Two-step Heat Treatment of Pineapple Crowns Increases Thermotolerance

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Summary. Thermotolerance of pineapple crowns ('Champaka 153') to 50C and above was increased with a 30-min first treatment at 30, 35, or 40C. Pineapple crowns receiving a 30-min heat treatment, before a second heat treatment at 50 or 55C, exhibited significantly less leaf damage than controls receiving no first treatments ($P \leq 0.05$). The degree of thermotolerance was dependent upon the season in which crowns were harvested; greater thermotolerance occurred in crowns harvested in April than those harvested in October. Maximum thermotolerance occurred after an interval of at least 8 h between the first treatment and the higher temperature heat treatment. Thermotolerance was stable for at least 24 h.

Pineapple is the second-largest agricultural commodity in Hawaii. At present, the Hawaiian pineapple industry employs 3700 people, uses 27,000 acres, and produces 460,000 tons of fruit. One of the most serious threats to the Hawaiian pineapple industry is mealybug wilt

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of pineapple (MWP) (German et al., 1992; Rohrbach et al., 1988). MWP is a serious problem for pineapple producers worldwide and is associated with the presence of mealybugs (*Dysmicoccus brevipes* and *D. neobrevipes*) (German et al., 1992; Rohrbach et al., 1988; Wakman et al., 1995). Although MWP has been studied for more than 60 years (Carter, 1933; Gunasinghe and German, 1989; Hu et al., 1992; Unman et al., 1989; Unman et al., 1991; Wakman et al., 1995), the etiology of this disorder is still unknown. In 1988, a closterovirus was found in pineapple in Hawaii (Gunasinghe and German, 1989). Since then, pineapple closterovirus (PCV) has been observed to be widespread in pineapple in several countries (Unman et al., 1989; Wakman et al., 1995). Recently, a pineapple badnavirus (PBV) was found in Australia (Wakman et al., 1995) and in Hawaii (unpublished data). The etiological role of these viruses in MWP is unknown.

To examine the etiological significance of these pineapple viruses, it is essential to have virus-free pineapple plants for experimentation. Previously, Unman et al. (1991) heat-treated pineapple crowns at 50, 55, and 60°C and tested for the presence of PCV and subsequent growth of plants in the greenhouse and field. This work demonstrated that heat treatment resulted in PCV-free pineapple plants and that such plants had improved growth rates and fruit yields (Unman et al., 1991). However, heat treatment at 50°C damaged leaf tissue, and temperatures of 55 and 60°C drastically reduced crown survival rate. Due to variability in crown damage, tremendous variability in plant growth occurred following heat treatments at 50°C. Uniformity of growth is essential to pineapple production because plants are forced to flower simultaneously. Because fruit size is dependent on plant size at the time of forcing of fruit development, usually with ethylene or ethephon (Bartholomew and Malezieux, 1994), uniform fruit size can be achieved only with uniform stands of plants.

The objective of this study was to reduce crown damage by optimizing the conditions of heat treatment and thus achieve uniformity in plant growth. We have developed a procedure that uses a moderate temperature to induce thermotolerance, followed by a high temperature known to result in virus-free plants (Unman, et al., 1991).

Materials and methods

Plant materials. Crowns collected from fruits of the 'Smooth Cayenne' pineapple (*Ananas comosus* L. Merr.) clone 'Champaka 153' were used for heat treatments. Crowns treated in October 1993 were medium size grade with an average weight of 288.7 ± 54.9 g (mean $\pm$ SD). Crowns used in April 1994 experiments were small size grade with an average weight of 144.7 ± 36.5 g. Crowns received no preplant chemical dip treatment before the heat treatments.

Experimental design and statistical analysis. Pineapple crowns were assigned at random to groups of 20 for treatments. The two-step heat treatment experiments were conducted twice using crowns harvested in Oct. 1993 or Apr. 1994. In both experiments, the interval between the first and second heat treatments was 8 h. Statistical comparisons were made for each experiment using 20 multiple observations for each treatment. Unequal variances between treatment groups necessitated using two-tailed *t* tests for unequal variances. Treatment groups were replicated for the 0- to 24-h interval experiments using crowns harvested in Apr. 1994. Replicates of treatment groups containing 20 crowns each were compared using *F* tests to ensure that the replicates could be compared for analysis. After pooling of the replicates, *t* tests for unequal variances were performed between treatments. A significance level of $P \leq 0.05$ was used for all statistical comparisons.

Induction of thermotolerance (first heat treatment). A waterproof thermocouple (8 cm $\times$ 1.5-cm-diameter shanks) was inserted in the bottom center to a depth of ≈ 4 cm in eight randomly selected crowns in each group of 20 pineapple crowns. An additional thermocouple was placed in the bottom of the mesh container used to hold the crowns during treatment. A 10th thermocouple was placed in the water bath where water could circulate freely over it. Crowns were distributed randomly in a mesh container. Temperatures were monitored using a model 5100 Logger Lab (ECD). Crown ambient core temperature was generally 22 to 23°C before treatment. The desired water temperature (30, 35, or 40°C) was established before the addition of the crowns in a 200-liter

water bath equipped with a stirrer. The water bath temperature was maintained for the duration of the treatment within $\pm 0.2^\circ\text{C}$ of the desired temperature by adding hot water accompanied by vigorous stirring. Crown core temperature was monitored every 10 min until crown temperature was within $\pm 1^\circ\text{C}$ of the treatment temperature, after which monitoring was conducted every 2 min. Water temperature was monitored every 2 min throughout the treatment. All monitored crowns reached the treatment temperature within 4 min of one another, and batch-to-batch variation between like treatments averaged <6 min. Timing of the 30-min treatment began when a minimum of five of the eight thermocouples in crowns were within 0.2°C of the treatment temperature. When crown cores had been at the target temperature for 30 min, crowns were removed from the water bath and placed on shelves outdoors in a shaded area to cool and dry. Intervals ranging from 0 to 24 h between first treatment and second heat treatment were tested for presence of thermotolerance induced by the first heat treatment.

Second heat treatment. Crowns were prepared for the second temperature treatment and treated as described above at the target temperature (40, 50, 55, or 60°C). Water level was maintained a minimum of 20 cm above the highest crowns to avoid temperature differentials that may occur at the water surface. Intense crowding of crowns was avoided as it can result in bruising at higher temperatures. Temperature regulation and monitoring was as outlined previously. When the temperature of five of eight randomly chosen crowns was within 0.2°C of the target temperature, the crowns were maintained for 30 min at the target temperature, and placed on the shelves to cool until damage assessment.

Damage assessment. Crowns were rated for damage 7 days after the second treatment by viewing the crown from above and assigning an overall percentage of damaged leaf area. Using 10% intervals, crowns were selected that exhibited damage ranging from 0% to 100%. These crowns were used as standards for comparison to assist in maintaining assessment continuity.

Results

Thermotolerance was induced by 30-min first treatments at 30, 35, or

Table 1. Evaluation of pineapple crown leaf damage (mean percentage $\pm$ sd) 7 days after the second heat treatment.

Second heat treatment		First treatment temp (°C)		
temp (°C)	No first treatment	30	35	40
<i>October crowns</i>				
No treatment	0.8 ± 0.7 g ^y	1.4 ± 1.5 g	1.5 ± 1.6 g	4.7 ± 3.7 f
40	4.7 ± 3.7 f	1.3 ± 1.6 g	8.9 ± 5.4 e	10.8 ± 7.7 e
50	60.5 ± 19.9 c	9.2 ± 6.8 e	17.0 ± 10.3 de	19.6 ± 10.3 d
55	97.2 ± 4.3 a	91.8 ± 10.3 ab	90.5 ± 8.3 b	82.9 ± 12.2 b
<i>April crowns</i>				
No treatment	3.5 ± 2.6 f	1.8 ± 2.4 g	3.4 ± 3.4 f	4.87 ± 2.4 e
40	4.8 ± 2.4 e	4.0 ± 4.0 ef	2.7 ± 2.4 fg	4.4 ± 2.6 e
50	19.5 ± 13.1 c	7.1 ± 10.2 de	9.5 ± 6.0 d	7.2 ± 3.7 de
54	48.8 ± 37.1 b	19.5 ± 13.5 b	23.3 ± 13.8 c	18.4 ± 11.4 c
60	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a

^yThe interval between the first treatment and second treatment was 8 h.

^zMeans followed by same letter are not significantly different from each other at $P = 0.05$ (Student's *t* test).

40C, with the greatest reduction in crown damage occurring when a 30C first treatment was used (Table 1). When subjected to second, higher temperature heat treatments, the induced thermotolerance reduced crown damage at 40–55C. At 60C, crowns suffered devastating damage with or without first treatment (Table 1). Induction of thermotolerance was observed in pineapple crowns harvested in different seasons. However, thermotolerance was greater in crowns harvested in April (Table 1).

Crowns receiving no heat treatment exhibited mean leaf damage ranging from 0.8% (October crowns) to 3.5% (April crowns) (Table 1). Treatment for 30 min at 30 and 35C did not cause any significant leaf damage. A single treatment at 40C resulted in damage just under 5%, slightly higher than the untreated controls. October crowns receiving no first treatment before exposure at 50C exhibited an average of 60.5% leaf damage. All groups receiving 30, 35, or 40C first treatment before 50C treatment exhibited significantly less leaf damage than untreated crowns exposed to 50C (Table 1). April crowns receiving no first treatment and exposed to 50C were more thermotolerant than October crowns (Table 1). April crowns receiving a first treatment before a 54C heat treatment suffered mean leaf damage of <24%, whereas crowns receiving no first treatment suffered nearly 49% damage (Table 1). The thermotolerance of April crowns broke down at 60C (Table 1). The best combination that induced thermotolerance and did not cause damage to

crowns harvested in both seasons was a 30C treatment followed by a second treatment at 50C.

The interval between first and second treatments significantly affected thermotolerance. April crowns treated at 30C and followed by a 52C treatment within 0 to 24 h exhibited a range of thermotolerance that was greatest after 8 h and remained stable for at least 24 h (Fig. 1).

Discussion

Thermotherapy has proven effective in eliminating bacteria, fungi, and viruses from peanut, potato, begonia, yam, garlic, and gladiolus (Conci and Nome, 1991; Dunbar et al., 1993; Grondeau and Samson, 1994; Hsieh, 1985; Kaiser, 1980; Raabe and Baker, 1971; Sinha and Singh, 1985). Also, pineapple crowns treated at 50–60C for 30–60 min exhibited increased vigor and fruit yield (Unman et al., 1991). The mechanism underlying the benefits of thermotherapy in pineapple is unknown. It may be due to elimination of viruses, other unknown pathogens, or a physiological response. Increases in growth rate and fruit yield are important, because they may enable the pineapple industry to shorten production cycles and increase yields. The disadvantages of previous attempts at thermotherapy were that crown damage was produced and plant growth variations occurred in treated plants. The approach we have developed induces thermotolerance and significantly reduces damage caused by the higher temperatures known to stimulate growth (Unman et al., 1991). This reduction in damage could result

in more uniform plant growth while increasing vigor and fruit yield. Assessment of plant growth is of continuing interest. In addition, this approach also might be useful for the elimination of fungi, bacteria, nematodes, insects, and viruses from other plants which are sensitive to thermotherapy.

The results presented here are consistent with the presence of heat shock response (HSR) observed in a variety of organisms (Veiriling, 1991). In the HSR a moderate heat shock treatment induces thermotolerance to subsequent higher temperature treatments. Heat shock protein (HSP) is a component of the HSR and has been linked to thermotolerance in wheat, soybean, and sugarcane (Hendershot et al., 1992; Krishnan et al., 1989; Moisyadi and Barrington, 1989; Shuster et al., 1988). HSP production can be induced by exposing plants or cell suspensions to temperatures 8–10C above normal growing conditions for 30 min to 2 h. Maximum HSP synthesis generally occurs after a 2-h treatment (Hendershot et al., 1992; Moisyadi and Barrington, 1989; Kimple and Key, 1985). The thermotolerant state following a 2-h HSP induction treatment requires 4 to 8 h to develop and disappears after 48 h in cultured sugarcane cells (Moisyadi and Barrington, 1989). Optimum growth temperatures for pineapple plants are 30C (day) and 20C (night) with a mean of 23 to 24C (Bartholomew and Malezieux, 1994). Therefore, it is likely that the heat shock response and maximum HSP synthesis in pineapple would occur between 30 to 40C. In this study, exposing pineapple crowns to a first treatment temperature, allowing thermotolerance to develop, then applying the heat treatments used previously (Unman et al., 1991) significantly reduced plant damage. The loss of thermotolerance observed at 55C (for October crowns) and 60C (for April crowns) indicates that tolerance can break down at higher temperatures. It is possible that production of HSP was responsible for the thermotolerance we observed.

The two-step heat treatment significantly reduces damage at 50C for April and October crowns. The effects of shorter or longer first treatments remain to be studied. Changes in duration of the second heat treatment may also have marked effects on the elimination or inhibition of pathogens.

Fig. 1. Mean percentage of leaf damage sustained by pineapple crowns has-vested in April after receiving a 30C pretreatment followed 0 to 24 h later by a 30-min heat treatment at 52C. Means followed by same letter at-e not significantly different from each other at $P = 0.05$ (Student's *t* test). Dotted lines appearing at 5.5% and 23.5% represent damage levels sustained by crowns receiving neither treatment or no pretreatment, respectively.

The effects of the two-step heat treatment on the pineapple closterovirus and badnavirus are being determined. It is not known why April crowns were more thermotolerant than October crowns. However, it is important to take these differences into account when conducting such treatments.

In conclusion, pineapple crowns receiving a 30-min heat treatment, before a second heat treatment, exhibited significantly less leaf damage than controls receiving no first treatment. The best combination that induced thermotolerance and did not cause damage to crowns was a 30C treatment followed by a second treatment at 50C with a 8-h interval between the treatments.

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