LIFE + BIODIVINE – Soil biological activity protocol

As agreed during the meeting in Beaune (October, 17th – 18th, 2011), each demonstration site will have to monitor soil invertebrates in different landscape features during 2012 and 2013. The soil invertebrates will be monitored using bait-lamina sticks.

Each bait-lamina stick contains 16 holes filled with cellulose powder, bran flakes and active coal. This mixture will be consumed by the soil invertebrates. The more holes are consumed, the more invertebrates are present on the monitored place.

▪ Number of monitoring sessions

Bait-lamina tests can be performed at any given time, except in periods of ongoing dryness and/or ongoing soil frost. Lowest thermal limit for faunal activities in soils is approximately 4°C.
We will proceed to 4 monitoring sessions: **May 2012 (postponed to May 2013), October-November 2012, May 2013 and October 2013.**

As bait sticks cannot be stored more than 2 months without risk of degradation, **each partner will have to order sticks for each monitoring session**. You cannot order them in advance. You should plan a **3 weeks delay** before receiving the sticks once you sent your order form.

NB: *For the next monitoring sessions (2013), we will have to discuss either we re-used the 124 initial sticks by filling those with bait or we re-buy the sticks already filled. We can choose the first option if:*

- *We found before the next session an organization that can provide us the bait to be filled in the sticks.*
- *The price of the bait alone is far cheaper than the filled sticks.*

The price is 2,5€ per stick (+ shipping costs) so we **will order exactly 124 sticks per session**.

- Prices are available through this link : http://www.terra-protecta.de/englisch/ks-bestell-en.htm
- More information available at http://www.terra-protecta.de/englisch/ks-info-en.htm

▪ Bait lamina sticks implementation on field:

- According to the budget available for the implementation of this protocol, each partner will have **124 sticks per session**. **This will determine the number of monitored sites, the number of replicates, the number of sampling stations into a replicate and the number of sticks into a sampling station.**

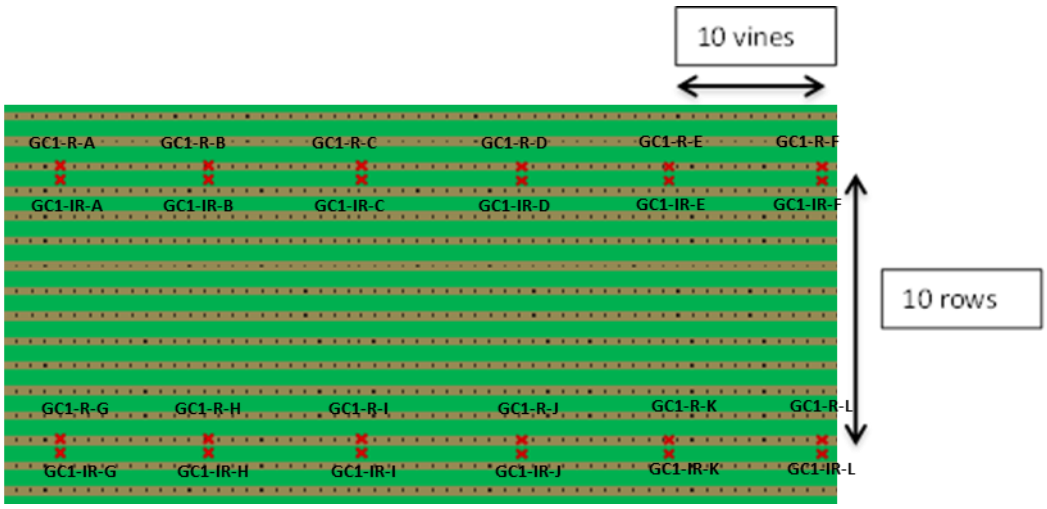
  - Nb of monitored sites: **2 types of vineyard** will be monitored:

    1 Vineyard plot with ground cover vs 1 Vineyard plot without ground cover

  - Nb of replicates: **2 of each monitored vineyard**.
    To implement replicates in the same pedoclimatic conditions, the 2 replicates of each monitored vineyard have to be on the same plot (if the plot is large enough) or in two adjacent plots.

  - Nb of sampling stations per replicate: **12 sampling station** each have to be found per plot: 12 on the row and 12 in-between the row, in order to have 12 "pairs" of values in each monitored plot.

  - Nb of sticks per sampling station: **2 bait-lamina stick will provide**: a value for the inter-row and a value for the row.



Example of disposition of bait lamina sticks in one plot (example for plot "ground-cover-1 = GC1; R=Row, IR= Inter-Row; A= First Sampling Station")

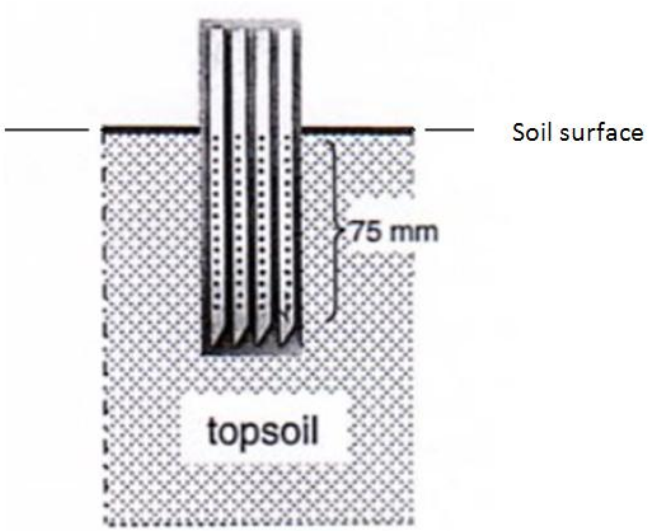
➔ Sum up table:

Nb of monitored vineyard	2
Nb of replicates	2
Nb of sampling stations per replicate	12
Nb of sticks into a sampling station	2
Total sticks needed for a year (2x2x2x12)	**96**

➔ Bait-sticks implementation scheme:

One « matrix » that shows approximately similar soil conditions (soil type, slope, soil temperature, soil moisture, …)

- In any case, **the first hole of each stick should be positioned short beneath the soilsurface (see drawing below). So, the part of the stick that doesn't present any hole will be out of the soil.**
- **The sticks will be placed vertically into the soil**. You can use a tool like a pretchel to make a proper slit in the soil.



Be sure that no tillage will be done by the farmer during the implementation of the bait-sticks.

- **Since the necessary exposure time depends on the site and on the moisture content of the soil, feeding activity assessment can require between 7 (in soils with good moisture conditions) and 20 days (dryer soils) exposure.**
  The most favourable conditions are easily obtained by a short pre-test.

➔ Pre-Test:

The pre-test aims to evaluate the speed with which the bait sticks will be eaten by the soil invertebrate fauna. This information is essential before starting the real test to know how often the sticks have to be checked in order not to find empty sticks that won't allow the measure of the soil invertebrate activity.

The pre-test has to be done in the middle of each sampling line (2 sampling lines per vineyard plot), a few days before each session with 24 sticks (3 for each sampling line). Every set of 3 sticks will be checked **every day or every two days to know the speed at which the sticks will be degraded.**

Each partner will keep 4 sticks (124-(96+24)) in case the material needs to be replaced.

▪ Bait lamina analysis:

The sticks are stored in polyethylene sheets or polyethylene bags to keep the baits from drying out and to prevent formation of cracks that could be interpreted as holes eaten by the soil fauna.

Once the sticks are implemented on field, the degradation rate has to be checked regularly (the frequency will depend on the pre-test results). This checking will be done on all sampling stations for all plots.

The monitoring will be stop as soon as **at least 5 sticks of 1 plot are degraded at 50%** : at least 8 eaten holes on (at least) 5 sticks on (at least) 1 plot. The **totality** of bait-lamina will be collected and the degree of degradation recorded.

- The number of eaten hole(s) has to be written down in the following table:
  - The column "Σ" of the following table = **the number of eaten holes on the whole stick.**
  - The columns n° 8 to n°1 of the following table = **the number of eaten holes on the 4cm upper layer**.
  - The columns n° 9 to n°16 of the following table = **the number of eaten holes on the 4cmlower layer**.
  - In each case of this table, note:
    - **"1"** if the bait has been eaten: "eaten"= light comes through the hole in the bait (shake the lamina in case of doubt).
    - **"0"** if the bait has not been eaten:"not-eaten" = no light comes through (even if the bait shows some transparency but doesn't present any hole).

The approximate time for one measuring replicate (12 bait-lamina sticks) analysis is 30 min.

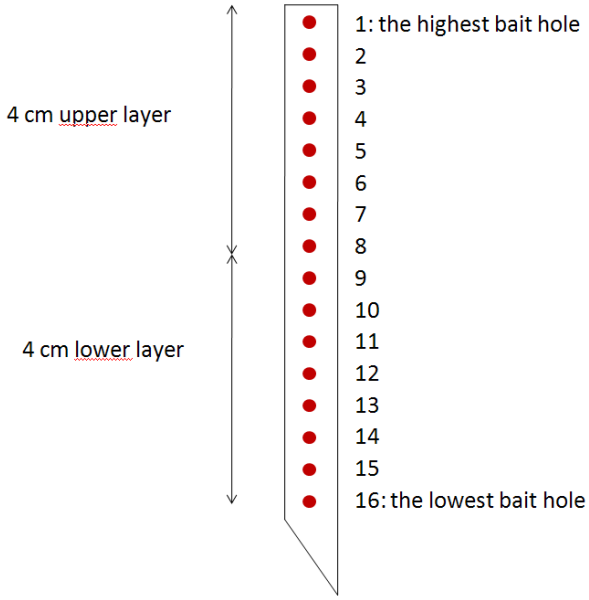
Bait-lamina number	Bait position on the stick (hole's number) (16= lowest bait / 1 = highest bait)																
	4cm lower layer								4cm upper layer								
	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	Σ
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10																	
11																	
12																	
Σ																	

Table 1 "raw data": Number and location of perforated hole per stick (Lines=number of the stick / Columns = hole's number)

The following drawing of a stick helps to know where to find:

- the 4 cm upper,

- the 4cm lower layer,
- the number of each hole from 1 to 16.



Drawing that helps understanding the functioning of a stick

An Excel version of this table is joined to this tutorial.

Fill in this table each day you measure the degradation of the sticks. These tables will be used to fill in the calculated data table (Excel file joined to this tutorial).

Thus, we willcalculate, for each situation (Ground-Cover-Inter-Row, Ground-Cover-Row, No-Ground-Cover-Inter-Row, No-Ground-Cover-Row):

- number of eaten holes per layer (lower or upper one) and per bait-lamina;

All data can be entered and comparedin **Table 2** (see Excel attached file).

- Comparison of the data by an appropriate statistic test (for example: the non-parametric Mann-Whitney U-test).
- Other variables can be considered according to each partner's competences (*i.e.* soil temperature, air moisture, soil type, slope, rainfall average, maximal and minimal air temperature…).