Research Report

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Final report on potential amendments to mango fruit handling for improvement of postharvest control of mango anthracnose.

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Abstract/summary of research

We integrated dilute ethanol (5 to 10% v/v) into a laboratory simulation of existing packinghouse practices as an effort to improve control of mango anthracnose currently provided by the standard quarantine hot-water treatment. Ethanol is a well-known for synergistically increasing the lethality of hot water to postharvest pathogens. In preliminary research conducted here at UF, Gainesville, heated, dilute aqueous solutions of ethanol (2 to 8%) completely inactivated the anthracnose pathogen in diseased fruit tissues, whereas unheated dilute ethanol or hot water alone were fungistatic (temporary inhibition). Hot-water treatment is already a feature of packinghouses involved in export trade. We proposed to design a dilute ethanol treatment that can achieve the same synergistic control with commercially packed fruit that we have observed in laboratory bench-top tests. Ethanol is a GRAS chemical and would not require a registration if used as a packing aid. Bacteria and fungi exposed to lethal levels of ethanol have not shown signs of developing resistance. Certain microbes may develop some resistance to injurious (but not lethal) levels of heat, but this tolerance does not appear to persist within the treated population.

Objectives:

Prevent further development of quiescent anthracnose infections that are not visible on mango fruit at the time of packing, and to do so by inexpensive modification of current packinghouse procedures and equipment.

Significance and Background:

Anthracnose is a major postharvest decay of mango fruits. Black lesions with an indefinite border and larger than 2 cm in diameter were reported to be common among ripe mango fruit intended for market (Aruaz, 2000). Disease incidences of nearly 100% of fruit are observed among fruit produced in wet to very humid growing conditions. Early lesions are mostly restricted to the peel, but the pathogen grows into the pulp as fruit ripen. At harvest, many fruit have

quiescent infections where the pathogen, *Colletotrichum gloeosporioides* exists as an appressorium attached to the epidermal cell layer, with or without an infection peg penetrating into that cell layer. Further fungal development in unripe fruit is inhibited by endogenous metabolites produced by the host. When the fruit begin ripening these fungistatic compounds dissipate and the pathogen resumes active colonization of fruit tissues. As such, pathogen structures are located on or quite near fruit surfaces during quiescent phases of infection cycles. This provides an opportunity to target postharvest control measures to surfaces of fruit, at a time when the pathogen is quiescent.

Hot water treatment of mango fruit is required by quarantine rules to prevent introduction of certain insects into protected areas, with protocols for importation of mangoes into U.S. markets proscribed by USDA-APHIS (Aruaz, 2000, USDA, 2013). Pathogen development is sensitive to elevated temperatures (>30°C/86°F). Infection occurs most rapidly at temperatures ranging from 20 to 30°C (68 to 86°F) and optimal temperatures vary with strain of the pathogen (Aruaz, 2000). By contrast, pathogen development is inhibited or prevented at temperatures outside this range. High temperatures can be lethal. Unfortunately, mango surfaces can be damaged by exposure to high temperatures, developing lenticel pitting, surface scald, starch layering, collapsed shoulders, and internal cavities.

Overall, a quarantine hot-water treatment at constant 46.1°C (115°F) as required by quarantine rules partially controls anthracnose, particularly when infection levels are low (McGuire, 1991). But, such treatment does not completely control the decay. In our previous research (Bartz, et al. unpublished), temperatures and duration of treatment required to inhibit anthracnose were reduced if the hot water contained ethanol. Ethanol vapor slows ripening of mango and reduces microbial development on mango slices (Plotto et al., 2003). Plotto's group also determined upper limits of ethanol vapor concentration that avoided off-flavor development in those slices.

In unpublished work performed in our laboratory (cited above), a visiting scholar from India treated whole 'Tommy Atkins' fruit with a 1-hour hot (46.1° C/115° F) water treatment simulating mango quarantine treatment and then stored the fruit at 24°C (75°F). When the storage was terminated (20 days after treatment), fruit that had been treated with hot water containing 4% ethanol were completely free of anthracnose. Those treated with hot water alone, hot water containing 2% ethanol or room temperature water (24°C) containing 2, 4 or 8% ethanol had lesions. Better control was achieved if fruit were stored at 40°C (104°F) for 24 h in addition to the hot water + ethanol treatment.

Gabler, et al. (2004) provide evidence that heated solutions of ethanol have a synergistic effect on spores of various decay pathogens in comparison to heat alone or ethanol alone. Gutiérrez-Martínez et al. (2012) reported use of a combination of ethanol and hot water to control two additional postharvest pathogens of mango, but concentrations used in their fruit tests ranged from 10 to 30%. However, we questioned the practicality of adding ethanol directly to the hot-water quarantine treatment tanks given the volume of those tanks and the likely evaporation of ethanol from the hot water. Moreover, we do not believe that a 24-h dry air heat storage treatment is viable in commercial mango packinghouses. Dilute ethanol could be applied to mango fruit in three possible ways that feature minimal changes in packinghouse design or operation:

1. A recoverable spray rinse applied to fruit as they exit the wash tank, prior to pre-sizing and the quarantine hot-water treatment. This treatment might feature a short delay between the rinse and quarantine hot-water treatment to allow the residual dilute ethanol to seep into fruit surfaces.
2. As a vapor treatment of fruit as they recover from the quarantine hot-water treatment (pull a plastic shroud over each bin of hot-water-treated fruit and inject ethanol under the shroud or spray the hot-water-treated fruit with ethanol just before shroud is placed over the pallet).
3. As an additive to hydrocooler water for those using hydrocooling after the quarantine hot-water treatment.

Methods:

In initial tests sections of fruit tissues showing early decay symptoms were excised and exposed to various levels of heat (up to 54°C) plus dilute ethanol, for different time intervals to establish pathogen structures' sensitivity to heat plus ethanol. Ethanol concentrations in ambient (24°C/75°F) and hot (46.1°C/115°F) water were based on our previous research as well as the scientific literature (range 2 to 40%). Vapor treatment was based on the report by Plotto et al. (2003), but we avoided using levels that led to off-flavors in that report. Controls included: (1) heat-treated without adding an ethanol treatment; (2) treated with ethanol without heat; (3) not treated with either heat or ethanol and (4) ethanol added to the quarantine treatment bath as in our preliminary experiments discussed above. Test efficacy was based on further development of lesions, pathogen grow-out onto media in Petri plates, or fruit quality in storage.

Treatment design and evaluation:

Three approaches were used to evaluate the effect of ethanol on standard hot water treatment (quarantine treatment) of mango fruit.

1. Ethanol was added to heated water to a final concentration of 10%.
2. Quarantine hot water-treated fruit were hydrocooled with 5 or 10% solutions of ethanol.
3. Higher temperature water-treated fruit were exposed to a rinse of ethanol in a simulation of a stand-alone vapor treatment.

Results:

Two large tests were conducted in the postharvest lab at Gainesville with fruit harvested from UF/IFAS Tropical Research and Education Center (TREC) groves in Homestead. In the first test, the fruit were contaminated with latex. Ethanol, particularly when combined with heat, appeared to dissolve the latex, which led to severe surface scald. The damage often appeared over surfaces that would be exposed to spurting latex during harvest (Fig. 1) and prevented an evaluation of anthracnose control.

Fruit obtained from TREC for the second test had severe anthracnose symptoms prior to treatment (Fig. 2). None of the experimental treatments stopped or slowed the further development of symptoms.

Figure 1. Evidence that surface injury by heated dilute ethanol was related to dried latex deposits on fruit surfaces—note arrows pointing to injuries.

Figure 2: Mangoes used in second large test. Severe anthracnose symptoms were observed prior to the treatments and continued to expand after treatment.

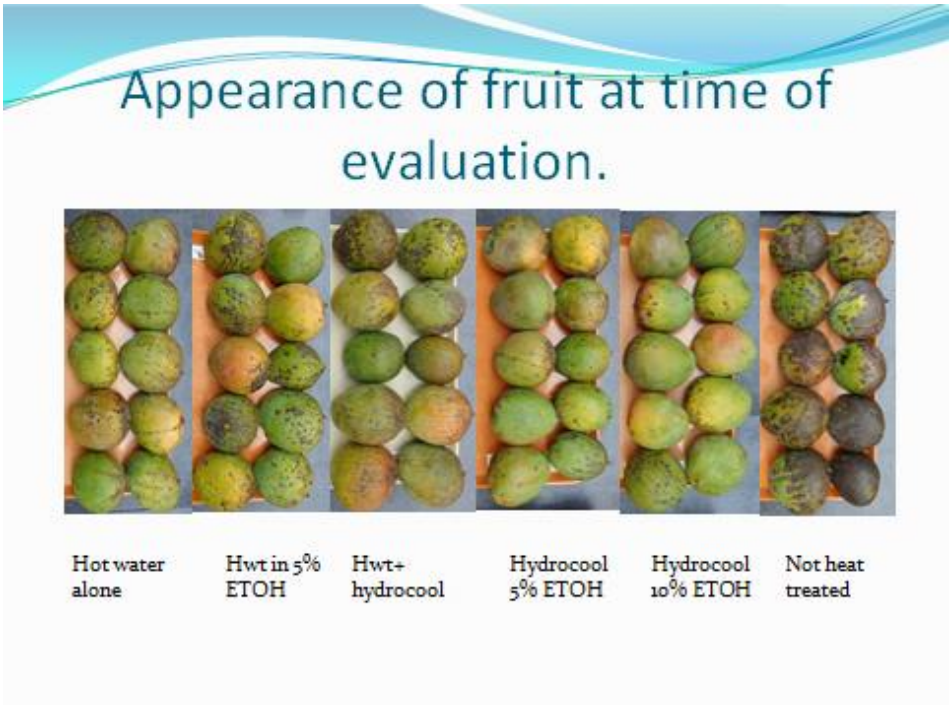
A third large test was conducted on fruit ('Keitt') purchased from a grower located on Pine Island, FL. Control fruit were severely diseased by the conclusion of the 16-day storage with an average of 74% of the surfaces of these fruit covered with lesions.

Table 1. Treatment of 'Keitt' obtained from Pine Island (Fall 2014)

Treatments	Surface area decayed	significance
Control	74.17	A
QHW with 5% ethanol	12.93	B
QHW plus HC	9.85	BC
QHW plus HC with 5% ethanol	7.83	CD
QHW alone	7.13	CD
QHW plus HC with 10% ethanol	6.00	D

Fruit stored for 16 days at 20°C
QHW = 60-min at 46.1°C quarantine hot water treatment
HC=water or water plus ethanol at 21°C applied to fruit for 20 min after the mandated 30 min resting period following QHW. Final pulp temperature = ca. 25°C.

The cleanest fruit at the end of the storage were those receiving the quarantine hot water treatment and those that had been hydrocooled with 5 or 10% ethanol after the standard quarantine treatment with from 6 to less than 8% surface area covered with lesions. Heat-treating (standard quarantine treatment) with 5% ethanol also led to a significant reduction in decay severity, but those receiving the standard quarantine hot water treatment without ethanol had significantly less anthracnose coverage (12.93 versus 7.13%, respectively). There was no evidence of ethanol treatment related surface damage.



During the off-season for mango production in Florida, lightly diseased fruit were obtained from local produce markets. Small lesions along with minimal surrounding tissues were removed from these fruit, exposed to various treatments, and then plated on a fungal growth medium (APDA) to determine if pathogen viability had been affected. A new, short-term (5-10 min) higher temperature heat treatment (54°C) was used because of its description in the literature as the highest temperature tolerated by mango fruit. Whole fruit (purchased from local markets) were not harmed by exposure to this temperature for up to 10 min. The pathogen was eradicated from sections of lightly diseased tissues by exposure of those tissues to 54°C-water for 5 or 10 min. Nothing grew out of these sections, whereas fungal growth was abundant from sections treated for a similar period in 4 or 8% ethanol at room temperature. The addition of ethanol to the 54° C water had no detectable effect on pathogen eradication, since the hot water alone was 100% effective.

During the second season, mango fruit were again obtained from the Homestead, FL production area. 'Keitt' and 'Kent' were harvested from a well-maintained grove located at TREC (Tropical Research and Education Center). 'Tommy Atkins' was purchased from a local Homestead area grower. Surface damage associated with heat treatment plus ethanol did not occur, which supported our evaluation that the damage after the initial treatments conducted the previous season was due to presence of latex on those fruits.

The incidence of anthracnose in control fruits was much lower than samples obtained from the second harvest of the previous season. Almost all treatments reduced anthracnose compared with the controls with the only exceptions occurring with 'Keitt.' Overall, the least level of anthracnose for all three varieties was observed on fruit that had received the standard hot water quarantine treatment, with the standard 30 min rest period and then hydrocooled with 10% ethanol to a pulp temperature of 25°C (Table 2), but was not significantly different from the control provided by various other treatments depending on variety. Anthracnose control for 'Keitt' and 'Kent' was not improved over that provided by the quarantine hot water treatment

when it was either preceded or followed by a 10-min exposure to 54°C-water, but that practice did show increased effectiveness for 'Tommy Atkins'.

Table 2. Percentage surface area of mango fruit with anthracnose at 7days after various treatments:

treatments	'Tommy Atkins'	'Keitt'	'Kent'
Control	31.3 A	9.5 A	11.6 A
QHW	8.2 B	5.1 B	6.3 B
QHW followed by HW	8.1 B	6.0 B	6.0 B
QHW then HW plus HC with 10% ethanol	4.4 C	10.0 A	6.9 B
QHW followed by HC with 10% ETOH	2.6 C	5.1 B	5.1 B
HW followed by QHW	2.5 C	9.4 A	5.4 B

Stored at 20°C.
QHW = 60 min at 46.1°C quarantine hot water treatment.
HW = submersed in 54°C water for 10 min.
HC = heat treated fruit allowed to drain for 30 min at ambient air temperature and then submersed in 10% ethanol at 21°C until fruit temperature fell to ca. 25°C
Averages not followed by the same letter were significantly different at P≤0.05.

Discussion:

Our observations generally confirm that heat treatments, including the standard quarantine hot water treatment, provide significant control of anthracnose, which is a major factor in successful marketing of mango produced in humid regions. We were unable to show that adding ethanol to the heated water consistently increases its efficacy as a mango anthracnose treatment. A caution is that, if latex has dried on fruit surfaces, ethanol treatment produces a surface scald. However, addition of ethanol to hydrocooler water at a concentration of 10% led to the lowest severity of anthracnose in one test with 'Tommy Atkins' fruit. Tests involving exposure to water at 54°C (129°F) were envisioned as a possible added value test, which could be employed by receivers that are observing the beginning of active anthracnose in new or fresh out of storage shipments. It certainly was effective on young lesions, but failed to completely control the disease if the infections were latent. Thus, the fungal apressorium and probable infection peg appear to be more resilient to heat than is developing mycelium. By contrast, well-developed infections also appear to be resilient to heat. Additional tests would be needed to confirm that the 54°C-treatment is indeed a potential added value treatment or if adding ethanol to the water will enable use of a slightly lower temperature.

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