CONCERNS WITH TEMPERATURE QUARANTINE TREATMENT RESEARCH

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Quarantine treatments demand a very high level of security. Relatively minor variations in that level of security when used on a large, commercial scale may result in pests surviving a quarantine treatment. Because of the low level of infestation present in most commodities, that failure may not become manifest for, some time. However, temperature quarantine treatment failures have occurred when the level of security demonstrated during the research phase of a treatment did not hold up during the commercial phase. There are many factors which may cause differences between the results of research and the results of commercial application. We discuss some that have caused problems or have been of concern. Researchers are encouraged to imagine their treatments being applied on a commercial scale and anticipate possible problems.

Assessing Mortality Even something so seemingly simple as assessing mortality can cause problems for quarantine treatment research. Two different approaches have been used for infesting fruit for quarantine treatment research: 1) fruits are exposed to insect oviposition and the insects are allowed to develop to the appropriate stage; after treatment, fruits are stored to allow any survivors to emerge, or 2) insects are reared on a diet and placed in fruits at the desired stage; after treatment, insects are removed from the fruits and mortality is assessed. The first approach more closely approximates the natural condition, whereas the second approach is advantageous in that numbers of insects tested are known, stage and infestation rate can be precisely controlled, and infestability of the commodity is not a problem. Studies comparing the two treatments have not been done.

The definition of mortality after a temperature treatment may differ. With fruit flies, many researchers considered any larvae that emerged from infested fruits after treatment as survivors, while others counted only puparia that developed. Some researchers counted only normal appearing puparia, and others did not count puparia as survivors if no adult emerged. Failure to move several days after treatment even following prodding with pins and bristles (larvae of Tortricidae) has been used to determine mortality. Still other researchers gave no definition of mortality. We feel that any insect that emerges from the fruit or is alive when the insects are removed should be counted as a survivor. This is because quarantine regulators will count any live insects they find as survivors. Furthermore, an insect which survives a temperature quarantine treatment may complete development and reproduce. Thomas & Mangan (1995) found that normal adults emerged from some abnormal tephritid puparia formed by heated last instars. Hallman (1990) found no relationship between percentage mortality of fruit fly larvae subjected to heat in fruits and adult development from surviving larvae. Furthermore, fruit fly larvae which survived a heat treatment that killed up to 99% still reproduced as well as controls.

Genotype of Insect Insects that have been reared in the laboratory for several generations are often different from their feral counterparts. Because genetics ultimately controls the ability of organisms to express tolerance to any environmental stress and laboratory-reared insects are usually maintained at constant moderate temperatures, it is conceivable that laboratory-reared insects might differ in their response to heat and cold quarantine treatments compared with feral insects. Most quarantine treatment research is done with laboratory-reared colonies because of the need for large numbers of insects of the same stage. Almost no information exists comparing laboratory and feral insects for response to quarantine treatment. The potential of feral insects being more tolerant of extreme temperatures than laboratory-reared insects should be examined. Feral fruit fly larvae removed from field-infested papayas and larvae taken from a laboratory colony reared on semi-artificial diet and placed in the center of papayas subjected to heated air showed no difference in mortality (Hansen et al. 1990).

Temperature Regime in Research vs. Commercialization The temperature regime to which insects are exposed before treatment can affect their response to that treatment. In most quarantine treatment research, insects have been reared under controlled conditions which are in many ways different from those they face in their natural setting (Hallman 1996). Rearing temperatures are usually constant and moderate while temperatures of fruits in the field fluctuate between quite cool and very warm. Hallman (1994) found that fruit flies reared at a constant 30°C were more tolerant of hot water immersion than those reared at constant lower temperatures. The effects of heat-shock proteins are well known (Denlinger & Yocum 1998). Holding last instar fruit flies at 35°C for 8 h prior to immersion in 46°C water for 4.5 min reduced mortality by more than two-thirds compared with larvae maintained at 25°C before immersion (Beckett & Evans 1997). Lester & Greenwood (1997) found that mild heat pre-treatments considerably increased tolerance of lightbrown apple moth larvae to subsequent immersion in 43°C water These types of heat pre-treatments are commonly and inadvertently applied to fruits by the sun and warm packing sheds. Because these effects seem to apply across all taxonomic groups and may result from relatively mild changes in temperature, and given that pre-treatment temperature changes on a commercial scale are often unavoidable, we wonder why this has not lead to failures in temperature quarantine treatments. Part of the reason may be that quarantine security is generally very conservative; this could become a problem if quarantine security is liberalized (Liquido et al. 1997). But also, it may be that when live larvae are found in a treated fruit shipment other reasons are blamed.

Because of the potential for failure of a quarantine treatment caused by pre-treatment temperature variation, it is recommended that researchers incorporate reasonable pretreatment temperature modification into research designed to develop temperature-based quarantine treatments. This has not been done intentionally for any confirmatory research on temperature quarantine treatments; on the contrary, researchers usually prefer to maintain pre-treatment temperatures at constant, mild levels.

variability of Commodity A two-stage hot water immersion of papayas was designed to kill only fruit fly eggs and early instar larvae near the surface (Couey & Hayes 1986). Papayas harvested not more than one-quarter ripe were not found to contain fruit flies more advanced than first instar. However, in March of 1987 nine of 16,000 papayas which were subjected to the treatment were found to be infested with third instar fruit fly larvae (Zee et al. 1989). It was discovered that some papayas had an opening at the blossom end which allowed for fruit flies to oviposit in the papaya at an earlier stage and for larvae to more quickly enter the center of the fruit where the lowered treatment severity could allow for survivors. Papayas from 5-3 1 % of trees from commercial orchards sampled in 1987 had that opening to some degree. During the research leading to this treatment natural infestation studies were done with fruits taken from only two orchards. Also, the large scale confirmatory tests (80,000 fruits) were conducted entirely with papayas from one orchard; that grower may have simply had a low incidence of the blossom end defect and/or a low incidence of fruit fly-infested papayas. The lesson to be learned from this incident is that fruits used in quarantine treatment research should be sampled from a wide range of the exporting area to increase the chances that any fruits which may yield different responses would be present in the experimental units. Also, researchers should not lightly dismiss unexpected anomalies in research results; six survivors of this treatment found during the research phase were considered "accidental reinfestation".

***Considerations of* Scale Although** a temperature treatment can be devised and function efficiently in a research facility, it may not operate as expected commercially because of problems related to scale. These problems are more acute for heat treatments than cold treatments because heat treatments are short. Also, they are more critical for heated air treatments than for heated water treatments because: 1) Heated air will take the path of least resistance in a fruit load and may leave areas with lower temperatures. 2) As air moves through a fruit load its temperature is reduced and the humidity changes. 3) Air speed is directly proportional to fruit heating rate and inversely related to density of fruit load. Williamson & Winkleman (1989) discuss challenges in developing a commercial-scale heated air treatment facility.

Rate Of Temperature Change A treatment applied quicker is often more effective than the same treatment applied at a slower rate. For example, Gould & Hennessey (1997) found a faster rate of mortality of fruit fly larvae in carambolas cooled in 40-45 min to a holding temperature of 1.1°C versus those cooled to 1.1°c in over 24 h. In this case, the faster cooling rate may have prevented cold hardening by the insect. Because the cooling rate of fruit in large, commercial coolers is usually slower than that achieved in small research coolers, it is possible that a cold treatment applied on a commercial scale may have lower mortality than that same treatment when performed on a research scale. Of course, the same can be said for heat treatments. If that proves to be a concern, then the heating or cooling rate during research should not be faster than that possible commercially.

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