PURE

TASK 3.3A – MAIZE "ON-STATION"

Research Protocol

OBJECTIVE
The final goal is to evaluate the main advanced and innovative IPM strategies in order to supply farmers with an effective and complete package suitable for protecting effectively maize with a very low environmental impact and reasonable costs.

BACKGROUND
The research protocol will be based on the following principles:

- focus on **grain maize** only (although deliverables will be useful for silage maize as well)
- a **multi-pest** approach will be followed;
- the protocol should be **as common as** possible between CNR, UDCAS, ARVALIS and DLO but IPM systems should be **adapted to local conditions**;
- at each experimental site the **pressure of major harmful organism** will be estimated by using the most suitable monitoring systems chosen on the base of a cost – benefit analysis;
- **rotation** is the key element of the systems tested;
- key element of the IPM strategies will be the identification and use of hybrids or varieties tolerant/resistant to one or possibly more pests and diseases;
- precision farming approach should be followed in the IPM systems tested;
- monitoring and control strategies should have low costs, proportional to grain maize incomes.

Areawide level monitoring (performed by using the arable crop bulletin that will provide information on pest pressures) should be a key element to achieve this. The bulletin is already produced in Italy and we will supply to other partners for adaptation according to their region.
- Stakeholders groups will have to be formed as soon as possible and be part of the identification-testing-evaluation circle for IPM tools and strategies.

The protocol will compare three levels (treatments) of crop protection: **conventional, advanced IPM and innovative IPM**
a) Conventional system will use the most common rotation in the region and the standard agricultural practices (i.e. commonly applied good agriculture practices).
b) Advanced IPM system will use more diversified rotation(s) always depending on the region Cultural practices like sowing date and false seed bed preparation, crop cultivars choice as well as use of monitoring and forecast systems, herbicide band applications coupled with mechanical weeding will be considered.
c) Innovative IPM system will implement the most recent innovations made available and make use of all tools that reduce the dependence on pesticides. It will deal with more complex rotations, (e.g. cover crops between two main crops), innovations in mechanical weeding, DSS, and innovative biological tools. Generally, non-chemical methods will be preferred (e.g. biofumigant defatted seed meals, biofumigant cover crops, Bt treatments), although they may be more expensive and/or less effective.

SITES

Italy, CNR
Hungary, UNIVERSITY OF DEBRECEN
France, ARVALIS
The Netherlands – DLO, (Reduced experiment on weeds comparing chemical based control with 2 levels of IPM based management) –

EXPERIMENTAL LAYOUT

Experimental design: Randomised block design
Replications : at least 3
Plot size: at least 300

PHASES

A) DEFINITION OF THE ROTATION: based on background described above the maize fields chosen for »on station« trials will be included in typical rotation (anyway no maize after maize); Cultivation: the best agronomic practises useful to »make healthy« the crop will be implemented: good preparation of »sowing bed«, in furrow fertilizer to favour early vigour, irrigation if applicable, ...

All the basic agronomic information (previous crops, organic matter content, other main soil characteristics, rainfall and its distribution, ...) will be recorded.

B) <u>IDENTIFICATION OF THE MOST TOLERANT/RESISTANT HYBRIDS</u> <u>(resistance to viruses, to main diseases,…)</u>
To find and prepare a list of the most well fitted high yield hybrids in the different areas with preference to new materials and choose the most resistant/tolerant ones to:

- viruses,
- diseases
- tolerance to ECB

mainly for using them in the advanced and innovative IPM systems that will be tested.

C) <u>PEST – DISEASE PRESSURE EVALUATION</u>

1) *SOIL INSECTS – Agriotes* spp. *or other local important pests* : bait traps and YATLORf) Annex
2) WCR- *Diabrotica virgifera virgifera* (YATLORf, Pherocon AM) for conventional Annex 2
3) Blackcurworm *Agrostis ipsilon, Agrostis segetum* traps (Delta- sticky, HARTSTACK) Annex 3
4) ECB- *Ostrinia nubilalis* (Sesamia) – light traps Annex 4
5) *Helicoverpa armigera* pheromone traps Annex 5

IPM practices in Advanced and Innovative scenarios will be based on Alert programmes using development models that indicate their implementation. These will be also used for practical reasons informing stakeholders so that a continuous farm updating will be possible. In order to make cost feasible Alert programmes will work at:

1) **AREAWIDE LEVEL** Areawide IPM strategies that gives general and specific information at county, area scale from monitoring networks and development models implementation optimized using statistical tools like geostatistics; this information can be used and disseminated by technicians

but at the same time can reach and be useful to the single farmers through internet and other media; this will identify areas where pests populations keep below threshold at very low cost and areas where field further assays are needed;

2) **AT FIELD LEVEL** On farm IPM strategies addressed to a single farm and parts of a single farm by using based on specific sampling and specific farmers' information; these can use tools used in ABOVE GROUND

PEST		**CONVENTIONAL**	**ADVANCED**	**INNOVATIVE**
SOIL INSECTS (Agriotes)		In furrow soil insecticide or seed treatment	Generally no treatments – decision based on risk assessment with/or not monitoring results	Generally no treatments – decision based on monitoring results
Diabrotica (WCR)		In furrow soil insecticide or seed treatment	Rotation no treatments	Rotation no treatments
Blackcutworm		Post emergence insecticide treatment at pest presence	Generally no treatments – decision based on development models	Generally no treatments – decision based on monitoring results
Aphids and other Heteroptera including vectors of viruses		Post emergence insecticide treatment at pest presence	Generally no treatments – decision based on monitoring results	Generally no treatments – decision based on monitoring results
ECB (Sesamia)		Broad spectrum Insecticide treatment just after maize flowering	Decision based on development models– if needed low impact insecticides (selective)	Decision based on development models – if needed biological treatments or really selective low impact insecticides
Helicoverpa armigera and other leaf pests		Insecticide treatment at presence	Decision based on development models– if needed low impact insecticides	Decision based on development models – if needed biological treatments

D) PLANT CROP INSPECTIONS AND MEASURES

Each plot should be scouted by choosing at random 2 areas of 20 m X 2 maize rows per field and observing all the plants. Plants with typical wireworm or black cutworm damage, will be individuated and all the larvae found near the collar will be collected and identified. Please indicate sampling areas used from the beginning till the end of the trial.

In each sample area the following observations will be done at emergence and 5-7 leaves :

- crop stand (number of normal plants/20 m);
- number of seeds damaged;
- number of emerged plants damaged by wireworms, cutworm or other soil pests per 20 m.

E) ECB AND OTHER PHYTOPHAGOUS INSECTS ASSESSMENTS

1) light trap captures (every day assessment or at least two times per week);

2) Assessments on ECB attacks and life cycle

2.1) delimitation of plot borders;
2.2) Evaluation of the plot's uniformity; if there are zones clearly different because of accidental factors (e.g. maize lodging due to adverse climatic conditions exclude them from the sampling;
2.3) ON THE AREAS OF EACH PLOT, FORM SAMPLING AREAS: exclude the 2 outer maize rows from each side and 1.5 m above and below to avoid any edge effects; in the centre form 2 sub-plots of 20 m x 2 maize rows each.
2.4) observations

In each sub-plot you count:

2.4 1) damage from 1st generation*: n° plants with symptoms of 1st generation ECB attack* (*e.g. holes on leaves) – at the second half of June;*

2.4 2) Development: *every week starting from the second half of June, on 40 plants with ECB damage, taken from the border rows of the plots, assess the number of larvae and pupae, by cutting along the maize plant in half; when more than 30% of the insects are pupae eggs are expected in 7 days; from next week start with egg clusters assessment;*

2.4 3) 2nd generation egg clusters: from 30% pupae on inspection of leaves just above and below the ear of at least 300 plants/field at random in order to find and count egg clusters laid over time; count parasitized and non parasitized egg clusters;

2.4 4) 2nd generation larvae presence: inspection of silk to assess young larvae and/or sign of presence (chewed tissues, feces, ..on 40 plants per subplot;

2.4 5) other herbivores (aphids, red mites): 20-30 days after treatments: *inspection od sub-plots observing at least 5° plants at random; plants to be divided into: aphids: : 0 = no presence; 1= 1-2 small colonies; 2=several and/or large colonies; red mites= as above.*

2.4 6) beneficials: after 30-40 days from the treatment collection of 100 leaves in the middle of the plot and observation of main predators/parasites (lacewing eggs, pupae of syrphidae, pupe of ladybirds, Orius, pupae and adults of Stethorus and Oligota, ….

2.4 7) Evaluation of ECB at harvest

At the same sampling areas as indicated above (2.3), measure:

a) Total number of plants (final stand)
b) Plants without ECB damage;
c) Plants without ears/cobs;
d) Plants with symptoms of ECB attack (e.g. holes on leaves, on cobs);
e) Plants broken above ear;
f) Plants broken below ear;

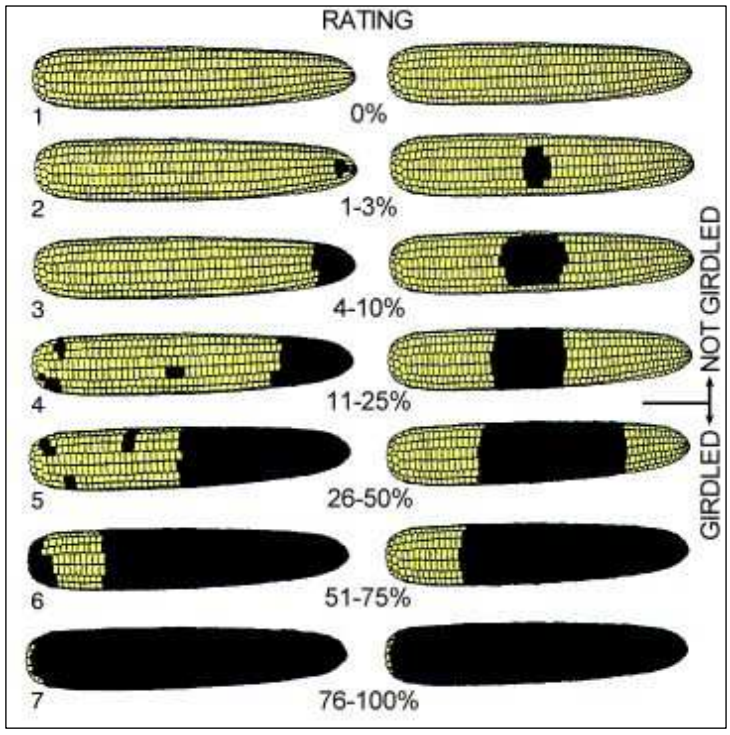
On 10 plants from each subplot measure :

g) plants with ECB damage on the cob: each cob of the 10 plants will be classified according to the percentage of surface damaged by ECB using a scale from 1 to 7, which corresponds to: 1 = non attacked, 2 = < 4%; 3 = 5-10 %, 4 = 11-25 %, 5 = 25-50%, 6 = 50-75%, 7 > 75%.

h) plants with *Fusarium* presence each cob of the 10 plants will be classified according to the percentage of surface covered by *Fusarium* using a scale from 1 to 7, which corresponds to: 1 = non covered; 2 = 1-3 %, 3 = 4-10%; 4 = 11-25 %, 5 = 25-50%, 6 = 50-75%, 7 > 75%.

i) larvae/plant;

j) number of tunnels per plant (recording the length; > 10 and < 10 cm);



F) WEED ASSESSMENT

Main objective: Evaluate and compare the effect of conventional, advanced and innovative weed management practices on weed densities and biomass

Materials and Methods

1. Sampling area/plot will be the area inside the plot excluding the 2 outer maize rows from each side and 1.5m above and below to avoid any edge effects (see example below).

2. Fixed rectangles will be used for the weed assessments placed in a way that allows weed density counts along and between rows (ideally the space between two maize rows, 0.75cm)

3. Locate rectangles in the centre of each plot along the crop rows of the sampling area, when the first measurement will take place after maize emergence

4. **VERY IMPORTANT**: Randomize rectangles throughout the sampling area according to the weed distribution in the field (e.g. check the plot for weed patches before placing rectangles) in order to get the best estimation of the weed density in each plot

5. Fixed rectangles should cover at least 1% of the sampling area/plot

6. When herbicide applications or mechanical weeding is performed, remove rectangles for convenience but keep indication where the rectangle was fixed e.g. by leaving a colored ribbon along the row on the spot (maize plant) that the left bottom corner of the rectangle was.

Weed density & biomass assessments

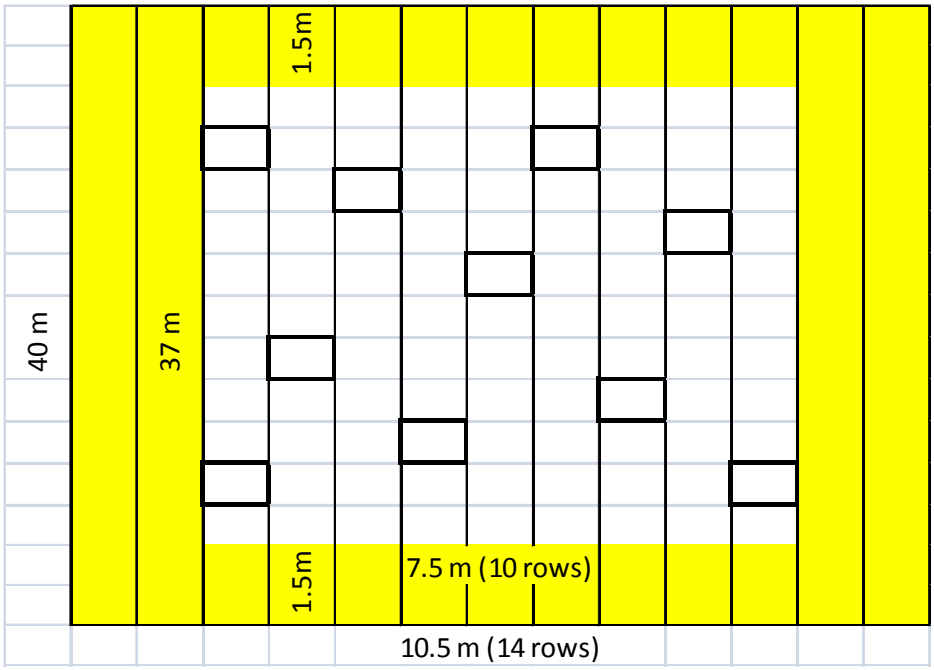
Weed density assessment: Weed seedlings/species are counted from fixed rectangles and not removed to check post-emergence weed control effect and final weed density.

- 1st measurement after maize emergence-2 leaves,
- 2nd at two-three weeks after post-emergence weed control (herbicide or mechanical weeding),
- 3rd before maize harvesting to identify final weed density.
- **Data collected**: Weed density per species as affected by different treatments as well as total final weed density/m^2/ plot.

Weed biomass assessment: From each plot, total weeds will be cut from the fixed rectangles and placed in a numbered bag corresponding to each plot. Fresh weight from each bag/plot will be measured by the use of a precision balance.

- 1 measurement before maize harvesting after the final density assessment.
- **Data collected:** Total fresh weed biomass /m^2/ plot.

. **Example of a plot (0.75cm between row spacing, vertical lines indicate the 14 rows)**



- 10 fixed rectangles, each of 40 cm x 75 cm dimensions, located in the centre of each plot, along the 10 middle crop rows and in a random position, when the first measurement takes place after maize emergence

- Sampling area of the plot excluding the borders around: 7.5m (10 rows in the centre) x 37m=277.5 m^2
- Of which sampled/plot: 10 (rectangles) x 0.30m^2 (40cmx75cm) =3 m^2 (1.08% of sampling area)

Maize biomass Assessment

1. Aboveground dry biomass:
    a. Plants will be cut at 15 cm from the ground, from the central 3 middle rows by 5 m long/plot = 11.25 m^2.
    b. The total fresh weight of the whole plants (including cobs) will be measured with the use of a balance.
    c. From these plants, 3 plants will be sub-sampled / plot, measured for their fresh weight, chopped in large pieces (also cobs) and put in the oven to obtain their dry weight and consequently the moisture content. Then this %MC value will be used to obtain the total dry matter/plot. Please transform final dry matter values in t/ha

G) YIELD AND QUALITY ASSESSMENT

Only on the sampling area of each plot (exclude the 2 outer maize rows from each side and 1.5 m above and below to avoid any edge effects), proceed with harvesting using a small combine or a specific harvester:

1) proceed with measuring width and length of the sample area also counting number of rows;

2) then harvest and measure weight and collect a random grain sample of 500 g for the moisture content (ISO 711:1997) (calculation of grain yield shall be expressed in t per hectare of grain with 14 % moisture content) and one of 2-3 kg for the mycotoxin analysis as follows:

- with a specific container you take in successive moments small grain samples that come from the cochlea of the harvester (at least 10 samples of 200 gr or better 20 samples of 100 gr and put them together in a plastic bag;
- close the bag in an air tight way so you avoid air as much as possible inside the bag;
- place a tag inside and one outside the bag;
- in maximum 6 hours place the samples in a freezer (-18°C).

ANNEX 1

Soil insect pressure - Wireworm monitoring

LARVAE
This will be done in September - October and/or March - April before the swarming period, when soil temperatures above 10°C .

1. *Bait traps*: 6 to 12 bait traps will be placed in each plot according to plot size, **provided the soil is bare** (traps will only work properly if there is no/low presence of CO2-producing roots). Each trap will be made and used according to the description given by Chabert and Blot (1992) — a modified version of traps described by Kirfman *et al*. (1986). These comprise a plastic pot 10 cm in diameter provided with holes in the bottom; the pots are filled with vermiculite, 30 ml of wheat seeds and 30 ml of corn (maize) seeds. The pots will be wetted before being placed into the soil just below the surface and covered with an 18 cm diameter plastic lid placed a few cm above the rim of the pot.

2. Traps will be checked by hand-sorting the contents after 10 - 15 day. Count and record the number of larvae found. The manually observed material will be put on Tullgren funnels and processed as described for soil cores. Place all larvae in airtight vials with a little of humid soil, and send to: Dr Lorenzo Furlan, via Q. Sella 12, 30027, San Donà di Piave VE, ITALY, for identification.

References

Chabert, A., Blot, Y. 1992: Estimation des populations larvaires de taupins par un piège attractif. *Phytoma* **436**, 26- 30

Kirfman, G.W., Keaster, A.J. & Story, R.N. 1986. An improved wireworm (Coleoptera: Elateridae) sampling technique for midwest cornfields. *Journal of the Kansas Entomological Society*, **59**, 37-41.

Monitoring of adults
Use the YATLORf traps with deep bottom if it is going to be used also for the monitoring of Diabrotica adults; baited with the sex pheromones of the various species, products can be supplied by the Plant Protection Institute of Budapest, and place inside a dispenser Kartel 730. The YF trap has to place just above the ground, with the lower rim of the brown trap body, 2-3 cm below the soil level (the deep bottom completely inside the soil).

The timing for management of the traps is as follows:

1 On **20th March** the trap will be placed, for convenience use an indicator for the place where the trap is, in the centre of the monitoring area with the sex pheromone bait for ***A. brevis*** in a **low** position with **the top facing below**; (or *A. sputator* in other region, see table 1)
2 On **10th April** the captured insects will be taken off[b] and the dispenser with the pheromone for ***A. sordidus/rufipalpis (Hungary)*** will be added in a **medium** position and with **the top facing below**.
3 On **10th May** ca. the captured insects will be at the edge of a field [b] and the pheromone bait[a] for ***A. sordidus*** (at ca. 30 days) will be substituted with a new one in a **medium** position and with **the top facing below, but also the bait for *A. litigiosus* will be added in a high position only in Italy**.
4 On **10th June** ca. the captured insects will be taken off[b] and the bait[a] for ***A. brevis*** will be substituted with the one for ***A. litigiosus*** (only Italy) in a **low** position and with **the top facing below;** substitute the bait for ***A. litigiosus*** in a high position with the bait for *A.*

ustulatus; **in a high position the pheromone for Diabrotica can also be added; in this case add an insecticide strip at the bottom of the trap.**

5 On **10th July** ca. the captured insects will be taken off[b] and the bait[a] for ***A. ustulatus*** will be substituted and placed at the same position. Substitute also the pheromone for Diabrotica.

6 On **10th August** the captured insects will be taken off[b] and the trap will be substituted for following year.

Example procedure; see table 1 for lure combination in each site.

In France, Germany, Slovenia, Hungary and The Netherlands a trap B baited with *A. obscurus* (in low position) and *A. lineatus* (in **medium** position) will be added in early April; the traps will be inspected with lure substitution every month until July (see Table 1).

[a] = capsule Kartel 730 for *A. brevis*, *A. sordidus*, *A. litigiosus*, *A. ustulatus; A. lineatus*, *A. obscurus*
[b] = insect collection from traps and counting
1- the trap is removed from the soil
2- Before opening, the trap is placed in a large trasparent bag, then the trap is opened and the insects fall inside the bag.
3- the bag should be closed immediately.
4- the trap is placed back into the soil.

Warning: never open lure cap.

Table 1. Lures for YATLORf traps in the different on station sites.

LURE COMBINATIONS	REGION
A. brevis, A. sordidus, (A. litigiosus), A. ustulatus	**Italy (North eastern)**
A. brevis, A. sordidus, , A. litigiosus	**Italy (other regions)**
A. brevis, A. sordidus, trap A ***A .lineatus, A. obscurus trap B***	**France**
A. sputator, A.rufipalpis (same lure of sordidus), A. ustulatus - trap A ***A. lineatus, A. obscurus trap B***	**Hungary**
A. sputator, A. ustulatus - trap A ***A. lineatus, A. obscurus trap B***	**Slovenia and The Netherlands**
A. sputator, A. sordidus, A. ustulatus - trap A ***A. lineatus, A. obscurus Trap B***	**Germany**

ANNEX 2

Soil insect pressure - Diabrotica

Since rotation is one of the characteristics of the IPM strategies under evaluation, *Diabrotica virgifera virgifera* (WCR) will not be a main target species. Just in case the conventional rotation under study is featured by a high presence of maize, WCR monitoring will be done. The monitoring procedures will vary with sites:

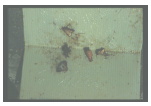
1. *WCR non established populations:* Yf traps, sex pheromone lure; procedure like ANNEX 1. One trap per “on station” site, one trap per “on farm” large-plot

2. *WCR established populations:* Yf traps, floral volatile lure; procedure like ANNEX 1. One trap per “on station” site, one trap per “on farm” large-plot. Possible addition of ®Pherocon AM traps.

ANNEX 3

BLACKCUTWORM

TRAPS TO USE (OPTIONS)

1) **HARTSTACK (photo**  **)**
2) **VARL (Csalomon) add strip of insecticide at the bottom (photo**  **)**
3) **Normal Trap test (photo)** 

LURES: sex pheromones Csalomon to be kept in the fridge (better freezer - 18°C) before use

INSTALLATION AND MANAGEMENT OF TRAPS

HEIGHT OF TRAPS: approximately at 1 m

PERIOD OF MONITORING: 25 February – beginning of June;

LURE REPLACEMENT: every 30 - 45 days;

INSPECTIONS: at least twice per week; at every control clean the dirt from traps if necessary; send the data, including the zeros if no adults found;

THE ADULTS WILL BE REMOVED AT EVERY CONTROL, PLACED IN A BAG WITH LOCATION AND DATE INDICATION for the following control.

ANNEX 4

ECB, *Ostrinia nubilalis*

TRAP TO USE

Light trap

4) (photo)

INSTALLATION AND MANAGEMENT OF TRAPS

- POSITION: at the edge of a field

- HEIGHT OF THE TRAPS: approximately at 1 m

- PERIOD OF MONITORING: early May – early September;

  - MANAGEMENT: the lamp has to be switched on at sunset and switched off at sunrise automatically or manually; add a piece of cotton soaked in ether in the bottom;

- INSPECTIONS: at least twice per week; at every inspection take out all the specimens and sort out all the *Ostrinia nubilalis* specimens; register the data including the zeros if no moths are found.

ANNEX 5

HELICOVERPA

TRAP TO USE

1) **VARL (Csalomon) add strip of insecticide at the bottom (photo**  **)**

LURES: sex pheromones Csalomon to be kept in the fridge (better freezer - 18°C) before use

INSTALLATION AND MANAGEMENT OF TRAPS

HEIGHT OF TRAPS: at the level of the top of the vegetation, it is advisable to set up traps on the branches of bushes or trees near fields

PERIOD OF MONITORING: mid-May;

LURE REPLACEMENT: every 30 - 45 days;

INSPECTIONS: at least twice per week; send the data, including the zeros if no moths found; occasionally some specimens of *Heliothis maritima* or *H. viriplaca* can be captured, however, these can easily be told apart from *H. armigera* based on the broad band across their forewings. Some specimens of the noctuid *Discestra dianthii* can also be caught, this is much smaller and dark brown in colour. Other non-target catches can include some microlepidoptera (Crambidae), these are much smaller and cannot be confounded with the cotton bollworm.

THE MOTHS WILL BE REMOVED AT EVERY CONTROL, PLACED IN A BAG WITH LOCATION AND DATE INDICATION for the following control.