Effect of Heat Treatment on a Closteroviruslike Particle Associated with Mealybug Wilt of Pineapple

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ABSTRACT

Ullman, D. E., German, T. L., McIntosh, C. E., and Williams, D. D. F. 1991. Effect of heat treatment on a closteroviruslike particle associated with mealybug wilt of pineapple. Plant Dis. 75:859-861.

Pineapple crowns (*Ananas comosus* 'Smooth Cayenne') known to be infected with a closteroviruslike particle associated with mealybug wilt of pineapple were heat-treated in water at 40, 50, and 60 C for 30, 60, and 120 min at each temperature. Plant survival was 80–100% at 40 and 50 C, and enzyme-linked immunosorbent assay (ELISA) demonstrated that 60–100% of these surviving plants were rendered free of closteroviruslike particles. Heat treatment at 60 C increased plant mortality and did not improve the percentage of plants rendered free of virus. Plant survival decreased to 70, 20, and 0% with treatments of 60 C for 30, 60, and 120 min, respectively. Plants placed in the field and greenhouse and maintained free of mealybugs were shown, with ELISA and subsampling for virus purification, to remain free of closteroviruslike particles through forcing for fruit production and harvest. In addition, these plants produced fruit, crowns, and ratoon growth free of closteroviruslike particles.

Additional keyword: serology

Mealybug wilt of pineapple (*Ananas comosus* (L.) Merr.) was first described in Hawaii in the early 1900s (12) and continues to be a serious problem in most areas of the world where pineapple is cultivated (1–3,5–8,12,14,15). Although the etiology of mealybug wilt of pineapple remains unknown, a biological agent, transmissible by mealybugs, has been implied as the cause of this devastating disease (3–6,8,11). A long, flexuous, rod-shaped virus has been isolated from pineapple (cv. Smooth Cayenne) with symptoms of mealybug wilt (9). Based on virus morphology, molecular weight of the coat protein, and the approximate size of the genomic RNA, this pineapple virus has been assigned tentatively to the type II closterovirus group (9). This closteroviruslike particle has been detected serologically in cultivated pineapple (Smooth Cayenne) throughout the Hawaiian islands (15).

A major hindrance to demonstrating the etiological significance of this virus has been the lack of test plants free of closteroviruslike particles for experimentation. Production of pineapple cv. Smooth Cayenne free of closteroviruslike particles will permit investigations into the etiological role of the closteroviruslike particle in mealybug wilt of pineapple and other effects the virus may have on pineapple growth and fruit production.

Heat treatments and therapies have been successfully used to produce virus-free planting material for potato and sugar cane production, as well as other crops (10,13). Successful hot water and hot air treatments at widely varying temperatures and treatment times have been used (35–54 C) (13). Unfortunately, the heat required to eliminate viruses frequently also causes severe plant damage (10,13).

The purpose of this investigation was to determine 1) if pineapple crowns could survive water bath heat treatments of 40, 50, and 60 C for varying times; 2) if water bath heat treatments could render pineapple plants free of closteroviruslike particles; 3) if plants remained free of closteroviruslike particles, grew vigorously, and produced fruit and ratoon growth free of closteroviruslike particles; and 4) if heat-treated, closteroviruslike particle-free pineapple grew more vigorously than nonheat-treated pineapple infected with virus.

MATERIALS AND METHODS

Plant material. Heat treatments in the laboratory were done using crowns harvested from commercially cultivated, closterovirus-infected pineapple from the Dole Pineapple plantation on the Hawaiian island of Oahu (provided by Susan Shenk, Dole Research Division). Crowns were taken to the laboratory immediately after harvest and did not receive any pesticide treatment.

Heat treatment of large numbers of pineapple crowns for field testing was done with crowns harvested from commercially cultivated, closterovirus-infected pineapple on the Hawaiian island of Maui. Crowns were provided by David D. F. Williams and L. Douglas MacClure of Maui Land and Pineapple Company, Ltd.

Heat treatment. A large waterbath was used to maintain constant water temperatures for heat treatments in the laboratory. Heat treatment was done at 40, 50, and 60 C. When the water bath reached the appropriate temperature, crowns were placed in the water. Crown core temperatures were taken with a thermocouple, and plants were treated for 30, 60, and 120 min after the crown core temperature reached the water temperature. This process was repeated at each temperature with five crowns in each treatment. Immediately after heat treatment, 15 untreated control crowns and all heat-treated crowns were planted in Sunshine Mix (Fisons Horticultural Inc., Vancouver, British Columbia, Canada) and maintained with mist irrigation (three times per day) in the greenhouse.

Based on the results of the laboratory heat treatments, large-scale heat treatments were conducted at 40 and 50 C for 60 and 30 min, respectively. Large (1,115 L) steam jacketed kettles (Lee Metal Products, Inc., Philpsburg, PA) were filled 75% full with water and brought to the appropriate temperature. Crowns (380 per net) were placed in large nets and lowered into the steam jacketed kettle with a forklift. One thermocouple (H40601K Ten Point Thermocouple Scanner, 1.6 mm diameter, 881564 Pentration Precision, Extech, Waltham, MA) was inserted in the core of a crown at the center of the batch and one in the water near the center of the kettle. Water temperature in the kettle was maintained by controlling steam flow into the kettle jacket as needed. When thermocouple readings indicated that crown cores had reached the water temperature, treatment timing began. Using this method, approximately 11,000

Accepted for publication 27 February 1991 (submitted for electronic processing).

pineapple crowns were treated, half at 40 C and half at 50 C.

Field planting, laboratory heat treatments. One year after laboratory heat treatments, four plants free of closteroviruslike particles were selected based on results from ELISA and electron microscopy observations. The plants were planted in cages covered with silk screen at the Poamoho Experiment Station of the University of Hawaii on the island of Oahu. Plants were placed on black plastic mulch with drip irrigation as in plantation practice (see next section). Before planting, soil was treated with fenamiphos (Nemacur, Mobay Corp., Baltimore, MD) against possible nematode infestations. Immediately after planting, the plants were treated with diazinon to prevent mealybug infestation and the area was baited with hydramethylnon (Amdro, American Cyanamid Company, Wayne, NJ) to prevent ant infestations. Observations for ant populations were made monthly and hydramethylnon treatments were repeated as needed.

Field planting, large-scale heat treatment. Crowns from large-scale heat treatments were planted immediately after heat treatment and dipping in 1,133 g of fosetyl-Al (Aliette, Rhone Poulenc Inc., Monmouth Junction, NJ) plus 198 g of benomyl (Benlate 50DF, E. I. du Pont de Nemours & Co., Inc., Wilmington, DE) in 446 L of water. These crowns were planted at the Maui Land and Pineapple Company, Ltd., Haliimaile Plantation. Crowns were planted after normal plantation practice, which includes the use of drip irrigation (T-Tape Down for bed center, placed 25 mm deep) (T-Systems International, Puunene, HI) and black plastic mulch (1-mil barrier polyethylene, 70 cm wide) (Shields Bag and Printing Company, Arthur Jollymour Packaging, Morage, CA). As a control, 500 crowns were witheld from heat treatment, dipped in fosetyl-Al + benomyl as decribed earlier and planted in the same block as heat-treated crowns. Rows were numbered and staked such that every plant was assigned a unique coordinate for future serological testing, virus purification, and growth evaluation.

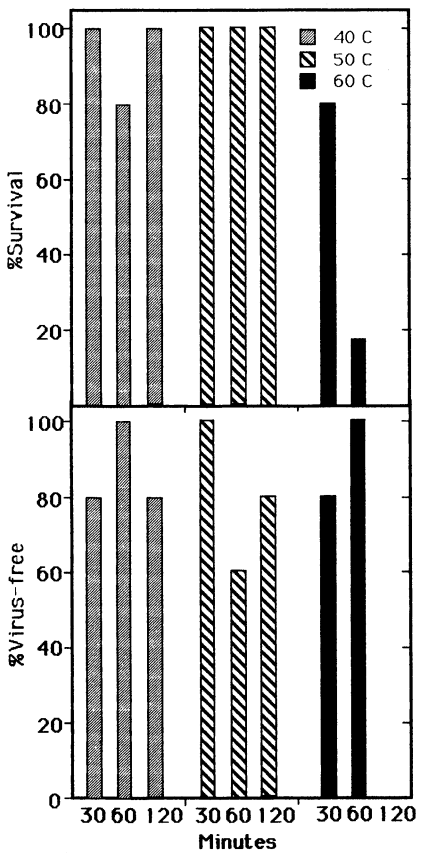

Fig. 1. Summary of plant survival and efficacy of various heat treatments for elimination of a closteroviruslike particle associated with mealybug wilt of pineapple.

Virus detection. Crowns heat-treated in the laboratory were tested for virus presence with enzyme-linked immunosorbent assay (ELISA) using previously described techniques (15). Plants were first sampled for ELISA testing when new growth appeared. To reduce destruction of leaves, samples were taken with a cork borer from four leaves and pooled for ELISA. Plants that initially tested free of closteroviruslike particles by ELISA were tested five more times over 18 mo. To verify the results from ELISA, purification of the closterovirus was attempted from three virus-free plants using previously decribed techniques (9). After purification, five grids from each plant were prepared and viewed using transmission electron microscopy (TEM) for virus particles as previously described (9,15).

Field-planted pineapple from laboratory heat treatments were tested by ELISA four more times over 18 mo after field planting. After harvest, the plants, their ratoon growth, and crowns were screened for presence of closteroviruslike particles using TEM and previously described virus purification techniques (15). Large-scale heat treatments were sampled when four to eight new leaves were produced, 3.5 mo after planting. A subsample of four plants from heat treatments falling into different size classes (1, 2, and 3) and untreated controls were screened for the presence of virus particles using TEM and virus purification techniques.

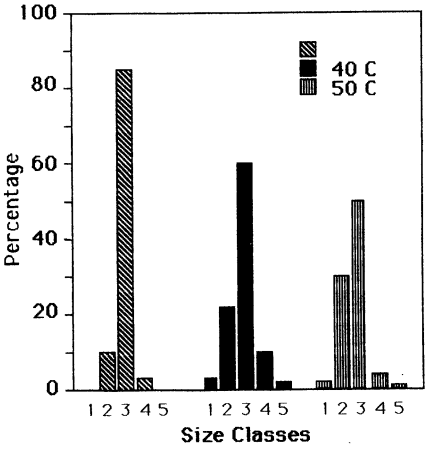

Fig. 2. Evaluation of plant size in untreated control and heat-treated field plantings. Ratings are based on a visual scoring system with size classes as follows: 1 = exceptionally large, 2 = slightly smaller, but well above average, 3 = average, 4 = smaller than average with signs of active growth, and 5 = stunted, no sign of active growth.

Quantification of plant growth rate. Plant growth rate was quantified in plants from large-scale heat treatments only. A size rating scale was developed by dividing the plants into six size classes where 1 = the largest, 2 = slightly smaller, 3 = average, 4 = stunted but growing, 5 = extremely stunted with little sign of growth, and 6 = dead. Using this rating, every plant in the experiment was visually scored 3.5 mo after planting. This method was selected because all other measures of growth and development in pineapple require destructive sampling techniques. Results were analyzed with chi-square contingency testing.

RESULTS

Laboratory heat treatments. Pineapple crowns readily survived heat treatments of 40 and 50 C, with 80–100% of the plants surviving at all treatment intervals (Fig. 1). Plant survival decreased dramatically when crowns were submitted to 60 C for more than 30 min (Fig. 1). All nonheat-treated control plants survived. Among the surviving heat-treated plants in each treatment, 60–100% were rendered free of closteroviruslike particles at the various temperature and time regimes (Fig. 1). Heat treatment at 50 C for 30 min permitted 100% plant survival and rendered 100% of the plants free of closteroviruslike particles. Plants in which closteroviruslike particles could not be detected with ELISA at the first sampling remained free of closteroviruslike particles for more than 18 mo in the greenhouse.

Data from ELISA indicate that the four plants selected from this cohort for field planting remained free of closteroviruslike particles through harvest, approximately an additional 18 mo. Furthermore, these plants produced crowns and ratoon growth free of closteroviruslike particles. Virus purifications from these plants, their crowns, and ratoon growth, as well as a subsample of the plants grown in the greenhouse, failed to yield any visible closteroviruslike particles. Although changes in growth rate were not measured among these plants, they appeared to grow faster and reach a far larger size than we observed in nearby plantation plantings.

Large-scale heat treatment and field planting. Visual scores for plant size are summarized in Figure 2. Heat treatment resulted in significantly more plants in the largest size classes of 1 and 2 than observed in the nonheat-treated control (40 C: $\chi^2 = 13.655$, df = 4, $P \leq 0.0085$; 50 C: $\chi^2 = 29.146$, df = 4, $P \leq 0.0001$). There was no significant difference between heat treatments ($\chi^2 = 5.337$, df = 4, $P \leq 0.254$), with 25 and 40% of the pineapple treated at 40 and 50 C, respectively, ranked in the largest size classes of 1 or 2. The untreated control was very uniform in size, with nearly 90% of the plants falling in the average size class of 3 and less than 10% in plant size classes 1 or 2. Plants tested in size classes 1 and 2 did not yield visible virus particles after virus purification. In contrast, heat-treated plants and untreated controls in size class 3 yielded virus particles upon purification.

DISCUSSION

Our data clearly show that pineapple crowns can readily survive heat treatments of 40 and 50 C for as long as 120 min. Pineapple can also clearly withstand temperatures as high as 60 C, but only for 30 min before severe plant damage is incurred. Heat treatment may also be a successful therapy for rendering pineapple free of the closteroviruslike particles associated with mealybug wilt of pineapple. In laboratory heat treatments, best results were achieved at 40 and 50 C for 60 and 30 min, respectively. This contrasts with what we would have expected, which is that heat treatment at longer intervals would produce the highest number of plants free of closteroviruslike particles. The reason why this occurred is currently unknown but may be explored further in future experimentation designed to refine heat treatments.

In order for heat treatment to be useful to the pineapple industry, treatment must be adapted to accommodate very large numbers of plants in a timely fashion to be considered cost effective. Based on laboratory heat treatments, treatments of 40 and 50 C for 60 and 30 min, respectively, were selected for testing on a large scale. Heat treatment on this much larger scale supported findings in the laboratory that best results were obtained at 50 C for 30 min. Like laboratory-treated plants, plants from large-scale heat treatment survived very well.

Our results also suggest that our success rate in rendering plants free of closteroviruslike particles was not as high as in the small-scale laboratory setting. Nevertheless, heat treatment apparently has potential for use on a commercial scale to render pineapple crowns free of closteroviruslike particles. Perhaps our most significant finding is that pineapple growth rates were profoundly affected by heat treatments. As subsamples of plants in the largest size classes, 1 and 2, were free of closteroviruslike particles, increased growth rates may be in response to elimination of closteroviruslike particles. It is also possible that increased growth rates occurred for another reason, such as elimination of some other endophytic organism.

These data are of tremendous importance to pineapple culture worldwide, as increased growth rates would allow earlier forcing for fruit production and significantly increase yields. Further experimentation to improve the efficacy of large-scale heat treatments is in progress, as well as continuing evaluation of closteroviruslike particle free plants mentioned in this paper, their crowns, and ratoon growth. Successful elimination of closteroviruslike particles from pineapple with heat treatment is also of great importance to our continuing efforts to determine the etiology of mealybug wilt of pineapple. Using heat treatment, we have created stocks of test plants free of closteroviruslike particles and are working toward determining the potential etiological significance of closteroviruslike particles in pineapple.

ACKNOWLEDGMENTS

We thank Maui Land and Pineapple Company, Ltd. for providing land and technical support and Richard Ebesu for technical assistance in laboratory heat treatment of pineapple. Investigations were supported in part by the State of Hawaii Governor's Agricultural Coordinating Committee (Contracts 87-12 and 86-8). Journal Series 3519 of the Hawaii Institute of Tropical Agriculture and Human Resources.

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