

Minimum requirements for the evaluation of fruit characteristics in the REFPOP

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1 General rules

- a) In each of the REFPOP planting sites, agricultural practice typically used at the site is applied. Calcium spraying should be avoided as it can influence bitter pit.
- b) Trunk diameter of each tree (see 2.) is measured before bloom.
- c) Flowers are not thinned, but flowering intensity and date of flowering begin are registered for each tree (see 3.).
- d) Fruits are hand-thinned after the June fruit drop; thereby up to two apples per fruit cluster are retained. Crop load may optionally be estimated before the thinning (see 4.).
- e) Fruits of an individual tree are harvested if more than 50% of them reach maturity (see 5.).
- f) Fruits from individuals of the same genotype (replicates) are collected individually based on harvest date of single trees, and therefore not necessarily on the same day.
- g) To avoid phenotyping the same tree more than once, it is advised to remove all fruits from the tree at harvest date or the plant should be clearly marked as harvested.
- h) All traits are evaluated per individual tree; fruits from replicates are not pooled for the assessment.
- i) At the harvest, all fruits from a tree are counted (see 6.). Fruits fallen to the ground due to, e.g., over-ripeness or strong wind, also need to be considered.
- j) Fruits that are not fully developed and of a very small size due to, e.g., aphids or second flowering, should not be considered but discarded before the traits are recorded. Their number is recorded (see 7.).
- k) Scoring of weight, color, bitter pit and fruit russeting (see 8.-11.) is performed on a minimum of 5 or more fruits for up to 20 fruits per tree; otherwise, a random sample of 20 fruits is selected for the assessment (e.g., by taking single apples out of a bag or a box with closed eyes). Random sampling will secure that each developed fruit from a tree will get an equal chance of being selected.
- l) Color, bitter pit and russeting are scored by eye on sample fruits at full maturity. Registered value is an approximation for the whole fruit sample, an average calculated in head.
- m) All traits are estimated in the management 1. If management 1 and 2 are identical, scoring of traits in the management 2 is optional.

2 Trunk diameter

Diameter of a tree measured 20 cm above the grafting point, scored with a digital caliper.

3 Flowering

3.1 Beginning of flowering

Date of a flowering begin of each tree when 10% of flowers open.

3.2 Flowering intensity

Flowering intensity in percent of present flowers compared to the maximum possible number of flowers. Plant shows the maximum possible number of flowers when flowering at each leafy shoot.

1 – 0%

3 – 25%

5 – 50%

7 – 75%

9 – 100%

4 Crop load after the June fruit drop (optional evaluation)

Estimation of the crop load after the June fruit drop (before the hand thinning) in percent of present fruits compared to the maximum possible number of fruits. Plant shows the maximum possible number of fruits when bearing fruits at each leafy shoot.

1 – 0%

3 – 25%

5 – 50%

7 – 75%

9 – 100%

5 Ripening and harvest date

5.1 Harvest date

Date at which more than 50% of the fruits of an individual tree are physiologically fully mature, determined by iodine coloration or expert knowledge.

5.2 Note on the status of harvest

Especially in case of too early or late harvest, this characteristic may be helpful for estimation of the true ripening date and planning of future harvests.

0 – harvest before full maturity of fruits

1 – harvest of fully mature fruits following 5.1

2 – harvest of overripe fruits

6 Number of fruits

Total number of fruits from a tree at harvest date.

7 Number of undeveloped fruits

Total number of undeveloped fruits from a tree discarded between the counting of fruits and weight measurement (see 1.j). This measure makes it possible to calculate average fruit weight after undeveloped fruits were discarded in trees with less than 20 developed fruits, else the number of fruits to divide the weight with would be unknown.

8 Fruit weight

8.1 Weight of a fruit sample

Weight of a fruit sample (see 1.k) at harvest date measured with scales or a sorting machine.

8.2 Weight and size heterogeneity

Note on size diversity within the measured fruit sample.

0 – sample rather homogeneous (more than $\frac{3}{4}$ of the fruits have similar size)

1 – sample rather heterogeneous

9 Fruit color

9.1 Ground color determination

Estimated ground color, given by the assignment of grades from zero to two (Figure 1), averaged per tree.

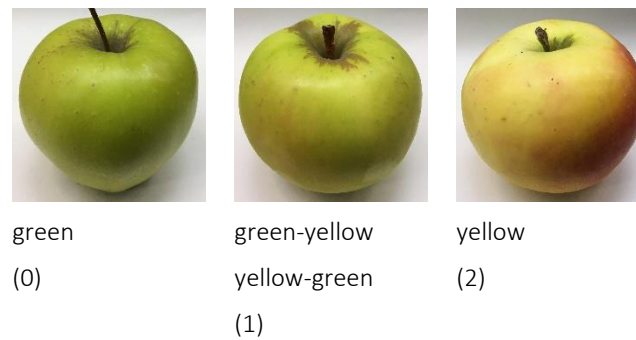


Figure 1: Scale for the assessment of ground color with corresponding grades

9.2 Estimation of the relative area covered by the red over color

Approximate percentage of area of red over color, given by the assignment of grades from zero to five (Figure 2), averaged per tree.

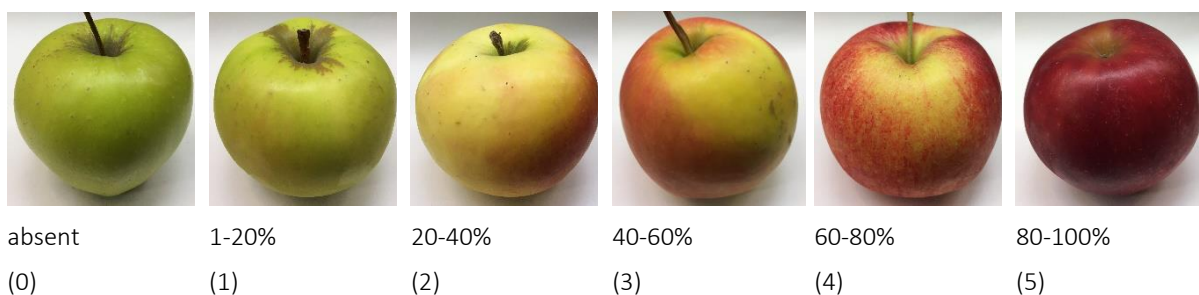


Figure 2: Scale for the assessment of red over color with corresponding grades

9.3 Red over color heterogeneity

Note on the red over color diversity within the measured fruit sample.

0 – sample rather homogeneous (more than $\frac{3}{4}$ of the fruits have similar color)

1 – sample rather heterogeneous

10 Bitter pit

10.1 Frequency of bitter pit presence

Number of fruits carrying the trait. Frequency equal to zero stands for absence of bitter pit in the whole fruit sample.

10.2 Grade of bitter pit

Assessment of bitter pit using an adapted scale from Buti et al. (2015) assigning grades from zero to two (Figure 3), averaged per tree.

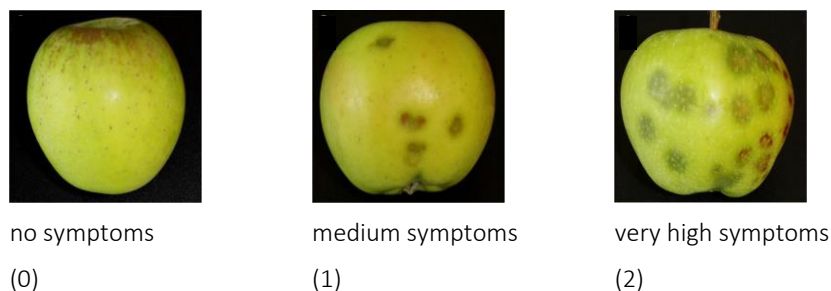


Figure 3: Bitter pit assessment scale with corresponding grades

11 Fruit russetting

11.1 Frequency of russetting presence

Number of fruits showing *russetting*. Frequency equal to zero stands for absence of russetting in the whole fruit sample.

11.2 Estimation of the relative area covered by russetting

Approximate percentage of fruit surface covered by the russet given by the assignment of grades from zero to five (Figure 4), averaged per tree. Very small patch of russetting deep in the stalk cavity, which is reaching up to 50% of the depth of the cavity (Figure 5) and in case of no other signs of russetting on the fruit skin, should be considered as absent russetting (grade 0).

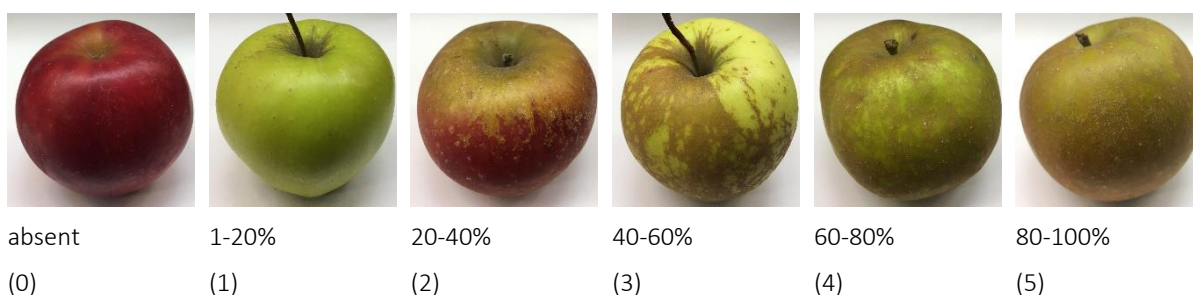


Figure 4: Scale for the assessment of russetting with corresponding grades

11.3 Additional optional frequency assessment of the trait presence per area

Area of the russetting presence, noted individually for stalk basin, cheeks and eye basin as a number of fruits carrying the trait in the given area. This characteristic may allow for additional modelling of fruit attractiveness.

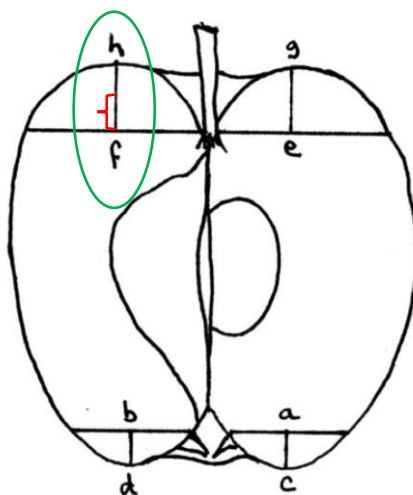


Figure 5: Visualization of fruit dimensions (UPOV Guidelines 2005); depth of stalk cavity (h-f) highlighted by the green ellipse, bottom 50% of the stalk cavity depth marked by the red curly bracket

References

Buti, M., et al. "Identification and validation of a QTL influencing bitter pit symptoms in apple (*Malus × domestica*).*" Molecular breeding* 35.1 (2015): 29.

UPOV – International union for the protection of new varieties of plants. "Apple (Fruit varieties): Guidelines for the conduct of tests for distinctness, uniformity and stability." (2005)