Elimination of Mycoplasmalike Organisms in Cabot Highbush Blueberry with High-Carbon Dioxide Thermotherapy

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ABSTRACT

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By means of fluorescent staining with the RNA-specific diamidino phenylindole (DAPI), mycoplasmalike organism (MLO)-related sieve tube fluroescence was detected in longitudinal sections of roots of Cabot highbush blueberry (*Vaccinium corymbosum*). Attempts to eliminate the MLOs by means of conventional heat therapy of whole plants in a growth chamber were unsuccessful because the plants failed to produce new growth and died within 4 wk when grown at a constant 38 C with ambient CO_2. However, when an enhanced CO_2 level (1,200 ppm) was used, Cabot plants survived at 38 C and produced new shoot growth for 6 wk, enabling softwood cuttings 10–20 mm long to be taken and propagated. After testing for the presence of MLOs with the DAPI staining technique, the number of DAPI-positive plants grown from heat-treated cuttings decreased with time that the source plants had been grown at 38 C, reaching zero at 5 and 6 wk. Stocks of clones of virus-tested, DAPI-negative Cabot (designated Cabot-86) and similarly tested Ivanhoe-86 and Blue Haven-86 highbush blueberry cultivars have now been established.

Highbush blueberry (*Vaccinium corymbosum* L.) is frequently infected in eastern United States by the blueberry stunt mycoplasmalike organism (BSMLO). The biology and control of blueberry stunt disease (BSD) have been recently reviewed (6). The highbush blueberry cultivar Cabot has been reported (8) to be susceptible to BSMLO and is recommended as a graft indicator host for BSD detection (1). During an examination of ungrafted Cabot plants intended for use as virus indicators, mycoplasmalike organisms (MLOs), were found in sieve tubes of roots but less reliably in shoots by electron microscopy and by use of the DAPI technique (7). The relationship of these MLOs to BSMLO has not been established.

Thermotherapy has been used successfully to eliminate MLOs from several clonally propagated crop plants (5). Artificial elevation of the CO_2 level during thermotherapy from ambient (350 ppm) to about 1,100 ppm was found to increase markedly the ability of grapevine (*Vitis vinifera* L.) and *Leea brunonia* Clarke to withstand prolonged thermotherapy (2).

When conventional thermotherapy (38 C, ambient CO_2) was used on the Cabot blueberry, plants failed to produce new shoots or to survive for more than 4 wk. Therefore, in this study, we examined the effects of thermotherapy under elevated CO_2 conditions on the growth and survival of Cabot blueberry and ultimately on the elimination of MLOs in order to produce virus-tested, MLO-free stock.

MATERIALS AND METHODS

Thermotherapy. High-CO_2 thermotherapy has not yet been widely used commercially to eliminate MLOs from clonally propagated plants. For this reason, we describe the high-CO_2 system we assembled in our greenhouse at Oregon State University. The equipment,

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schematically shown in Figure 1, prepares a CO_2, air, water-vapor mixture, introduces and allows it to flow through a commercial reach-in plant growth chamber, and measures the CO_2 level within that chamber. CO_2 from a standard commercial welding-grade 23-kg CO_2 cylinder (A) (letters in parentheses in this paragraph refer to Fig. 1), controlled by coarse and fine needle valves (B) and gauged by flow meters (C), is mixed with compressed air from a commercial 23-L, 50-psi air compressor (D) after the air has been humidified by bubbling it through a 20-L carboy of tap water (E). The resulting CO_2-enriched, humid air is led into the air circulation system of a growth chamber (122 × 58 × 107 cm) (F), where fans circulate the air around the plant (G). A 2.8-cm-diameter outflow tube (H) installed through one of the access ports of the growth chamber allows continuous air movement through the chamber at an approximate rate of 0.7 m^3/hr. The CO_2 is kept at the desired level (usually 1,200 ppm) by monitoring the chamber and appropriately adjusting the needle valves (B). CO_2 is monitored with a relatively inexpensive, dedicated, infrared CO_2 monitor (J) (Horiba Instruments Co., Model APBA-200, Irvine, CA) fitted with one or more condensate traps (I) and a water-jacketed condenser (L). A standard chart recorder (K) prints an ongoing record of CO_2 concentration in the chamber at the level of the leaves.

Blueberry plant management. Six Cabot blueberry plants were propagated from a parent block of Cabot plants kindly supplied by A. W. Stretch (USDA ARS, Chatsworth, NJ) and were used for the enhanced CO_2 heat therapy study. Sample plants were found to contain MLOs as determined by the DAPI test (7). The resulting plants were grown in 15-cm pulp pots for 6–9 mo before being double-potted with a layer of peat moss between the inner and outer pulp pots. Six plants were then placed in the growth chamber at 16-hr day lengths and a photosynthetic photon flux (fluorescent and incandescent lighting) at the leaf surfaces of 395 $\mu mol \cdot s^{-1} \cdot m^{-2}$. Temperature in the chamber was raised 2 C every 2 days from ambient to 38 C. Plants were manually watered daily with 1/10-strength Hoagland's solution no. 2 modified to include NH_4NO_3 and preequilibrated to growth chamber temperatures. The relative humidity of the chamber was maintained near 90%, and the CO_2 level was maintained near 1,200 ppm. A parallel group of six Cabot plants was grown under similar conditions in a growth chamber at an ambient CO_2 level (350 ppm).

Tissue culture. Shoot apices 10–20 mm long were excised from new growth after heat therapy periods of increasing duration. After surface sterilization in 0.2% NaOCl for 10 min, shoots were placed in test tube slants of sterile agar-based medium (4). Incubation conditions for explants were 45 $\mu mol \cdot s^{-1} \cdot m^{-2}$ fluorescent light 16 hr/day at 25 C. When unrooted explants had become well differentiated (3 cm high), they were transplanted to 1:1 peat/perlite in intermittent mist in the greenhouse to root. After sufficient root formation under mist (about 3–5 mo), the Cabot plants were grown in 15-cm pots under standard greenhouse conditions until their root systems were large enough to detect MLOs in sieve tubes by the DAPI test.

MLO testing. MLO presence was tested by the DAPI method, using 10-μm-thick freezing microtome longitudinal sections of roots, as previously described (7).

RESULTS

When grown in a growth chamber at a constant 38 C without CO_2 enrichment, Cabot plants invariably died within 4 wk without producing appreciable new growth. When the CO_2 level was increased to 1,200 ppm in the growth

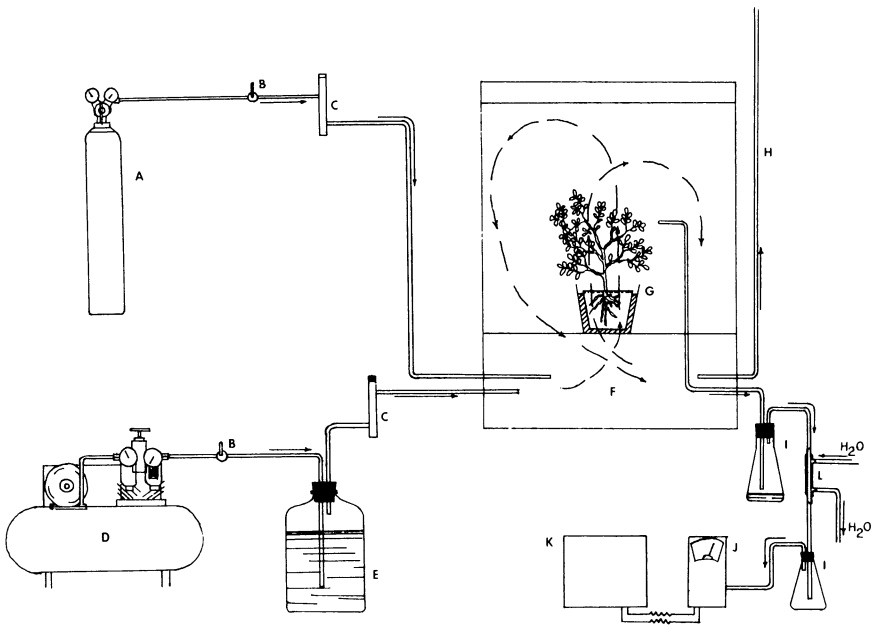

Fig. 1. Diagram of system used to maintain a constant high-CO_2 level in a growth chamber during thermotherapy of Cabot highbush blueberry plants. A = compressed CO_2 cylinder, B = needle valve, C = flow meter, D = air compressor, E = humidifier (20-L water carboy), F = growth chamber, G = double-potted Cabot blueberry plant, H = air outflow tube, I = water trap, J = Horiba CO_2 analyzer, K = chart recorder, and L = water-jacketed condenser.

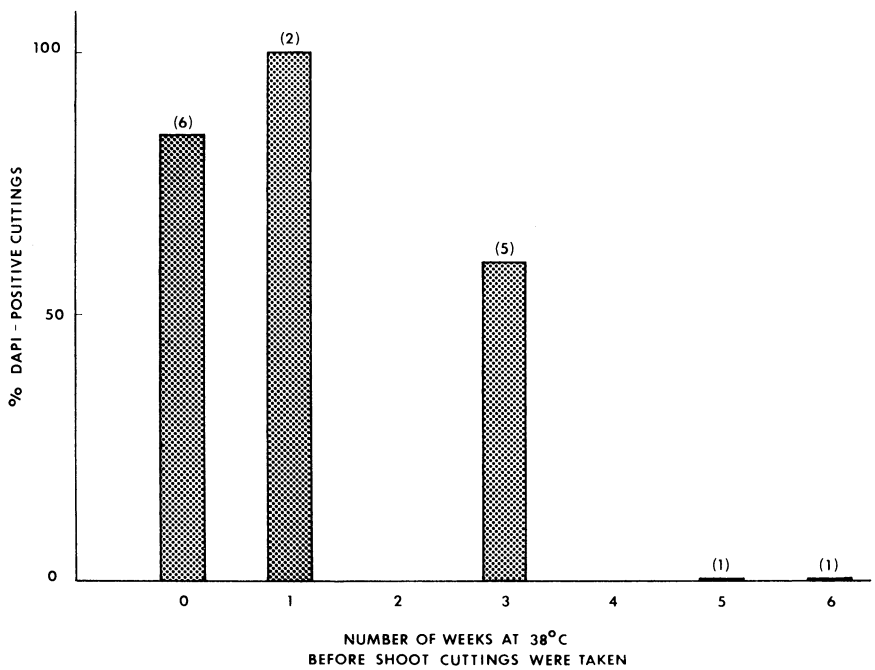

Fig. 2. Percentage of Cabot blueberry plants regenerated from shoot cuttings 10–20 mm taken after 0–6 wk at 38 C (1,200 ppm CO_2-enriched atmosphere) containing mycoplasmalike organisms, as determined by the DAPI test in root sieve tubes. (Numbers in parentheses are total numbers of plants tested. Lines at 5 and 6 wk both indicate 0/1 DAPI-positive plant.)

chamber at a constant 38 C, Cabot plants continued to grow, although old leaves died prematurely and young growth was chlorotic. The DAPI ratings of root sieve tubes from the rooted cuttings taken from Cabot plants after 0–6 wk of growth at 38 C in an atmosphere enriched to 1,200 ppm CO_2 are presented in Figure 2. DAPI ratings of infected root sieve tubes from plants grown at room temperature (0 wk at 38 C in Fig. 2) indicated the reliability of this MLO detection method to be 83%. Cuttings rooted after 5–6 wk at 38 C were free of MLOs as evaluated by the DAPI test.

The clone of Cabot established from a plant that was freed of MLOs after 6 wk in high-CO_2 heat therapy has been designated Cabot-86. The number 86 represents the year of release of this clone of Cabot as one that has been virus- and MLO-indexed by the USDA, ARS. Highbush blueberry clones of Ivanhoe-86 and Blue Haven-86 found in parallel unpublished tests to be free of the ELISA-detectable viruses, blueberry red ringspot virus, blueberry shoestring virus, peach rosette mosaic virus, tobacco ringspot virus, and tomato ringspot virus, were selected from the collections available to us and were found by DAPI root testing to be free of MLOs. Clones of these three cultivars have been propagated in a screenhouse for distribution to requesting public agencies.

DISCUSSION

MLOs generally are eliminated from plant material after 2–4 wk at 35 C or higher (5). False blossom MLO was eliminated from infected cranberry plants (*V. macrocarpon* Ait.) after 8 days at 42 C (3). Dry heat (52 C for 2 hr) eliminated BSMLO in the few highbush blueberry cuttings that survived that treatment (8).

Because the MLO-infected Cabot plants studied had enlarged stipules but lacked other typical BSMLO disease symptoms and required an unusually long period of heat therapy for elimination, these data are not necessarily applicable for predicting the requirements for BSMLO eradication in highbush blueberry plants by thermotherapy. These data indicate that MLOs like those we studied should be successfully eliminated from infected *Vaccinium* clones propagated from softwood cuttings 10–20 mm long obtained from plants grown for 5 wk or more at a constant 38 C in an atmosphere containing 1,200 ppm CO_2. The study also offers additional evidence that the DAPI test in the hands of an experienced worker is a reasonably reliable, rapid method of determining the presence of MLOs in root sieve tubes of suspect *Vaccinium* plants.

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