Task 3.3b: On-farm experimentation 2011

Experimental protocol

Partners involved: KIS, CNR, JKI, UDCAS and Biotop

Objectives and perspectives:

On-farm experiments will test/validate single IPM tools, or one-year solutions to specific problems or "small IPM packages" (e.g. specific weed problems, specific control tools or small IPM packages to manage ECB and/or soil insects) in real field conditions (ideally plots should be at least **5.000 m^2**).

Tools and/or solutions to be tested will be chosen after discussion with the stakeholders groups (the first year will be a bit difficult) or they may arise from on-station experiments.

On-farm experiments will have to be managed with commercially available or technologically mature equipment which is suited for field scale applications.

Experimental area (the minimum no. of farms (i.e. replicates) where an IPM tool is tested in each region is 2**):**

On-farm experiments will be carried out in southern conditions by CNR (4 trials per year) and Biotop (2 trials per year), in central conditions by JKI (2 trials per year - with input of expertise from DLO) and in eastern conditions by KIS (2 trials per year) and UDCAS (4 trials per year), for a total of 14 experiment per year. Replications will be achieved by involving several farms rather than replicating within farms. IPM solutions designed and tested on-station may have to be adapted to the specific farming conditions and constraints.

Experimental facilities and equipment - within a common experimental approach, all materials and methods will be locally chosen according to their relevance for IPM implementation (if applicable as many as possible (at least two) different locations with similar experimental design). The trials will run for four years: start 2011 – finish 2014.

Tools that will be assessed (at least 2 IPM tools (or 3 if possible) will be assessed):

- tools to control weeds;
- *Trichogramma* against ECB on each location (where possible)

Experimental design – to assess the effectiveness of the proposed IPM solution three plots: A, B and C (each plot will be at least **5.000 m^2**) will be established on each location (2 IPM tools + conventional should be tested in the same farm) – the plots will be arranged on the same field (the same maize cultivar and the same technology carried out in previous years). The chosen field will be divided on the 3 plots: A, B and C.

On plot A (control plot) conventional technology based on existing knowledge and tools (technology conducted according to local practices for maize production – technology that is actually used by farmers).

On plot B IPM tools for weeds control will be validated **(Annex 3: weed protocol**):

- in SLO and HUN stale + harrowing before maize sowing + in maize growth stage 2-3 leaves harrowing and if needed broadcast spraying with reduced doses. In maize growth stage 6-7 leaves correctional broadcast spraying as needed

- in ITA, FRA and GER: goosefoot hoe + band spraying

On plot C, which should be situated at least 100 m from the plot A (control plot) *Trichogramma* release against ECB will be tested (**Annex 2**).

On plot C soil insects (wireworms) will be also followed (where possible) – maize will be sown on alternate strips (with 6 rows) treated with soil insecticide (or seed dressing) and untreated in order to assess the reliability of soil insect alert programme and the actual effect of soil insecticides. Soil insects will be followed as proposed in **Annex 1**.

Experimental conditions (weather, temperature, precipitations) as well as all other critical parameters (crop variety or hybrid; soil management: water, fertility; planting date; seeding rate/plant population; row spacing; chemical use) will be followed on each location.

Methodology to measure the effectiveness of the chosen IPM solution is based on the detection/occurence of the target pests as well as on visual inspection of the damage that is caused by the target pest. Yield of maize will also be measured (**Annex 4**).

Therefore the weed species composition will be determined as proposed in Annex 3.

Additionally the targeted pest species (ECB) will be monitored on each location/plot using light traps (as agreed). Visual inspection to investigate the damage caused by targeted pest will be also conducted (i.e. number of maize stalks infested by ECB, ECB damage to crop ears, yield of maize will also be observed). Materials for monitoring have to be standardised and used by all. If feasible the same materials will be used as in the on-station experiments.

Evaluation of ECB at harvest: total plants; plants without ears; % of attacked ears with a notation of damage by a class system (4-5 levels), and in the same time a notation of the *Fusarium* development also with a class system; number and the position of ECB larvae (in stalks, peduncles or ears); plants broken above ear; plants broken below ear.

Demonstation activities

Field days and demonstration activities will be organized in each participant country (IT, GER, SLO, HUN, NL) on at least one location.

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 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**

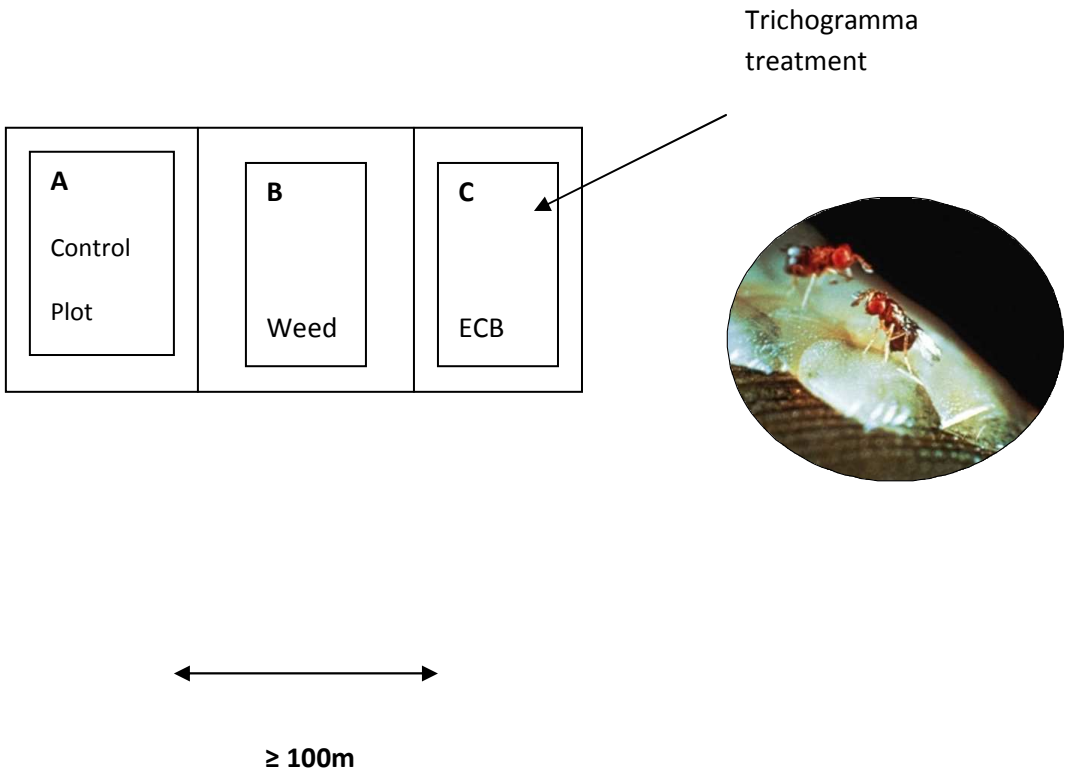
ANNEX 2: ECB assessment

PURE - Task 3.3b – On-farm experiment

Biological control against ECB using *Trichogramma brassicae*

Experimental design

In the same grain maize field, 3 plots A, B, C ≥ 5000 m²



Trichogramma treatment will be done against the second generation of ECB **(G2)** in Italy, Hungary, Slovenia and France as it is the most damageable for the crop, and against the single generation of ECB in Germany (that corresponds to the 1rst one (G1) in the southern countries).

Sub-task 1: ECB monitoring (see annex 4 of TASK3.3a protocol).

Adults' flight(s) - one in Germany and two in other countries G1+G2 - will be followed using a light trap. The trap has to be installed before the beginning of the ECB flight.

Sub-task 2: *Trichogramma* release assessment (see ECB Doc 1).

- In Germany: the date of release will be planed according to information given by the Plant Protection Services of the country (if not available, Biotop will try to give this information)
- In the others countries: the release date is based on the observation of the **ECB pupation**, which have to be done after the first flight is finished. The release date is planed one week after the threshold of 25-30% of pupation is reached. This information has to be sent to Biotop in order to reactivate *Trichogramma* and to prepare the conditioning. The product will be delivered to each participant by express carriage, in insulated boxes keeping the product in good conditions of temperatures.

Sub-task 3: *Trichogramma* treatment (see ECB Doc2)

For this first year experiment, we propose releasing trichogramma two times to be sure that the egg laying period will be properly covered (only one release in France where the forecasting system is effective for long time).

In Germany: according to the usual practice in the location of experiment (to be defined with Arnd)

Others southern countries:

Release 1: one week after 25-30 % of pupation. Dose: 375000 T/Ha conditioned in 50 dispensers.

Release 2: 2 weeks after the release 1. Dose: 375000 T/Ha conditioned in 50 dispensers.

Releases will be done according to the product specifications.

Sub-task 4: Pest pressure evaluation and parasitisation assessment (see ECB Doc 3)

In plots A and C:

- In southern countries only: % of attacked plant by the ECB G1 (visual damages). To be done just before the 2nd flight beginning, on 100 plants per plot.
- In all countries: scouting of egg masses under the maize leaves on 100 plants per plot. Each egg mass will be noted as: Fresh (F), Parasitized (P) or Hatched (H). Then percentages of infestation and of parasitisation are calculated. Scoutings are carried out at least 2 times during the egg laying period: 10 days and 20 days after the 1st release.

Sub-task 5: ECB damages assessment at harvest (see ECB Doc 3)

In plots A and C:

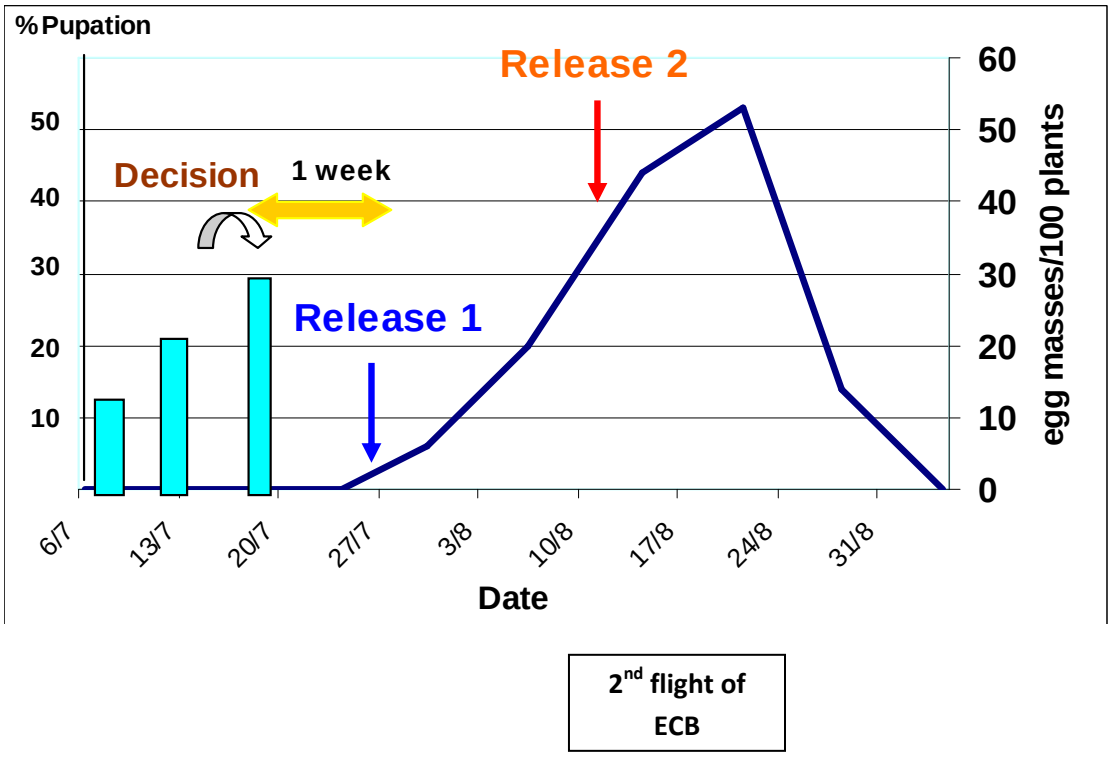
- On 100 plants per plot:
    - % of plants without ears, % of attacked plants (any visuals damages), % of broken plants above ear, % of broken plants below ear (including broken peduncles with fallen ears)
    - % of attacked ears with a notation of damage by a class system, and in the same time, notation of the *Fusarium* development also by a class system (4 levels).
- On 50 plants per plot, in the same sampling areas but only on the first 5 plants in each: dissection of the entire plants and counting of the number and position of ECB larvae (Stalk, peduncle, ear)

<u>Doc 1:</u> Trichogramma release assessment

In Germany: depending on the Plant Protection Services informations.

In southern countries: Italy, Hungary, Slovenia and France

Principle: the date of release is based on the % of ECB G1 pupation which allows forecasting the beginning of the 2nd flight.



The evaluation of % of pupation starts when the first flight is finished, by cutting attacked plants and counting larvae and pupae of ECB inside the stalks. For each counting, the total number of larvae and pupae should be at least **30 alive individuals** to be accurate enough, the number of plants to be cut is depending of the infestation (usually < 30 infested plants).

Calculation of the % of pupation:

L = number of alive larvae

P = number of alive pupae

% of pupation = P / (L+P) x 100 with L+P ≥ 30

A pupae in a maize stalk

This work is done in the experimental field, and if possible, in at least 2 others fields in the same area and with the same sowing date (3 fields in total to have an over view of the local situation). If possible, the best is choosing high infested fields to save counting time.

Usually several countings are needed, the frequency is once or twice a week depending of the temperatures (total counting 2 to 4), to reach the right percentage (about 25-30% of pupation) to take the decision of the first Trichogramma release (to be placed one week after).

Time evaluation: 1 counting for 30 individuals = 1.5 hour

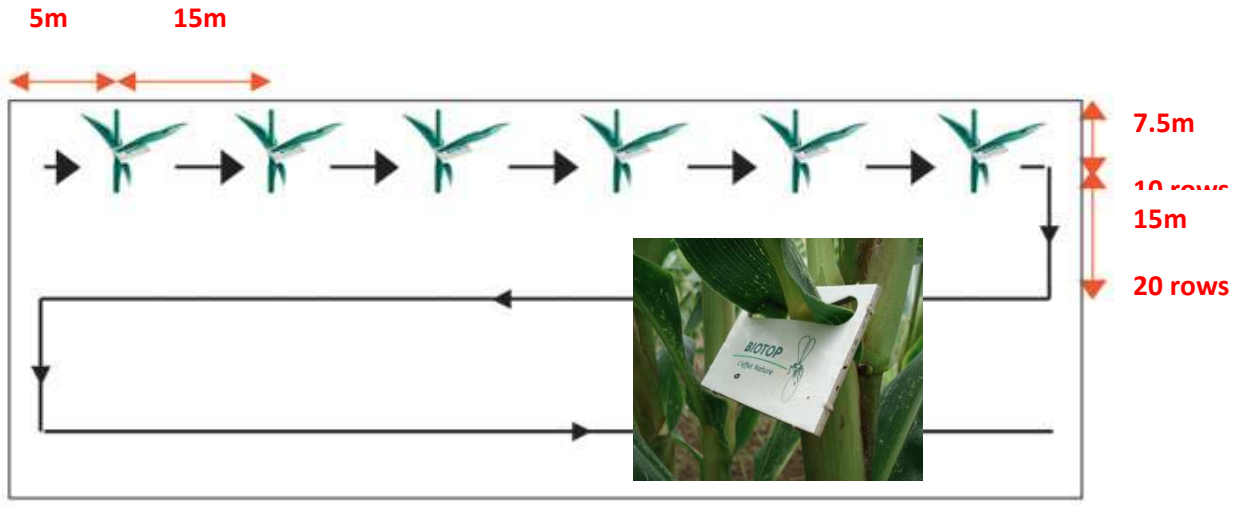
Doc 2: Trichogramma treatment

In Germany: depending on the available product.

Against the 2nd generation of ECB only in southern countries: Italy, Hungary, Slovenia and France

2 releases at 2 weeks interval, each one with 375000 T/Ha in 50 dispensers/ha (the product will be ready to use).

Scheme of release:



Time evaluation: 1 ha treated = 20 minutes

Warning!

The product is delivered by express carriage, and has to be used immediately or within 24 hours after delivery (storage between 15 and 20°C, in a ventilated room far away from pesticides or cigarette's smoke).

Do not expose the product to the sunlight and do not leave it in a closed car staying in the sun (very high temperature inside = high mortality of beneficials).

Use the product early in the morning or late in the evening to avoid high temperatures.

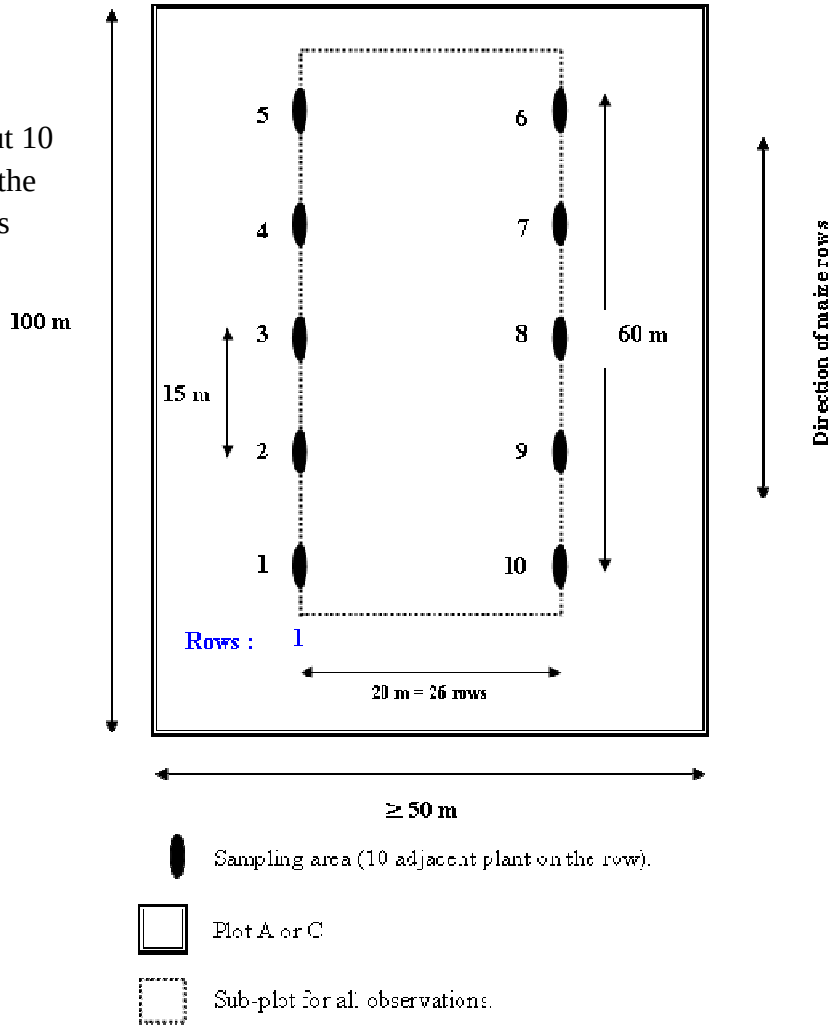
No risk with rain falls or irrigation.

<u>Doc 3:</u> pressure and damages assessment of ECB; Trichogramma parasitism evaluation

Only in plots A and C

<u>Sampling areas:</u>

In the centre of each plot, mark out 10 areas of 10 adjacent plants where the observations will be carried out, as shown on the schema:



<u>a/ Pest pressure</u>:

- 1st generation (G1) in southern countries only: before the beginning of 2nd flight: % of damaged plants on 100 plants (all visuals damages on leaves and on stalks).

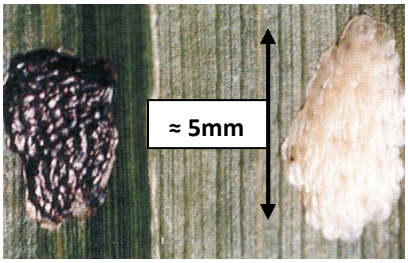
Time evaluation: 1 hour/plot

- In all countries: 10 days and 20 days (2 times at least) after the 1st Trichogramma release, counting of all egg masses found on 100 plants, and notation as Fresh/white (F), Parasitized/black (P) or Hatched (H) egg masses (see pictures below).

Results: **total egg masses/100 plants = (F+P+H)**

Time evaluation: 2 hours/control/plot

Fresh egg mass under a leave= F



Left: Parasitized (Totally black) = P

Right: Fresh

Black head stage = no parasitized = H

(Head of larvae visible)

Egg mass just hatching (on left) = H

(With young larvae)

Warning! Do not mistake parasitized egg mass (totally black eggs) and black head stage that means no parasitized egg mass (small black points are easily visible through the chorion).

b/ Trichogramma parasitism:

- Results: **% of apparent parasitism = P/(F+P+H) x 100**

NB: this calculation under-evaluate the actual % of parasitism as we do not know if fresh egg masses are parasitized or not (after being parasitized eggs need 4-5 days to turn black).

c/ ECB damages assessment at harvest

Counting on **100 plants** by plot:

- % of plants without ears
- % of attacked plants (any visuals damages)
- % of broken plants above ear, % of broken plants below ear (including broken peduncles with fallen ears)
- % of attacked ears with a notation of damage by a class system (4 levels)
- On each ear, notation of the *Fusarium* development also by a class system.

Class of attack for ECB or *Fusarium*:

- 0 = no attack
- 1 = low attack (1-5 attacked grains)
- 2 = moderate attack (6-15 attacked grains)
- 3 = high attack (more than 15 attacked grains)

Counting on **50 plants** by plot:

Only on the first 5 plants in each sampling areas: dissection of the entire plants and counting of the number and position of ECB larvae (Stalk, peduncle and ear).

Time evaluation: 2,5 hours/plot (Plus 1,5 hours for harvesting the collected ears)

ANNEX 3: Weed assessment

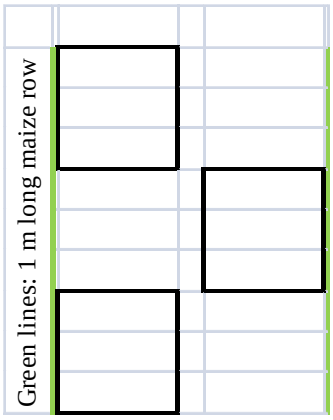
Creating subplots:
Shortly after sowing of maize we select and mark randomly 14 subplots (sampling areas) per plot A and per plot B (A = control/conventional plot; B = IPM tools for weeds control), each area of a size of 0,75 m²: width 0,75 m (rows distance from plant to plant) x length 1 m. These subplots should be selected according the weed distribution/condition in the field (a short scouting across the plot will give you the idea of distribution) in order to get the best estimation of the weed density in each plot

For each plot (A+ B) there will be:
14 subplots (sampling areas) x 0,75 m² = 10,5 m²/plot sampled (0,21% of 5000 m²).

The following parameters should be reported:
a) weed species (according to the EPPO-Code, see http://cipm.ncsu.edu/names/index.cfm)
b) total weed coverage (%) and coverage (%) of dominant species visually assessed
c) total weed biomass (dry matter), only estimated at the last evaluation
d) weed density/number per species (where feasible and for who wants to have more data - **OPTIONAL**)*

Weeds should be estimated at 3 times:
a) just before the first treatment (herbicide or other method)
b) 3 weeks after the last treatment (herbicide or other method)
c) cca. 3 months after the (last) treatment, when weed biomass is at maximum, e.g. more than 50 % of the weeds are flowering (BBCH 61-65) - before harvesting

Weed biomass assessment: For each plot (A + B), total weeds will be cut at the soil surface from the sampling areas and placed in a numbered bag corresponding to each plot. Total weed biomass/plot will be dried in an oven and weighed (kg/ha).
***OPTIONAL Weed density/number assessment**: Weed seedlings/species should be counted from all 14 subplots/sampling areas /plot (in case of very high weed densities, sample from 3 quadrates (each 0,1 m² = 0,33 x 0,33 cm) for each subplot and place them as shown below for the weed assessments).
Example of quadrats positioning inside a subplot (1m x 0,75cm)



ANNEX 4: Yield estimation

Yield shall be assessed at all experimental field/plots (control, weed and ECB) separately.

The yield shall be estimated using combine harvester. The weight of harvested grains shall be separately assessed for each experimental field/plot, whereas a random grain sample of 500 g for the moisture content determination and one of 2-3 kg for the mycotoxin analysis will be collected 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).

Calculation of grain yield shall be expressed in tonnes per hectare grain with 14 % moisture content.

**** Alternatively (not obligatory – see below):***

On each field/plot 4 subplots (randomly chosen) shall be created.

Harvest

Subplots shall be randomly determinate in all experimental fields/plots

Number of subplots in each field/plot: 4

Dimension of subplots: cca. 10 m^2, (2 rows x 7 m length)

Type of harvest: manually – all the cobs from each subplot shall be detached from the plants and immediately taken from the field for further evaluation

Yield estimation

Separation of grain from all cobs harvested from each subplot with parcel grain harvester (if applicable; if not grain shall be separated by hand from the sample of 10 cobs. From the ratio of grain and ears the grain yield is assessed – weight all cobs from separated subplot and using the ratio of grain and ears calculate the grain yield → *Grain yield = weight of cobs x ratio of grain and ears of the sample of 10 cobs*)

Grain moisture determination (ISO 711:1997)

Calculation of grain yield which shall be expressed in kg per hectare grain with 14 % moisture content.