, slope, exposure) and management (same seed source or same genetic material, same cropping system).

When it is not possible to find the full complement of fields that are located in similar environments (topography, soil, slope, exposure), and managed in a uniform way (same seed source or same genetic material; same cropping system), it is possible to use a design in pairs in which the two fields within a pair should be as similar as possible while differences between pairs are allowed. Within a pair, there will always need to be one field that will serve as control while the other field will be treated so as to have potentially improved pollination (Figure 3.5). With such a design, the number of pairs to find will be equal to half of the total complement of fields. Still, the two paired fields will need to be at least 2 km apart from each other.

Figure 3.5

LOCATING PAIRED PLOTS IN A LANDSCAPE



Demonstration of a paired design (when it is not possible to find the full complement of fields located in similar environments). The two fields within a pair should be as similar as possible while differences between pairs are allowed. Within a pair, one field will serve as control (in this case, without hives) while the other field will be treated so as to have potentially improved pollination.

Figure 3.6

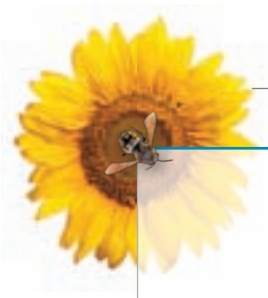
HOME GARDENS AS STUDY FIELDS

Home gardens with cucurbits in Chitwan, Nepal.

Home gardens with cucurbits in Kakamega, Kenya.

When there is no 'field' as such, for example when cucurbits such as pumpkins (*Cucurbita* spp., probably *Cucurbita moschata* (Duch.)) are grown around houses, a study field can be composed of a set of one or several patches, each patch including one or several plants of the focal crop. The identification of these sites will still need to be set in a uniform environment and being similarly managed so that the pollinator treatment will be the main difference between the set of patches that will be compared.

When there is no 'field' as such, for example for cucurbit plants such as pumpkin, *Cucurbita* spp., that are grown around houses in many rural areas all over the world, a study 'field' will be composed of a set of one or several patches, each patch including one or several plants of the focal crop (Figure 3.6). The selection of such a study 'field' will still need to take into account all the requirements laid out above, especially in terms of being set in a uniform environment and being similarly managed so that the pollinator treatment will be the main difference between the set of patches that will be compared. For example, one study 'field' may consist of patches of cucurbit plants around houses located far way from the closest beehives and/or patch of natural habitat, while the other study 'field' will consist of cucurbit plants around houses with beehives nearby and/or close to a patch of natural habitat.



SECTION 4
TREATMENTS TO VARY THE LEVEL OF POLLINATION SERVICE

Improved pollination can result from improved pollen transport, deposition and fertilization effectiveness. Hand pollination would be the obvious method to achieve full control of the amount, viability and origin of the pollen used for pollination. However, for most crops it is essentially impossible to undertake hand pollination at the whole plant scale. In order to achieve improved pollination, there are still many other possible approaches. A few of them are considered here in that they are simple, can be applied over a wide range of situations and are amenable to manipulation over a short time scale for experimental purposes. For each, the pros and cons, and the implementation modalities are examined below. Those applying the protocol can select amongst these treatments to attain potentially improved pollination. These treatments are:

4.A POLLINATOR (BEE) SUPPLEMENTATION

Most crops are pollinated by bees, especially honey bees (Klein *et al*. 2007; Rader *et al*. 2009). Eusocial bees, such as honey bees – whether Western honey bees (*Apis mellifera* L.) or Eastern honey bees (*Apis cerana* F.) – as well as bumble bees such as *Bombus terrestris*, and solitary gregarious species such as leafcutter bees (*Megachile rotundata*) and mason bees (*Osmia* spp.) have been domesticated and their nests can be moved around for crop pollination (Delaplane and Mayer 2000). It is therefore possible to supplement the local pollinator fauna by introducing colonies, nests or cocoons of these species (Figure 4.1). **Use of non native species should be strongly discouraged** as they could have severe negative impacts on the local pollinator fauna and, indeed, whole ecosystems (Hingston and McQuillan 1999, Goulson 2003, Kato and Kawakita 2004; Figure 4.2).

Figure 4.1

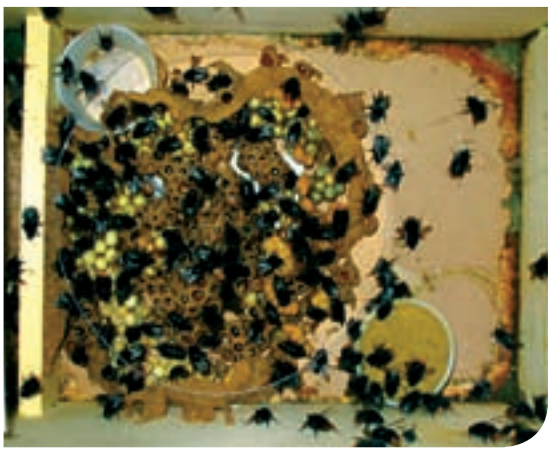
POLLINATOR SUPPLEMENTATION

An apiary in Kenya, on the grounds of an export green bean production company (left) and a meliponary in Brazil, on the farm of an Açai farmer (right).

Supplemention of the local pollinator fauna (as an experimental treatment) can be carried out by introducing colonies, nests or cocoons of pollinating species. Apiaries, or melioponaries, can be established close to study fields. Use of non native species should be strongly discouraged as they could have severe negative impacts on the local pollinator fauna.

Figure 4.2

RISKS OF INTRODUCTION OF FOREIGN POLLINATORS



Bombus terrestris *nest box.*

The introduction of foreign pollinator species has led to severe problems in nearly all the countries where it has been tried, whether it be the spread of pathogens from the imported stock to the wild colonies of the same or other species with *Nosema ceranae* from honey bees to local bumble bees in Argentina (Plischuk *et al.* 2009), the enhanced spread of weeds pollinated by the introduced species (as with *Lupinus arboreus* by *Bombus terrestris* in Tasmania ; Stout *et al.* 2002) or the escape of the imported species and its replacement of the local species with ecological consequences that still remain to be assessed as with colonies of *Bombus terrestris* in Japan (Matsumura *et al.* 2004, Inoue *et al.* 2008).

Pros and cons:

- → Applicable regardless of the location of the crop.
- → Applicable regardless of the crop production process (e.g. greenhouse, open field).
- → Builds on what is already known about the effective pollinators of the crop.

- ← Pollination depends upon pollinator species introduced.
- ← Limitation to managed pollinators.
- ← Unclear relationship between stocking rate of introduced pollinators and forager density on focal crop (it is usually a good idea to record pollinator density and diversity at least once just before pollinator introduction).
- ← Effect of pollinator addition is usually not additive in relation to existing pollinator foraging populations.
- ← Possible negative effects of high pollinator density.
- ← Use of non-native species could have detrimental impacts on native species (Figure 4.2).

IMPLEMENTATION MODALITIES AND INDEPENDENT VARIABLE RECORDING

DESCRIPTION OF IMPLEMENTATION ACTION	NUMBERS REQUIRED
Introduce managed pollinators in or nearby half of the study fields at onset of effective flowering (flowering that will produce crops). The stocking rate of introduced pollinators (number of colonies or of bee nests or cocoons per unit area of study field) should be the same in all treated fields. Its value should be set based on the reproductive biology of the crop and the literature (e.g. usually 1 to 10 honey bee colonies per ha of focal crop ; McGregor, 1976; Delaplane and Mayer 2000) Record the stocking rate of introduced pollinators (number of colonies or of bee nests or cocoons per unit area of study field) in each study field.	5 fields with and 5 fields without pollinators introduced.
In large fields with length > 450 m long, introduce pollinators along a single side perpendicular to its length to get a gradient of pollinator density (Vaissière *et al*. 1984, Aras *et al*. 1996). Record the stocking rate of introduced pollinators (number of colonies or of bee nests or cocoons per unit area of study field) and the distance to the closest introduced pollinator unit at each experimental site (i.e. each location of measurement - see below) in each study field.	5 fields > 450 m long with pollinators introduced on a single side to get a gradient of pollinator density from near to far from side with introduced pollinators (usually one experimental site for recordings can be set at each 150 m distance of the pollinator front).

4.B LANDSCAPE CONTEXT

Pollinator abundance and diversity vary with landscape context, in such a way that wild bee populations are generally greater close to natural habitat and in areas with a high cover of natural habitat (Blanche *et al*. 2006; Chacoff and Aizen 2006, Ricketts *et al*. 2008; Figure 4.3). Thus the distance of the focal field to an area of natural habitats or the relative surface occupied by natural habitats within a 2 km radius around the study field can be used to create differing levels of pollination service, especially since recent results suggest that a guild of pollinators is often more effective than a single species (Klein *et al*. 2003; Hoehn 2008). This approach can also be used for unmanaged wild pollinators such as beetles on atemoya *Annona squamosa* L. x *A. cherimola* Mill. (Blanche and Cunnigham 2005) and *hawkmoth* on papaya *Carica papaya* L. (Martins and Johnson 2009) and other crops (Figure 4.4).

Figure 4.3

LANDSCAPE CONTEXT

Sacred grove in southwestern Ghana; these many groves in agricultural landscapes provide patches of natural habitat.

Wild bee populations are generally greater close to natural habitat and in areas with a high cover of natural habitat. Thus the distance of the study field to an area of natural habitats or the relative surface occupied by natural habitats within a 2 km radius around the study field can be used to create differing pollinating fauna density and diversity, thereby probably leading to differing levels of pollination service.

Figure 4.4

UNMANAGED POLLINATORS

Agrius Cingulatus

Isognath

mangaba flower

mangaba fruit

Heliconius – Nymphalidae, visiting a mangaba flower

all photographs: © Clemens Schlindwein

Two sphingid moth pollinators (first and second row) and one butterfly pollinator (bottom row) of Mangaba (*Hancornia speciosa* Gomez), an important native fruit crop in central and northern Brazil, and associated plants. The pollinators of this crop are highly diverse - including butterflies, bees and moths - and often require different host or food plants at different stages. Thus, the pollinators cannot be "managed" directly, but can be encouraged by preserving remnants of natural vegetation in agricultural landscapes.

Source: Oliveira, Schlindwein *et al.* 2006

Pros and cons:

- → Realistic variations of pollinator abundance and diversity.
- → Takes into account all pollinator fauna and can therefore be especially useful when the pollinating species are unknown.
- → Useful for crops for which pollination is achieved only or mainly by unmanaged pollinators: e.g. for oil palm *Elaeis guineensis* Jacq. and atemoya or custard apple, *Annona squamosa* L. x A. *cherimola* Mill. pollinated by beetles; cocoa, *Theobroma cacao* L., pollinated by Ceratopogonidae midges; and papaya, *Carica papaya* L., pollinated by moths.
- → Consistent with farming policy in some areas (Figure 4.5).

- ← Potential correlated factors that affect yield and its components can confound results (e.g. fields along river bottom may all benefit from better soil conditions).
- ← Requires landscape heterogeneity to locate fields in contrasting situation.
- ← Repeatability may be limited over the years due to year-to-year fluctuations in pollinator populations.

IMPLEMENTATION MODALITIES AND INDEPENDENT VARIABLE RECORDING (REFER TO FIGURES 3.2 AND 3.4)

DESCRIPTION OF IMPLEMENTATION ACTION	NUMBERS REQUIRED
In a uniform area (similar topography, soil, slope, exposure), locate fields in landscape of predominantly intensive agriculture and fields in landscape dominated by natural habitats. Habitats must be assessed locally at least on the general level of classification of natural habitat (forest, natural grassland, brush, etc), agricultural habitat (annual crops, orchards), and urban habitat. Record the proportion of natural habitat around each study field within a 1 km radius.	5 fields in landscape of predominantly intensive agriculture, and 5 fields in landscape dominated by natural habitats.
Locate fields close to (≤ 200 m) and far from (> 1 km) the closest patch of natural habitat. The patches of natural habitat should be as large as possible so as to provide as diverse a pollinator fauna as possible. For small bees, area should be ≥ 0.5 ha; for large bees, a larger patch is needed. Record distance to closest patch of semi-natural habitat in each study field.	5 fields close to (≤ 200 m) and 5 fields far from (> 1 km) the closest patch of natural habitat
Locate long fields (> 450 m long) with a single side perpendicular to its length adjacent to a patch of natural habitat, so as to have a gradient of distances from the edge of this patch across the field. Record distance to edge of natural habitat at each experimental site (i.e. each location of measurement – see below) in each study field.	5 fields > 450 m long to have a gradient of pollinator density from near to far from edge with natural habitat

Figure 4.5

USING LEGISLATED CONSERVATION PRACTICES AS A BASIS FOR EXPERIMENTAL DESIGN

The landscape context for identifying pollination deficit is consistent with farming practices policy in a number of countries that require some portion of farmland to be "set aside" in the service of biodiversity. For example, agricultural policy in Switzerland since 1998 encourages farmers to adopt environmentally friendly methods. Farmers receive financial support only if they meet certain requirements. A key element of proof of ecological performance requires farmers participating in support schemes for multifunctional agriculture to set aside a minimum of 7 percent of land area as ecological compensation areas (ECA). Studies have shown that establishing ECA is an effective method of enhancing both pollinator species richness and abundance and pollination services to nearby intensely managed farmland (Albrecht *et al*. 2007).

In Brazil such "set asides" are mandatory. Called Reserva Legal (legal reserves), a portion of each property or settlement must have an area established for the conservation and rehabilitation of the ecological processes and biodiversity, protection of the native fauna and flora, and sustainable use of natural resources (such as rubber extraction or Brazil nut harvesting in the Amazon forest). Thus, the Reserva Legal must be a natural area with indigenous species, managed in a sustainable way. The size of the RL varies according to the biome in which it is found:

1) **80 percent of the rural propriety when it is in the forested area of the Legal Amazon biome;**
2) **35 percent of the rural propriety when it is in the Cerrado area of the Legal Amazon biome;**
3) **20 percent of the rural propriety when it is in the area of forests or other native vegetation formations in the other regions of Brazil;**
4) **20 percent of the rural propriety when it is in the area of native prairies in any region of the country.**

4.C COMBINED TREATMENT – INTRODUCED POLLINATORS AND LANDSCAPE CONTEXT

The two treatments listed previously to enhance pollinator populations are only the two main types used in the literature. But there are a few other means to reach maximum pollination or increase pollinator populations on some specific crops. For example on kiwifruit, artificial pollination with machine-harvested pollen is possible and can be used as a reference (Gonzalez *et al*. 1998). Also, when the most effective pollinator species are known at a given location along with some elements of its biology, it may be possible to provide adapted nesting sites or other management tools to enhance their population density. This has been effective, for example, with artificial nests for carpenter bees (*Xylocopa* spp.) in orchards of passion fruit vines *Passiflora edulis* Sims. (Freitas *et al*. 2003), or *Forcipomya* spp. midges in cocoa plantations (Kaufmann 1975).

This treatment to secure a range of pollination services combines the introduction of managed pollinators together with naturally occuring variation in pollinator populations due to landscape diversity. Recent results suggest that the combination of the two approaches can be more effective than either one alone. For example, Greenleaf and Kremen (2006) showed that wild bees that were more abundant and diverse near wild habitat enhanced honey bee pollination effectiveness on sunflower (*Helianthus annuus* L.) for hybrid seed production (Figure 4.6). Using this experimental design could produce some interesting results in disaggregating the respective contributions of managed versus wild pollinators to crop yields.

Figure 4.6
COMBINATORIAL TREATMENTS

A combinatorial approach to secure a range of pollination services combines the introduction of managed pollinators together with naturally occuring variation in pollinator populations due to landscape diversity. The combination of the two approaches can be more effective than either one alone. Recent research both from California (Greenleaf & Kremen 2006) and from South Africa have shown that the presence of wild bees enhance honey bee pollination effectiveness on sunflower (*Helianthus annuus* L.) for hybrid seed production. It is suggested that using this a combinatorial design could help to increase the understanding of the respective contributions of managed versus wild pollinators to crop yields.

This dual approach will have the same pros and cons as the two treatments described in A and B above. However, it is especially important to remember the minimum distance between treated and untreated fields when planning the experimental design here so as to combine but not to confound the effects of both approaches. For example, if managed pollinators are introduced along one edge of a field, even a large one, while natural habitat is present along an adjacent or the opposite edge, it will not be possible to draw a conclusion as to which pollinator population led to the observed result (Figure 4.7). Also, if one wants to draw management conclusions from the proposed experiment, then the use of a factorial design is recommended, that is fields close and far from natural habitats should be combined with fields with and without pollinator introduction with 5 fields for each treatment combination (which gives a total of 20 fields; see Figure 3.3). It may be very hard, indeed, to find such a large number of fields separated by the required isolation distance of 2 km as a minimum and yet located in environments that are similar (topography, soil, slope, exposure) and managed in a uniform way (same seed source or same genetic material; same cropping system). In this case, one could locate five quartets of fields, that is five sets of 4 fields (one for each treatment combination) and the 4 fields within a quartet should be as similar as possible while differences between quartets of fields are allowed (each quartet will then be treated as a block for statistical analyses).

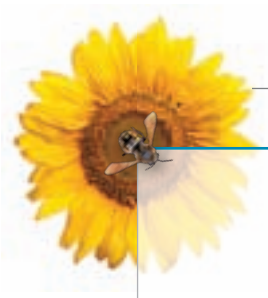
Figure 4.7

COMBINING TREATMENTS TO CREATE A POLLINATOR FRONT

In this cowpea (*Vigna unguiculata* (L.) Walp.) field in the Ceara state of Brazil, it is proposed to use the combined treatment of landscape context and introduction of hives. In this case, hives should be placed along the pollinator front provided by natural vegetation, in the far edge of the field. Placing hives along another side (for example, where people are standing) would confound rather than combine the effects of the treatments.

IMPLEMENTATION MODALITIES AND INDEPENDENT VARIABLE RECORDING (REFER TO FIGURE 3.3)

DESCRIPTION OF IMPLEMENTATION ACTION	NUMBERS REQUIRED
Locate fields in intensive agricultural area located > 1 km from closest patch of natural habitat without supplementation by managed pollinators and fields adjacent to patch of natural habitat (≤ 200 m) and introduce managed pollinators along side of field closest to natural habitat. Record distance to closest patch of semi-natural habitat and stocking rate of introduced pollinators (number of colonies or of bee nests or cocoons per unit area of study field) for each study field.	5 study fields of each kind (total of 10 fields)
Select 10 fields in intensive agricultural area located > 1 km from closest patch of natural habitat and 10 fields nearby (≤ 200 m) natural habitat or in landscape dominated by natural habitats. Supplement half of each of these with managed pollinators along edge closest to natural habitat. Record distance to closest patch of natural habitat and stocking rate of introduced pollinators (number of colonies or of bee nests or cocoons per unit area of study field) for each study field.	Factorial design (5 study fields for each combination of treatment => 20 study fields)
Locate 5 long fields (> 450 m long) with a single side perpendicular to its length adjacent to a patch of natural habitat, so as to have a gradient of distances from the edge of this patch across the field. Supplement these fields with managed pollinators along side close to natural habitat. Record the stocking rate of introduced pollinators (number of colonies or of bee nests or cocoons per unit area of study field) in each field. In addition, record at each experimental site (i.e. each location of measurement – see below) in each study field, the distance to the pollinator front.	5 fields > 450 m long



SECTION 5
LAYOUT OF EXPERIMENTAL SITES

Once the pollination treatment has been selected and the study fields have been located in agreement with the farmers, an experimental site will be established in each field for data collection (refer back to Figure 3.1 for terminology of study fields, experimental sites, recording plots, etc.). In long fields with a gradient of distances to the pollinator front, several sites will be established in each field. For fields that are large enough and planted with an herbaceous crop, the experimental site will cover a nominal area of 50 m x 25 m aligned along the rows and set in a representative area of each field following a basic design (Figures 5.1 and 5.2). For crops planted in rows, it is best to lay this experimental site along the rows to make it easier to set the plots for data collection (Figure 5.1). For fields large enough that are broadcast-sown, the layout of Figure 5.1 can also be used with the long axis of the site aligned with the longest axis of the field.

For fields > 450 m long for which the goal is to obtain a gradient of pollinator density, the experimental sites should be set perpendicular to the length of the field and at fixed distances from the edge with the pollinator front with 150 m increments (e.g. 25, 175 and 325 m from edge).

For fields that are not large enough or when the shape of the field does not allow for the establishment of such an experimental site – for example in the case of a long field planted on a terrace along mountain side – then the whole field will be used as an experimental site.

On the other hand, for very large fields, the experimental site should be set halfway between the geometric center of the field and its edge so as to represent an 'average' situation assuming a linear gradient of pollinators between the edge and the center of the field.

For orchard crops, it is the tree planting pattern that will dictate the size of the experimental site as an area 50 m x 25 m may be far too small and not encompass but a single tree. By using the tree as the individual unit, rather than a distance of row or an area, it is possible to lay out an experimental site that will permit the establishment of plots for data collection (see next page).

FIGURE 5.1

LOCATION OF THE EXPERIMENTAL SITE FOR DATA COLLECTION IN A STANDARD FIELD PLANTED WITH ROWS

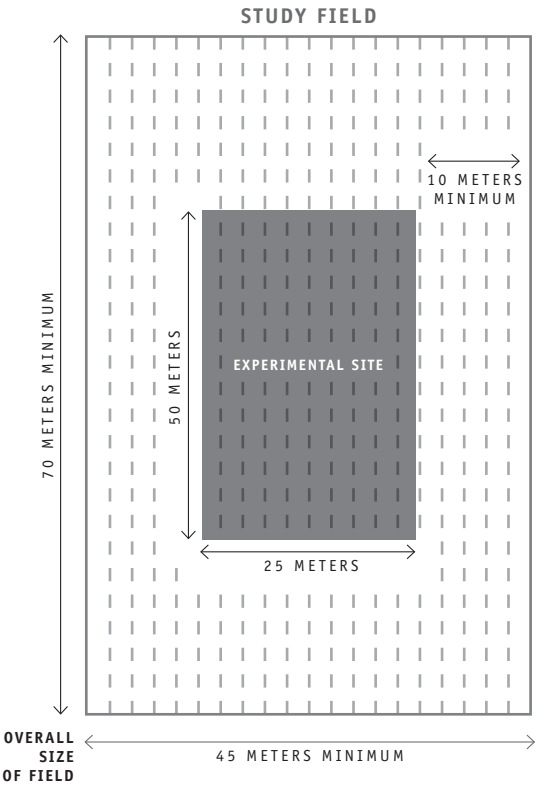
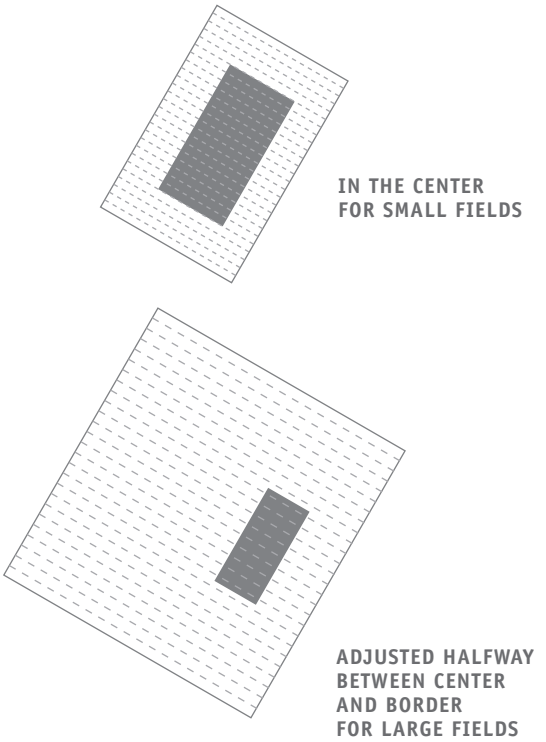


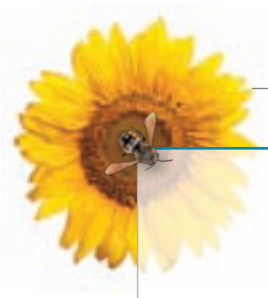
FIGURE 5.2

LAYOUT OF THE EXPERIMENTAL SITE IN RELATION TO THE SIZE OF THE STUDY FIELD



Finally, when the study 'field' consists of a set of patches of plants of the focal crop species – such as for cucurbits grown in home gardens (see Figure 3.7) – the experimental site will consist of a subset or all of these patches, the actual number of patches being adjusted so as to enable the collection of data over an adequate number of sampling units as indicated in the next section.

As a reminder, it is very important that the management of all experimental units (field or plot or plant) be as similar as possible (except for the pollinator treatment). This means that they are planted with the same crop variety at more or less the same time, are managed in a uniform fashion and receive the same level of inputs (fertilizer, weeding, pest control, etc).



SECTION 6
POLLINATOR DEPENDENT VARIABLES AND DATA COLLECTION

In Section 4, the kind of data that should be recorded to characterize each study field (namely the stocking rate of pollinator units, the distance to the closest patch of natural habitat, and/or the proportion of natural habitat in a 1 km radius around the study field), is indicated for each treatment. These will provide the values of the independent variables that are used at each site.

For each study field, it will be essential to record all information deemed important to characterize this field as well as the cropping system used so as be able to justify that all or a subset of the study fields can validly be compared among themselves: field size, soil type and preparation, field immediate surrounding (hedge bordering the field or not), fertilizer application, planting date, genetic material (variety, source of seeds), planting density, planting pattern (for dioecious and self-incompatible species), list of main weed species in bloom and percent soil cover of these weeds at the time of crop flowering, main management practices (irrigation, pesticide applications), and harvesting date (see data recording sheet in Annex 1).

Even when using well contrasted treatments either based on pollinator supplementation or landscape context, there is no guarantee that the response on the crop will match the intensity of the treatment exactly in either pollinator abundance or diversity. For this reason, data will have to be recorded on a regular basis to assess the impact of the pollinator treatment on the abundance (pollinator density) and the diversity (species richness or broader categories) of pollinators in the focal crop throughout its main blooming period. The response of the crop plants in terms of production output will then have to be recorded to be able to measure the effects of the pollinator treatment.

DEPENDENT VARIABLES	NUMBERS REQUIRED
Pollinator density (pollinator/floral unit)	Pollinators (usually bees) /100 to 500 flowers or flowering units depending upon the focal crop (flower size) and the density of open flowers (scan sampling)
Pollinator diversity (species richness or broader categories)	Pollinator catch along fixed transect on the flowers of the focal crop (with insect net)
Agronomic yield	Production per unit area (expressed as kg of output and number of produce units – fruits and/or seeds – per m^2, acre or ha)
Quality of production	Any characteristics of the produce that may affect its price and marketability (e,g, average weight or size for fruit such as apple (*Malus x domestica* Borkh) or seeds such cashew nut (*Anacardium ocidentale* L.); grade for strawberry; oil content and oil quality for seed from oilseed crops; germination rate for planting seeds)
Economic yield	Expressed in local currency; production per unit area multiplied by the sale price paid to the producer per unit production

6.A DATA COLLECTION FOR MEASURING POLLINATOR DENSITY

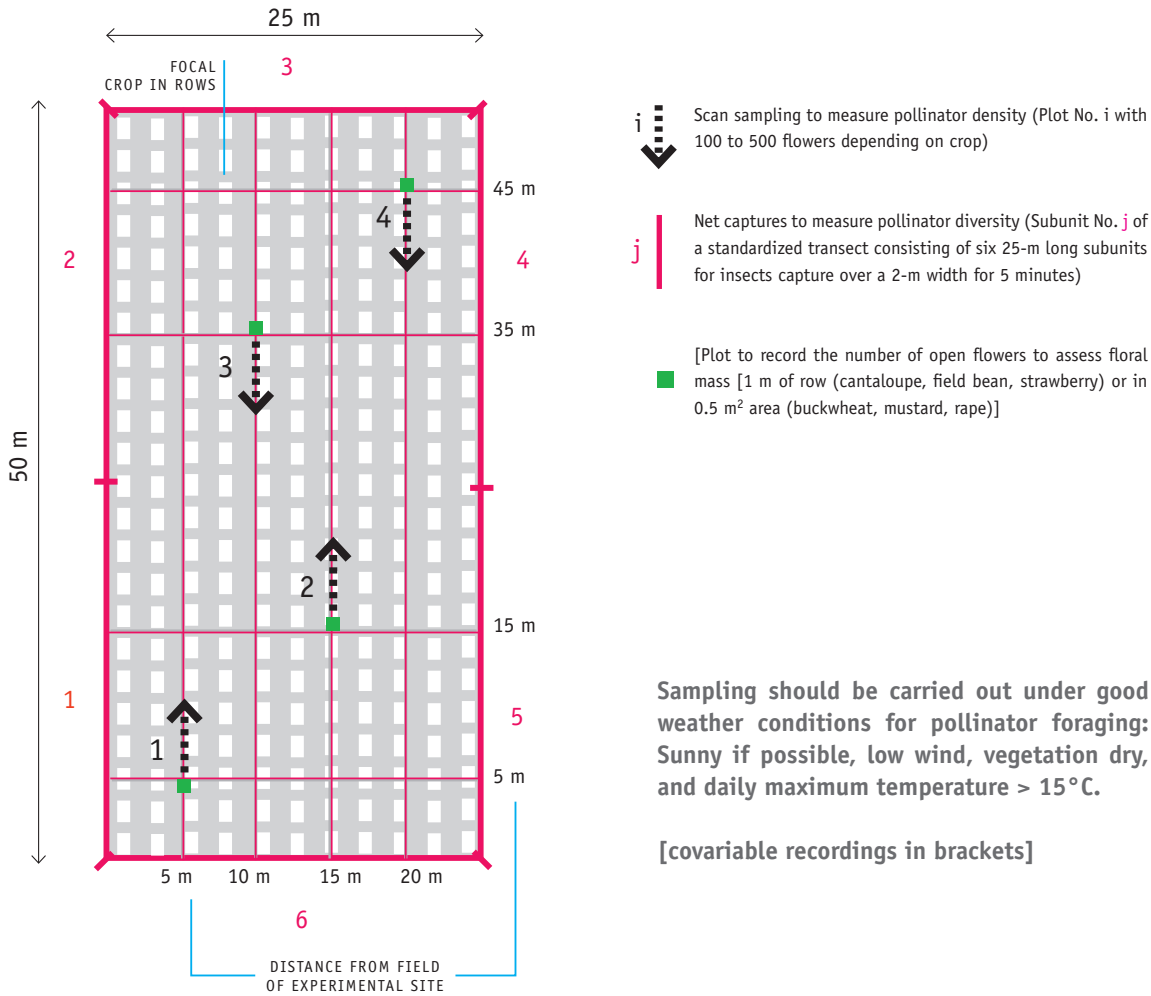
These measurements should be recorded in the experimental sites only under good weather conditions for foraging bees: temperature ≥ 15°C, low wind, no rain, and dry vegetation (Westphal *et al*. 2008). Recordings should be made from the onset of the main blooming period, that is when ≥ 10% of the plants have started to bloom with flowers at anthesis (that is with open corolla).

Pollinator density will be measured by scan sampling a fixed number of open floral units in each of the 4 plots located in each experimental site (Figures 6.1 and 6.2 - see symbols for scan sampling) and the data will be recorded in appropriate data sheets on at least 4 dates during the main flowering period (Annexes 2, 3, 4 and 5). For orchards, a plot will consist of at least 2 trees (Annex 3), and when a pollenizer variety is present, a plot will consist of at least 2 trees of each type (Annex 4). When there is no plot, the required number of flowers will be surveyed over the whole experimental site - that is, on the selected patches of plants (Annex 5).

The recordings will be done by scan sampling as there is no duration attached to the observations but rather an insect will be recorded or not depending on whether it is present at the time a given flower is first seen. Scan sampling was selected because it provides the most reliable way to assess pollinator density on flowers (Levin *et al*. 1968). This sampling will be done by walking slowly along a set path, in between rows when rows are present, and recording the numbers of pollinators seen when looking at individual floral units one by one in sequence (Figure 6.4). The term 'floral unit' is used here to mean an individual flower whenever practical.

Figure 6.1

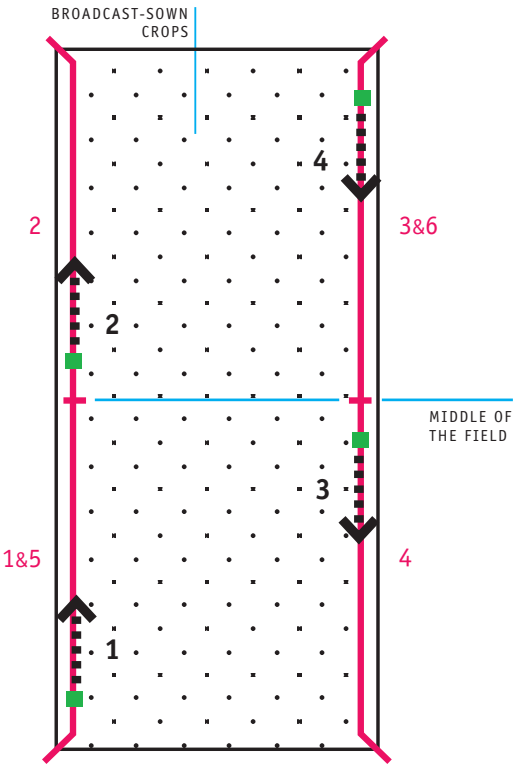
SAMPLING LAYOUT TO MEASURE POLLINATOR ABUNDANCE AND DIVERSITY



Whenever individual flowers are too small or too tight together to be observed one at a time, the floral unit will be an inflorescence like a flower head for crops with a tight inflorescence such as sunflowers (*Helianthus annuus* L.) or buckwheat (*Fagopyrum esculentum* Moensch) or even a loose panicle such as cashew nut trees or mango trees (Table 6.1). The number of floral units to scan in each plot will be set at the start of the experiment. However, it should be adjusted based on the density of floral units so that it does not take more than 15 minutes to scan a plot and should also be adjusted to take into account the size and relative attractiveness of the floral units to avoid having too many null values. For example, for large nectariferous flowers such as those of cotton (*Gossypium hirsutum* L.), passionfruit (*Passiflora edulis* Sims) or pumpkin (*Cucurbita maxima*

Figure 6.2

LAYOUT OF SAMPLING AREAS TO MEASURE POLLINATOR DENSITY AND DIVERSITY IN SMALL FIELD WITH A BROADCAST-SOWN CROP (E.G. MUSTARD/RAPE OR BUCKWHEAT)



i — Scan sampling to measure pollinator density (Plot No. i with 100 to 500 flowers depending on crop; if needed, plots to measure flower density can be placed before the beginning of these plots)

j — Net captures to measure pollinator diversity. (Subunit No. j of a standardized transect consisting of six 25-m long subunits for insects capture over a 2-m width for 5 min (subunits 1 and 5 are the same with 15 min minimum in between their surveying; idem for subunits 3 and 6)

[Plot to record the number of open flowers to assess floral mass of 0.5 m^2 area]

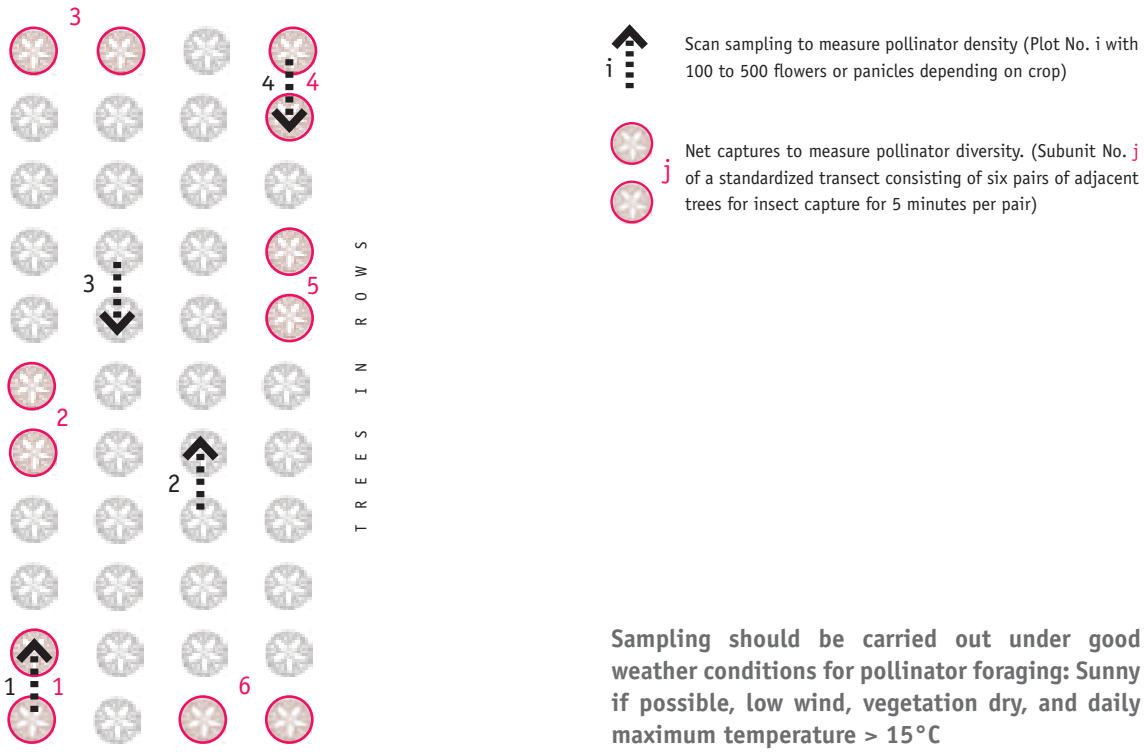
Sampling should be carried out under good weather conditions for pollinator foraging: Sunny if possible, low wind, vegetation dry, and daily maximum temperature > 15°C

[covariable recordings in brackets]

Duch.), the scan of 100 flowers per plot usually provides reliable estimates (Annex 5). It is also the case for the large inflorescence such as those of mango trees or sunflowers, and for crops which often have few open flowers per plant on a given day even at peak bloom such as French beans (*Phaseolus vulgaris* L.), or strawberry (*Fragaria ananassa* Duchesne ex Rozier) (Annex 3). For crops with smaller and more abundant flowers such as apple and cantaloupes (*Cucumis melo* L.) as well as smaller inflorescence such as those of buckwheat (*Fagopyrum esculentum* Moench.) 200 to 250 floral units per plot are usually needed (Annexes 2 and 4). Finally for crops with small flowers such as those of most Brassicaceae like canola (*Brassica napus* L.) or mustard (*Brassica campestris* L.), the number of floral units scanned per plot should be increased to 400 or 500 to avoid too many zero values. Pollinator density will be recorded in reference to a fixed number of floral units at anthesis rather than a fixed area or length of row so as to take into account the level of flowering and also be able to draw management recommendations subsequently by linking pollinator density on a per flower basis with production results.

Figure 6.3

SAMPLING LAYOUT TO MEASURE POLLINATOR ABUNDANCE AND DIVERSITY IN AN ORCHARD WITHOUT POLLENIZER TREES



In practical terms, this monitoring will be done by an observer with two hand counters, one in each hand, who scans the flowers that are well exposed as well as those that may be somewhat hidden (Figure 6.4). For orchard trees, depending on their height, the use of binoculars might be useful so as to be able to identify the broad categories of foragers in all parts of the trees. It is essential that there be no bias resulting from the observer in recording pollinator density in control versus treated fields or when moving along a potential gradient of pollinator density. To this end, the same observer should do the recording in all the study fields of a given focal crop in a given location, or on all the plots along a gradient when a gradient design is used. When this is not possible and several observers are doing the recording, they should alternate between the fields with the different treatments so as to even out any difference due to the observer. One hand counter will be used to record the number of observed floral units while the other counter will be used to record the number of pollinators seen in these floral units. If possible, this basic method can be refined by using several hand counters to record separately different pollinator groups when these are of particular interest

Figure 6.4

METHODOLOGY FOR RECORDING POLLINATOR DENSITY

Pollinator abundance should be measured by scan sampling: walking slowly along a set path, in between rows when rows are present, and recording the numbers of pollinators seen when looking at individual floral units one by one in sequence. The recorder scans the flowers that are well exposed as well as those that may be somewhat hidden while holding two hand counters, one in each hand. One hand counter will be used to record the number of observed floral units while the other counter will be used to record the number of pollinators seen in these floral units.

for the focal crop (e.g. Annex 5: the data sheet for recording pollinator density in Nepal on squash flowers has different columns to record separately Western honey bees, Eastern honey bees, bumble bees, and other wild bees, while syrphid flies and other pollinators have been pooled under a single 'Other' column because they are known to be of lower pollination effectiveness). These measurements should be taken once on a sampling day following a rotating schedule amongst a set of two to four fixed times per day (e.g. 1 000 h, 1 200 h, 1 400 h and 1 600 h local time for apple flowers). The fixed times will depend upon the length of the period of anthesis of the focal crop flowers and the period when pollinators are active. For squash (*Cucurbita pepo* L.) and pumpkin (*Cucurbita maxima* Duch.) flowers that wilt by noon and sometimes earlier, it is usually not possible to go beyond two recording periods per day (Annex 5), while for flowers that stay open and are visited over the whole day and which are easily scanned, such as cantaloupe and mango flowers, recordings can be done over four periods during a day – (Annexes 2 and 3). Apple flowers usually do not open very early and so their scanning can be done only twice during the day (Annex 4). In all cases, the standard time closest to the solar time should be used so as to have comparable results among countries.

Table 6.1

CHOICES OF FLORAL UNIT FOR MEASURING POLLINATOR DENSITY

The term 'floral unit' is used here to mean an individual flower whenever practical. Whenever individual flowers are too small or too tight together to be observed one at a time, the floral unit will be an inflorescence like a flower head for crops with a tight inflorescence such as sunflowers (*Helianthus annuus* L.) or buckwheat (*Fagopyrum esculentum* Moensch) or even a loose panicle such as cashew nut trees or mango trees. Examples of appropriate choices for the floral unit by crop are given in the table below

CROP	APPROPRIATE FLORAL UNIT
Apples, Melon, or Squash	individual flowers
Sunflower	compound flower head
Cashew, Mango	floral panicles
Mustard	inflorescences

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Each study field will be monitored only once on a recording day, but the time of recording of pollinator density will change among the different fields on each date of recording so that every study field has its pollinator density recorded at least once over all time periods during its blooming season. For this reason, the interval between two consecutive recordings will vary depending upon the flowering phenology of the crop. For determinate crops with a short flowering cycle that lasts only 10 to 15 days such as apple trees, for example, bee counts should be done every 3 to 4 days, while for indeterminate crops such as cotton or mustard, bee counts can be done on a weekly basis so as to cover the whole flowering season. This counting frequency should also be adjusted based on the weather since bee counts can only be made whenever the conditions are adequate for bee foraging (maximum daily temperature ≥ 15°C, low wind and no rain, and crop plants dry).

6.B DATA COLLECTION FOR MEASURING POLLINATOR DIVERSITY

These recordings will be made with an insect net right after the recording of pollinator density inasmuch as possible and they should also be conducted in the experimental sites only under good weather condition for foraging - that is, temperatures ≥ 15°C, low wind, no rain, and dry vegetation (Figure 6.5). Because honey bees can be very abundant and their presence and abundance will be recorded with the pollinator density, *Apis* bees can be caught during the net captures to assess pollinator diversity to make sure that are, indeed, *Apis* bees, but they will not be recorded in the appropriate sheets. Some examples of these data sheets are presented in Annexes 6 and 7 for an herbaceous row crop and Annex 8 for an orchard crop.

Figure 6.5

COLLECTING POLLINATORS WITH A SWEEP NET

To assess pollinator diversity in herbaceous crops, insects visitors that are suspected to be effective pollinators (most commonly bees – Apiformes – and syrphid flies that are also called drone flies – Syrphidae) will be caught with insect nets along six 25 m long and 2 m wide transects over 5 minutes each, for a total of 30 minutes per study field (Figures 6.2, 6.3 and 6.4; see symbols for net captures).

To assess pollinator diversity in herbaceous crops, insects visitors that are suspected to be effective pollinators (most commonly bees – Apiformes – and syrphid flies that are also called drone flies – Syrphidae) will be caught with insect nets along six 25 m long and 2 m wide transects over 5 minutes each, for a total of 30 minutes per study field (Figures 6.1 and 6.2 - see symbols for net captures). In orchard crops, insects visitors that are suspected to be effective pollinators, will be caught with insect nets in six plots of a pair of adjacent trees (Figure 6.3). Again, five minutes of surveying will be spent on each plot, for a total of 30 minutes per study field, and the surveying will be done by walking slowly around each tree. Depending on the height of the tree, the use of a telescopic net or a small ladder in the field might be useful so as to be able to sample the foragers in all parts of the trees.

The insects will be killed with killing jars using either potassium cyanide and/or ethyl acetate (the former kills the insects very quickly but is dangerous to use while the second takes more time, but has the advantage of making the bees pull their tongue prior to death and tongue length and characteristics are important characters to identify bees; it is also possible to use a cyanide killing jar with a few drops of ethyl acetate placed on tissue paper inside the jar so as to have the advantages of both methods). After capture, each specimen will be mounted in the evening following collection or, if available, placed in a fridge for 24-28 hours to get rid of the cadaveric stiffness and subsequently mounted. Mounting will be done on pins following usual entomological procedures and each specimen will receive a tag with the collection date, exact location of collection, focal crop name and name of collector as follows:

22 February 2010
Kosi Katarmal
Uttarakhand, INDIA
on flowers of *Brassica campestris*
Ranbeer S. RAWAL

If immediate mounting is not possible, specimens will be pooled by study field and date of capture and placed in a small jar along with the tag information listed above written in soft pencil on a small piece of paper. All such jars will then be stored either in a freezer at -20°C or in 70 percent ethanol until they can be mounted adequately. Freezer storage should be preferred if at all possible as specimens stored in 70 percent ethanol need a special procedure to dry them and mount them in a way that they can be identified properly (for further help on this, see the videos on http://www.youtube.com/swdroege and also the PDF document at http://bio2.elmira.edu/fieldbio/beemanual.pdf).

Once mounted, specimens will then be identified to the species level if possible or else at least to the same taxonomic level as used to record the density of insect pollinators (Annexes 2, 3, 4 and 5). Because taxonomic expertise on bees is not readily available in most places, it may be necessary to send the specimens to various experts. The precise data on the diversity of non-*Apis* pollinators will therefore usually not be readily available after specimens are caught and initial analyses may have to be done on the categories listed in the data sheet rather than on species diversity. It is noteworthy that a further step is now available as a key to the bee families of the world is available on the internet (http://www.yorku.ca/bugsrus/BFoW/Images/Introduction/Introduction.html). This resource should be used as much as possible to better assess bee diversity in the 'wild bee' category. All specimens should be properly mounted, curated and stored safely to make a reference collection (Figure 6.6).

Figure 6.6
INSECT COLLECTING AND LABELING

With respect to the sampling of pollinator diversity, it is important to maintain a properly curated and mounted collection of insect specimens. Mounting will be done on pins following usual entomological procedures and each specimen will receive a tag with the collection date, exact location of collection, focal crop name and name of collector.

6.C DATA COLLECTION FOR COVARIABLES

Covariables are variables that are usually not related to the independent variables, but which may contribute to explain the values of the dependent variables and also help in the interpretation and analyses of the results. By collecting information on covariables, the investigator may gain a more precise picture of the level and key characteristics of the pollination service and this is why they are also listed below. Their recording will depend upon the time available for the experiment, in particular the flower density may be quite time consuming to assess, but, together with forager density data, it will provide some independent assessment of the characteristics of each field in terms of overall plant vigor and yield potential as well as overall crop attractiveness.

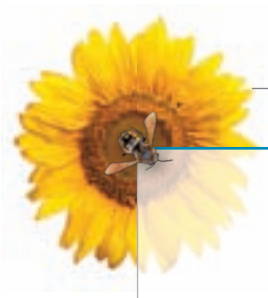
POSSIBLE COVARIABLES	DETAILS
Flower density or phenology: the number of floral units at anthesis (with corolla open) per unit area of study field on a given date (Annexes 9 and 10)	Provides an assessment of the quantity of flowers to be pollinated and also, together with the size of the field and the pollinator density, a mean to assess the total floral mass present, the total amount of resources (nectar and pollen) available to pollinators on the study field, and the total size of the pollinator population foraging in the field
Age of trees (or diameter of trunk at given height)	Assessment of the production potential
Weather conditions (included in the data sheet to record forager density – see Annexes 2, 3, 4 and 5)	Impact on foraging activity of pollinators

If deemed important, the recording of flower density needs to be done at the same time as the recording of the pollinator density and diversity so that the three variables can be related to assess the overall population of pollinating insects in the study field. This measurement is usually best done after the other two and when the flowers at anthesis can easily be distinguished from buds as well as wilted flowers. Flowering units are defined here as previously in Section 6.A and a flowering unit is considered at anthesis whenever at least one of its flower is at anthesis. From that day on, a flowering unit is considered to be at anthesis until all of its constitutive flowers are wilted and therefore no longer at anthesis. Wilting is often noticeable by the closing of the corolla (as in cucurbits and liguliflorae Asteraceae such as chicory *Cichorium intybus* L. and lettuce *Lactuca sativa* L. or the dropping of the petals (as in almond *Prunus dulcis* (Mill.) D. A. Webb), apple *Malus domestica* Borkh., kiwifruit *Actinidia deliciosa* (A. Chev.) C. F. Liang & A. R. Ferguson, rape *Brassica napus* var. *napus* L., and strawberry *Fragaria ananassa* Duchesne ex Rozier) though in some species the stigma can remain receptive after the corolla has dropped (e.g. strawberry, personal observation). Wilted flowers should not be included in the count. It is noteworthy that in some species, most noticeably Asteraceae such as sunflowers *Helianthus annuus* L., the wilting

of the disc florets is not straightforward to see and one usually considers that the anthesis of a head is over when all the ray florets have their stigma exposed (Asteraceae are protandrous). For herbaceous crops planted in rows and where rows are well defined throughout the season, the number of floral units at anthesis is recorded on plots that cover a set length of row. This length varies with the planting density and the floribundity of the crop, but it is best set so that at peak bloom the numbers of floral units per plot can be recorded within 15 min at most by a trained observer. This usually amounts to 1 m of row for crops such as strawberries *Fragaria ananassa* Duchesne ex Rozier and cantaloupes *Cucumis melo* L., while plots of 3 to 5 m of row can usually readily be examined in crops like cotton *Gossypium hirsutum* L. and sunflower *Helianthus annuus* L. that have a low floribundity or large inflorescences. When the rows are no longer identifiable at the flowering stage, it is best to record the number of floral units at anthesis in the fixed area of a square or circular frame. Just as for the length of row, the size of this area will depend upon the plant density and the crop - for squash, a frame of 1 or even 2 m^2 is usually necessary to avoid having too many null values. For crops with many smaller flowers such as buckwheat Fagopyrum esculentum Moench or rape *Brassica napus* var. napus L., a frame of 0.5 m^2 is usually large enough.

Orchard trees are a real challenge to assess the floral mass and there is no easy way to solve it. But for these crops, it is not always necessary to have absolute numbers of floral units per unit area, and often a relative assessment of the flowering stage is what is really important. The recording plots are usually made of a single or two trees (a production tree and a pollenizer tree for self-incompatible species). If branches are easily accessible, the flowering may be followed over one main branch or two on each tree in the plot. If this is not possible, then a photograph taken at a fixed spot can be taken on the occasion of each recording of pollinator density to assess the flowering in rough relative terms.

The layout of the plots or area for quadrat location to measure the flower density is presented in Figures 6.1 and 6.2. Also an example of data sheets to record the flower density of an herbaceous crop and the flowering phenology of an orchard crop are provided in Annexes 9 and 10, respectively.



SECTION 7
PRODUCTION DEPENDENT VARIABLES AND SAMPLING UNITS

Many variables have been used to assess the impact of pollination level on crop output. These include variables related to pistil characteristics (e.g. number of conspecific pollen grains per stigma, number of pollen tubes per style, and the proportion of fertilized ovules), to the initiation of fruits (e.g. fruit set and seed set), to agronomic yield expressed in weight or number of produce per unit area, and economic yield expressed as gross or net return per unit area in local currency.

7.A. AGRONOMIC YIELD

Yield variables are usually not available until a long lag time after flowering and many factors not related to the pollination level during flowering can interfere with the production output and thereby confound the effects of the pollinator treatment. Also yield data are not always easy to record. In particular, plants with indeterminate flowering may require repeated harvesting of the marketable produce over the whole production season (e.g. vegetables such as green bean *Phaseolus vulgaris* var. *vulgaris* L., eggplant *Solanum melongena* L., pepper Capsicum annuum var. annuum L.), tomato *Lycopersicon esculentum* var. *esculentum* Mill. and zucchini *Cucurbita pepo* L., and also some fruits such as mango *Mangifera indica* L. and strawberry *Fragaria ananassa* Duchesne ex Rozier). Also, for perennial crops, the harvest should be measured over two seasons to avoid the confounding effect of alternate bearing on different trees and orchards. Nonetheless, because the protocol is aimed at gathering data meaningful for farmers, despite their shortcomings yield variables will be the only ones considered here.

As indicated previously (Section 2), crop plants can compensate for pollen limitation with longer flowering periods and greater flower production. In addition, fruit set and seed set can be resource-limited, and thereby the results obtained by increasing pollination levels on a subset of

flowers on a plant may result in larger fruit and more seeds from those flowers, but not greater overall production on a plant basis (Knight *et al*. 2005). As a consequence of these two important mechanisms, **it is essential when considering agricultural output that the whole plant be used as the smallest sampling unit rather than individual flowers or a sample of flowers regardless how large**. Therefore, the proposed yield measurements are based on the whole plant as smallest sampling unit, that is the yield will be calculated on the basis of a sample of individual plants, a set of plots or the whole field. For each, the pros and cons are examined below. Those applying the protocol can select the best sampling units for their focal crop and study fields to measure the agronomic yield and the quality of the output, the only requirement being that the same sampling units be used in all study fields inasmuch as possible.

7.A.1 Individual plants

Pros and cons

→ Natural yield unit from a farmer's standpoint (especially for trees).
→ Biological unit, reflecting an integrated response to the treatment.
→ Applicable in mixed cropping systems.
→ Provides intrafield variability (usable with gradient within field).

← Needs plant density at harvest to calculate yield.
← Does not control for resource allocation between years unless recorded over several years.
← Not possible for some crops when plants are highly intermingled at harvest (buckwheat, rape).
← Variability among plants often very large.
← Mechanical harvest usually not possible except for some tree crops.

7.A.2 Recording plot (unit length of row or unit area of study field)

Pros and cons

→ Useful when individual plants are too intermingled (buckwheat, rape).
→ By recording plot size, result are directly expressed in yield units meaningful for farmers.
→ Amenable to mechanical harvest.
→ Provides intrafield variability (usable with gradient within field).

← May require more work than individual plants for harvesting.
← Not applicable in mixed cropping systems.

7.A.3 Whole study field

Pros and cons

- → Data can often be obtained directly from farmer.
- → Direct measurement of commercial yield over the whole study field.
- → Meaningful for farmers and the public.

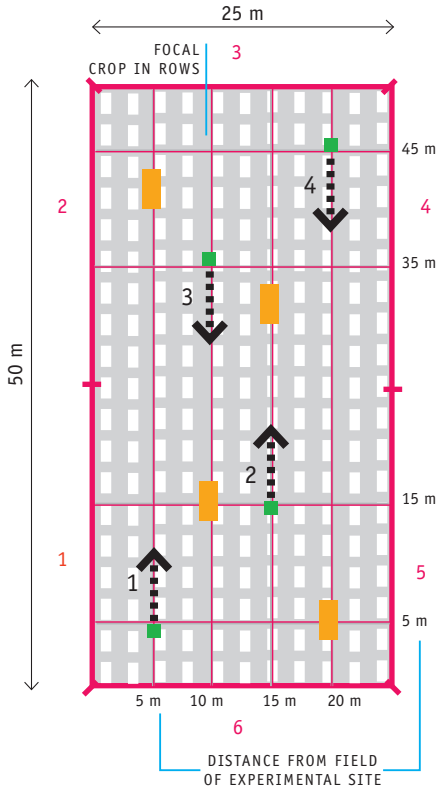
- ← Farmers may be reluctant to provide data.
- ← No measurement of intrafield variability (not usable for gradient within field).
- ← Between field variability can easily confound the link to pollination level (water availability; fertilizer; pest control).

From a practical standpoint, whenever possible, it is best to obtain the yield data from individual plants or from plots that are a given length of row (e.g. Aras *et al*. 1996, Vaissière *et al*. 1984). For instance, in a melon *Cucumis melo* L. field where it is difficult to distinguish plants, the yield plot could be taken as 2 meters of a row. The layout for such sampling units is presented in Figures 7.1, 7.2 and 7.3 and some examples of data sheets to record such data are provided in Annexes 11 to 14. When individual plants are harvested as in mixed planting systems, it is best to harvest adjacent plants that are located in the same general area as the proposed plots (Figures 7.1 and 7.2). In general, it is best to harvest a minimum of 2 plants per plot (e.g. trees for orchard crops) and up to 10 plants or more per plots for herbaceous determinate crops. Produce should be harvested when fully mature and right before commercial harvest.

Once a produce is harvested, it may be possible to measure quality characteristics of all or a sample of the production units if time, budget and available technology permits. No special protocol will be provided here for these measurements as they will clearly vary from one crop to another, be a function of the analytical tools available locally for these analyses, and may also be context specific, that is dependent upon the requirements of a specific market. For example in Kenya, the pods of export-grade runner beans (*Phaseolus coccineus* L.) must be straight shaped and measure between 24 and 27 cm in length and anything smaller or beyond this range is considered a reject. Poor pollination leads to missing seeds resulting in sickle-shaped beans that are no longer acceptable for the export market.

Figure 7.1

LAYOUT OF YIELD PLOTS IN FIELD PLANTED WITH ROW CROP



Yield plot (length of 1 to 5 m of row, or 5 to 10 adjacent plants)

LOCATION OF PREVIOUS SAMPLES, FROM FIGURE 6.1:

i Scan sampling to measure pollinator density (Plot No. i with 100 to 500 flowers depending on crop)

j Net captures to measure pollinator diversity (Subunit No. j of a standardized transect consisting of six 25-m long subunits for insects capture over a 2-m width for 5 minutes)

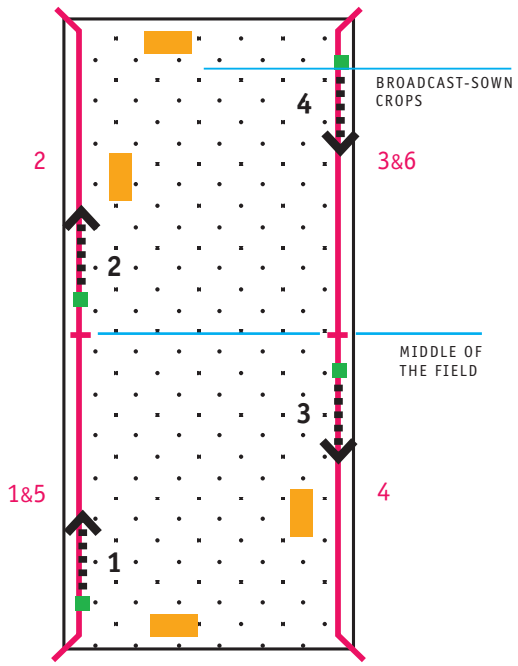
[Plot to assess floral mass]

Sampling should be carried out under good weather conditions for pollinator foraging: Sunny if possible, low wind, vegetation dry, and daily maximum temperature > 15°C

[covariable recordings in brackets]

Figure 7.2

LAYOUT OF YIELD PLOTS IN SMALL FIELD WITH A BROADCAST-SOWN CROP (E.G. MUSTARD/RAPE OR BUCKWHEAT)



Yield plot (l1 m^2 area, or 5 to 10 adjacent plants) located at least 2 m from edge of field inasmuch as possible

LOCATION OF PREVIOUS SAMPLES, FROM FIGURE 6.2:

i Scan sampling to measure pollinator density

j Net captures to measure pollinator diversity

[Plot to assess floral mass}

[optional recordings in brackets]

Examples of data on quality than can be recorded are:

- For fruit and vegetable crops: average size (e.g. diameter, circonference, shape, weight; Figures 1.4, 1.8, 7.4 and 7.5), number of filled seeds (e.g. apple; cucurbits), quality of the flesh consumed (sweetness, flavor; e.g. with tomato Hogendoorn *et al*. 2010).
- For nut crops: average size (e.g. diameter, circonference, weight).
- For oilseed crops: seed size, oil content, quality parameters of the oil (Barbier *et al*. 1967).
- For seed crops for planting: germination rate, quality parameters for seed industry (Kevan and Eisikowitch 1990).

7.B ECONOMIC YIELD

If the price paid to the producer per production unit is known, it may also be possible to assess the yield of each harvesting unit (plant, plot or field) in economic terms, that is expressed in local currency or an international standard.

Pros and cons

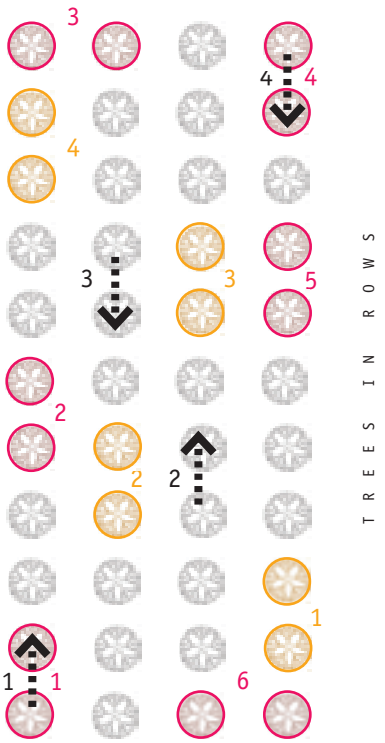
→ Meaningful variable for farmers and consumers.
→ Meaningful for government and policy makers.
→ May assist farmers to record proper documentation.
→ May also include non-market values, e.g. nutritional valu.

← Farmers may be unwilling to share the price at which they sold their crop.
← Very context specific.
← Can be very volatile from one season to the next.
← Lack of accepted methodology (interdisciplinary).
← Link to pollination deficit may be tenuous and difficult to establish.
← Usually beyond the control of individual farmers.

If at all possible, the producer price should be obtained for the production of each study field so as to provide some input data for the economic analyses of the impact of adopting pollinator-friendly practices.

Figure 7.3

LAYOUT OF YIELD PLOTS IN AN ORCHARD WITHOUT POLLENIZER TREES



k — Yield plot (2 adjacent trees of the production variety), located at least 1 row away from the edge of the orchard inasmuch as possible

LOCATION OF PREVIOUS SAMPLES, FROM FIGURE 8:

i — Scan sampling to measure pollinator density (Plot No. i with 100 to 500 owers or panicles depending on crop)

j — Net captures to measure pollinator diversity: Subunit No. j of a standardized transect consisting of six 25-m long subunits for insects capture over a 2-m width for 5 min

Figure 7.4

IMPACT OF POLLINATION LEVEL ON STRAWBERRY QUALITY

© Kristine Kreweka Agroecology, Göttingen, Germany

Strawberries after open insect-pollination (left), passive self-pollination (center), and passive self-pollination plus 75 percent of the incident airborne pollen flow (right). Pollination can have a strong impact on agronomic yields and produce quality.

Figure 7.5

IMPACT OF POLLINATION ON MARKET VALUE

Because adequate pollination has a direct and positive effect on fruit size, symmetry and overall appearance, it is especially important for small farmers who sell their produce at a road stand as here nearby Nairobi in Kenya.

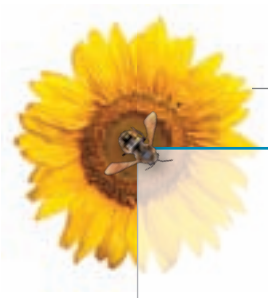
SECTION 8
STATISTICAL ANALYSES

With a completely randomized design with one factor with two levels (e.g. Figure 3.2), control sites without introduced pollinators will have a factor value set at 0 while fields with introduced pollinators using a fixed stocking rate will have a factor value set at 1. The values of the dependent variables for the two groups will then be easily contrasted using usual one-way ANOVA procedures. When pairs or blocks of fields are used, similar methods can be used using adapted ANOVA procedures. When a factorial design is used for a combination of two treatments (e.g. Figure 3.3), a two-way ANOVA should be used so as to be able to test the effect of interaction between the two factors.

This will probably not be so with the distance to the closest patch of natural habitat or the proportion of natural habitat in a 2 km radius around each study field as those values are continuous and will probably vary from field to field along a gradient so that regression analyses may be more appropriate to analyze the results of the landscape treatments.

For large fields with a gradient of distances from the pollinator front, ANOVA with contrasts or regression methods should be used depending on the number of distances set from the pollinator front.

In all cases, it will also be of interest to look at the correlation between forager density and diversity on one hand and the yield variables on the other, as in Hoehn *et al.* (2008). This will be especially important in drawing appropriate management conclusions from the studies conducted using the proposed protocol.



SECTION 9
GENERAL CONCLUSIONS

The present protocol was developed for use by the seven countries in the GEF/UNEP/FAO project on the "Conservation and Management of Pollinators for Sustainable Agriculture through an Ecosystem Approach": Brazil, Ghana, India, Kenya, Nepal, Pakistan, and South Africa. It is by no means meant to be restricted to these countries. Indeed, this protocol has been developed so as to encompass the largest array of crops and situations possible. It is anticipated that it can be used over a wide range of crops and in many countries so that it becomes possible to better document the pollination situation for as many animal-pollinated crops as possible on a worldwide basis. It is therefore hoped that many people will find this protocol useful and will adopt it and share their experience with it in return and provide feedback so as to improve it. This protocol can be downloaded for free on the web site at

http://www.internationalpollinatorsinitiative.org/jsp/documents/documents.jsp;

a discussion forum on the use of this protocol is available at

http://www.fao.org/agriculture/crops/core-themes/theme/spi/gppp/gppp-home/en.

Finally, it should be stressed that this protocol is aimed to address pollination as a production factor at the farm scale level. As such, one should always remember that, as a production factor in its own right, pollination management needs to be fully integrated into the overall farm management system to optimize production in a holistic and sustainable way.

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POLLINATION DEFICIT ASSESSMENT STUDY – FIELD CHARACTERISTICS

COUNTRY: _____ **SITE :** _____ **YEAR :** _____

FIELD NUMBER _____ **COMMON NAME OF FOCAL CROP** _____

FIELD LOCATION & SURROUNDINGS

(GPS COORDINATES) LONGITUDE _____ LATITUDE _____ ALTITUDE (M) _____

TOPOGRAPHICAL SITUATION (HILL TOP, ON SLOPE, VALLEY BOTTOM, PLATEAU,…)

TYPE OF HOLDING (SMALL FARMER OR LARGE FARM) _____ SOIL TYPE _____

SOIL PREPARATION

HEDGE SUROUNDING THE FIELD YES / NO

MAIN PLANT SPECIES IN THE HEDGE

DISTANCE OF FIELD TO CLOSEST PATCH OF NATURAL HABITAT (M ; PATCH ≈ > 0.4 ha = 1 ACRE IN SURFACE)

FIELD SIZE (HA OR ACRE) & APPROXIMATE DIMENSIONS

FOCAL CROP

SPECIES SCIENTIFIC NAME

PRODUCTION VARIETY

VARIETY POLLENIZER IF PRESENT (FRUIT TREES)

ORIGIN OF SEEDS OR SEEDLINGS (GRAFTED ?)

CULTURAL PRACTICES

PLANTING DATE **PLANTING DENSITY** (# PLANTS OF THE FOCAL CROP /UNIT AREA)

TYPE OF PLANTING ROWS / BROADCAST / RANDOM * **TYPE OF STAND** MONOCULTURE / MIXED PLANTING*

RATIO OF POLLENIZER TREE / PRODUCTION TREE (FOR DIOECIOUS CROPS & FRUIT TREES)

DISTANCE BETWEEN ROWS (M)

DISTANCE AMONG PLANTS WITHIN ROWS (M)

** CROSS ITEM THAT DOES NOT APPLY*

SHEET NUMBER____

ANNEX 2:
DATA SHEET TO RECORD POLLINATOR DENSITY ON PLOTS OF AN HERBACEAOUS CROP

Density of insect pollinators in open pollinated flowers (scan sampling)

COUNTRY: **BRAZIL** SITE : **PETROLINA** FOCUS CROP : **CANTALOUPE (*Cucumis melo*)** YEAR :

				RECORDING CONDITIONS					NUMBER OF FLOWER VISITORS				
									HONEY BEES		OTHER BEES	OTHER	
Field number & size (ha)	**TREATMENT**	**Date & observer**	**Recording number**	**Time at start**	**Period**	**Weather conditions**	**Plot number**	**Number flower observed**	***Apis mellifera***	**Bumble bees**	**other wild bees**	**(including drone flies Syrphidae)**	**Remarks**
1 to **10**	Honey bee colony nearby **present / absent***		1, 2, 3, 4	**1000 h, 1200 h, 1400 h, 1600 h**	**Morning or Afternoon**	Sunny, overcast, wind level (instantaneous temperature if available)	**1** or **2** or **3** or **4**		**scan sampling** : the insect(s) must be present at the very time when the inside of the flower is first seen				
			0 ≈ prior to colony introduction *(if applicable)*				1	200					
							2	200					
							3	200					
							4	200					
			1				1	200					
							2	200					
							3	200					
							4	200					
			2				1	200					
							2	200					
							3	200					
							4	200					

* Indicate the number of colonies of *Apis mellifera* nearby the study field & whether these colonies were present or introduced at onset of flowering

SHEET NUMBER____

ANNEX 3:

DATA SHEET TO RECORD POLLINATOR DENSITY ON ORCHARD TREES IN PLOTS LOCATED ALONG A GRADIENT OF DISTANCES TO POLLINATOR FRONT

Density of insect pollinators in open pollinated flowers (scan sampling)

COUNTRY: **GHANA** SITE : _____ FOCUS CROP : **MANGO** (*Mangifera indica)* YEAR : _____

PRODUCTION VARIETY : _____

						RECORDING CONDITIONS					NUMBER OF FLOWER VISITORS					
											HONEY BEES	OTHER BEES		FLIES	OTHER	
Orchard number & size (ha)	**Location**	**TREATMENT**	**MEAN DISTANCE OF TREES IN EXPERIMENTAL PLOT TO POLLINATOR FRONT**	**Date & observer**	**Recording number**	**Time at start**	**Period**	**Weather conditions**	**Plot number** (2 adjacent trees / plot)	**Number of inflorescence with ≥ 1 open flower surveyed**	***Apis mellifera***	**Stingless bees**	**other wild bees** (including carpenter bees)	**FLIES** (Syrphidae & others)	**OTHER** (including wasps)	**Remarks**
1 to 5		Honey bee colonies present or brought or not*	m		0 ≈ Prior to colony introduction (if applicable), 1, 2, 3, 4	0800-1000 h, 1000-1200 h, 1200-1400 h, or 1400-1600 h	Morning or Afternoon	Sunny, overcast, wind level, (intantaneous temperature if	**1** or **2** or **3** or **4**		**scan sampling** : the insect(s) must be present at the very time when the flower is first seen					
* Indicate the number of colonies of *Apis mellifera* nearby the study orchard & whether these colonies were present or introduced at onset of flowering																
			60						1	100						
									2	100						
									3	100						
									4	100						
			210						1	100						
									2	100						
									3	100						
									4	100						
			360						1	100						
									2	100						
									3	100						
									4	100						
			510						1	100						
									2	100						
									3	100						
									4	100						

SHEET NUMBER____

Density of insect pollinators in open pollinated flowers (scan sampling)

COUNTRY: **INDIA** SITE : **MOHAL, KULLU** FOCUS CROP : **APPLE (*Malus x domestica*)** YEAR :

					RECORDING CONDITIONS						NUMBER OF FLOWER VISITORS						
											HONEY BEES		OTHER BEES		DRONE FLIES (Syrphidae)	OTHER	
Orchard number & size (ha)	Location	TREATMENT	Date & observer	Recording number	Time at start	Period	Weather conditions	Tree type*	Plot number (2 adjacent trees / plot**)	Number of open flowers surveyed	*Apis cerana*	*Apis mellifera*	Bumble bees	other wild bees			Remarks
1 to 10		Honey bee colonies brought or present or not*		1, 2, 3, 4	1000–1230 h or 1330–1600 h	Morning or Afternoon	Sunny, overcast, wind level, (intantaneous temperature if available)		**1** or **2** or **3** or **4**		**scan sampling** : the insect(s) must be present at the very time when the flower is first seen						
* Indicate the number of colonies of *Apis cerana* and/or *Apis mellifera* nearby the study orchard & whether these colonies were present or introduced at onset of flowering																	
				0 ≈ prior to colony introduction *(if applicable)*				production	1	250							
								pollenizer	**1**	**250**							
								production	2	250							
								pollenizer	**2**	**250**							
								production	3	250							
								pollenizer	**3**	**250**							
								production	4	250							
								pollenizer	**4**	**250**							
				1				production	1	250							
								pollenizer	**1**	**250**							
								production	2	250							
								pollenizer	**2**	**250**							
								production	3	250							
								pollenizer	**3**	**250**							
								production	4	250							
								pollenizer	**4**	**250**							

SHEET NUMBER____

Density of insect pollinators in open pollinated flowers (scan sampling)

COUNTRY : **NEPAL** SITE : **CHITWAN, TERAI** FOCUS CROP : **SQUASH (*Cucurbita moschata*)** YEAR :

				RECORDING CONDITIONS				NUMBER OF FLOWER VISITORS					
								HONEY BEES		OTHER BEES		OTHER	
House	**Pollinator treatment***	**Date & observer**	**Recording number**	**Time at start**	**Period**	**Weather conditions**	**Number of flowers observed**	***Apis cerana***	***Apis mellifera***	**Bumble bees**	**other wild bees**	**(including drone flies Syrphidae)**	**Remarks**
	House with honey bee colony **present** or **absent***		1, 2, 3, 4	**0800–1000 h or 1000–1200 h**	**Early morning or late morning**	Sunny, overcast, strong wind (instantaneous temperature if available)	(staminate and pistillate flowers observed together)	**scan sampling** : the insect(s) must be present at the very time when the inside of the flower is first seen					
			1				100						
			2				100						
			3				100						
			4				100						
							100						
							100						

* Indicate the number of colonies of *Apis cerana* and/or *Apis mellifera* nearby the study orchard & whether these colonies were present or introduced at onset of flowering

SHEET NUMBER____

ANNEX 6:
DATA SHEET TO RECORD POLLINATOR DIVERSITY IN PLOTS OF AN HERBACEOUS CROP

Diversity of non-*Apis* insect pollinators in open pollinated flowers (sweep net captures)

COUNTRY: **INDIA** SITE : **KOSI KATARMAL** FOCUS CROP : **MUSTARD (*Brassica campestris*)** YEAR :

					RECORDING CONDITIONS				TYPE (SPECIES) & NUMBER OF SPECIMENS PER TYPE				
									BEES OTHER THAN HONEY BEES				
Field number & size (ha)	**Location**	**TREATMENT**	**Date & observer**	**Recording number**	**Time at start**	**Period**	**Weather conditions**	**Transect number in a day**	**Bumble bees (*Bombus* spp.)**	**other wild bees**	**DRONE FLIES (Syrphidae)**	**OTHER**	**Remarks**
	Valley, mid-mountain, hill top	Honey bee colonies nearby **present / absent***		1, 2, 3, 4	1000–1200 h or 1400–1600 h	Morning or Afternoon	Sunny, overcast, strong wind (instantaneous temperature if available)	1, 2, … 6	Flower visiting insects that are likely pollinators caught by sweep net over a 5 minute period along a 25 m transect within or along side the field with a total of 6 transects				
				0 ≈ prior to colony introduction *(if applicable)*				1					
								2					
								3					
								4					
								5					
								6					
				1				1					
								2					
								3					
								4					
								5					
								6					

* Indicate the number of colonies of *Apis cerana* and/or *Apis mellifera* nearby the study field & whether these colonies were present or introduced at onset of flowering

SHEET NUMBER____

ANNEX 7:
DATA SHEET TO RECORD POLLINATOR DIVERSITY ON PLOTS IN AN ORCHARD WITH POLLENIZER PLANTS

Diversity of non-*Apis* insect pollinators in open pollinated flowers (sweep net captures)

COUNTRY: **INDIA** SITE : **MOHAL, KULLU** FOCUS CROP : **APPLE (*Malus x domestica*)** YEAR :

					RECORDING CONDITIONS				TYPE (SPECIES) & NUMBER OF SPECIMENS PER TYPE			
									BEES OTHER THAN HONEY BEES			
Orchard number & size (ha)	**Location**	**TREATMENT**	**Date & observer**	**Recording number**	**Time at start**	**Period**	**Weather conditions**	**Plot number** (2 adjacent trees / plot**)	**Bumble bees**	**other wild bees**	**DRONE FLIES (Syrphidae)**	**OTHER**
1 to 10		Honey bee colonies brought orpresent or not		1, 2, 3, 4	1000–1230 h or 1330–1600 h	Morning or Afternoon	Sunny, overcast, strong wind (Temperature if available)	1, 2, … 6	Flower visiting insects that are likely pollinators caught by sweep net over a 5 minute period on 2 adjacent trees within experimental site or along side for small orchards; **one recording of 5 min should focus on two pollenizer trees (indicated in grey)**			
* Indicate the number of colonies of *Apis cerana* and/or *Apis mellifera* nearby the study orchard & whether these colonies were present or introduced at onset of flowering												
				0 ≈ prior to colony introduction *(if applicable)*				1 2 3 4 5 **6 (pollenizer)**				
				1				1 2 3 4 5 **6 (pollenizer)**				

SHEET NUMBER____

ANNEX 8:
DATA SHEET TO RECORD POLLINATOR DIVERSITY ON ORCHARD TREES IN PLOTS LOCATED ALONG A GRADIENT OF DISTANCES YO POLLINATOR FRONT

Diversity of non-*Apis* insect pollinators in open pollinated flowers (sweep net captures)

COUNTRY: **GHANA** SITE : FOCUS CROP : **MANGO (*Mangifera indica)*** YEAR :

					RECORDING CONDITIONS				TYPE (SPECIES) & NUMBER OF SPECIMENS PER TYPE				
									BEES OTHER THAN HONEY BEES				
Orchard number & size (ha)	**Location**	**TREATMENT**	**MEAN DISTANCE OF TREES IN EXPERIMENTAL PLOT TO POLLINATOR FRONT**	**Date & observer**	**Recording number**	**Time at start**	**Period**	**Weather conditions**	**Plot number** (2 adjacent trees / plot)	**Stingless bees**	**other wild bees**	**Flies (including drone flies Syrphidae)**	**OTHER (including wasps)**	**Remarks**
1 to 5		Honey bee colony brought or present or not*	m		0 = Prior to colony introduction (if applicable), 1, 2, 3, 4	0800-1000 h, 1000-1200 h, 1200-1400 h, or 1400-1600 h	Morning or Afternoon	Sunny, overcast, strong wind (Temperature if available)	1, 2, … 6	Flower visiting insects that are likely pollinators caught by sweep net over a 5 minute periodon two adjacent trees within the experimental site or along orchard for small units				
			60						1					
									2					
									3					
									4					
									5					
									6					
			210						1					
									2					
									3					
									4					
									5					
									6					

* Indicate the number of colonies of *Apis mellifera* nearby the study orchard & whether these colonies were present or introduced at onset of flowering

SHEET NUMBER____

Flower density (number of open flowers per unit area) **& flowering phenology**

COUNTRY: **INDIA** SITE : **KOSI KATARMAL** FOCUS CROP : **BUCKWHEAT (*Fagopyrum esculentum*)** YEAR :

Field number & size (ha)	Location	TREATMENT	Date & observer	Recording number	0.5 m^2 quadrat number	Number of inflorescence with ≥1 open flowers per quadrat	Remarks	Recording number	0.5 m^2 quadrat number	Number of inflorescence with ≥1 open flowers per quadrat	Remarks
1 to 10	Mountain top	Honey bee colony nearby present / absent*		1, 2, 3, 4				1, 2, 3, 4			
				0 ≈ prior to colony introduction *(if applicable)*	1			3	1		
					2				2		
					3				3		
					4				4		
				1	1			4	1		
					2				2		
					3				3		
					4				4		
				2	1				1		
					2				2		
					3				3		
					4				4		

* Indicate the number of colonies of *Apis mellifera* nearby the study field & whether these colonies were present or introduced at onset of flowering

SHEET NUMBER____

ANNEX 10:
DATA SHEET TO RECORD FLOWERING PHENOLOGY IN AN ORCHARD CROP WITH POLLENIZER TREE

Flowering phenology

COUNTRY: **INDIA** SITE : **MOHAL, KULLU** FOCUS CROP : **APPLE (*Malus x domestica*)** YEAR :

Orchard number & size (ha)	Location	TREATMENT	Date & observer	Recording number	Tree type*	Plot number (2 adjacent trees / plot*)	Branch number in each tree**	Total number of flower buds on branch (to be recorded only once at or prior to onset of bloom)	Number of opened flowers on branch	Remarks
1 to 10		Honey bee colony brought or not*		1, 2, 3, 4	**Indicate the variety**	**1** or **2** or **3** or **4**				
				0 ≈ prior to colony introduction (if applicable)	production	1	1			
							2			
					pollenizer	**1**	**1**			
							2			
					production	2	1			
							2			
					pollenizer	**2**	**1**			
							2			
					production	3	1			
							2			
					pollenizer	**3**	**1**			
							2			
					production	4	1			
							2			
					pollenizer	**4**	**1**			
							2			

** For pollenizer trees, use tree closest to the two production trees of the plot surveyed

** Select & tag one representative branch on each opposite side of each tree; each branch should be large like about 2 thumbs at its base & record all buds & opened flowers on each branch at each reading

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ANNEX 11:
DATA SHEET TO RECORD YIELD ON PLOTS OF AN HERBACEOUS CROP PLANTED IN ROWS AND IN MONOCULTURE

Yield data

COUNTRY: **KENYA** SITE : **NANYUKI** FOCUS CROP : FRENCH BEAN (***Phaseolus vulgaris***) YEAR :

					YIELD COMPONENTS			
Field number & size (ha)	**Location**	**TREATMENT**	**Date harvest**	**plot number (length of row in m)**	**Number of plants harvested in the plot**	**Weight of fresh pods**	**Number of fresh pods**	**Remarks**
1 to **10**	(indicate large farms or small holder)	**Close** or **far** from patch of natural habitat				g / plot		
				1				
				2				
				3				
				4				

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ANNEX 12:
DATA SHEET TO RECORD YIELD OF INDIVIDUAL PLANTS OF AN HERBACEOUS CROP ON PLOTS WITH MIXED PLANTING

Yield data

COUNTRY: **INDIA** SITE : **KOSI KATARMAL** FOCUS CROP : **MUSTARD (*Brassica campestris*)** YEAR :

							YIELD COMPONENTS			
Field number & size (ha)	**Location**	**TREATMENT**	**Date harvest**	**plot number (0.5 m² quadrat)**	**Number of plants harvestable in plot**	**Plant number**	**Number of pods per plant**	**Weight of seeds per plant**	**Number of seeds per plant**	**Remarks**
1 to **10**	**Mountain top / Plaine**	Honey bee colony nearby **present / absent***						g / plant		
				1		1				
						2				
						3				
						4				
						5				
				2		1				
						2				
						3				
						4				
						5				
				3		1				
						2				
						3				
						4				
						5				
				4		1				
						2				
						3				
						4				
						5				
						1				
						2				
						3				
						4				
						5				

* Indicate the number of colonies of *Apis cerana* and/or *Apis mellifera* nearby the study orchard & whether these colonies were present or introduced at onset of flowering

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Yield data

OUNTRY: **INDIA** SITE : **MOHAL, KULLU** FOCUS CROP : **APPLE (*Malus x domestica*)** YEAR :

							YIELD COMPONENTS		
Orchard number & size (ha)	**Location**	**TREATMENT**	**Tree type***	**Date harvest & observer**	**Plot number** (2 adjacent trees / plot)	**Tree number**	**Number of fruits per tree**	**Weight of fruits per tree**	**Remarks**
1 to 10		Honey bee colony brought or not*	Indicate the variety		**1** or **2** or **3** or **4**			kg / tree	
			Production variety		**1**	1			
						2			
					2	1			
						2			
					3	1			
						2			
					4	1			
						2			

* Indicate the number of colonies of *Apis cerana* and/or *Apis mellifera* nearby the study field & whether these colonies were present or introduced at onset of flowering

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ANNEX 14:
DATA SHEET TO RECORD YIELD ON ORCHARD TREES IN PLOTS LOCATED ALONG A GRADIENT OF DISTANCES TO POLLINATOR FRONT

Yield data

OUNTRY: **GHANA** SITE : Modest Step farm FOCUS CROP : **MANGO (*Mangifera indica*)** YEAR :

Orchard number & size (ha)	Location	TREATMENT	MEAN DISTANCE OF TREES IN EXPERIMENTAL PLOT TO POLLINATOR FRONT	Tree type*	Date harvest & observer	Plot number (2 adjacent trees / plot)	Tree number	YIELD COMPONENTS: Number of fruits per tree	YIELD COMPONENTS: Weight of fruits per tree	Remarks
1 to 10		Honey bee colony brought or not*	m	Indicate the variety		**1** or **2** or **3** or **4**			kg / tree	
			60	Production variety		**1**	1			
							2			
						2	1			
							2			
						3	1			
							2			
						4	1			
							2			

* Indicate the number of colonies of *Apis cerana* and/or *Apis mellifera* nearby the study field & whether these colonies were present or introduced at onset of flowering

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Printed in Italy on ecological paper - January 2011

Design: studio@bartoleschi.com

As a contribution to the International Pollinators Initiative, FAO and its partners have collaborated with INRA (Institut National de la Recherche Agronomique, a public research body of the French government) to develop a protocol for assessing and detecting if a crop production system is suffering a pollination deficit. This document thus presents a handbook for the application of the protocol, outlining the underlying concepts, the hypothesis to be tested, and the modification and application of the protocol to a variety of circumstances in developing countries, such as small fields, home gardens, and high environmental variability.

GLOBAL ACTION ON POLLINATION SERVICES FOR SUSTAINABLE AGRICULTURE

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I1929E/1/01.11