Castanea sativa Mill.

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Propagation

Seed propagation

Castanea sativa flowers from May to early July according to climate and latitude, and the flowers remain highly receptive for 3–4 weeks. Pollination involves both wind and insect vectors, especially bees, and it is influenced by climatic conditions. In dry conditions, wind disperses pollen very effectively, while in wet conditions, pollen viscosity limits dispersal (Cuenca Valera and Majada Guijo, 2012). Strong and persistent precipitations can limit pollination.

Seeds (nuts) are enclosed in a prickly, hard involucre (bur). Most seeds fall to the ground free from the burs and can be collected manually, using special vacuum cleaners, or by self-propelled harvesters from late September to early November. Manual harvesting can be done directly with rakes and gloves or with nets placed under the trees during thinning or before to catch spontaneous falls. Nets, which can be laid on the ground or suspended, speed up collection and cleaning but involve installation costs and can be damaged by wildlife (Cuenca Valera and Majada Guijo, 2012). Sweeping machines or vacuum cleaners are attached to a tractor, are self-propelled, or in some cases portable. Their use will depend on the characteristics of the terrain and the plantation (access, slope, size of the plot, arrangement of the trees, soil cleanliness) (Cuenca Valera and Majada Guijo, 2012).

Damaged seeds (e.g., those affected by insects) can be removed using densitometric methods in water. Cuenca Valera and Majada Guijo (2012) suggested the following procedure to prevent insect larval development and enhance nut preservation: a) immersion in hot water (48–50°C) for 30–45 min followed by 8–12 h in cold water to restore initial thermal conditions and avoid overheating; b) immersion in cold water for 5–8 days (nuts to water volume ratio, 2:3) to create low-oxygen conditions. This eliminates aerobic microorganisms causing molds and rots, and promotes anaerobic microorganisms that induce slight lactic fermentation, reducing the sugar content and thus the food source for rot fungi. The presence of characteristic CO_2 bubbles on the water surface indicates that the treatment is complete. Water renewal, usually partial, depends on both the quantity of nuts and the renewal frequency (more frequent renewals use smaller amounts of water).

The seeds are recalcitrant (Pence, 1992) and to preserve them they are typically stratified in moist sand or peat moss at 1–4°C for 2–3 months before sowing. Seeds cannot be stored for long periods even under refrigeration, as they will lose their germination ability. *C. sativa* nuts can also be stored for a few months by sealing them in a polyethylene, 50-μm thick bag at 4°C. Alternatively, seeds can be stored at 50%–55% relative humidity at 0–3°C overwinter (Cuenca Valera and Majada Guijo, 2012). When stored in dry conditions, germination capacity rapidly declines. In addition, when stored at temperatures below 0°C, nuts freeze and become unusable (Stilinović, 1987).

A high germination rate occurs after cooling in humid conditions to break seed dormancy (Mencarelli, 2001; Fernandes et al., 2022). To achieve more synchronized germination, nuts are stratified in moist sand or peat moss at 1–2°C for at least 2 months, but no more than 4–5 months. At the end of stratification, higher temperatures promote germination (Beccaro et al., 2019a).

Germination tests, following ISTA standards, involve placing seeds on filter paper under high humidity conditions at a night-day temperature of 20–30°C. The first count is performed after 7 days, with the final count after 21 days. The quantitative characteristics of *C. sativa* seeds are shown in Table 11.4.

Vegetative propagation

Traditional *C. sativa* orchards consist of grafted plants, as grafting is the most effective method for vegetative propagation of selected fruit varieties. Other propagation methods are less suitable for large-scale production of trees intended for fruit production, due to cost, low effectiveness, or both, as mature trees (those bearing fruits) are usually unable to root. Techniques such as layering and rooting cuttings (both soft- and hardwood), and micropropagation are used to produce self-rooted rootstocks or self-rooted plants for orchards.

Grafting is done in different seasons according to the technique used: in winter, whip graft; in winter–spring, triangle (inlay) graft, splice (slice) graft; in early spring, lateral graft and Cadillac; in spring, under bark graft and crown graft; in spring-summer, T-budding; in summer, chip budding, and semisoft scion graft (Bounous, 2014). The scions used for winter grafting are collected from healthy and well-lignified one-year-old branches. If the mother plants are old, severe pruning can induce new vigorous shoots suitable for scions. The scions are generally cut when the trees are completely dormant in winter and are stored in closed polyethylene bags at 1–2°C. The scions

Table 11.4 Characteristics of seed lots of *Castanea sativa*.

Fruit to seed yield (% in weight)	Purity (%)	No. seeds/kg	Germination capacity (%)
100 (80–100)	100 (100)	50–257 (120)	60–90 (79)

Mean values in brackets.
Gradi (1980), Piotto (1992), Berrocal et al. (1998), Catalán (1991), Ribeiro et al. (2001), Navarro Cerrillo and Gálvez (2001), Louro and Pinto (2011), Cuenca Valera and Majada Guijo (2012), Moraldi (2024).

used for summer grafts are normally cut from the mother trees right before the moment of grafting. Graft wounds must be protected to prevent tissue dehydration and pathogen attack. Cold (for field use) or warm (for nursery use) commercially available dressings are composed of fats, natural and synthetic resins, or vegetable oils. To prevent *Cryphonectria parasitica* infection, grafts can be treated with formulations containing biocontrol agents, copper, or benzimidazole fungicides (Beccaro et al., 2019a). Hot water treatment has been tested to control pest and diseases in propagation processes and it is very effective for the disinfection of scions against *Dryocosmus kuriphilus* (Ciordia et al., 2020).

Vegetative propagation uses the low layering or stooling method (Cuenca Valera and Majada Guijo, 2012; Beccaro et al., 2019a; Serdar et al., 2019). Briefly, the plant is cut at ground level and new shoots formed in spring are covered with soil to promote rooting. The plant is cut annually, and the best shoots are selected while removing the rest. Selected shoots are defoliated at the base between mid-May and mid-June, lightly scraped, and treated with a paste containing rooting hormones (mainly auxins). Traditionally, pastes for rooting have been made by dissolving indolebutyric acid (IBA) in petroleum jelly, with concentrations ranging from 4%–8% based on shoot lignification. Shoots are then banded with a wire ring below the hormone-treated area to enhance photosynthate accumulation and promote root formation. To improve rooting, select young shoots and use loose, moderately acidic, and well-drained soils. Rooted sprouts are detached form mother plants and lifted from mid-November, after leaf drop. These plants often have poorly balanced root systems, with peripheral shoots showing base curvature, which can be corrected by cultivating the plants for an additional season in the nursery (Cuenca Valera and Majada Guijo, 2012).

Cuttings are an alternative vegetative propagation method that requires less land for the deployment of mother plants, but it needs more specific and engineered facilities, such as air-humidity control systems. Semiherbaceous stems are collected in June from mother plants grown in big pots under shade, and they are cut into one to three bud sections. The base of these sections is dipped in a hormonal solution (1000 ppm of IBA) for some minutes and placed in trays filled with a highly draining substrate to root inside rooting tunnels in the greenhouse. A fog system in the summer ensures high air humidity to prevent dehydration of cuttings during the rooting process. Rooted cuttings are transplanted to pots the next spring to resume growth. These plants develop a higher-quality rooting system compared with those produced by layering. In addition, as they are potted, they have a longer period for commercialization (Beccaro et al., 2019a; Serdar et al., 2019).

Micropropagation is another vegetative propagation method that allows quick propagation starting with very little material, although it is limited to technologically advanced nurseries (Beccaro et al., 2019a). Successful results require healthy, juvenile shoots (Vieitez, 1992), but the plants produced by this method are free from viruses and diseases, making it an ideal system for moving plants around the world.

The micropropagation protocol for *C. sativa* through axillary shoots can be divided into four stages: establishment, shoot proliferation, shoot rooting, and hardening. Various media have been used, but the most adopted are the GD mineral medium (Gresshoff and Doy, 1972) and the MS medium (Murashige and Skoog, 1962). Apical necrosis and chlorosis can be avoided by using half-strength nitrate in the culture media (Vieitez et al., 1986).

Seedling cultivation

To establish forest stands, sexually propagated trees are used in compliance with legal requirements (Directive 1999/105/CE). *Castanea sativa* seeds are typically collected from coppice stands or orchards (Ciordia et al., 2012), and rarely from wild populations. However, it must be taken into account that orchard stands exhibit less genetic differentiation compared with coppices and old high forest stands (Seabra et al., 2001). For fruit orchards, nursery production includes seedlings used as rootstocks, grafted plants, and vegetative copies of selected material (e.g., clonal rootstocks, grafted plants, or other asexually propagated plants of selected cultivars). Clonal rootstocks are produced by rooting shoots from clones selected for their tolerance to root diseases, dwarfing effects, and grafting compatibility.

There is scant literature on the quality of *C. sativa* reproductive forest material. This is because the species rarely loses its ability to produce new shoots from stumps, which ensures its persistence and regeneration in forests. Moreover, many *C. sativa* plantations have been established for creating or restoring orchards, where grafted nursery material is more commonly used. This has led to a lack of an approach for evaluating the material that aligns with the target plant concept or assesses the field performance of the material (Clark et al., 2023). Moreover, planting *C. sativa* is not currently a priority for species maintenance as it is for *Castanea dentata* in the US. Since *C. sativa* is widely distributed across Europe, the main objective is growing forest reproductive material to restore stool density to suitable levels in coppice stands (Manetti et al., 2022).

The main problem in establishing nursery seedlings of *C. sativa* is the availability of healthy nursery material free from the pests *Phytophthora cambivora* and *Cryphonectria parasitica* that are the two most influential factors hindering the success of reforestation projects. For this reason, the breeding and production of planting material is mostly directed toward the creation of tolerant genotypes, both for rootstocks and for cultivars (which are mainly propagated by grafting).

Container plants

High germination rates occur after cooling in humid conditions to remove seed dormancy. To synchronize germination, the nuts should be stratified in moist sand or peat moss at 1–2°C for at least 2 months (maximum 4–5 months). At the end of the stratification, increased temperatures promote germination and both the primordial root (radicle and hypocotyl), and the young shoot (epicotyl) emerge from the torch at the top of the seed. After removing the root apex, the pregerminated nuts are sown (flat side down) in raised beds, trays, or pots. The soil should be soft and well drained. The sowing depth is generally no more than 3–5 cm. In *C. sativa*, as in other recalcitrant, large, nutrient-rich seeds; the weight of the seed affects seedling development (Tumpa et al., 2021). Nursery cultivation can begin in March–April, though if the cultivation is done in a greenhouse, sowing can start in February. The latter is useful to obtain larger 1-year-old rootstock.

The volume of the container can range between 400 and 1000 mL to grow 1- or 2-year-old seedlings. The growing medium can be 70%–80% peat (or coir) mixed with 20%–30% perlite to improve aeration. Compost can replace peat up to 50%, obtaining plants of the same size and quality. Pine bark is not recommended due to the occurrence of *Fusarium circinatum* (Beccaro et al., 2019a).

Cultivation density is crucial for promoting healthy growth and producing high quality seedlings. If it is too high, overlapping leaves can result in shading and reduce water availability in each container cell. An optimal density is 100 plants/m^2 (Beccaro et al., 2019a) and density should not exceed 250 plants/m^2 (Cuenca Valera and Majada Guijo, 2012). Using hydrogels mixed with the substrate can help maintain water levels between irrigations (Cuenca Valera and Majada Guijo, 2012).

Although slow-release fertilizers mixed in the initial substrate can be used, fertilization of the container plant is usually carried out through fertigation systems; as an example, NPK (14:7:14) mineral fertilizer can be applied once a month at a rate of 23.3 mg/plant of N in each application (Fuertes-Mendizábal et al., 2021). Initial fertilization should be high in nitrogen to support plant growth and phosphorus to establish the root system. During the hardening phase, we recommend reducing nitrogen and increasing potassium to enhance resistance to water and heat stress. High nitrogen fertilization should be avoided to prevent strong leaf growth, which can expand the canopy and make irrigation uniformity difficult (Cuenca Valera and Majada Guijo, 2012). Plant growth-promoting (PGP) biostimulants can complement fertilization since these microorganisms provide multiple benefits to plant growth; in addition, they can significantly improve plant tolerance to water deficit (Fuertes-Mendizábal et al., 2021).

Inoculation with ectomycorrhizal fungi has been proven to improve water uptake and mineral nutrition and to prevent fungal root diseases (Crawford, 1995). Inoculation with *Boletus* spp. and *Morchella conica* can improve seedling quality (Gonzalez et al., 2010); valuable *Tuber* spp. have also been tested with some success (Crawford, 1995).

For container-grown plants, observed and recommended values of morphological attributes are reported in Table 11.5. A straight stem with no branches can be recommended for reforestation or forest restoration projects (Fig. 11.3).

Table 11.5 Observed morphological and physiological attributes and recommended values of high-quality 1 + 0 container seedlings of *Castanea sativa*.

	Container seedlings		Bare root seedlings	
Attributes	**Observed values**	**Recommended values**	**Observed values**	**Recommended values**
Shoot height (cm)	33–77	(40) 50–70	10–86	40–60
Root collar diameter (cm)	0.96–1.15	>0.6	0.52–1.34	>0.6
Shoot dry weight (g)	1.4–9.3		3.1–28.5	
Root dry weight (g)	2.2–8.7		14.7–22.2	
Plant dry weight (g)			35.1–58	
Shoot/root dry weight ratio			1.1–1.8	

Hipps et al. (1997), Çiçek and Tilki (2007), Álvarez-Lafuente et al. (2018), Tumpa et al. (2021), Fuertes-Mendizábal et al. (2021).

Figure 11.3 One-year old seedlings of *Castanea sativa*. *(Barbara Mariotti.)*

Bare root plants

The same recommendations as for container cultivation apply to seeds. The soil should be soft and well drained, with a seeding depth generally not exceeding 3–5 cm, and covered with a sterile substrate, such as peat, to prevent dehydration and weed growth (Cuenca Valera and Majada Guijo, 2012). The soil should be compacted with a roller to eliminate air pockets that could hinder germination. It is advisable to protect the sowing from predators using repellents or nets, which can also provide shade. Depending on climatic conditions and the seed stratification period, germination will typically be completed within 4–6 weeks (Cuenca Valera and Majada Guijo, 2012). For producing 1-year-old seed rootstock, sowing in raised beds is recommended, with row spacing typically 30–40 cm—at least 20 cm (Cuenca Valera and Majada Guijo, 2012) - and rows 80–100 cm apart (Beccaro et al., 2019a). Irrigation, fertilization, and control of weeds and pathogens are crucial. Hoeing or mechanical weed control methods are used, although plastic mulch can also be employed; however, this is not considered an environmentally sustainable practice (Beccaro et al., 2019a). Root pruning can improve root system structure, making it shorter and more fibrous, which enhances plant lifting and survival (Cuenca Valera and Majada Guijo, 2012). Root pruning also favors the ratio between the shoots and roots by slowing shoot growth. Lifting is carried out in winter, and planting should be done during the same period before the growing season starts. Small plants can be transplanted and cultivated for an additional year in the nursery.

Observed and recommended values of morphological attributes of bare root plants are reported in Table 11.5. Plants with straight stems and no branches can be recommended for reforestation or forest restoration projects where timber is the main objective.

Field establishment

C. sativa plantings for silvicultural purposes are established to create new high-forest stands (Balandier and Dupraz, 1999; Bourgeois et al., 2004). Artificial regeneration is not needed when stumps are present, as this species has a strong and long-lasting resprouting capacity. In such cases, seedlings grown from seeds cannot survive the competition. In Italy, *C. sativa* has been introduced into both pure and mixed productive plantations to provide high-value timber (Buresti-Lates and Mori, 2009), with recommendations to use protective measures against browsing. Similar plantings have been implemented in other EU countries with funding from European Community Support Frameworks (Bourgeois et al., 1991; Patrício, 2006; Patrício and Nunes, 2017). The afforestation rate of *Castanea sativa* in Portugal, northern Spain, and France was estimated at 4500 ha/year (Alvarez-Alvarez, 2004). In France, for timber production, a recommended planting density is up to 1200 plants/ha (IDF, 1990; Patrício, 2006; Benedetti-Ruiz et al., 2023).

C. sativa trees thrive in deep, cool, well-drained, and limestone-free soils. Therefore, conducting a soil analysis is recommended. Ideal soils should be light to medium-textured to prevent the risk of fungal infections like *Phytophthora* sp., which causes ink disease. Soils should be at least 40 cm deep and moderately acidic, with a pH no lower than 4.2 (Cuenca Valera and Majada Guijo, 2012). While *C. sativa* can tolerate frost, they are sensitive to late spring frosts. The ideal water supply is at least 600 mm during the vegetative period.

It is possible to establish plantations by direct seeding, sowing at a depth of 4–5 cm, typically with two to three seeds per hole. Spring sowing is recommended with stratified seeds to minimize the time the seeds remain in the soil without germinating, thus reducing damage from rodents and birds. Seed shelter, a new tool to protect seeds from rodents (Castro et al., 2015), can promote seeding success.

For timber plantations, one seedling per hole is preferred as it is more robust than two seedlings. *C. sativa* is challenging to establish, so taking great care and monitoring the seedlings during their initial 2–3 years is essential. Fertilizing the planting hole, ensuring adequate water during the first summers, and shielding the plants from herbivores and sunburn with 1.20 m protective tubes are recommended (Cuenca Valera and Majada Guijo, 2012). The optimal planting time is late autumn or early winter, while the plant is dormant, and avoiding planting on excessively windy or frosty days is crucial.

The choice of planting density depends on whether the objective is to produce timber in monospecific or mixed plantations, timber and fruit, or only fruit. Planting density can be high, medium or low, with or without intercropping. Low-density plantations require more intensive early intervention to develop straight trunks and balanced crowns. Timber plantations generally have higher densities, while fruit plantations are more widely spaced to promote flowering and fruit production. In timber plantations, an ideal density is one that yields 180–250 high quality stems by the end of the rotation period (Cuenca Valera and Majada Guijo, 2012).

For timber production, using average quality plants with minimal pruning typically requires a density of 950–1100 plants/ha, with spacings of 3.5 × 3 m, 4 × 2.5 m, or 3 × 3 m (Cuenca Valera and Majada Guijo, 2012). If high-quality plants are used, density can be lower, but pruning needs to be more frequent. At densities of 400–830 plants/ha, the recommended spacing is 5 × 5 m, 4 × 4 m, or 4 × 3 m (Cuenca Valera and Majada Guijo, 2012). Currently, the 4 × 4 m spacing is common, which reduces planting costs though it requires more intensive pruning. Bourgeois et al. (2004) suggested a minimum row distance of 4 m, with planting densities of 625–1200 plants/ha on agricultural land and 1100 to 1430 plants/ha on forestland. For fruit production, new plantings densities can be as low as 100 trees/ha (10 × 10 m) or much more (250–300 trees/ha) in intensive management. Agroforestry schemes can also be done in lines with the tress spaced 3 m, and the rows separated by 10–12 m, allowing grazing or crop production between the rows (Cuenca Valera and Majada Guijo, 2012).

Several ecological factors can hinder planting success, including early frosts, summer droughts during the first 2–3 years, high soil lime, and temporary waterlogging. In addition, young trees are also highly sensitive to weed competition. Initially, herbicides should be used to control weeds, as mechanical methods can damage roots and potentially spread diseases like ink disease in areas with high soil moisture (Cuenca Valera and

Majada Guijo, 2012). Disc harrows, cultivators, or rotovators are not recommended due to the shallow root system of *C. sativa* trees. Instead, control weeds with manual brush cutters or herbicides in early summer, taking care to damaging the tree if using systemic products. Mulching with cut grass or brush around the base of the trunk helps suppress weeds and maintain soil moisture. In addition to grass, it is essential to control woody shrubs in the early years, possibly using a chain brush cutter. In some cases, such as with brambles, ferns, or broom, competition is minimal, and they can be beneficial by keeping the soil cool, providing shade, and protecting against large game damage (Cuenca Valera and Majada Guijo, 2012). Avoid carrying out these operations during very hot periods to prevent trunk damage and burn issues (Cuenca Valera and Majada Guijo, 2012). Timber plantations are not commonly fertilized, although leaving leaf litter is beneficial for organic matter contribution. Fertilization is crucial in agricultural soils with nutrient deficiencies, however, particularly in fruit production plantations. Recommendations can be found in Cuenca Valera and Majada Guijo (2012) and Beccaro et al. (2019b).

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