Plant Growth Regulation 5: 141–154 (1987)

Physiological effects of temperature and growth regulators on foliar chlorophyll, soluble protein, and cold hardiness in citrus*,**

C.S. MAUK
Fruit Crops Department, University of Florida, Gainesville, FL 32611, USA

M.G. BAUSHER and G. YELENOSKY
U.S. Department of Agriculture, Agricultural Research Service, 2120 Camden Road, Orlando, FL 32803, USA

(Submitted 30 July 1986; revised and accepted 4 January 1987)

Key words: citrus, protein, cold hardiness, paclobutrazol, benzyladenine, napthaleneacetic acid, gibberellic acid, abscisic acid, minerals

Abstract. Leaf chlorophyll (Chl, A, B) and total soluble protein were assayed in greenhouse-grown 1.5-year-old trees of 2 citrus types, trifoliate orange (*Poncirus trifoliata* (L.) Raf.) and sour orange (*Citrus aurantium* L.) exposed to 12 h (day/night) photoperiods in growth chambers under high (30 °/21 °C, day/night; noncold-hardening) and low (16 °/5 °C; cold-hardening) temperature regimes. Trees were sprayed 2 × per week for 5 weeks with one of the following solutions at 100 μM: napthaleneacetic acid (NAA), paclobutrazol (2RS, 3RS)-1-(4-chlorophenyl-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol) (PPP333), benzyl-adenine (BA), abscisic acid (ABA), gibberellic acid (GA_3), minerals only (N, P, K, S, Ca, Mg) and BA (+) minerals. NAA, PP333, ABA and GA_3 decreased Chl A, B and soluble protein in both citrus types under cold-hardening conditions in contrast to increases with the use of BA and BA (+) minerals especially in trifoliate orange. Both BA and GA_3 increased Chl A, B and protein synthesis under high temperature in both citrus types. Under noncold-hardening temperatures, GA_3 enhanced Chl A, B but sharply reduced foliar protein concentration. Dieback of both cultivars following exposure to temperatures down to −6.7 °C was decreased 7% by NAA sprays during noncold-hardening temperatures. Cold tolerance of noncold-hardened trifoliate orange trees was also improved with ABA and PP333. Foliar sprays of NAA (sour orange) and PP333 and BA (+) minerals (trifoliate) increased cold tolerance of cold-hardened trees by 8%. Results indicate that spray applications of growth regulators influence physiological factors associated with foliar functioning and cold tolerance in citrus during different temperature regimes.

Introduction

Citrus trees can tolerate temperatures as low as −7 °C [23] when preconditioned to gradually decreasing temperatures. This cold-hardening environment exists naturally in northern California where cool night temperatures trigger cold-

* University of Florida Agricultural Experiment Station Series No. 7446.
**This paper reports the results of research only. Mention of a trademark of a proprietary product does not constitute a recommendation for use by the U.S. Department of Agriculture to the exclusion of other products that may also be suitable.

hardening induction. The subtropical climate of Florida, however, is typified by sporadic warm and cold periods that severely reduce or negate environmentally-induced cold hardening. Searches for more cold-tolerant genotypes or root-stock:scion combinations that are also commercially productive have been relatively unsuccessful [28], and orchard heating has become too expensive. Therefore, the use of plant growth regulators as potential cryoprotectants has received increased attention.

The use of chemicals to induce cold tolerance in cold-sensitive crops has been investigated in many species [10, 15, 18] and citrus [25, 28]. Results have been variable and difficult to interpret due to interactions among physiological factors involved in the cold-hardening process in citrus. Increased cold tolerance in citrus has been reported with the use of growth-suppressing chemicals such as AMO-1618 (2-isopropyl-4-dimethylamino-5-methyl-phenyl-1-piperdinecarboxylate methyl chloride), a gibberellin biosynthesis inhibitor [25].

Several products of plant metabolism have been studied in conjunction with low temperature response in plants [11, 18, 20]. Protein metabolism has been linked to cold tolerance through studies of individual amino acids [2, 10, 18, 21] and isolated proteins [5, 7, 17] in the foliage of plants exposed to temperature extremes. Chlorophyll-protein complexes have also been studied in this regard [7]. Citrus protein research [1, 6] has recently been expanded in include the effects of cold stress. Preliminary results [24] showed significantly higher proline levels in citrus foliage following exposure to cold-hardening conditions [24]. Further research, however, found no significant differences in the soluble, protein complexes [26].

The overall objective of this study was to examine the interaction of temperature and exogenously-supplied growth regulators on foliar chlorophyll, soluble protein and cold tolerance of deciduous (trifoliate orange) and nondeciduous (sour orange) citrus types. Information gained would be beneficial towards critically evaluating the potential to hormonally manipulate cold hardening in citrus in lieu of the environment.

Materials and methods

Plant material

Orange trees of sour orange (*Citrus aurantium* L.) and trifoliate orange (*Poncirus trifoliata* (L.) Raf.) were grown in a greenhouse in Pro-mix, a composite of peat and loam, and fertilized monthly with a water-soluble mixture of N, P, K, S, Ca, Mg and micronutrients. The 1.5-year-old trifoliate orange and sour orange trees had 51 to 54 and 39 to 42 nodes, respectively, and were approximately 70 cm in height at the start of the experiment.

Controlled environment conditions

Trees of each type were randomly separated into 2 groups of 24 to 36 plants each and placed in walk-in growth chambers under 12 h photoperiod for 5 weeks. The high temperature chamber was 30 °/21 °C, day/night and the low temperature unit which was 21 °/10 °C the first week, followed by 16 °/5 °C for 4 weeks. The light intensity was 500 μmol-m^{-2}-sec^{-1} at the uppermost nodes of the trees to 300 μmol-m^{-2} sec^{-1} at pot level 25 cm above the chamber floor. Lighting was supplied by a mixture of fluorescent and incandescent sources. Chamber air was circulated at 60 m/min and automatic steam injection maintained the relative humidity at 60 $\pm$ 5%.

Freeze testing

Trees of both types and each temperature:growth regulator treatment were placed in a separate chamber following 5 weeks' exposure to growth chamber conditions. Freeze tests were conducted in the dark at 50 $\pm$ 5% relative humidity under a temperature profile previously reported [23, 25]. The trees were kept at 4.4 °C for 2 h followed by a decrease of 1.1 °C/h to -6.7 °C which was maintained for 4 h. The temperature was then increased 1.1 °C/h to 4.4 °C, after which plants were held at 23 °C for 4 h and placed in greenhouses at 30 °C/23 °C. Plants were evaluated every 2 weeks for a 6-week period to assess the degree of freeze injury using percentage of leaf kill, dieback of the mainstem and recovery in growth (i.e. viable buds and nodes with renewed vegetation) as indices of freeze damage.

Foliar application of growth regulators

Foliar spray treatments consisted of BA (Sigma Chemical Co., St. Louis, MO, USA), GA_3 (Aldrich Chemical Co., Milwaukee, WI, USA), PP333 (ICI Americas Inc., Goldsboro, NC, USA), NAA, and ABA (Nutritional Biochemical Corp., Cleveland, OH, USA), each at 100 μM in 40% methanol and 0.1% Triton X-100 (surfactant). Two additional foliar spray treatments, applied to trifoliate orange trees under low temperature, were minerals only (N, P, K, S, Ca, Mg) at previously reported concentrations [13] and BA (+) minerals. Foliar sprays were applied 2 $\times$ /week for 5 weeks to abaxial and adaxial surfaces until runoff.

Foliar chlorophyll extraction and determination

Three subsamples of fresh tissue (0.5 g for trifoliate orange and 1.0 g for sour orange) were taken from each of 3 replicates thereby totalling 9 chlorophyll

(Chl) determinations for each foliar spray treatment:temperature combination. Tissue was harvested at time zero (T_O) when the plants were placed in the growth chamber and at 3 and 5 weeks. The tissue was finely minced and suspended in 3 ml dimethylformamide (DMF) for 8 h in the dark at 4 °C [14]. The supernatant was then obtained by filtration, and the remaining leaf material was resuspended in 4 ml DMF. The supernatants were then pooled 4 h later and brought to 15 ml with additional DMF prior to reading absorbance at $^{OD}664$, $^{OD}646$ and $^{OD}626$ for Chl A, B, and proto-Chl, respectively. Concentrations for total Chl A, B were obtained by comparing sample ODs to those of Chl A and B standards (Sigma Chemical Co., St. Louis, MO, USA) dissolved in DMF. Sample OD readings were performed using a LKB Model 4050 spectrophotometer equipped with a 4.5 ml cuvette and 10 mm pathlength.

Foliar protein extraction and determination

Samples of leaf tissue were taken as described in the previous section. Each sample was combined with 4 ml 0.2 M phosphate buffer (pH 7.2), 3% PVPP (w/w) and homogenized with a polytron at 4 °C. The mixture was centrifuged at 10,000 g for 20 min. Total protein was assayed in each sample using a standard procedure [3] in which 5 ml reagent dye (Coomassie Brilliant Blue G-250 (Biorad Laboratories, Richmond, CA USA), 4.7% (w/v) ethanol and 8.5% (w/v) phosphoric acid) was added to 0.2 ml sample aliquots. The solution was mixed by inversion and the absorbance read at $^{OD}595$ after 4 min and before 1 h with a LKB spectrophotometer. Sample $^{OD}595$ was measured against a reagent blank consisting of 0.2 ml phosphate buffer in 5 ml of protein dye reagent. Protein level (μg) was then plotted versus $^{OD}595$ to produce a standard curve for subsequent protein determinations in unknown samples. Bovine serum albumin was used as the standard protein source in the range of 10 to 140 μg.

Results

Foliar Chl A, B levels increased during the 5-week period of exposure to high temperature, noncold-hardening conditions (NCH) in both citrus types, although this increase was more pronounced in sour orange (Fig. 1A). Levels of Chl in both remained relatively constant over the first 3 weeks of exposure to low temperature, cold-hardening (CH) conditions (Fig. 1A). During the next 2-week period, foliar Chl doubled in sour orange while the level in trifoliate orange decreased.

Protein content in the foliage of both was twice as great under NCH than CH conditions (Fig. 1B), however, after 3 weeks protein levels in trifoliate orange under NCH conditions decreased compared to a 70% increase under CH conditions.

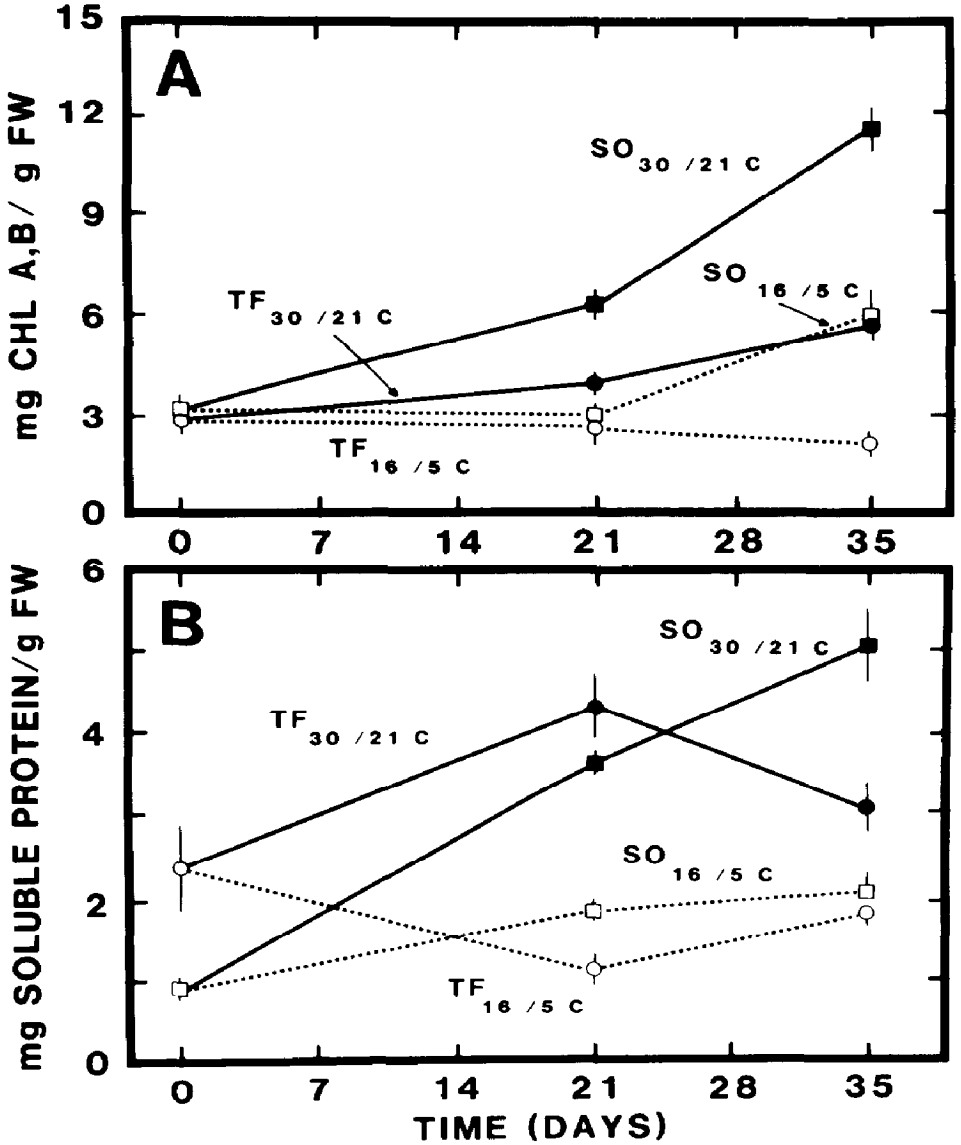

Fig. 1. Changes in foliar chlorophyll, Chl A + B, (A) and soluble protein (B) in 1.5-year-old greenhouse-grown sour orange and trifoliate orange trees during 5 weeks' exposure to noncold-hardening (30 °/21 °C) or cold-hardening (16 °/5 °C) temperatures. Vertical bars represent standard error for each mean.

Multiple sprays of GA_3 and ABA increased foliar Chl, A, B of sour orange versus the control after 5 weeks (Fig. 2A), whereas NAA decreased Chl. Under CH conditions, all treatments decreased Chl compared to the control at 5 weeks (Fig. 2B). Chl in ABA-, GA-, and NAA-treated trees was sharply reduced.

Under NCH conditions, ABA and BA were most effective in increasing foliar protein by 47% and 27%, respectively, compared to the control in sour orange at 5 weeks (Figure 3A). In contrast, GA_3 decreased foliar protein by 26%. PP333

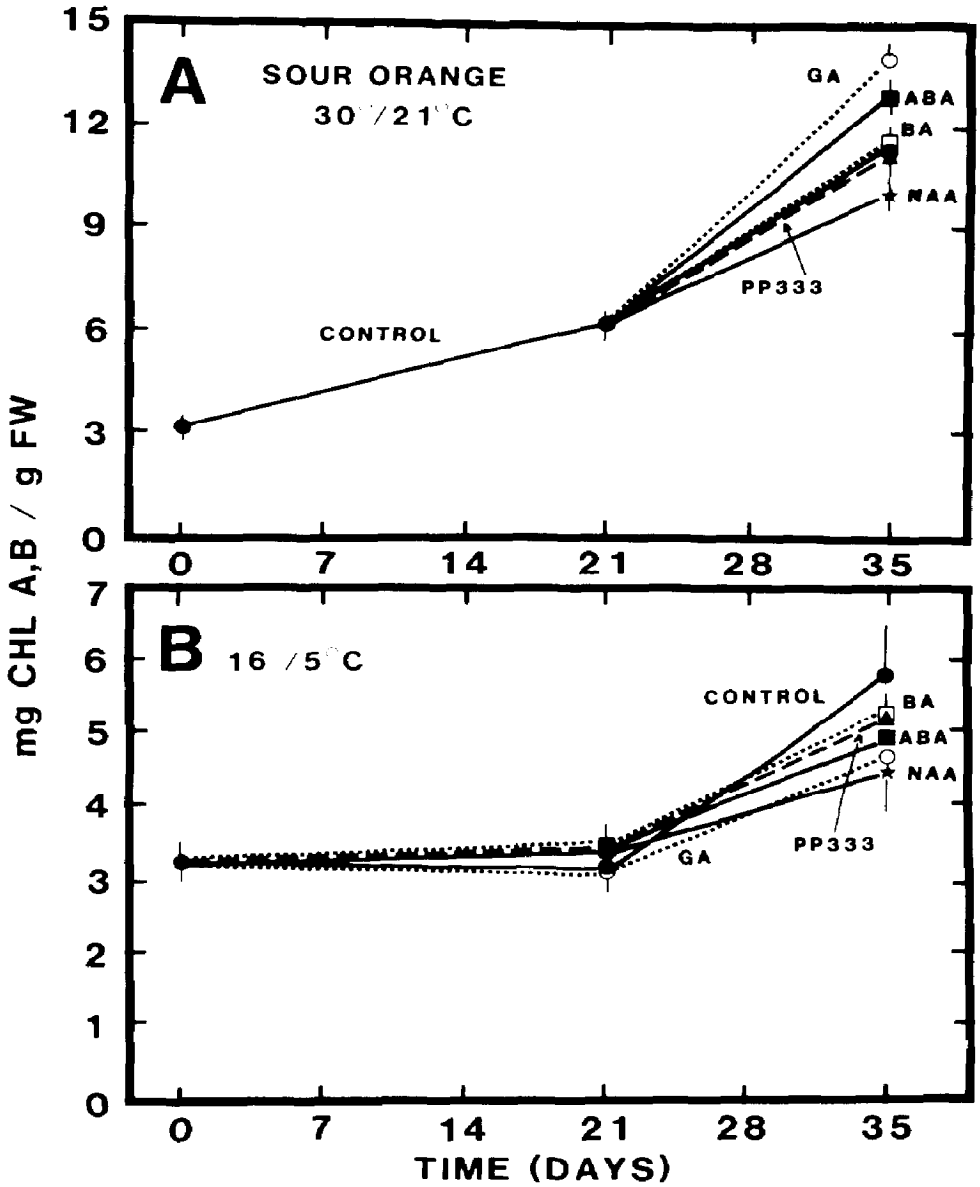

Fig. 2. Effect of growth regulator sprays (each at 100 μmol; 2 ×/week for 5 weeks) on foliar chlorophyll (Chl A + B) levels in sour orange trees during exposure to noncold-hardening, 30 °/21 °C (A), and cold-hardening, 16 °/5 °C (B) temperatures. Spray designations: GA (gibberellic acid), ABA (abscisic acid), BA (benzyladenine), PP333 (paclobutrazol), and NAA (napthaleneacetic acid). Vertical bars represent standard error for each mean.

and NAA increased protein in sour orange under CH conditions at 3 weeks (17% and 13%, respectively) compared to a 19% decline with GA_3 (Fig. 3B). The only major treatment difference at 5 weeks was the GA-induced 25% protein loss.

ABA and BA increased in Chl A, B by 12% after 5 weeks' exposure of trifoliate orange trees to NCH conditions (Fig. 4A), whereas, GA_3 and PP333

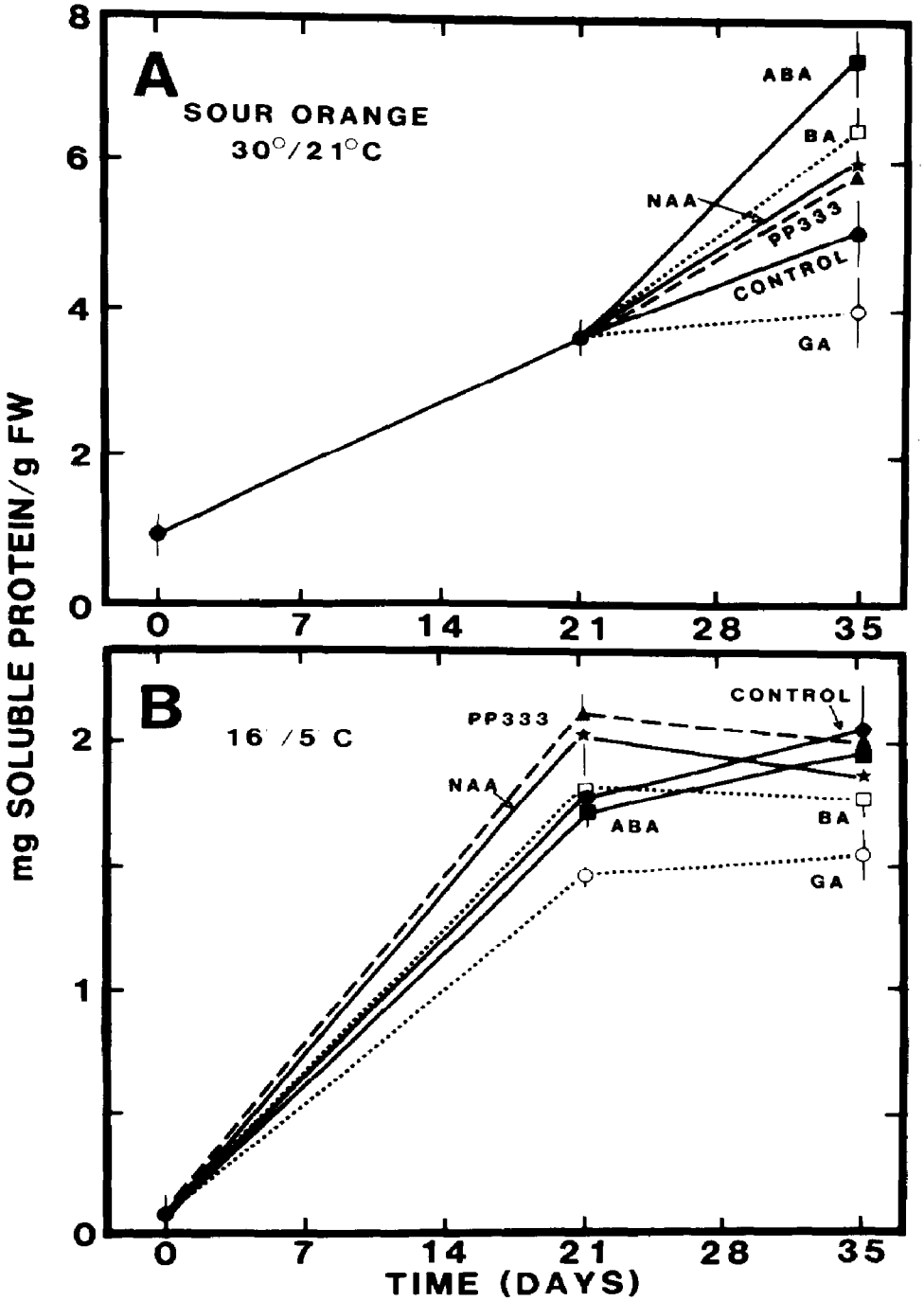

Fig. 3. Influence of growth regulator sprays (each at 100 μmol; 2 ×/week for 5 weeks) on foliar soluble protein levels in sour orange trees exposed to noncold-hardening, 30 °/21 °C (A) and cold-hardening, 16 °/5 °C (B) temperatures for 5 weeks. Spray treatment designations defined in Fig. 2. Vertical bars represent standard error for each mean.

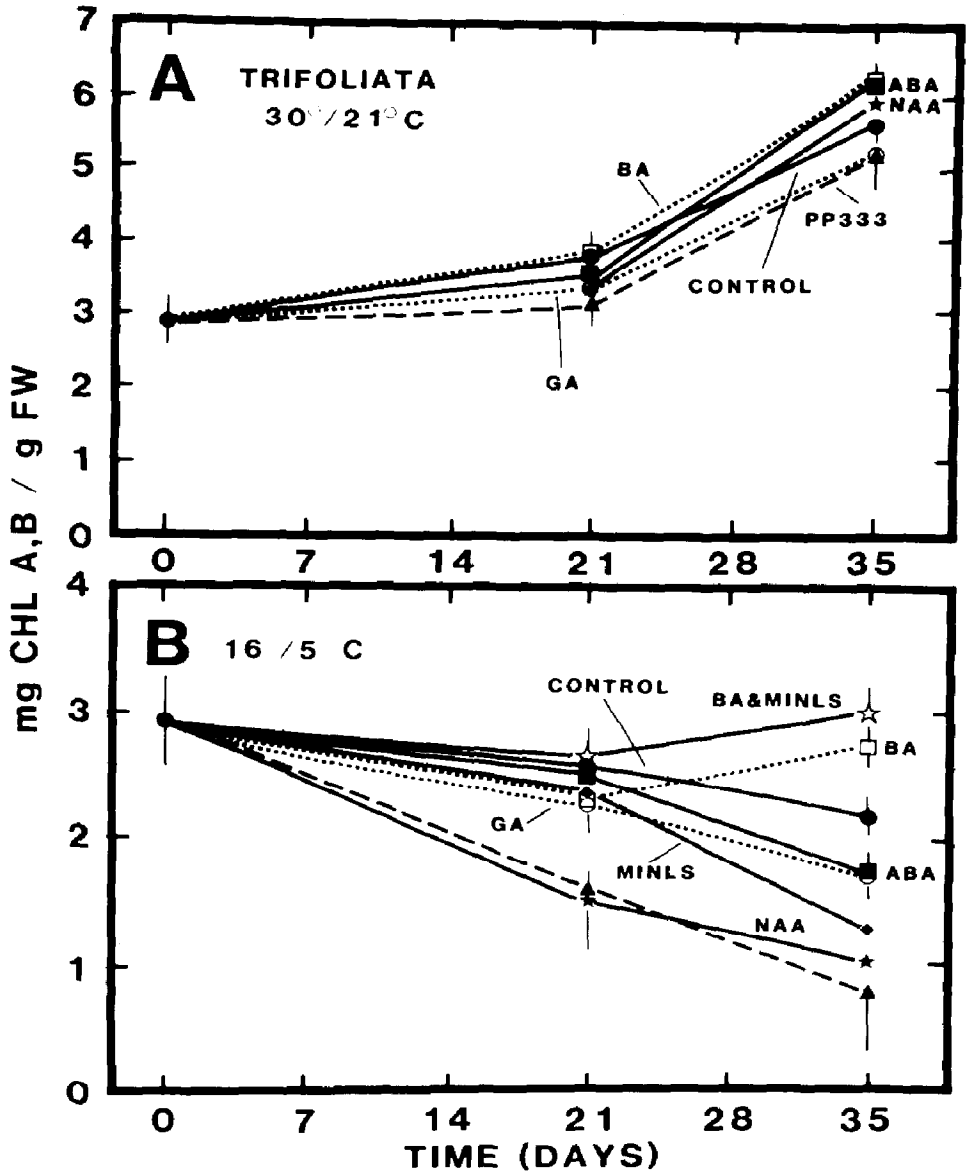

Fig. 4. Response of foliar chlorophyll levels (Chl A + B) in trifoliate orange trees to growth regulator sprays (each at 100 μmol; 2 ×/week for 5 weeks) during exposure to noncold-hardening, 30 °/21 °C (A), and cold-hardening, 16 °/5 °C (B) temperatures for 5 weeks. Spray treatment defined in Fig. 2 with additional treatments of minerals (MINLS) and benzyladenine plus minerals (BA + MINLS). Vertical bars represent standard error for each mean.

decreased Chl by 8%. Under CH conditions, BA and BA (+) minerals resulted in 25% and 37% increases, respectively, after 5 weeks (Fig. 4B). ABA and GA_3 treatments were inhibitory (22%), with NAA and PP333 sharply reducing Chl at 3 weeks (40%) and especially after 5 weeks (53% and 64%, respectively).

Protein levels in trifoliate orange after 3 weeks' exposure to NCH conditions

were sharply reduced by NAA (27%) and GA (51%) treatment (Fig. 5A). The GA-induced inhibition was also evident at 5 weeks (32%), however, PP333 increased protein 23%.

The influence of PGR's on trifoliate orange protein content under CH conditions was greatest with BA (+) minerals which resulted in nearly twice as much protein versus the control at both 3 and 5 weeks (Fig. 5B). BA alone increased protein (14%) at 5 weeks. NAA was inhibitory at 3 weeks (24%) as well as GA_3 at 5 weeks (16%).

NAA was the only foliar treatment which resulted in an increase in cold tolerance (i.e. reduction in mainstem dieback) of sour orange trees exposed to NCH conditions prior to freeze testing (Table 1). Trees exposed to a CH environment were more cold tolerant than those under NCH conditions. Hormonal effects were also more prevalent under the CH regime where NAA and ABA sprays reduced mainstem dieback by 64 and 42 percentage units, respectively, versus the control. These treatments produced 3 × more new growth (i.e. sprouted nodes) compared to the other sprays at 6 weeks following freeze testing.

Hormonal effects on cold tolerance of trifoliate orange trees exposed to NCH conditions were slight, however, there was some promotion due to NAA and PP333 (Table 2). The overriding influence under CH conditions was the low temperature effect which resulted in only 7% mainstem dieback compared to 99% for the NCH regime. PP333, minerals, and BA (±) minerals all resulted in no mainstem dieback. The most pronounced effect under cold temperature in trifoliate orange was the substantial promotion of new growth by BA (77%) and BA (+) minerals (59%).

Discussion

Increases in soluble protein levels in the foliage of woody perennials have been associated with the capacity to cold acclimate [4, 18, 21, 22]. Soluble foliar proteins varied with citrus type as shown by the decrease in trifoliate orange and increase in sour orange during cold-hardening temperatures (Fig. 1B). Protein levels of cold-hardened trees of both, however, were substantially less than their noncold-hardened counterparts. Since cold-hardened trees exhibited greater cold tolerance (Tables 1 and 2), quantitative differences in soluble protein were not indicative of the potential for cold tolerance in citrus. It has not been determined, however, if continued exposure in cold-hardening temperatures for longer periods (5 to 8 weeks) would have resulted in greater soluble protein levels and increased cold tolerance compared to noncold-hardening conditions. Qualitative studies [26] involving SDS-PAGE separations of soluble proteins from cold and noncold-hardened citrus foliage have not shown distinct differences. Exposure of other plant species [4, 17] to cold-hardening temperatures has resulted in the formation of different soluble proteins. Nonsoluble, membrane-bound protein fractions have not been investigated in citrus to determine

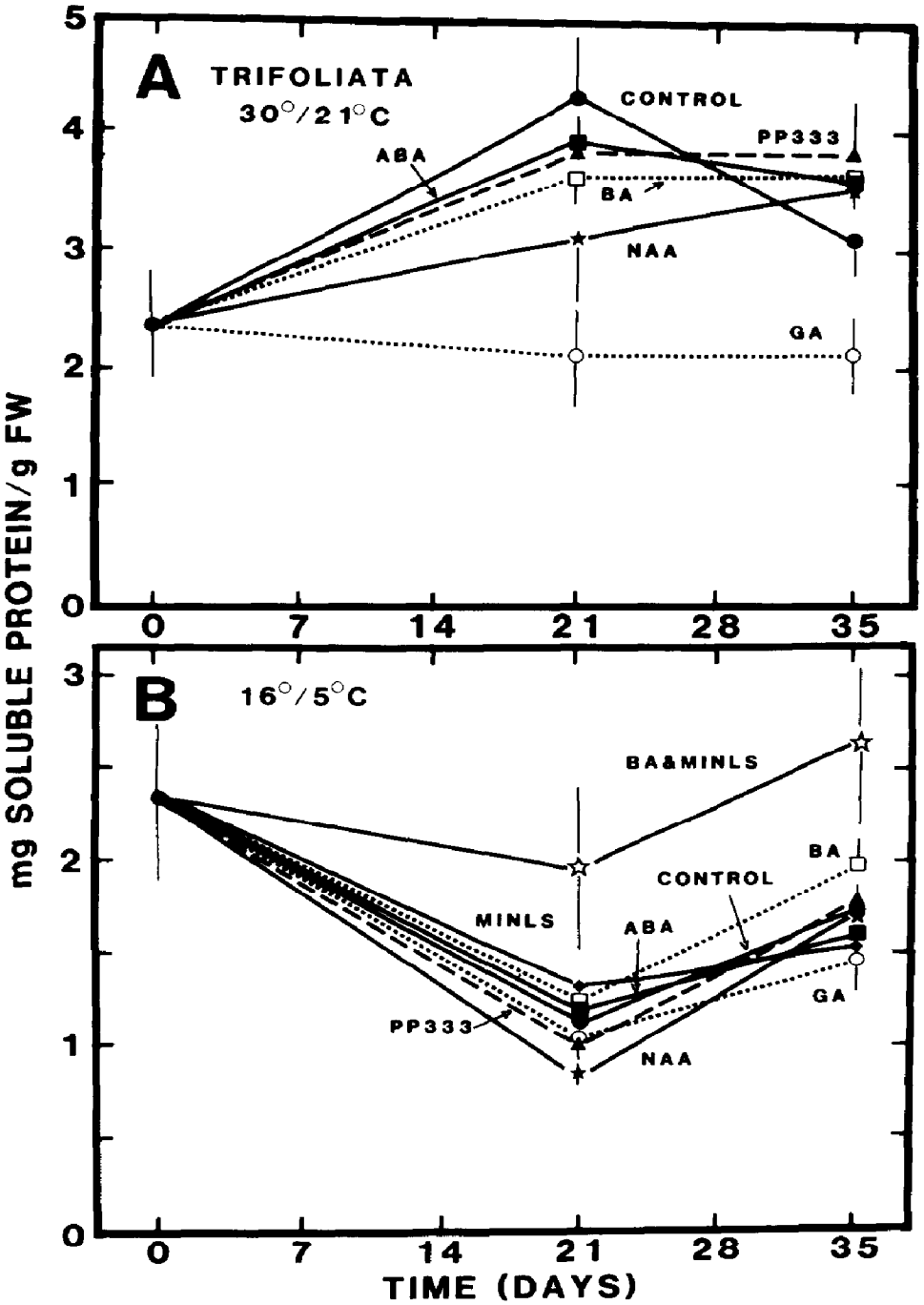

Fig. 5. Changes in foliar soluble protein levels in trifoliate orange trees as affected by growth regulator sprays (each at 100 μmol; 2 ×/week for 5 weeks) during exposure to noncold-hardening, 30 °/21 °C (A), and cold-hardening, 16 °/5 °C (B) temperatures for 5 weeks. Spray treatment defined in Fig. 4. Vertical bars represent standard error for each mean.

Table 1. Interaction of temperature (cold and noncold-hardening conditions) and foliar growth regulator sprays on percentage of mainstem dieback and percentage of sprouted nodes in 1.5-year-old sour orange trees 6 weeks after freeze testing.*

Temperature regime	Foliar spray treatment**	% mainstem dieback ± SE	% sprouted nodes ± SE
Noncold-hardening	Control	100 ± 0	0 ± 0
30 °/21 °C	ABA	100 ± 0	0 ± 0
	PP333	100 ± 0	0 ± 0
	NAA	95 ± 6	4 ± 4
	GA	100 ± 0	0 ± 0
	BA	100 ± 0	0 ± 0
Cold-hardening	Control	78 ± 4	18 ± 6
21 °/10 °C (week 1)	ABA	36 ± 21	42 ± 22
16 °/5 °C (week 2–5)	PP333	59 ± 27	33 ± 17
	NAA	14 ± 4	48 ± 11
	GA	83 ± 4	16 ± 4
	BA	90 ± 1	12 ± 1

* Freeze tests were conducted in a dark chamber at 50% relative humidity. Plants were held at 4.4 °C for 2 h followed by a temperature decline of 1.1 °C/h to − 6.7 °C which was held for 4 h. The temperature was then increased 1.1 °C/h to 4.4 °C followed by transfer to greenhouse.
**Sprays were at 100 μM in 40% methanol and 0.1% Triton X-100 and applied 2 ×/week for 5 weeks until foliar runoff.

Table 2. Interaction of temperature (cold and noncold-hardening conditions) and foliar growth regulator sprays on percentage of mainstem dieback and percentage of sprouted nodes in 1.5-year-old trifoliate orange trees 6 weeks after freeze testing.*

Temperature regime	Foliar spray treatment**	% mainstem dieback ± SE	% sprouted nodes ± SE
Noncold-hardening	Control	99 ± 1	0 ± 0
30 °/21 °C	ABA	95 ± 2	0 ± 0
	PP333	93 ± 4	0 ± 0
	NAA	93 ± 3	0 ± 0
	GA	100 ± 0	0 ± 0
	BA	99 ± 1	0 ± 0
Cold-hardening	Control	7 ± 2	23 ± 1
21 °/10 °C (week 1)	ABA	7 ± 2	10 ± 2
16 °/5 °C (week 2–5)	PP333	0 ± 0	20 ± 1
	NAA	4 ± 2	6 ± 1
	GA	4 ± 4	22 ± 4
	BA	0 ± 0	77 ± 2
	Minerals	0 ± 0	35 ± 5
	BA + Minerals	0 ± 0	59 ± 7

* Freeze tests were conducted in a dark chamber at 50% relative humidity. Plants were held at 4.4 °C for 2 h followed by a temperature decline of 1.1 °C/h to − 6.7 °C which was held for 4 h. The temperature was then increased 1.1 °C/h to 4.4 °C followed by transfer to greenhouse.
**Sprays were at 100 μM in 40% methanol and 0.1% Triton X-100 and applied 2 ×/week for 5 weeks until foliar runoff.

their response to cold-hardening temperatures. Previous research with nonsoluble protein fractions in black locust [4] did not show any effect due to cold-hardening conditions.

The growth regulator sprays exhibited varying effects on soluble foliar protein, chlorophyll and cold tolerance. The most noticeable were due to NAA and BA which both showed the potential to increase cold tolerance (Tables 1 and 2), even though their impact on foliar chlorophyll and protein drastically differed. NAA, for example, enhanced cold tolerance in both citrus types under noncold-hardening temperatures, while decreasing protein and chlorophyll. NAA-enhanced cold hardening has been reported in citrus , however, the physiological effects have received little attention. The cessation or retardation of foliar functioning and growth in general due to NAA treatment has been associated with an increase in cold hardiness and may be related to an overall decrease in relative water content of plant tissues [18]. Cold-hardened citrus trees [22] have shown a 4-fold increase in foliar water potential compared to those exposed to noncold-hardening conditions [22].

The influence of BA on foliar chlorophyll and protein was generally the opposite of NAA, although both showed the potential to promote cold hardiness. BA application retarded chlorophyll loss in barley [16], and delayed senescence in soybean [13], while inhibiting foliar mineral loss. Foliar sprays of BA also promoted photosynthate translocation from leaves to young fruitlets in citrus [12]. The relationship between these events and cold hardiness remains unresolved. NAA and BA most likely act independently on physiological processes related to foliar functioning and cold hardiness in citrus. BA may function more directly on cold hardiness via foliar maintenance, whereas NAA has an indirect effect through a decline in growth and increase in tissue dehydration.

ABA has been shown to promote while GA_3 inhibited cold hardiness [10, 15]. In citrus, ABA promoted cold hardiness (Table 1, CH; Table 2, NCH), in comparison to GA_3 which displayed no effect. ABA stimulated chlorophyll production under noncold-hardening conditions in both citrus types (Figs. 2A and 4A), but resulted in a decline under cold-hardening temperatures (Figures 2B and 4B). In this case, the decline in foliar functioning was associated with an increase in cold tolerance. ABA-treated apple trees have shown increased cold tolerance and reduced asparagine levels [10] which supported a possible ABA-protein interaction, but this has not been demonstrated in citrus.

GA_3 hastened the decline in soluble protein (Figs. 3A-B and 5A-B) which has correlated highly with a decrease in cold hardiness in other crops [10, 15] as well as citrus [27]. Inhibitors of GA_3 synthesis, such as AMO-1618, have promoted cold tolerance in citrus [25]. Even though the decline in GA-induced cold hardiness was slight in this study, it has been more pronounced in similar experiments with citrus involving the same citrus types (data unpublished). Foliar application of PP333, another GA_3 synthesis inhibitor , enhanced cold tolerance of trifoliate orange (Tables 1 and 2) which further supported the inhibitory nature of GA_3 on the process.

Summary

Growth promoters (BA) and inhibitors (NAA) have the potential to promote cold hardiness through either a strong stimulatory effect on foliar physiology or a marked inhibition of growth in general. This suggests that each growth regulator may possess an independent role in the cold-hardiness phenomenon and may also interact with physiological processes other than soluble protein and chlorophyll metabolism. The relationship between soluble protein levels in citrus foliage and the degree of cold hardiness remains uncertain and is essentially unresolved pending more specific qualitative research.

References

1. Agusti JA and Beltran JP (1982) Quantitative determination of the protein content of citrus leaf extracts: A comparative study. Analyt Biochem 127:368–371
2. Benko R (1968) The content of some amino acids in young apple shoots in relation to frost resistance. Biol Plant (Praha) 11(5):334–337
3. Bradford RR (1976) A rapid and sensitive method in the quantification of microgram quantities utilizing the principle of protein-dye binding. Analyt Biochem 72:248–254
4. Brown GN and Bixby JA (1975) Soluble and insoluble protein patterns during induction of freezing tolerance in black locust seedlings. Physiol Plant 34:187–191
5. Chou JC and Levitt J (1972) The hydrophobicity of proteins from hardy (freezing resistant) and nonhardy species of grains. Cryobiology 9:268–272
6. Clements RL (1966) Disc electrophoresis of citrus fruit proteins. Phytochem 5:243–249
7. Elfman B, Huner PA, Griffith M, Krol M, Hopkins GW and Hayden DB (1984) Growth and development at cold-hardening temperatures: Chlorophyll-protein complexes and thylakoid membrane polypeptides. Can J Bot 62:61–67
8. Goren R and Goldschmidt EE (1970) Regulative systems in the developing citrus fruit. I. The hormonal balance in orange fruit tissues. Physiol Plant 23:937–947
9. Hendry NS, Van Staden J and Allan P (1982) Cytokinins in citrus. II. Fluctuations during growth in juvenile and adult plants. Sci Hort 17:247–256
10. Holubowicz T and Boe AA (1970) Correlation between hardiness and free amino acid content of apple seedlings treated with gibberellic acid and abscisic acid. J Amer Soc Hort Sci 95(1):85–88
11. Levitt K (1966) Cryochemistry of plant tissue. Cryobiology 3(3):243–251
12. Mauk CS, Bausher MG and Yelenosky G (1986) Influence of growth regulator treatments on dry matter production, fruitlet abscission and C^{14}-assimilate partitioning in citrus. J Pl Gr Reg 5:111–120
13. Mauk CS, Brinker A and Nooden LD (1986) Synergistic action of cytokinin and mineral nutrients on foliar senescence and pod development in soybean explants. Bot Gaz 145: (in press)
14. Moran R and Porath D (1980) Chlorophyll determination in intact tissues using N,N-dimethylformamide. Pl Physiol 65:478–479
15. Rikin A, Walsman M, Richmond AE and Dorvat A (1975) Hormonal regulation of morphogenesis and cold resistance. I. Modification by abscisic acid and by gibberellic acid in alfalfa (*Medicago sativa* L.) seedlings. J Exp Bot 26(91):175–183
16. Sabater B, Rodriguez MT and Zamorano A (1981) Effects and interactions of gibberellic acid and cytokinins on the retention of chlorophyll and phosphate in barley leaf segments. Physiol Plant 51:361–364

17. Shomer-Ilan A and Waisel Y (1975) Cold hardiness of plants: Correlation with changes in electrophoretic mobility, composition of amino acids, and average hydrophobicity of fraction-1-protein. Physiol Plant 34:90–96
18. Simonovitch D (1981) Common and disparate elements in the processors of adaptation of herbaceous and woody plants to freezing: A perspective. Cryobiology 18:166–185
19. Smillie RM and Hetherington SE (1983) Stress tolerance and stress-induced injury in crop plants measured by chlorophyll fluorescence in vivo. Pl Physiol 72:1043–1050
20. Taborsky G (1979) Protein alterations at low temperatures: An overview. Adv Chem Ser 180:1–25
21. Tamassy I and Zayan M (1982) Soluble proteins and amino acids as related to cold hardiness in some apricot varieties belonging to different groups. Acta Hort 121:141–152
22. Vigue J, Li PH and Oslund CR (1974) The effect of low temperature on the polyribosome, nucleic acid and protein content of potato leaves. Pl and Cell Physiol 15:1055–1062
23. Yelenosky G (1978) Cold hardening 'Valencia' orange trees to tolerate – 6.7 °C without injury. J Amer Soc Hort Sci 103:449–452
24. Yelenosky G (1979) Accumulation of free proline in citrus leaves during cold hardening of young trees in controlled temperature regimes. Pl Physiol 64:425–427
25. Yelenosky G (1985) Extension growth and cold hardening of young 'Valencia' orange trees sprayed with AMO-1618. Pl Gr Reg 5:47–53
26. Yelenosky G and Guy CL (1982) Protein scans of cold-hardened and freeze-induced 'Valencia' orange leaves. Cryobiology 19:646–650
27. Yelenosky G and Wutscher HK (1985) Growth capacity of 'Valencia' orange buds on different rootstocks during cold-hardening temperatures. J Amer Soc Hort Sci 110(1):78–83
28. Young R (1971) Effect of growth regulators on citrus cold hardiness. J Amer Soc Hort Sci 96:708–710