Effect of Mechanical and Moisture-stress Conditioning on Growth and Cuticle Composition of Broccoli Transplants

Joyce G. Latimer

Department of Horticulture, Georgia Station, University of Georgia, Griffin, GA 30223

Ray F. Severson¹

Tobacco Safety Lab, USDA–ARS, Russell Research Center, Athens, GA 30605

ADDITIONAL INDEX WORDS. brushing, *Brassica oleracea* L. Group *Italica*, epicuticular wax, mechanical stress

ABSTRACT. Epicuticular waxes were analyzed to explain the visible differences in the waxy bloom of conditioned broccoli (*Brassica oleracea* L. Group *Italica* 'Green Duke') transplants. Seedlings (22 days old) were subjected to brushing (40 cycles per minute, 1 minute twice daily), wind (7 m·s⁻¹ for 5 minutes twice daily), or moisture-stress conditioning (MSC; visible wilt for 2 to 4 hours daily) for 16 (1987) or 21 (1988) days during transplant production in the greenhouse. The epicuticular waxes of the uppermost fully expanded leaves were removed by dipping detached leaves into methylene chloride. The extract was derivatized with trimethylsilyl reagents and subjected to capillary gas chromatography. The primary epicuticular wax components were the nonpolar C₂₉ compounds nonacosane, nonacosan-15-ol, and nonacosan-15-one, which were identified by mass spectrometry. In a Summer 1987 experiment, cuticle samples taken over time of treatment indicated acclimation to the conditioning treatments relative to untreated plants. After 9 days of treatment, the amount of total epicuticular waxes present on the leaves was reduced 38%, 31%, or 11% by wind, brushing, or MSC, respectively. However, after 15 days of treatment, the amount of cuticle present was reduced 15% by brushing but only 6% by wind and was 17% greater in MSC-treated plants. Two weeks after transplanting to the field there were no differences in the amount or composition of the epicuticular waxes. In Fall 1988, all treatments reduced plant growth, but only MSC tended to increase the amount of C₂₉ epicuticular components during greenhouse production. Differences in the amounts of epicuticular waxes were no longer significant after 8 days in the field.

To ensure high survival and rapid reestablishment, greenhouse-grown vegetable transplants must be adequately hardened or conditioned before field planting. Acclimation of plantlets produced in tissue culture has proven that cuticle composition and development are among the factors affecting transplant stress tolerance and reestablishment (Johansson et al., 1992; Sutter, 1984; Sutter and Langhans, 1982). Epicuticular waxes on the surfaces of leaves may play a role in frost hardiness, drought tolerance, and disease and insect resistance (Eigenbrode and Espelie, 1995; Kolattukudy, 1980, 1987; Yang et al., 1992), all factors important in the establishment of transplanted crops like broccoli. Although relatively small variations in environmental conditions affect the morphology of epicuticular waxes on developing leaves, larger differences are required to affect wax composition, e.g., a 20 °C rise in temperature reduced the hydrocarbon content of the cuticle of brussels sprouts (*Brassica oleracea* L. Group *gemmifera*) leaves by 40% (Baker, 1982). Water-deficit stress increased cuticle thickness by 33%, altered the epicuticular wax composition, and increased total wax content of cotton (*Gossypium hirsutum* L.) leaves (Oosterhuis et al., 1991). The composition of surface waxes of various *B. oleracea* crops (cabbage, broccoli, and cauliflower) is remarkably similar and the major component of the extract is nonacosane (C₂₉ hydrocarbon) (Kolattukudy, 1965).

Mechanical conditioning is a nonchemical means of controlling plant growth in the greenhouse and of conditioning transplants to the rigors of transplanting to the field (Latimer, 1991). Moisture-

stress conditioning (MSC), the controlled exposure of plants to nonlethal water deficits (Eakes et al., 1991), has been moderately successful in controlling broccoli transplant growth in the greenhouse (Latimer, 1990). During experiments using brushing, wind, and MSC treatments to control greenhouse growth of broccoli transplants (Latimer, 1990), we observed that the treatments varied in how they affected the appearance of the waxy bloom of the plants. Preliminary analysis of the amount of cuticular waxes deposited on the most recently matured leaves at the end of treatment in the Spring 1987 resulted in reductions of 19%, 25%, or 13% by wind, brushing, or MSC, respectively, relative to untreated broccoli plants (unpublished data). Therefore, we conducted the following studies to examine the effect of these treatments on the amounts and composition of epicuticular waxes during conditioning in the greenhouse and after subsequent field establishment of broccoli transplants.

Materials and Methods

Broccoli seeds were sown directly into plastic cell-packs (cell dimensions, 3.8×3.8×6.0 cm, 48 cm³) containing a peat–pine bark medium (Metro-Mix 300; Grace Horticultural Products, Cambridge, Mass.) on 16 July 1987 and 12 Sept. 1988. The seedlings were thinned to one per cell, selecting for uniformity of leaf area and plant height. The final plant density was 485 plants/m². Seedlings were watered with a water-soluble fertilizer (20N–8.7P–16.6K; Peter's 20–20–20; Grace Horticultural Products) at 200 mg·L⁻¹ N twice weekly beginning after seedling emergence. Greenhouse day/night temperatures averaged 30/20 °C in Summer 1987 and 26/16 °C in Fall 1988 over the treatment period.

In 1987 and 1988, treatments were initiated 21 d after sowing, when the second true leaf was beginning to expand. Treatments included 1) untreated plants that were not intentionally disturbed;

Received for publication 8 May 1997. Accepted for publication 7 July 1997. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Deceased.

Table 1. Growth measurements of broccoli transplants treated with mechanical conditioning by wind or brushing or with moisture-stress conditioning (MSC) in the greenhouse during Summer 1987 (16 d of treatment) or Fall 1988 (21 d of treatment). Treatments were initiated 21 d after seeding.

Treatment	Stem length (cm)	Leaf area (cm ²)	Leaf dry mass (mg)	Specific leaf mass (mg·cm ⁻²)	Shoot dry mass (mg)
Summer 1987					
Untreated	11.5 b ²	62.4 a	177 a	2.84 a	334 a
Wind	11.8 ab	57.9 a	155 b	2.67 ab	310 a
Brushing	12.3 a	50.5 b	133 c	2.62 b	269 b
MSC	10.6 c	59.4 a	166 ab	2.83 a	312 a
Fall 1988					
Untreated	9.8 a	87.2 a	311 a	3.56 b	524 a
Wind	8.6 b	75.2 b	266 b	3.53 b	443 b
Brushing	8.0 c	68.3 c	237 bc	3.46 b	397 b
MSC	6.8 d	43.8 d	206 c	4.71 a	327 c

²Mean separation within columns and year by LSD, 5% level.

2) a wind stress obtained by placing the flat of plants in front of a fan to apply a wind of 7 m·s⁻¹ toward the long side of the flat for 5 min twice daily; 3) brushing the tops of the seedlings with a piece of cardboard for ≈1 min (40 cycles, back and forth) twice daily; and, 4) MSC, wherein plants were allowed to wilt for up to 2 to 4 h daily. A flat of 72 plants was assigned to each treatment and treatments were replicated four times with flats arranged in a randomized complete block in the greenhouse. The brushed and wind-stressed flats were removed from the bench for the treatment period and returned to the experimental design immediately after treatments were applied. Six plants were collected randomly to measure leaf area and dry mass (after 72 h in a 70 °C forced air oven), stem length, and shoot dry mass at the end of the greenhouse experiment. The remaining plants were transplanted to the field (Cecil sandy loam soil, Typic Halpludult) where day/night air temperatures over the sampling period averaged 32/19 °C in 1987 and 19/8 °C in 1988 and day/night soil temperatures (at 5 cm) averaged 28/24 °C in 1987 and 17/14 °C in 1988. Data were subjected to analysis by SAS general linear models procedure (SAS Institute, Cary, N.C.) with mean separation by protected least significant difference LSD, *P* < 0.05).

Samples for epicuticular waxes were collected from plants over the treatment period during both experiments. Four plants were sampled from each treatment flat in each of the four blocks. The two most recently fully expanded leaves of each plant were removed, weighed, and extracted as described. After the epicuticular waxes were extracted, leaf area was determined by placing the leaf between layers of clear plastic and running it through a leaf area meter (LI-3000; LI-COR, Lincoln, Nebr.). Epicuticular waxes were removed by dipping each leaf into methylene chloride eight times at about 1 s/dip. The extract was stored at 0 °C until analyzed, at which time a subsample was derivatized with 1 TMSI : 3 pyridine (by volume) at 76 °C and analyzed by capillary gas chromatography (SE-54, 0.3 mm i.d. × 30 m, oven temperature 150 °C to 250 °C at 10 °C/min followed by 250 °C to 290 °C at 4 °C/min) using gas chromatography (GC) (model 5840; Hewlett Packard) fitted with a flame-ionization detector (Severson et al., 1984). Component identities were verified by mass spectrometry (MS).

Initial cuticle samples were collected after

9 d of treatment at the time maximum treatment effects were visible on the leaves present at treatment initiation. Additional samples were collected after 15 (Summer 1987) or 21 (Fall 1988) d of treatment, when leaves developing during treatment had expanded and the plants appeared to have adapted to the treatments. The final samples were collected at 14 or 8 d after transplanting the plants to the field in 1987 or 1988, respectively. Transpiration of four comparable plants in each subplot was measured at each collection time in 1988 with a steady-state porometer (LI-1600).

Results and Discussion

GROWTH RESPONSES. Treatment effects on plant growth in Summer 1987 were fully described previously (Latimer, 1990) but are summarized for comparison with the Fall 1988 data (Table 1). In Summer 1987, stem length was reduced only by MSC and leaf area and shoot dry mass only by brushing, relative to untreated controls. However, leaf dry mass was reduced by both forms of mechanical conditioning. In 1988, wind, brushing, and MSC reduced stem length, leaf area, dry mass, and shoot dry mass. Specific leaf mass, an indicator of leaf thickness, showed little

Fig. 1. Chromatogram of C₂₉ cuticular components of broccoli leaves. Compounds were derivatized with 3 TMSI : 1 pyridine and identities were verified by GC-MS: C₂₉ hydrocarbon = nonacosane, C₂₉-ol = nonacosan-15-ol (trimethylsilyl ether), and C₂₉-one = nonacosan-15-one.

Table 2. The C_{29} components of the cuticle samples taken over time of treatment with mechanical conditioning by wind or brushing or with moisture-stress conditioning (MSC) in Summer 1987. Treatments were not imposed after transplanting to the field.

Sampling time and treatment	C_{29} cuticular components ($\mu\text{g}\cdot\text{cm}^{-2}$)			
	C_{29}	$C_{29}\text{-ol}$	$C_{29}\text{-one}$	Total
After 9 d of treatment				
Untreated	18.0 a ^z	6.22 a	16.4 a	40.6 a
Wind	11.5 b	4.05 b	9.7 c	25.2 b
Brushing	12.8 b	4.78 b	10.4 c	28.0 b
MSC	16.7 a	5.72 a	13.8 b	36.2 a
After 15 d of treatment				
Untreated	18.6 b	6.04 b	15.6 b	40.2 b
Wind	17.4 bc	5.83 b	14.4 bc	37.6 bc
Brushing	16.1 c	5.79 b	12.3 c	34.2 c
MSC	21.1 a	6.81 a	19.1 a	47.0 a
After 14 d in the field				
Untreated	22.7	6.63	14.2	43.5
Wind	22.8	6.54	15.5	44.8
Brushing	23.6	6.79	15.4	45.8
MSC	21.9	6.38	14.6	42.9
	NS	NS	NS	NS

^zMean separation within sampling time and column by LSD, 5% level.

^{NS}Nonsignificant at $P = 0.05$.

response to treatment under summer conditions in 1987, but in Fall 1988 was increased by MSC. The higher temperatures in the greenhouse in 1987 contributed to the greater stem length measured for the 5-week-old plants than the 6-week-old plants in 1988 and probably accounted for the reduced growth response to brushing in 1987. Mechanically stressed tomato (*Lycopersicon esculentum* Mill.) seedlings grown at high (32 °C day/28 °C night) temperatures were less responsive to shaking than were plants grown at moderate (27 °C day/23 °C night) temperatures (Heuchert and Mitchell, 1983).

EPICUTICULAR WAX ANALYSIS. Preliminary characterization of the epicuticular wax samples determined that all the extracted components partitioned into the nonpolar phase. The C_{29} compounds—nonacosane (the C_{29} hydrocarbon), nonacosan-15-ol (C_{29} alcohol), and nonacosan-15-one (C_{29} ketone)—were the dominant components of broccoli cuticle (Fig. 1), which is consistent with Kolattukudy (1980).

On the first sampling date in Summer 1987, mechanical conditioning by wind or brushing reduced the amounts of all three C_{29} components of the cuticle (Table 2). The amount of hydrocarbon from wind-treated plants was reduced 36% relative to that of untreated plants, while brushing reduced hydrocarbon content 29% on the first sampling date. MSC reduced only the amount of the C_{29} ketone, down 16% relative to the untreated plants. On the second sampling date, only brushed plants continued to exhibit lower levels of hydrocarbon and ketone components, relative to the control. After 14 d in the field, no significant differences remained among the treatments (Table 2).

The total amount of the C_{29} epicuticular waxes was reduced by wind and brushing after 9 d of treatment, but only brushing caused a significant reduction after 15 d of treatment (Table 2). MSC increased the amount of the total C_{29} epicuticular components after 15 d of treatment.

In the Fall 1988 experiment, the distribution of C_{29} components was similar to that determined in 1987, and, as with the 1987 results, we saw little change in the distribution of the components in response to conditioning treatments. However, the total epicuticular waxes of plants subjected to MSC were higher than those of untreated plants on the first sampling date, but the total C_{29} surface

waxes were reduced by brushing or by wind on the first or second sample dates, respectively (Fig. 2). After 8 d in the field (29 d after initiation of treatment), there were no differences in the total amount of epicuticular waxes on the sampled leaves.

In Fall 1988, transpiration rate was not affected by wind or brushing of the transplants at any sample date (data not shown), but after 9 d of treatment, MSC plants transpired at <50% the rate of controls (MSC 6.8 $\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ vs. 14.0 $\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ for controls, $P < 0.05$). Although not as great, differences in transpiration between MSC and untreated plants were still significant after 21 d of treatment (MSC 8.07 $\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ vs. 10.2 $\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ for controls, $P < 0.05$), but were no longer significant at 8 d after planting to the field. Brushing increased whole-plant transpiration of tomato (van Iersel, 1997) and increased cuticular transpiration of detached leaves of cucurbit transplants (Latimer and Beverly, 1994). Rubbing (a thigmic stress) the waxy bloom off of *B. oleracea* plants also increased cuticular transpiration, but the amount of wax on the

Fig. 2. Total epicuticular waxes of broccoli transplants after 9 and 21 d of treatment in the greenhouse and at 8 d after transplanting to the field in Fall 1988.

leaf did not correlate with cuticular water loss, suggesting that water-barrier properties of the cuticle are affected also by the arrangement of the wax particles (Denna, 1970). Epicuticular wax morphology is significantly affected by abrasive treatments like rubbing leaf surfaces (Hoad et al., 1993).

Although mechanical conditioning reduced the amounts of C_{29} cuticle components, the relative distribution of these components was generally unchanged, indicating that the biosynthetic pathway of the C_{29} components is inhibited or that the response is simply due to the physical removal of the wax. This is logical for brushed plants. However, we observed that mechanical conditioning by wind resulted in a combination of physical disturbance and dehydration stress during the first 5 to 7 d of treatment. Leaves sampled after 9 d of treatment were almost fully expanded when treatment was initiated.

Hall and Jones (1961) suggested that, when growth is interrupted by a stress experience, the wax removed by stress or by normal weathering is not replaced fast enough to maintain normal wax levels. Epicuticular wax is primarily deposited during leaf expansion (Schieferstein and Loomis, 1956), and leaves developing under stress produce waxes to survive under the existing stress conditions (Saneoka and Ogata, 1987), which would explain the acclimation of the broccoli plants to the conditioning treatments.

Perhaps, brushed plants were not able to replace waxes as fast as they were physically removed by the treatment. Wind-treated plants were more responsive to the treatment early in the experiment due to the multiple stressors observed during treatment, physical removal of waxes due to leaf-to-leaf contact in the wind, and physiological (drought) stress. As the plants adapted to the wind treatment, they no longer wilted during treatment, but the physical contact (leaf-to-leaf) was less than that experienced by the brushed plants. Therefore, we expected more direct removal of epicuticular waxes by brushing than by wind.

Brushed plants also are resistant to some insect pests during greenhouse production. Brushing reduced thrips populations on tomato and bell pepper (*Capsicum annuum* L.) transplants (Latimer and Oetting, 1994). The structure and composition of epicuticular waxes also function in mediating plant resistance to insect (Eigenbrode and Espelie, 1995) and disease pests (Kolattukudy, 1987; Yang et al., 1992). The mechanism of induced pest resistance in response to brushing is not known but may relate to changes in cuticle morphology or epicuticular wax composition (Eigenbrode and Espelie, 1995).

Mechanical conditioning or MSC can effectively control plant growth nonchemically. Although the conditioning treatments affected broccoli leaf appearance, none of the treatments increased plant transpiration. In addition, mechanical conditioning may be beneficial in reducing plant height and insect pest pressure during greenhouse production. Furthermore, neither mechanical conditioning nor MSC reduced plant survival or performance in the field (Latimer, 1990).

- Baker, E.A. 1982. Chemistry and morphology of plant epicuticular waxes, p. 139–165. In: D.F. Cutler, K.L. Alvin, and C.E. Price (eds.). *The plant cuticle*. Academic, New York.
- Denna, D.W. 1970. Transpiration and the waxy bloom *Brassica oleracea* L. *Austral. J. Biol. Sci.* 23:27–31.
- Eakes, D.J., R.D. Wright, and J.R. Seiler. 1991. Moisture stress conditioning effects on *Salvia splendens* 'Bonfire'. *J. Amer. Soc. Hort. Sci.* 116:716–719.
- Eigenbrode, S.D. and K.E. Espelie. 1995. Effects of plant epicuticular lipids on insect herbivores. *Annu. Rev. Entomol.* 40:171–194.
- Hall, D.M. and R.L. Jones. 1961. Physiological significance of surface wax on leaves. *Nature* 191:95–96.
- Heuchert, J.C. and C.A. Mitchell. 1983. Inhibition of shoot growth in greenhouse-grown tomato by periodic gyratory shaking. *J. Amer. Soc. Hort. Sci.* 108:795–800.
- Hoad, S.P., C.E. Jeffree, J. Grace, L. Skarby, and S. Fink. 1993. Effects of wind and abrasion on cuticular integrity in *Fagus sylvatica* L. and consequences for transfer of pollutants through leaf surfaces. *Agr. Ecosystems Environ.* 42:275–289.
- Johansson, M., E.C. Kronstedt-Robards, and A.W. Robards. 1992. Rose leaf structure in relation to different stages of micropropagation. *Protoplasma* 166:165–176.
- Kolattukudy, P.E. 1965. Biosynthesis of wax in *Brassica oleracea*. *Biochemistry* 4:1844–1855.
- Kolattukudy, P.E. 1980. Cutin, suberin, and waxes, p. 571–645. In: P.K. Stumpf (ed.). *Lipids: Structure and function*. Academic, New York.
- Kolattukudy, P.E. 1987. Lipid-derived defensive polymers and waxes and their role in plant-microbe interaction, p. 291–314. *The biochemistry of plants*, vol. 9. Academic, New York.
- Latimer, J.G. 1990. Drought or mechanical stress conditioning affect broccoli seedling growth and transplant establishment, but not yield. *HortScience* 25:1233–1235.
- Latimer, J.G. 1991. Mechanical conditioning for control of growth and quality of vegetable transplants. *HortScience* 26:1456–1461.
- Latimer, J.G. and R.B. Beverly. 1994. Conditioning affects growth and drought tolerance of cucurbit transplants. *J. Amer. Soc. Hort. Sci.* 119:943–948.
- Latimer, J.G. and R.D. Oetting. 1994. Brushing reduces thrips and aphid populations on some greenhouse-grown vegetable transplants. *HortScience* 29:1279–1281.
- Oosterhuis, D.M., R.E. Hampton and S.D. Wulschleger. 1991. Water deficit effects on the cotton leaf cuticle and the efficiency of defoliant. *J. Prod. Agr.* 4:260–265.
- Saneoka, H. and S. Ogata. 1987. Relationship between water use efficiency and cuticular wax deposition in warm season forage crops grown under water deficit conditions. *Soil Sci. Plant Nutr.* 33:439–448.
- Schieferstein, R.H. and W.E. Loomis. 1956. Wax deposits on leaf surfaces. *Plant Physiol.* 31:240–247.
- Severson, R.F., R.F. Arrendale, O.T. Chortyk, A.W. Johnson, D.M. Jackson, G.R. Gwynn, J.F. Chaplin and M.G. Stephenson. 1984. Quantitation of the major cuticular components from green leaf of different tobacco types. *J. Agr. Food Chem.* 32:566–570.
- Sutter, E.A. 1984. Chemical composition of epicuticular wax in cabbage plants grown in vitro. *Can. J. Bot.* 62:74–77.
- Sutter, E.A. and R.W. Langhans. 1982. Formation of epicuticular wax and its effect on water loss in cabbage plants regenerated from shoot-tip culture. *Can. J. Bot.* 60:2896–2902.
- van Iersel, M.D. 1997. Tactile conditioning increases water use by tomato. *J. Amer. Soc. Hort. Sci.* 122:285–289.
- Yang, J., P.R. Verma and G.L. Lees. 1992. The role of cuticle and epidermal cell wall in resistance of rapeseed and mustard to *Rhizoctonia solani*. *Plant and Soil* 142:321–325.