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Rapidly Diagnosing Grapevine Corky-bark by in Vitro Micrografting

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Abstract. **Grapevine *(Vitis vinifera* L.) explant shoots indexed forcorky-bark and rootstocks from healthy LN33 indicator plants were sterilized and maintained in vitro. When infected shoot tips were micrografted onto LN33 shoots, typical corky-bark symptoms appeared in 8 to 12 weeks. We suggest developing this method further to replace the regular, 2-year indexing procedure.**

Grapevine corky-bark is a widespread virus-like disease present in many grape-producing countries. It was first recognized in California (Hewitt, 1954) and named rough bark. Since then, the disease has been reported in many countries (Beukman and Goheen, 1970; Tanne and Dubizki, 1985). It is graft-transmissible and viral in nature (Beukman and Goheen, 1970). Recently, various closterovirus particles of various lengths have been found in association with diseased tissues (Namba et al., 1991). The disease is latent in many European cultivars and American rootstock; however, in susceptible cultivars, such as 'Gamay', 'Cabernet Franc', 'Petite Sirah', 'Thompson Seedless', and 'Cardinal', it retards leaf growth and causes irregular wood maturity and soft, rubbery canes with longitudinal cracks at the base. Leaves are often smaller than normal and roll downward in summer, and leaf veins of red cultivars remain green. In many cultivars, pits and grooves develop in the xylem and plant vigor is reduced (Beukman and Goheen, 1970).

The disease is diagnosed by grafting cultivars onto indicator plants, the most common of which is LN33 ('Couderc 1613' × 'Thompson Seedless'). LN33 typically reacts with internode swelling, longitudinal splitting of spongy and soft bark, and the development of pits and grooves in the woody cylinder. Diagnosing the disease by indexing is laborious and time-consuming, requiring as long as 2 years to complete. Rapid diagnosis is important to determine the disease status of grapevine material assumed virus-free by meristem culture or heat treatment and that of introduced cultivars and suspected field material.

Micrografting has been used to eliminate citrus (Jonard et al., 1990) and grape viruses (Ayuso et al., 1978; Martinet rd., 1987) and to follow the phenomenon of incompatibility (Chimot-Schall et al., 1986; Jonard et al., 1990). Our paper reports on an in vitro micrografting procedure for grapevines and demonstrates its use to diagnose grapevine corky-bark rapidly.

LN33 and corky-bark-diseased 'Thompson Seedless' (isolate CB-LK) shoots were sterilized by immersing them in 2.5% sodium hypochlorite plus one drop of Tween-20 for 10 min. Sterilized shoots were maintained on a Murashige and Skoog (MS) (1962) medium supplemented with 3% sucrose and 0.8% agar (Difco Bitek; Difco Laboratories, Detroit). Shoots were rooted on MS medium containing 0.3 μM α– naphthaleneacetic acid. Cultures were kept in a growth chamber at 25C, and 16 h of light was provided by cool-white fluorescent bulbs (45 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

Shoots used as scions were excised to a wedge-like shape (Fig. 1), and the epidermis was peeled off the cut surface (1 cm) with a surgical blade. Scions were kept on wet absorbent papers in a petri dish until grafting. LN33 (serving as rootstock) shoots were topped 2 cm above the root system. A 0.5-cm sloping cut was made in the rootstock. The scion was inserted in the cut and held in place with sterile aluminum foil (Fig. 2). The micrografted plants were inserted in test tubes and later transferred and maintained on rooting medium in GA7 culture vessels (Magenta Corp., Chicago). After 4 to 5 weeks, the plants were transferred to Jiffy-7 (Jiffy Products, Norway) pots and

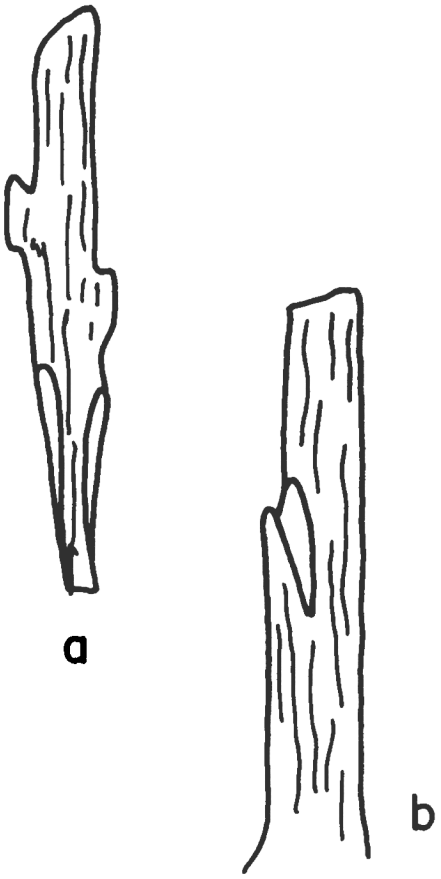

Fig. 1. Schematic presentation of micrograft technique: **(a)** scion, **(b)** rootstock. Note slanting cut.

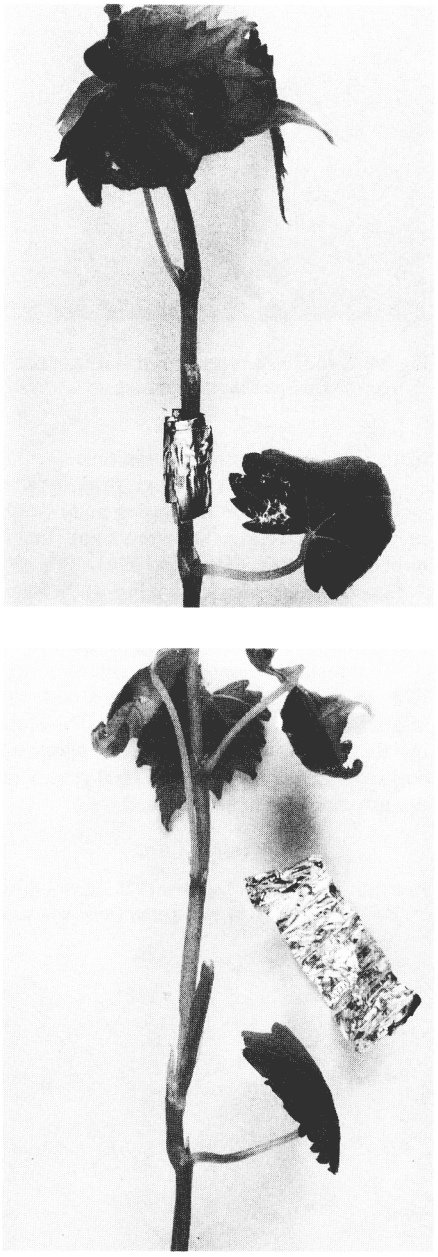

Fig. 2. Micrografted grape: **(top)** wrapped with aluminum foil, **(bottom)** after removing foil.

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Fig. 3. Longitudinal cracks formed in stem of LN33 rootstock 12 weeks after grafting.

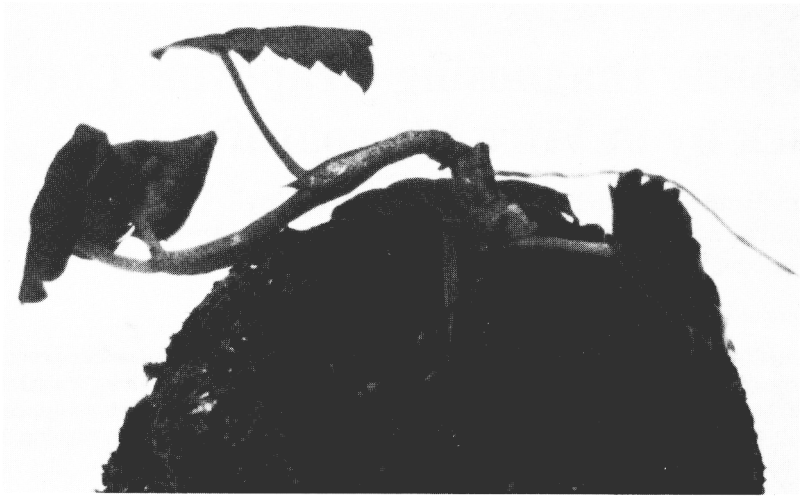

Fig. 4. Leaf down-rolling and node swelling in grafted LN33. Notice stunting of the plant.

Table 1. Number and percentage of successful grafts and percentage of grafts showing corky-bark symptoms on grapevine shoots.

	Grafts				
	Performed	Successful		Showing symptoms	Symptom
Expt.	(no.)	(no.)	(%)	(%)	consistency
1	10	7	70	35	+
2	5	4	80	25	+
3	4	3	75	66	+
4	10	9	90	22	+
Control[z]					
1	10	9	90	0	---
2	10	9	90	0	---

[z]Control = virus-free 'Thompson Seedless' grafted on healthy LN33 indicator.

maintained under aseptic conditions.

Eight to 12 weeks after grafting, typical corky-bark symptoms, including node swelling, leaf down-rolling, and some small, longitudinal cracks, developed on LN33 (Fig. 3). Affected plants were stunted (Fig. 4). Seventy to ninety percent of the grafts was successful and symptoms were consistent (Table 1).

This technique could help detect corky-bark in 8 to 12 weeks compared with the standard 2-year indexing procedure. Developing the method for large-scale diagnosis of corky-bark and possibly other viral grapevine diseases remains to be achieved.

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