INFLUENCE OF TEMPERATURE ON SUSCEPTIBILITY OF *TRIBOLIUM CONFUSUM* (COLEOPTERA: TENEBRIONIDAE) POPULATIONS TO THREE MODIFIED DIATOMACEOUS EARTH FORMULATIONS

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ABSTRACT

The influence of temperature on the insecticidal effect of 3 commercially available modified diatomaceous earth (DE) formulations and their mixture against adults and larvae of *Tribolium confusum* Jacquelin du Val originating from different European geographical locations was evaluated in laboratory tests. The temperatures tested were 20, 25, and 30°C. The *T. confusum* populations (strains) originated from Greece, Italy, Portugal, United Kingdom, Germany, France, and Denmark. Mortality counts were carried out after 7 d of exposure of *T. confusum* individuals to DE-treated wheat. The 3 modified DEs tested were PyriSec, Insecto, Protect-It, and a mixture of the three. The dose rates tested were 500 and 1000 ppm. The strains from Portugal and France were the least susceptible to DEs, while the most susceptible one was the strain from the United Kingdom. The increase of temperature in many of the combinations tested increased the efficacy of DE formulations against both adults and larvae, but there were some cases in which temperature had no effect on DE efficacy. Moreover, adults were more tolerant than larvae to the DEs tested as well as to their mixture, regardless of the strain or temperature. The present study clearly indicates that there are serious variations in the efficacy of DEs against *T. confusum* individuals originating from different geographical regions, and several factors affect this efficacy, such as the type of formulation and the temperature level prevailing.

Key Words: Modified diatomaceous earth, populations, temperature, *Tribolium confusum*, wheat

RESUMEN

La influencia de la temperatura sobre el efecto de los insecticidas en 3 formulaciones comerciales disponibles de suelo de diatomita (SD) modificada y su mezcla en contra de adultos y larvas de *Tribolium confusum* Jacquelin du Val provenientes de diferentes regiones geográficas de europa fue evaluada en pruebas del laboratorio. Las temperaturas probadas fueron 20, 25 y 30°C. Las poblaciones de razas de *T. confusum* provenieron de Grecia, Italia, Portugal, Reino Unido, Alemania, Francia y Dinamarca. Se realizaron los recuentos de mortalidad después de 7 d en que los individuos de *T. confusum* fueron expuestos a trigo tratado con SD. Los 3 SD probados fueron PyriSec, Insecto, Protect-It y una mezcla de los tres. Las dosis probadas fueron 500 y 1000 ppm. Las razas de Portugal y de Francia fueron las menos susceptibles a SD, mientras que la mas susceptible fue la raza del Reino Unido. El aumento en la temperatura en muchas de las combinaciones probadas aumento la eficacia de la formaciones de SD contra los adultos y larvas, pero hubo unos casos en que la temperatura no tuvo ningun efecto sobre la eficacia de SD. Además, los adultos fueron mas tolerantes que las larvas hacia los SD probados igual que la mezcla de los 3 SD, sin importar la raza o temperatura. Este estudio claramente indica que hay variaciones serias en la eficacia de los SD contra individuos de *T. confusum* que provienen de diferentes regiones geográficas, además de varios factores que afectan dicha eficacia, como la clase de formulación y el nivel de temperatura prevaleciente.

One of the promising alternatives to traditional insecticides in stored-product protection is the application of diatomaceous earths (DEs). DE is composed of fossilized remains of phytoplanktons (diatoms) (Korunic 1998). DEs have a physical mode of insecticidal action, because they "inactivate" the epicuticular lipids of the insects' cuticle and insects die due to water loss and desiccation (Ebeling 1961, 1971; Korunic 1998; Subramanyam & Roesli 2000). DEs have low mammalian toxicity and, as inert materials, can provide long-term protection against stored product insects (Athanassiou et al. 2005a; Vayias et al. 2006c). Several studies document that the efficacy of DE is affected by several factors such as grain moisture and relative humidity, temperature, commodity, target species, and life stage (Korunic 1998; Arthur 2000b; Subramanyam & Roesli 2000; Fields & Korunic 2000; Mewis & Ulrichs 2001; Vayias & Athanassiou 2004; Athanassiou et al. 2003, 2005b; Vayias et al. 2006c, Korunic & Fields 2006). In addition, the insect strain is another factor that can affect significantly the insecticidal efficacy of DE and its further assessment as a grain protectant (Fields et al. 2003; Arnaud et al. 2005).

The confused flour beetle, *Tribolium confusum* Jacquelin du Val, is one of the most serious stored product insect pests worldwide (Aitken 1975). Although this species develops best in processed amylaceous commodities, both larvae and adults can infest, feed, and develop in sound kernels (Aitken 1975). This species has now developed resistance to many chemical insecticides (Zettler 1991; Arthur & Zettler 1992; Zettler & Arthur 1997). Many studies have shown that several newer DE formulations are effective against both *T. confusum* adults and larvae (Arthur 2000b; Vayias & Athanassiou 2004; Athanassiou et al. 2004, 2005b; Vayias et al. 2006c). According to these studies, at the adult stage, *T. confusum* is one of the most tolerant insect species to DE, and according to Arthur (2000b) and Athanassiou et al. (2005b), it can survive dose rates that can be lethal for other species (Korunic 1998; Athanassiou et al. 2005b). On the other hand, its larvae are very susceptible to DEs (Vayias & Athanassiou 2004). In a recent work, Vayias et al. (2006b) found considerable variations in adult mortality when different populations of *T. confusum*, originating from different parts of Europe, were exposed to DE-treated grain. Thus, even if similar experimental conditions are followed, results obtained from studies from different parts of the world may not be comparable, because different populations were used. Vayias et al. (2006b) used only adults at 1 temperature level; hence, additional experimentation is needed to examine other life stages at a broader range of temperatures. In the present study, 3 commercially available modified DE formulations were evaluated against larvae and adults of *T. confusum* populations originating from different geographical locations of Europe. The impact of temperature on the effect of DEs on these populations also was assessed.

Materials and Methods

Formulations and Commodity

Three modified and enhanced DE formulations were used in the tests: (a) PyriSec (Agrinova Gmbh, Obrigheim/Mühleim, Germany) that contains 1.2% natural pyrethrum (25%), 3.1% piperonyl butoxide and 95.7% of SilicoSec, which is a DE of freshwater origin containing 92% SiO_2. The particle size distribution is between 8 and 12 µm (Athanassiou et al. 2004), (b) Insecto (Insecto Natural Products, Inc., Costa Mesa, CA, USA), a DE of marine origin containing 86.7% SiO_2 and 10% food-grade additives. The particle size distribution is between 0.82 and 52.33 µm (Subramanyam et al. 1994), (c) Protect-It (Hedley Technologies Inc., Mississauga, Ontario, Canada) a DE that contains 83.7% SiO_2 with 10% silica aerogel. The particle size distribution is between 5 and 6 µm (Korunic & Fields 1995), and (d) a mixture of the 3 above-described DE formulations. The mixture, which is not commercially available, was prepared at our laboratory only for experimental purposes by admixing equal quantities of the tested DE formulations. The commodity tested was untreated, clean, hard wheat (var Mexa) harvested in 2004. The dockage of the wheat was minimal (<0.6%) and the moisture content as determined by a Dickey-John moisture meter (Dickey-John Multigrain CAC II, Dickey-John Co., Lawrence, KS, USA) was approx. 11.4%.

Insects and Rearing Cultures

Tribolium confusum adults less than 14 d old and 3-4th instars were used in the tests. There were 7 different populations obtained from Danish Pest Infestation Laboratory (Lyngby, Denmark), Central Science Laboratory (York, UK), Benaki Phytopathological Institute (Kifissia, Greece), Institute for Stored Product Protection (Berlin, Germany), University of Molise (Campobasso, Italy), Laboratoire Denrées Stockées (Cenon Bordeaux, France), and Tropical Scientific Research Institute (Lisbon, Portugal) (the above strains are abbreviated in the text as DK, UK, GR, GER, IT, FR and POR, respectively). All strains had been initially collected from the local fauna of each country. Both adults and larvae of the populations were reared on wheat flour plus 5% brewers yeast (by weight) at 27 ± 1°C and 65 ± 5% RH. The GR strain was kept at the Benaki Phytopathological Institute for >10 years, while the rest were reared in the same laboratory for >7 generations, after introduction from their respective countries of origin.

Bioassays

Exposure studies were carried out at 20, 25, and 30°C and 70 ± 1.5% relative humidity (RH). Six 1-Kg wheat lots were prepared and placed in 6 cylindrical glass jars (18 cm in diameter, 30 cm in height). Three of these jars contained wheat treated with 500 ppm of each DE (one jar per DE), whereas each of the other 3 jars contained wheat treated with 1000 ppm of DE. Previous studies have shown that *T. confusum* adults are not susceptible at doses lower than 500 ppm of some of these DEs (Arthur 2000a, b; Athanassiou et al. 2005b; Vayias & Athanassiou 2004). In addition, 2 cylindrical jars contained wheat treated with an equivalent mixture of all of the DEs at the same dose rates. An additional jar of untreated wheat was used as control. All jars were shaken manually for approx. 5 min to achieve distribution of the dust in the entire grain mass. For each strain's life stage, 4 samples of 30 g each were taken from each jar, and each sample was placed in a cylindrical glass vial (7 cm in diameter, 12 cm in height). The vials were closed, except for a hole (3 cm in diameter) in the cap, which was covered with organdy to allow sufficient aeration. Then, 30 *T. confusum* adults or larvae from each strain were separately introduced into each vial, and all vials were placed in incubators set each time at the aforementioned conditions. Mortality of the exposed individuals (adults or larvae) was measured after 7 d of exposure. The entire procedure was repeated 3 times, by preparing new lots of wheat each time. During the experimental period the RH level was maintained with saturated potassium iodine solution, as recommended by Greenspan (1977).

Data Analysis and Statistics

Preliminary analysis for both adults and larvae showed homogeneity among the replicates of each assay according to the Levene test (Levene 1960). The data were initially corrected by Abbott's (1925) formula. The arcsine transformed data were separately analyzed for adults and larvae. For a given life stage, mortality counts were analyzed by the GLM Procedure of SAS (1998) with adult (or larval) mortality as the response variable and DE formulation, dose rate, strain, and temperature as main effects. Control mortality ranged between 2-8% and 3-12% for adults and larvae, respectively. Means were separated by Tukey-Kramer (HSD) at $P = 0.05$ (Sokal & Rohlf 1995).

RESULTS

Tribolium confusum Adults

All main effects and associated interactions were significant at $P < 0.001$ level (formulation: $F = 174.83$, $df = 3, 2015$, dose rate: $F = 15313.22$, $df = 1, 2015$, strain: $F = 134.07$, $df = 6, 2015$, temperature: $F = 1142.84$, $df = 2, 2015$, formulation × dose rate: $F = 24.75$, $df = 3, 2015$, formulation × strain: $F = 2.15$, $df = 18, 2015$, formulation × temperature: $F = 4.54$, $df = 6, 2015$, dose rate × strain: $F = 9.44$, $df = 6, 2015$, dose rate × temperature: $F = 39.20$, $df = 2, 2015$, strain × temperature: $F = 2.22$, $df = 12, 2015$).

Significant differences in the mortality of adults were noted among the *T. confusum* strains as well as among the temperatures tested. At 20°C, mortality of adults at the dose rate of 500 ppm, was less than 20% for all the DEs tested whereas the respective figures at 1000 ppm ranged between 37.5% and 83.8%. At this temperature, in most cases, the POR and GR strains were significantly less susceptible than the other strains at 500 ppm, while at 1000 ppm the least susceptible strains were the POR and FR, with the exception of Protect-It where the least susceptible strains were POR and IT (Tables 1, 2).

The increase of temperature from 20°C to 25°C led to a significant increase in mortality of adults in most of the cases tested. However, in the case of Insecto, significant differences between 20 and 25°C were noted only with the GR and GER strains. At 25°C, as noted for 20°C, the most tolerant strains were the POR and FR. Although the POR was more tolerant than the FR at 25°C, significant differences in adult mortality between those 2 strains were not noted at either dose levels of Pyrisec and at 1000 ppm of Protect-it. At 500 ppm DEs, significant differences in adult mortality were not observed between the IT and GR strain, except for the case of mixture of DEs (Tables 1, 2).

At 30°C, mortality values of *T. confusum* adults were higher than 90%, regardless of the strain or formulation. This was noted at 1000 ppm, while at 500 ppm, adult mortality did not exceed 60%. At this temperature, strains POR and FR were the most tolerant strains, whereas the most susceptible one was the UK strain. Generally, of the formulations tested, Protect-it and the mixture of DEs were the most effective, and the least effective one was Insecto (Tables 1, 2).

Tribolium confusum Larvae

All main effects and associated interactions were significant at $P < 0.001$ (formulation: $F = 213.85$, $df = 3, 2015$, dose rate: $F = 2191.16$, $df = 1, 2015$, strain: $F = 157.64$, $df = 6, 2015$, temperature: $F = 747.59$, $df = 2, 2015$, formulation × dose rate: $F = 4.64$, $df = 3, 2015$, formulation × strain: $F = 2.62$, $df = 18, 2015$, formulation × temperature: $F = 29.66$, $df = 6, 2015$, dose rate × strain: $F = 28.95$, $df = 6, 2015$, dose rate × temperature: $F = 6,77$, $df = 2, 2015$, strain × temperature: $F = 4, 515$, $df = 12, 2015$).

As noted for adults, larval mortality increased with temperature irrespective of the *T. confusum* strain, DE formulation, or dose. In most cases, at temperatures higher than 25°C and at the highest dose rate tested, larval mortality was 100%. Regardless of temperature, DE formulation, and dose rate, significantly more larvae of the UK strain were dead in comparison with the other 6 strains. On the other hand, the most tolerant

TABLE 1. MEAN MORTALITY (± SE) OF DIFFERENT STRAINS OF *T. CONFUSUM* ADULTS EXPOSED TO WHEAT TREATED WITH 500 PPM OF 3 DE FORMULATIONS AND THEIR MIXTURE, AT 3 TEMPERATURE LEVELS.

		Temperature (°C)		
Formulation	Strain	20	25	30
Pyrisec	GR	7.5 ± 2.6 Ab	20.5 ± 1.3 Bb	38.5 ± 2.2 Cc
	IT	20.1 ± 3.7 Af	21.1 ± 1.6 Ab	27.0 ± 2.7 Bb
	DK	13.5 ± 3.4 Ad	28.9 ± 2.6 Bc	40.5 ± 2.8 Cc
	POR	2.5 ± 4.5 Aa	12.8 ± 1.5 Ba	15.0 ± 2.6 Ba
	UK	18.5 ± 5.4 Aef	35.7 ± 4.8 Bd	54.2 ± 4.2 Cd
	FR	10.8 ± 1.4 Ac	13.2 ± 1.4 ABa	15.4 ± 2.6 Ba
	GER	16.2 ± 3.9 Ae	27.8 ± 2.9 Bc	37.8 ± 4.2 Cc
Insecto	GR	5.0 ± 1.5 Ab	12.8 ± 1.5 Bb	15.4 ± 1.3 Bb
	IT	10.4 ± 2.1 Ac	10.5 ± 3.1 Ab	13.5 ± 1.1 Bb
	DK	2.7 ± 5.4 Aa	5.3 ± 1.7 Aa	37.8 ± 2.7 Bd
	POR	10.0 ± 5.4 Ac	7.7 ± 1.1 Aa	10.0 ± 2.1 Aa
	UK	11.1 ± 2.8 Ac	25.0 ± 3.6 Ac	41.7 ± 2.5 Ae
	FR	10.8 ± 2.9 Ac	7.9 ± 1.7 Aa	10.3 ± 1.3 Aa
	GER	1.5 ± 1.2 Aa	22.2 ± 3.3 Bc	32.4 ± 2.7 Cc
Protect-It	GR	2.5 ± 1.3 Aa	23.1 ± 1.3 Bb	41.0 ± 1.6 Cc
	IT	5.0 ± 1.5 Aa	21.1 ± 1.5 Bb	29.7 ± 2.5 Cb
	DK	13.5 ± 2.3 Ac	34.2 ± 2.3 Bd	45.9 ± 1.6 Cd
	POR	2.5 ± 1.3 Aa	17.9 ± 1.3 Ba	25.0 ± 3.4 Ca
	UK	14.8 ± 4.6 Ac	39.3 ± 4.6 Be	54.2 ± 1.5 Ce
	FR	10.8 ± 2.7 Ab	21.1 ± 2.7 Bb	25.6 ± 4.1 Ca
	GER	13.5 ± 2.3 Ac	27.8 ± 2.3 Bc	40.5 ± 7.4 Cc
Mixture	GR	5.2 ± 1.3 Aa	25.6 ± 1.2 Bc	43.6 ± 1.6 Cc
	IT	20.0 ± 1.5 Ac	21.1 ± 2.9 Ab	32.4 ± 2.5 Bb
	DK	5.4 ± 2.3 Aa	31.6 ± 3.5 Bd	48.6 ± 1.5 Cd
	POR	2.5 ± 1.3 Aa	17.9 ± 1.3 Ba	25.0 ± 3.4 Ca
	UK	18.5 ± 4.6 Ac	35.7 ± 5.9 Be	58.3 ± 1.5 Be
	FR	13.5 ± 2.7 Ab	18.4 ± 1.6 Ba	30.8 ± 4.1 Cb
	GER	18.9 ± 2.3 Ac	30.6 ± 2.3 Bd	45.9 ± 7.8 Ccd

For a given formulation, means in the same column followed by the same lowercase letter are not significantly different, while means in the same row followed by the same uppercase letter are not significantly different; lowercase letters for strains; uppercase letters for temperature; temperature $df = 2, 36$, strain $df = 6, 71$, Tukey-Kramer test at 5%; for abbreviations of strains see materials and methods.

strains, in terms of larval mortality, were the POR and FR, with the former strain being more susceptible than the latter. The GER strain was more tolerant than the GR, IT, and DK strains. Significantly more larvae of the DK strain were dead in comparison with the GER, GR, and IT strains at all formulations and temperatures tested. Significant differences in larval mortality between the GR and IT strain were not noted, except for the case of 500 ppm of Protect-It, in which larvae of the GR strain were more susceptible than those of the IT strain (Tables 3, 4).

DISCUSSION

The results of the present study indicate that DEs could be used with success against *T. confusum*, but their insecticidal efficacy is highly influenced by several factors such as temperature, life stage, type of DE formulation, and dose rate. These findings seem to support those obtained from recent studies (Rigaux et al. 2001; Arnaud et al. 2005; Vayias et al. 2006b) indicating that the efficacy of DEs against a given species depends on the geographical location of the species origin. For instance, Arnaud et al. (2005) found significant differences in susceptibility to DE formulations among adults of the red flour beetle, *Tribolium castaneum* (Herbst) originating from different geographical locations of the world. In a recent study, Vayias et al. (2006b) in tests of several DEs against the same strains of *T. confusum* found significant variations, which were not always consistent for all formulations. However, in that study, the strains were tested only at the adult stage, and only at 1 temperature level. In light of our findings, it is clearly evident that the observed difference in susceptibility among the strains tested is determined by both formulation and temperature, indicating the complexity of this

TABLE 2. MEAN MORTALITY (± SE) OF DIFFERENT STRAINS OF *T. CONFUSUM* ADULTS EXPOSED TO WHEAT TREATED WITH 1000 PPM OF 3 DE FORMULATIONS AND THEIR MIXTURE AT 3 TEMPERATURE LEVELS.

		Temperature (°C)		
Formulation	Strain	20	25	30
Pyrisec	GR	65.0 ± 4.5 Ad	76.9 ± 1.3 Bb	92.3 ± 1.5 Ca
	IT	55.0 ± 1.5 Ac	65.8 ± 4.2 Ba	94.6 ± 2.2 Ca
	DK	75.7 ± 3.5 Ae	81.6 ± 1.6 Bc	100 ± 0.0 Cb
	POR	42.5 ± 2.5 Aa	66.7 ± 2.7 Ba	92.5 ± 2.3 Ca
	UK	74.1 ± 4.1 Ae	89.3 ± 4.3 Bd	100 ± 0.0 Cb
	FR	48.6 ± 1.6 Ab	65.8 ± 2.2 Ba	94.9 ± 1.4 Ca
	GER	73.0 ± 4.4 Ae	80.6 ± 2.1 Bc	100 ± 0.0 Cb
Insecto	GR	47.5 ± 5.6 Ac	69.2 ± 3.1 Bc	100 ± 0.0 Ca
	IT	45.0 ± 3.4 Abc	60.4 ± 1.4 Bb	100 ± 0.0 Ca
	DK	70.3 ± 2.8 Ae	84.2 ± 2.7 Bd	100 ± 0.0 Ca
	POR	37.5 ± 1.3 Aa	56.4 ± 3.9 Ba	100 ± 0.0 Ca
	UK	74.1 ± 1.9 Af	85.7 ± 1.3 Bd	100 ± 0.0 Ca
	FR	43.2 ± 2.7 Ab	60.5 ± 4.1 Bb	100 ± 0.0 Ca
	GER	59.5 ± 1.4 Ad	83.3 ± 2.9 Bd	100 ± 0.0 Ca
Protect-It	GR	77.5 ± 3.9 Ac	87.2 ± 2.6 Bb	100 ± 0.0 Ca
	IT	72.5 ± 2.5 Ab	81.6 ± 1.4 Ba	100 ± 0.0 Ca
	DK	81.1 ± 4.2 Ad	92.1 ± 1.3 Bc	100 ± 0.0 Ca
	POR	65.0 ± 1.5 Aa	79.5 ± 3.1 Ba	100 ± 0.0 Ca
	UK	85.2 ± 2.3 Ae	96.4 ± 1.9 Bd	100 ± 0.0 Ca
	FR	73.0 ± 3.6 Ab	78.9 ± 2.2 Ba	100 ± 0.0 Ca
	GER	83.8 ± 3.8 Ade	91.7 ± 1.5 Bc	100 ± 0.0 Ca
Mixture	GR	75.0 ± 1.5 Ac	89.7 ± 1.2 Bc	97.4 ± 1.3 Ca
	IT	70.0 ± 3.7 Ab	84.1 ± 1.6 Bb	100 ± 0.0 Ca
	DK	78.4 ± 1.3 Ad	94.7 ± 1.6 Bd	100 ± 0.0 Ca
	POR	62.5 ± 2.5 Aa	76.9 ± 2.6 Ba	100 ± 0.0 Ca
	UK	81.5 ± 1.9 Ae	100 ± 0.0 Be	100 ± 0.0 Ba
	FR	64.9 ± 4.2 Aa	83.9 ± 1.5 Bb	100 ± 0.0 Ca
	GER	73.0 ± 1.6 Ac	86.1 ± 4.4 Bb	100 ± 0.0 Ca

For a given formulation, means in the same column followed by the same lowercase letter are not significantly different, while means in the same row followed by the same uppercase letter are not significantly different; lowercase letters for strains; uppercase letters for temperature; temperature *df* = 2, 36, strain *df* = 6, 71, Tukey-Kramer test at 5%; for abbreviations of strains see Materials and Methods.

phenomenon. For instance, at 500 ppm, Insecto was equally effective among temperatures against 3 of the *T. confusum* strains at the adult stage. Rigaux et al. (2001) examining the susceptibility of different *T. castaneum* strains to Protect-It, found that mortality was directly related to insect mobility, and that the less mobile strains were the least susceptible. It is generally accepted that low mobility decreases the contact with DE particles (Korunic 1998; Subramanyam & Roesli 2000). Apart from mobility, potential variations in the composition of the epicuticular lipids and cuticle thickness may be responsible for these variations. Since the use of DEs is currently limited, probably the strains examined have never been in contact with DEs. Consequently, the development of resistance after previous exposure to DEs is unlikely. Since many DE formulations are now in the process of registration in several parts of the world, these variations should be taken into account. Fields et al. (2003), reporting the results from an international working group with several DEs, found considerable variations among strains originating from different geographical regions. Although the use of standardized strains is probably a solution to this variation, especially among working groups conducting tests with DEs, the use of a local strain is practically more preferable since this is adapted to local conditions.

The effect of life stage on the insecticidal efficacy of DE against a given insect species has been examined in previous studies (Subramanyam & Roesli 2000; Mewis & Ulrichs 2001; Vayias & Athanassiou 2004). Vayias & Athanassiou (2004) found that larvae of *T. confusum* were much more susceptible than adults when exposed to SilicoSec-treated wheat. Our findings support this report not only for Silicosec, but also for the other DEs tested. As above, these differences among adults and larvae of a given species could be

TABLE 3. MEAN MORTALITY (± SE) OF DIFFERENT STRAINS OF *T. CONFUSUM* LARVAE EXPOSED TO WHEAT TREATED WITH 500 PPM OF 3 DE FORMULATIONS AND THEIR MIXTURE AT 3 TEMPERATURE LEVELS.

		Temperature (°C)		
Formulation	Strain	20	25	30
Pyrisec	GR	70.0 ± 2.1 Abc	82.5 ± 3.3 Bb	87.5 ± 2.5 Cb
	IT	2.5 ± 2.5 Ac	80.0 ± 2.1 Bb	85.0 ± 2.6 Cb
	DK	70.4 ± 2.1 Abc	97.3 ± 1.3 Bc	97.5 ± 1.3 Bc
	POR	67.5 ± 1.3 Ab	80.1 ± 2.1 Bb	85.1 ± 1.5 Cb
	UK	77.5 ± 3.9 Ad	97.5 ± 1.3 Bc	100 ± 0.0 Bc
	FR	67.6 ± 2.5 Ab	70.0 ± 2.1 ABa	72.5 ± 2.5 Ba
	GER	62.5 ± 2.5 Aa	72.5 ± 2.5 Ba	87.3 ± 2.4 Cb
Insecto	GR	55.0 ± 1.5 Abc	75.0 ± 1.5 Bc	80.0 ± 3.7 Cc
	IT	52.5 ± 1.3 Ab	77.5 ± 1.3 Bc	85.0 ± 1.5 Cd
	DK	65.1 ± 1.5 Ad	82.5 ± 1.4 Bd	87.5 ± 1.3 Cd
	POR	55.4 ± 2.6 Abc	70.0 ± 2.1 Bb	75.0 ± 1.5 Cb
	UK	75.0 ± 3.4 Ae	85.0 ± 1.5 Bd	87.6 ± 2.6 Bd
	FR	47.5 ± 2.5 Aa	57.5 ± 3.9 Ba	62.5 ± 3.3 Ca
	GER	57.5 ± 4.5 Ac	67.5 ± 1.3 Bb	77.5 ± 1.3 Cbc
Protect-It	GR	70.0 ± 3.7 Ac	82.6 ± 4.5 Bc	90.0 ± 3.7 Cd
	IT	65.0 ± 1.5 Ab	80.0 ± 3.7 Bc	87.5 ± 2.5 Ccd
	DK	77.5 ± 2.5 Ad	90.0 ± 3.6 Bd	95.0 ± 2.6 Ce
	POR	60.0 ± 3.1 Aa	75.0 ± 4.6 Bb	82.5 ± 1.3 Cb
	UK	82.5 ± 1.3 Ae	100 ± 0.0 Be	97.5 ± 1.3 Be
	FR	57.5 ± 3.9 Aa	65.0 ± 4.5 Ba	72.5 ± 2.5 Ca
	GER	67.5 ± 2.5 Abc	82.5 ± 2.5 Bc	85.0 ± 1.5 Bbc
Mixture	GR	75.0 ± 3.4 Abc	87.5 ± 2.5 Bcd	87.3 ± 3.3 Bb
	IT	72.5 ± 4.5 Ab	87.8 ± 2.9 Bcd	87.5 ± 4.5 Bb
	DK	85.0 ± 2.6 Ad	90.0 ± 1.2 Bd	92.5 ± 1.9 Bc
	POR	70.0 ± 4.5 Aab	85.0 ± 1.5 Bbc	87.1 ± 1.6 Bb
	UK	85.6 ± 1.5 Ad	97.5 ± 1.3 Be	97.5 ± 1.5 Bd
	FR	67.5 ± 3.4 Aa	67.5 ± 4.9 Aa	72.5 ± 2.5 Ba
	GER	77.5 ± 2.5 Ac	82.5 ± 2.5 Bb	85.0 ± 4.5 Bb

For a given formulation, means in the same column followed by the same lowercase letter are not significantly different, while means in the same row followed by the same uppercase letter are not significantly different; lowercase letters for strains; uppercase letters for temperature; temperature $df = 2, 36$, strain $df = 6, 71$, Tukey-Kramer test at 5%; for abbreviations of strains see Materials and Methods.

attributed to several factors, such as differences in morphological traits, epicuticular composition, cuticle thickness, and agility. One possible explanation is that adults or larvae of the same developmental stage may have a different thickness of cuticle or even different composition of epicuticular lipids. Interestingly, for many of the combinations tested here, the rank of susceptibility is similar for adults and larvae. This may suggest that behavioral characteristics may be among the dominant reasons for these variations. Further experimental work is needed to examine the basis of this hypothesis.

The effect of temperature on the insecticidal effect of DEs against stored grain insects has been investigated by several researchers (Aldryhim 1990; Arthur 2000b; Dowdy & Fields 2000; Vayias & Athanassiou 2004; Athanassiou et al. 2005b). For the majority of the stored-product beetle species examined increase in temperature increases the DE efficacy (Fields & Korunic 2000; Subramanyam & Roesli 2000; Athanassiou et al. 2005b). Increase of temperature enhances internal water loss through the insects' body surface, and thus, it is positively related to increased desiccation caused by DE particles (Fields & Korunic 2000). Moreover, at high temperatures insects are more mobile, resulting in increased contact with the DE particles. However, in the case of *T. confusum*, the effect of temperature is often contradictory. Aldryhim (1990) found that *T. confusum* adults were more susceptible at 20°C than at 30°C on wheat treated with the DE Dryacide. In contrast, Vayias & Athanassiou (2004) found that both adults and larvae of *T. confusum* strain noted here as GR were more susceptible to Silico-Sec-treated wheat and flour at elevated temperatures. Similar results also were reported by Arthur (2000b) for Protect-It, for both adults of *T. confusum* and *T. castaneum*. Nevertheless, by

TABLE. 4. MEAN MORTALITY (± SE) OF DIFFERENT STRAINS OF *T. CONFUSUM* LARVAE EXPOSED TO WHEAT TREATED WITH 1000 PPM OF 3 DE FORMULATIONS AND THEIR MIXTURE AT 3 TEMPERATURE LEVELS.

		Temperature (°C)		
Formulation	Strain	20	25	30
Pyrisec	GR	87.5 ± 3.3 Ad	100 ± 0.0 Ba	100 ± 0.0 Ba
	IT	85.0 ± 1.5 Acd	100 ± 0.0 Ba	100 ± 0.0 Ba
	DK	92.4 ± 1.3 Ae	100 ± 0.0 Ba	100 ± 0.0 Ba
	POR	80.0 ± 2.3 Ab	100 ± 0.0 Ba	100 ± 0.0 Ba
	UK	95.0 ± 1.5 Ae	100 ± 0.0 Ba	100 ± 0.0 Ba
	FR	75.0 ± 2.6 Aa	100 ± 0.0 Ba	100 ± 0.0 Ba
	GER	82.5 ± 2.5 Abc	100 ± 0.0 Ba	100 ± 0.0 Ba
Insecto	GR	70.0 ± 3.1 Ac	92.5 ± 1.3 Bd	100 ± 0.0 Ca
	IT	72.8 ± 2.5 Ac	95.0 ± 1.5 Bde	100 ± 0.0 Ca
	DK	80.0 ± 0.6 Ad	97.5 ± 1.3 Bef	100 ± 0.0 Ba
	POR	67.5 ± 1.3 Ab	80.0 ± 0.9 Bb	100 ± 0.0 Ca
	UK	85.0 ± 1.6 Ae	100 ± 0.0 Bf	100 ± 0.0 Ba
	FR	62.5 ± 1.3 Aa	72.5 ± 2.5 Ba	100 ± 0.0 Ca
	GER	72.5 ± 1.4 Ac	87.5 ± 1.3 Bc	100 ± 0.0 Ca
Protect-It	GR	97.4 ± 1.4 Ad	100 ± 0.0 Aa	100 ± 0.0 Aa
	IT	97.3 ± 1.3 Ad	100 ± 0.0 Aa	100 ± 0.0 Aa
	DK	97.5 ± 1.4 Ad	100 ± 0.0 Aa	100 ± 0.0 Aa
	POR	85.0 ± 3.4 Ab	100 ± 0.0 Ba	100 ± 0.0 Ba
	UK	100 ± 0.0 Ad	100 ± 0.0 Aa	100 ± 0.0 Aa
	FR	72.5 ± 1.3 Aa	100 ± 0.0 Ba	100 ± 0.0 Ba
	GER	92.5 ± 1.4 Ac	100 ± 0.0 Ba	100 ± 0.0 Ba
Mixture	GR	100 ± 0.0 Ac	100 ± 0.0 Aa	100 ± 0.0 Aa
	IT	97.5 ± 1.3 Ac	100 ± 0.0 Aa	100 ± 0.0 Aa
	DK	100 ± 0.0 Ac	100 ± 0.0 Aa	100 ± 0.0 Aa
	POR	90.0 ± 3.4 Ab	100 ± 0.0 Ba	100 ± 0.0 Ba
	UK	100 ± 0.0 Ac	100 ± 0.0 Aa	100 ± 0.0 Aa
	FR	75.0 ± 3.5 Aa	100 ± 0.0 Ba	100 ± 0.0 Ba
	GER	92.5 ± 1.3 Ab	100 ± 0.0 Ba	100 ± 0.0 Ba

For a given formulation, means in the same column followed by the same lowercase letter are not significantly different, while means in the same row followed by the same uppercase letter are not significantly different; lowercase letters for strains; uppercase letters for temperature; temperature *df* = 2, 36, strain *df* = 6, 71, Tukey-Kramer test at 5%; for abbreviations of strains see Materials and Methods.

testing the same strain, Athanassiou et al. (2005b) found that the influence of temperature is affected by the exposure interval. Hence, in that study, *T. confusum* adults were more susceptible to SilicoSec, at 30°C than at 32°C at exposures ≤48 h, while the reverse occurred at exposures ≥7 d. The age/instar of the adults/larvae tested may be among the crucial factors for these dissimilar results, and this is why previous studies suggest the use of standardized-age individuals of *Tribolium* spp. (De Paula et al. 2002; Vayias & Athanassiou 2004), while for other species, age is not very important (Athanassiou et al. 2006a). In an extensive study with various DEs and stored-product insect species, Fields and Korunic (2000) found negative or positive correlations with temperature. Although the authors did not test *T. confusum*, their data indicate that from, the species tested, *T. castaneum* were slightly less susceptible to DEs at elevated temperatures. Increased temperature is expected to increase feeding and as a result, metabolic water. Furthermore, the synthesis of cuticular waxes may be faster at higher temperature levels through temperature-mediated biochemical pathways (Fields & Korunic 2000). Since, as inert materials, DEs are not affected by temperature, it seems that other, physiological factors are responsible for the variations recorded in the present work. Also, as noted above, the source of DEs seems to have a certain effect on insect mortality, although Korunic (1997, 1998) reported that there are more important factors than the diatom species that compose each DE; these factors are chiefly tapped density, SiO_2 content, oil absorbency, particle size, and secondarily pH (Korunic 1997). Nevertheless, the additives that each DE contains may differentiate the DE efficacy. Hence, the observed difference in relation to temperature for Insecto could be attributed to the presence of food additives, which may attract insects regardless of the temperature level; food attractants may lead to DE consumption, causing internal desiccation. The present findings partially support the aforementioned reports, since, in the combinations tested here, the strains examined had either positive relation or no relation to temperature. In contrast, none of the combinations showed a negative correlation with temperature. Consequently, our findings support the admission that, like most stored-product insect species, *T. confusum* is generally more vulnerable to DEs at high temperatures.

Vayias et al. (2006a) reported the first results for the effect of the combination of natural pyrethrum with DE against *T. confusum*, but there is still inadequate information on the effect of this combination against stored-grain pest species. Generally, the efficacy of most pyrethroids is negatively related to temperature (Snelson 1987; Arthur 1996). In the present tests, PyriSec was more effective at high temperature levels which may suggest that the DE particles moderated the effect of temperature on natural pyrethrum. Vayias et al. (2006a) found that natural pyrethrum was equally effective against *T. confusum* pupae at 25 and 30°C.

The type of the DE formulation is also an important factor affecting insecticidal effect (Korunic 1998; Fields & Korunic, 2000; Subramanyam and Roesli, 2000). Our findings are in compliance with this claim since efficacy of the tested DEs varied for a given *T. confusum* adult or larvae strain. As noted above, this fact could be attributed to the different SiO_2 content of the tested DEs as well as to the different substances that have been added to the DE formulations in order to enhance their efficacy. Due to these variations, Arnaud et al. (2005) proposed as a possible solution the use of a DE mixture, in which more than one DE is present. In the same study, the mixture of the tested DEs was more effective than the application of each DE alone. Generally, a mixture could combine all the positive characteristics of different DEs, such as the use of low insecticidal rates and the presence of food additives. Athanassiou et al. (2007) found that a mixture of Protect-It, PyriSec and Insecto were more effective than a single DE application on both wheat and maize, against adults of *T. confusum*, the rice weevil, *Sitophilus oryzae* (L.) and the lesser grain borer, *Rhyzopertha dominica* (F.).

The increase of dose from 500 to 1000 ppm moderated or eliminated the differences among DEs or strains. This is due to the fact that, at 1000 ppm, the mortality level was 100% or close to 100%, which may have concealed any differences. However, 1000 ppm is a dose rate that is considered too high, and affects negatively to a great degree the physical properties of the grains, particularly bulk density (Korunic et al. 1996; Subramanyam & Roesli 2000; Athanassiou et al. 2006b). According to Fields and Korunic (2000), this is the main problem in presenting efficacy data with DEs: mortality ranges between 0% and 100%, so any increase of dose results in 100% mortality, covering possible interaction with other factors that may affect mortality. As a secondary pest, which cannot infest easily sound grain seeds directly, the damage on kernels by *T. confusum* is more gradual in comparison with other pests, such as the internal feeders *S. oryzae* (rice weevil) and *R. dominica* (lesser grain borer) (Aitken 1975). Hence, it would take several generations for this species to cause considerable grain damage. Although *T. confusum* adults are tolerant to DEs, larvae are highly susceptible. Consequently, the residual applications of DEs may gradually eliminate *T. confusum* populations through the high immature mortality. As inert materials, DEs can persist for a long period in grain without loss in insecticidal efficacy (Athanassiou et al. 2005a; Vayias et al. 2006c).

Our focus was to evaluate the efficacy of DEs under a wide range of conditions, as well as the potential interactions of these conditions. DEs are now in the first steps of acceptance and registration as grain protectants in many parts of the developed world. The use of DEs in stored-product protection meets with several drawbacks as regards some properties of the treated grain such as reduced bulk density, reduced flowability, and dusty appearance (Korunic 1997, 1998; Subrmanyam & Roesli 2000). These drawbacks have led researchers to assess the efficacy of DEs at lower dose rates by evaluating the combined use of DEs with other methods, such as heat, botanicals or entomopathogenic fungi (Dowdy & Fields 2000; Athanassiou et al. 2006b; Kavallieratos et al. 2006; Michalaki et al. 2006; Vassilakos et al. 2006). This new DE-based approach, which needs also additional investigation, is likely to provide the inferences necessary for a more extensive use of DEs as grain protectants in future.

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