Factors affecting quarantine heat treatment efficacy

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Abstract

Heat quarantine treatments have been used for 70 years on a variety of fresh agricultural commodities and pests. Despite the high level of efficacy demanded of these treatments and the very low levels of infestation usually found in commodities traded across quarantine barriers, heat treatments have sometimes failed. Key differences between investigation and application which may affect treatment efficacy against the pest include method of assessing efficacy, genotype and most tolerant stage of the insect, affinity of the research setting to commercial reality, preconditioning of fruit, pre- and post-temperature regimes experienced by fruits in research versus commercial settings, and size of operation. Researchers must be cognizant of the differences between the research versus commercial setting, the practical restraints faced by industry, and the effect these contrasts could have on treatment efficacy. Published by Elsevier Science B.V.

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1. Introduction

Various methods have been devised for applying heat to commodities post-harvest in order to kill quarantined pests which may be infesting the commodities. This article concentrates on concerns with heat treatment research and their impact on commercial application. Most of the citations refer to fruit flies (Diptera: Tephritidae) because most heat quarantine treatments applied on a commercial scale are against this important pest group. For an overview of heat treatments, their history, and comparisons of treatments readers are referred to other articles in this special issue plus recent reviews (Lurie, 1998; Mangan and Hallman, 1998). The term ‘quarantine treatment’ is preferred over other terms that have been used, such as ‘disinfestation’ or ‘phytosanitary’ treatment, because of its precision. That is, the treatment is being done because an organism that has been quarantined by the importing country may be present. The words disinfection and phytosanitary could be perceived to be applicable to any situation where the commodity simply needs to be ‘cleaned up’, not necessarily that any organisms present are of quarantine importance nor that the level of control achieves quarantine security.
2. Procurement of quarantined test organisms

Quarantine treatment research is almost always performed with insects which have been reared in a controlled laboratory setting for many generations because of the ease of handling and the large numbers needed. A laboratory-reared population is known to rapidly change its gene frequency to adapt to rearing conditions of constantly mild environmental conditions, vast overpopulation, grossly reduced space, and reduced proficiency for seeking food, mate, and ovipositional sites (Chambers, 1977). Consequently, a reasonable hypothesis is that a laboratory population might respond differently to heat than feral populations because of variations in the titer of heat shock proteins (hsps) among populations and other inherited adaptations which might undergo differential selection pressures in the laboratory than in agroecosystems. Although known to occur for laboratory insects such as *Drosophila spp*. (Denzlinger and Yocum, 1998), this aspect has hardly been studied for quarantined pests. The only controlled study found during this review which compared a laboratory population against feral insects for quarantine treatment purposes was with oriental fruit fly, *Bactrocera dorsalis* (Hendel), third instars in heated ‘Papayas’; no differences in mortality were noted (Hansen et al., 1990). Feral insects should be tested more frequently for their response to quarantine treatments compared with the laboratory populations used to develop these treatments.

In addition to heritable differences between feral insects and laboratory populations used to develop quarantine treatments, there may also be phenotypical differences between insects infesting commodities in the field and those used to infest the commodities in the laboratory (Hallman, 1994). Caribbean fruit flies reared at a constant 30°C were more tolerant of hot water immersion than those reared at lower temperatures (Hallman, 1994). Insects infesting fruits in the field are often exposed to higher temperatures than those used in many laboratory experiments where human comfort is a primary concern! Furthermore, insects in field situations will arguably produce hsps more often due to temperature fluctuations in the field than insects in a controlled laboratory environment, resulting in the former’s increased ability to tolerate heat compared with the latter. Consequently, lacking information that shows that insects reared under stable, mild conditions are not more susceptible to heat, researchers would be advised to incorporate temperature fluctuations which occur in the field and packing-house into heat quarantine treatment research.

3. Commodity infestation

The method used to infest a commodity can affect the response of the insect to a treatment. Studies on mortality of insects when they are not physically on or in the regulated commodity may be useful in determining relative differences between insect growth stages or other factors but should not be used to set treatment parameters. With interior pests, such as fruit fly larvae, various infestation techniques have been used: (1) placement of larvae reared on diet in a cavity inside the fruits which is then sealed (Fig. 1); (2) placement of eggs under fruit surfaces and allowing them to develop to larvae before treatment; or (3) placement of fruits into cages with adults which lay eggs in the fruits; larvae are allowed to develop. In the latter case, the peels of difficult to infest fruits have sometimes been perforated to facilitate oviposition. The advantages of placing larvae in the fruits prior to treatment are (1) difficulty of the insect in infesting the fruits is not a problem; (2) except for the hole used to insert the larvae, the fruit will be of commercial condition, as opposed to overly soft or partially decomposed when treated; fruit condition might affect heating rate and insect survival; and (3) the number of insects infesting each fruit can be precisely known and controlled. The chief disadvantage is the artificial nature of the infestation with its unknown effects on insect response to a heat treatment. This technique has been used without comparing the response to natural infestation. A reasonable hypothesis would be that larvae placed in fruit would be more tolerant of heat for two reasons: (1) all of the larvae would be near the center of the fruit and receive less heat than larvae
distributed more naturally in the fruit, many of which are near the fruit surface; and (2) larvae taken out of their diet generally enter the post-feeding stage with changed behavior and physiology compared with the feeding stages (Fraenkel and Bhaskaran, 1973). Because post-feeding larvae (fruit flies and many lepidopterous and coleopterous borers) are preparing to exit the fruit and seek a pupariation site in the ground, it may be reasonable to expect that they are more tolerant of environmental extremes (including heat) than feeding larvae. Indeed, Mangan and Ingle (1994) cite unpublished data where nonfeeding third instar Mexican fruit fly, Anastrepha ludens (Loew), were nearly twice as tolerant to 47°C as feeding third instars. This is the only reference located during this review (and it is unpublished!) that gives information on possible differences in mortality response between natural and artificial infestation.

Alternatively, post-feeding larvae placed in the center of fruits might be less tolerant of heat than feeding larvae. Post-feeding larvae may be less adapted to low oxygen conditions in fruit, and heat in the presence of low oxygen is more deadly to insects than heat in the presence of ambient levels of oxygen (Whiting et al., 1995). An intimate relationship of feeding larvae in their host environment perhaps would induce greater tolerance to temperature extremes than post-feeding larvae placed en masse in otherwise sound fruit.

4. Insect population density

Quarantine treatment research is usually done on heavily infested commodities whereas commercially treated commodities will have very low infestation levels. Crowding might affect treatment efficacy by changing the consistency of the fruit and causing stress to the insects due to competition for food and toxicity of waste and breakdown products. Hansen and Sharp (1997) found that in a system using diet-reared Caribbean fruit fly third instars in 5 cm long pieces of 2.5 cm diameter metal tubes (unspecified electrical con-
duit) immersed in 40°C water for 67 min, mortality increased considerably when > 50 larvae were placed in the tubes, but not at < 50 larvae per tube. However, quarantine treatment research is not done at densities that high (≥ 50 larvae per 24.5 cm³ of host material). Hallman and Sharp (1990b) present data where the infestation density of Caribbean fruit fly third instars in different lots of carambolas immersed in 46–46.4°C water varied from a mean of 2.8 to 34 per fruit. At the two lowest densities mortality was 100% after 20 min, whereas at the two highest densities mortality was 97.8 and 99.9% after 20 min. That study indicates that higher density was related to greater tolerance to heat.

5. Measuring efficacy

One of the key decisions researchers face when developing a quarantine treatment is how to measure treatment success. The first and seemingly most logical measurement is mortality of all insect stages which might be present in the commodity. In fact, researchers often state or imply mortality is measured without being specific as to how this was done. This can make a difference in treatment severity and acceptance by importing regulatory agencies. With fruit flies, for instance, researchers have based treatment efficacy on such diverse criteria as finding all insects dead inside opened fruits one day post-treatment, no emergence of insects from the fruit, failure of any emerging larvae to pupariate, failure of any larvae to form normal-looking puparia, and failure of any adults to emerge from puparia formed from insects treated inside of the fruits. These criteria are arranged in descending order of severity of treatment; i.e. a more severe treatment is needed to kill all insects inside fruits within one day of treatment than prevent any insects from later emerging from the fruits. For example, Japan reportedly did not accept hot water immersion of mangoes because live fruit fly larvae were found in mangoes treated in Mexico. The measure of efficacy used in the research on hot water treatment of mangoes against fruit flies was usually failure of any larvae emerging from treated fruits to form a normal-looking puparium (Thomas and Mangan, 1995). During the confirmatory stage of the research supporting hot water immersion of mangoes an unknown proportion of larvae were alive in the mangoes some time after treatment, some emerged from the mangoes, and some even pupariated (Sharp et al., 1988), although the puparia may not have been normal and adults did not emerge. Therefore, under conditions deemed successful, live larvae could be found by inspectors inside ‘properly’ treated mangoes. Indeed, live larvae have been found in hot water treated mangoes by inspectors in the United States on several occasions.

Another problem is pointed out by the mango example. Failure to form a normal-looking puparium does not mean that an adult fruit fly will not emerge from that puparium; this was an untested assumption in the mango hot water treatment research (Sharp, 1988). Thomas and Mangan (1995) found 50% adult emergence from one type of abnormal puparium. This is logical; the puparium is an inanimate case formed by the shed larval exoskeleton in which the fly pupates. As long as it is structurally sound and roomy enough for the pupa to develop and has an operable exit for the adult to emerge, it should not matter that it does not look normal.

With heat treatments and, indeed, any treatments where finding live insects is unacceptable, it is recommended that the criterion for efficacy be mortality at the earliest time that inspection could occur. A good rule of thumb would be no live insects found 24 h after treatment.

6. Selection of commodities for treatment

Commodities used in research must be representative of the total range of cultivars, maturity classes, management systems, agroecosystems, and growing seasons that could be treated for export. Any of these factors might affect the results. An example is development of the ‘double dip’ treatment for Hawaiian papaya against fruit flies (Couey and Hayes, 1986). Papayas not more than one-quarter ripe were immersed in 42°C water for 30 min followed by 49°C water for 20
min and then cooled with an ambient water spray. This treatment was designed to kill only eggs and early instar fruit fly larvae near the peel. Papayas not more than one-quarter ripe were not found to contain older larvae or any larvae deeper in the fruit pulp, both of which had a greater chance of surviving the treatment. In March, 1987 nine of 16 000 treated papayas were found to contain live oriental fruit fly larvae (Zee et al., 1989). Further investigation revealed that some papayas had an opening to the seed cavity at the blossom end which allowed fruit flies to oviposit in the papaya at an earlier stage than usual and for larger larvae than first instar to be found deeper in the pulp than usual. A survey in 1987 revealed that papayas from 5 to 31% of trees in commercial orchards in the Puna district of Hawaii had the blossom end opening. Zee et al. (1989) cited P.J. Ito and H.Y. Nakasone who said that the opening was not present in the original commercial papaya cultivars planted in Hawaii, but must represent inadvertent cross pollination of these otherwise highly self-pollinated hermaphrodite inbred lines. In any case, the opening must have been present to some extent when the research on the treatment was done just a few years before the opening was discovered. Couey and Hayes (1986) did note six surviving larvae during the research phase of the treatment, but dismissed them as ‘accidental reinfestation’. Natural infestation studies were done with fruits taken from only two orchards. The large scale confirmatory tests using 80 000 fruits were conducted entirely with papayas from one grove. That orchard may have simply had a low incidence of the blossom end opening and/or a low incidence of fruit fly infested papayas. An additional lesson to be learned from this incident, besides the fact that research commodities must be taken from the whole range of possibilities, is that researchers should not lightly reject unexpected anomalies in results!

7. Effect of fruit conditioning on insects

A quarantine treatment must satisfy two conflicting goals: kill all quarantined insects present and prevent significant damage to the commodity, which, in the case of fresh produce and nuts, is also alive. A quarantine heat treatment is almost always detrimental to the quality of fresh agricultural commodities. This is commercially acceptable if that damage is not extensive enough to curtail marketing possibilities. Variations on heat treatments have been suggested to reduce damage. Long ago, Jones (1940) found that holding fruits at 38–43°C for several hours before a heated air treatment reduced the amount of damage subsequently suffered by the fruits. However, anything that lessens damage to the commodity might have a similar effect on the quarantined insects. For example, Hallman (1990) found that cooling heat-treated carambolas, Averrhoa carambola L., in 13°C water increased Caribbean fruit fly, Anastrepha suspensa (Loew), survival compared with slower-cooled fruits left in ambient air. Fig. 2 shows internal temperatures for mangoes cooled in relatively still ambient air and cooled in circulating water. The mangoes cooled in air remained at temperatures lethal to fruit flies ( > 40°C) longer than those cooled in water. Therefore, any modification of a quarantine treatment designed
to maintain commodity quality should be tested for its effect on the insect.

8. Research environment versus commercial

Quarantine treatment research is usually done under carefully controlled conditions of temperature, fruit quality, and other parameters in order to identify, quantify, and regulate factors which might affect efficacy. Commercial conditions, however, can rarely be so carefully controlled; commodities to be treated will come from different and evolving production systems and will be exposed to varying temperatures before and after treatment. The variations between research methodology and commercial reality can affect treatment efficacy. Hallman (1994) found that tolerance of Caribbean fruit fly third instars to heat increased in direct proportion to the rearing temperature. Waddell et al. (2000) demonstrated that non-lethal heat spikes increase the tolerance of the Queensland fruit fly, *Bactrocera tryoni* (Froggatt), to heat. The extremes of commercial conditions, especially the temperatures to which fruits may be exposed, should be incorporated into the large-scale testing done to confirm quarantine treatment efficacy.

9. Commercial scaling-up of research

Small quantities of commodities in a research laboratory setting can be heated much faster than possible in a large-scale commercial treatment facility. This is especially true of heated air treatments because air is compressible, does not carry as much heat as water, and its heat-holding capacity is affected by humidity. Neven (1998) noted that a faster heating rate generally resulted in greater mortality of codling moth, *Cydia pomonella* (L.). Researchers should be aware of industrial limitations, such as slower heating rates and changes in other parameters which may affect treatment efficacy. These variables should be incorporated into the research protocols.

10. Conclusions and recommendations

Research methodology can change the efficacy of heat treatments and must be considered in light of what is commercially feasible. Heat shock has long been suspected to affect the susceptibility of quarantined insects to heat treatments. Artificial infestation methods have been used without consideration that they may lead to more severe treatments than necessary with consequentially reduced commodity quality. The genotype of the test population versus feral populations has hardly been examined although it underlies response of the organism to any stimulus such as heat. The precise method used to measure efficacy is often not stated and may differ from inspectors’ assumptions. Even the fruit selected for the experiments may affect treatment efficacy, as in the case of the papaya ‘double-dip’ treatment. Therefore, researchers must envision how their treatments will be applied as they are performing the research and evaluate the effect of variations in application, such as slower heating rates and variable fruit temperatures, on efficacy. Perhaps the main reason temperature treatments have not failed more often is that they are quite robust. Treatments are designed to provide a very high level of control (such as ‘probit 9’ or 99.9968%), and the levels of infestation usually found in fresh agricultural commodities traded internationally are extremely low resulting in low probabilities of finding live insects after an insufficient quarantine treatment.

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