SECTION 1 DEFINITIONS AND CONCEPTUAL FRAMEWORK

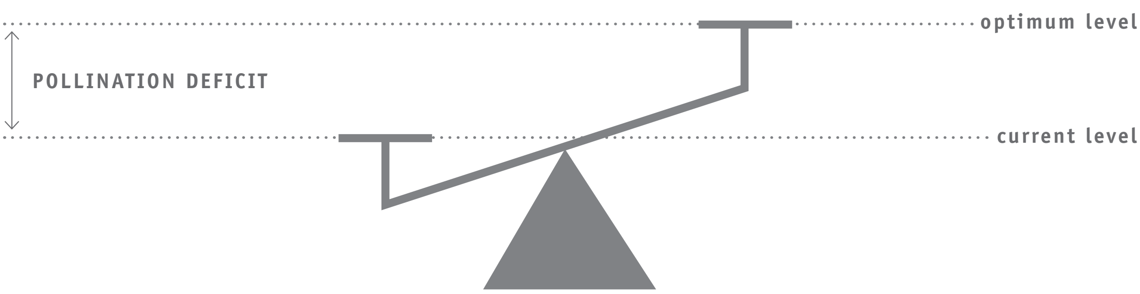
The following conceptual framework underlies the protocol; the definitions of terms often lead to the need for further definitions, in a logical sequence. The terms defined are underlined.

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

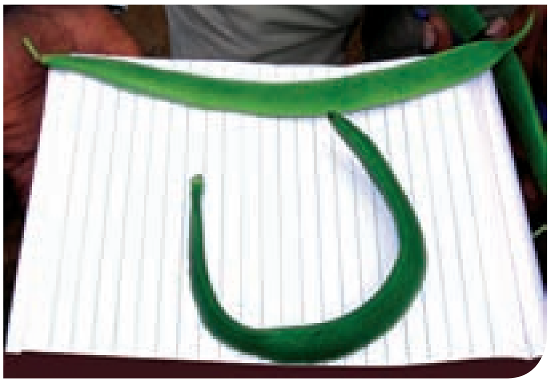
POLLINATION DEFICIT IN RELATION WITH OPTIMUM POLLINATION LEVEL



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.

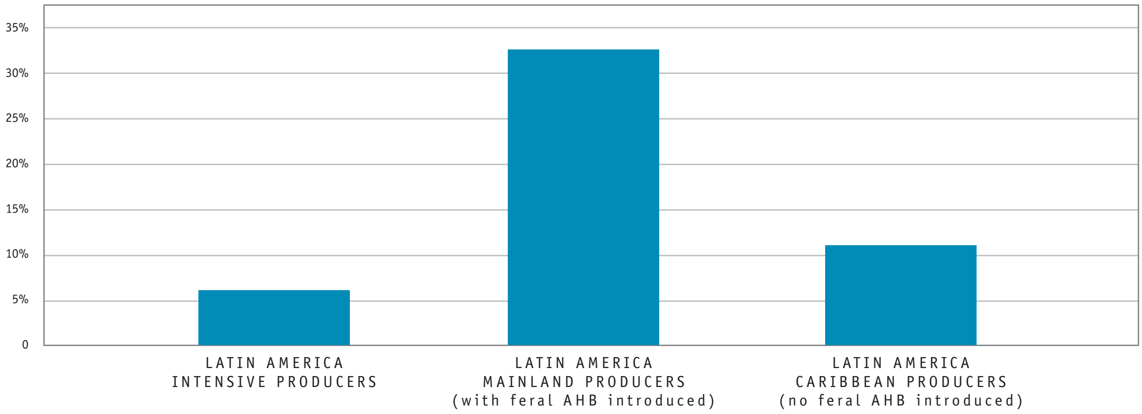
A **quantitative pollination deficit** is an insufficient number of conspecific pollen grains deposited onto the stigma during the **effective pollination period** (see below). It is often the result of an insufficient number of visits by pollinators (Figure 1.5).

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



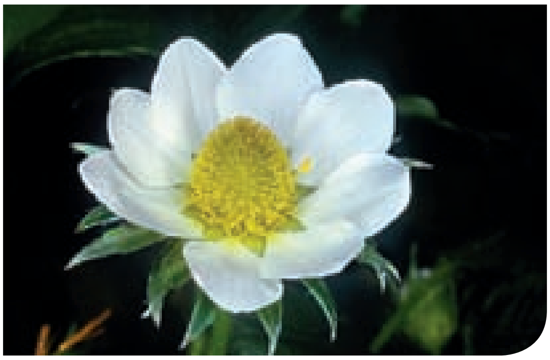
PERCENT CHANGE IN COFFEE PRODUCTION FROM 1961-1980 (BEFORE AHB) TO 1981- 2001 (AFTER AHB)

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



© Nicolas Morison

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

© Nicolas Morison

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

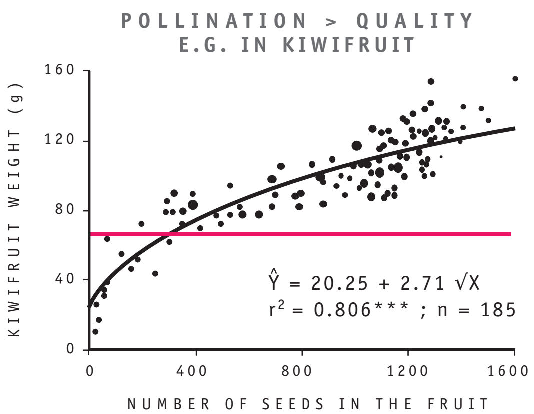
CROP POLLINATION DEFICIT: STRAWBERRIES IN KENYA

© Bernard Vaissière

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

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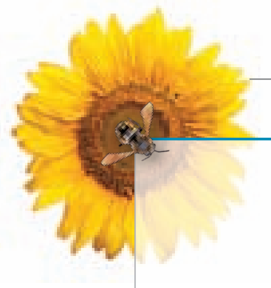
Farmers in northern India and in the Chitwan district of Nepal are choosing to grow less of their traditional crops, such as mustard (*Brassica rapa* L.), because yields have declined. The crop is important for both food security and animal feed. In the Chitwan region, farmers recognise that the bee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

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

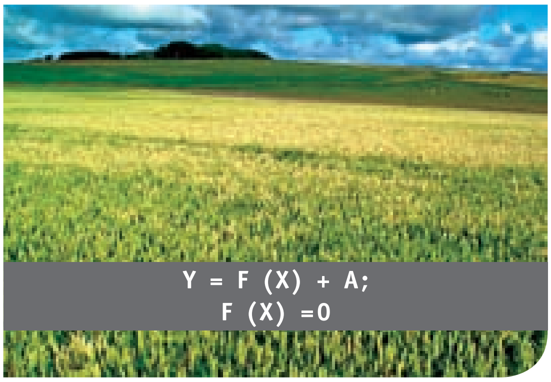
where:

- 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



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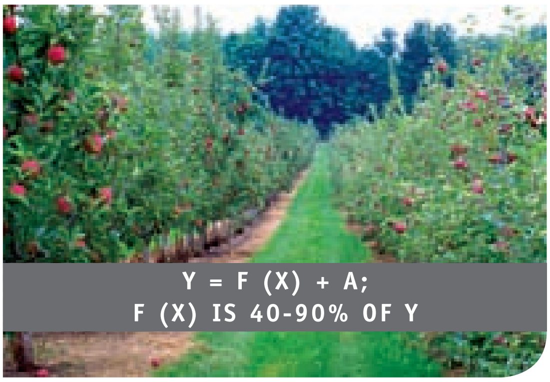


© New York Apple Association

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)

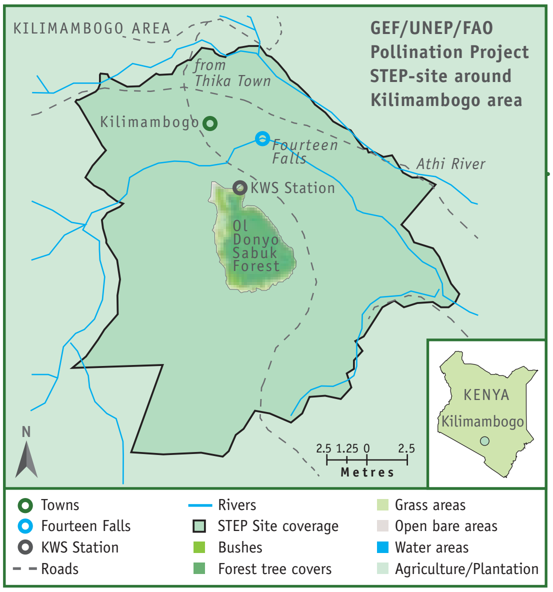
SECTION 3
GENERAL CONSIDERATIONS FOR EXPERIMENTAL DESIGN & STUDY FIELD SELECTION

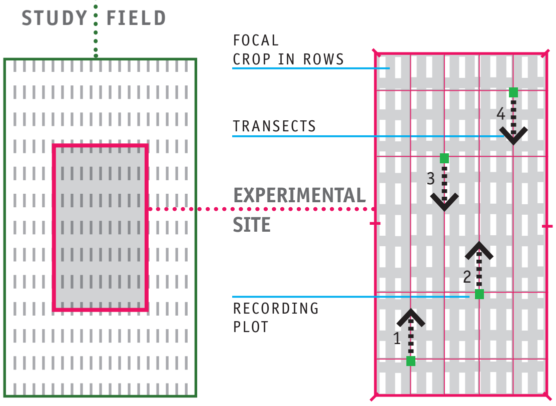
Within the GEF/UNEP/FAO project on the "Conservation and Management of Pollinators for Sustainable Agriculture through an Ecosystem Approach", demonstration sites have been selected, termed "STEP" sites, where STEP stands for Study, Training, Evaluation and Promotion Sites (Figure 3.1). In this project, 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

STEP (STUDY, TRAINING, EVALUATION AND PROMOTION) SITE





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

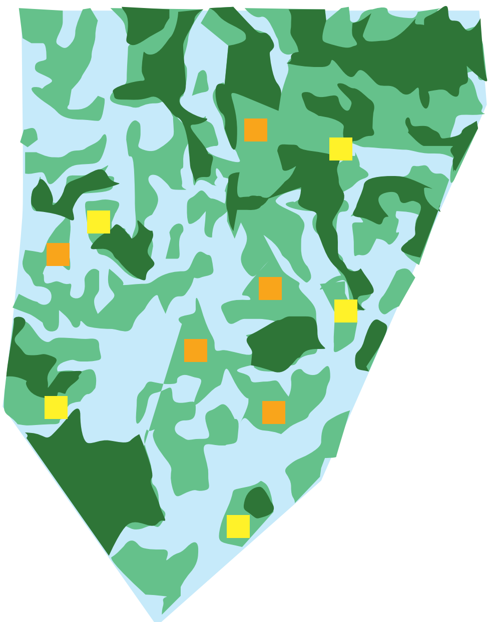
Figure 3.2

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

LAND USE CLASSES

- Agriculture
- Forest
- Open/Built up
- Study fields near natural habitat
- Study fields far from natural habitat

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).

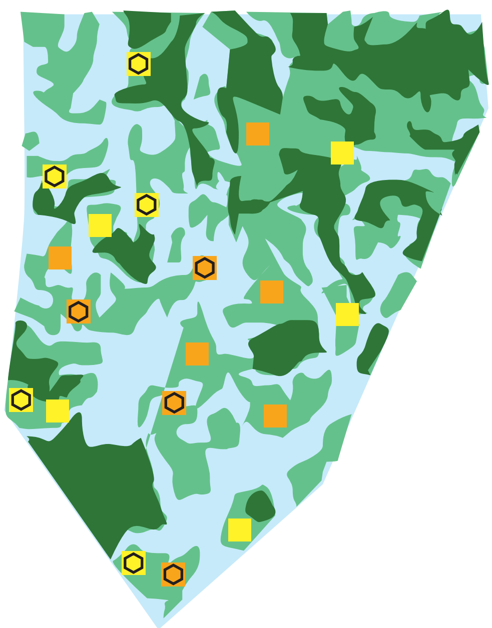
Figure 3.3

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

LAND USE CLASSES

- Agriculture
- Forest
- Open/Built up
- Study fields near natural habitat
- Study fields with hives, near natural habitats
- Study fields far from natural habitat
- Study fields with hives, far from natural habitats

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

© Natacha Chacoff

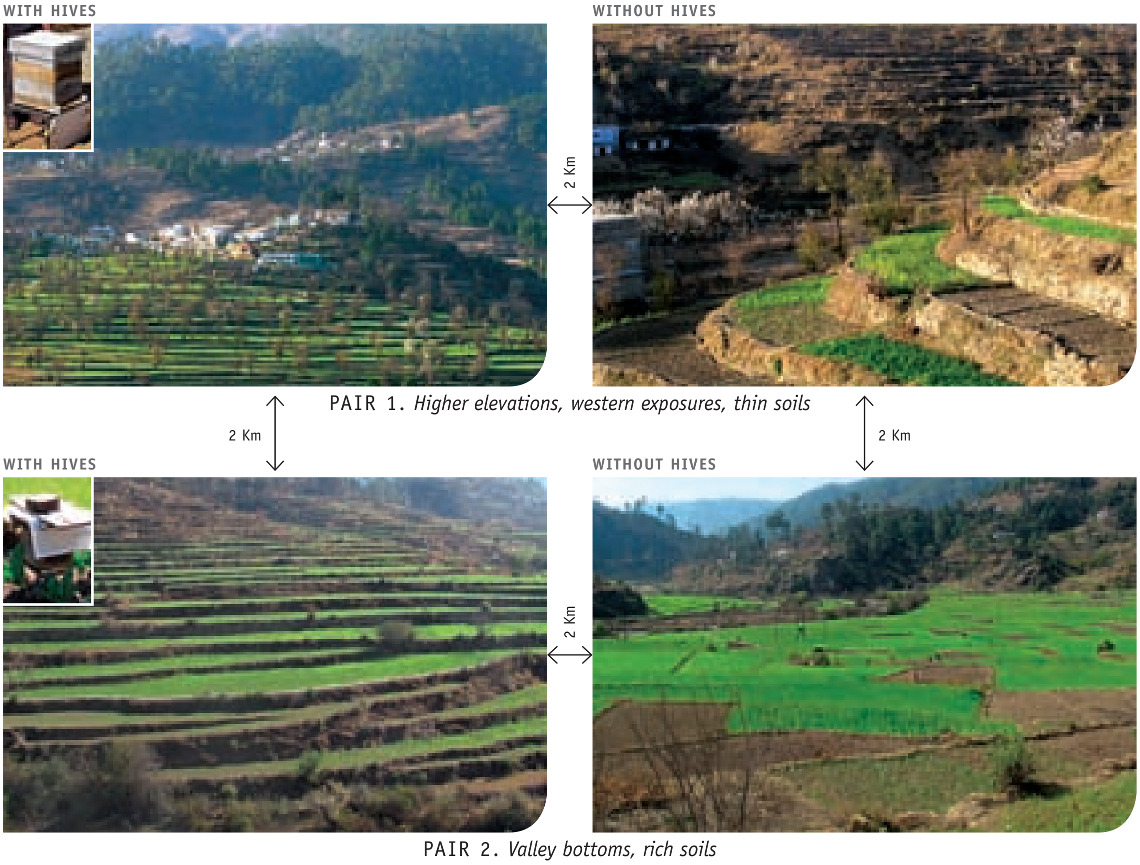
Remnants of semi-natural habitat along one edge of intensive grapefruit (Mach) plantation in the Northwest of Argentina.

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

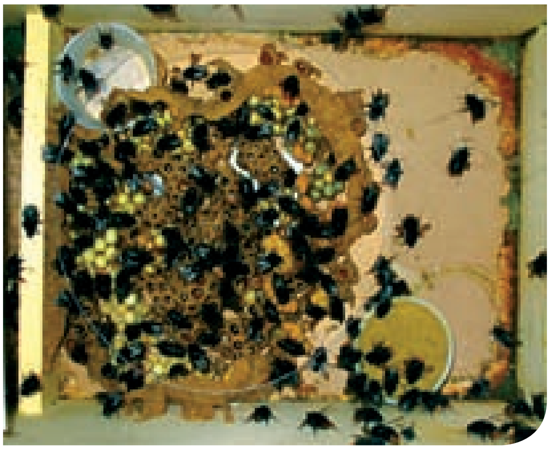
POLLINATOR SUPPLEMENTATION

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

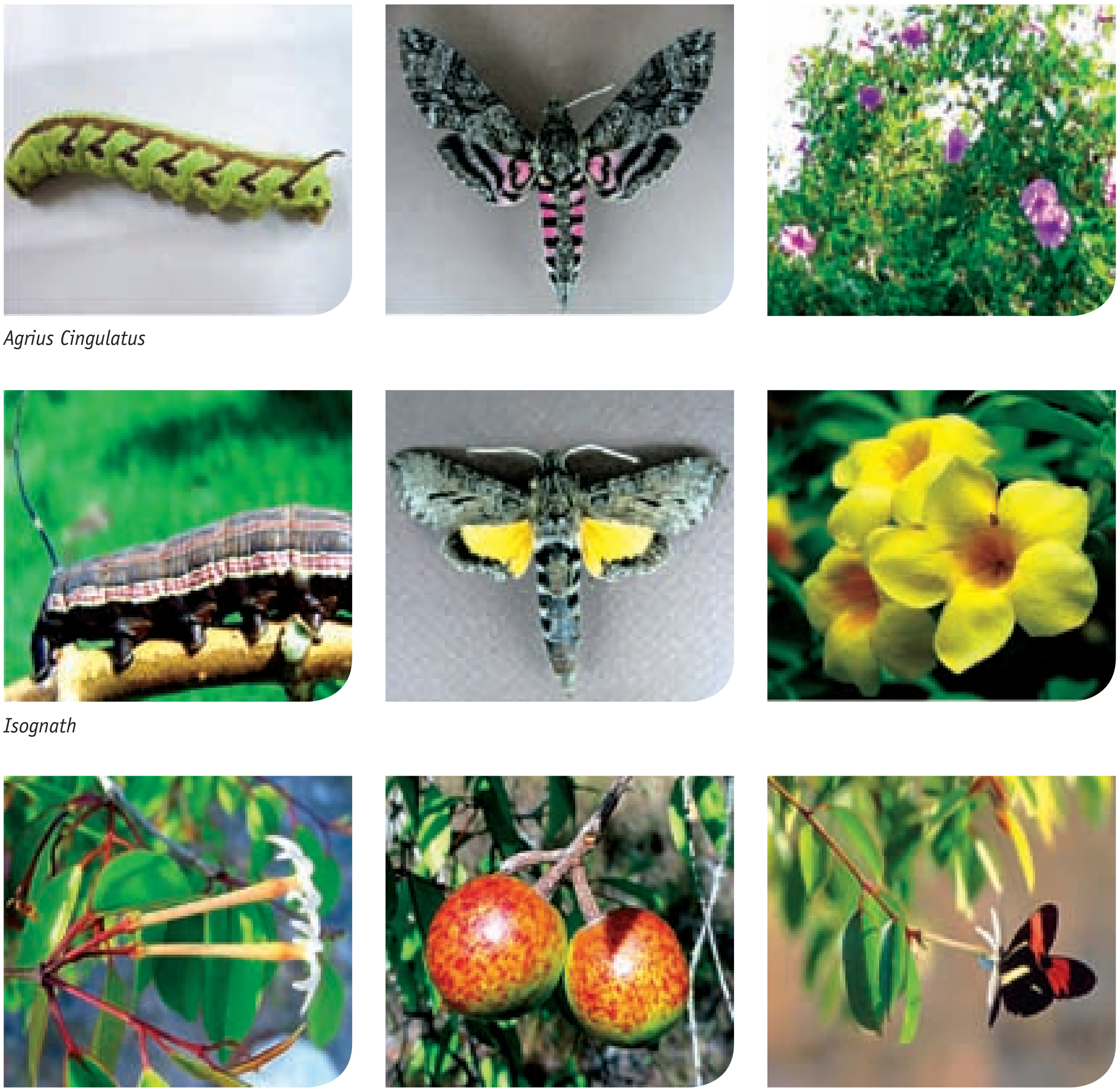
© Peter Kwapong

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