INHIBITION OF FRUIT FLY (DIPTERA: TEPHRITIDAE) DEVELOPMENT BY PULSED ELECTRIC FIELD

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ABSTRACT

Pulsed electric field (PEF) has been studied as a means to inactivate microorganisms in liquid prepared foods to prolong shelf life and prevent food poisoning. PEF is thought to inactivate microbes by permeabilizing the cell membrane and has less adverse effects on nutritional quality and flavor of the food than traditional thermal pasteurization or sterilization methods. The goal of quarantine treatments are similar to the goal of food pasteurization in that any quarantined insects present in the commodity must be prevented from reproducing using techniques which are not significantly detrimental to the quality of the commodity. Traditional quarantine treatments include fumigation, heat, cold, and ionizing irradiation. PEF was applied to Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), eggs and feeding third instars. The treatment disintegrated some of the eggs. Percentage egg hatch was progressively reduced to a minimum of 2.9% as voltage was increased to a maximum of 9.2 kV/cm^2 delivered in ten 50 µs pulses. Nevertheless, no first instars treated as eggs with ≥5.0 kV (ten 50 µs pulses) survived to the third instar. PEF did not kill third instars immediately; however, they displayed a variety of pathological symptoms including sluggishness, elongated, larviform, and partial pupariation, and development of necrotic spots throughout the body. No third instars treated with >2.0 kV survived to the adult stage. Therefore, PEF has been shown to control insects, although considerable entomological and engineering work would be needed before a PEF-based treatment might become practical.

Key Words: pupariation, Mexican fruit fly, *Anastrepha ludens*

RESUMEN

Se ha estudiado el campo eléctrico pulsado (CEP) para inactivar microorganismos en alimentos líquidos preparados con el objetivo de conservar su calidad y prevenir intoxicación. Se postula que el CEP inactiva los microorganismos al permeabilizar la membrano de la célula. El CEP causa menos daño a la calidad nutritiva y sabor del alimento que métodos termales tradicionales. Los tratamientos cuarentenarios son similares a la pasteurización del alimento en que hay que inhibir la reproducción de todo insecto cuarentenado presente en la mercancía. Tratamientos cuarentenarios tradicionales incluyen fumigación, calor, frío, e irradiación. El CEP se aplicó a posturas y terceros ínstares de la mosca mejicana de las frutas, *Anastrepha ludens* (Loew) (Diptera: Tephritidae). El tratamiento disintegró algunos de las postucon. El porcentaje de eclosión fue el 2.9% el máximo voltaje usados (diez pulsos de 50 µs al 9.2 kV/cm^2). Sin embargo, ningún primer ínstar tratado con ≥5.0 kV llegó al tercer ínstar. El CEP no mató a los terceros ínstares de inmediato; no obstante, mostraron una variedad de síntomas patológicos incluyendo pereza, desarrollo de puntos necróticos, y pupariación alargada, larviforme, y parcial. Ningún tercer ínstar tratado con >2.0 kV sobrevivió al estado del adulto. Por eso, el CEP se ha sido capaz de controlar insectos, aunque falta mucho trabajo entomológico y de ingenería antes de poder ser práctico.

Insects inside harvested agricultural commodities must often be killed, prevented from completing development, or from reproducing without significantly harming the commodity or leaving residues of potentially harmful chemicals. Such treatments are needed not only to prevent continued increase of the pest population levels and associated losses of commodity quantity and quality, but also preclude the importation of exotic pests. This task has been made more difficult in recent years by the loss and pending loss of key fumigants. Use of ethylene dibromide was halted a decade ago because it was deemed a cancer risk. Methyl bromide is currently scheduled to be phased out within several years because it is considered a significant stratospheric ozone depleter. Other techniques for postharvest control of insects, such as exposure to extreme temperatures, are replacing these fumigants (Mangan & Hallman 1997, Mason & Strait 1997).

High voltage electric field pulses delivered in microseconds can deactivate vegetative stages of microorganisms (Grahl & Märkl 1996). Pulsed electric field (PEF) is being studied as a nonthermal means of fluid food preservation. The mode of action of PEF is thought to be related to increased permeability of the cell membrane due to compression caused by an electrical potential across the membrane when an external electrical field is applied. Electrical pulses of 25 kV or more may be needed to inactivate bacteria (Zhang et al. 1995). According to PEF theory, smaller voltages should suffice to inactivate organisms with larger cells, such as insects, because the electrical potential between the interior and exterior surfaces of the cell membrane, ΔV, is positively related to the size of the cell by the following equation:

$$\Delta V = \left(\frac{l}{l - 0.67a}\right)a \cdot E$$

where *l* is the length of the cell, *a* is the radius, and E is the external electric field applied. Hence, the greatest possible transmembrane potentials, ca. 1.5($a \cdot E$), occur to the largest cells of spherical shape [condensed from Grahl & Märkl (1996)]. Because of the presence of an electrically-influenced nervous system and complex multicellular organs in insects, it is conceivable that PEF may kill insects with a lower voltage than that needed to inactivate the insect cells by electropermeabilization.

The objective of this research was to determine if PEF could be used to kill insects; this is the first published record of the reaction of a multicellular organism to this treatment.

Materials and Methods

Pulsed Electric Field Generator

Experiments were conducted with a PEF generator at the Ohio State University, Department of Food Science & Technology. The PEF generator consists of a high voltage (≤15 kV DC) power supply (Cober Electronics 1450-4) which transmits voltage through a pulse generator (Cober Electronics Model 2829) to a static fluid treatment chamber containing the insects. The treatment chamber (a cylinder 1 cm long by 1 cm diameter or 0.8 cm^3) was bored out of a block of polycarbonate and placed between two stainless steel electrodes. Temperature of the electrodes was measured with type J, 20 gauge thermocouples and a data logger (John Fluke model 52).

Insects

Mexican fruit fly, *Anastrepha ludens* (Loew), originated in Montemorelos, Nuevo Leon, Mexico and were reared on a semi-artificial diet at Weslaco for 4-6 generations (Spishakoff & Hernandez-Davila 1968). Eggs (about half way through development) and feeding late-third instars were placed in the PEF treatment cylinder containing 0.05-0.2% NaCl in water. 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. Insects were treated in groups of about 100-200 eggs or ten larvae. Egg treatments were replicated twice and larval treatments 2 or 3 times. Controls were placed in 0.05-0.2% salt solution for a few minutes. After treatment eggs were placed on moist filter paper and larvae in petri dishes to observe development. First instar larvae emerging from eggs were placed on a semi-artificial diet; after seven days the diet was strained and large (third instar) larvae recovered.

Results

Eggs

Percentage egg hatch declined progressively to a low of 2.9% as voltage increased to the highest dose, 9.2 kV (Fig. 1). 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 formed a brown gel several hours later. Probit analysis of egg mortality gave a y-intercept of -0.58 and a slope of 0.31. The estimate of $LD_{99.9968}$, a level of control often demanded of quarantine treatments against tephritids (Shannon 1994), was 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 ≥5.0 kV and placed on diet, while only few developed from those treated with 4.0 kV.

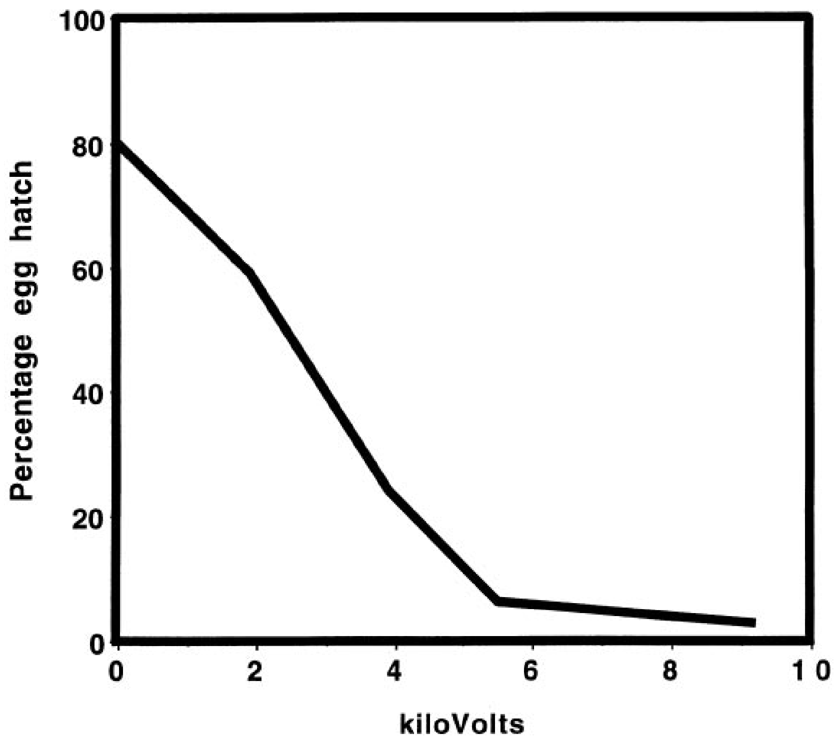

Fig. 1. Percentage Mexican fruit fly egg hatch as a consequence of voltage applied by pulsed electric field (ten 50 µs pulses per cm^2).

Larvae

Larvae contracted slightly but noticeably when the pulses passed through the treatment chamber. This reaction became less pronounced with each successive pulse. Treated larvae were very sluggish after the treatment; however, none were dead. Some of the larvae regained a measure of activity a few hours after treatment, but most remained sluggish. By about 10 hours after treatment, larvae were pupariating, and all of the control puparia were normal coarctate (Tables 1 and 2). Only 11 of 60 puparia treated as larvae with one or two 50-µs pulses at 2.0 kV, were normal (Tables 2). Nineteen of 90 puparia of larvae treated with one to three 50-µs pulses at 2.0 kV were of the elongated coarctate form (Fig. 2) as was one of the puparia treated at 1.9 kV for 10 pulses (Table 1). Most of the PEF-treated larvae formed larviform puparia (Fig. 3). 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 (Fig. 4). Many other treated larvae never began to pupariate but necrotic areas formed throughout the bodies, and eventually they died (Fig. 5). Some larvae were still moving after the entire body was black. The first treated insects to die did so about 24 hours after treatment. Although some treated insects lived several days, they were never as active as untreated larvae. Before 66 hours post-treatment, all larvae treated

TABLE 1. CONDITION OF *ANASTREPHA LUDENS* ABOUT 42 HOURS AFTER BEING SUBJECTED AS FEEDING THIRD INSTARS TO 10 PULSES OF 50 µS EACH AT VARIOUS VOLTAGES/CM².

	No. of insects of a total of 20 in each stage					
	Larvae		Puparial appearance			
Voltage (kV)	Normal	Necrotic	Normal	Elongated	Larviform	Partially pupariated
0	5	0	15	0	0	0
1.9	0	5	0	1	14	0
3.5	0	6	0	0	7	7
5.3	0	0	0	0	9	11
7.4	0	0	0	0	0	20
8.0	0	0	0	0	0	20

at 3.5-8.0 kV were dead. Forty-six of 50 control larvae developed into normal adults. Also, one male larvae treated with 1.9 kV developed first into a slightly elongated puparium and then into an apparently normal adult. From 30 larvae treated with one pulse of 2.0 kV, two apparently normal females emerged from seemingly normal puparia.

TABLE 2. CONDITION OF *ANASTREPHA LUDENS* ABOUT 46 HOURS AFTER BEING SUBJECTED AS FEEDING THIRD INSTARS TO 2.0 KV/CM² FOR 1-10 PULSES OF 50 µS EACH.

	No. of insects of a total of 30 in each stage					
	Larvae		Puparial appearance			
No. pulses	Normal	Necrotic	Normal	Elongated	Larviform	Partially pupariated
0	2	0	28	0	0	0
1	7	0	10	11	2	0
2	11	2	1	6	10	0
3	7	7	0	2	14	0
4[1]	4	3	0	0	22	0
5	9	6	0	0	15	0
6	3	7	0	0	20	0
7	3	8	0	0	17	2
8	1	8	0	0	19	2
9	0	8	0	0	19	3
10[1]	0	19	0	0	6	4

[1]Total number of larvae with 4 and 10 pulses was 29.

Fig. 2. Varying degrees of elongation of *Anastrepha ludens* puparia subjected as third instars to pulsed electric field (2 kV, one 50 µs pulse). Untreated puparium is shortest one (far left).

Fig. 3. One normal and eight larviform *Anastrepha ludens* puparia. Larviform puparia treated as third instars with pulsed electric field (2 kV, four 50 µs pulses).

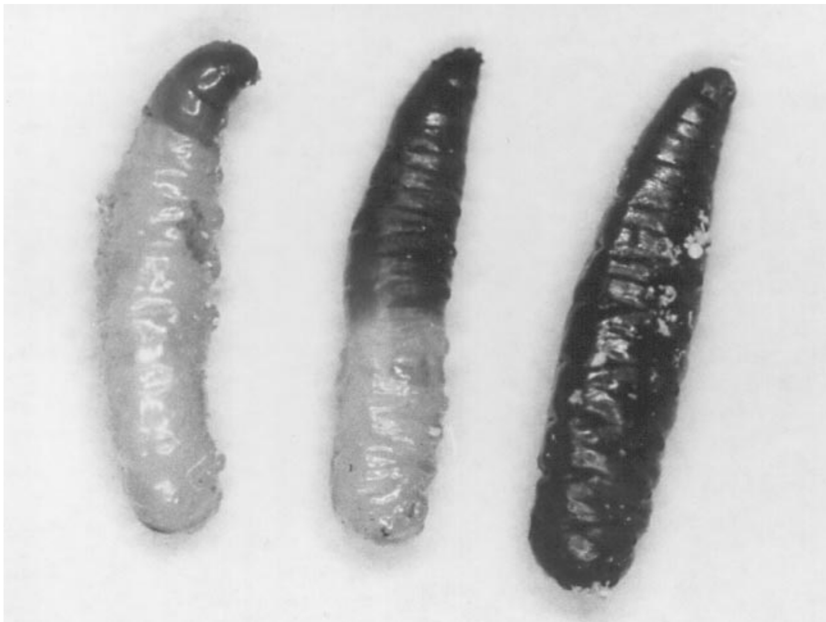

Fig. 4. Varying degrees of larviform pupariation by *Anastrepha ludens* treated with pulsed electric field (2 kV, ten 50 µs pulses) while third instars.

Temperature increase of the electrodes was never more than 0.1°C, confirming that PEF is a nonthermal treatment.

Discussion

Cumulative prevention of eclosion of *A. ludens* eggs by PEF was reduced only slightly after 3.8 kV and did not reach 100% at the highest dose, 9.2 kV. Although the estimated dose to achieve $LD_{99.9968}$ egg mortality was 14.7 kV, no late-instar larvae developed from eggs treated with ten pulses at ≥5.0 kV. Only larviform puparia formed from larvae subjected to 10 pulses at ≥3.5 kV or ≥4 pulses at 2.0 kV. Therefore, although acute mortality of *A. ludens* did not occur to any appreciable extent at the PEF doses used in this study, complete metamorphosis was stopped with much lower doses than those needed to inactivate microorganisms. A quarantine treatment need not cause acute mortality to be used commercially. Irradiation does not cause significant acute insect mortality at the doses used on fruits and vegetables, although completion of insect development can be averted (Burditt 1994). Consequently, PEF could be employed as a quarantine treatment under the same criteria used for irradiation. In fact, the efficacy of a PEF treatment would be easier to assess than that of irradiation. Irradiated third instar tephritids move normally and usually form normal puparia at the doses used on fruits. PEF-treated third instars, however, remain lethargic and, for the most part, do not pupariate normally.

Some of the pathological symptoms shown by the larvae were similar to those caused by irradiation of larvae of cyclorrhaphous flies. Irradiation at ≥25 Gy prevented inversion of the larval head of *Sarcophaga bullata* Parker (Diptera: Sarcoph-

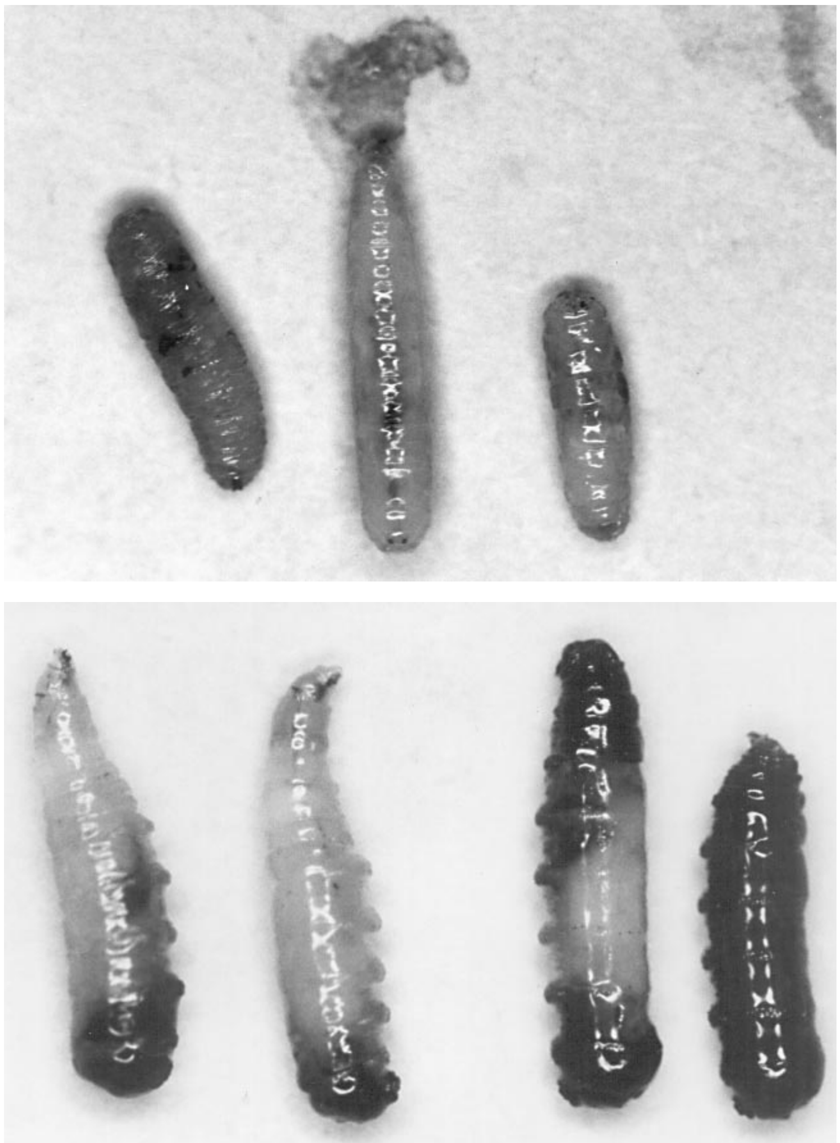

Fig. 5. Varying degrees of necrosis of third instar *Anastrepha ludens* 28 (a) and 46 (b) hours after treatment with pulsed electric field (2 kV, seven to ten 50 µs pulses).

agidae), resulting in the formation of a larviform puparium (Sivasubramanian et al. 1974). Ligation of postfeeding cyclorrhaphous larvae in the mid-body area before molting hormones were produced and tight enough to prevent translocation of the hormones from the anterior central nervous system to the posterior half of the body produced normal pupariation in the anterior end while maintaining the posterior larval (Fraenkel & Zdarek 1970). We hypothesize that in those larvae which partially pupariated in the anterior end of the body, hormones initiating puparial sclerotization and melanization were not translocated but simply diffused from the central nervous system at the anterior end of the body. This was substantiated by our observation that in certain individuals the heart was not pumping.

Larviform puparia failed to retract the anterior prespiracular segments and contract longitudinally. This is consistent with a general paralysis of the musculature (Zdarek & Fraenkel 1987). The fact that larvae never recovered their former level of activity after PEF treatment denotes that the paralysis was permanent. Elongated puparia failed to retract the anterior prespiracular segments in varying degrees but were more successful at contracting longitudinally, indicating that PEF more easily paralyzed the former muscular system (retractors), which are also used in everting the anterior spiracles, than the latter (contractors), which are used in larval locomotion. In one case an adult with normal appearance emerged from a slightly elongated puparium (Table 1), confirming that normal puparia are not necessary for successful development of adult tephritids (Thomas & Mangan 1995).

Finally, because of the diverse reactions observed, PEF may prove to be a useful tool in the study of insect developmental biology.

ACKNOWLEDGMENTS

We thank Donghui Song and Yiping Su for assisting in the operation of the PEF equipment, Shaoyi Zhang for making the treatment chamber (all three of the Ohio State Univ. Dept. of Food Sci. and Tech.), and Julia Olivarez, USDA-ARS, Weslaco, Tex., for rearing *A. ludens.* Waldemar Klassen, Univ. of Florida, Tropical Research & Extension Center, Homestead, provided valuable comments which improved the manuscript.

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