

Table 1. Distance of total weed control between Nov. 4, 1973, and Oct. 1974, in peach seedling nursery rows using string impregnated with simazine, atrazine, and diuron.

Treatment ²	Distance of total weed control (cm)			
	One strand of string		Two strands of string	
	Treated side of row	Untreated side of row	Treated side of row	Untreated side of row
No string	0c ^y	0d	0b	0a
String on soil surface	23a	8ab	38a	10a
15 cm above soil surface	22a	9ab	42a	7a
30 cm above soil surface	12b	4bc	34a	2a
In tree tops (30-75 cm)	2c	1cd	0b	0a

²Seedlings were 8 months-old at the start of the experiment.

^yMean separation within columns by Duncan's multiple range test, 5% level.

about 15 cm apart.

Herbicide was removed from the string during the period of exposure. The amount of herbicide remaining on the string was 12, 30, 37 and 50% of the original content for the 0, 15, 30 and 30-75 cm locations, respectively; indicating the effect of placement on the amount of leaching. The amount of simazine, atrazine and diuron leached to the soil was 4.05, 1.35, and 1.35 kg/ha for the soil surface location.

There was no injury to the tree where the string came in contact with the bark. Temporary, localized leaf discoloration occurred where direct string contact was made with an expanding leaf tip. No injury was observed due to root uptake of the herbicides. Although injury from these herbicides has been reported by some researchers (2, 7) good tolerance has been shown by others (3, 6). Skroch (7) indicated careful consideration should be given to the placement of herbicides in relation to the tree root systems. Marriage and Saidak (4) observed this for eight peach cultivars in relation to dichlobenil in-

jury. The string method of application apparently prolonged the released of herbicide allowing greater tree safety. In addition, this method and time of application gave preemergence control of biennial or winter annual weeds before they reached later stages of growth when control becomes more difficult.

A wheat bioassay in the fall showed no injury indicating that the amount of soil residue of each herbicide had decreased to less than a phytotoxic concentration within the year following treatment.

No problems were encountered with the use of the string when it was placed on the soil surface. However, when placed above the surface some stretching occurred over the 3.6 m distance. In two of the latter plots the string broke as a result of wind action after four months.

The cost of using herbicide impregnated string is approx one third that of hand weeding but more expensive than spraying. The purchase of expensive machinery and the possibility of injury

to the young plants by the machines moving through the nursery rows during herbicide application is avoided with the string method. The tolerance of young seedlings to overall sprays of herbicides, particularly where winter annual weeds occur, has not been well established. A herbicide treated string offers considerable promise for use in other nursery stock, sensitive to herbicide sprays, and for weed control when plants are in leaf. We conclude that, simazine, atrazine, and diuron impregnated string provides a safe as well as effective method of weed control for 2 *Prunus* species.

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Clonal Propagation of *Coffea arabica* L. from Callus Culture¹

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Abstract. Large numbers of coffee organoids were produced from callus cultures derived from leaf blade segments. Organoids developed from callus grown on a modified Linsmaier-Skoog medium, containing 0.1 mg per liter kinetin and 0.1 mg per liter 2,4-dichlorophenoxyacetic acid (2,4-D), typically consisted of rudimentary stems and leaves only. Roots were formed and the organoids developed into coffee plants upon transfer of the organoids to a Gresshoff-Doy medium containing 0.1 mg per liter naphthaleneacetic

acid (NAA) but no kinetin. The plants continued to grow after being transferred to soil.

Coffea spp. have been grown in tissue culture from fruit pericarp and endosperm (3) and from stem fragments (1, 6, 7, 8). Keller et al. (3) reported production and release of caffeine from primary callus cultures derived from endosperm and pericarp of *C. arabica*. Buckland (1) grew viable, transferable suspension cultures from stem sections of *C. arabica* and recently Townsley (7) reported production of coffee "aroma" from such suspension cultures. Staritsky (6) obtained embryoids (organoids) and rudimentary plantlets

consisting of leaf, hypocotyl, and primary root from callus derived from young shoot stem sections of *C. canephora* Pierre ex Froehner, but was unable to induce embryoid formation in *C. arabica* cultures. Sharpe et al. (5) grew callus cultures from seeds, shoots, leaves, and anthers of *C. arabica*. Shoots and primary roots were obtained from orthotropic shoot explants, but no further development occurred.

This study was undertaken in order to investigate the possibilities for asexual propagation of Arabian coffee by tissue culture methods.

Leaves obtained from lateral growth of *C. arabica* tree³ were surface-sterilized by immersion for 20 sec in 70% ethanol then 20 min in a 3% calcium hypochlorite solution. The leaves were cut into segments of about 25 mm² after 3 rinses with sterile isotonic (0.85%) saline solution. The segments, consisting of portions of the blade with lateral veins, were explanted onto 10 ml of Staritsky agar (6) containing 0.1 mg per

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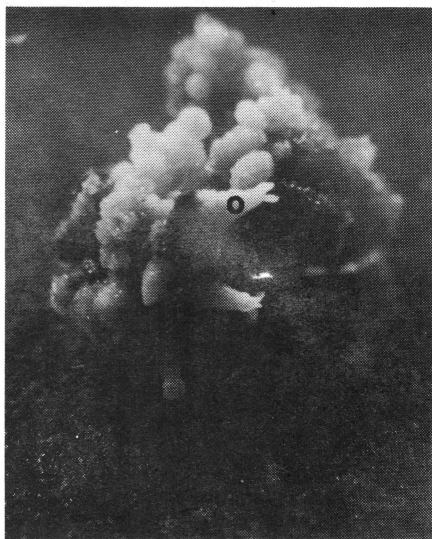


Fig. 1. Coffee callus and newly formed organoids (o) after 60 days of incubation on Staritsky agar containing 0.1 mg/liter kinetin and 0.1 mg/liter 2,4-D.

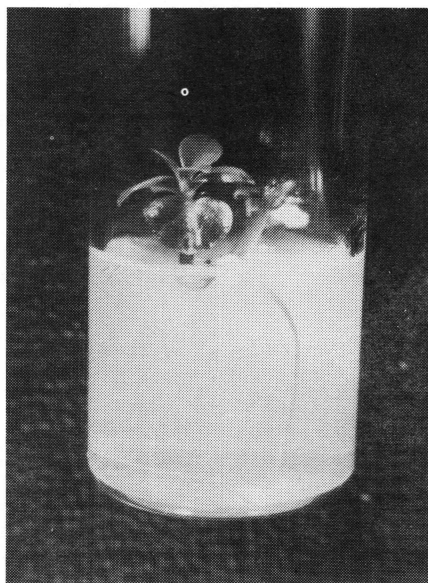


Fig. 2. Root development after 60 days of incubation on Gresshoff-Doy medium.

liter kinetin and 0.1 mg per liter 2,4-D in 9 cm high glass screw cap vials. Cultures were grown in the dark on a diurnal cycle of 16 hr at 30°C and 8 hr at 25°C.

Slow callus growth occurred. Numerous organoids formed at the periphery of the callus after 60 days of incubation. Organoids had either pink stems with white leaves or consisted of white primary rootlets (Fig. 1). Organoids were removed from the dark and cultured under 5300 lux provided by combined fluorescent and incandescent lighting for a cycle of 16 hr daily at 30°C followed by dark for 8 hours at 25°C. Most of the shoots and leaves turned green under these lighting conditions but complete plantlets were not formed, contrary to Staritsky's findings (6). Transfer of the shoots to fresh medium did not produce plantlet formation from the organoids and they often reverted to callus in the Staritsky medium. The organoids also showed no geotropism, as many were inverted in the agar. Many of the organoids showed abnormal leaf formation, e.g. small or oddly shaped, fused, or albino leaves.

Twenty selected organoids, consisting of a green shoot with normal leaves, were transferred in an upright position to Gresshoff-Doy Medium #4 (2) with 0.1 mg per liter NAA but no kinetin. Roots began to form after 2 months of further incubation in the new medium at the temperature and light conditions described above. Light intensity was increased to 10,000 lux after 4 months of incubation. This appeared to increase the rate of growth of the plantlets.

Fragments of callus with organoids, originally grown on Staritsky's medium, continued to form new organoids after being transferred to the new medium. Those with roots but without leaves did not form plantlets on the Gresshoff-Doy medium. Plants that developed after 60 days of incubation in the Gresshoff-Doy medium had 1 or 2 roots with no root hairs (Fig. 2). Attempts at transferring these plantlets to soil were unsuccessful.

Almost all of the plantlets were growing vigorously after 7 months of

total incubation and had developed root systems in the agar. Plantlets were then aseptically removed from the medium. Agar was washed from the roots by repeated pipetting of sterile isotonic saline solution. Plantlets were transferred to a sterile system consisting of 50 g of a light potting soil mixed with an equal amount of vermiculite in 250 ml flasks stoppered with Dispo plugs. Ten ml of Gresshoff-Doy medium #4 without hormones was added to each flask. Plantlets were incubated under the same conditions as the organoids, with water added as needed. Sterile conditions were no longer maintained after the plants reached a height of 3 to 4 cm. Plants grew vigorously under non-sterile conditions. Plants were transplanted to larger pots in a greenhouse when they attained a height of 4 to 5 cm and extensive normal roots were visible at the bottom of the flasks. Growth of coffee plants, normal in appearance, has continued 3 months after transfer to greenhouse conditions. The method presented here could be used for clonal propagation of Arabian coffee plants in those types of research where possible genetic variations among seedlings might be detrimental. Further experimentation to determine feasibility is needed with robusta (*C. canephora*), Liberian (*C. liberica* Bull ex Hiern), excelsa (*C. excelsa* Chev.) and other coffees which must be propagated vegetatively.

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Fig. 3. Coffee plant differentiated *in vitro*, 3 months after transfer to greenhouse conditions.