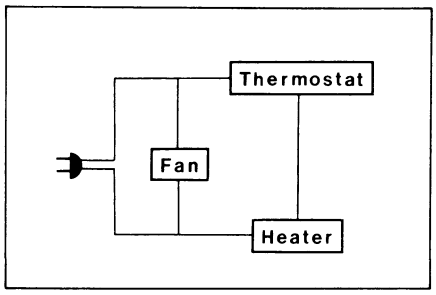

Fig. 2. Electrical wiring diagram showing the connections between the fan and the thermostat and heater.

a 30-cm distance directly in the path of the forced-air outlet. The necessity of proper positioning can be reduced by conveying the warm air to the opposite side of the chamber from the fan intake with a plastic or metal pipe. Spacing 1-cm-diameter holes every 10 cm on the upper surface of the pipe also helps to eliminate "hot spots" in the chamber. Since heat escapes through the sides of the enclosure, plant material in direct contact with the sides of the chamber will be colder than the air temperature. These "cold spots" can be eliminated by ensuring that air can circulate completely around the test material.

If deemed necessary, additional control and safety devices may be added to the chamber. Electric junction boxes with on/off switches and indicator lights could be located on an outside wall. Low wattage fluorescent and incandescent lights may also be installed within the chamber. However, care must be taken so that their heat output does not interfere with the proper functioning of the temperature-regulating elements. Temperature alarms may be justified if very rare or expensive plant materials are used. While increasing the sophistication of the chambers, these additional items also increase their cost.

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Inhibitory Effects of Confection Sunflower Hulls[1]

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Abstract. **Sunflower hull material from Sunflower (*Helianthus annuus* L.) was unsuccessful as an amendment in potting mixtures because of inhibition to top and root growth. Aqueous and methanol extracts from sunflower hulls reduced the rate of germination with lettuce and radish seeds and reduced primary root elongation with lettuce in bioassay tests.**

The initial purpose of this study was to test confectionery sunflower hulls, which are in plentiful supply locally, as a soil amendment in potting mixtures. The hulls were used as half-shells, as well as processed through a hammer mill (Prater equipped with a 4.76-mm, round, hole-perforated half-screen) and through a cracking mill (Ferrel-Ross with 0.5 mm clearance and differential roller speeds of 700 and 1100 rpm). The sunflower hulls were used in ratios of 1:9, 2:8; 3:7, 4:6, and 5:5 with a Bradwell clay loam.

Inhibitory effects soon became apparent on transplanted snapdragon (*Antirrhium majus* L.) seedlings with severely reduced root and top growth as the amount of hull and fineness of grind increased. Newly rooted transplanted coleus (*Coleus blumei* Benth) cuttings showed the same trend, accompanied by increased basal defoliation. Even direct-seeded sunflower (*Helianthus annuus* L.) plants showed the same trend, although not as marked as with the other 2 plants.

Since the first 3 test plants used showed declining root and top growth, the amendment phase of the study was discontinued and the emphasis switched to bioassay tests of extracts from the hulls. The fact that finer-ground material gave increased inhibition suggested that the more broken surfaces allowed more release of inhibitory sustance(s).

Accordingly, 24-hr Soxhlet extractions were made using 250 ml of either distilled water or methanol for 35 g of finely ground (Wiley Mill) hulls. The solvent was evaporated to dryness under vacuum and the final extract was obtained by redissolving the water-soluble portion in 100 ml of distilled water. Seed germination of lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativum* L.), as well as primary root elongation of lettuce, was carried out on the extract and on 8:2, 6:4, 4:6, and 2:8 dilutions (extract: distilled water by volume). Distilled water was used as the control. Six ml of the test solution was used in each of the 4 replicates to moisten the

Table 1. Average germination and mean germination rate of lettuce and radish seed, subjected to various dilutions of aqueous extracts of sunflower hulls.[z]

Extract: distilled water ration (v/v)	Germination (%)					Mean germination rate (days)
	Day 2	Day 3	Day 4	Day 5	Day 6	
			Lettuce			
10:0		17	50	72	87	4.3
8:2		59	84	95	93	3.4
6:4		61	73	82	86	3.5
4:6		70	82	89	94	3.3
2:8		88	90	95	95	2.9
0:10		95	97	98	98	2.2
LSD 5%		17.9	15.1	9.2	3.8	0.4
1%		24.5	20.9	12.8	N.S.	0.6
			Radish			
10:0	22	89	97			2.9
8:2	24	82	97			2.9
6:4	41	94	100			2.7
4:6	45	91	100			2.6
2:8	82	97	99			2.2
0:10	74	94	100			2.3
LSD 5%	20.2	7.7	N.S.			0.2
1%	28.0	N.S.	N.S.			0.3

[z]25 seeds on blotting paper moistened with 6-ml aliquot.
[y]Extract was 100 ml from 35 gm finely ground hulls soxhlet extracted for 24 hr.

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Table 2. Average root elongation of germinated lettuce seed measured in the second 24-hr period when subjected to various dilutions of aqueous and methanol extracts of sunflower hulls.[z]

Extract:[y] distilled water ratio (v/v)	Avg root elongation (cm)	
	Distilled water	Methanol
10:0	0.9	1.5
8:2	1.3	1.9
6:4	1.0	1.3
4:6	1.3	1.2
2:8	2.2	3.9
0:10	8.6	8.6
LSD 5%	0.8	2.9
1%	1.1	4.6

[z]5 seedlings on blotting paper moistened with 6-ml aliquot.
[y]Extract was 100 ml from 35 finely ground hulls soxhlet extracted for 24 hr. Both distilled water and methanol extracts were evaporated to dryness under vacuum and redissolved in distilled water.

blotting paper in Petri dishes. Four replications of 25 seeds were placed on the moist blotting paper, the Petri dishes were taped closed, and germination tests were conducted in the dark at a temperature of 22°C. Counts were made daily until germination was deemed complete. In the root elongation test, lettuce seed germinated on distilled water-moistened blotting paper until radicle protrusion was visible. Four replications of 5 germinated seeds were then transferred onto blotters moistened (6 ml) with the test solutions. The Petri dishes were again taped closed and placed in racks in a near vertical position in the dark at 22°. The test plants were allowed to stabilize for 24 hr, and then the primary root elongation during the next 24-hr period was measured.

Split-plot analyses indicated that there were no significant germination differences in the response to distilled water and methanol extracts. Using the water extract as an example (Table 1), there was a pronounced inhibitory effect on germination of both lettuce and radish, with lettuce being slower to germinate than radish, and the inhibitory effect more prolonged.

In the root elongation bioassay (Table 2), the distilled water and methanol extracts yielded significantly different results (split-plot analysis P = 5%) with the distilled water extract giving greater inhibition of root elongation. Both extracts and dilutions severely reduced primary root elongation compared to the control (0:10 ratio).

Wilson and Rice (2) have reported that certain associated species show reduced growth which was not due to competition when grown with sunflower (*H. annuus*) under field conditions. They also noted inhibitory effects of extracts from various parts of the sunflower plant on the germination of sunflower seeds and the seeds of numerous other plants. Although they showed inhibitory effects from extracts of the inflorescence, no specific mention was made of the seeds or hulls of sunflower. Later, Anderson et al. (1) reported similar allelopathic inhibition of growth on radish, *Raphanus sativus* L., and wheat, *Triticum aesativum* L., when associated with *Helianthus mollis* Lam.

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In Vitro Propagation of *Castanea sativa* Mill. through Meristem-tip Culture[1]

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Abstract. **Meristem tips of *Castanea sativa* Mill. from *in vitro* cultures derived from seedling explants initiated multiple shoot-buds and developed shoot-bud in the presence of 1 mg/liter 6-benzylamino purine (BA) + 0.01 mg/liter indolebutyric acid (IBA). Root formation was readily achieved within 20 to 25 days when excised single shoots were transferred onto a fresh medium supplemented with IBA (1 mg/liter).**

Castanea sativa, widely distributed throughout northern regions of Spain (2), is highly valued for its economical and ecological importance (3). Unfortunately, habitat has been decreasing in recent decades due to several fungal and viral diseases. Propagation of chestnut by tissue culture would be desirable since meristems from *in vitro* cultures are often disease-free and genetically stable.

Explants from chestnut seedlings were cultured on a basal medium: Cheng's mineral salts (1) and (per liter) thiamine 0.25 mg, inositol 0.25 g, sucrose 30 g, and bacto-agar 6 g plus 5 mg BA. All media were adjusted to pH 5.5 before autoclaving. Cultures were maintained at a constant temperature of 25°C and 18-hr photoperiod with a light intensity of 2.5 klx.

Explants formed multiple shoots from which meristem tips (0.3 × 0.2 mm) were excised and cultured for 2 weeks with 1 mg/liter BA + 0.1 mg/liter IBA and then transferred to various concentrations of IAA or IBA (0.01, 0.1, 1, or 10 mg/liter) alone and in combination with zeatin or BA (0.1, 1, 5, or 10 mg/liter). There were 5 cultures per treatment.

Meristem tips developed as a single shoot in the presence of 1 mg/liter IBA; however, with 1 mg/liter BA + 0.01 mg/liter IBA, several new shoots (3 to 5 per explant) originated directly from the meristem tip (Fig. 1). The new shoots formed could be transferred for rooting after 20 days in culture (Fig. 2). To increase the number of shoot-buds generated, the amount of BA applied was increased to 2 and 5 mg/liter (Fig. 3). The number of shoots initiated increased greatly, but callus also developed.

Single shoots longer than 2 cm were excised from proliferating cultures and explanted onto half-strength basal medium supplemented with 1 mg/liter IBA (Fig. 4a). Under these conditions, the single shoots rooted readily within 20 to 25 days; shoots grew normally showing perfect leaf enlargement. Although IBA at higher concentrations of 2, 5, and 10 mg/liter stimulated some rooting, more callus originated from the base of the excision zone along with roots. Furthermore, the shoots grew more slowly and frequently had necrosis and malformations on the shoot tips.

Plants were established in soil despite some transplanting shock and plants grew normally. These results indicate that large-scale micropropagation of chestnut trees is feasible.

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