HortScience 11(2):100–101. 1976.

Cold Storage Maintenance of Strawberry Meristem Plantlets[1]

R. H. Mullin and D. E. Schlegel
Department of Plant Pathology, University of California, Berkeley, CA 94720

Additional index words. tissue culture, *Fragaria* X *ananassa, Fragaria virginiana, Fragaria vesca.*

Abstract. **Long preservation of potentially valuable germplasm of strawberry (*Fragaria* spp.) was facilitated by cold storage of "pathogen-free" meristem plantlets. More than 50 different cultivars have been maintained for up to 6 years as meristem plantlets in sterile culture tubes at 4°C air temperature in darkness.**

The production and maintenance of clean planting stocks for use by nurserymen and growers is basic to disease control in vegetatively propagated crops. The meristem-tip culture technique, currently utilized to produce "disease-free"[2] stock of many plants, rids individual plants of injurious fungi, bacteria, viruses, and nematodes, and thereby actually produces a "pathogen-free" plantlet (7). However, no adequate method has previously been developed for long term maintenance of these plantlets in the "pathogen-free" state.

The conventional method of maintaining "disease-free" nuclear stocks is by isolating meristem-derived plants in containers of sterilized soil in greenhouses and/or screenhouses with rigid insect control and sanitation. Since some possibility of reinfection or contamination exists despite the rigid control efforts, repeated yearly tests on each plant are still necessary to insure their freedom from known pathogens. The expensive space and labor required for this maintenance method severely limit the number of individual plants or cultivars which can be maintained. Thus, early in the meristem-tip culture program of strawberries, we recognized the need for a simple system whereby a reserve of several hundred "pathogen-free" meristem plantlets could be maintained free from chance reinfection.

Small culture tubes (13 x 100 mm) containing "pathogen-free" strawberry meristem plantlets about 0.6 cm high with roots at least 1.3 cm long and large enough for transplanting were placed in a dark refrigerator maintained at 4°C air temperature. The caps were sealed by a strip of parafilm to slow loss of nutrient medium during storage. The procedure for producing such plantlets, described in detail elsewhere (2), involved culturing excised aseptic meristematic tissues 0.5–0.67 mm high on defined nutrient media (Table 1) with constant illumination. Initially, the tissue was placed on a Whatman No. 1 filter paper bridge 2.9 cm high in small glass test tubes containing 2.5 ml medium A. After 4 weeks, all cultures were transferred to similar tubes containing 2.5 ml medium B. Plantlets were produced within 1–3 months. During storage, the tubes were checked every 3 months, and 1 or 2 drops of sterilized medium B were added to cultures showing evidence of dessication. Addition of larger amounts of medium killed the plantlets.

Table 1. Formulation of media used to grow plantlets from strawberry meristematic tissue.

Components	Medium A	Medium B
Knop's[z]	1 liter	1 liter
Berthelot's[y]	0.5 ml	0.5 ml
Iron-EDTA[x]	0.5 ml	0.5 ml
Dextrose	30 g	30 g
Thiamine·HCl	1.0 mg	1.0 mg
Nicotinic Acid	–	0.5 mg
Pyridoxine·HCl	–	0.5 mg
Glycine	–	2.0 mg
Meso-Inositol	–	100.0mg
Indole acetic acid	1.0 mg	2.5 mg
6-furfurylaminopurine (kinetin)	–	0.1 mg

[z]Knop's Solution: $Ca(NO_3)_2 \cdot 4H_2O$, 1.0 g/liter; KNO_3, 0.25 g/liter; $MgSO_4 \cdot 7H_2O$, 0.25 g/liter; KH_2PO_4, 0.25 g/liter.

[y]Berthelot's Solution: $Fe_2(SO_4)_3$, 50 g/liter; $MnSO_4 \cdot 7H_2O$, 2.0 g/liter; H_3BO_3, 0.05 g/liter; KI, 0.5 g/liter; $NiCl_2 \cdot 6H_2O$, 0.05 g/liter; $CoCl_2 \cdot 6H_2O$, 0.05 g/liter; $ZnSO_4 \cdot 7H_2O$, 0.1 g/liter; $CuSO_4 \cdot 5H_2O$, 0.05 g/liter; H_2SO_4 (32 N), 1.0 ml/liter.

[x]Iron Solution: Na-EDTA, 24.7 g/liter; $FeSO_4 \cdot 7H_2O$, 26.6 g/liter; $Na_2CO_3 \cdot H_2O$, 16.0 g/liter.

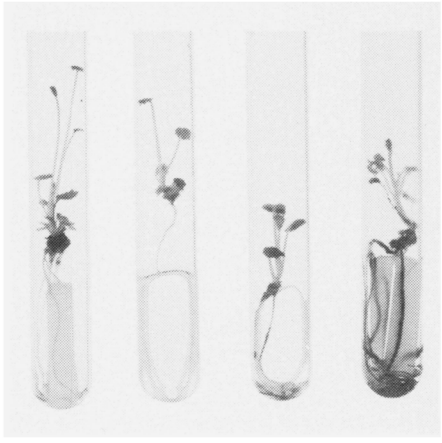

Fig. 1. Disease-free strawberry meristem plantlets after storage in the dark at 4°C show etiolated top growth and substantial root growth.

Plantlets have been maintained viable and healthy for up to 6 years under these conditions (Fig. 1). Fifty plantlets which were moved to partially sterilized soil at yearly intervals during 6 years all survived transplanting and grew into normal plants (Fig. 2). The remaining 700 plantlets appear vigorous and able to tolerate further cold storage for several more years (Fig. 3). The strawberry cultivars maintained include:

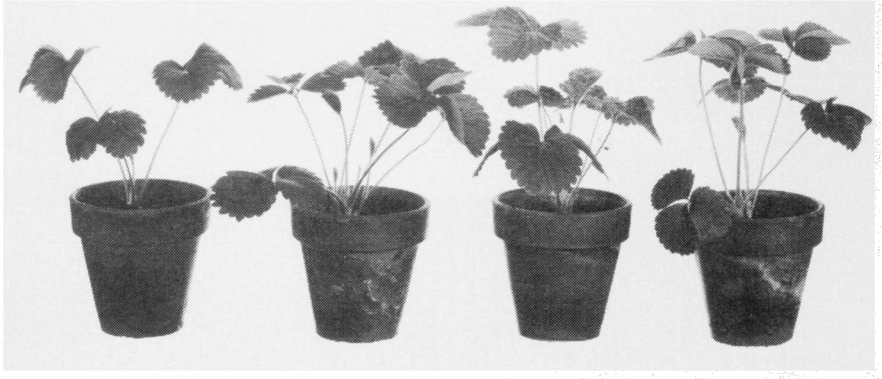

Fig. 2. The meristem plantlets shown in Fig. 1 all survived transplanting to partially sterilized soil.

[1]Received for publication December 15, 1975.

[2]The terms "disease-free" and "pathogen-free" are defined as free from known diseases and free from known pathogens.

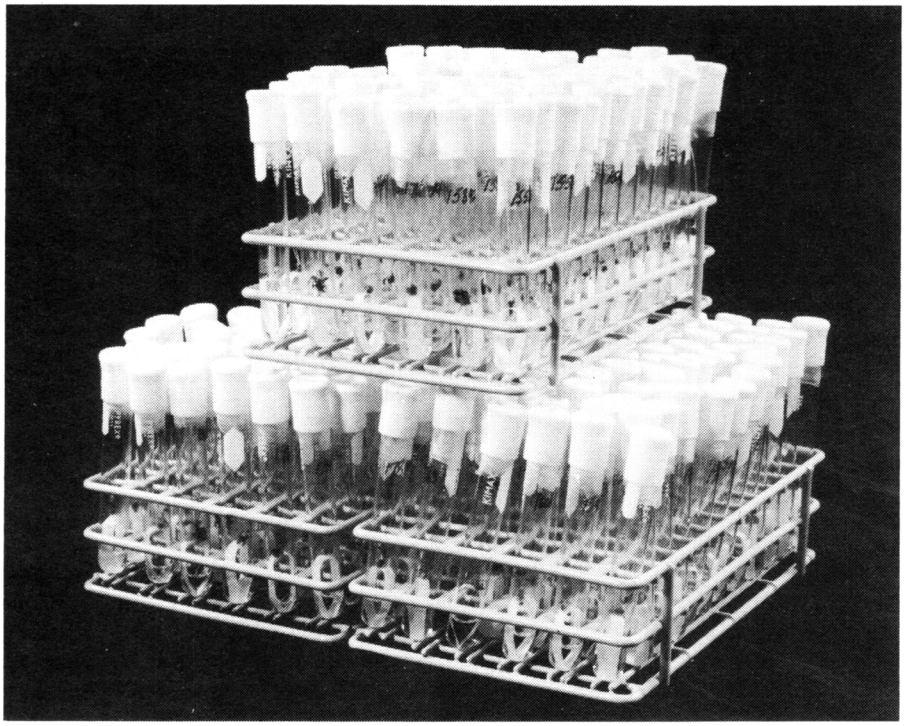

Fig. 3. Several hundred "pathogen-free" strawberry meristem plantlets were stored at 4°C air temperature in individual test tubes held in 22 x 13 cm racks.

Fragaria X *ananassa* Duch. cvs., Aiko, Aliso, Benton, Cruz, Donner, Fresno, Hood, Lassen, Marshall, Northwest, Olympus, Puget Beauty, Salinas, Sequoia, Shasta, Sierra, Solana, Tioga, Toro, Torrey, Tufts, and 30 experimental selections; *F. vesca* L. cvs. UC 1, 3, 4, 5 and 6; and *F. virginiana* Duch. cvs. UC 10, 11 and 12.

The maximum duration and optimal conditions of storage have not been determined except that, whereas plantlets stored better at 1°C than at 4°, they were killed by slow freezing. Cryogenic storage was not attempted.

This relatively inexpensive storage method has reduced costly greenhouse maintenance of strawberry nuclear foundation stock in California. In the future, this long storage of "pathogen-free" meristem plantlets may facilitate germplasm preservation of other plants, such as fruit trees and potatoes (1, 4), which can be cultured from meristem-tips (3, 6) or from tuber discs (5). This system may also be adaptable for preservation of many other plants, currently maintained as budsticks, scions, or cuttings, when suitable culturing systems have been developed.

Literature Cited

1. Fridlund, P. R. 1968. IR-2, a germplasm bank of virus-free fruit tree clones. *HortScience* 3:227-229.
2. Mullin, R. H., S. H. Smith, N. W. Frazier, D. E. Schlegel, and S. R. McCall. 1974. Meristem culture frees strawberries of mild yellow edge, pallidosis, and mottle diseases. *Phytopathology* 64:1425-1429.
3. Quoirin, M. 1974. Permiers resultats obtenus dans la culture "in vitro" du meristeme apical de sujets portegreffe de pommier. *Bul. Rech. Agron. Gembloux* 9:189-192.
4. Rowe, P. R. 1966. The United States potato collection. *Amer. Potato J.* 43:278-285.
5. Skirvin, R. M., S. Lam, and J. Janick. 1975. Plantlet formation from potato callus *in Vitro*. *HortScience*. 10:413.
6. Stace-Smith, R. and F. C. Mellor. 1968. Eradication of potato viruses X and S by thermotherapy and axillary bud culture. *Phytopathology* 58:199-203.
7. Wilhelm, S. 1962. Symposium on pathogen-free stock. *Phytopathology* 52:1234-1235.

HortScience 11(2):101–103. 1976.

Field Performance of Cold-stored Plants of Strawberry Cultivars and Selections in the Pacific Northwest[1]

Hugh A. Daubeny, Jack A. Freeman[2], and H. S. Pepin[3]
Research Station, Agriculture Canada, 6660 N. W. Marine Drive, Vancouver, British Columbia

Additional index words. Benomyl, *Fragaria* X *ananassa*, dormancy

Abstract. **Survival and vigor, after -1°C storage, of Totem strawberry (*Fragaria* x *ananassa* Duch.) plants dug at 8 different dates, from November 15, 1973 to April 1, 1974, was affected adversely only when dug April 1. Predigging treatment with Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate(benomyl) did not affect survival or vigor from any of the dates. In 1975, digging date (March 1 vs April 1) did not affect the survival of plants of 4 cultivars or 6 selections. However, plants of 'Totem' and 'Northwest' and 2 selections dug April 1 showed reduced vigor compared to those dug March 1.**

Dormant strawberry plants, previously treated with fungicide, are commonly dug and stored until spring planting (2, 3, 4). Such plants will remain in excellent condition at -1°C for extended periods (3). However, in the Pacific Northwest non-dormant plants are often dug and stored at -1°C because more favourable conditions for digging can exist in the spring, after growth has commenced, than during the winter. Moreover, Freeman and Pepin (1) found that non-dormant plants of 'Northwest' did not grow as well as those of 'British Sovereign' or 'Siletz' after -1° storage.

In recent years 'Totem' has become an important cultivar in the region. Under commercial conditions spring-dug (non-dormant) plants of the cultivar have appeared less vigorous and, in some instances, have had lower survival rates than winter-dug (dormant) plants after -1°C storage. In the present study, vigor and survival were measured after -1° storage of 'Totem' plants which have been sprayed with the systemic fungicide benomyl compared to those which had not been sprayed from several digging dates through the winter and early spring of 1973-1974. In addition the growth responses of 'Totem' plants and those of 3 other cultivars grown commercially in the Pacific Northwest and of 6 advanced selections from the British Columbia strawberry breeding program were compared subsequent to digging at 2 different dates in 1975 and storage at -1°. The first date was chosen to ensure dormant plants and the second to ensure non-dormant plants.

1973-1974 treatments. Sixty-four field plots, each consisting of 12 'Totem' plants, were established at Abbotsford, British Columbia in May, 1973. Individual plots were designated with proposed 2 week digging dates beginning Nov. 15 and ending April 1 (except

[1]Received for publication October 25, 1975.

[2]Research Station, Agriculture Canada, Agassiz, British Columbia. (Contribution No. 222, Agassiz).

[3]We wish to thank C. Borno, S. Clements and D. Stary for their assistance.