

Research Note

Inhibition of fungal infection using sulfite pads prior to initiation of callus from *Vitis labruscana* cv. Concord

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S u m m a r y : Incubation of plant material with potassium metabisulfite was found to inhibit fungal infections of explants from grapevines. Grapevine tissue of *Vitis labruscana* cv. Concord was incubated with sulfite pads containing 0.4 g of potassium metabisulfite for one and two days prior to culturing and evaluated against a control that had been surface sterilized with 0.5 % NaOCl and 70 % ethanol after one week for losses due to microbial contamination. Sulfite fumigation of plant material reduced the incidence of mold infection, particularly in tissue cultures developed from fruit explants which had reductions in contamination as high as 10 fold. Continued attempts to isolate contaminants from cultures initiated from these explants showed no signs of infection.

Introduction: Chemical disinfectants containing NaOCl are commonly used to control bacteria and other microorganisms (TANNER 1989) including surface sterilization of plant material prior to culturing in liquid or solid media (DODDS and ROBERTS 1985). The use of these chemicals under heavy fungal pressure can prove inadequate in eliminating mold infection of cultures. Research programs involved in tissue culture frequently grow plants in greenhouses to reduce levels of contamination upon culturing. Preliminary work in the authors laboratory with store bought table grapes had low incidence of infection as did cultures prepared with field material early in the season using typical methods of surface sterilization with 0.5 % NaOCl (10 % chlorox) and 70 % ethanol. As the season progressed into the warm humid summer of upstate New York, mold infection of tissue cultures became very frequent and attempts to culture material from the field became futile. During discussions it was suggested that storage with sulfite pads normally used for preservation of grapes during shipping could have been responsible for the lack of infection seen in the store-bought fruit and might be helpful in reducing infection rates in field material. Sulfite has proven to be an effective inhibitor of yeast (PILKINGTON and ROSE 1988) and molds (RAMAKRISHNA *et al.* 1991). Sulfite fumigation of plant material was tested for its ability to limit mold infections in tissue culture.

Materials and methods: **P l a n t m a t e r i a l :** Petioles and immature berries from Concord grape vines were harvested in late August, 1992 from Cornell Vineyards at the New York State Agricultural Experiment Station.

R e a g e n t s : All media salts, vitamins and growth regulators were purchased from Sigma Chemical Co. Disposable, presterilized petri dishes were from Fisher Scien-

tific Co. Bacto-agar was purchased from Difco Laboratories. The sulfite pads were Uvas Quality Grape Guards manufactured in Chile. These contained approximately 0.40 g of potassium metabisulfite per pad.

S u r f a c e s t e r i l i z a t i o n : Explants were washed with moderate agitation in a water/soap solution - about 500 ml distilled or deionized H₂O with 5 drops of Micro soap. The tissue was then rinsed thoroughly in distilled water once followed by a 2 min dip in 70 % ethanol. All plant material was then allowed to soak in 10 % chlorox solution for 10 min after which there were 3 rinses in sterile, distilled water. All work from the point of chlorox submersion was performed under a laminar flow hood.

S u l f i t e i n c u b a t i o n : 15 petioles cut to 10 cm or 25 immature berries still on the cluster were placed in a 500 ml beaker containing one sulfite pad at the bottom and sealed with aluminum foil on the top. Two beakers for each tissue type were prepared. One beaker each from the petioles and from the fruit was incubated for 24 h prior to surface sterilization and the remaining two were incubated for 48 h. All incubations took place at 1 °C. A control group of fruit and petiole tissue was harvested at the same time as the incubated tissue. The control was surface sterilized and cultured without any sulfite incubation within a few hours of harvest from the field. 20 plates were prepared for each treatment and the control. Loss of individual explants and entire petri plates to fungal infections were evaluated after 1 week in culture. Uninfected fruit explants propagated ample callus which was used to initiate cell suspensions in liquid media. These were evaluated every 2 months for contamination on YM and MLB media.

C u l t u r i n g o f p l a n t m a t e r i a l : The petioles had 0.5 cm cut off each end to get rid of disrupted tissue exposed to sterilization chemicals before cutting into 3 mm pieces and plating on media at 5 pieces per plate. The berries were cut into quarters and placed 4 pieces to a plate. Culture media utilized Gamborgs B5 inorganic salts and vitamins (GAMBORG *et al.* 1968) supplemented with 3 % sucrose, 0.5 mg/l 2,4-D and 0.5 mg/l BA for the fruit. Media for the petioles was the same except it utilized 0.5 mg/l IAA as an auxin. 0.8 % Bactoagar was used as a gelling agent. Plates were stored in the dark at 23 °C and callus that developed was transferred to new media every 4-6 weeks.

Results and discussion: Figure indicates that incubation of fruit explants with sulfite pads for 24 h prior to surface sterilization lowered the incidence of mold infections by 4 fold. Incubating for 48 h cut the infection rate of fruit explants by 10 fold. All uninfected fruit explants initiated callus growth within 3-6 weeks. Callus from the fruit was soft and friable and was off-white to light brown in color. The petiole explants had fewer mold infections due to the treatment, however callus growth was limited. Suspensions initiated from fruit callus showed no signs of contamination on YM or MLB media as late as 7 months after sulfite fumigation took place.

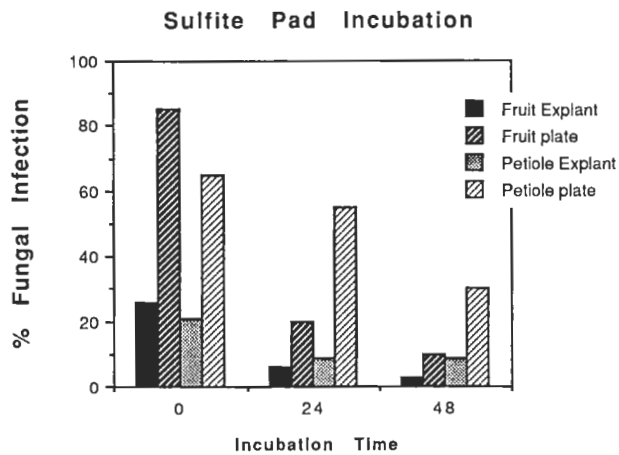


Figure: Percent mold infection noted for individual explants and entire plates for both fruit and petiole material for the control, and after 24 and 48 h incubation periods.

The wet summer season of 1992 created ample opportunity for molds to thrive in the vineyards of upstate New York. As the season progressed it became evident that action other than standard chemical surface sterilization needed to be taken if any explants prepared were to avoid bringing fungal growth into culture. The use of sulfite pads for (sterilization) fumigation of the plant material allowed for continued culture of material from the field. Treatment with the pads worked best with fruit, but also inhibited infection in culture with petiole tissue. One possible reason for the slight difference between the two tissues could be the cutting of petiole tissue that had to occur upon harvest whereas the fruit went in to incubation as a cluster. Exposed internal tissue could have served as refuge for any molds or molds spores which contaminated the petiole after cutting. The lack of callus growth from petiole may be due to the date the material was harvested. Control plates that did remain uninfected also experienced reduced callus initiation. Longer initiation times or failures of callus initiating with explants taken late in the season has been noted previously (HAWKER *et al.* 1973; HONG *et al.*

1989). The berries, still green at the time this material was harvested, did not initiate callus as quickly as fruit picked earlier in the season but all explants not infected by fungus did eventually produce ample callus.

Examination of the rate of plate infection (at least one explant infected) shows that infection of the explants was approximately uniformly distributed throughout the plates within each set. This distribution resulted in essentially a complete loss of the control plates for the fruit. The plates with the sulfite treated explants that did not show infection in the initial one week culture period remained sterile and produced callus which is still being propagated. Repeated attempts to isolate contaminants on YM and MLB media from suspensions initiated using this callus showed no evidence of fungi or bacteria.

This study presents evidence that the use of sulfite fumigation is a simple and effective adjunct to the use of sodium hypochlorite and ethanol for explant sterilization and could prove useful for research programs that are limited to field material in humid climates.

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