Five, 6, and 7 consecutive applications of benomyl at 6.72 kg/ha and 7 consecutive sprays at 1.12 and 3.36 kg/ha significantly reduced oxidant stipple compared to that occurring on control vines. Three early benomyl applications (before July 24) reduced oxidant stipple at the same degree as additional applications (July 24 and after) indicating the need for chemical coverage of the foliage at a stage when vines were most sensitive to O_3 (unpublished data). The leaves of the basal 6 nodes which were most severely affected had emerged and attained full size by June 12.

Monitoring data (2) show that a major O_3 episode occurred in New York State on July 18 and 19, 1972, when O_3 concn reached 28 pphm for short durations. However, until accurate prediction of episodes is possible, season-long coverage may be necessary to provide protection against O_3 injury by strategic timing of benomyl sprays.

Although complete protection was not provided to grapevines in these studies, the alleviation of oxidant stipple symptoms by benomyl in 1972 indicates promise of viticulturally important control after further experimentation.

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Callus and Cell Culture from Grape Berries[1]

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Abstract. **The development of callus from immature grape berries of *Vitis vinifera* L. cvs. Sultana, Muscat Gordo Blanco, Cabernet Sauvignon, Shiraz, Clare Riesling and Rhine Riesling is described. Callus has been successfully subcultured on solid and liquid media.**

The growth and use of callus tissue from stems and petioles of grapevine plants has recently been discussed by Staudt et al. (8). Routine development and culture of callus from grape berries has not been described. Grape berries themselves showed some growth when cultured *in vitro* (5, 7) and occasionally 'Sultana' berries burst and callus formed slowly (5). After 6 months, callus was observed on sections of berries of 'Cabernet Sauvignon' (D.R. Barras, personal communication). Many species or organs of species require special media before callus or cell cultures can be initiated and maintained (3). Also tissue cultures from different organs of the same plant can have different properties, and embryogenesis and organogenesis in some instances may only occur with tissue cultures developed from a particular organ of a plant (4). Neither process has ever been reported in tissue cultures of grapevine.

The present paper describes conditions for the rapid formation and prolonged culture of callus from berries of several cultivars of *V. vinifera*. Cell suspension cultures have been started and maintained from some of the callus.

Berries of vinifera cultivars as indicated in the text were used as starting material. Young green hard berries of approx 5 mm diam and containing soft seeds were washed in distilled water and then shaken in a 7% aqueous solution of $CaOCl_2$ for 15 min. After 3 washes in sterile distilled water, the berries were cut into quarters, the seeds removed and quarters of berry were placed on a sterile medium (Table 1) based on that of Gamborg and Eveleigh (2). The vials or flasks were kept in the dark at 26°C.

The conditions for optimum development of callus from 'Cabernet Sauvignon' berries were determined by experiments with combinations of inorganic and organic compounds, growth substances, light regimes, temp and pH. High growth rates occurred at 26°C in the dark on a 1% agar medium containing 0.2 – 0.6% casein hydrolysate in addition to inorganic salts, vitamins, 2 – 5% sucrose, 0.1 ppm naphthaleneacetic acid (NAA) and 0.2 ppm kinetin at pH 5.5–6.0 (Table 1). When coconut milk which had not undergone repeated freezing and thawing was used in place of casein hydrolysate the growth rate was often doubled, but casein hydrolysate was used routinely for convenience.

Callus tissue was only produced in massive amounts from grape berries at certain stages of development. Very small berries, berries approaching maturity with soft flesh and reddening skins, and mature berries, did not readily produce callus tissues. Young berries (approx 5 mm diam) were found to produce the maximum amount of callus tissue.

Table 1. Composition of medium for growth of grape callus.

Component	mg/liter
Macronutrients	
KNO_3	2,500
$(NH_4)_2SO_4$	150
$CaCl_2 \cdot 2H_2O$	150
$NaH_2PO_4 \cdot 2H_2O$	150
$MgSO_4 \cdot 7H_2O$	250
Micronutrients	
H_3BO_3	3
$MnSO_4 \cdot 4H_2O$	10
$ZnSO_4 \cdot 7H_2O$	2
KI	0.75
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.25
$CoCl_2 \cdot 6H_2O$	0.25
Vitamins	
myo-inositol	100
nicotinic acid	1.0
pyridoxine HCl	1.0
thiamine HCl	10
Others	
Casein hydrolysate[z]	2,000
FeNaEDTA[y]	30
Sucrose	20,000
Agar	10,000
Kinetin	0.2
Napthalene acetic acid	0.1

[z]Calbiochem, Los Angeles, U.S.A.

[y]Fluka, Switzerland.

The growth of callus from berries of some cultivars produced little or no callus while others consistently produced large amounts (Table 2). 'Rhine Riesling' produced the most callus under the conditions found optimal for 'Cabernet Sauvignon' callus growth. More recently callus cultures also were established of 'Chenin Blanc', 'Waltham Cross' and the 'Sultana' mutant 'Bruce's Sport' (1).

Pieces of callus (approx 500 mg)

Table 2. Growth of callus from quarters of grape berries for 42 days on solid culture medium. The initial berry tissue weighed between 20 and 30 mg.

Cultivar	No. started	No. sterile	No. with callus	Mean fresh wt of callus (g)
Sultana	10	6	6	0.99
Muscat Gordo Blanco	10	6	2	0.02
Wortley Hall	10	6	0	–
Cabernet Sauvignon	10	9	8	0.98
Shiraz	10	9	8	0.66
Clare Riesling	10	8	4	0.60
Rhine Riesling	10	8	8	4.68

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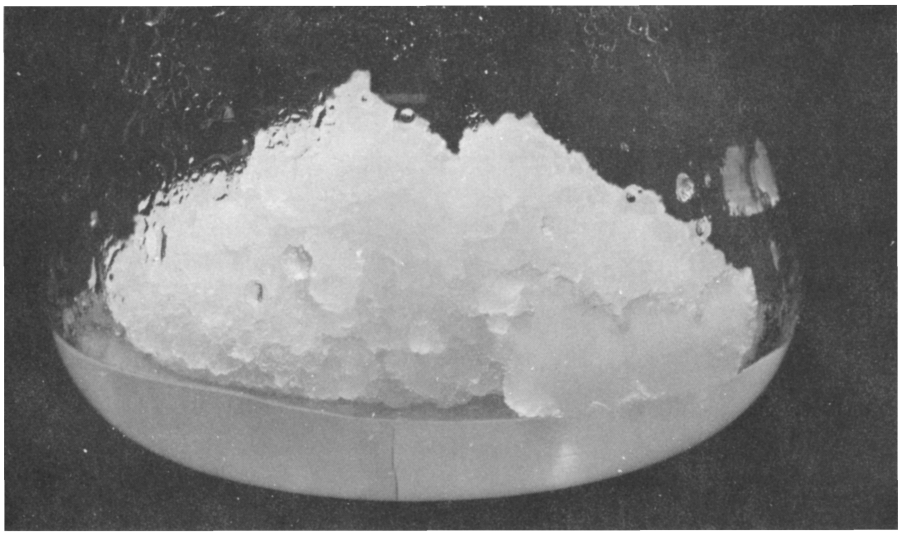

Fig. 1. Callus derived from a 'Clare Riesling' berry. The culture shown is a 5th subculture of the original callus. The 125 ml flask contains 25 ml of nutrient medium in 1% agar.

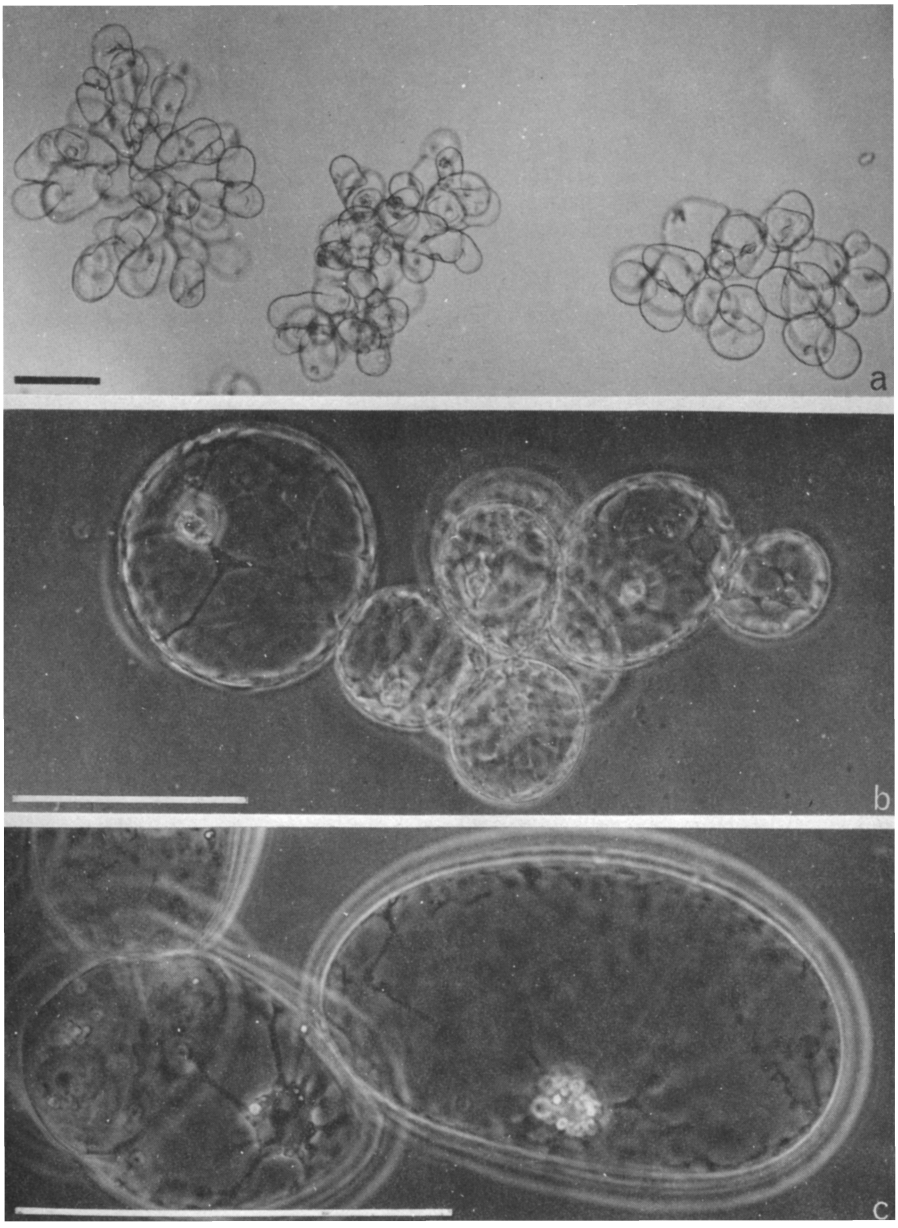

Fig. 2. Suspension cultures of *Vitis vinifera.* Callus was grown on solid medium from young berries and subsequently transferred to liquid medium. Scale lines represent 0.1 mm. a) 'Rhine Riesling'. b) 'Sultana'. Phase contrast. c) 'Clare Riesling'. Phase contrast.

were subsequently transferred to fresh medium in 125 ml conical flasks. Callus of 'Sultana', 'Rhine Riesling', 'Shiraz' and 'Clare Riesling' were routinely subcultured at least 8 times. Figure 1 shows callus of 'Clare Riesling' which was subcultured 5 times.

Pieces of callus (approx 500 mg) from the second subculture of 'Sultana', 'Clare Riesling', 'Rhine Riesling' and 'Shiraz' were also transferred to liquid medium and swirled at 110 cycles/min at 26°C in the dark. Single cells and cell aggregates were produced in these liquid suspension cultures (Fig. 2). Cells were of different sizes and many showed starch grains densely packed around the nucleus. Cytoplasmic streaming was observed. No obvious differentiation occurred. After 10 weeks, subcultures were made and new cells were rapidly produced. Filamentous forms were commonly seen. Many pieces of callus ranging in size from barely visible up to 5 mm in diam were found after a 4–6 week culture period. Cells can be rapidly generated by starting the liquid suspension culture with 2 or 3 pieces of callus each weighing about 750 mg from a 4th or 5th subculture. In the case of 'Sultana' the 25 ml of liquid medium became solid with cells about 2 weeks after inoculation. A spoonful of these cells (approx 1.5 g) is sufficient to initiate a subculture which also solidifies in about 2 weeks. These rapidly growing cells contain only traces of starch.

The cell cultures described herein provide new material for developmental and biochemical studies. Cells cultured from berries of 'Bruce's Sport', a mutant of 'Sultana' whose berries are largely deficient in the enzyme polyphenol oxidase (6), could supply a genetic marker useful in research on hybridization of grapevines by parasexual means.

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