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# Insecticidal Effect of Diatomaceous Earth Against Three Species of Stored-Product Psocids on Maize, Rice, and Wheat

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**ABSTRACT** We evaluated the efficacy of three diatomaceous earth (DE) formulations, Dryacide, Protect-It, and Insecto, against three Psocoptera species, *Liposcelis entomophila* (Enderlein) (Liposcelididae), *Lepinotus reticulatus* Enderlein (Trogiiidae), and *Liposcelis decolor* (Pearman), in the laboratory. Bioassays were conducted in three grain commodities, wheat, rice and maize, at 30°C and 75% RH, and the DEs were applied at the recommended dose rates of 1,000, 400, and 500 ppm for Dryacide, Protect-It, and Insecto, respectively. Differences in adult mortality were found among grains and DEs for *L. entomophila* and *L. reticulatus*, but these trends were not consistent for all combinations tested. Type of grain and DE did not affect *L. decolor* mortality significantly. Moreover, mortality increased with increasing exposure time for *L. entomophila* and *L. reticulatus*, but there was no effect of exposure time on *L. decolor*. After 7 d of exposure, mortalities of *L. entomophila*, *L. reticulatus*, and *L. decolor* were 56, 55, and 40%, respectively, and the respective mortality levels after 14 d were 63, 71, and 42%. Progeny production after 30 d was significantly suppressed for all species in the treated grains. However, progeny production was still high in the treated grains and reached 54, 42, and 76 individuals/10 g of grain for *L. entomophila*, *L. reticulatus*, and *L. decolor*, respectively. Progeny production did not vary with commodity. Our results suggest that DEs, when used alone, will not provide effective control of psocids.

**KEY WORDS** *Liposcelis entomophila*, *Lepinotus reticulatus*, *Liposcelis decolor*, grains, inert dusts

Psocoptera, also known as psocids, until recently were considered pests of minor economic importance in stored products partly because of their small size, which makes them difficult to detect in storage facilities, and because little information is available on their eco-biology. However, psocids have become an emerging problem during the last decade in both stored grain and grain processing facilities (Nayak 2006, Throne et al. 2006). The key psocid species associated with grain and related products in several parts of the world are *Liposcelis entomophila* (Enderlein) (Psocoptera: Liposcelididae), *Liposcelis decolor* (Pearman), *Liposcelis bostrychophila* Badonnel, *Liposcelis paeta* Pearman, and *Lepinotus reticulatus* Enderlein (Psocoptera: Trogiiidae) (Sinha 1988; Rees and Walker 1990; Lienhard and Smithers 2002; Nayak et al. 2002a, b; Kucerova and Horak 2004; Nayak 2006; Throne et al. 2006; Nayak and Daglish 2007).

Until recently, the control measures that were used against other stored product insects, such as Coleoptera and Lepidoptera, were considered sufficient for the simultaneous control of psocids, despite the fact that no data were available for the evaluation of specific control measures against psocids. Recent studies clearly indicate that psocids show a remarkable tolerance to some of the currently used insecticides at application rates that are usually effective against other stored product insect species. For example, the insect growth regulator (IGR) methoprene, which is very effective against the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), which is a major beetle pest of stored wheat (Arthur 2004b), was not very effective against *L. bostrychophila*, *L. entomophila*, and *L. paeta* (Nayak et al. 1998). Also, *L. paeta* and *L. entomophila* were found to be resistant to fenitrothion, chlorpyrifos-methyl, pirimiphos-methyl, deltamethrin, bioresmethrin, and carbaryl, whereas a survey of field populations of these species suggested that psocid tolerance to protectant insecticides is a natural phenomenon and not related to resistance (Nayak et al. 1998). Other studies have shown that *Liposcelis* spp. are resistant to permethrin and pirimiphos-methyl applied as structural treatments (Collins et al. 2000, Nayak et al. 2002a). Spinosad, which effectively controls *R. dominica*, is unable to completely control *L. entomophila*, *L.*

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*bostrychophila*, *L. paeta*, and *L. decolor* adults and progeny (Nayak and Daghli 2007). Psocids can develop high levels of resistance to phosphine, and in Australia, this has elevated their pest status to that of the major beetle pests (Nayak et al. 2003). There is currently no information available on the effectiveness of contact insecticides or fumigants against *L. reticulatus*.

One of the most well-studied alternatives to traditional neurotoxic grain protectants is the use of diatomaceous earths (DEs). DEs have been evaluated by several researchers against a wide range of stored product insect pests, such as beetles (Fields and Korunic 2000; Subramanyam and Roesli 2000; Arthur 2000, 2003, 2004a, b; Athanassiou et al. 2003, 2004, 2005; Kavallieratos et al. 2005), moths (Subramanyam et al. 1998, Athanassiou 2006), and mites (Cook and Armitage 1999, Palyvos et al. 2006). DEs act in the cuticle, causing desiccation and death through water loss (Korunic 1998). Therefore, it is generally expected that soft-bodied species or life stages of the same species will be more susceptible to DEs. For example, Vayias and Athanassiou (2004) found that larvae of the confused flour beetle, *Tribolium confusum* Jacquelin Du Val (Coleoptera: Tenebrionidae), were considerably more susceptible than adults after exposure to the DE formulation SilicoSec on wheat or flour. Because psocids are considered soft-bodied insects, DEs could be good candidates for their control. Despite this, there is almost no information on the effect of DEs on psocids. In this study, we evaluated three commercially available DE formulations against *L. entomophila*, *L. decolor*, and *L. reticulatus* on maize, rice, and wheat.

### Materials and Methods

**Commodities.** Wheat (variety Mexa), rice (variety Thaibonnet), and maize (variety Dias) were used in the test. The initial moisture content of the three grains, as determined by a moisture meter (Multigrain CAC II; Dickey-John, Aurora, IL), ranged between 11.9 and 12.6%. All grains were placed in incubators for 21 d to equilibrate to the desired conditions before the beginning of the experiments (see below). After this interval, the mean moisture content of the grains was 13.2%.

**DE Formulations.** Three commercially available DE formulations were used in the tests. These were (1) Insecto (Insecto Natural Products, Costa Mesa, CA), a DE that contains 10% food-grade additives; (2) Protect-It (Hedley Technologies, Mississauga, Ontario, Canada), which contains 90% DE and 10% silica aerogel; and (3) Dryacide (Entosol, Roselands, New South Wales, Australia), which also contains 90% DE and 10% silica gel. The label rates for Insecto, Protect-It, and Dryacide are 500, 400, and 1,000 ppm, respectively; these rates were used in the tests. The above DE formulations are registered in the United States and in other parts of the world as grain protectants and have been proven effective against several stored product insect species.

**Insects.** The cultures of *L. entomophila*, *L. reticulatus*, and *L. decolor* were obtained from the Biological Research Unit, Grain Marketing and Production Research Center, USDA-ARS, Manhattan, KS. The cultures were maintained at 30°C, 75% RH, and continuous darkness on a mixture of 97% cracked wheat, 2% rice krispies, and 1% brewer's yeast. Two-week-old adult females were obtained by putting late-instar nymphs in 0.5-liter glass canning jars containing 250 g of psocid diet. After 3 wk, the adults in the jars were  $\approx$ 2 wk old, and females were removed and used in the bioassays. Psocids used for starting cultures of *L. entomophila* and *L. reticulatus* were obtained in 2004 from steel bins containing wheat at the Grain Marketing and Production Research Center; psocids for cultures of *L. decolor* were obtained in 2006 from the same location in steel bins containing wheat. Voucher specimens of *L. reticulatus*, *L. entomophila*, and *L. decolor* were deposited in the Kansas State University Museum of Entomological and Prairie Arthropod Research under lots 181, 182, and 205, respectively.

**Bioassays.** All tests were conducted at 30°C, 75% RH, and continuous darkness. The desired relative humidity was maintained by using a saturated sodium chloride solution (Greenspan 1977). Lots of 0.5 kg of the three grains were placed in 0.5-liter cylindrical glass jars, treated with the respective DE quantities, and shaken manually for 3 min to achieve equal distribution of the dust to the entire grain mass (Kavallieratos et al. 2005). From each lot, nine 10-g samples were taken and placed in cylindrical glass vials (2.5 cm in diameter, 8 cm high), which were closed, apart from a hole (1 cm in diameter) covered with fine mesh at the top to allow aeration. Ten females of *L. entomophila* were placed in each vial. The same procedure was repeated for the other psocid species. The vials were placed in incubators set at the conditions mentioned above. An additional set of jars and vials were used for each grain, which served as untreated controls. After 7 d, three of the nine vials were opened, and psocid mortality was determined, whereas the mortality was checked 1 wk later in another three vials. Progeny production was measured 30 d after the introduction of the parental adults in the last three vials. Temperature and humidity during the experiment were monitored using HOBO data loggers (HOBO H8; Onset Computers, Bourne, MA). The entire experiment was repeated twice for a total of three replications.

**Data Analysis.** Generally, control mortality ranged between 0 and 20% (0–2 adults/vial, in most cases 0), and treatment mortalities were corrected using the Abbott's formula (1925). The mortality data were analyzed separately for each species in a three-way analysis of variance (ANOVA) for commodity, exposure interval, and DE treatment as main effects by using the JMP IN software v.5.1 (Sall et al. 2001). The same procedure was followed (without exposure) for progeny production data, but progeny production in the control (untreated) grains was included in the analysis. Means were separated by the Tukey-Kramer hon-

**Table 1.** ANOVA results for main effects and interactions for mortality of three psocid species (total df = 53)

Source	df	<i>L.</i>					
		<i>entomophila</i>		<i>reticulatus</i>		<i>decolor</i>	
		F	P	F	P	F	P
DE	2	8.6	<0.01	3.4	0.04	2.5	0.09
Grain	2	3.4	0.04	9.4	<0.01	0.8	0.44
Exposure	1	12.6	<0.01	38.7	<0.01	1.8	0.19
DE × grain	4	2.8	0.04	5.5	<0.01	0.2	0.94
DE × exposure	2	0.7	0.48	1.6	0.21	0.1	0.89
Grain × exposure	2	9.2	<0.01	3.4	0.04	0.6	0.53
DE × grain × exposure	4	5.7	<0.01	5.7	<0.01	0.6	0.64

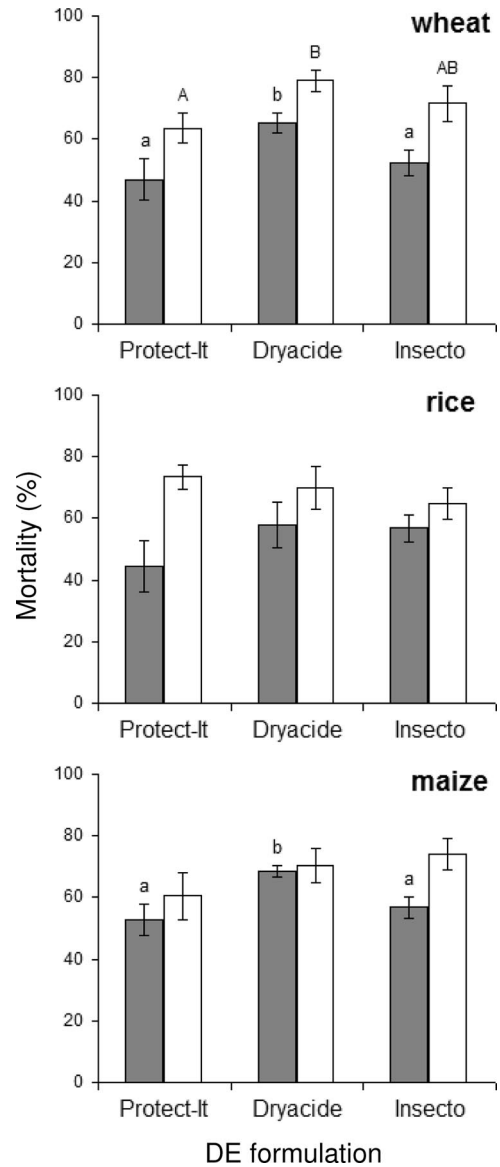
estly significant difference (HSD) test at  $\alpha = 0.05$  (Sokal and Rohlf 1995).

**Results**

***Liposcelis entomophila.*** For mortality counts, all main effects and all interactions were significant at  $P < 0.05$ , with the exception of DE × exposure (Table 1). Generally, mortality increased with increasing exposure (Fig. 1). After 7 d of exposure, mortality on maize and wheat treated with Dryacide was significantly higher than for the other two DEs (Fig. 1). However, ≈35% of the exposed adults were still alive. No significant differences were found among DEs on rice. Mortality of *L. entomophila* reached 79% for Dryacide applied to wheat after 14 d of exposure (Fig. 1). No significant differences among DEs were found on the other two grains, where mortality ranged between 60 and 75%. Only DE treatment was significant for progeny production (Table 2). Significantly fewer progeny were produced in the treated grains than in the controls, but there were no significant differences among the three DEs (Table 3).

***Lepinotus reticulatus.*** As in the case of *L. entomophila*, all main effects and interactions for mortality were significant, with the exception of DE × exposure (Table 1). No significant differences were noted among the three DEs after 7 d of exposure on wheat, and mortality reached 79% (Fig. 2). However, mortality did not exceed 47 and 59% for rice and maize, respectively. Mortality was increased after 14 d, even for rice and maize (Fig. 2). No significant differences were noted among DEs on wheat and rice, and mortality ranged between 81 and 89%. Significantly more adults were dead in maize treated with Dryacide than with Protect-It; mortality after a 14-d exposure to Dryacide applied to maize reached 93%. As in the case of *L. entomophila*, only treatment was significant for progeny production (Table 2). Significantly fewer progeny were produced in the treated than in the untreated commodities, but there were no significant differences among the three DEs (Table 3).

***Liposcelis decolor.*** For mortality, neither main effects nor interactions were significant (Table 1). Generally, this species was less susceptible to DE than the other two species (Fig. 3). Mortality did not exceed 44 or 47% after 7 or 14 d, respectively, on any of the commodities tested. As for the other two species, only



**Fig. 1.** Mean (%) mortality ( $\pm$ SE) of *L. entomophila* adults on three grains treated with three DE formulations after 7 (gray columns) and 14 d (white columns) of exposure (within each grain and exposure, means followed by the same letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at  $\alpha = 0.05$ ).

treatment was significant for progeny production (Table 2). Significantly fewer progeny were produced in the treated than in the untreated commodities, but there were no significant differences among the three DEs (Table 3).

**Discussion**

DEs when used alone will not provide effective control of psocids, and differences exist in the insecticidal efficacy of the different DEs. For example, grain

**Table 2.** ANOVA results for main effects and interaction for progeny production of three psocid species (total df = 35)

Source	df	<i>L. entomophila</i>		<i>L. reticulatus</i>		<i>L. decolor</i>	
		F	P	F	P	F	P
		Treatment (DEs or control)	3	18.3	<0.01	36.8	<0.01
Grain	2	1.5	0.23	0.6	0.52	0.9	0.39
Treatment × grain	6	0.7	0.61	1.0	0.42	1.2	0.34

type and DE used and exposure time did not affect *L. decolor* mortality significantly, but this was not the case for *L. entomophila* and *L. reticulatus*. Differences in insecticidal efficacy of different DEs has been shown for other stored product pests (Korunic 1997, 1998; Fields and Korunic 2000; Subramanyam and Roesli 2000). Using adults of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), which are considered extremely tolerant to DEs (Stathers et al. 2002), Athanassiou et al. (2007) found that an abamectin-enhanced DE was more effective than Protect-It or PyriSec (a pyrethrum-enhanced DE). Korunic (1997) noted that some of the most crucial factors that determine the efficacy of a given DE are silica content, particle size, pH, and diatom shape.

Generally, DEs are considered to be less effective on maize than on wheat or rice against stored grain beetle pests (Subramanyam and Roesli 2000; Athanassiou et al. 2003, 2005). Athanassiou et al. (2003) showed that the DE SilicoSec was less effective on maize than on rice and barley against adults of the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae). Reduced retention of DE particles in the maize kernels in comparison with other grain commodities may be one of the basic reasons for these dissimilar results among grains (Kavallieratos et al. 2005).

Our study has shown that DE suppressed progeny production (19–39% in comparison with the progeny in the controls) for all species over a 30-d exposure interval. Despite this, progeny production in the treated grain remained rather high even when parental mortality was high. *L. reticulatus* females were very susceptible to DEs, with an average mean mortality for all grains and DEs of 85% after 14 d, yet progeny

production was still rather high at 28–42 progeny per vial. In fact, progeny production for this species in the treated commodities was comparable to that of *L. entomophila*, despite the greater parental mortality for *L. entomophila* compared with *L. reticulatus*. In addition, it should be noted that we observed progeny for all species during the 14-d mortality counts.

Opit and Throne (2008) found that larger numbers of both *L. entomophila* and *L. reticulatus* are produced on rice and wheat than on maize. In this study, a similar pattern was observed in the control vials, but commodity had no effect in the treated grains, indicating that progeny suppression was primarily caused by the effect of DE and not caused by a complementary effect between DE and the type of grain. Practically, DEs may not be effective as long-term protectants against psocids, even in the most susceptible species, because one of the key characteristics of a successful protectant is progeny suppression.

In the only report thus far on the effect of DEs against psocids, Yang et al. (2003) reported 100% mortality of *L. entomophila* after exposure to surfaces treated with Protect-It at 65% RH or lower, whereas mortality was reduced with increase of RH above this level. However, ours is the first study in which efficacy of DEs as an admixture with grain is tested against psocids. These results indicate that the three species tested vary in their susceptibility to DEs, with *L. decolor* being the most DE-tolerant species. It should be noted that the doses tested here cause 100% mortality to other stored grain insect species. Athanassiou et al. (2005) reported that 500 ppm of the DE SilicoSec caused 100% mortality and complete suppression of progeny production on wheat against *S. oryzae*. Nevertheless, we found that some individuals of even the most susceptible psocid species could survive 14 d of exposure to DE.

DEs are generally very effective for another group of soft-bodied stored-product pest species, mites. Palyvos et al. (2006) found that 500 ppm of the DE SilicoSec on wheat was highly effective against *Tyrophagus putrescentiae* (Schrank) (Astigmata: Acaridae), whereas mortality was low for its predator *Cheyletus malaccensis* (Oudemans) (Prostigmata: Cheyletidae). Cook and Armitage (1999) found that 1,000 ppm of Dryacide provided 100% inhibition of

**Table 3.** Mean progeny production (no. of individuals/vial ± SE) for three psocid species on three commodities (means for a species within each column followed by the same letter are not significantly different; HSD test at  $\alpha = 0.05$ )

Species	Treatment	Wheat	Rice	Maize
<i>L. entomophila</i>	Control	153.7 ± 36.3a	201.1 ± 28.4a	117.2 ± 20.2a
	Protect-It	37.3 ± 4.6b	54.4 ± 12.8b	40.0 ± 5.2b
	Dryacide	43.5 ± 6.9b	42.6 ± 7.4b	32.3 ± 4.1b
	Insecto	49.1 ± 9.0b	38.8 ± 7.7b	29.4 ± 4.4b
<i>L. reticulatus</i>	Control	128.0 ± 21.2a	172.4 ± 39.0a	92.9 ± 17.7a
	Protect-It	34.9 ± 4.1b	28.0 ± 6.3b	27.7 ± 3.3b
	Dryacide	30.1 ± 3.9b	37.4 ± 6.8b	36.3 ± 4.5b
	Insecto	41.9 ± 8.8b	34.6 ± 5.6b	32.4 ± 4.0b
<i>L. decolor</i>	Control	194.9 ± 41.5a	217.5 ± 34.7a	181.0 ± 28.5a
	Protect-It	60.3 ± 12.3b	79.3 ± 18.4b	53.4 ± 9.5b
	Dryacide	76.2 ± 17.3b	49.2 ± 13.2b	59.4 ± 12.4b
	Insecto	61.3 ± 10.2b	64.3 ± 16.7b	42.0 ± 8.7b

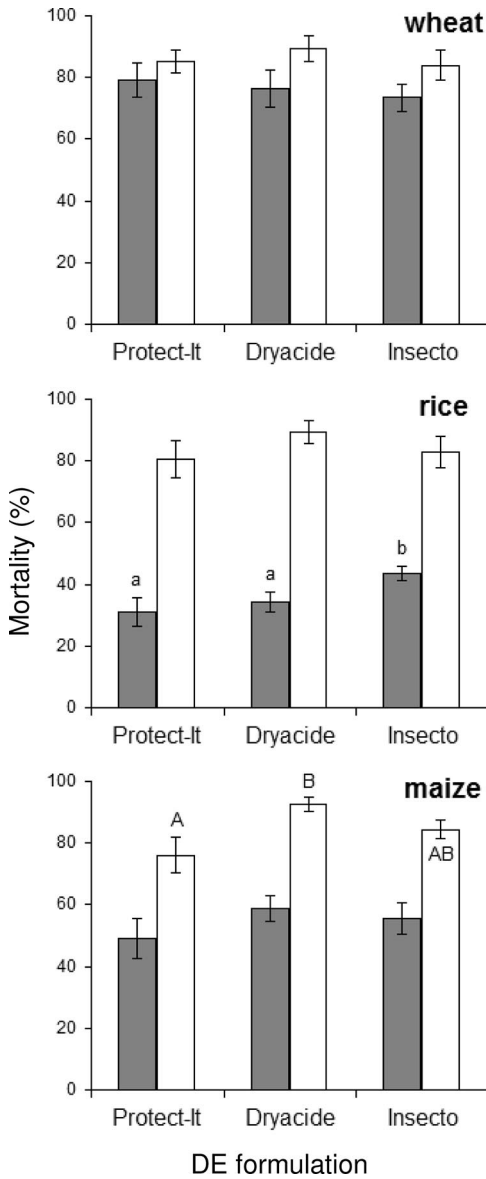


Fig. 2. Mean (%) mortality ( $\pm$ SE) of *L. reticulatus* adults on three grains treated with three DE formulations after 7 (gray columns) and 14 d (white columns) of exposure (within each grain and exposure, means followed by the same letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at  $\alpha = 0.05$ ).

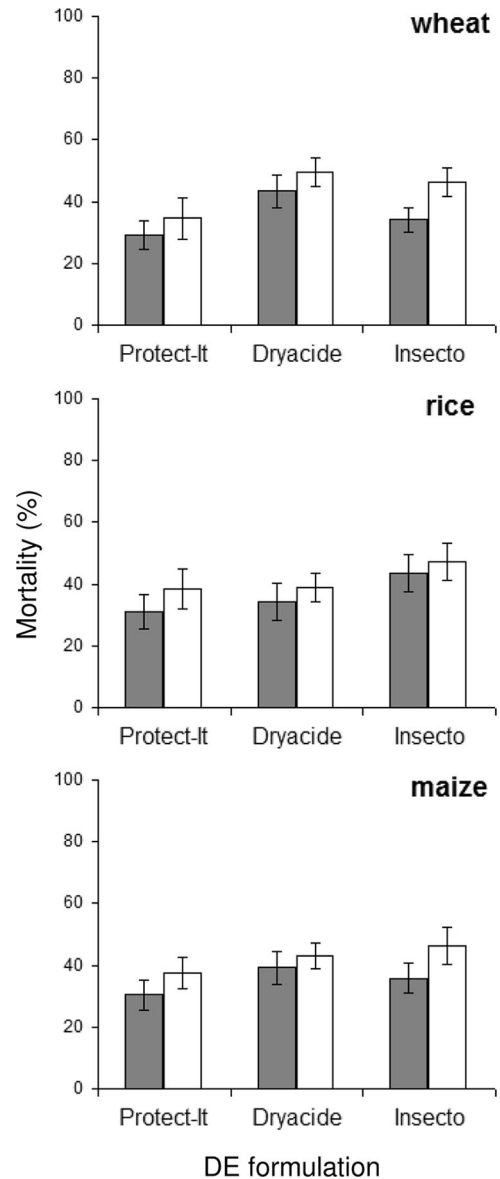


Fig. 3. Mean (%) mortality ( $\pm$ SE) of *L. decolor* adults on three grains treated with three DE formulations after 7 (gray columns) and 14 d (white columns) of exposure.

population growth of *Acarus siro* L. (Astigmata: Acaridae), but they reported that higher doses were needed for *Lepidoglyphus destructor* (Schrank) (Astigmata: Glycyphagidae). Hence, cuticle thickness may not be the major parameter that affects susceptibility to desiccants, such as DEs.

Research needs to be conducted to determine the reasons for the low susceptibility of psocids to DEs. However, the nature of the cuticle in psocids may be one possible reason. Lord and Howard (2004) found

that *Liposcelis bostrychophila* was remarkably tolerant to three species of entomopathogenic fungi at doses that were lethal to other stored product insects, such as beetles (Kavallieratos et al. 2006, Athanassiou and Steenberg 2007). The authors suggest that the unusual cuticular lipid composition of this species may play a role in antifungal defense. Moreover, Howard and Lord (2003) noted that this composition may protect psocids from surface abrasion and that low molecular weight compounds may help the insects in sloughing off dust.

Another possible reason for low susceptibility to DEs in psocids is the fact that some insect species can

moderate water loss. For example, using a water-loss moderation mechanism located in the rectal portion of the hindgut, the larvae of the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), remain unaffected after exposure to DE-treated substrates (Machin 1975, Mewis and Ulrichs 2001). For other species, this mechanism is located in the mouth, such as in the cockroach *Arenivaga investigata* Friauf and Edney (Blattaria: Polyphagidae) (O'Donnell 1977). In the case of psocids, Rudolph (1982b), using *Psyllipsocus ramburi* Selys-Longchamps (Psocoptera: Psyllipsocidae) as a model species, reported that Psocoptera also have an active water vapor uptake mechanism, which is located in the mouthparts and that the hypopharynx is exposed to ambient atmosphere during absorption causing water vapor condensation. Also, this uptake seems to characterize a large number of species of this order, and it is a continuous uptake process directly related to relative humidity levels (Rudolph 1982a). For species of the genus *Liposcelis*, Knulle and Spadafora (1969) reported that water absorption from the air occurs at relative humidity levels <58%.

The order of tolerance to the DEs tested was found to be *L. decolor* > *L. entomophila* > *L. reticulatus*. Nayak et al. (2002b) also noted that *L. decolor* was more tolerant to azamethiphos plus carbaryl compared with *L. entomophila*, *L. bostrychophila*, and *L. paeta*, whereas the same species was also tolerant to chlorpyrifos-methyl. It is also important to point out that even though we used DEs at the labeled application rates during this study, doses between 400 and 1,000 ppm negatively affect some of the physical properties of grains, particularly bulk density (Korunic et al. 1998), which seems to be the main drawback for a more extensive use of DEs (Korunic 1998, Subramanyam and Roesli 2000).

Although this study showed that DEs when used alone will not provide effective control of psocids, it is possible that DE combined with other insecticides may be much more effective. DEs might be best used in conjunction with other protectants, such as organophosphorus insecticides, which are effective against several psocid species (Nayak et al. 1998, 2002b). Thus far, enhanced DE formulations have proven effective for the control of several stored product beetle species (Arthur 2004a, Athanassiou et al. 2007). Additional experimentation is needed to evaluate the parameters that affect the efficacy of the simultaneous use of neurotoxic insecticides and inert dusts against stored product psocids.

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