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RESEARCH ARTICLE

Insecticidal effect of diatomaceous earth and pirimiphos-methyl against phosphine-susceptible and phosphine-resistant populations of two stored product beetle species

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Abstract

In the present study, we evaluated the insecticidal efficacy of diatomaceous earth (DE) and pirimiphos-methyl for the control of phosphine-susceptible and phosphine-resistant populations of *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L.). Insecticides were applied on wheat or rice at two doses: DE was applied at 1000 and 2000 ppm and pirimiphos-methyl at 1 and 5 ppm. Adult mortality was measured after 7, 14, and 21 days of exposure, and progeny production capacity on the treated substrates was evaluated 65 days later. For *T. castaneum*, we found that DE, at 2000 ppm, was able to provide 100% control of two of the three populations tested, while for the third population mortality reached only 84%. Similarly, there were differences in mortality levels after exposure to DE-treated grains between the two *S. oryzae* populations tested. At 1 ppm, pirimiphos-methyl was not effective for any of the *T. castaneum* populations tested, but complete mortality was recorded for all populations at 5 ppm. In general, populations of *S. oryzae* were more susceptible than those of *T. castaneum*, for both commodities. Our data indicate that both insecticides can be used with success in phosphine resistance management programs, but there are populations of a given species that may be less susceptible, which constitutes a preliminary screening essential.

Keywords Phosphine resistance · Diatomaceous earth · Pirimiphos-methyl · Grain protectants · Stored-product beetles

Introduction

Phosphine is the major fumigant of disinfestations of stored product insects worldwide, and is currently considered as a viable and effective control measure in a wide range of facilities and durable commodities (Cato et al. 2017; Afful et al. 2018; Nayak et al. 2020). Nevertheless, the extensive use of phosphine has contributed to the development of resistance in several stored product insect species, such as the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) (Collins et al. 2005; Afful et al. 2018), the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Daglish et al. 2014), the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) (Toon et al. 2018), and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Chen et al. 2015). Phosphine resistance has been documented worldwide in different parts of the world, while the catalogue of resistant populations is dramatically increased (Saglam et al. 2015; Gautam et al. 2016; Cato et al. 2017; Agrafioti et al. 2019; Nayak et al. 2020). The increased frequency of this phenomenon necessitates the adoption of management strategies that have a dual role: to successfully control phosphine-resistant populations and to mitigate the occurrence of resistance.

One of the main measures that should be taken towards this direction is the use of insecticides that have a different mode of action than that of phosphine (Nayak et al. 2020). For example, Collins (2009) suggest the efficacy of different contact insecticides (e.g. diatomaceous earth) for the control of stored-product insect populations that were resistant to

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phosphine, with good results, which could lead to guidelines for specific active-ingredient (AI)-dose combinations per species and population. Bajracharya et al. (2013) reported that the bacterial-based insecticide spinosad and a combination of chlorpyrifos-methyl with deltamethrin could be used successfully for phosphine-resistant populations of *R. dominica* and *S. oryzae*. Moreover, Nayak et al. (2005) tested spinosad and found that was effective against phosphine-resistant populations at 1 mg/kg of grain for *R. dominica* but not for *S. oryzae*, *T. castaneum* and the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae).

One promising method for the control of stored product insects, which is considered as a viable alternative to traditional insecticides, is the use of diatomaceous earth (DE) (Korunic 1998; Subramanyam and Roesli 2000). Korunic et al. (1996) reported that DEs could be applied in the same way with residual chemicals, can be easily removed from the grain and does not affect the final product, such as flour or pasta. The effect of DEs against stored product insects has been broadly evaluated by different research groups (Fields and Korunic 2000; Arthur 2000; Vayias and Athanassiou 2004; Athanassiou et al. 2004, 2005). DEs have a purely mechanical mode of action, since they cause desiccation and eventually death through water loss, and can serve as a good alternative over the use of neurotoxic insecticides and fumigants. In the case of phosphine, Conceição et al. (2012) tested DE for the control of phosphine-resistant and phosphine-susceptible populations of *T. castaneum* and *R. dominica* and found no significant variation in mortality for both species, suggesting the absence of cross-resistance.

One of the most used grain protectants is the organophosphorus (OP) compound pirimiphos-methyl (Daglish et al. 2018), which has been thoroughly evaluated for the control of many stored-product insect species (Huang and Subramanyam 2005; Rumbos et al. 2013, 2016; Kavallieratos et al. 2017, 2019). For instance, Rumbos et al. (2013) tested pirimiphos pirimiphos-methyl against *S. oryzae* and found that this AI could provide complete (100%) control at 1 ppm, which was far lower than the label dose. Similarly, Athanassiou et al. (2016) reported 100% mortality of *S. oryzae* even at 0.5 ppm. Despite the fact that there are numerous studies that have examined the factor that affect the efficacy of pirimiphos-methyl, there are disproportionally few data on the efficacy of this AI for the control of populations that are resistant to phosphine. In a recent study, Agrafioti and Athanassiou (2018) tested pirimiphos-methyl at 0.1, 1, and 10 ppm against phosphine-susceptible and phosphine-resistant populations of *O. surinamensis*, and have found noticeable variations among populations. In that study, the authors suggested that a differential susceptibility may not be directly linked with resistance to phosphine, but, in general, this hypothesis merits additional investigation, under the possibility of cross resistance (Agrafioti and Athanassiou 2018; Nayak et al. 2020).

From a practical point of view, the evaluation of grain protectants as alternatives to phosphine needs to be expanded to a wider range of cases, as different populations may exhibit different levels of resistance to phosphine. In this context, it is essential to also screen populations that are "weakly resistant" or "strongly resistant" to phosphine, under comparable protocols, on the basis of adaptation and fitness cost parameters (Nayak et al. 2020). Such an effort should include AIs with dissimilar modes of action, to draw the inferences necessary for a judicious utilization of old and newer AIs on a rotation basis, towards a phosphine management strategy. In this regard, the aim of the present work was to evaluate DE and pirimiphos-methyl, for the control of different populations of *T. castaneum* and *S. oryzae*, which had different susceptibility levels to phosphine. Moreover, there are limited related studies which evaluate DE and pirimiphos-methyl in two different commodities and different doses against resistant and susceptible phosphine populations and especially *S. oryzae*. For this purpose, we have carried out a series of laboratory bioassays on two types of commodities, wheat and rice.

Material and methods

Insects

We used three populations for *T. castaneum*, one susceptible and two resistant to phosphine, and two populations for *S. oryzae*, one susceptible and one resistant to phosphine. For each species, the standard susceptible population, namely *T. castaneum* QTC4 and *S. oryzae* LB, have been maintained for several years at laboratory conditions, and their susceptibility to phosphine has been confirmed in previous works (Daglish et al. 2015; Athanassiou et al. 2019). The other three populations, named as *T. castaneum* BTS, *T. castaneum* QTC931 and *S. oryzae* G1, were field populations collected from Serbia, Australia and Italy, respectively, and reared at the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agriculture, Crop Protection and Rural Environment, University of Thessaly, since 2017 for QTC931, G1 and 2016 for BTS.

Detection of phosphine resistance

The Food and Agriculture Organization (FAO) protocol, as described by the FAO Plant Protection Bulletin (FAO 1975), and modified by Agrafioti et al. (2019), was used for the evaluation of the presence of phosphine resistance. In brief, twenty adults of each of the tested species and populations were placed in a 1.5-L glass jar and exposed to phosphine concentration of 30 ppm for 20 hours, as suggested by Cato et al. (2017) and Agrafioti et al. (2019). After the termination of the exposure interval, active adults, i.e. capable of coordinated movement, and immobilized adults, i.e. not capable of coordinated movement, were recorded, as suggested by Athanassiou et al. (2019). The whole procedure was repeated three times, which were considered as replicates, with three sub-replicates each, with new phosphine production on each replicate, as indicated by Agrafioti et al. (2019).

Contact insecticides and commodities

The DE formulation was SilicoSec, a DE that contains 92% SiO_2 (Biofa GmbH, Münsingen, Germany), which was used at two doses, 1000 and 2000 ppm, corresponding to the protective and curative label doses of the formulation. The pirimiphos-methyl formulation was Actellic 50 EC (Syngenta Crop Protection AG, Switzerland), which contains 500 g of active ingredient per liter, and used at 1 and 5 ppm, while the label dose of this formulation is at 4 ppm (Rumbos et al. 2013).

Untreated, clean and uninfested soft wheat and paddy rice kernels were used in the tests. Before the experiments, the wheat and rice were kept in cold storage (−20 °C) for at least 2 weeks to eliminate possible insect infestation. The moisture content of the grains before the experiments was determined by a moisture meter (Multitest, Gode SAS, Le Catelet, France) and found to be 13.5%.

Bioassays

Lots of 500 g of wheat or rice were placed in glass jars of 1000 ml in capacity and treated with the DE at the doses indicated above. An additional series of lots without DE was used as a control. The jars were then shaken for 5 min to ensure that the formulation was equally distributed throughout the grain mass. For the tests, we used plastic cylindrical vials (3 cm in diameter, 8 cm high, Rotilabo Sample tins Snap on lid, Carl Roth, Germany), with the “neck” covered with Fluon (polytetrafluoroethylene; Northern Products, Woonsocket, USA) to avoid insects’ escape. Each vial contained 20 g of treated or untreated wheat or rice, and then 20 adults were placed into each vial, using different vials for each insect species and dose. There were three vials for each dose—insect species—combination, while the same procedure was repeated three times, by preparing new lots of treated and untreated grains (3 × 3 = 9 vials for each combination). All vials were maintained in incubators set at 25 °C, 55% relative humidity (r.h.) and continuous darkness. The mortality of the exposed beetles was recorded after 7, 14 and 21 days. At the end of the 21st day, all adults (dead and alive) were removed from the vials and the vials remained in the same conditions for an additional period of 65 days in order to record progeny production. After this interval, the vials were opened and the individuals found were measured.

For pirimiphos-methyl, lots of 500 g of wheat or rice were sprayed with pirimiphos-methyl at two dose rates, 1 and 5 ppm, using different lots per dose. An additional series of lots was sprayed with distilled water and served as a control. All doses were applied on the grains by using a Kyoto BD-183K airbrush (Grapho-tech, Japan), using a total volume of 1 ml spraying solution per kilogram of grain. The sprayed grains were left for 1 day at ambient conditions before handling. Then, the treated grains were placed in glass jars and shaken manually to achieve equal distribution of the insecticides in the entire grain mass. The conditions, replicates, etc., as well as the mortality and progeny production measurements were performed as in the case of DE.

Data analysis

Control mortality was generally low for both insect species and commodities, and did not exceed 10%. The same vials were examined for mortality at the different exposure intervals (7, 14, and 21 days), so mortality data were analyzed by using a repeated-measure MANOVA with population, dose, and commodity as the main effects, and exposure as the repeated measure by using the JPM 8 software (SAS Institute Inc., Cary, NC, USA). For progeny production, the data were analyzed by using a one-way analysis of variance (ANOVA), with dose, population, and commodity as the main effects and number of progeny as the response variable. Means were separated by using the Tukey-Kramer honestly significant difference (HSD) test, at 0.05.

Results

Detection of phosphine resistance

Regarding immobilization after exposure to phosphine, all adults of both susceptible populations (*T. castaneum* QTC4 and *S. oryzae* LB) were found to be immobilized after the 20-h exposure at 30 ppm. In contrast, for the other populations, all individuals were still active after the termination of the exposure interval.

Efficacy of DE

For *T. castaneum*, mortality was affected by population and dose, but not by commodity (Table 1). At 1000 ppm on wheat, differences in mortality levels were noted among populations for all exposure intervals (Table 2). After 7 days, there was no mortality for QTC4, while mortality for BTS reached 49%. At the 21-day interval, mortality was 100% only for BTS. At 2000 ppm on wheat, at the 21-day exposure, mortality for

Table 1 Repeated-measures ANOVA parameters for main effects and associated interactions for mortality levels of adults of different populations of *T. castaneum* (error *df*= 96) and *S. oryzae* (error *df*= 64) to DE and pirimiphos-methyl

	T. castaneum					*S. oryzae*				
Source		DE		Pirimiphos-methyl			DE		Pirimiphos-methyl	
Source between variables	*df*	*F*	*P*	*F*	*P*	*df*	*F*	*P*	*F*	*P*
All between	11	115.8	<0.001	130.8	<0.001	7	24.3	<0.001	15.2	<0.001
Intercept	1	4523.6	<0.001	3930.8	<0.001	1	15884.8	<0.001	4268.9	<0.001
Population	2	378.3	<0.001	3.1	0.048	1	7.4	0.008	17.2	<0.001
Commodity	1	7.5	0.007	0.1	0.713	1	21.2	<0.001	14.6	<0.001
Dose	1	272.2	<0.001	1361.5	<0.001	1	131.3	<0.001	29.8	<0.001
Population × commodity	2	5.1	0.008	16.0	<0.001	1	1.1	0.301	6.3	0.015
Population × dose	2	112.6	<0.001	3.2	0.043	1	0.5	0.483	17.8	<0.001
Commodity × dose	1	0.2	0.682	0.1	0.690	1	8.9	0.004	14.1	<0.001
Population × commodity × dose	2	1.3	0.288	16.2	<0.001	1	0.1	0.815	6.3	0.015
Source within interactions										
All within interaction	22	12.9[a]	<0.001	10.9[a]	<0.001	14	10.1a	<0.001	5.3[a]	<0.001
Exposure	2	349.0	<0.001	65.2	<0.001	2	192.2	<0.001	16.4	<0.001
Exposure × population	4	40.3[a]	<0.001	6.3[a]	<0.001	2	6.4	0.003	6.9	0.004
Exposure × commodity	2	0.2	0.828	5.0	0.009	2	9.1	<0.001	5.9	0.004
Exposure × dose	2	6.5	0.002	64.7	<0.001	2	64.3	<0.001	15.1	<0.001
Time × population × commodity	4	1.8[a]	0.123	9.2[a]	<0.001	2	1.2	0.291	1.6	0.210
Exposure × population × dose	4	17.2[a]	<0.001	6.3[a]	<0.001	4	4.1	0.020	7.9	0.001
Exposure × commodity × dose	2	10.4	<0.001	5.0	0.008	2	3.0	0.055	4.9	0.010
Exposure × population × commodity × dose	4	2.7	0.034	9.1[a]	<0.001	4	0.7	0.484	2.4	0.100

[a] Wilks' lamda approximate *F* value

Table 2 Mean mortality (% ± SE) of adults of three populations of *T. castaneum* exposed for 7, 14, and 21 d to DE, applied on wheat and rice at two doses (1000 and 2000 ppm) (*df*=2, 26)

Dose	Commodity	Insect population	Day 7	Day 14	Day 21
1000 ppm	Wheat	QTC4	0.0 ± 0.0B	6.7 ± 2.8C	30.0 ± 5.6C
		BTS	49.4 ± 7.6A	94.4 ± 1.7A	100.0 ± 0.0A
		QTC931	3.3 ± 1.4B	23.3 ± 3.8B	62.8 ± 5.1B
		F	38.6	257.6	63.0
		P	<0.001	<0.001	<0.001
	Rice	QTC4	1.7 ± 0.8B	16.7 ± 5.8B	65.6 ± 3.9B
		BTS	60.6 ± 8.2A	97.2 ± 1.7A	100.0 ± 0.0A
		QTC931	1.1 ± 0.7B	11.1 ± 2.5B	65.0 ± 5.4B
		F	51.3	164.8	26.9
		P	<0.001	<0.001	<0.001
2000 ppm	Wheat	QTC4	57.8 ± 7.5B	87.8 ± 3.4A	100.0 ± 0.0A
		BTS	82.8 ± 3.1A	94.4 ± 2.8A	100.0 ± 0.0A
		QTC931	0.6 ± 0.6C	25.6 ± 4.4B	83.9 ± 3.1B
		F	80.2	111.3	27.1
		P	<0.001	<0.001	<0.001
	Rice	QTC4	67.2 ± 10.2B	98.3 ± 1.2A	100.0 ± 0.0A
		BTS	95.6 ± 2.6A	99.4 ± 0.6A	100.0 ± 0.0A
		QTC931	1.7 ± 1.2C	31.7 ± 10.0B	75.0 ± 10.4B
		F	62.0	44.4	5.7
		P	<0.001	<0.001	0.009

Within each column, dose, and commodity, means followed by the same uppercase letter are not significantly different according to the Tukey-Kramer HSD test at 0.05; where no letters exist, no significant differences were noted

Table 3 ANOVA parameters for progeny production counts for *T. castaneum* on wheat and rice treated with DE and pirimiphos-methyl (total *df* =144)

Source		DE		pirimiphos-methyl	
	df	*F*	*P*	*F*	*P*
Whole model	17	8.0	<0.001	34.3	<0.001
Intercept	1	34.0	<0.001	360.3	<0.001
Population	2	2.0	0.141	3.1	0.049
Commodity	1	12.1	0.001	15.8	<0.001
Dose	2	16.2	<0.001	233.2	<0.001
Population × commodity	4	5.0	0.008	5.5	0.005
Population × dose	2	5.2	0.001	7.6	<0.001
Commodity × dose	2	22.6	<0.001	21.5	<0.001
Population × commodity × dose	4	2.9	0.024	2.5	0.046

QTC4 and BTS was 100%, but only 84% for QTC931 (Table 2). On rice treated with 1000 ppm, mortality was generally lower than that on wheat, and after 21 days was 100% only for BTS. In contrast, at 2000 ppm, mortality levels at this interval were similar with those of wheat.

Regarding progeny production of *T. castaneum*, only commodity and dose had a significant effect (Table 3). Progeny production was negligible for all doses in both commodities. Moreover, no significant differences were noted among populations (Table 4).

For *S. oryzae*, mortality was significantly affected by dose and commodity, but not by population (Table 1). On wheat treated with 1000 ppm, mortality for both populations was 100% after 21 days of exposure (Table 5). At 2000 ppm, mortality was high even from the 7-day exposure interval, and exceeded 99% 7 days later. Similarly, for rice, at 1000 ppm, adult mortality after 21 days of exposure was >97%, and reached 100% at 2000 ppm (Table 5).

Regarding progeny production of *S. oryzae*, all main effects were significant (Table 6). For both commodities, progeny production was much higher on wheat than on rice (Table 7). On wheat and rice treated with 1000 ppm, LB showed low levels of progeny production than G1 (Table 7). At 2000 ppm, progeny production was comparable with that at 1000 ppm, but there were no significant differences between populations.

Efficacy of pirimiphos-methyl

For *T. castaneum*, mortality was only affected by dose (Table 1). At 1 ppm on wheat, after 7 days no significant differences were recorded among populations. After 21 days of exposure, mortality was 30, 28, and 53% for QTC4, BTS, and QTC931, respectively (Table 8). At 5 ppm on wheat, adult mortality was complete (100%) after 7 days of exposure for all populations.

On rice treated with 1 ppm on rice, after 7 days no significant differences were noted among populations, but at the 21-day exposure interval adult mortality varied remarkably (Table 8). However, at 5 ppm after 7-day mortality was 99–100% for all populations.

Regarding progeny production, commodity and dose had a significant effect (Table 3). However, progeny production was negligible for all treatments, while in the controls both populations gave similar numbers.

For *S. oryzae* mortality, all main effects were significant (Table 1). At the 7-day exposure interval, mortality was 100%, but only for LB (Table 9). At the 21-day exposure, G1 mortality reached 92.8%. Moreover, on wheat treated with 5 ppm, all adults were dead at the 7-day exposure, for both populations.

At 1 ppm of pirimiphos-methyl on rice, there were significant differences in mortality levels between the two *S. oryzae* populations, where LB was more susceptible than G1 (Table 9). The increase of dose to 5 ppm resulted in mortality levels that reached 99–100% at the 21-day exposure interval.

Regarding progeny production of *S. oryzae*, commodity and dose had a significant effect, but not population (Table 6). In general, in the untreated wheat, G1 gave higher progeny production levels than LB, but progeny on untreated rice was much lower for both populations (Table 7). Moreover, on wheat treated with 1 ppm, progeny production of G1 was significantly higher than that of LB, but no significant differences were noted at 5 ppm. Moreover, no significant differences were recorded between populations on rice, for either dose.

Discussion

For both species, and especially for *T. castaneum*, we identified considerable variations in susceptibility among the populations tested, for the two insecticides examined here. Our overall data for DE show that sufficient control can be achieved at 2000 ppm for *S. oryzae* in terms of parental individuals, but progeny production in the treated substrate could not be avoided, and in some cases can be high. Conversely, despite the fact that there were remarkable variations in mortality levels for *T. castaneum* in the DE-treated grains, progeny production was low. This is probably due to the fact that this species is a secondary colonizer that cannot develop easily in sound grain kernels (Rees 1995; Athanassiou et al. 2005). Athanassiou et al. (2005) noted that DEs are affected by temperature, and an increase of temperature increases the effectiveness of DEs. The differential susceptibility of populations of *T. castaneum* to DEs has been also reported by Rigaux et al. (2001), who tested fourteen *T. castaneum* populations and

Table 4 Progeny counts (number of adults per vial ± SE) of *T. castaneum* on untreated wheat and rice, or wheat or rice treated with DE at two doses (1000 and 2000 ppm), or pirimiphos-methyl at two doses (1 ppm and 5 ppm), 65 days after the removal of the parental adults (*df*=2, 26)

Dose	Commodity	Insect population	DE	Pirimiphos-methyl
Control	Wheat	QTC4	7.4 ± 2.0A	21.5 ± 1.9
		BTS	7.2 ± 1.9A	19.0 ± 2.8
		QTC931	0.8 ± 0.3B	16.8 ± 1.3
		F	5.3	1.3
		P	0.012	0.287
	Rice	QTC4	0.3 ± 0.3	16.8 ± 0.7A
		BTS	0.1 ± 0.3	5.1 ± 1.6B
		QTC931	0.0 ± 0.0	12.0 ± 2.0A
		F	0.7	14.7
		P	0.506	<0.001
1000 ppm/1 ppm	Wheat	QTC4	0.0 ± 0.0	2.0 ± 1.0
		BTS	0.1 ± 0.1	3.1 ± 1.2
		QTC931	0.1 ± 0.1	0.3 ± 0.2
		F	0.5	2.4
		P	0.613	0.113
	Rice	QTC4	0.0 ± 0.0	0.2 ± 0.2
		BTS	0.0 ± 0.0	0.8 ± 0.4
		QTC931	0.0 ± 0.0	0.3 ± 0.2
		F	-	1.515
		P	-	0.240
2000 ppm/5 ppm	Wheat	QTC4	0.0 ± 0.0	0.2 ± 0.1
		BTS	0.3 ± 0.2	0.2 ± 0.1
		QTC931	0.3 ± 0.2	0.2 ± 0.1
		F	1.3	-
		P	0.282	1.000
	Rice	QTC4	0.7 ± 0.5	0.0 ± 0.0B
		BTS	0.2 ± 0.1	0.4 ± 0.2A
		QTC931	0.4 ± 0.3	0.0 ± 0.0B
		F	0.7	6.4
		P	0.523	0.006

Within each column, dose, and commodity, means followed by the same uppercase letter are not significantly different according to Tukey-Kramer HSD test at 0.05, where no letters exist, no significant differences were noted

found 7-day mortality levels that ranged between 5 and 100%. Similarly, for the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), Vayias et al. (2009) found different susceptibility levels of various populations from Europe, when exposed to commercial DE formulations. Apparently, the differential susceptibility among the populations tested to DE is probably attributed to behavioural and biological factors, such as DE particle uptake and water loss stress, and not to resistance to phosphine. Therefore, the different mortality rates and the different susceptibility to phosphine do not necessary mean that all the other attributes are the same. However, Conceição et al. (2012) found that phosphine resistant and susceptible populations of *T. castaneum* and *R. dominica*, have similar susceptibility levels on DE. The fact, however, that some populations were more susceptible than others should be further investigated on the basis of the evaluation of alternative AIs for resistance management.

As in the case of DE, the increase of the concentration of pirimiphos-methyl from 1 to 5 ppm increased efficacy against all populations tested, but progeny production was not totally suppressed. However, the application of 1 ppm of pirimiphos-methyl revealed, for both commodities, wide differences among the populations tested. In general, both species can be considered as susceptible to this AI (Rumbos et al. 2013, 2016). Resistance of different stored-product insects, including *T. castaneum* and *S. oryzae* to different OPs, has been extensively studied (Subramanyam and Hagstrum 1995; Arthur 1996; Guedes et al. 1996; Daglish 2008). Daglish (2008) found that binary combinations of the OP

Table 5 Mean mortality (% ± SE) of adults of two populations of *S. oryzae* exposed for 7, 14, and 21 days to DE, applied on wheat and rice at two doses (1000 and 2000 ppm) (*df*=1, 17)

Dose	Commodities	Insect population	Day 7	Day 14	Day 21
1000 ppm	Wheat	LB	63.3 ± 3.2	98.9 ± 0.7	100.0 ± 0.0
		G1	47.8 ± 8.2	95.0 ± 2.2	100.0 ± 0.0
		F	2.9	2.8	-
		P	0.105	0.114	-
	Rice	LB	31.7 ± 5.3	96.1 ± 1.6	97.8 ± 1.7
		G1	27.8 ± 4.0	90.0 ± 2.8	98.9 ± 0.7
		F	0.3	3.6	0.4
		P	0.565	0.075	0.555
2000 ppm	Wheat	LB	93.9 ± 2.5	99.4 ± 0.6	100.0 ± 0.0
		G1	82.8 ± 5.6	98.9 ± 1.1	100.0 ± 0.0
		F	3.2	0.2	-
		P	0.091	0.661	-
	Rice	LB	83.9 ± 3.6	99.4 ± 0.6	100.0 ± 0.0
		G1	78.9 ± 5.7	100.0 ± 0.0	100.0 ± 0.0
		F	0.5	1.0	-
		P	0.469	0.332	-

Within each column, dose, and commodity, means followed by the same uppercase letter are not significantly different according to Tukey-Kramer HSD test at 0.05; where no letters exist, no significant differences were noted

chlorpyrifos-methyl with other AIs could be used successfully to control OP-resistant populations of different stored-product beetle species. Regarding populations with different susceptibility levels to phosphine, Agrafioti and Athanassiou (2018) used pirimiphos-methyl for the control of phosphine-susceptible and resistant populations of *S. oryzae* and found complete mortality of both species at 0.1 ppm, suggesting the absence of cross resistance. In contrast, in our study, we found that there are considerable differences in efficacy of this AI between the two *S. oryzae* populations tested. Our data, in conjunction with those reported by Agrafioti and Athanassiou (2018), clearly suggests that a grain protectant selection for use in phosphine resistance management should be regarded as a population-specific, rather than a more generic AI-specific strategy. In this context, even if different populations of the same species respond uniformly in a given AI, there are cases where there may be tolerant populations, which should be controlled with an alternative insecticide or method. Pilot laboratory bioassays can be helpful towards this direction, before the wider adoption of a given AI in a certain area with increased phosphine-resistance frequencies. Apart from contact insecticides, other methods that have been tested with success for the control of phosphine-resistant populations are heat treatment (Agrafioti et al. 2019), ozone (Xinyi et al. 2017) and the fumigant sulfuryl fluoride (Nayak et al. 2016).

Table 6 ANOVA parameters for progeny production counts for *S. oryzae* on wheat and rice treated with DE and pirimiphos-methyl (total *df* =96)

Source		DE		Pirimiphos-methyl	
	df	*F*	*P*	*F*	*P*
Whole model	11	19.3	<0.001	36.8	<0.001
Intercept	1	119.9	<0.001	143.0	<0.001
Population	1	21.7	<0.001	10.0	0.002
Commodity	1	78.0	<0.001	97.3	<0.001
Dose	2	19.4	<0.001	76.8	<0.001
Population × commodity	1	15.4	<0.001	10.1	0.002
Population × dose	2	3.8	0.025	2.3	0.102
Commodity × dose	2	22.0	<0.001	60.1	<0.001
Population × commodity × dose	2	3.1	0.049	4.7	0.012

Table 7 Progeny counts (number of adults per vial ± SE) of *S. oryzae* on untreated wheat and rice, or wheat and rice treated with DE at two doses (1000 and 2000 ppm), or pirimiphos-methyl at two doses (1 ppm and 5 ppm), 65 days after the removal of the parental adults (*df*=1, 17)

Dose	Commodity	Insect population	DE	Pirimiphos-methyl
Control	Wheat	LB	194.2 ± 41.7A	59.1 ±11.1A
		G1	308.1 ± 36.1B	136.1 ± 24.1B
		F	4.7	8.4
		P	0.045	0.010
	Rice	LB	24.8 ± 2.6A	3.2 ± 1.4
		G1	13.3 ± 3.5B	4.3 ± 1.1
		F	6.9	0.4
		P	0.018	0.541
1000 ppm/1 ppm	Wheat	LB	8.5 ± 3.3A	15.7 ± 5.0A
		G1	51.8 ± 8.2B	80.8 ± 15.7B
		F	24.0	15.6
		P	<0.001	0.001
	Rice	LB	3.0 ± 0.9	4.3 ± 2.3
		G1	15.8 ± 7.7	6.3 ± 1.5
		F	2.7	0.5
		P	0.120	0.483
2000 ppm/5 ppm	Wheat	LB	18.8 ± 7.4	4.8 ± 2.8
		G1	31.4 ± 7.2	1.3 ± 1.0
		F	1.5	1.4
		P	0.237	0.260
	Rice	LB	4.7 ± 1.2	0.0 ± 0.0
		G1	1.8 ± 1.0	9.2 ± 4.8
		F	3.4	3.7
		P	0.084	0.071

Within each column, dose, and commodity, means followed by the same uppercase letter are not significantly different according to Tukey-Kramer HSD test at 0.05; where no letters exist, no significant differences were noted

Phosphine resistance is also strongly related with fitness cost. Sousa et al. (2009) showed reduced population growth and developmental rates of phosphine-resistant populations of *T. castaneum*, *R. dominica* and *O. surinamensis*, as compared with susceptible ones, suggesting that reduced progeny production can potentially compromise phosphine dispersal. However, for some populations, the authors found that there were no differences in progeny production of resistant and susceptible populations, underlying the importance of population-related adaptations. Similar results have been also reported by Bajracharya et al. (2013) for phosphine-resistant and phosphine-susceptible populations of *T. castaneum* and *R. dominica*. In the present study, we noticed that the field population of *S. oryzae* (G1) was able to produce more offspring than the laboratory population (LB). We are unaware of the degree that these differences in fecundity are correlated with a fitness benefit in the resistant population, if any.

The type of the commodity played a major role on the efficacy of different grain protectants, including DEs and pirimiphos-methyl. For *S. oryzae*, Athanassiou et al. (2003) found that the efficacy of SilicoSec varied among different grains, and that this DE was more effective on barley than on maize. Athanassiou and Kavallieratos (2005) noted that the reduced efficacy of DE in some grains is partially due to reduced retention of the DE particles in the external part of the kernel, which reduces insect contact with DEs. Similarly, Rumbos et al. (2016) found that pirimiphos-methyl was more effective on wheat than on rice for the control of *S. oryzae*. This increased efficacy on wheat as compared with rice for the control of major stored-product beetles has been also reported in the case of other insecticides as well, with different modes of action (Fang et al. 2002; Athanassiou et al. 2008; Kavallieratos et al. 2010; Vassilakos and Athanassiou 2012). On the other hand, despite the fact that there was a decreased mortality of the populations tested here on rice, progeny production was low, suggesting that wheat may be more suitable for insect reproduction than rice, and can compensate, eventually, any losses from increased parental mortality. Apart from the differences in progeny production between the two commodities in the treated substrates, we have noticed that the

Table 8 Mean mortality (% ± SE) of adults of three populations of *T. castaneum* exposed for 7, 14, and 21 days to pirimiphos-methyl, applied on wheat and rice at two doses (1 ppm and 5 ppm) (*df*=2, 26)

Dose	Commodity	Insect population	Day 7	Day 14	Day 21
1 ppm	Wheat	QTC4	9.4 ± 2.7	21.7 ± 4.8AB	30.5 ± 4.6
		BTS	9.4 ± 3.4	19.4 ± 4.7B	28.3 ± 5.0
		QTC931	6.1 ± 3.9	47.8 ± 11.5A	53.3 ± 11.1
		F	0.3	4.2	3.4
		P	0.723	0.027	0.051
	Rice	QTC4	8.3 ± 3.0	26.7 ± 4.2B	39.4 ± 9.6B
		BTS	16.1 ± 4.5	47.2 ± 4.0A	77.8 ± 4.6A
		QTC931	6.1 ± 3.9	7.8 ± 4.2B	10.5 ± 4.2C
		F	1.9	13.5	25.8
		P	0.175	<0.001	<0.001
5 ppm	Wheat	QTC4	100 ± 0.0	100 ± 0.0	100 ± 0.0
		BTS	100 ± 0.0	100 ± 0.0	100 ± 0.0
		QTC931	100 ± 0.0	100 ± 0.0	100 ± 0.0
		F	-	-	-
		P	-	-	-
	Rice	QTC4	100 ± 0.0	100 ± 0.0	100 ± 0.0
		BTS	99.4 ± 0.5	100 ± 0.0	100 ± 0.0
		QTC931	100 ± 0.0	100 ± 0.0	100 ± 0.0
		F	1.0	-	-
		P	0.383	-	-

Within each column, dose, and commodity, means followed by the same uppercase letter are not significantly different according to Tukey-Kramer HSD test at 0.05; where no letters exist, no significant differences were noted

two sets of control commodities, i.e. the ones that were used as controls for each insecticide, gave different progeny numbers. We assume that this is probably due to the fact that these two control sets were treated in a different way, i.e. they were sprayed with water only in the case or pirimiphos-methyl, which might have differentiated their microenvironment.

Our study shows that both label rates of DE and pirimiphos-methyl were more effective on wheat than on rice

Table 9 Mean mortality (% ± SE) of adults of two populations of *S. oryzae* exposed for 7, 14, and 21 days to pirimiphos-methyl, applied on wheat and rice at two doses (1 ppm and 5 ppm) (*df*=1, 17)

Dose	Commodity	Insect population	Day 7	Day 14	Day 21
1 ppm	Wheat	LB	100.0 ± 0.0A	100.0 ± 0.0A	100.0 ± 0.0A
		G1	86.7 ± 3.4B	91.7 ± 2.9B	92.8 ± 3.0B
		F	15.0	8.3	5.7
		P	0.001	0.011	0.029
	Rice	LB	90.0 ± 6.6A	93.3 ± 4.4A	94.4 ± 3.8A
		G1	46.1 ± 9.4B	55.5 ± 10.3B	63.3 ± 9.7B
		F	14.5	11.3	8.8
		P	0.001	0.004	0.009
5 ppm	Wheat	LB	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
		G1	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
		F	-	-	-
		P	-	-	-
	Rice	LB	99.4 ± 0.5	99.4 ± 0.5	100.0 ± 0.0
		G1	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
		F	1.0	1.0	-
		P	0.332	0.332	-

Within each column, dose, and commodity, means followed by the same uppercase letter are not significantly different according to Tukey-Kramer HSD test at 0.05; where no letters exist, no significant differences were noted at least in the populations that were tested here. The results of the present work underline the need to follow individualized scenarios, based on specific populations that are to be controlled with grain protectants, when a phosphine resistance mitigation strategy is implemented. Therefore, grain protectants that are able to control phosphine-resistant populations in one given area may not control other populations in another area and thus, generalizations should be avoided.

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Data Availability The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate Not applicable.

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