CORE organic DOMINO

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DYNAMIC SOD MULCHING AND USE OF RECYCLED AMENDMENTS TO INCREASE BIODIVERSITY, RESILIENCE AND SUSTAINABILITY OF INTENSIVE ORGANIC FRUIT ORCHARDS AND VINEYARDS

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DOMINO PROJECT GUIDELINES FOR EXPERIMENTAL PRACTICE

Evaluating innovations for a sustainable intensification of organic orchard and vineyard

The aim of this handbook of experimental guidelines is to level out analyses run during the Domino project on practices for sustainable management of organic apple orchard and vineyard in field condition.

Analysis refer to the main crop and to the performances of species introduced ad living mulches. A second section reports protocol for soil chemical, physical and biological fertility evaluations.

Indication are provided for activities run either in structured experimental stations as well as in farm trials.

The standard levels of accuracy allowing to collect reliable information are exposed for both experimental condition.

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SOIL CHEMICAL, PHYSICAL AND BIOLOGICAL FERTILITY
CROP GROWTH AND FRUIT ANALYSES

1 Grapes

1.1 Assessments of pest damage

Vineyard pest include mammals, birds, insects, mites, fungi, bacteria, viruses, and other organisms. Depending on replicate size, mark a minimum of 15 clusters or 15 leaves per replicate for observation. In some cases, repeated observations on the same clusters or leaves are necessary to determine the progression of damage over time.

Pest ratings are important to measure during the growing season, but the most important time to collect these data is directly before harvest. Pest damage can be recorded as **incidence**, which is presence or absence of the damage, or as **severity**, which is typically the percentage of surface area on a leaf or cluster affected. In the case of severity, record **observed percentage** area rather than using a rating scale for ease of future calculations (e.g., 50% of the cluster is covered in mildew, or 30% of the leaf is mottled due to leafhopper feeding). Incidence data allows you to determine how widespread a particular type of pest damage is in a vineyard; severity data allows you to determine how bad that damage is on individual clusters or leaves. Since the human eye is naturally drawn to differences, tagging clusters that will be rated should be done ahead of any treatment application to avoid bias in selection.

1.2 Assessments during crop growth

Trunk diameter See chapt. 3.3.

Shoot length Measurements can be taken as the rate of shoot growth: measurements will have to

be made selecting critical times of the season when shoot growth may be important to measure: flowering, veraison, pre-harvest and post-harvest. Total growth: if you are interested in the total growth then it is possible to make just one measurement in the season, at post-harvest. Select six shoots from the middle vines in each treatment plot. Select shoots on the basis of their developmental stage and appearance that is representative of the whole vine. Avoid particularly short, undeveloped shoots. Measure the length of the tagged shoot from its base (where it joins onto the spur or cordon) to its tip. Count the number of nodes from the base of the shoot (the first leaf that emerged at bud burst) to the tip (the most recent leaf that emerged and unfolded from the growing tip of the shoot).

Mean shoot length (cm) = sum of shoot length / n. shoots

Shoot growth rate (cm/week) = current shoot length - previous shoot length / interval between measurements in weeks.

Mean node number = sum of nodes / n. shoots

Mean internode length (cm) = Mean shoot length / Mean node number

Pruning weight as estimate of vine vigor. This can be done yearly by collecting pruning weights in winter. Hand-prune the middle vines in each treatment plot. Remove and discard old wood from the current season's canes. Count pruned canes of the middle vines per treatment. Do not count or weigh small canes less than 5 nodes. Record the number of canes. Bundle canes together so that they can be easily weighed. Record the total pruning weight (kg) of the middle vines in the field using scales.

Mean cane weight (grams) = total pruning weight x 1000 / cane number

Pruning weight per vine (kg) = total pruning weight / number of vines pruned in each plot.

Yield to pruning weight ratio = Yield per vine (kg) / Pruning weight per vine (kg).

1.3 Assessments at harvest

All the following parameters should be recorded yearly

Yield Information required:

- Number of bunches per vine. Select a random sample of vines (equivalent to 5% of the vineyard is recommended), count the number of bunches for each vine and determine the average number of bunches per vine (sample at least 20 vines).
- Number of bearing vines per hectare (e.g. 1090 vines/ha at 2.4m x 3.6m spacing or 1300 vines/ha at 2.4 x 3m spacing).
- The average cluster weight for that cultivar from that vineyard (in kg).

Yield = Clusters per vine x vines hectare x weight per cluster

Fruit quality Samples should always be collected at the same time of day and processed as soon as possible after collection, preferably on the same day. When collecting clusters or berries for analysis, be sure to include fruit from both sides of the canopy as well as from sun-exposed and shaded positions in the canopy, unless canopy side or sun exposure are factors you are specifically trying to differentiate between.

To minimize changes in composition before processing, samples should be collected into a cooler and stored on ice or in a refrigerator. Store samples in a freezer (0ºF or colder) if you are planning to process them after more than a few days.

Soluble solids In ripening grapes, soluble solids is a reliable and simple measure of fruit sugar concentration and, thus, of fruit maturity. It is usually measured using a hand-held, temperature-compensated refractometer and expressed in °Brix (equivalent to % w/w or g/100 g of liquid). Grape berries typically undergo the color change associated with véraison once they have reached about 9–10 °Brix, and, depending on variety, they continue to accumulate sugar up to 23–25 °Brix.

pH The pH is a measure of the total free hydrogen ions available in a solution ($pH = -\log 10 [H+]$, where [ ] denotes concentration in mol/L); it provides information on fruit acidity (and hence maturity) and the favorability of the juice for the growth of microorganisms. The pH of pre-véraison grape berries is below 3.0 but increases during ripening. The pH of mature fruit is

around 3.5 and can sometimes exceed 4.0 in overripe fruit (Keller 2015). The typical pH range for grape juice is 3.4 to 3.8 for red wine grapes and 3.2 to 3.6 for white wine grapes. However, this can change based on wine style preference. The pH is measured using a pH meter or litmus paper. A pH meter is the preferred and more accurate method. To avoid inaccurate readings, make sure the temperature of the grape juice and the buffers used for calibration is the same (Iland et al. 2013). Note that due to the log scale, it is mathematically meaningless to directly calculate pH averages. The error is small for pH values that are similar but gets larger if the samples are more variable. For example, the average pH of two juices with pH 3.0 and pH 4.0 is 3.26 and not 3.5.

Titratable acidity (TA) The TA is an approximate marker of the concentration of organic acids (mainly tartaric and malic acids) and measures all the hydrogen ions (free and bound) in a solution. The TA of grape juice at maturity ranges from less than 4 g/L to over 10 g/L depending on variety and environmental conditions. The typical TA range for white wine grape juice is 4 to 10 g/L; the typical TA range for red wine grape juice is 4 to 8 g/L. These ranges can change, however, based on wine style preference

Further parameters to measure might include:

- The **size** and the **form** of the berries.
- **Color** of berries.
- The **timing of key phenological** stages, such as bloom, lag phase, véraison, and commercial fruit maturity;

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2. Apple and apricot

2.1 Assessments during crop growth

Trunk diameter Calipers are held at always at the same distance from the soil on the uphill side of the tree. The arms of the caliper are placed on either side of the tree trunk, perpendicular to the sides of the tree, and the diameter between the two arms can be read from the scale The calipers must be held at the same angle of lean of the tree, if lean is present.

Two measurements, at right angles to each other, should be observed on each tree. The first measurement is usually the largest diameter for non-circular trees, followed by the measurement made at right angles to the first. The arithmetic or geometric mean is taken of the two measurements and recorded as trunk diameter. Alternatively, the trunk circumference is measured with a measuring tape 20 cm above grafting. In both case the position is marked to make sure it stays the same for all following measurements. Measure trunk diameter at least at the beginning of the trial and at the end of each experimental year.

Photosynthetic activity Uptake of CO_2 can be measured with the means of an IRGA (Infra-Red Gas Analyser). Measure at least 2 fully expanded leaves per plant. Set the LED for a standard of 1000 $\mu mol\ m^{-2}\ s^{-1}$. Measure plant photosynthetic activity at least once per year when the level of stress or competition with for plant is supposed to be higher. In fertilization trial measure immediately after supply to check for plant stress due to toxicity.

Leaves macro nutrients (P, K, Ca, Mg, Na) and micro nutrients (B, Fe, Mn, Zn) The nutrient level in plant tissue depends on the plant part that is tested and the stage of plant growth. Do not include plants that have been showing visible deficiency symptoms. Sample always the leaves in the same stage of development and in similar expositions on the tree. Dry leaves after harvest at 40°C to avoid dample deterioration from occurring. In processing samples use microwave digestion, then analysis with ICP-OES. Analyses on sample leaves for nutrient content should be performed at least yearly between July and August.

2.2 Assessments at harvest

All the parameters below should be measured yearly, at least at full maturation stage. It is possible to add analysis at different maturation stages.

Days after full bloom (DAFB). DAFB should be used as a general reference to standardize data collection over years and among groups. Record full bloom by block and cultivar each spring.

Fruit size randomly harvesting 20 fruit per tree, measure the maximum diameter

Fruit redness and base color To evaluate the lot selected for inspection, take a sample of at least 10 fruits randomly selected . Fruits should be free from defects such as sun scorch and pest or disease damage, which may have affected the normal ripening process. The color of the fruits (typically background color of the individual fruit) will be determined according to one of the following methodology: Sorting Machine Greefa MSE 2000 with program Greefa 2 Excel, base color and red area (%) or Sorting machine AWETA with program AWETA. Measure fruit color yearly, at least at full maturation stage.

Fruit firmness For apples, use the 11 mm tip supplied with the pressure tester and penetrate to a depth of 7.9 mm as marked on the plunger. The tip of 8 mm diameter is preferable for apricot.

Calibration of mechanical tester :

- make sure pressure gauge is clean and lubricated
- loosen up the test by working the plunger in and out about a dozen times
- hold the tester in a vertical position on the pan of a reliable bench scale
- press down slowly until the scale registers a weight close to your anticipated readings

adjust tester if needed according to instrument directions and test again

Test each apple on both the blush side and the non-blush side, then average both readings. Another method is to do one measurement per fruit between blush and non-blush side. Use flat area on cheek, halfway between calyx and stem ends, rather than on shoulders or calyx end. Before testing remove a disk of skin between the size of a nickel and a quarter. A peeler on a stand that is handy if you are doing a lot of samples.

If there is no automatic penetrometer (fruit texture analyzer) available, the most critical feature of firmness testing is the speed with which you apply force to the plunger. The proper speed is about 2 seconds, and to regulate your speed you might say to yourself, “one, one thousand, two, one thousand” as you insert the plunger into the fruit. Applying pressure too fast is probably the most common way of getting a false reading.

It takes some care and practice to operate the pressure tester the same each time to get consistent results. If possible, have the same person do all the pressure tests.

The presence of watercore will give higher readings that are inaccurate. Therefore, discard firmness measurements of apples that have watercore. Large apples are usually softer than smaller ones, so for firmness measurements try to choose apples of a relatively uniform diameter and that are representative of the fruit in the block. Measure the fruit firmness yearly, at least at full maturation stage.

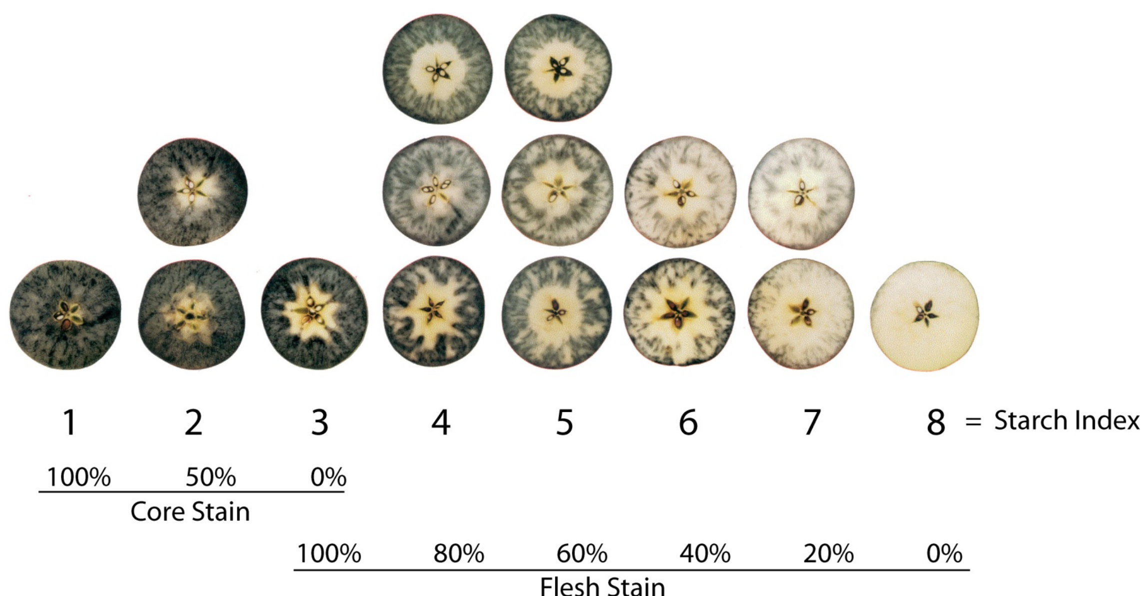
Percent soluble solids (or sugar levels). To measure the percentage of Brix, or sugar, in a solution, a refractometer can be used. As fruit matures, refractometer readings increase, indicating fruit maturity is progressing.

Fruit from trees with a heavy crop will have lower readings than fruit from trees with a light crop under similar growing conditions. Sugar content will be higher in years of reduced moisture availability, high temperatures, and high sunlight. As with firmness, refractometer readings will also vary by fruit position within the tree and nutritional status. Fruits located in exposed areas, where considerable photosynthesis is taking place, have higher soluble solids. Fruits heavily shaded and located inside the tree or on weak spurs have the lowest soluble level of fruit on that tree.

Measurements are made by squeezing a small amount of juice from the fruit onto the prism of the refractometer. For apple from each fruit two longitudinal slices (from stem end to calyx-end) are taken, one from the most colored side and one from the opposite. The core is removed. The slice is squeezed longitudinally to get a mixture of juice from all regions. For apricot Cut the fruit in half and measure half. Hold the instrument up to the light and read the percentage of soluble solids by looking through the lens. After each sample of juice, rinse the prism face off and wipe with a soft tissue to avoid contamination among samples. One can calibrate refractometers by zeroing with distilled water and at 10 percent with a solution of 10 grams of sucrose dissolved in

90 grams of water. Measure the fruit soluble solids yearly, at least at full maturation stage.

Starch levels/Stage of maturity can also be assessed by performing the starch-iodine test to document starch disappearance. Applying an iodine solution to the cut surface of fruit stains the starch a blue black. The iodine solution can be made by dissolving 10 grams of iodine crystals and 25 grams of potassium iodide in 1 liter of water.



The pattern of starch disappearance is specific for each variety. Fruit used for firmness testing and soluble solids readings can also be used for the starch-iodine test. Cut the fruit at right angles to the core, approximately halfway from the stem to the calyx end. Apply the iodine solution to the cut surface, drain away any excess, and rate the fruit after 2 minutes. The reaction of iodine and starch is temperature-dependent. Under cold conditions, the reaction will take longer. An external heating source will speed up the reaction in cold environments. **Avoid contact and be cautious when mixing and applying iodine solution.** Test a minimum of 10 fruits per block, preferably 20.

A commonly used rating system is a scale of 1 to 8, as follows:

1 = full starch (100% blue-black)

2 = 50% of core stained

3 = 0% of core stained; 100% of flesh stained

4 = 80% of flesh stained

5 = 60% of flesh stained
6 = 40% of flesh stained
7 = 20% of flesh stained
8 = free of starch (no stain)
Measure the fruit starch level yearly, at least at full maturation stage.

Apples macro nutrients (P, K, Ca, Mg, Na, N, C) 25 to 30 fruits should be collected from a sun exposed positions, between 1.60 and 1.90 m from the soil and the diameter should represent the fruit diameter-mean of the tree. The apples will be grinded and treated with sulfuric acid and hydrogen peroxide and mineralized with the help of a copper catalysator (380°C). Nitrogen and phosphorus will be determined with a colorimetric method, while the other nutrients will be measured with a ICP-OES. Another method is to digest apple in the microwave and determinnitrogen and carbon by the element analyzer. Measure the fruit nutrient content yearly, at least at full maturation stage.

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2.3 Production costs

Record yearly working time and input costs.

3. Fragaria vesca and other mulching species

3.1 Assessments during crop growth

3.1.1 Plant growth

Assessments could be carried out soon after planting, during flowering and just before or at harvest

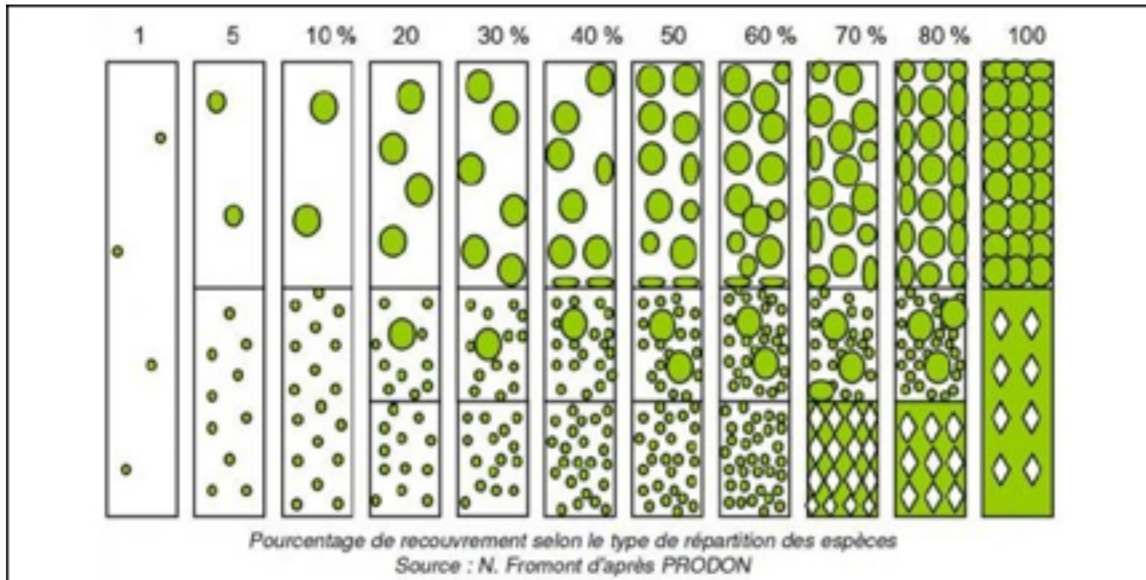
- **Plant diameter** to be measured two ways across each plant.
- **Plant height, vigor, growth and health** (by visual indexes) and number of leaves (foliage density).
- **Runners** (stolons) production, to be measured as number/plant.
- Visual estimation of **Verticillium wilt and Powdery mildew** can be carried out by counting the number of plants showing disease symptoms. Other pests and diseases are likely to be more localized and could just be noted if seen, unless a full assessment of pest / disease infestation and effect on quality and yield is required.
- Living mulches development **key stages** (flowering, recovery time after mowing, runners production)

All the above parameters should be measured at least after the mains two growth peak at the end of spring and during autumn.

3.1.2 Soil cover

Plant density (from seedling to first true leaves stage). It is possible to count the individuals per species in 50X50 frame - quadrat positioned exactly under the tree in the row, at both site of the tree.

Soil cover (when plants are well developed) Measure a vertical projection of exposed leaf area. The cover would equal the shadow cast if the sun was directly overhead. Estimate the soil covered, by dividing weeds from mulches, in a 50X50 frame - quadrat positioned exactly under the tree in the row, at both site of the tree. Express the data as a percentage of ground-cover, using,



Pourcentage de recouvrement selon le type de répartition des espèces
Source : N. Fromont d'après PRODON

to evaluate this percentage, the graphs that classically accompany the Braun-Blanquet scale. This, in order to make it possible to make graphs of the evolution of the cover crop composition, with, for each date, a total which makes 100 %, including the bare ground.

Minimum recommended frequency of observation will be 1 date during winter rest and 2 dates between April and September

Cover is thought to be more ecologically significant than density or frequency because it is an estimate of how much a plant dominates an ecosystem:

1. Cover is more highly related to biomass than density or frequency and therefore reflects the amount of CO_2 and light that the plant capture and turn into phytomass (above-ground plant biomass)
2. Cover also reflects the amount of soil water and nutrients that the plant can harvest and use (best estimated by canopy cover)

Living mulch species competitive power: index proposed by A Ferré, ASREDHOR, France:

Competitive power =

$$\frac{\ln\left(\ln\left(\frac{\text{Living mulch's cover percentage}}{\text{Weeds cover percentage} + 1} + 1\right) + 1\right)}{\ln(\ln(50) + 1)}$$

The calculated indicator is between 0 and 1. Ground covers are considered competitive when the indicator is greater than about 0.33 (value for which the percentages of ground-cover and weed coverages are similar).

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3.1.3 Compatibility with the main crop

Recording of the key stages of both primary and secondary crops. Record the timing of each input supply to the main crop and the preharvest interval. Record harvest time for mulches fruit or leaves in case of medicinal plants and compare with the data above. To be measured yearly and over the whole season.

3.2 Assessments at harvest

Generation of additional income/technical sustainability (exclusively in case an income is expected from the living mulch: edible plants, medical plants, etc) and economical sustainability of product valorization

Compatibility of fruit consumption with the supply of external inputs on the main crop: recording of the timing of all treatments and preharvest interval of each product.
Recording of the working time required by harvesting the complementary crop.
Commercial performance of the complementary crop: yield (using relevant indicators : fruit number per commercial class, dry matter, fruit weight, pest/disease damages etc.) ; financial income.
Plants need to be hand-picked every 2-3 days for approximately four weeks, until harvest ceases. It is generally easier to record the yield of Class 1° and 2° fruits from each plot, taking sub-samples from a smaller number of plants to examine fruit quality in more detail. Rotten/diseased/damaged fruit must be removed to avoid contaminating ripening berries. Fruit size, shape, color, firmness and sugars/soluble solids (oBrix) can be recorded for a detailed trial.

4. Root density determination

4.1. Soil coring methodology

While setting the layout, the distance from the stems should be consistent. Define the distance and depth according to plant age and vigor. A distance of 20 cm from the stem can be taken for young plants. The decision on sampling either the row or the inter-row is related to the research hypothesis and it has to be kept consistent over the trial.
After removing the superficial layer of organic soil, the coring can be carried out at two different depths of 0-20 and 20-40 cm.
Soil cores are acquired using a steel corer of know volume*. Once extracted the soil, to avoid root loss from decomposition, soil cores should be stored at 4 °C if roots will be cleaned within 1 week, or at -20 °C for longer term storage.
Dry the soil samples at 60°C until constant weight . Weight the soil.
Soak the soil samples in water for 30 minutes and gently wash the roots under running water using a 0.21 mm sieve to collect the largest amount of root fragments**. Pick up the root by using tweezers. Clean carefully the roots and divide the whole root sample into "fibrous roots" (approximately diameter <2mm) and "pioneer roots" (diameter >2mm) ***
Dry the root samples back at 60°C to constant weight. Weight the roots.
Root density is calculated in the literature as the ratio root weight: soil volume. Often while coring we perturb the soil so that soil bulk density might be altered. This is the reason why it is preferable to express the root density also as the ratio root weight : soil weight.

<u>The measurement must be carried out at least once over the experiment at the end of the last year of activity, thus assuming an uniform condition at starting time.</u> In case of a non-homogeneous initial situation an analysis at T0 would be also required.

*Depending on the soil characteristics, pounding in the soil core can be quite difficult: the possibility to irrigate might be taken carefully into account although the risk is high to get a sticky soil even harder to manage.

**If the soil is sandy you might find easier to extract most of the root by sieving the dry soil before soaking it into water

*** the reason for dividing pioneer from fibrous is that they have a very different specific root length. By weighting them all together it will be very hard to catch small differences in weight, that, in the case of fibrous root, might correspond to relevant differences in terms of length

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