PULSED ELECTRIC FIELD: A FUTURE QUARANTINE TREATMENT?

Guy J. Hallman* & Q. Howard Zhang
USDA-ARS, Weslaco, TX, and Ohio State Univ., Columbus

High voltage electric field pulses delivered in microseconds can deactivate vegetative stages of microorganisms (Grahl & Märkl 1996). This technique, known as pulsed electric field (PEF) is being studied as a nonthermal means of fluid food preservation which is less damaging to the food than other techniques, such as heat. The mode of action of PEF is thought to be related to increased permeability of the cell membrane due to compression by an electrical potential across the membrane when an external electrical field is applied. Electrical fields of 25 kV or more may be necessary to inactivate bacteria (Zhang et al. 1995). Smaller voltages should inactivate insects because they have larger cells, which are more susceptible to electropermeability, complex organ systems providing more possibilities for fatal damage, and a nervous system which is susceptible to electrical interference. The objective of this research was to determine if PEF would kill insects.

Presently no PEF machinery exists which can treat a whole fruit; therefore, we conducted our studies, the first ever involving PEF of a multicellular organism, in a small chamber (0.8 cm^3) *in vitro*. Studies on insect kill inside of fruit and tolerance of fruit to PEF await future equipment designs. Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae) eggs and feeding late-third instars were placed in water (0.05-0.2% salt) in the treatment chamber drilled out of a polycarbonate block. The insects were subjected to 1-10 pulses of 1.9-9.2 kV lasting 50 μs each with a lapse of about 30 seconds between pulses. After treatment insects were placed on moist filter paper in petri dishes to observe development. First instars hatching from eggs were placed on semi-artificial diet (Spishakoff & Hernandez-Davila 1968); after seven days the diet was strained and larvae recovered.

Results and discussion: Percentage egg hatch was progressively reduced as voltage increased and reached 2.9% at the highest dose (ten 50-μs pulses at 9.2 kV). Some of the eggs disintegrated during treatment, and the number that disintegrated seemed to be directly related to the voltage. The contents of these eggs apparently formed a brown gel several hours later. The estimate of $LD_{99.9968}$ (probit 9) to prevent egg hatch was ten 50-μs pulses at 14.7 kV with 95% fiducial limits of 10.6-31.4 kV. However, no third instars developed from first instars hatched from eggs treated with $\geq$ten 50-μs pulses at 5.0 kV and placed on diet, while few developed from those treated at 4.0 kV.

The larvae contracted slightly when pulses passed through the treatment chamber. This reaction became less pronounced with each successive pulse. Treated larvae were very sluggish for a few hours after the treatment; however, none were dead. Some of the larvae became somewhat active a few hours after treatment, but most remained sluggish. About 10 hours after treatment, larvae were pupariating, and all of the untreated puparia were normal. Only 11 of 60 puparia treated as larvae with one or two 50-µs pulses at 2.0 kV, were normal. Most of the PEF-treated larvae formed larviform puparia. Many others,

especially those treated with ten 50-µs pulses at 7.4 or 8.0 kV, formed partial larviform puparia always commencing at the anterior end. Many other treated larvae never began to pupariate but formed necrotic areas throughout the body and eventually died. The first treated insects to die did so about 24 hours after treatment. Although some treated insects lasted several days before dying; they were never as active as untreated larvae. Before 66 hours post-treatment, all larvae treated at 3.5 kV or more were dead. Forty-six of 50 control and one male larvae treated with ten 50-µs pulses at 1.9 kV developed to normal-looking adults. Two normal-looking females of 30 larvae treated with one pulse of 2.0 kV emerged from normal puparia.

PEF did not provide acute mortality; in this regard it is similar to irradiation which does not kill insects acutely in the doses applied to fresh commodities (<1 kGy). However, like irradiation, PEF can prevent formation of adult fruit flies and could, thus, satisfy the requirements of quarantine security. In this study, complete insect development was stopped with ten 50-µs pulses at 5.0 kV, and eggs were harder to inactivate than larvae.

References Cited

Grahl, T. & Märkl, H. (1996) Killing of microorganisms by pulsed electric fields. *Applied Microbiology and Biotechnology* **45**, 148-157.

Spishakoff, L.M. & Hernandez-Davila, J.G. (1968) Dried torula yeast as a substitute for brewer's yeast in the larval rearing medium for the Mexican fruit fly. *Journal of Economic Entomology* **61**, 859-860.

Zhang, Q., Monsalve-González, A., Qin, B.-L., Barbosa-Cánovas, G.V. & Swanson, B.G. (1994) Inactivation of *Saccharomyces cerevisiae* in apple juice by square-wave and exponential-decay pulsed electric fields. *Journal of Food Process Engineering* **17**, 469-478.