

A Handbook of Field Sampling Protocols for Biodiversity Indicator Monitoring

Produced by the James Hutton Institute for surveys of above-ground
biodiversity within and immediately surrounding arable fields.

August 2011

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Contents

1. Seedbank.....	4
Introduction	4
References	5
Field Sampling Protocol - Seedbank	6
i) Timing.....	6
ii) Sampling Intensity.....	6
iii) Sample Locations	6
iv) Soil Collection	6
v) Lab Processing	6
2. Within-Field Arable Weeds	8
Introduction	8
References	8
Field Sampling Protocol – Within-field weeds	9
i) Timing.....	9
ii) Sampling Intensity.....	9
iii) Sample Locations	9
iv) Quadrat Counts.....	9
v) Biomass Collection.....	10
vi) Lab Processing	10
3. Field Margins.....	11
Introduction	11
References	11
Field Sampling Protocol – margin vegetation	12
i) Timing.....	12
ii) Sample Locations	12
iii) Quadrat Counts.....	13
4. Invertebrates – Pitfall Trapping.....	14
Introduction	14
References	14
Field Sampling Protocol – pitfall trapping.....	15
i) Timing	15
ii) Sample Locations	15
iii) Sampling.....	16

5. Invertebrates – Vortis Sampling	17
Introduction	17
References	18
Field Sampling Protocol – vortis sampling	19
i) Timing.....	19
ii) Sampling Intensity.....	19
iii) Sample Locations	19
iv) Sample collection	19
v) Lab Processing	20
6. Invertebrates – Pollinators.....	21
Introduction	21
References	22
Field Sampling Protocol – bee and butterfly sampling.....	23
i) Timing.....	23
iii) Sample Locations	23
iv) Sample collection	23
BEAUFORT WIND SCALE.....	25
Acknowledgements	26

1. Seedbank

Introduction

Arable seedbanks are increasingly seen as an important source of biological diversity, and crucial to the functioning of arable systems (Squire *et al.*, 2003). Seedbanks present both a cost and a benefit to managed systems. High seed population densities can lead to a serious reduction of crop yield, but the buried seeds and emerged plants provide a range of ecosystem services including soil stabilisation, pollution control and a resource for arable food webs. The seeds and plants of the arable seedbank provide a greater diversity of form, composition and function than the few crop species that dominate arable land (Hawes *et al.*, 2003; Norris & Kogan, 2000).

The seedbank is therefore a valuable reference in studies of ecological impact (Heard *et al.*, 2003; Firbank *et al.*, 2003a; Perry *et al.*, 2003) and in the conservation and restoration of the commoner arable flora. In particular, the persistence of seeds in the seedbank make them far less sensitive than the emerged flora to immediate conditions of the field or weather and therefore confer a certain degree of resilience to the arable weed community. The weed flora has declined over the past 50 years, along with other indicators of diversity, primarily due to the increased use of herbicide and the competitive suppression of weeds by autumn-sown crops (Marshall *et al.*, 2003; Robinson & Sutherland, 2002). The 100 common weeds listed by Brenchley (1918) have remained widespread, but the rarer arable weeds have been severely reduced, even to extinction in some parts of the UK (e.g. Preston *et al.*, 2002). High seed population densities (of 10^5 m^{-2}) and species richness (of around 40 species per field) were recorded throughout the century in ploughed land under poor weed control (Brenchley & Warrington, 1933; Roberts & Stokes, 1958; Squire *et al.*, 2000). Very low seedbank populations of around 10^2 m^{-2} and as low as 10 species in a field, were recorded whenever management suppressed weeds for many years, whether by mechanical cultivation (Brenchley, 1918) or the use of herbicides (Marshall & Arnold, 1994). Populations have remained capable of rapid dynamics, readily increasing if control is relaxed over several years, or declining by up to 50% a year if seed return is severely reduced or prevented (Brenchley & Warrington, 1933; Roberts, 1958; 1962; Roberts & Feast, 1972; Wilson & Lawson, 1992).

The sporadic and largely uncoordinated studies of the UK arable seedbank since 1915 still provide a mostly unambiguous account of the general frequency and abundance of the commoner species in the arable seedbank. However, if seedbanks are to be a valuable indicator for changes in the status and health of arable systems, then a consistent sampling of sites over time is essential, as used for the emerged flora in countryside surveys in the UK for instance (Firbank *et al.*, 2003b). Only then can we confidently predict the impact of future changes in arable management practices on the resilience and community dynamics of arable weeds.

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Field Sampling Protocol - Seedbank

i) Timing

For an estimate of arable weed seedbank diversity from a single sample collection in a given year, soil should be collected in early spring (end of March) after the seedbank has been exposed to a winter chill and preferably before a spring crop is sown.

If resources allow, a second set of samples should be collected in the autumn after harvest, but before subsequent cultivations stimulate a flush of emergence in the field (September).

ii) Sampling Intensity

Between 15 and 20 samples should be collected from the cropped area of each field to be assessed. This is based on the assumption that there are around 50 fields included in the survey (Perry et al 2003). If fewer sites are to be surveyed, the within-field sampling intensity would need to be increased accordingly.

iii) Sample Locations

To capture the within-field variability in seedbank density and species distribution, collect soil from a grid of sample points across the whole field. To standardise the sampling locations across fields, aim to work off tramlines, if they are visible: select 4 tramlines that are roughly equally spaced along the field headland, and then collect samples from 5 points spaced equidistantly along each. At each sample point, soil should be collected approximately 1m away from the tramline into the cropped area of the field to avoid any peculiarities created by farm traffic within or between the wheelings.

iv) Soil Collection

1. Place a 50x50cm quadrat 1m into the crop from the tramline to the west of the fixed sampling locus
2. Clear surface debris (any plant material or compost)
3. With a trowel, mix the soil within the quadrat to a depth of 15cm
4. Fill a 2 litre pot to the top with the mixed soil, avoiding large stones
5. Empty the pot into a plastic bag labelled with the field name and sample number
6. Record the date, field condition and observer
7. Return samples to the lab for processing
8. If it is necessary to store samples before processing, keep the soil at approx 5°C, out of direct sunlight. Do not freeze the samples.

v) Lab Processing

Seedbank diversity is assessed using the germination method:

First Flush

1. Pass the soil through a 10mm sieve
2. Fill a seed tray (16.5cm x 22cm x 5cm) with the sieved soil to a depth of 4cm. Soil should be evenly spread across the tray and pressed to provide a reasonably solid substrate for the weeds when they germinate - i.e. not too loose.

3. Clearly label the tray with the field name, sample number and date.
4. If further analyses are to be carried out, all remaining soil should be returned to its polythene bag and passed over to soil biologists/chemists (for DNA extraction, nitrification/denitrification activity and analysis of soil chemistry etc).
5. The seed tray containing the seedbank sample should be placed in a glasshouse on benches and should be kept moist (standard glasshouse conditions are listed below).
6. Record the number of individuals of every weed species that germinate. This should be carried out twice a week through the initial flush, removing those that have been correctly identified and marking those where the identification is uncertain. Unidentified species should be recorded as such, labelled, and grown on as specimens in separate pots until identification is possible. This procedure should be continued until no further weeds germinate. The number of seedlings of each species should be recorded for up to 15 weeks after sample preparation.
7. Any seedlings that are hard to identify at this stage should be removed from the tray, labelled, potted up and grown on until they are at a stage where a positive identification can be made.

Second flush

1. After germination has ceased (no further seedlings germinating for 2 weeks), allow the trays to dry out for a week
2. Place each tray in a paper bag and lay out in stackable plastic trays
3. Set the trays outside over winter for a cold treatment (December to February)
4. At the beginning of March, bring the trays back into the glasshouse repeat the procedure described above for the first flush of seedlings.

Glasshouse conditions:

- Light intensity: $300\mu\text{mol.m}^2.\text{sec}^{-1}$
- Light duration: 12 hour day length
- Minimum Day temp: 18°C
- Minimum Night temp: 15°C
- Shade screens operative at $600\mu\text{mol.m}^2.\text{sec}^{-1}$ set at 22°C .

2. Within-Field Arable Weeds

Introduction

Since the 1920s, agricultural intensification has caused a decline in the diversity of arable plants and associated fauna (Marshall et al 2003), and has had a detrimental impact particularly on non-target species that are present at moderate or low abundance (Brenchley and Warrington 1933, Roberts 1958). This loss of biodiversity has important implications for the diverse array of associated herbivores, predators and parasitoids that depend on them for food and shelter (Altieri et al 1999, Haddad 2001, Hawes et al 2003, Koricheva et al 2000, Siemann et al 1998). Weeds, and dicotyledon weeds in particular, represent a highly valuable resource to primary consumers within arable fields, supporting up to ten times the biomass of herbivores per unit plant mass compared to crop plants (Karley et al 2011). Diversity in this weed layer results in increased diversity of herbivore consumers (Marshall et al. 2003), and some studies have shown that this effect is propagated through the food web to the third trophic level (e.g. Hawes et al. 2003, 2009, Taylor et al. 2006). The presence of specific weed species can also stabilise pest and beneficial natural enemy populations through provision of a greater range of microhabitats and sustained resource availability throughout the growing season.

Tolerance of some weed cover within fields is therefore essential for the maintenance of the within-field functions, including decomposition, soil nutrient retention, pest and disease population control, pollination and ultimately, in the long-term, primary productivity. The challenge is to define the optimal biomass and composition of the weed flora that supports a healthy arable foodweb but with minimum agronomic impact.

This protocol provides a guide for the assessment of within field arable weed diversity for monitoring biodiversity impacts of changes in agronomic practices and conducting surveys of arable weed species abundance in fields across the landscape.

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Field Sampling Protocol – Within-field weeds

i) Timing

Surveys of the above-ground weed flora are taken on 4 occasions through the growing season in winter sown crops and on 3 occasions in spring sown crops. These are timed to capture the effects of the main management events and weed development stages through the year, as follows:

Weed Count	Timing (calendar)	Timing (management)	Reason
1	Mid October (winter crops)	After sowing of winter crops	To capture first flush of weed seedlings after cultivation
2	Mid April (all crops)	After sowing of spring crops, before post-emergence herbicide applications	To capture the first flush of seedlings after cultivation in spring sown crops and the extent of winter kill in winter crops
3	Mid-end June (all crops)	After post-emergence herbicide applications	Mid-season count to capture effects of herbicide applications when weeds are starting to mature
4	End August (all crops)	Before harvest	To assess the weed density and accumulated weed biomass at the end of the growing season

ii) Sampling Intensity

Approximately 20 quadrat counts should be carried out from the within the cropped area of each field to be assessed. This is based on the assumption that there will be around 50 fields included in the survey (Perry et al 2003). If fewer sites are to be surveyed, the within-field sampling intensity would need to be increased accordingly.

iii) Sample Locations

Counts should be conducted from a point adjacent to the soil sampling locations if the seedbank is also being monitored. Weed counts should be taken from a grid of sample points across the whole field in order to capture the variability in weed density and species distribution. To standardise the sampling locations across fields, aim to work off tramlines, if they are visible. Select 4 tramlines, roughly equally spaced along the field headland, and then conduct counts at 5 points spaced equidistantly along each. At each sample point, place the quadrat approximately 1m from the tramline into the cropped area of the field to avoid any peculiarities created by farm traffic within or between the wheelings.

iv) Quadrat Counts

1. Record the date, field condition, crop and observer
2. Place a 50x50cm quadrat 1m into the crop from the tramline
3. Record the number of individual plants of each species present
4. Record the percentage cover of the dicot weeds, monocot weeds and crop
5. Record crop height and growth stage
6. On the last count before harvest, collect the weed and crop biomass as described below:

v) Biomass Collection

1. After the weeds have been counted and the cover estimates made, collect all the weeds from the 50x50cm quadrat into a polythene bag labelled with the field name, sample number and quadrat size.
2. From the same 50x50cm quadrat, collect all the crop plants and place into a larger polythene bag, labelled with field name and sample number.
3. Place a 1x1m quadrat over the 50x50cm quadrat so that the small quadrat sits in the bottom right hand corner of the large quadrat.
4. Collect all the weeds from the larger quadrat into a third polythene bag, labelled with the field name, sample number and quadrat size.
5. Return all plant material to a cold store at 5° C for immediate processing.

vi) Lab Processing

1. From each weed bag, separate the plants into species and place in a paper bag labelled with field name, sample number and weed species.
2. From each crop bag, divide the crop plants into stems and grain and place in separate paper bags labelled with field name, sample number and plant part.
3. Place all paper bags in drying ovens at 70° C for a minimum of 24 hours.
4. Record the dry weights for the contents of each bag.

3. Field Margins

Introduction

Vegetation is an important component of biodiversity in its own right. The vegetation associated with arable farming comprises the in-field weeds of the cropped area, the sown or managed margins or strips of usually perennial species, the boundary vegetation of perennials and hedges, assorted woodland and parcels of unused land. The balance between these types differs greatly across Europe, and between different types of farms in the landscape (Hawes et al 2010).

Much of the plant diversity in arable land is in the first 1m of the crop and/or in the field edges. The purpose of sampling the crop margin is to record changes in the arable weed population at the edge of cultivated fields. It is believed that the non-crop plant diversity increases towards the edge of a field and the field edge may contribute an important source of biodiversity. Edge vegetation may be affected directly by herbicide spray drift, or it may experience indirect effects mediated by animals.

The flowers and seeds on the field edge are a resource for bees, butterflies, birds and many herbivorous invertebrates. Reduction in the availability and quality of plant food resources (Biesmeijer et al, 2006), through the combined effects of habitat loss, habitat fragmentation and intensive agricultural practices (Kremen et al, 2002) has had a major impact on the beneficial insects that rely on them. Decreasing pollinator and natural enemy numbers could have consequences not only for the productivity of agricultural crops but also for population sizes of wild plant species. In Britain, parallel declines in pollinators and insect-pollinated plants have been demonstrated and a reduction in pollination and seed set of fragmented native plant populations has been reported (Rathcke & Jules, 1993). Alternative low intensity management systems that increase field margin resource availability and quality for pollinators, including extended seasonal resource abundance, are likely to be important in promoting pollinator diversity through niche differentiation.

This protocol describes the assessment of margin vegetation diversity and resource availability for beneficial invertebrates, particularly insect natural enemies and pollinators.

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Field Sampling Protocol – margin vegetation

i) Timing

Surveys of the vegetation in the field margins are conducted on 3 occasions during the growing season to capture the main vegetative, flowering and seeding stages of the perennial vegetation present. These should coincide with the pollinator surveys so that data on flowering plants can be related directly to pollinator abundance.

Count 1: May (record flowering only)

Count 2: July (record cover, flowering and seeding)

Count 3: September (record flowering and seeding only)

ii) Sample Locations

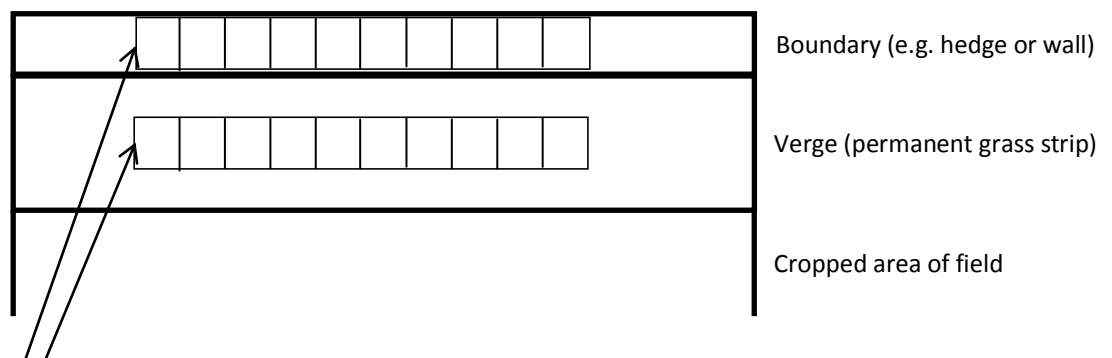
Field margin vegetation surveys are conducted in a 10m x 1m plot in the centre of each margin. Each plot will include a boundary sample and a verge sample.

Field Margin Verge Sample – V

The verge is classified as the grassy or herbaceous vegetation between the edge of the plough and the boundary. Where this feature is over 1m wide, a 10 x 1m quadrat is recorded. If it is wider than 2m, sample from 50 cm to 150 cm from the edge of the plough.

Field Margin Boundary Sample - B

A boundary is taken to be any physical feature that is an interface between the field and any other land cover type. A boundary may be a hedge, wall, fence, ditch, embankment etc. Where a ditch is found adjacent to the hedge, the bank of the ditch adjacent to the hedge is included in the quadrat, provided that at least 40 cm width of hedge is also included. If the boundary is a grassy track or even quite a bare track, the plot runs along the centre of the track. Where the boundary is a deep ditch which cannot readily be crossed, sample the near side of the ditch. If the ditch is shallow, run the sample along the middle.



10m x 1m quadrats in the verge and along the boundary, divided into 1m sections for vegetation assessments

iii) Quadrat Counts

1. Identify a point that is roughly the centre of the field margin to be assessed, and run a tape measure out to mark a 10m strip along the verge.
2. Following the instructions given below, record (a) the frequency of flowering species (May), (b) the cover of all species and the frequency of flowering (July), and (c) the frequency of seeding and flowering (September).
3. Repeat this procedure for the boundary sample.

(a) Flower sampling

Each 10×1m margin plot (boundary and verge) is divided into 10 segments. Species are given two-digit codes according to both frequency (in segments) and cover of blooms within segments.

- 1 <10 individual blooms & <1% cover of blooms
- 2 ≥10 individual blooms & <1% cover of blooms
- 3 = 1-5% cover
- 4 = >5% cover

For example:

- 31 = flowering in 3 out of 10 segments, with <10 individual blooms overall,
- 63 = flowering in 6 out of the 10 segments, with 1-5% cover of blooms in those 6 segments
- A4 = flowering in 10 out of 10 segments, with >5% cover of blooms. A represents "All"

Vertical area of blooms (e.g. flowers on a hawthorn hedge, or flowers up the stem of *Lamium album*) should be included in the overall estimate of cover. Do not agonize over estimating these values. They are inevitably only semi-quantitative; do not agonize over the number of segments either; they should be estimated by eye, not marked out individually. Flowers of grasses, sedges and rushes are not recorded to species.

(b) Cover sampling

Species cover sampling is a much lengthier process than recording flowering, because a full species inventory is required. Cover sampling is done only once, in June. The 6-point Braun-Blanquet scale is used:

- + = present, cover <1%
- 1 = cover 1-5%;
- 2 = cover 5.1-25%;
- 3 = cover 25.1-50%;
- 4 = cover 50.1-75%;
- 5 = cover >75%

All vascular plant species should be recorded for cover assessments. An overall estimate is made of grass cover. Cover of individual grass species is not recorded separately.

(c) Flower sampling

Seed is sampled in the same locations as cover, but with difference that seed presence is recorded as a frequency out of 10 segments. Sampling for seed is done in July and August. If no seeding plants of any species are present, this fact will be recorded because the feature will be marked as present on the form but no seed entry will be made for that feature. N.B. Grass seed is to be recorded separately for each species. Grasses are at this stage relatively easy to recognize.

4. Invertebrates – Pitfall Trapping

Introduction

Ground beetles (Carabidae) are frequently used as bioindicators (Rainio & Niemela, 2003) and tools for monitoring habitat diversity (Luff 1996) because the relative abundance and spatial distribution of different functional types summarise habitat features important to other components of the system, while requiring relatively low sampling effort. At a local, within-habitat scale, spatial heterogeneity in the type and extent of vegetation cover, temperature and humidity at the ground surface, pH and fertility of the soil, depth of the litter layer, and density of litter- and soil-dwelling prey are considered prime determinants of carabid species distribution and abundance patterns (Loreau & Nolf 1994, Thiele 1977, Niemela *et al.* 1996). They are an important component in arable agricultural ecosystems are thought to be potentially important in the biological control of certain agricultural pest insects and are a valuable food source for vertebrates. They are also a well-studied species-rich group with a tractable taxonomy.

Much work on the effects of both environmental change and agricultural management practices on flora and fauna, in particular the use of pesticides has shown that beetles, especially carabids, are good indicators when assessing environmental conditions in agricultural ecosystems (Luff & Woiwod, 1995).

In order to assess the effects of management practice the standard 'pitfall trap' method is used. This method has been used widely in environmental studies and provides a quick and relatively easy method of obtaining sufficient numbers of beetles for statistical analysis of species abundance and diversity.

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Field Sampling Protocol – pitfall trapping

i) Timing

Most Carabids are long-lived in the adult stage and therefore do not show the strong seasonal fluctuations of many other insects. This means that a fairly exhaustive investigation of an area within a short period of time is possible. It is, however, necessary to distinguish between spring and autumn breeding/active species.

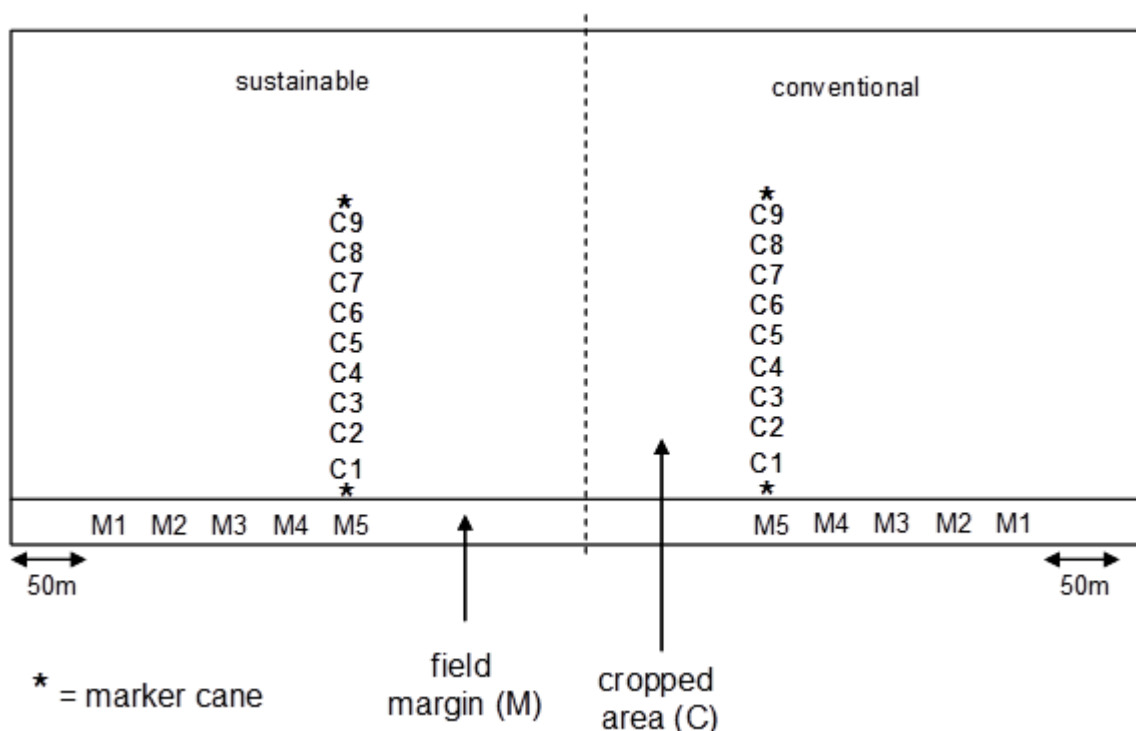
Traps should therefore be set for two sampling periods each year: May (after the spring crops are sown) and September (after harvest). Traps should be run for a period of four weeks for each sampling occasion.

ii) Sample Locations

Five pitfall traps should be positioned in one field margin of each field to be surveyed. Locate the first approximately 50 m from the field corner and space the remaining four at 10m intervals along the margin.

Nine traps should be positioned into the cropped area in a line perpendicular to the 5th margin pitfall trap (see diagram). These should also be spaced 10m apart. To re-locate the traps at the end of the 4 week sample period, mark the position of the 5th pitfall trap in the margin and the 9th pitfall trap in the crop with a 2m flexi-cane. All other traps can be located from these reference points.

Field layout using an example of a split-field experiment comparing two management regimes:

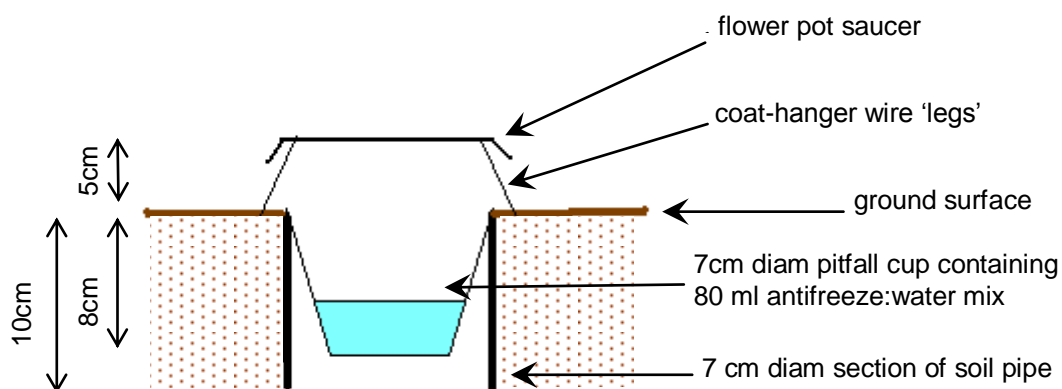


iii) Sampling

1. At each pitfall location, dig a hole and insert a section of soil pipe (7 cm diam x 10 cm long).
2. Put the pitfall cup containing a 50:50 blue antifreeze:water mix into the soil pipe ensuring that the top of the cup is level with the ground surface and there are no gaps between the rim and the soil.
3. Place a rain cover over the top of the trap. The rain covers are made from an upturned flower pot saucer held approx 5 cm above the ground surface with wire legs (coat-hanger grade).

[See diagram below for trap construction.]

4. After 2 weeks, check each trap to make sure that there is no damage or flooding and whether the antifreeze needs topping up.
5. After 4 weeks, collect the traps in, putting a labelled lid on each cup for transit back to the lab.
6. On return to the lab, drain the antifreeze off by pouring the contents of the trap through a sieve, then transfer the insects trapped into one (or several) large tubes containing 70% alcohol. Make sure that the proportion of insect to alcohol is no more than 50:50 in each tube. Use more than one tube per sample if necessary.
7. Store in a cold store at 5°C until the samples can be processed.
8. Identify adult Carabids to species where possible. Spiders, Staphylinids and Collembola should be identified to family. All other groups should be counted to order level.
9. Record the number of individuals within each of these taxonomic classes.



Pitfall trap construction.

5. Invertebrates – Vortis Sampling

Introduction

Insects and other arthropods are often the primary consumers of the aerial parts of crops and other plants within the crop canopy and in the field margins, and so will reflect the ecological impact that agronomic practices may have on the wider environment. A pilot study conducted at Rothamsted Research, where various sampling protocols were tested for their sensitivity to detect relatively rapid ecological changes, suggested that the most useful ‘indicator’ species for detecting direct effects of management change in arable fields are plant bugs (Heteroptera), springtails (Collembola), carabid beetles (Carabidae), and the larvae of butterflies and moths (Lepidoptera) and sawflies (Haughton pers com).

Plant bugs (Hemiptera: Heteroptera), comprise a widespread group of insects encompassing a wide variety of ecological strategies and life-histories (Southwood and Leston, 1959). Species range from monophagous herbivores to generalist predators. Duelli and Obrist (1998) have shown that plant bugs are one of the best single correlates for total biodiversity in agricultural areas. The group is also important as a preferred chick food for farmland birds, notably the grey partridge (Panek, 1997). The characteristically high species richness: abundance ratio of plant bug communities means that ecologically meaningful data can be obtained very efficiently from samples.

In farmland, high species richness is associated with non-crop habitats, such as field margins (Maudsley *et al.*, 1996), although some species inhabit arable fields for at least part of their life cycle. Whilst many adults are capable of sustained flight, immature plant bugs have restricted mobility and are therefore sensitive to within-field changes in habitat quality during the crop growing season. Plant bugs are therefore responsive to changes in botanical diversity, in both field margins and within crops, as a result of herbicide applications. Furthermore, herbivorous species may be measurably affected by subtle changes in the vegetation community, and predatory species by differences in invertebrate pest populations resulting from differences in crop management.

Epigeal **Collembola** are associated with the consumption of dead or dying vegetation, and nutrient recycling (Richards, 1974), and are thus most likely to reflect any differences in the flora that result from the different methods of weed control employed in GM herbicide-tolerant crops. Results from recent European field studies suggested that the more common species of epigeal Collembola associated with arable cropping could be useful indicators of the detrimental effects of pesticides (Frampton, 1994), and so could probably also be used to investigate the side effects of herbicide use. Recommended sampling methods include the combined use of suction sampler and pitfall traps (Berbiers *et al.*, 1989; Frampton, 1989) and both methods will be employed in this study. Suction sampling can measure the comparable effects of changes in micro-habitat on the species composition of diurnal species over the time scale of the study, whilst pitfall traps have the capacity to monitor both diurnal and nocturnal species (Frampton, 1994). One sample per month was considered suitable periodicity for long-term studies (Frampton & Cigi, 1993).

Lepidoptera (moths and butterflies) and **sawflies** (Hymenoptera :Symphyta) are species rich insect groups and valued components of the biodiversity of agricultural systems. Only a few species are agricultural pests, most feeding on non crop plants. Both groups are plant feeders in the larval stage and have very similar caterpillars that are known to be important large prey items for birds and mammals. For example, in extensive long-term studies by the Game Conservancy, sawfly populations have been shown to be an important component of the diet of the Grey Partridge and the decline of this species has been related to declines in such insects in response to increased pesticide use and changes in cultivation practices over the last 50 years (Potts, 1977). Long-term data from the Rothamsted Insect Survey also suggests that there has been a large decline in farmland moth populations over the same period, probably for similar reasons (Woiwod, 1992).

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Field Sampling Protocol – vortis sampling

i) Timing

Vortis sampling should be timed to coincide as closely as possible with weed counts if these are being conducted as part of the survey work, but should only be carried out when the weather is dry. If the vegetation is wet, the suction efficiency falls dramatically and the data will not give a reasonable representation of the invertebrate community present. Sampling periods should be roughly the middle to the end of:

- Sample 1. April
- Sample 2. June
- Sample 3. August

ii) Sampling Intensity

Three or four samples should be taken from each field area to be surveyed: ideally, the sampling should be stratified across margins, headlands and field centres to cover the range of variability in insect numbers across the field. This should generate 9-12 separate samples from each field to be surveyed.

iii) Sample Locations

Non-crop field margin vegetation, between field boundary and crop, on each field edge should be sampled at or within 1 metre (if the verge is narrower) from the crop edge, where such vegetation exists. Each verge should be sampled at roughly the middle of the field edge (i.e. avoiding corners and gates), preferably at the sample locations as the field margin vegetation survey points.

Parallel samples should be taken at the same locations as the margin samples but 2m into the crop headland from the field margin.

A further set of parallel samples should be taken at least 30m into the crop where access is easy, or at the junction of the headland with the main cropping area of the field where access is difficult e.g. with oilseed rape when fully grown. The latter will vary from 12-18m depending on the width of the headland in each field.

iv) Sample collection

1. Record weather conditions, date and field surveyor
2. Locate the first sample point and secure a small, labelled plastic bag to the output tube of the vortis machine with an elastic band
3. Lower the vortis onto the weed vegetation and 'suck' for 10 seconds, holding the machine approximately 2cm above the ground surface.
4. Move 1m along the margin (or parallel to the margin if sampling within the crop) and repeat
5. Repeat 3 more times to generate a bulked sample of 5 sub-samples of 10 secs each
6. Knock any insects that may still be in the tube down into the bag
7. Carefully remove the bag, tie securely and place in a cool bag for transport back to the lab

v) Lab Processing

1. On return to the lab, put all samples in a -20 freezer overnight to kill the insects sampled.
2. Sort through each sample, picking out all insects from the debris and storing them in labelled tubes of 70% ethanol. Ensure that there is no more than a ratio of 50:50 insects to ethanol otherwise they will rot.
3. Return tubes to a cold store at 5° C until ready for processing.
4. After the end of the field season, identify the Carabidae to species and count the number of insects in all other orders/families as follows:
 - Collembola,
 - Heteroptera (species, if possible, otherwise separate into Auchenorrhyncha, Aphids, other Hets),
 - Diptera (separated into Syrphidae, other Diptera),
 - Araneae,
 - Coleoptera (separated into Staphylinidae, Curculionidae, Chrysomelidae, Cantharidae, Elateridae, other Coleoptera),
 - other insects

6. Invertebrates – Pollinators

Introduction

Declining insect pollinator numbers in the UK and worldwide (Goulson et al, 2008) have the potential to seriously affect the productivity of insect-pollinated crops and have implications for the conservation and functioning of non-cultivated habitats (Kevan & Phillips, 2001). For example, the annual global economic value of insect pollination is estimated to be \$153 billion (Gallai et al. 2009) and loss of pollinator services could reduce worldwide crop production up to 8%, necessitating greater agricultural intensification (Aizen et al, 2009). Pollinator decline is also a natural heritage concern. Many species of the main insect pollinator groups are listed as priority species in the UK. For example, the majority of bumblebees, which are important pollinators of commercial crops (e.g. soft fruit, oilseed rape) and wild plants, have shown recent population declines. The UK hosts 27 species of bumblebees, of which three species are now extinct. Seven species of bumblebees are listed as UK priority species and two of them, the great yellow bumblebee (*Bombus distinguendus*) and the shrill carder bee (*Bombus sylvarum*), are particularly under threat. Scotland remains, nonetheless, a stronghold for many bumblebee species (Goulson, 2007). Decreasing pollinator numbers could have consequences not only for the productivity of agricultural crops but also for population sizes of wild plant species. In Britain, parallel declines in pollinators and insect-pollinated plants have been demonstrated (Biesmeijer et al, 2006) and a reduction in pollination and seed set of fragmented native plant populations has been reported (Rathcke & Jules, 1993). Arable vascular plants are particularly vulnerable: between the mid 20th Century and the period from 1987-99, the distribution of a quarter of the vascular plants in Scotland decreased, predominantly arable plants and species associated with grassland and upland habitats (Preston et al, 2006). Rare plants in arable or natural habitats may share pollinators with more common plant species and therefore may depend on the management of the common plants in their community for provision of insect pollinators (Gibson et al, 2006).

Pollinators are often resource-limited and major declines are driven by both land-use and climate change (Williams et al, 2009). A major cause of pollinator decline is likely to be the reduction in the availability and quality of plant food resources (Biesmeijer et al, 2006), through the combined effects of habitat loss, habitat fragmentation and intensive agricultural practices (Kremen et al, 2002) on vegetation diversity and abundance. Alternative low intensity management systems that increase within-field and field margin resource availability and quality for pollinators, including extended seasonal resource abundance, are therefore likely to promote pollinator diversity through niche differentiation. However, there is little quantitative data on the impact of alternative management strategies in the arable environment to inform policy and to advise landowners and conservation groups on improving insect pollinator diversity, abundance and pollination services.

This protocol describes standard transect walks to measure diversity and abundance a) on the field edges (at the edge of the cropped area, incorporating margin, verge and boundary) and b) on the flowering crop itself and on any flowering weeds in that crop. The transect is the least disturbing and statistically the most appropriate method for estimating bee forager diversity and abundance in a variety of habitats (Banazak, 1980; Teras, 1983). Such transects have already been used to

examine bees in oilseed rape crops and have proved practical and appropriate for examining differences between crops (Osborne, unpublished data). National recording schemes in Britain and elsewhere in Europe use the transect method for monitoring butterflies (Pollard and Yates, 1993).

Bees are “central place foragers” feeding at large distances (hundreds of metres) from their colony before returning. Monitoring bee species and abundance in a crop or habitat using transects, does not necessarily give information on population dynamics: we have no record of how many colonies are in an area and how productive they are in terms of queens and males – which will determine populations in the following year. Rather, since bees have strong preferences for different plants, the transect counts illustrate how attractive and useful a field is in terms of resources for bees nesting in the vicinity. This emphasises the importance of only scoring foraging or nest-searching bees: not bees in flight.

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Field Sampling Protocol – bee and butterfly sampling

i) Timing

Timing of transects will vary according to crop and conditions, but should be carried out once during May, June, July and August.

The May and July bee/butterfly transects should be walked on the same day as margin vegetation surveys, if these are also being carried out as part of the survey work. This is essential if the abundance and diversity of flower resources are to be related to pollinator abundance.

Walks should be carried out between 10.00h and 17.30h ideally when weather conforms to BMS standards (temperature above 13°C with at least 60% clear sky and above 17°C in any sky conditions, apart from heavy rain). If conditions are dry but marginal with respect to temperature, transects should be performed anyway.

iii) Sample Locations

Four 100m transect should be walked along the field margins (one on each margin, in an ideal field with 4 sides), avoiding corners and gates if possible.

A further four 100m transects should be walked along 4 tramlines into the crop. Select 4 tramlines roughly evenly space along the field. If the field is not 100m wide, split the crop transects into 50 m sections in two adjacent tramlines. Make sure the overall length walked is 400m.

It is suggested that the transect is walked in one direction to count bees (in a 2m wide strip to one side of observer) and in the opposite direction to count butterflies (in a 5m wide strip spread in front of the observer).

iv) Sample collection

1. Record weather conditions (maximum and minimum shade temperatures, percentage cloud cover and wind speed (using Beaufort scale, given below), date and field surveyor)
2. Note the flowering stage of the crop if it is flowering
3. Walk the transect at an even pace, covering approximately 15-20m per minute.
4. Bee recording – use tally marks to score all the bees seen within 2m width to one side, taking care not to shadow the transect.
 - a. Score the number of honey bees and each “colour type” of bumble bee and cuckoo bee (listed below) for each 100m section of the transect,
 - b. list the plant species on which they are foraging,
 - c. score all solitary bees together as present.
 - d. Bees should only be scored if they are actively foraging (or nest-searching queens) and not if they just fly straight past the observer.
5. Butterfly recording - each individual butterfly which comes within 5m in front of the recorder should be noted and “scored” with a tally mark as they are seen.

Bumble bee colour groups

There are 5 key banding patterns for workers:

black body, red tail	<i>B.lapidarius</i>
brown/ginger all over	<i>B.pascuorum</i>
yellow bands & red tail	<i>B.pratorum</i>
2 yellow bands & white tail	<i>B.terrestris</i> or <i>B.lucorum</i>
3 yellow bands & white tail (long body & head)	<i>B.hortorum</i>

The queens are MUCH bigger and follow the same banding patterns, except:

2 yellow bands & white tail = *B.lucorum*

2 yellow bands & buff tail = *B.terrestris*

BEAUFORT WIND SCALE

Beaufort No.	Description	Wind speed (knots)	Land signs	Sea signs
0	calm	<1	smoke rises vertically, leaves still	mirror smooth
1	light air	1-3	smoke drifts	scaly ripples
2	light breeze	4-6	leaves rustle, flags not extended	small wavelets, crests don't break
3	gentle breeze	7-10	light flags extended	large wavelets, crests may break
4	moderate breeze	11-16	all flags extended	small waves, some white horses
5	fresh breeze	17-21	trees in motion	moderate waves, many white horses
6	strong breeze	22-27	tree branches in motion	large waves with foam crests
7	near gale	28-33	walking into wind difficult	white foam from breaking waves blown in streaks
8	gale	34-40	twigs break from trees	high long waves, spin drift
9	strong gale	41-47	roof & fence damage	high waves, dense streaks of foam

Acknowledgements

This booklet was put together using information derived from protocols developed for the DEFRA-funded Farm-Scale Evaluations of GMHT crops (Firbank *et al* 2003, Perry *et al* 2003, and all papers in the Philosophical Transactions of the Royal Society of London, 2003, special issue volume 358) and subsequently adapted for a range of BBSRC and RERAD funded studies carried out at SCRI (2003-2010) and the JHI (from 2011).