



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

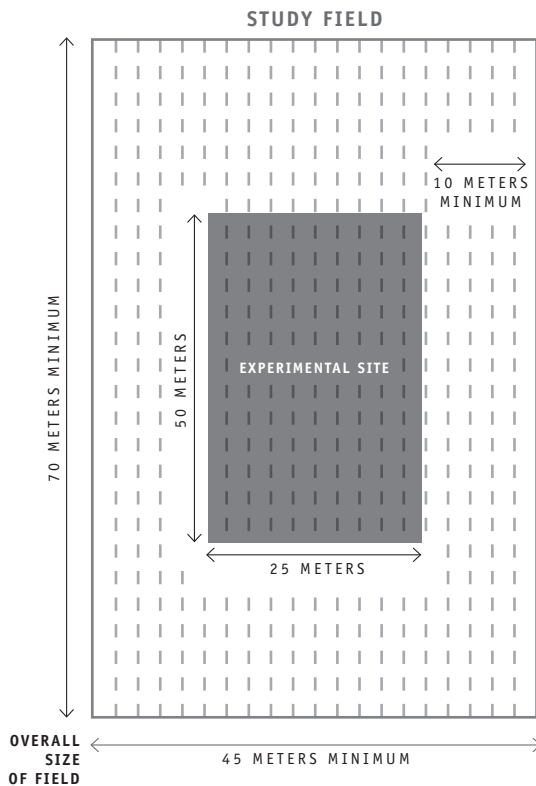
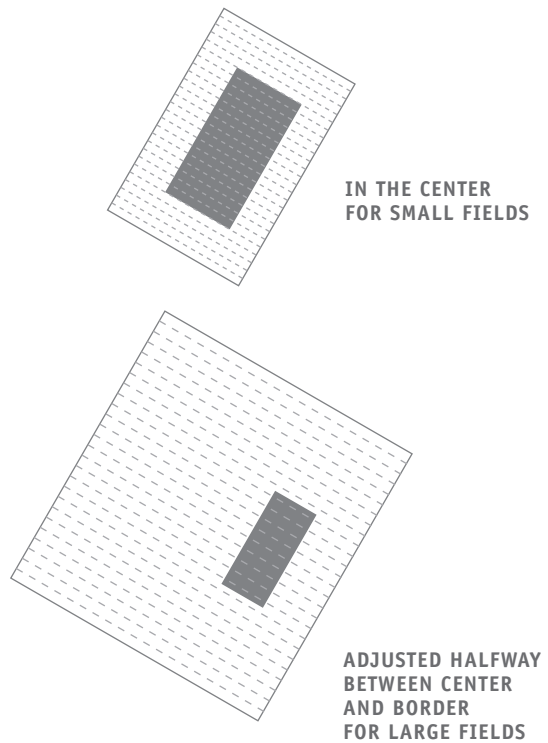


FIGURE 5.2
LAYOUT OF THE EXPERIMENTAL SITE IN RELATION
TO THE SIZE OF THE STUDY FIELD



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 to 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 (<i>Malus x domestica</i> Borkh) or seeds such cashew nut (<i>Anacardium occidentale</i> 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

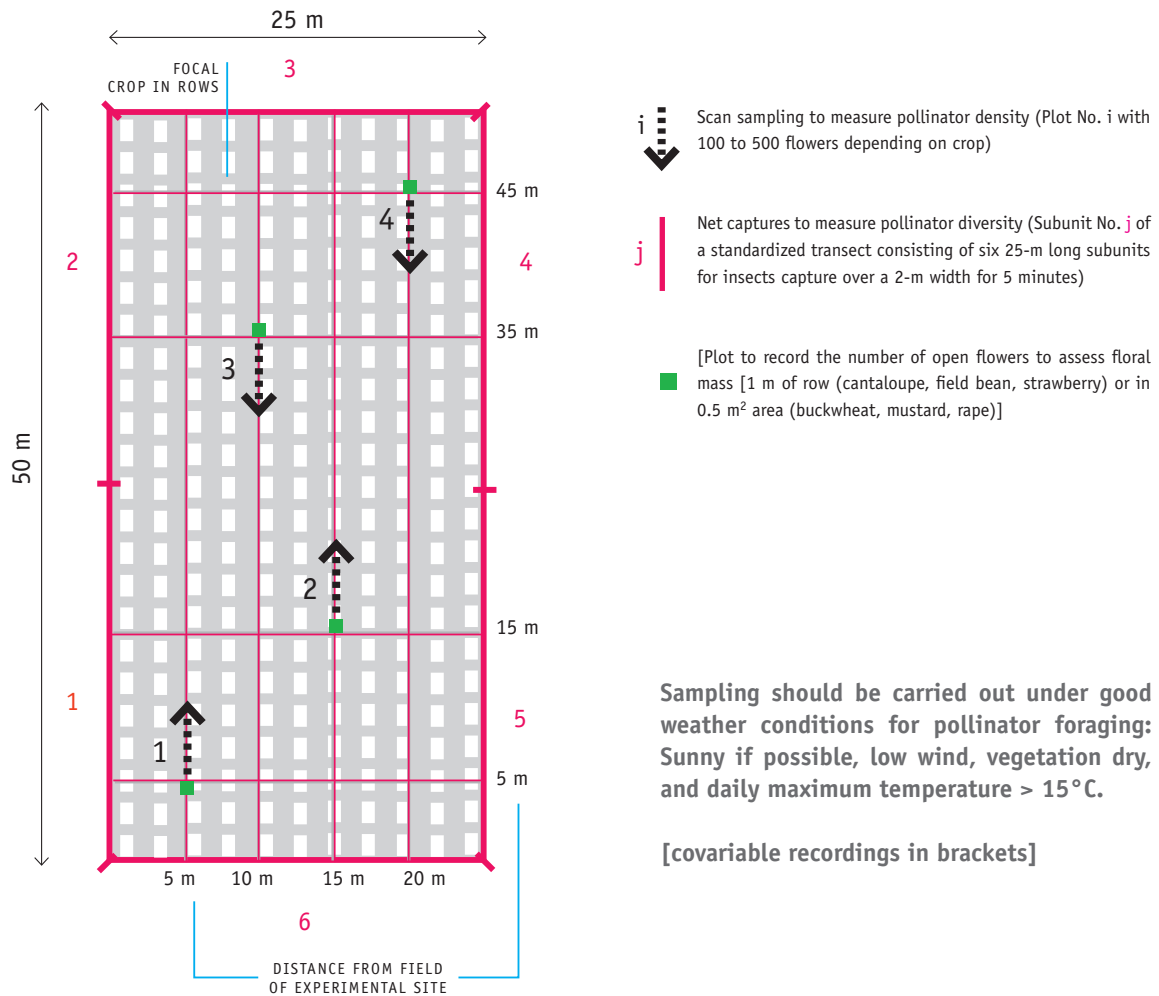
These measurements should be recorded in the experimental sites only under good weather conditions for foraging bees: temperature $\geq 15^{\circ}\text{C}$, low wind, no rain, and dry vegetation (Westphal *et al.* 2008). Recordings should be made from the onset of the main blooming period, that is when $\geq 10\%$ 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

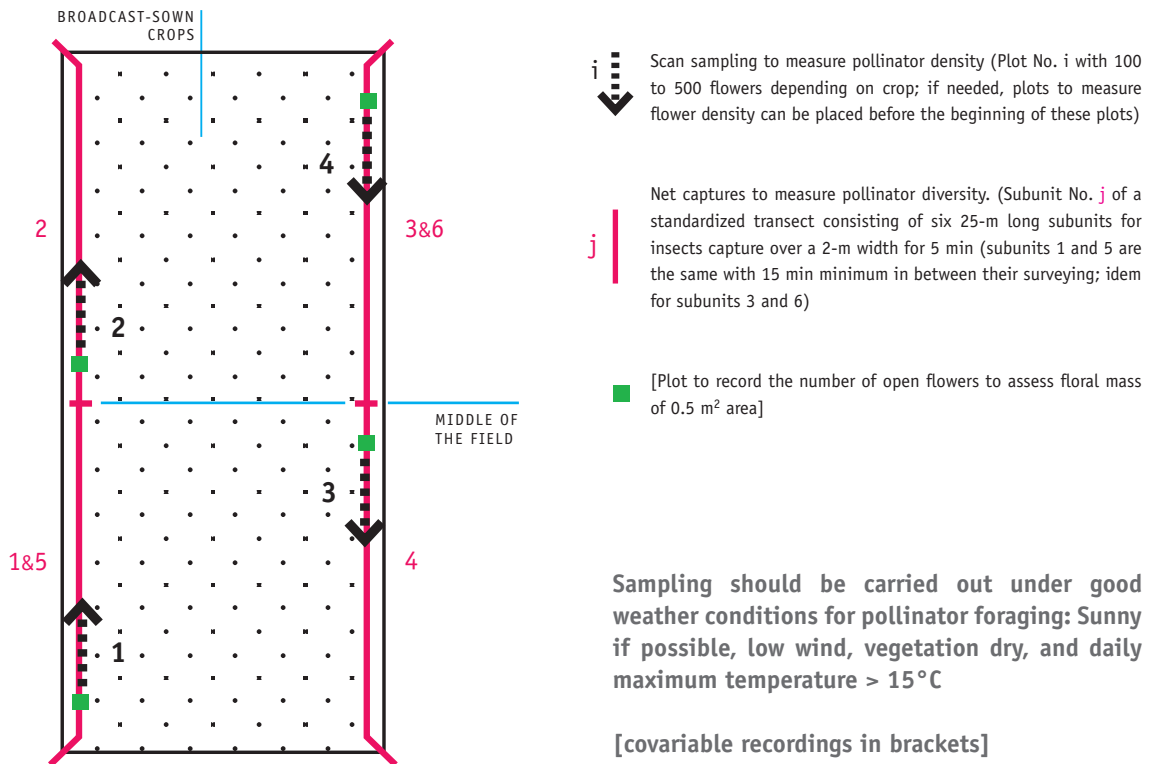
SAMPLING LAYOUT TO MEASURE POLLINATOR ABUNDANCE AND DIVERSITY



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* Moench) 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

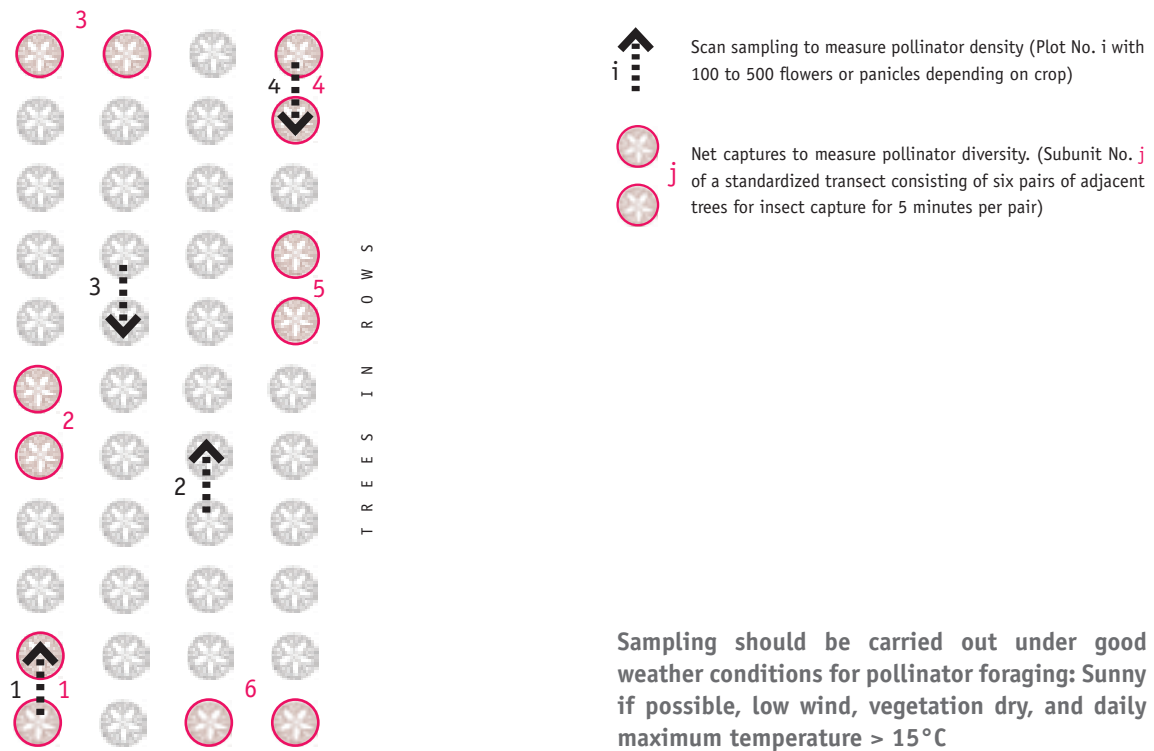
LAYOUT OF SAMPLING AREAS TO MEASURE POLLINATOR DENSITY AND DIVERSITY IN SMALL FIELD WITH A BROADCAST-SOWN CROP (E.G. MUSTARD/RAPE OR BUCKWHEAT)



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 $\geq 15^{\circ}\text{C}$, 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 $\geq 15^{\circ}\text{C}$, 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 m² 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 m² 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 pollinizer 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.