HEMUA

Higher Education Modernization to boost Uzbekistan Agricultural system and promote excellence and regional development

ERASMUS+ GA number 101179517

PROPAGATION

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PROPAGATION

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- Process by which plants can be perpetuated across space and time
- Can be **seed propagation** or **vegetative propagation**

Usually used for rootstocks and genetic improvement

GAMIC PROPAGATION

Usually used for varieties

AGAMIC PROPAGATION

SEED STRUCTURE and MORPHOLOGY

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The seed is formed of three parts: **embryo**, **endosperm** and **seed coat**

The **embryo** come from the fusion between the male and the female gametes. It is the result of the egg cell's fertilization by one of the two generative nuclei of the pollen generating the zygote. Is represented by an axis that develops the *hypocotyl* on one side and the *epicotyl* on the other side.

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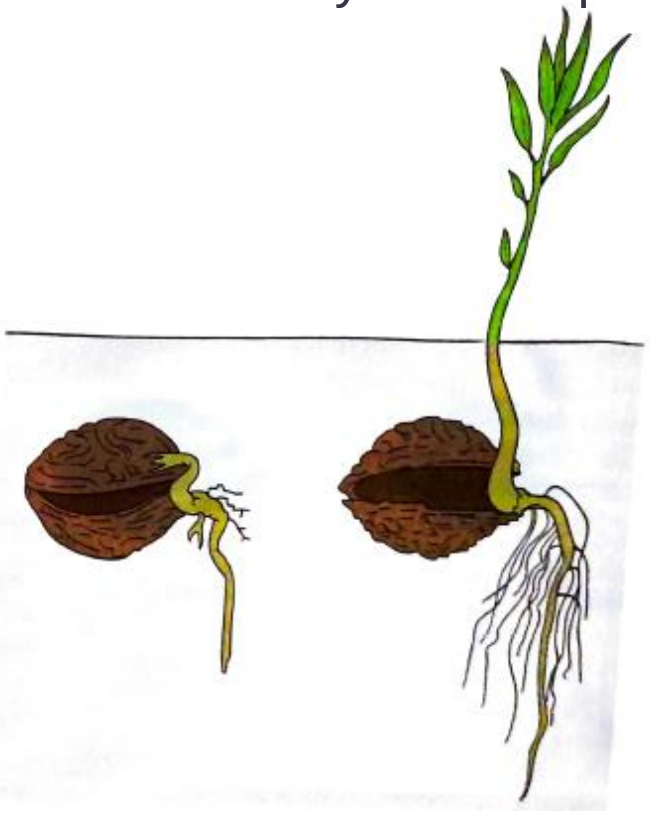
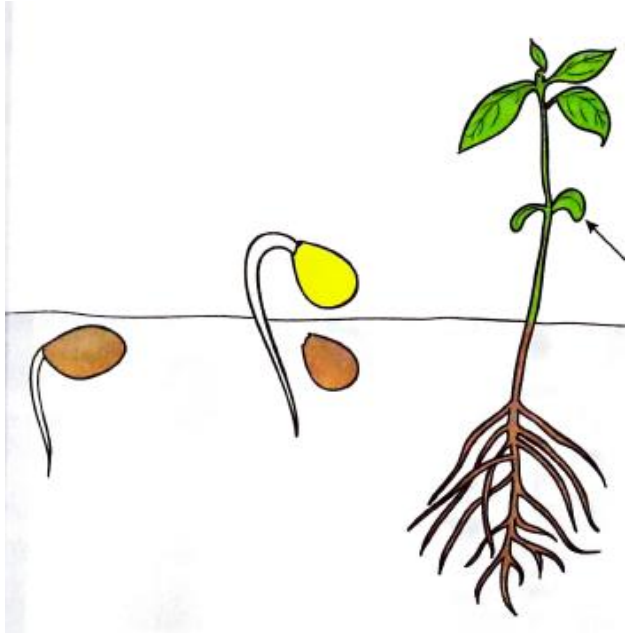
After the primary radicle has emerged outside the seed, the embryo continue growing with an intense mitotic activity until the plumule emerge from the soil with a **epigeus** or a **hypogeus** model.



Cotyledon remains buried and the rapid growth of the epicotyl pushed the plumule outside the soil.



Radicle and hypocotyl elongate until the cotyledons and plumule emerge few centimetres above the germination substrate

Sansavini et al. 2019. Principles of modern fruit Sciences

APOMIXIS and POLYEMBRYONY

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Apomixis → embryo formation without fertilization. These embryos are produced in a vegetative way and are identical to the mother. Was observed in *Citrus*, *Juglans*, *Corilus* e *Malus*

Polyembryony → formation of different embryos, in the same seed, one has a gamic origin, the others are nucellar. Due to the embryos resulting from the same egg, they are identical to one another, but are genetically diverse from the parents.

PROPAGATION BY SEED

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- ✓ Frequently used to produce seedlings or for genetic improvement (breeding)
- ✓ Each new plant is different from the other plant due to cross-fertilization
- ✓ Plants deriving from seeds go thought a period called *juvenile phase*

The length of juvenile period can vary from few month (rose) to 6-8 years (apple and pear) and even to 24-40 years (oak, beech)

Presence of thorns (brachy-blasts modification)

Sterility

Small and apex pointed leaves

Less developed cortex

Increased root activity

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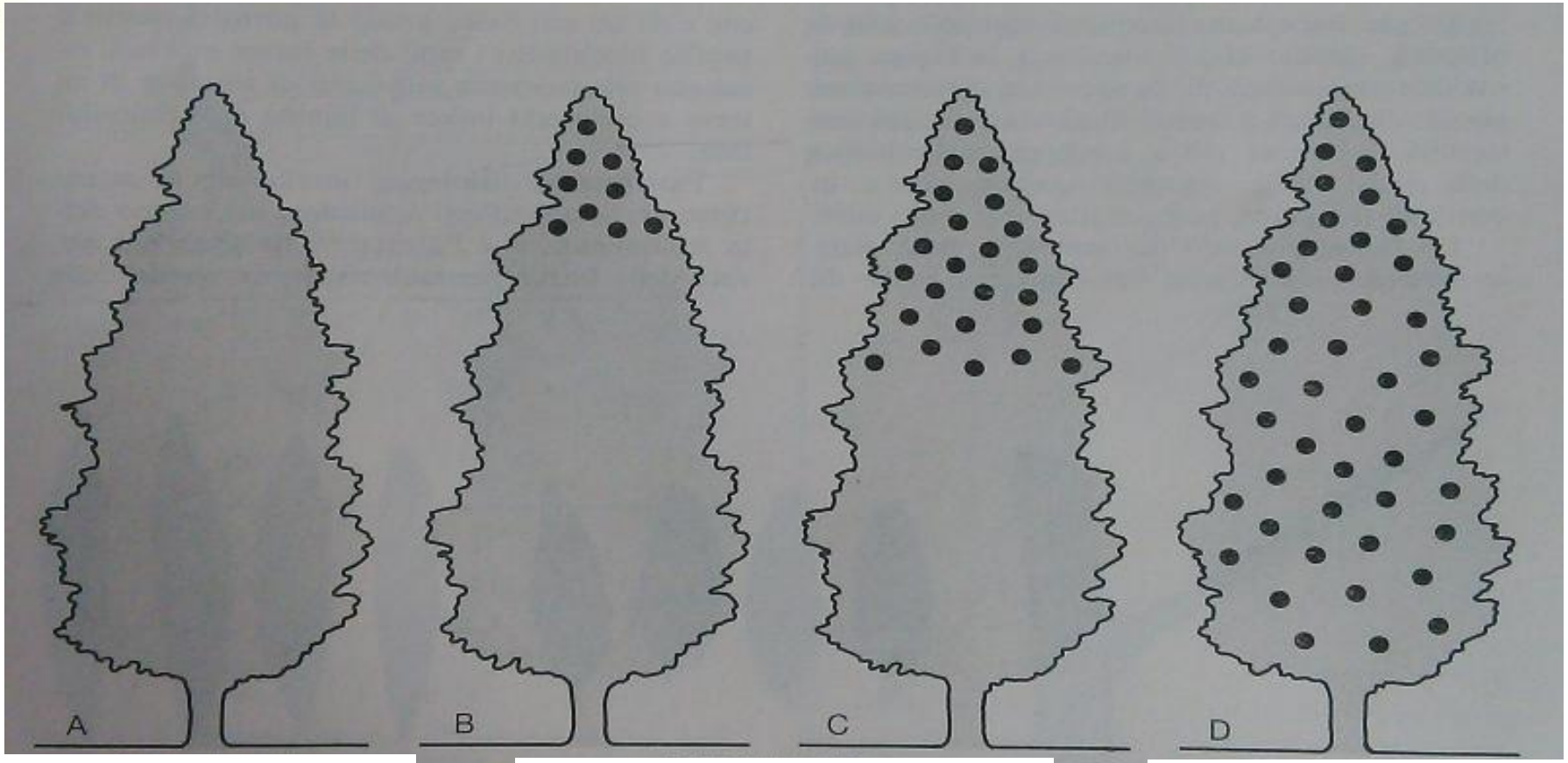

JUVENILE PERIOD TRANSITION PERIOD ADULT PLANT

Baldini, 1986

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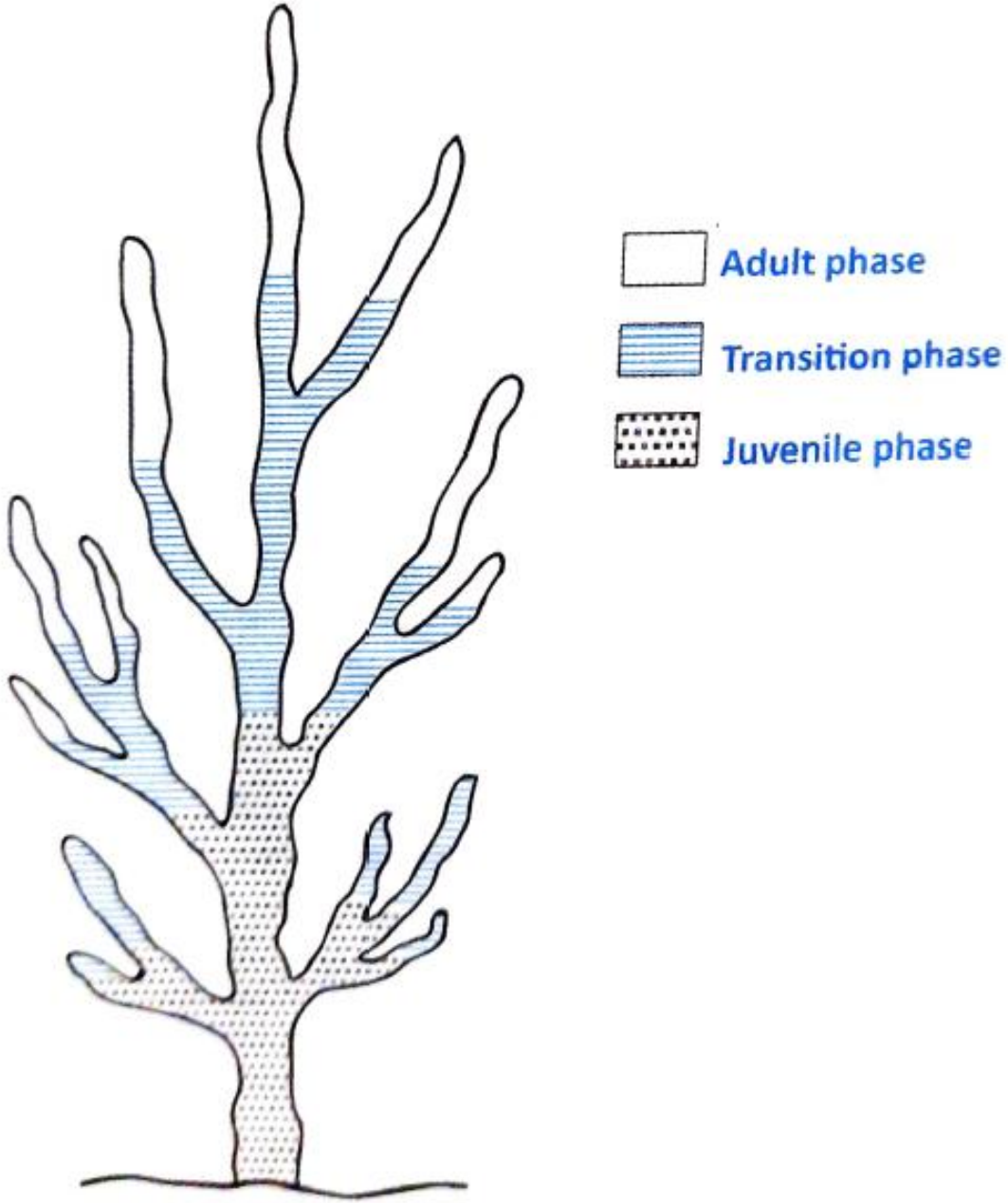
The first signal of juvenile phase termination appear on the canopy periphery



The length of juvenile period can vary from few month (rose) to 6-8 years (apple and pear) and even to 24-40 years (oak, beech)

Sansavini et al. 2019. Principles of modern fruit Sciences

PHYSIOLOGICAL ASPECTS

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- ✓ Embryo dormancy begins during fruit growth
- ✓ Dormancy last different period according to species and environmental conditions

SPECIES	GERMINABILITY
Apple	6 months
Pear	6 months
Loquat	15 days
Peach	6 months
Almond	4 months
Olive	3 years
Citrus	1 month
Grape	3 years
Hazelnut	8 months
Walnut	6 months
Chestnut	3 months

PHYSIOLOGICAL ASPECTS

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- ✓ Dormancy is a physiological syndrome that permits the seed to overcome periods of adverse growth conditions
- ✓ Carbon is mainly accumulated in the endosperm and in cotyledons
- ✓ At the end of the season there is a decrease of auxin and an increase of abscisic acid that induce **embryonal dormancy**
- ✓ An increase of promotors (auxin, gibberellins and cytokines) and a decrease of inhibitors induce the end of dormancy

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- ✓ Seeds have a high capacity of survival (**germinability**)
- ✓ Seed germination occurs when the factors inducing endo- and eco-dormancy are removed
- ✓ Germination is mainly controlled by environmental factors:

• *Water* → hydrate embryo cell colloids thus induce the beginning of metabolic process

• *Temperature* → influence embryo metabolic activity; the most effective temperature range rom 15°C to 30°C

• *Oxygen* → fundamental for tissue metabolic activity and fast removal of CO_2 produced by embryo respiration

• *Light* → germination of most seed is favoured by dark

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- During the transition between the end of dormancy and the beginning of germination the hormonal status of the seed is strongly modified:
  - *Abscisic acid* → responsible for the induction and maintenance of endo-dormancy
  - *Gibberellins* and *cytokinin* → solubilize nutrients stored in the endosperm and cotyledons sustain the shoot until it becomes autonomous
  - *Ethylene* → induce increase of respiration rates and promotes cell elongation processes

SEED GERMINABILITY

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- ✓ Dormancy can be removed through *stratification* (softening of seed covering) or *scarification* (scratching of seed cover) to make it permeable to water and gases (mainly O_2)
- ✓ Stratification is more effective if seeds are previously weeded in cold water for 1 day or hot water (30-70°C) for few hors and the scarified (e.g. peach)
- ✓ Treatment with gibberellin
- ✓ Mechanical or chemical (NaOH, H_2SO_4) scarification

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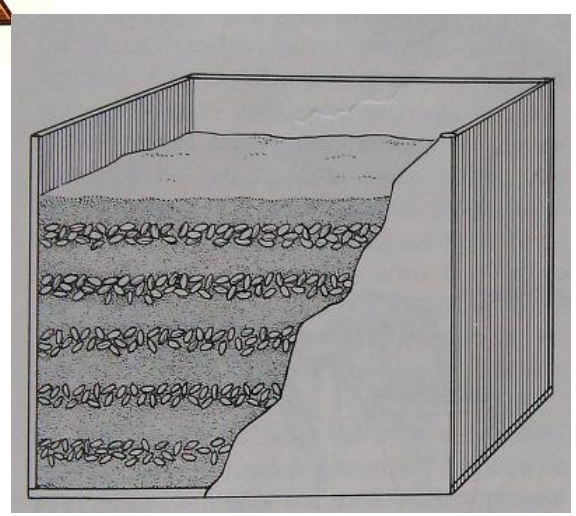

Dormant seed are usually stratified in wet sand or other inert material

- ✓ Time of seedling depends on climatic conditions of the geographical area: spring better for cold winters, autumn for milder climate
- ✓ Usually done in seedbeds arranged outdoors when external temperature is about 15°C or in a greenhouse with warm bed
- ✓ Small seed (apple, pear) → scattering and mixing with sand (20-25 g m^{-2})
- ✓ Big seed (peach) → sown in rows

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Species	Stratification period	Stratification duration
Almond	September	4–6 weeks
Apple and Pear	10–15 days after harvesting	8–10 weeks
Apricot	August	5 months
Carob	Without stratification	October
Cherry	August	6 months
Chestnut	October–November	5 months
Citrus	December	December
Grapevine	October–November	3–4 months
Hazelnut	October–November	6–7 months
Loquat	Without stratification	May
Olive	June–July	18 months
Peach	October	5 months
Persimmon	Without stratification	December
Pinus pinea	Without stratification	November
Plum	July–August	5 months
Walnut	October	5 months

Sansavini et al. 2019. Principles of modern fruit Sciences

ADVANTAGES OF SEED PROPAGATION

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- ✓ Seeds are free from virus and mycoplasmas
- ✓ Low costs
- ✓ Genetic variability useful for tree breeding

VEGETATIVE PROPAGATION

or

MULTIPLICATION

or

AGAMIC or ASEXUAL PROPAGATION

Based on the capacity of some organs (shoot, leaves, roots) to regenerate roots or adventitious buds, or join together (grafting) to generate a new plant genetically identically to mother and with no juvenile period.

PROPAGATION

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Grafting

Root sucker

Aerial, moud or trench layering

Cuttings

In vitro propagation

Union of two pieces of living plant

Plant parts still attached to the mother plant

Plant parts separated from the mother plant

GRAFTING

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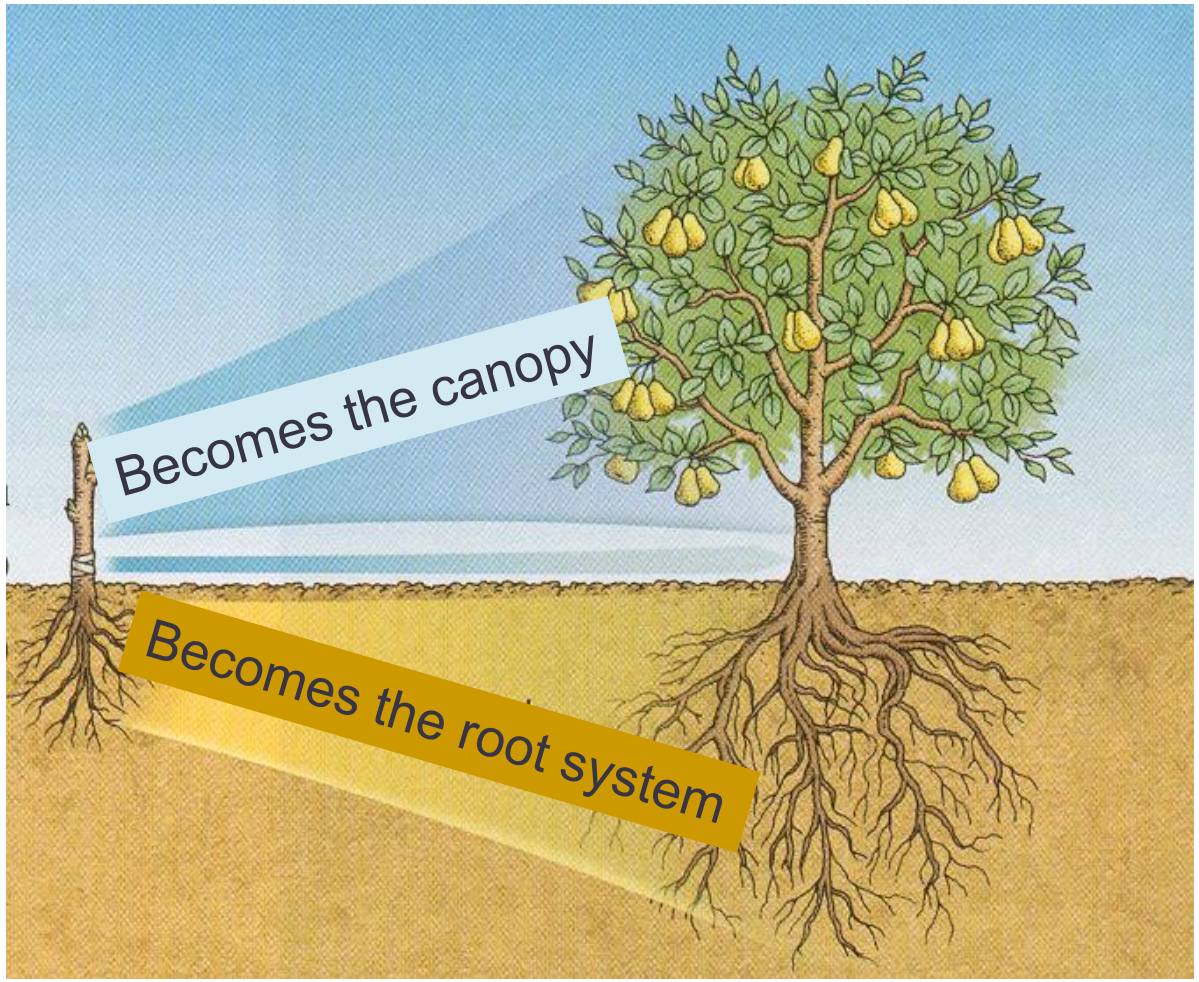
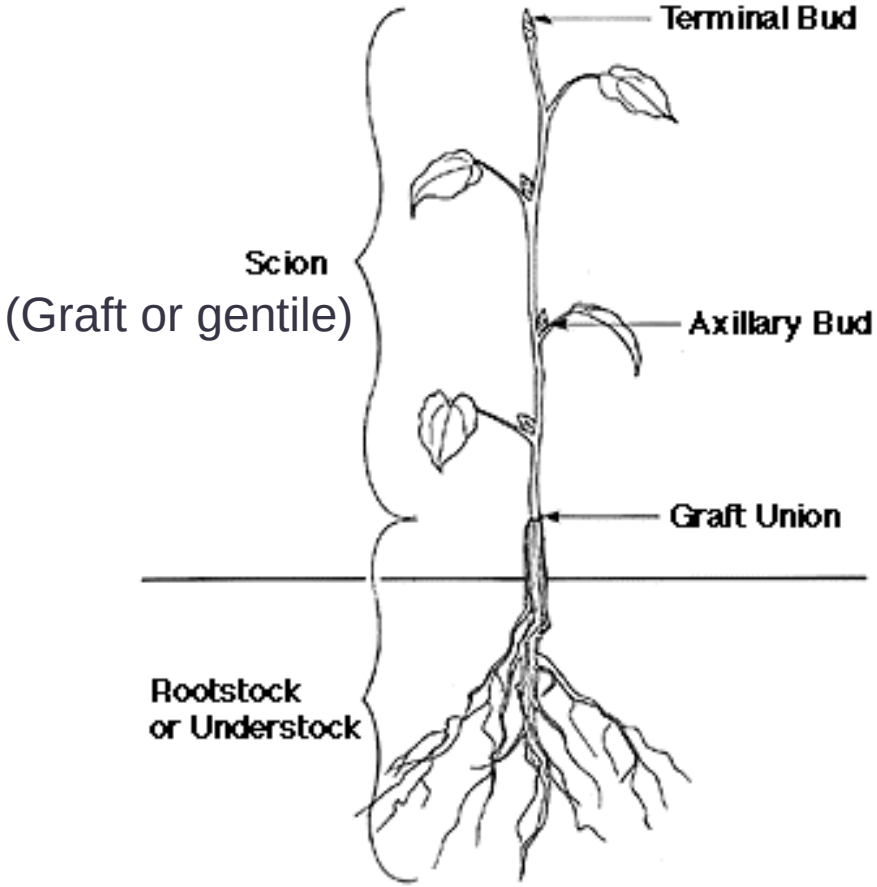
Propagation method that make it possible to join two or more distinct genotype with complementary function into a single plant. .





PURPOSE OF GRAFTING

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- ✓ adaptation to different pedoclimatic conditions;
- ✓ control of tree size (dwarfing rootstock);
- ✓ prevention of some pathogen attack
- ✓ Insert pollinizers inside the orchard (top grafting)
- ✓ replacement of old varieties;

GRAFT FUSION

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Grafting methods can differ depending on the technique used BUT they all have one thing in common: an immediate reaction to wound determined by the cut to produce new parenchyma cells (**callus**) starting from the cambium of the graft and rootstock

The speed of the process depends on *temperature* (12-28°C) and adequate humidity around the grafting area

When the two mass of callus tissue unite, a chain of parenchyma cells differentiates to reacquire meristematic characteristics

The process begins in the two cambium zones and goes on until the two the tissue converge.

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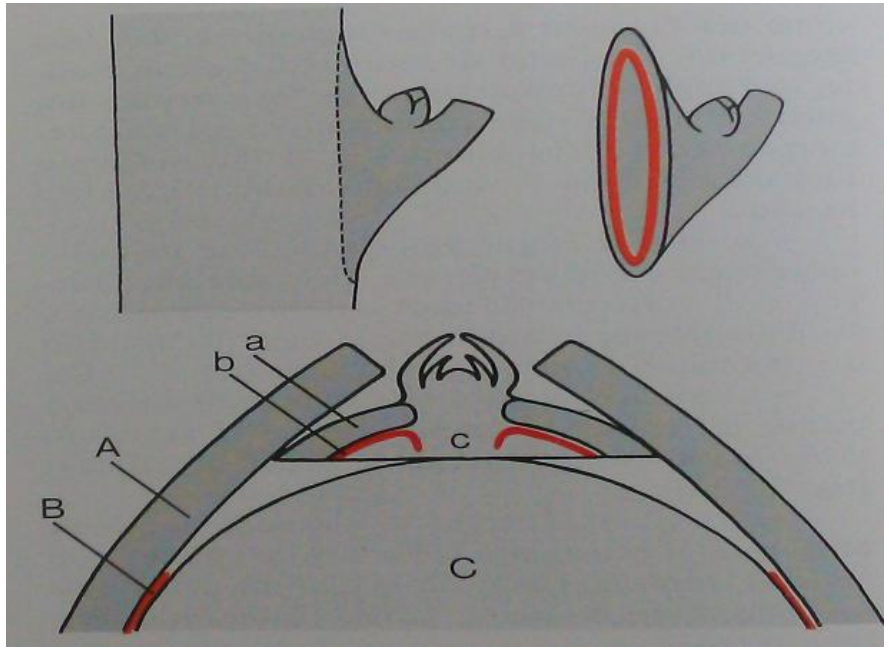
The vascular cambium of the scion must be aligned with the vascular cambium of rootstock

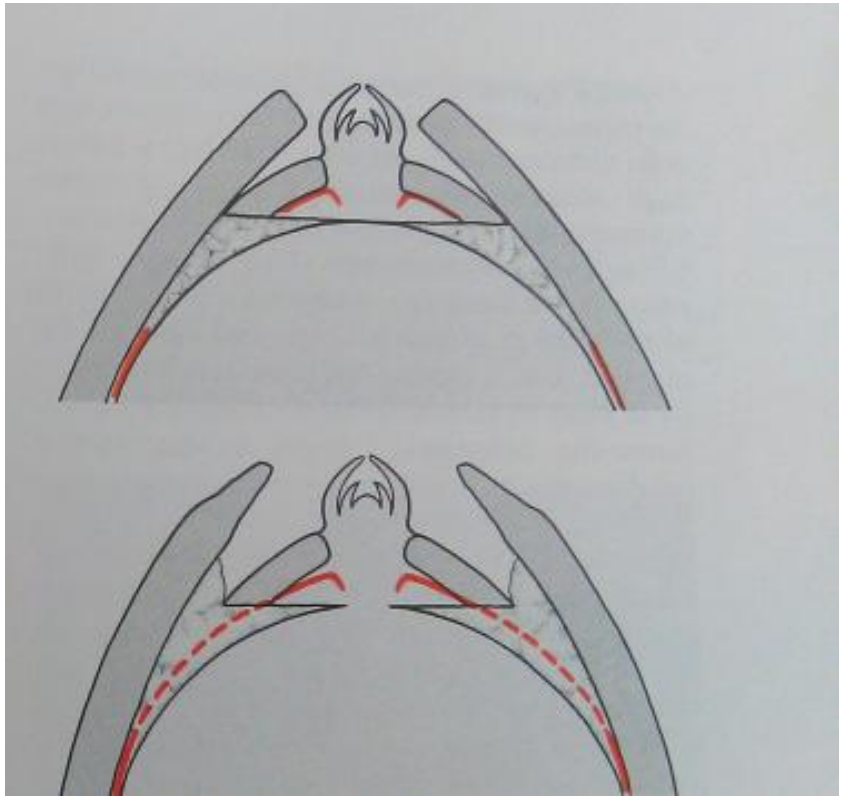


A= rootstock cortex
a = scion cortex

C = rootstock wood
c = scion wood

B = rootstock cambium
b = scion cambium

Baldini, 1986



Dotted line is the formation of new meristematic cells

TYPE of GRAFT

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A) Depending on the state of grafted buds → *sprouting buds* characterized by material taken after having gone thought the period of dormancy (late winter); *dormant buds* made with material taken during dormancy (late summer/autumn)

Before vegetative growth

Beginning of vegetative growth

Chip budding – dormant bud

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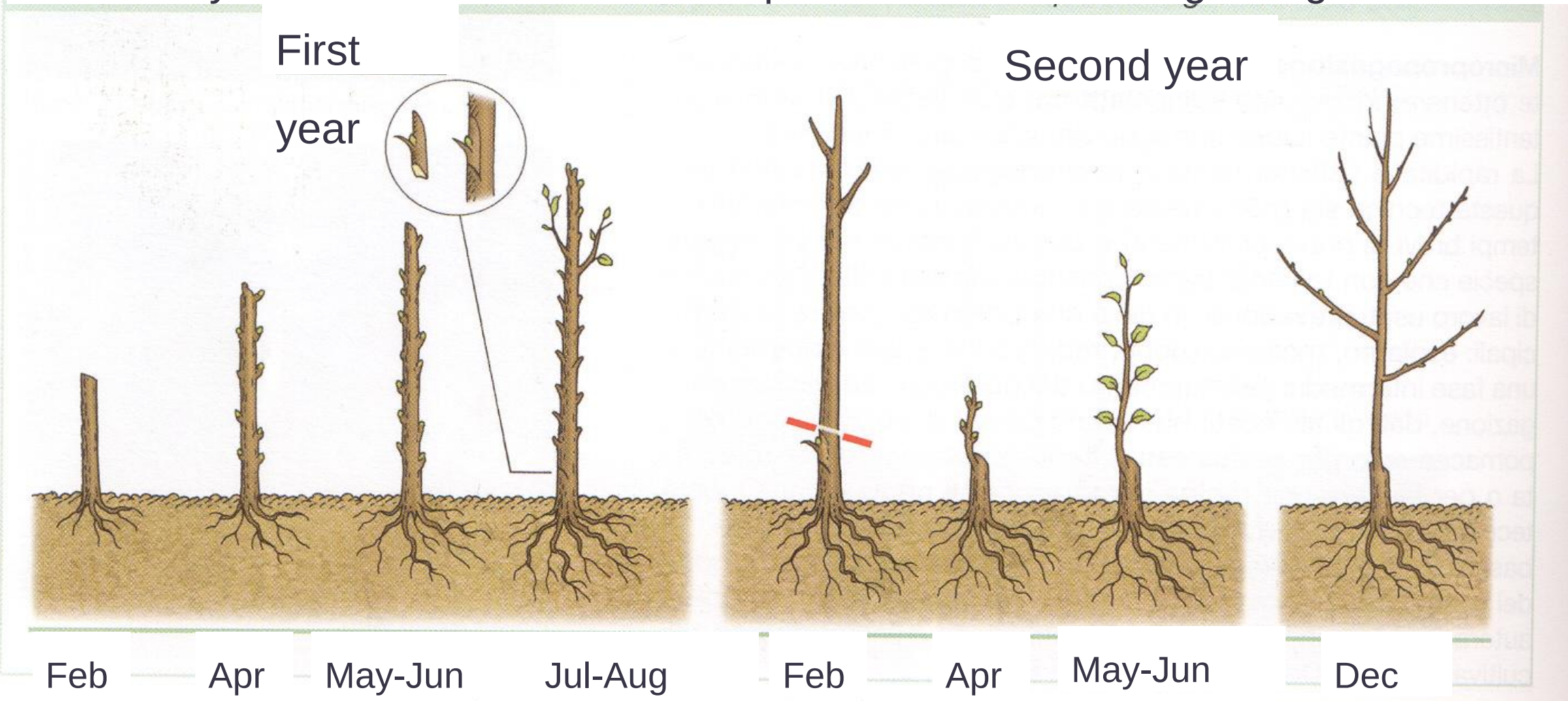
Biennial cycle for the production of whip with a dormant bud grafting



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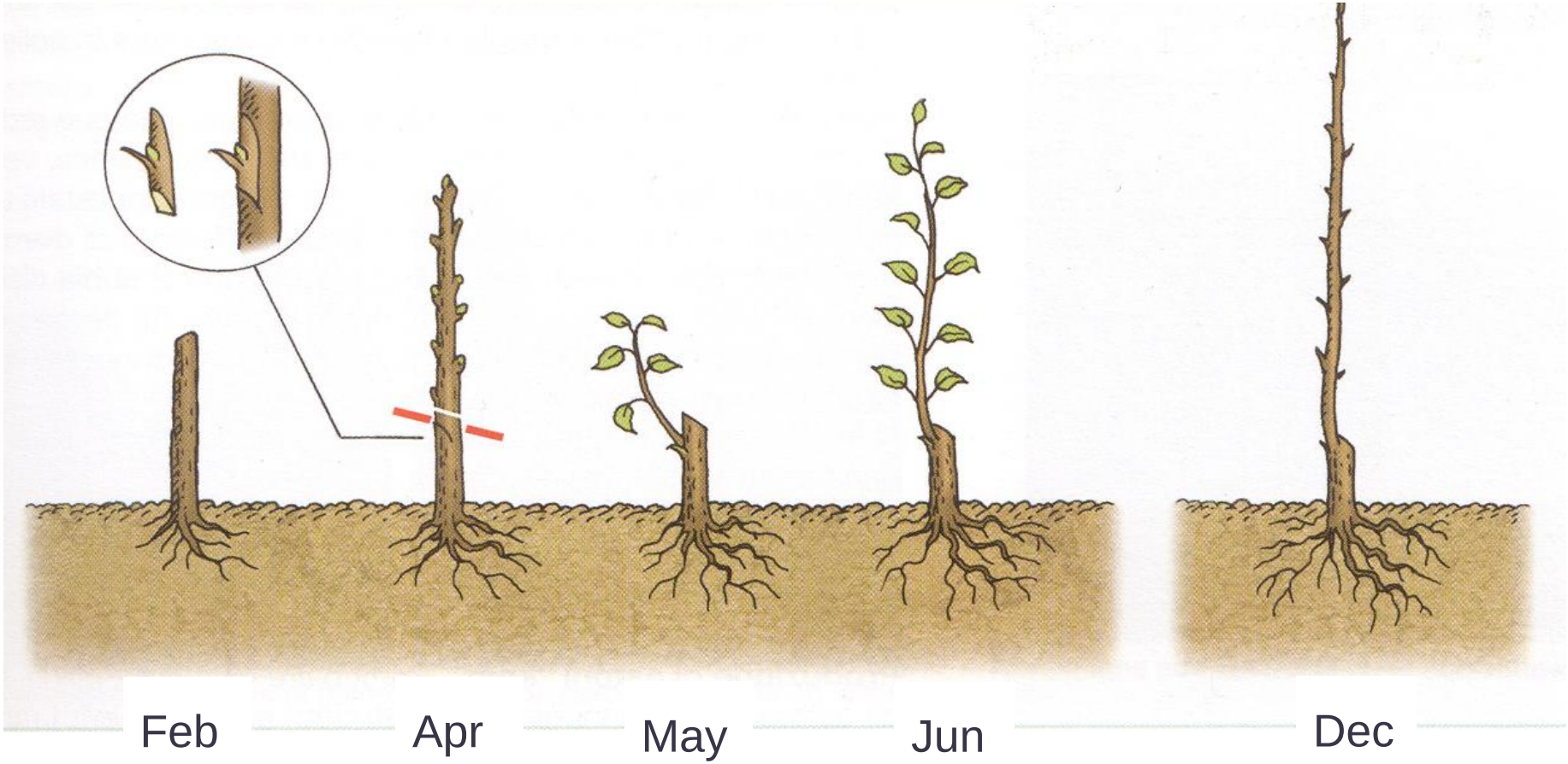
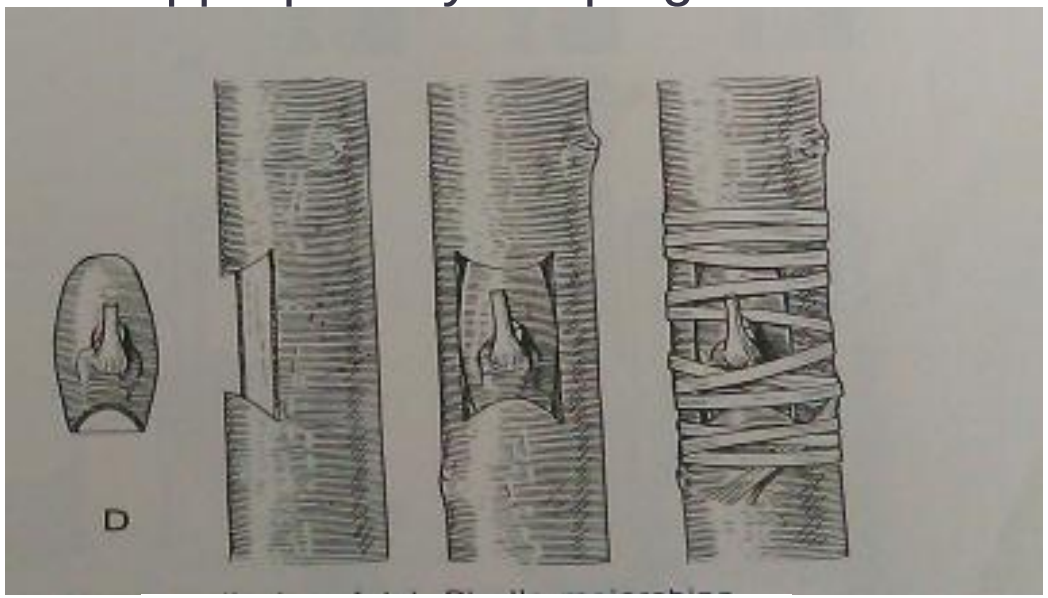
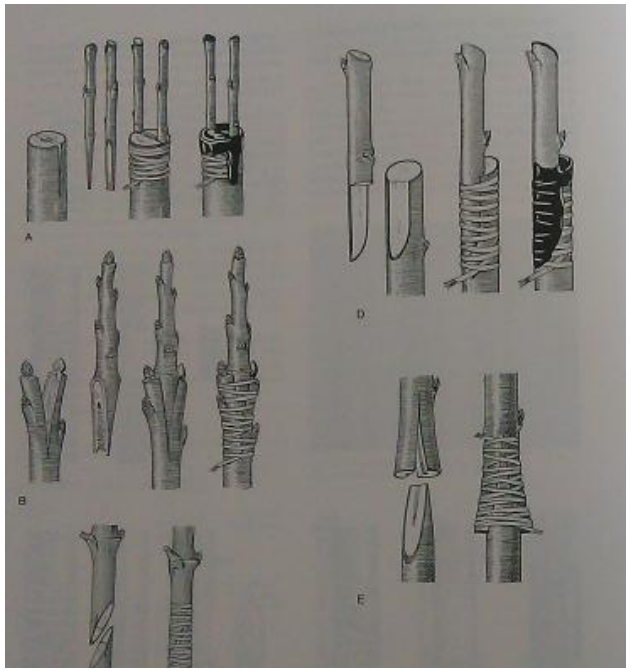
Biennial cycle for the production of whip with a sprouting bud grafting



B) Depending on the location of the scion on the rootstock → *subcortical graft* when the graft is inserted between the bark and wood of the rootstock. This is possible when there is active vegetative growth since the bark need to be easily detachable from the woody tissue underneath. *Intraxylem graft* when the graft is inserted into the stock by appropriately shaping the connecting areas of the two parts.

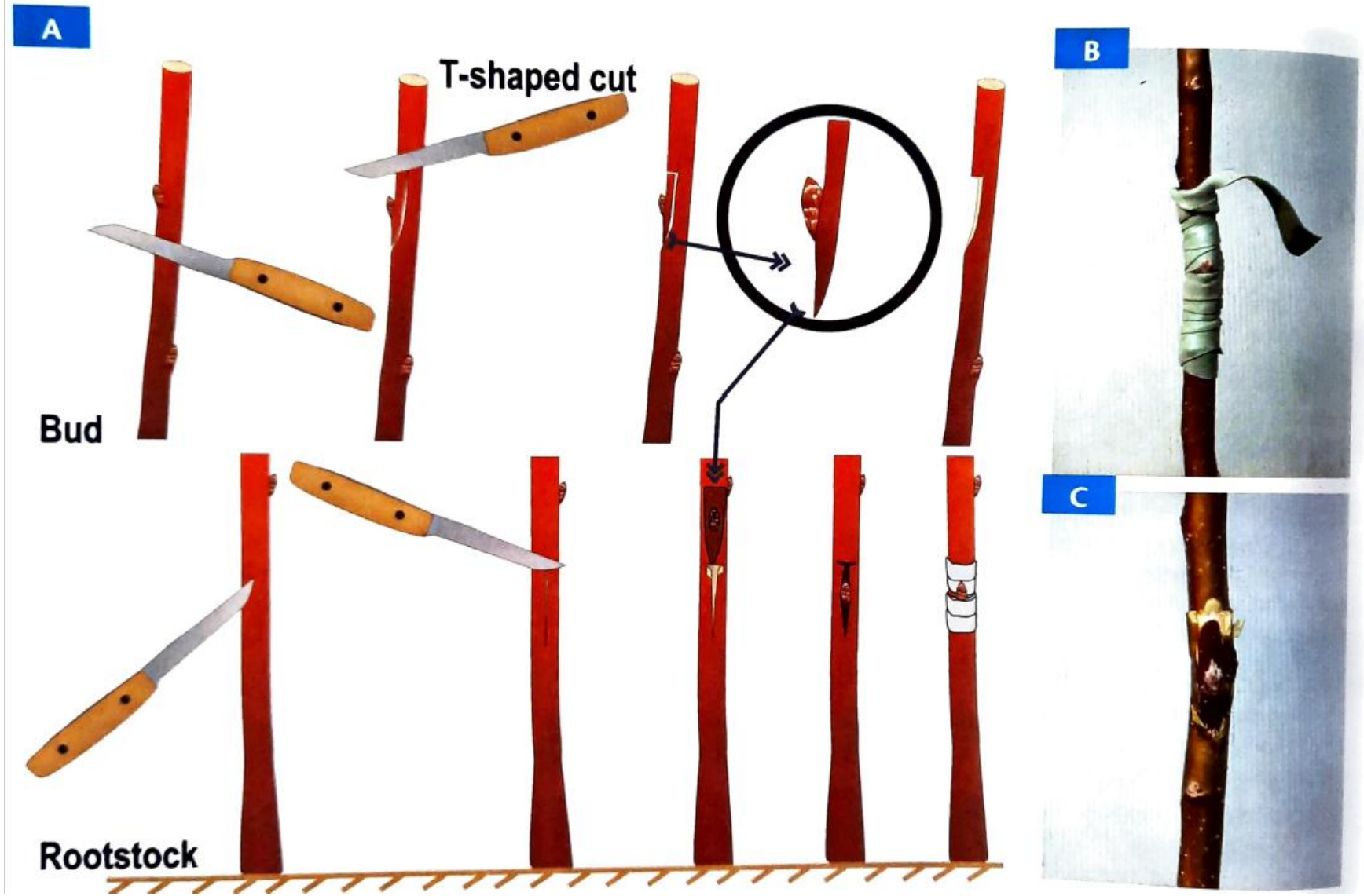


Baldini, 1986 Majorca graft (intraxylem)



Feather (subcortical)

T-shaped (subcortical)



Sansavini et al. 2019. Principles of modern fruit Sciences

C) <u>Depending on the size of the scion</u> → *micrografting* when the apical meristem of a bud is grafted on the rootstock (*in vitro*). If one or more nodes are used and the entire section of the branch is grafted including the central xylem cylinder, this is called *grafting*.

SUBCORTICAL (BARK) GRAFT
- budding (T or shield budding; patch budding)
- grafting (feather, beak, crown)

INTRAXYLEM GRAFT
- budding (chip budding, Majorca graft))
- grafting (cleft, whip, wedge)

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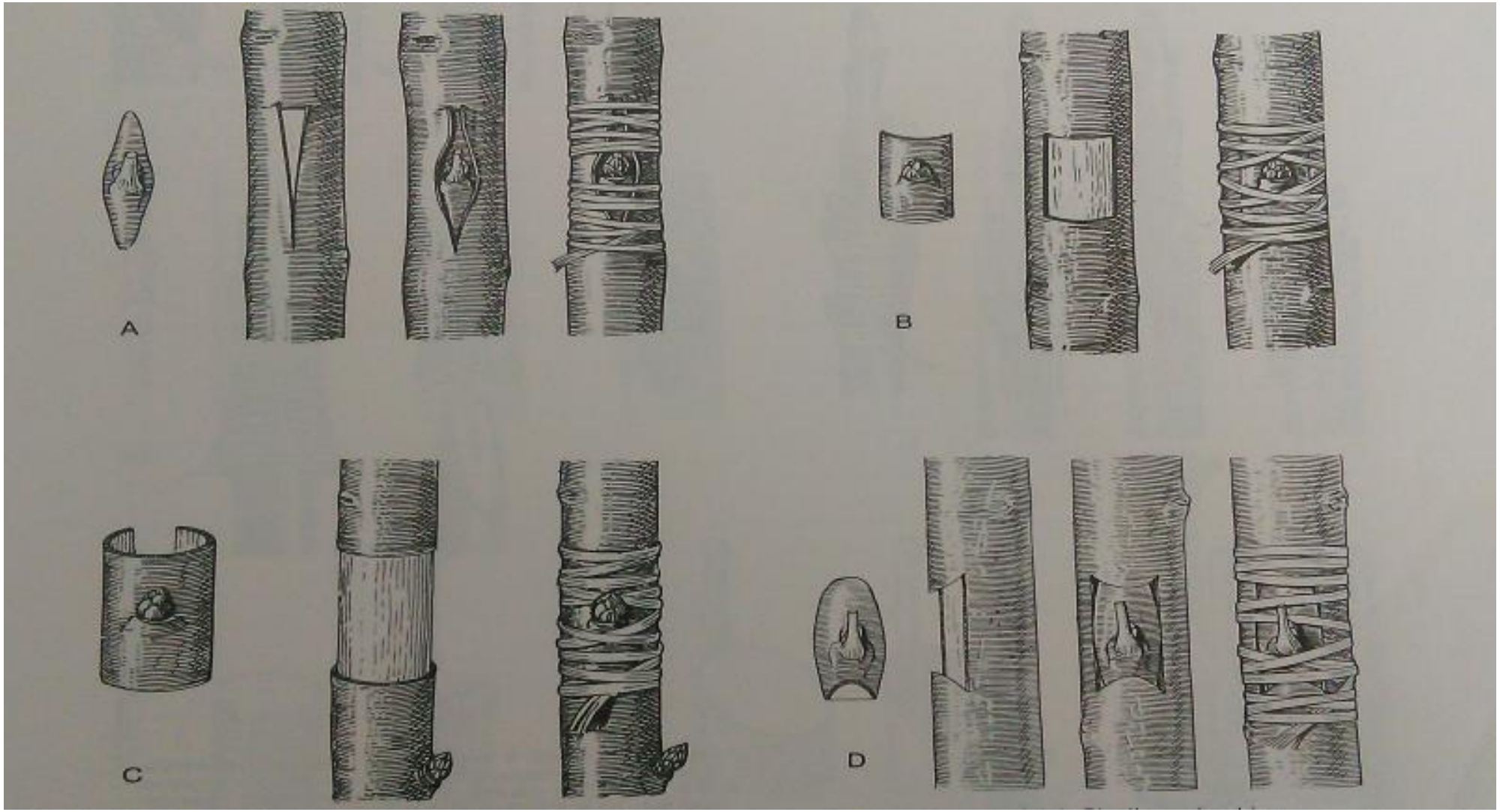

Different type of bud grafts

Baldini, 1986

WHIP GRAFT

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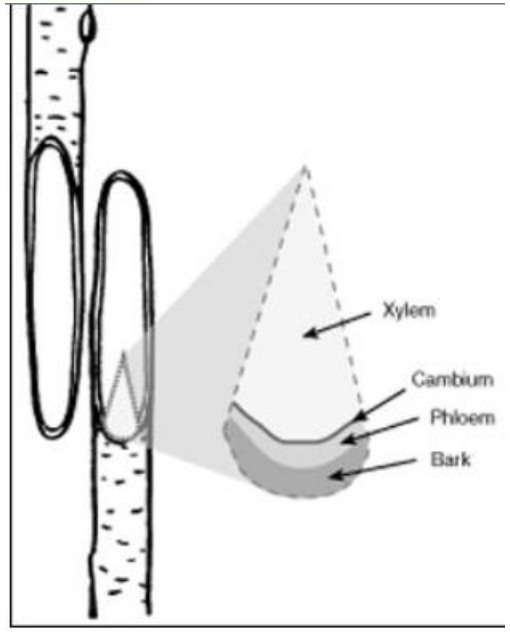
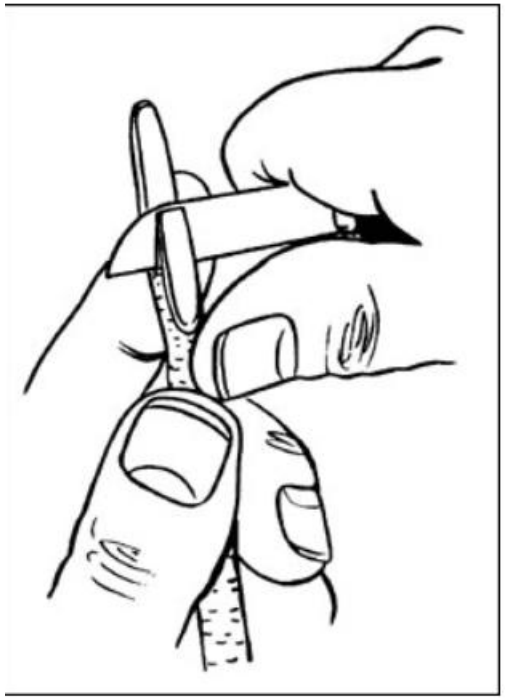
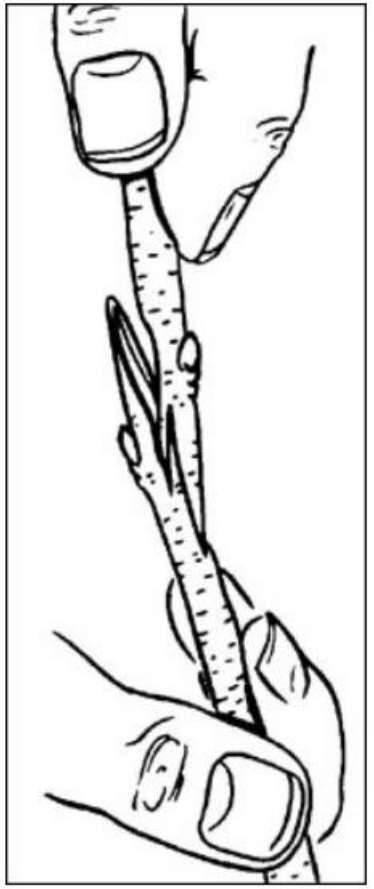


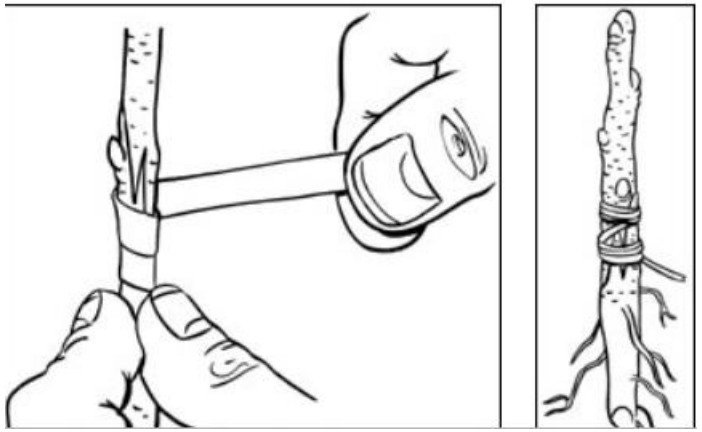
Cuts for the whip graft must be smooth and straight.



Cut again to form the tongue.



Push stock and scion tightly together.



Wrap graft to keep cuts tight and to prevent drying

https://extension.missouri.edu/publications/g6971

CLEFT GRAFT

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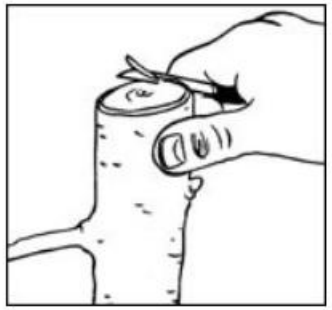
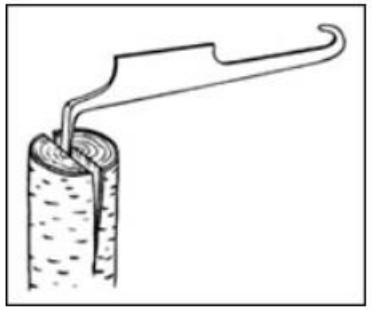
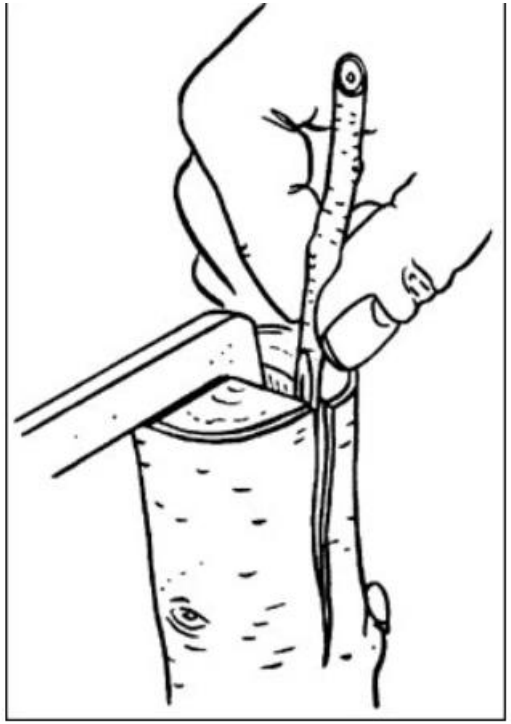
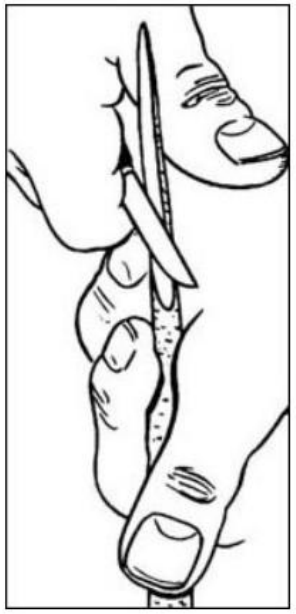


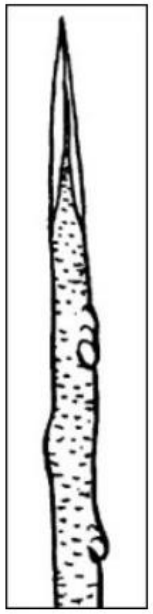


Cut stock smoothly. Trim any rough edges with a knife. Split stock, and open with a cleft grafting tool.

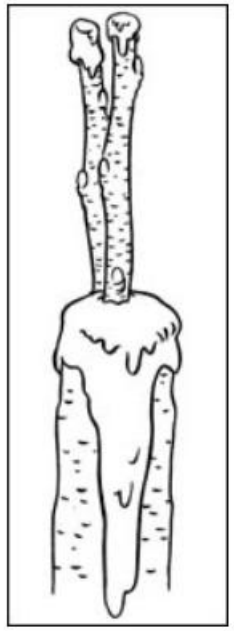


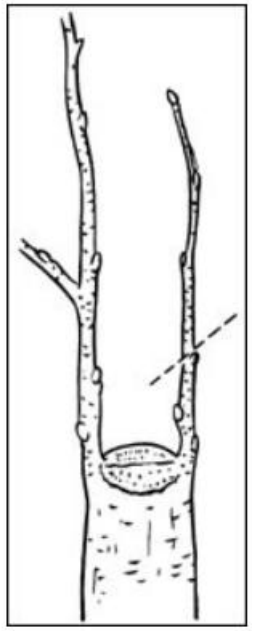
Promptly insert scion into stock after cutting





Make a long, smooth cut to prepare scion. Cut again to make a pie-shaped wedge.





After insertion, wax thoroughly to prevent drying. After the first year, shorten one scion to allow the other to develop.

https://extension.missouri.edu/publications/g6971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GRAFT INCOMPATIBILITY

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- TOTAL INCOMPATIBILITY→ failure at the graft union or early death of buds and of any shoot (e.g. nectarine/damasco plum)
- DELAYED INCOMPATIBILITY → decay and death of the scion in adult plants

Localized incompatibility → can be overcome by interposing a third genotype (*interstock*) that is compatible to both the rootstock and the cultivar (pear/Beurra hardy/quince)

Translocated incompatibility → can **NOT** be overcome with the use of an interstock (apricot/GF677; peach/almond).

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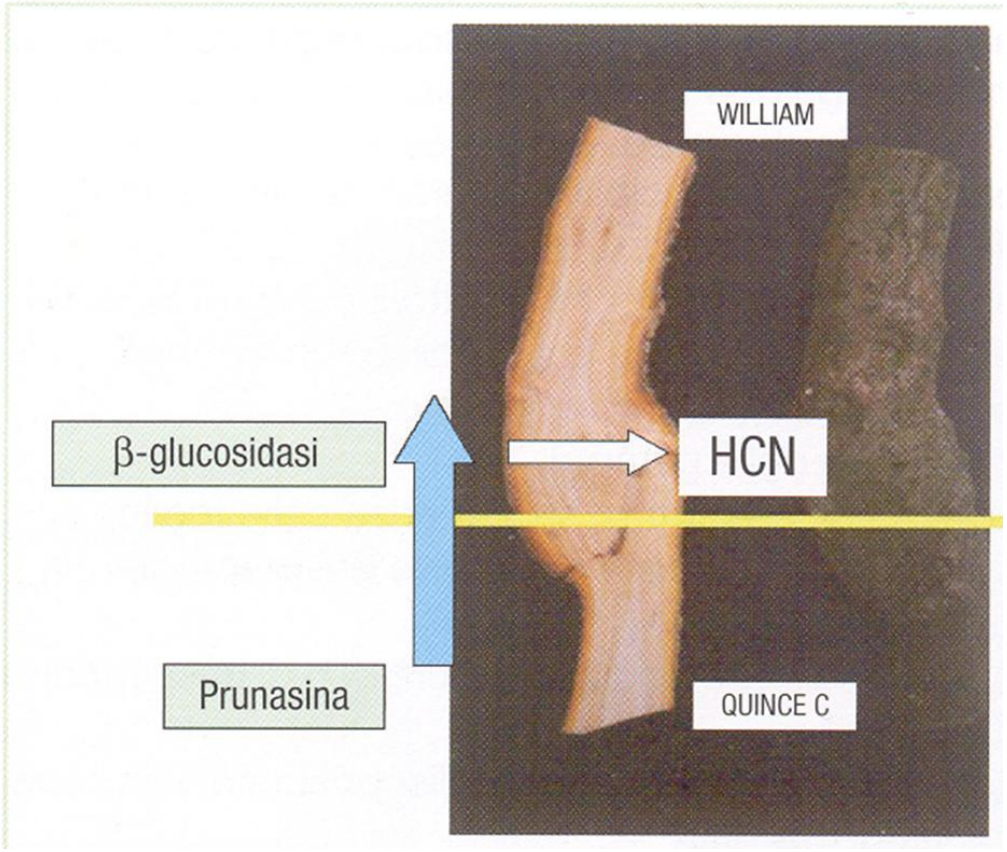
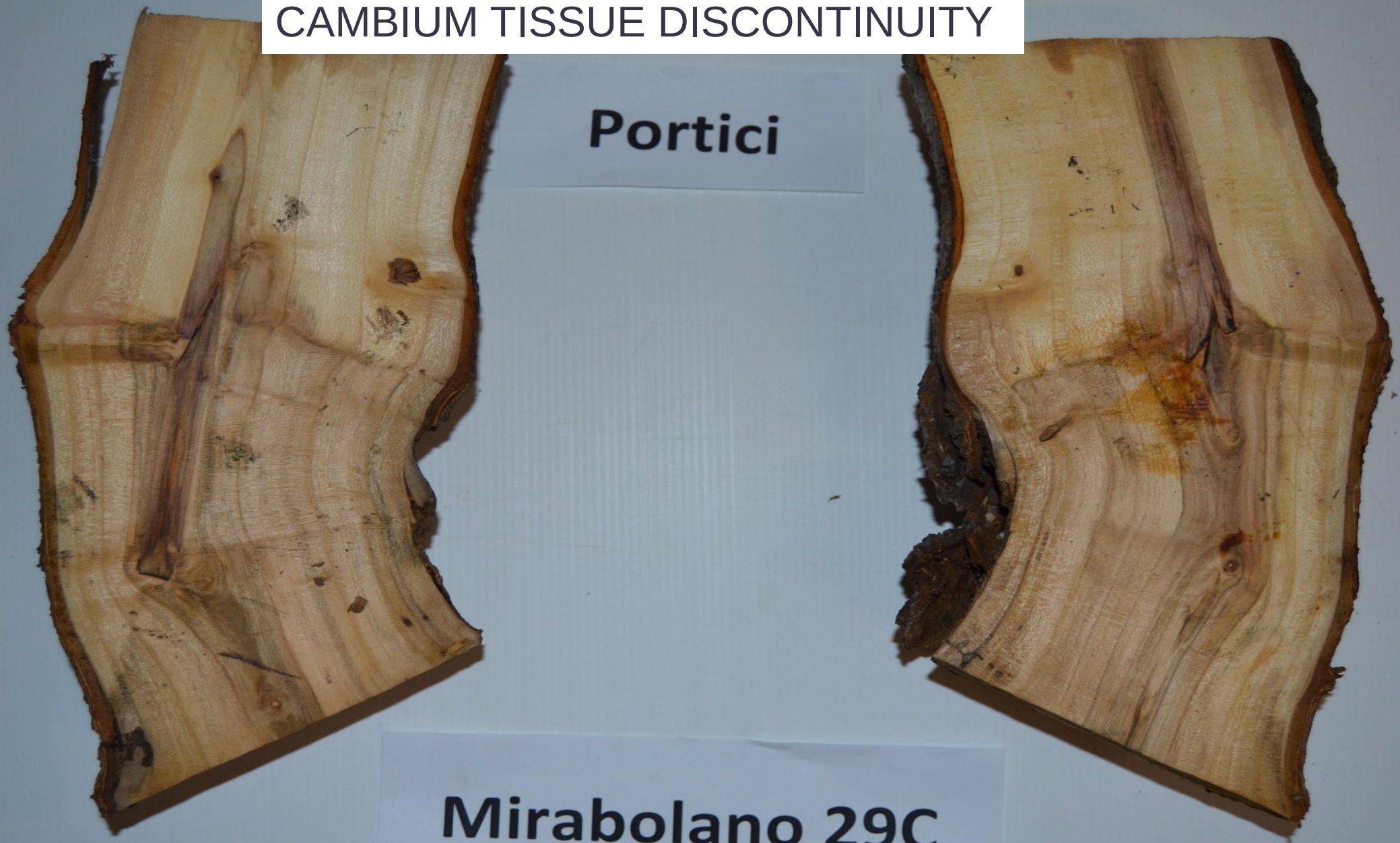
- INCOMPATIBILITY for CAMBIUM TISSUE DISCONTINUITY → occurs when to cambium do not join the graft union. The two genotypes remain separated and the grafting point is very weak (apricot/Mirabolano)
- INCOMPATIBILITY for GROWTH RATE DIFFERENCES BETWEEN GENOTYPES → diameter of the rootstock smaller than the variety (sweet cherry/sand cherry)
- PATHOGEN INDUCED INCOMPATIBILITY → virus, viroids and phytoplasmas in specific grafting combinations (tristeza on sweet orange/bitter orange)



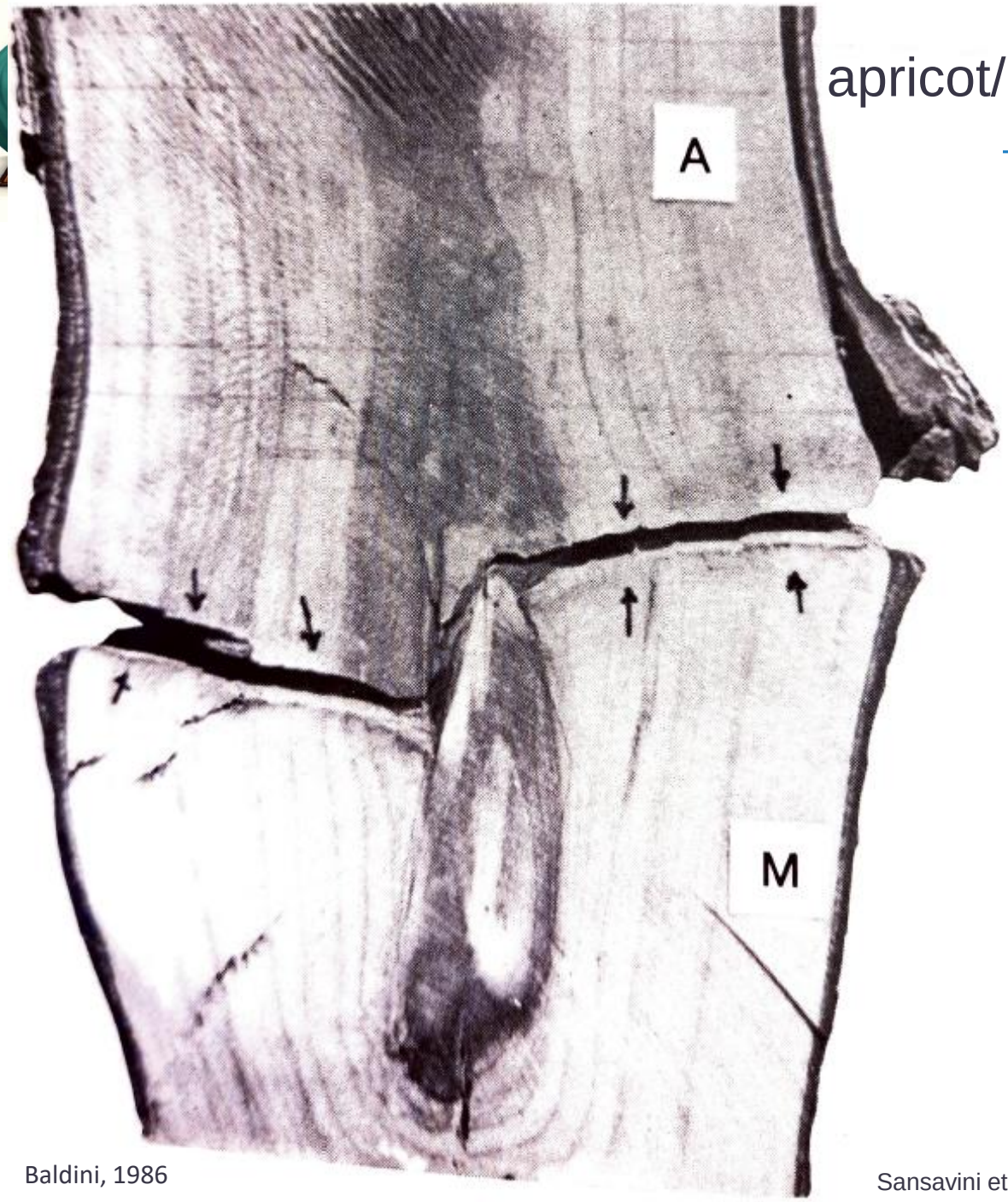
Prunasine, a glucoside, is produced by the rootstock and thanks to the enzyme β-glocosidase produce cyanuric acid that is toxic for plants

CAMBIUM TISSUE DISCONTINUITY



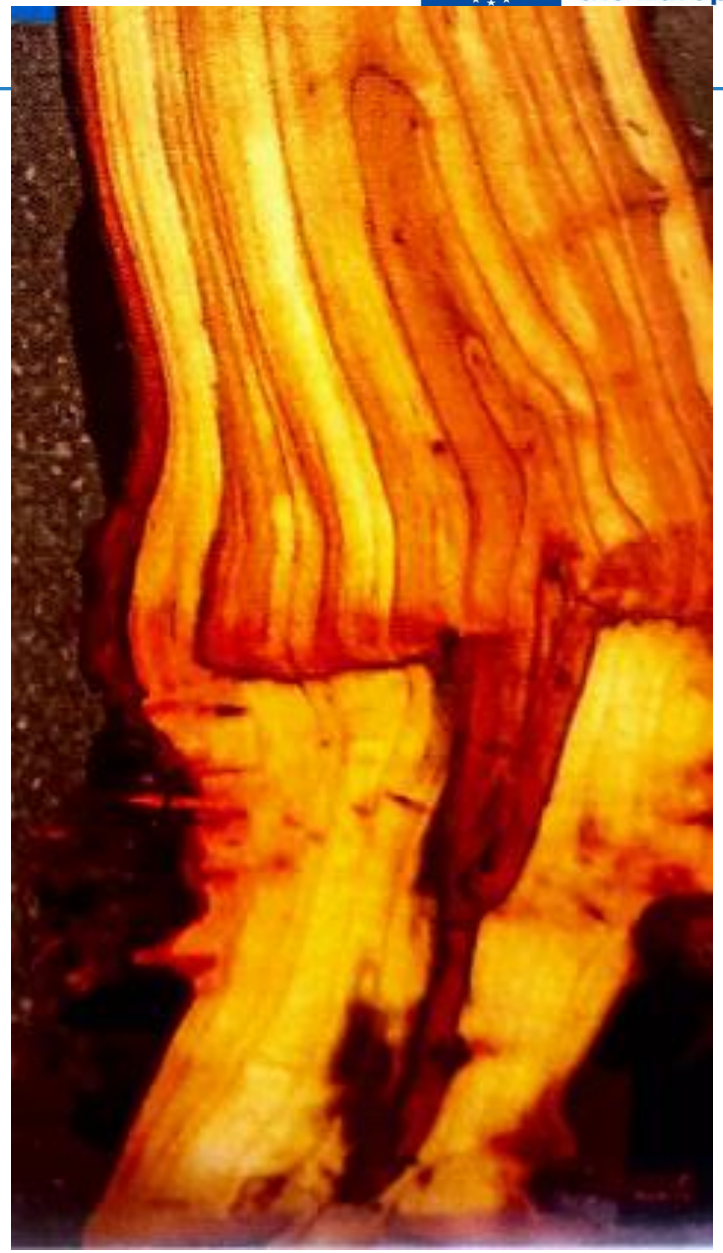
(*P. cerasifera*) Photo Sorrenti

apricot/mirabolano



Baldini, 1986

apricot/peach



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CAMBIUM TISSUE DISCONTINUITY

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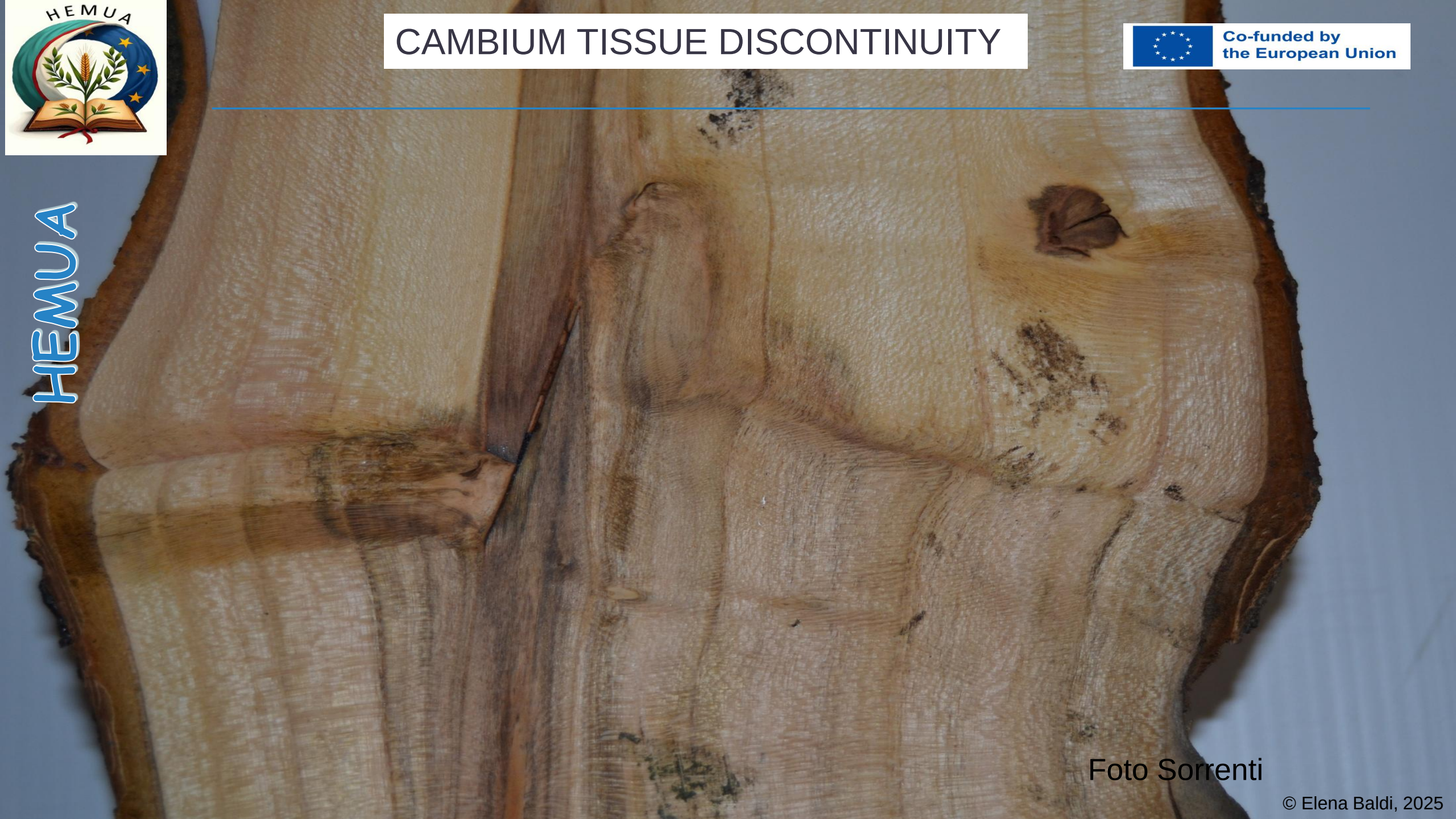

Foto Sorrenti

Malus domestica/Marubakaido (M. prunifolia)

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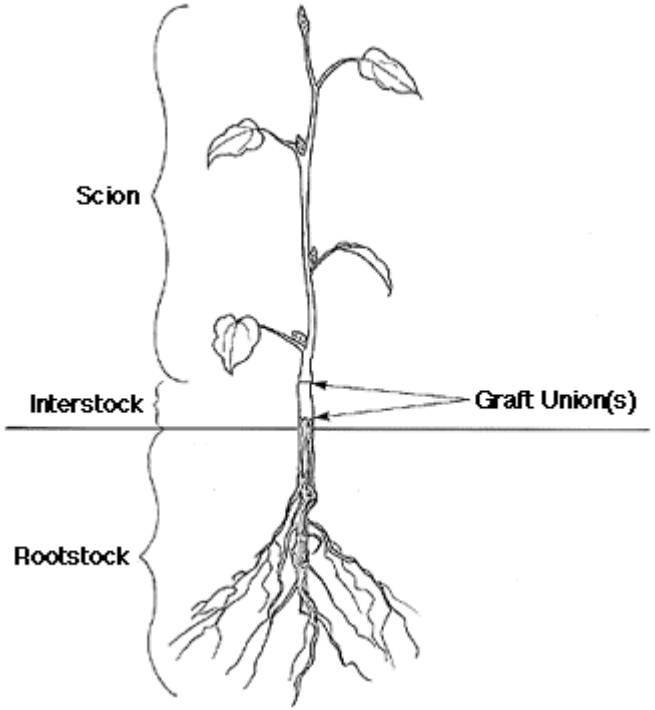
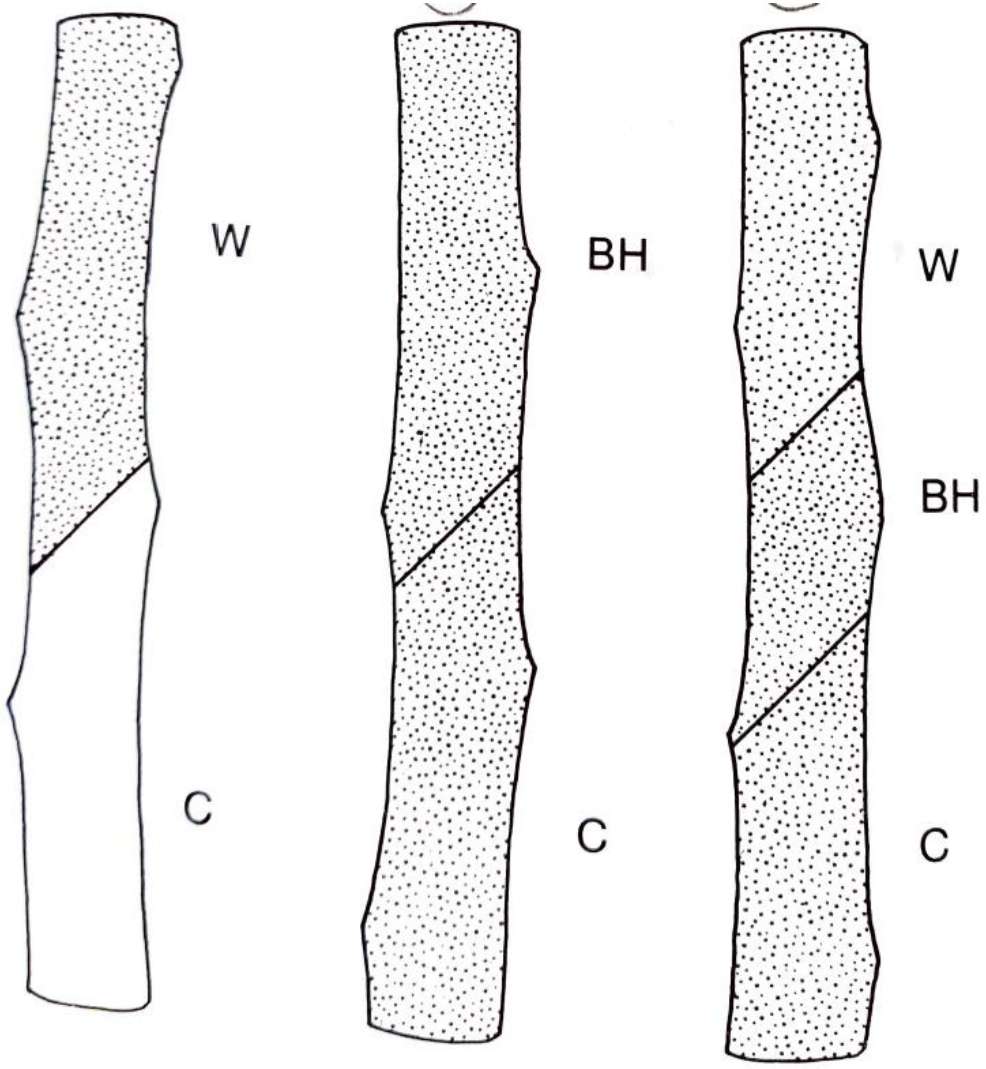


Double-worked graft.

https://content.ces.ncsu.edu/grafting-and-budding-nursery-crop-plants



Baldini, 1986

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Rootstocks can influence plants vigour; a dwarfing rootstock induce an early beginning of yield; but, in the long time lower yield

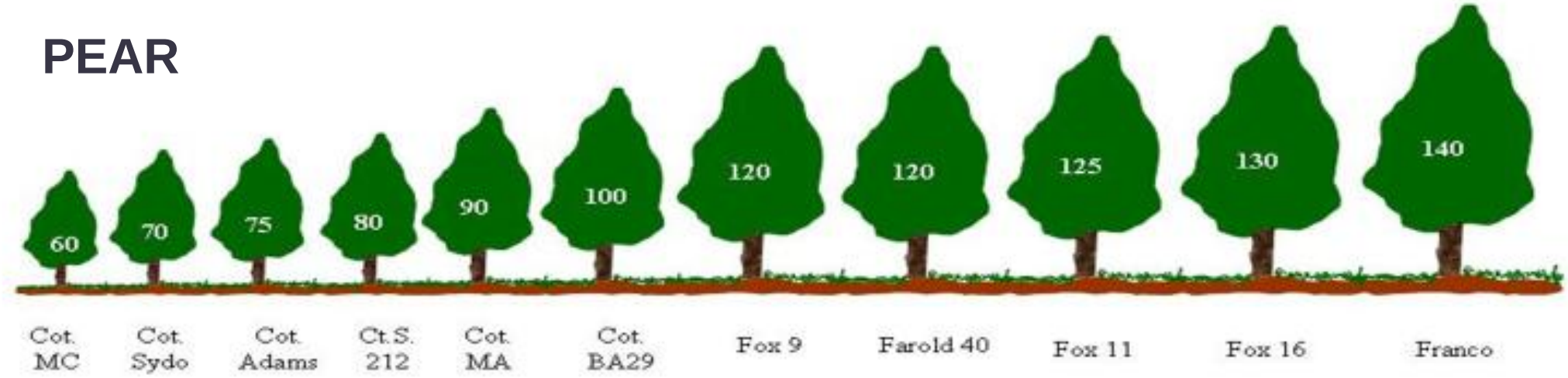
APPLE

DWARFING → M27, M9, M26

AVERAGE → MM106

VIGOROUS → M4, M16, MM111

PEAR



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One-year old grafted tree

www.geoplantvivai.com

CUTTING

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- Based on the capability of some part plants to regenerate roots
- Can be softwood (herbaceous), hardwood or semi-hardwood
- Plants in the juvenile phase produce roots more easily; all agronomic technique (fertilization, irrigation) that keep mother plant in vegetative stage facilitate rooting
- Excessive vigorous shoot (water sprouts or sucker) are very rich in N and are more difficult to root
- Often treated with auxin

HEMUA

ilmondoinungiardino.blogspot.com

HERBACEOUS CUTTING

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- Attention to leaves transpiration
- Sun light to facilitate auxin production
- High environmental humidity (mist and wetting of leaves)
- Bottom heated system

http://www.ecoriscaldamento.com/riscaldamento-elettrico/riscaldamento-serre/

http://www.archiexpo.com/prod/shresth-import-export/product-65375-561523.html

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How to stimulate rooting

1. auxins

2. bottom-heated system (soil T=20-22 °C; air T 5-10 °C)

3. humidification (100% humidity)

4. intermitted mist in order to keep leaves always wet to reduce transpiration and maintain good level of photosynthesis

5. bottom-heated+mist

6. acclimatization

FACTORS INFLUENCING ROOT PRODUCTION

1. presence of adventitious roots
2. cortex structure
3. shoot polarity
4. presence of bud and leaves
5. auxins supply (IAA)
6. ethylene stimulates rooting
7. gibberellins reduce formation of adventitious roots, but stimulate elongation
8. carbohydrate concentration in cuttings
9. cuttings age
10. mother plant physiological status

MICROPROPAGATION (*in vitro culture*)

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- Development of new shoots from dormant buds, shoot tips or axillary buds, thanks to the effect of cytokinin's in unblocking apical dominance
- The regeneration of plants from somatic cells (totipotency) through the differentiation process
- The main phases are:
    1. Explant collection and sterilization
    2. Shoot multiplication phase
    3. Rooting phase
    4. Acclimatization

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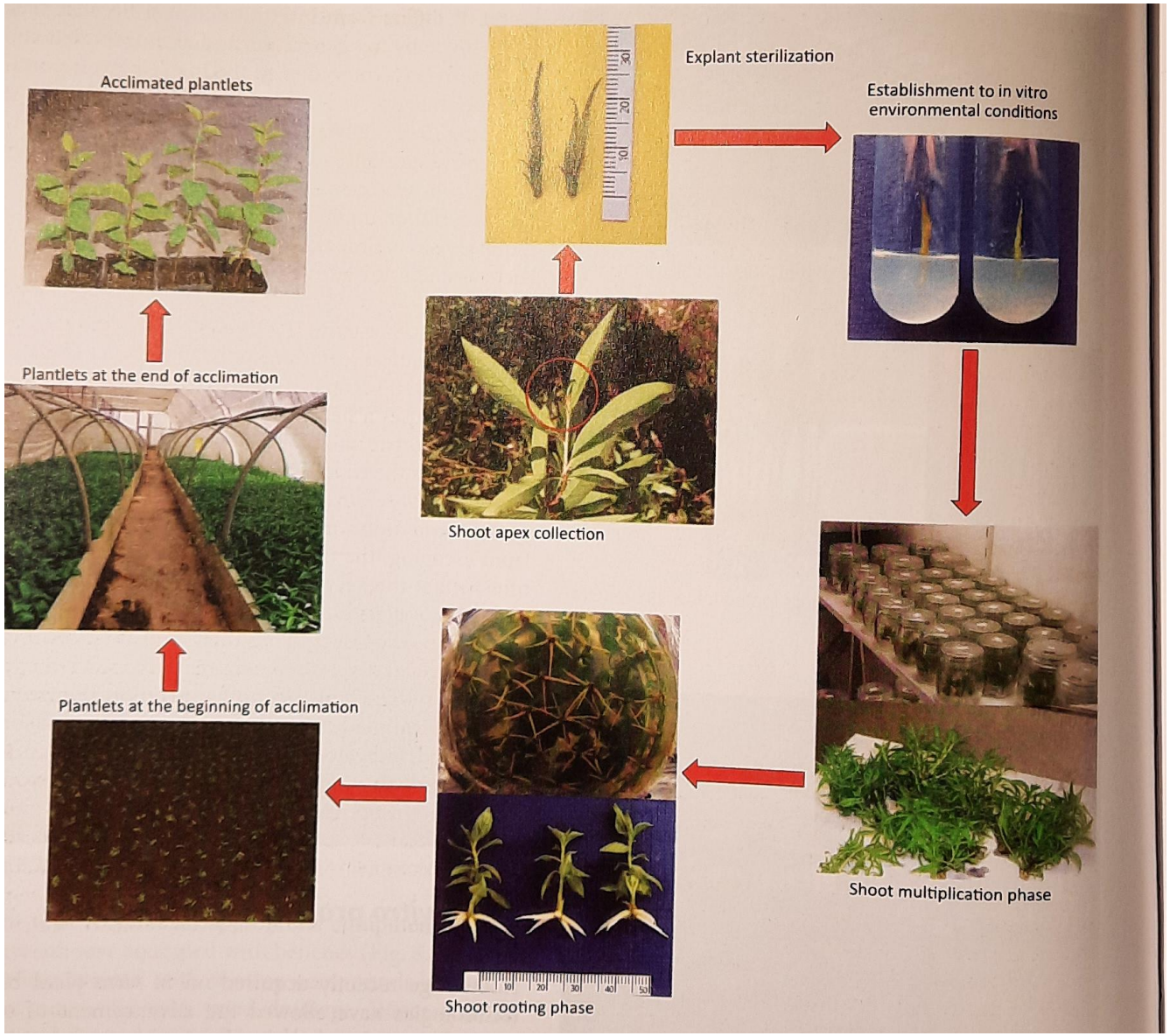
1. Explant collection and sterilization → dormant buds, shoot tips and axillary buds are collected from young shoots from mother plants, washed with sterile water and sterilized with EtOH or NaClO
2. Shoot multiplication phase → climatic cells (20-25°C), 16 h light, cultures no longer than 1 month and no more than 12 cycle; use of cytokinin and gibberellins
3. Rooting phase → root inductions with auxins
4. Acclimatization → transplanted in peat substrate, greenhouse conditions, change from heterotrophy to autotrophy



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MICROPROPAGATION ADVANTAGES

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- ✓ Fast propagation
- ✓ Recovery from infections
- ✓ Reduced production cost
- ✓ Possibility to propagate species that do not respond to other techniques
- ✓ Good sanitary conditions of plants
- ✓ Easy scheduling of plant production independent from natural environmental conditions
- ✓ Uniformity of plants
- ✓ Short multiplication period

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MICROPROPAGATION DISADVANTAGES

- X risk of mutation
- X come species can not be propagated
- X high cost for laboratory and labour

LAYERING

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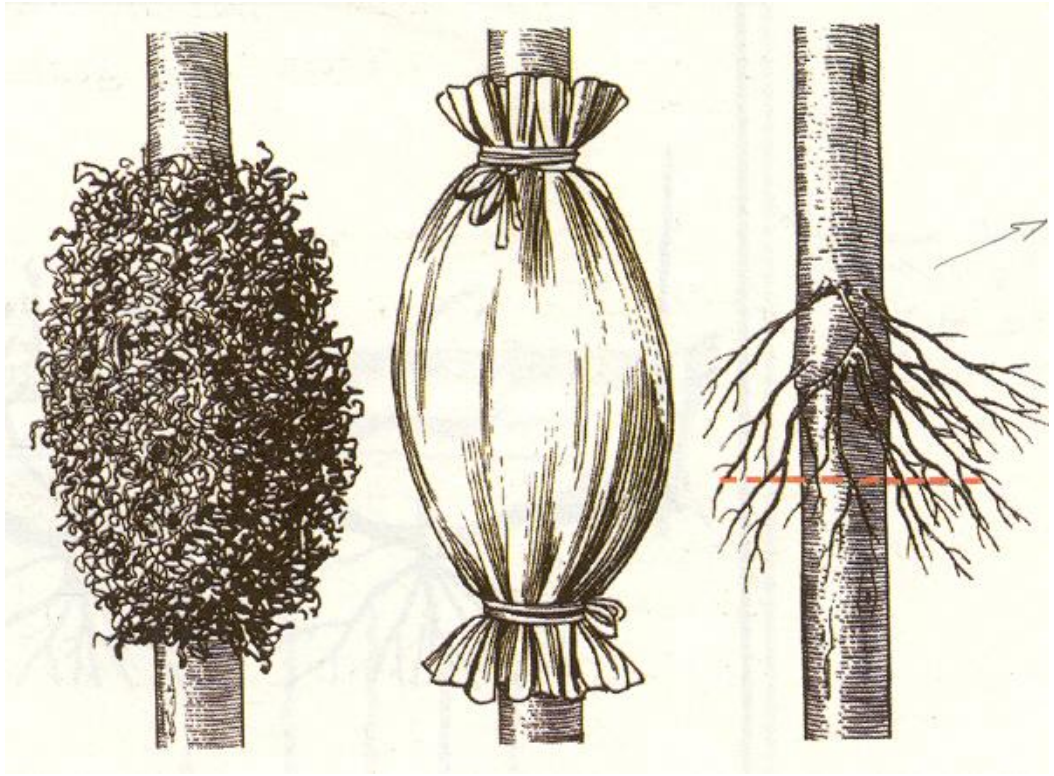
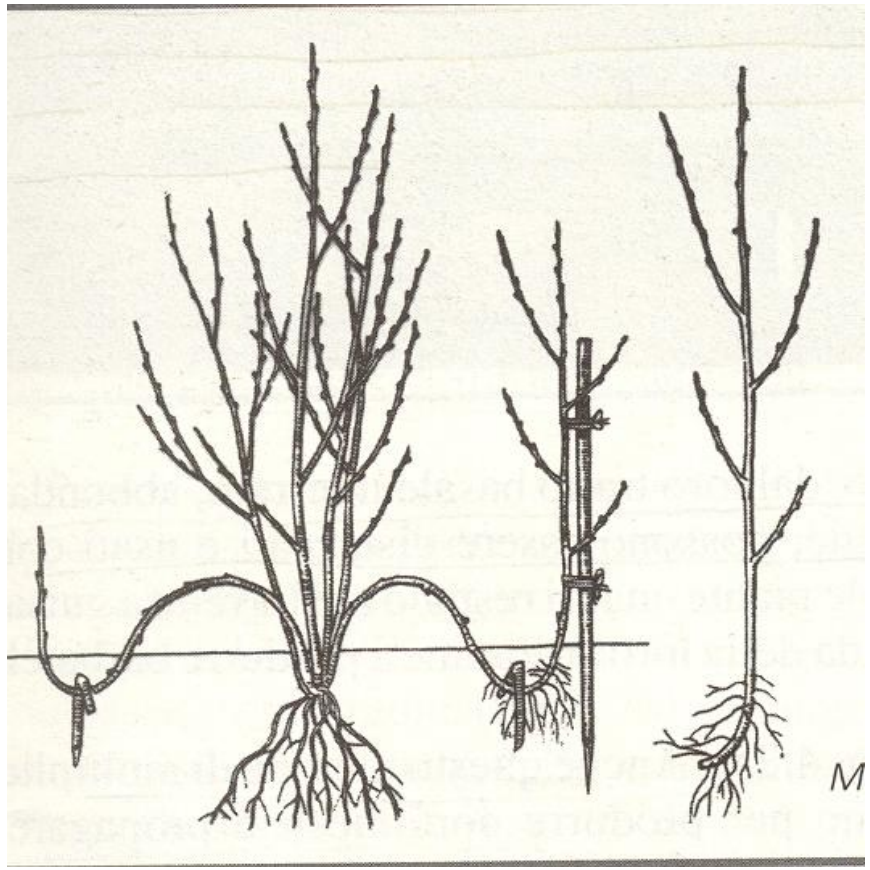
- Shoots or branches are deprived of lights in order to force the formation of roots.



AERIAL LAYERING → a piece of branch is wrapped in a layer of moist peat, held in a plastic cover



ARCHED LAYERING

Baldini, 1986

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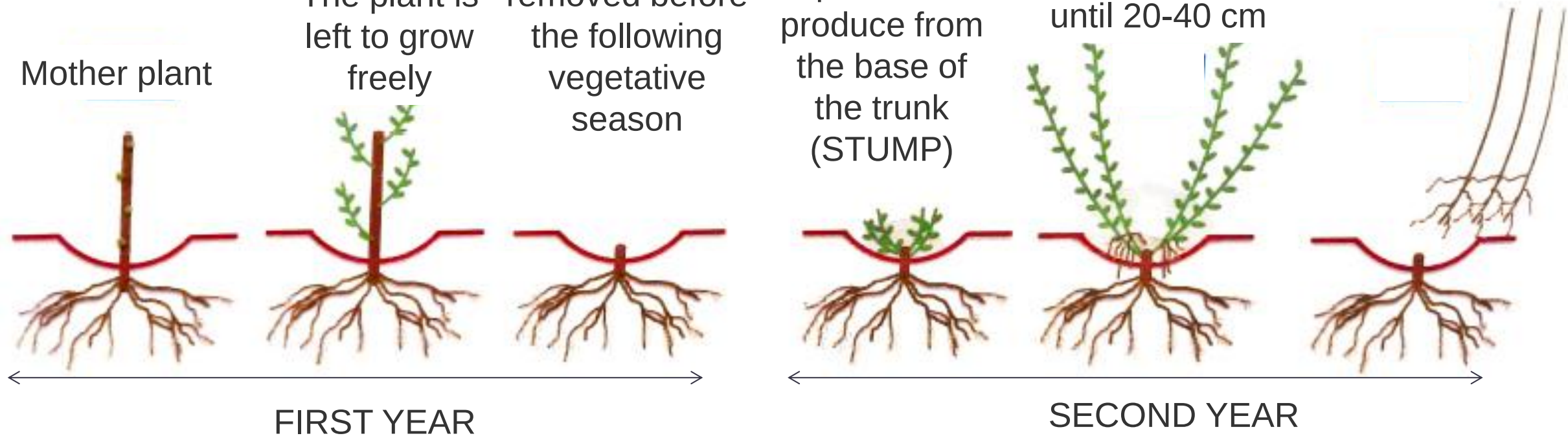
STUMP LAYERING → widely used for apple and quince rootstock.

This technique used the ability of some species to generate meristems (adventitious roots) on shoots in active growth



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TRENCH LAYERING → new shoots form along the full length of the branch upon vegetative recovery of the buds

The maiden is positioned at an oblique angle

Buds closed to the surface are fostered to sprout

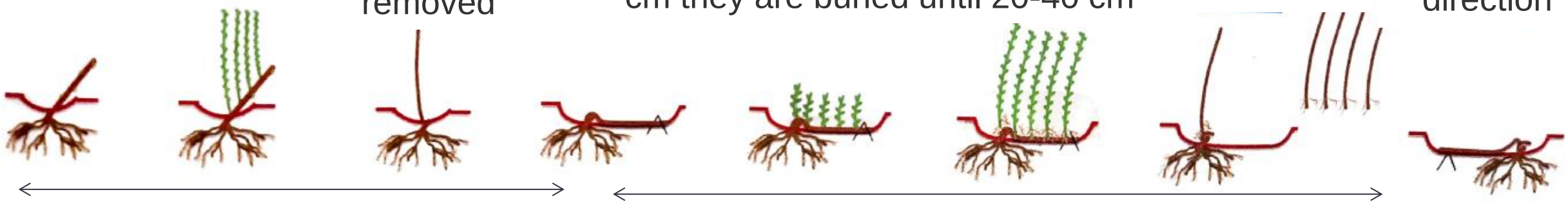
In autumn all the upper part is removed

The branch is bent down into a ditch along the row

When new sprouts reach 15-20 cm they are buried until 20-40 cm

After leaves fall plants are taken off

In the following year the shoot is bent in the opposite direction



FIRST YEAR

SECOND YEAR

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