Environ Sci Pollut Res (2018) 25:10407–10417
DOI 10.1007/s11356-017-9565-5

PLANT-BORNE COMPOUNDS AND NANOPARTICLES: CHALLENGES FOR MEDICINE, PARASITOLOGY AND ENTOMOLOGY

Insecticidal effect and impact of fitness of three diatomaceous earths on different maize hybrids for the eco-friendly control of the invasive stored-product pest *Prostephanus truncatus* (Horn)

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Received: 27 March 2017 / Accepted: 14 June 2017 / Published online: 15 July 2017

Abstract Diatomaceous earths (DEs) are able to successfully protect grain commodities from noxious stored-product insect and mite infestations; however, their effectiveness may be moderated by the grain hybrid or variety they are applied to. There is a gap of information on the comparison of the efficacy of different DEs when are applied on different maize hybrids against *Prostephanus truncatus* (Horn). Therefore, here we tested three commercially available DEs (DEA-P at 75 and 150 ppm, Protect-It at 500 ppm, and PyriSec at 500 ppm) on five different maize hybrids (Calaria, Doxa, Rio Grande, Sisco, and Studio) for the control of *P. truncatus* adults in terms of mortality (at 7 and 14 days), progeny production, properties of the infested maize hybrids (number and weight of kernels with or without holes, number of holes per kernel) and the adherence level of the tested DEs to the kernels. DEA-P was very effective at 75 ppm while a considerable proportion of the exposed *P. truncatus* adults was still alive after 14 days of exposure on all maize hybrids treated with 500 ppm of Protect-It or PyriSec, even though it was 3.3 times higher than the maximal application tested dose of DEA-P. Apart from parental mortality, DEA-P was able to reduce *P. truncatus* progeny production in all hybrids contrary to Protect-It or PyriSec. The adherence ratios were always higher for DEA-P than Protect-It or PyriSec to all maize hybrids. The highest numbers of kernels (or weight of kernels) without holes were noticed after their treatment with DEA-P. Doxa and Sisco performed better than Calaria, Rio Grande, or Studio based on the differences found concerning the numbers of kernels without holes at treatments with DEA-P and Protect-It. Overall, the findings of our study indicate the high potentiality of DEA-P as protectant of different maize hybrids to *P. truncatus* infestations at low doses, a fact that could help the eco-friendly management of this noxious species in the stored-product environment.

Keywords Biosafety · Grain protectants · Progeny production · Stored maize · Hybrids

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Introduction

The larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) is a serious insect pest of stored maize and cassava tubers in Africa (Hill 2003, Muatinte et al. 2014) and falls under regional quarantine concerns (Tyler and Hodges 2002, Myers and Hagstrum 2012). After its accidental introduction in Africa from Mesoamerica four decades ago, this species is now spread in a wide zone, which includes numerous countries in Africa (i.e., Benin, Burkina Faso, Burundi, Ghana, Guinea, Kenya, Malawi, Niger, Nigeria, Rwanda, South Africa, Tanzania, Togo, Zambia), Central

America (i.e., Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama), North America (i.e., Mexico, USA), South America (Colombia), and Asia (China, India) (Dunstan and Magazini 1981; EPPO, European and Mediterranean Plant Protection Organization 2017). The adults are long-lived and can be devastating for several commodities, as *P. truncatus* can rapidly develop extremely high populations, within only a few weeks (Nansen and Meikle 2002; Hill et al. 2002). Moreover, this species is also present in high numbers outside of the storage ecosystems, such as in forests, feeding from wood as in the case of most members of the family Bostrychidae (Borgemeister et al. 1998; Hill et al. 2002; Muatinte et al. 2014), while it is particularly abundant in maize right before harvest (Hill et al. 2002). Its presence cannot be easily detected during the harvest period, which means that maize is already infested during its introduction on the storage facility (Borgemeister et al. 1994; Hill et al. 2002).

The control of *P. truncatus* is negatively affected by the fact that this species is tolerant to several organophosphorus compounds (OPs), at dose rates that are usually very effective against other major stored-product species (Golob 2002; Rumbos et al. 2013). Interestingly, this tolerance might be linked with the ease of *P. truncatus* to develop resistance to insecticides (Golob 2002). In a recent study, Rumbos et al. (2013) reported that the OP pirimiphos-methyl was unable to control *P. truncatus* and another species of major importance for grains at their post-harvest stages, the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). For this purpose, the common practice to control this species in Africa is the simultaneous use of more than one active ingredients, of which usually one is pyrethroid (Golob 2002). Apparently, the continuous use of neurotoxic compounds meets with several major drawbacks that are related to environment and public health (Arthur 2012).

Diatomaceous earths (DEs) are efficacious alternatives to traditional grain protectants that have been evaluated with success in the case of most major stored-grain pests (Cook and Armitage 1999; Korunic 1998; Arthur 2001; Arnaud et al. 2005; Chanbang et al. 2007; Doumbia et al. 2014; Frederick and Subramanyam 2016). They are composed by the fossils of phytoplanktons (diatoms) and are known to have low mammalian toxicity (Round et al. 1990; Korunic 1998). At the same time, DEs are able to provide long-term protection against noxious stored-product insects (Vayias et al. 2006). However, considerable differences have been found among different commercially available DEs regarding their efficacy for the control of *P. truncatus*. In an earlier study, Stathers et al. (2004) found that Dryacide and Protect-It provided low (<0.25%) and average (<70%) mortalities respectively to *P. truncatus* adults while both DEs could not suppress offspring emergence on maize treated with 1000 g/ton at 27 °C and 50% relative humidity (RH) while values of mortality and progeny production were significantly lower and higher respectively at 60% RH. Similarly, Athanassiou et al. (2007) reported that some DEs were not effective at either lower and higher temperatures or lower and higher RH values. In contrast, the new DE, DEA-P (mixture of two natural substances, i.e., abamectin, freshwater DE) was used as a powder in the tests and was very effective against adults of *P. truncatus*, while this efficacy was not affected by temperature and RH at considerably lower doses as compared to other DEs. Furthermore, Kavallieratos et al. (2015) found that DEA-P was very effective for the control of other major stored-product beetle species, i.e., the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and *R. dominica*.

Previous studies have clearly documented that the type of commodity highly affects the efficacy of a given DE. For instance, Athanassiou and Kavallieratos (2005) reported that the DE PyriSec was not equally effective against *R. dominica*, when tested on eight different grains. Apart from different grain species, Kavallieratos et al. (2010) found that DEs provide different efficacy levels when applied at different wheat varieties, and this trend is manifested in more than one DE. In that study, the DEs Insecto, SilicoSec, and Protector differed in their efficacy against *R. dominica*, *S. oryzae*, and the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), regardless of the tested dose and the exposure interval. This characteristic is likely to be expressed more vigorously in the case of maize, due to the reduced efficacy of DEs on this commodity (Vayias and Stephou 2009). Thus, despite the fact that a given DE may provide a satisfactory level of efficacy, its effectiveness may be seriously moderated by the specific properties of the maize hybrid on which the DE is applied. In this context, there is still inadequate information on the comparison of maize hybrids in conjunction with DEs against *P. truncatus*. Therefore, the objective of the present study was to test three commercially available DEs on five different maize hybrids, for the eco-friendly control of *P. truncatus* adults. In addition to the efficacy levels, we also examined the progeny production of *P. truncatus* in both treated and untreated hybrids, the effect of the presence of *P. truncatus* to some properties of the infested maize hybrids and the adherence level of the tested DEs to the kernels.

Materials and methods

Insects and commodities

Adults of *P. trunctatus*, <1 week old, were used in the tests from a colony that was started in 2003 at the Laboratory of Agricultural Entomology, Benaki Phytopathological Institute and later was kept at the Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens since 2014. The insects were reared on whole maize at 30 °C, 65% RH at continuous darkness. The following untreated, clean, and free of infestations hybrids of maize *Zea mays* L. were used in the tests: the late Calaria (FAO 620), having 122–127 days biological cycle (i.e., days till physiological maturity), the late Doxa (FAO 750), having 138–140 days biological cycle, the late Rio Grande (FAO 700), having 130–135 days biological cycle, the semi-early Sisco (FAO 400), having 102 days biological cycle, and the late Studio (FAO 700), having 130–135 days biological cycle. All hybrids were provided by the Institute of Plant Breeding and Phytogenetic Resources, Hellenic Agricultural Organization "Demeter" (Thermi, Greece). Prior to the initiation of the experimentation, the maize hybrids were kept at 30 °C and 65% RH for 2 weeks to equilibrate their moisture content (Athanassiou et al. 2007).

DEs

The following three DEs were used in the experiments: (a) DEA-P, (b) Protect-It, and (c) PyriSec. DEA-P (Research and Consulting Inc., Toronto, ON, Canada) is a mixture of abamectin (MSD Agvet Division Merck and Co., Rahway, NJ, USA) and freshwater DE that contains 92.4% SiO_2 (Kavallieratos et al. 2015). Protect-It (Hedley Technologies Inc., Mississauga, ON, Canada) is a DE that contains 83.7% SiO_2 with 10% silica aerogel (Korunic and Fields 1995). PyriSec (Agrinova Gmbh, Obrigheim/Mühleim, Germany) contains natural pyrethrum, piperonyl butoxide, and SilicoSec, which is a DE of freshwater origin containing 92% SiO_2 (Kavallieratos et al. 2007).

Bioassays

DEA-P was applied at 75 and 150 ppm while Protect-It and PyriSec at 500 ppm. For this purpose, lots of 1-kg grains each were prepared for each maize hybrid, DE, and dose. The lots were separately placed into 5 l glass jars. The DEs corresponding to each dose were placed into the jars. Following this, each jar was shaken manually for 5 min to achieve equal distribution of the DE particles on maize kernels. An additional series of untreated maize hybrid lots were used as controls. The three maize hybrid samples, of 50 g each, were taken from each treated or untreated lot and separately put into three glass vials, of 100 ml capacity each, with a different scoop that was inside each jar. All quantities of maize hybrids were weighed with a Precisa XB3200D compact balance (Alpha Analytical Instruments, Gerakas, Greece). Then, 50 *P. truncatus* adults were introduced into each vial. To prevent insects to escape, the internal "necks" of the vials were covered by polytetrafluoroethylene (60 wt% dispersion in water) (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). The closure of each vial had a 2-cm diameter hole in the center that was covered by gauze to allow sufficient aeration inside the vial. All vials were placed into incubators set at 30 °C and 65% RH during the entire experimental period. The mortality of adult individuals was determined after 7 and 14 days of exposure. At each mortality check date, the content of each vial was carefully moved onto a different plastic dish (145 mm diameter) where it was spread as a layer with a brush. The internal "necks" of the dishes were covered by polytetrafluoroethylene as in the case of vials. Then, mortality was evaluated under an Olympus stereo microscope (SZX9, Bacacos S.A., Athens, Greece) by prodding each individual with a brush to detect any movement. After the examination of each dish, its content was carefully relocated back to the corresponding vial. Different brushes were used for each DE treatment and untreated controls. Dead individuals were discarded. After the 14-day mortality counts, all parental adults were discarded and vials returned into the incubators at the same conditions for 46 days. Then, the vials were opened and progeny production was estimated as described above. After insect counting, the number of kernels with or without holes and the number of holes per kernel made by *P. truncatus* were counted. Also, the kernels with or without holes were weighed using the Precisa XB3200D compact balance. The experiments were repeated three times, by preparing new lots, jars, and vials each time.

Adherence of DE to kernels

For the estimation of the adherence level of the tested DEs to the kernels, 500 g of each clean maize hybrid kernels were weighed. Next, the maize kernels of each hybrid were mixed with 0.5 g DEA-P or Protect-It or PyriSec in a well-closed glass vial and manually shaken for 1 min. Following this, each treated maize hybrid was sieved using a No. 10 sieve (2 mm openings, Retsch GmbH and Co., KG, Germany), with a closed cap and base, for 1 min, and the dust was collected and weighed. This amount was expressed as the percentage of DE adherence to each maize hybrid. The whole procedure was repeated six times for each DE and maize hybrid combination (Korunić 1997).

Data analysis

Control mortality was low (<5%), thus no correction was done for the mortality counts. The data were analyzed according to the repeated measures model (Sall et al. 2001). The repeated factor was the exposure interval, while mortality was the response variable. Maize hybrid and DE were the main effects. Progeny production counts were subjected to a two-way ANOVA, with maize hybrid, and DE as main effects. Progeny production in the untreated control vials was included in the analyses. Data for DEs adherence were subjected to a two-way ANOVA, with adherence (%) as response variable and maize hybrid and DE as main effects. For holes in the kernels and weight of kernels, two-way ANOVA was used, with number of kernels without holes, amount (g) of kernels without holes, number of kernels with holes, amount (g) of kernels with holes, and number of holes per kernel as response variables, and maize hybrid, and DE as main effects. In all cases, the associated interactions of main effects were incorporated in the analyses. All analyses were conducted by using the JMP 11 software (SAS Institute Inc. 2013). Means were separated by the Tukey-Kramer (HSD) test at 0.05 probability (Sokal and Rohlf 1995).

Table 1 MANOVA parameters for main effects and associated interactions leading to mortality of *P. truncatus* adults between and within exposure intervals (error df = 100)

Between exposure intervals Source	df	*F*	*P*
Intercept	1	1462.2	<0.01
Maize hybrid	4	1.4	0.23
DE	3	75.4	<0.01
Maize hybrid x DE	12	0.6	0.81
Within exposure intervals			
Source	df	*F*	*P*
Exposure	1	162.5	<0.01
Exposure x maize hybrid	4	1.7	0.16
Exposure x DE	3	44.1	<0.01
Exposure x maize hybrid x DE	12	1.9	0.04

Results

Mortality and progeny of *P. truncatus* adults

Between exposure intervals, the main effect DE was significant (Table 1). Within exposure intervals, the main effect exposure x DE and the associated interaction exposure x maize hybrid x DE were significant. After 7 days of exposure, mortalities of *P. truncatus* adults were very high (≥98%) on all maize hybrids treated with DEA-P at any dose and significantly higher than those caused by Protect-It or PyriSec (Table 2). Indicatively, the maximum mortalities caused by Protect-It and PyriSec were 55.1 and 45.1% on Sisco and Studio, respectively. After 14 days of exposure, all *P. truncatus* adults died on Calaria, Doxa, Sisco, and Studio treated with 150 ppm DEA-P while the mortality did not exceed 61.6 and 56.9% in the cases of Sisco and Studio that were treated with Protect-It and PyriSec, respectively. No significant differences among *P. truncatus* adult mortality were observed on maize hybrids treated with any DE and exposure interval.

Concerning progeny production, the main effect DE was significant ($F_{4,\ 224} = 20.8$, $P < 0.01$) while the main effect maize hybrid and the associated interaction maize hybrid x DE were not significant ($F_{4,\ 224} = 1.1$, $P = 0.34$ and $F_{16,\ 224} = 0.1$, $P = 0.97$, respectively). No or very low overall progeny production was noted on maize hybrids treated with DEA-P at any dose while the progeny was elevated in the cases of Protect-It (range, 61.0–104.6 adults per vial) or PyriSec (range, 32.6–111.9 adults per vial) (Table 3). No significant differences in offspring production were observed among maize hybrids treated with any DE.

Holes in kernels and weight of kernels by the activity of *P. truncatus*

For number of kernels without holes, the main effects were significant (Table 4). Significantly more kernels without holes were noted when all maize hybrids were treated with DEA-P at both doses than with Protect-It and PyriSec or remained untreated (Table 5). Treated Doxa and Sisco with either 75 or 150 ppm DEA-P showed significantly more kernels without holes than Calaria, Rio Grande, and Studio.

For weight of kernels without holes, the main effect DE was significant (Table 4). The kernels of all maize hybrids that were treated with both doses of DEA-P and found without holes were significantly heavier than the corresponding ones treated with Protect-It and PyriSec or the control kernels (Table 6). The overall weight of the former case of kernels ranged between 44.7 and 49.0 g while in the latter weight ranged between 23.0 and 37.5 g.

Concerning the number of kernels bearing holes, the main effect DE was significant (Table 4). Less than one kernel per vial with holes was found in vials that contained any maize hybrid treated with DEA-P (Table 7). No kernels with holes were found when Rio Grande was treated with DEA-P at both tested doses. In contrast, in the cases of all untreated hybrid maize kernels or treated with Protect-It or PyriSec, the numbers of kernels bearing holes per vial ranged from 28.1 to 53.0 and were always significantly higher than the corresponding numbers dealing with DEA-P.

For weight of kernels with holes, the main effect DE was significant (Table 4). The overall weight values of kernels with holes per vial did not exceed 0.2 g for all maize hybrids that had been treated with DEA-P at both doses and always were significantly lower than the values measured for maize hybrids remained untreated or treated with Protect-It or PyriSec (Table 8). No significant differences within maize hybrids were noted with the exception of DEA-P at 150 ppm.

For numbers of holes per kernel, all main effects and the associated interaction were significant (Table 4). Maize hybrid kernels that had been treated with DEA-P at both doses suffered by significantly less holes per kernel than kernels that remained untreated or treated with Ptotect-It and PyriSec

Table 2 Mean mortality (% ± SE) of *P. truncatus* adults exposed for 7 and 14 days on five maize hybrids treated with three DEs

	7 days					
DE	DEA-P		Protect-It	PyriSec		
Dose (ppm)	75	150	500	500		
Maize hybrid					*F*	*P*
Calaria	99.6 ± 0.3 A	100.0 ± 0.0 A	32.4 ± 12.8 B	30.4 ± 12.0 B	20.2	<0.01
Doxa	98.0 ± 1.5 A	99.8 ± 0.2 A	19.6 ± 7.3 B	22.9 ± 9.4 B	56.1	<0.01
Rio Grande	99.3 ± 0.5 A	99.8 ± 0.2 A	49.1 ± 13.3 B	42.4 ± 13.3 B	11.0	<0.01
Sisco	98.0 ± 1.4 A	99.3 ± 0.3 A	55.1 ± 13.3 B	37.3 ± 8.7 B	15.3	<0.01
Studio	98.7 ± 0.9 A	99.8 ± 0.2 A	35.1 ± 9.9 B	45.1 ± 12.8 B	18.0	<0.01
F	0.5	1.1	1.5	0.6		
P	0.75	0.35	0.23	0.64		
	14 days					
Calaria	99.8 ± 0.2 A	100.0 ± 0.0 A	44.0 ± 13.8 B	40.7 ± 14.6 B	11.0	<0.01
Doxa	100.0 ± 0.0 A	100.0 ± 0.0 A	32.7 ± 10.8 B	40.9 ± 8.5 B	28.7	<0.01
Rio Grande	100.0 ± 0.0 A	99.8 ± 0.2 A	58.4 ± 13.8 B	53.1 ± 13.1 B	7.2	<0.01
Sisco	99.8 ± 0.2 A	100.0 ± 0.0 A	61.6 ± 12.7 B	54.2 ± 11.2 B	8.3	<0.01
Studio	99.8 ± 0.2 A	100.0 ± 0.0 A	55.6 ± 12.3 B	56.9 ± 11.8 B	8.7	<0.01
F	0.5	1.0	0.9	0.4		
P	0.74	0.42	0.48	0.80		

For each exposure interval, within each row, means followed by the same uppercase letter are not significantly different. For each exposure interval, within each column, means followed by the same lowercase letter are not significantly different; df = 4, 40; df = 3, 32. Tukey-Kramer (HSD) test at $P = 0.05$. Where no letters were provided, no significant differences were recorded

(Table 9). Significantly less holes were counted on Sisco kernels than on Calaria kernels treated with Protect-It.

DE adherence

The main effects maize hybrid and DE and the associate interaction maize hybrid x DE were significant ($F_{4, 75} = 18.4$, $P < 0.01$; $F_{2, 75} = 22.9$, $P < 0.01$; $F_{8, 75} = 2.5$, $P = 0.01$, respectively). Significantly higher retention of DEA-P than Protect-It and PyriSec was recorded in Doxa and Rio Grande (Table 10). Although no significant differences in the degree of adherence were found among the three tested DEs in Calaria, Sisco, and Studio, DEA-P always adhered more on these maize hybrids than Protect-It and PyriSec.

Discussion

It is well addressed that DEs have certain major advantages, i.e., they have nontoxic mode of action, they reach consumers cleansed from possible residues due to the overall procession

Table 3 Progeny production of *P. truncatus* (adults per vial ± SE) on five maize hybrids treated with three DEs 60 days after the insertion of the parental adults

Formulation	Control	DEA-P		Protect-It	PyriSec		
Dose (ppm)	0	75	150	500	500		
Maize hybrid						*F*	*P*
Calaria	140.1 ± 41.3 A	0.2 ± 0.2 B	0.0 ± 0.0 B	104.6 ± 39.3 A	111.9 ± 45.3 A	4.2	<0.01
Doxa	124.3 ± 53.2 A	0.2 ± 0.2 C	0.0 ± 0.0 C	103.4 ± 43.3 A	32.6 ± 11.6 B	3.5	0.02
Rio Grande	111.7 ± 31.5 A	0.0 ± 0.0 C	0.0 ± 0.0 C	61.0 ± 20.3 AB	63.4 ± 17.8 AB	6.6	<0.01
Sisco	136.7 ± 32.0 A	0.0 ± 0.0 C	0.1 ± 0.1 C	78.4 ± 28.2 AB	63.9 ± 18.5 AB	7.7	<0.01
Studio	79.4 ± 31.5 A	0.2 ± 0.2 B	0.0 ± 0.0 B	65.6 ± 28.3 A	46.8 ± 23.7 A	2.9	0.04
F	0.4	0.8	1.0	0.4	1.3		
P	0.81	0.53	0.42	0.82	0.28		

Within each row, means followed by the same uppercase letter are not significantly different. Within each column, means followed by the same lowercase letter are not significantly different; in all cases df = 4, 40; Tukey-Kramer (HSD) test at $P = 0.05$. Where no letters were provided, no significant differences were recorded

Table 4 ANOVA parameters for main effects and associated interactions for number of kernels without holes, weight of kernels without holes, number of kernels with holes, weight of kernels with holes, and number of holes per kernel recorded in vials 60 days after the insertion of the parental *P. truncatus* adults (error df = 200)

Number of kernels without holes			
Source	df	*F*	*P*
Maize hybrid	4	9.1	<0.01
DE	4	32.1	<0.01
Maize hybrid x DE	16	0.6	0.92
Weight of kernels without holes			
Source	df	*F*	*P*
Maize hybrid	4	0.4	0.78
DE	4	32.1	<0.01
Maize hybrid x DE	16	0.4	0.98
Number of kernels with holes			
Source	df	*F*	*P*
Maize hybrid	4	1.0	0.40
DE	4	32.9	<0.01
Maize hybrid x DE	16	0.3	1.0
Weight of kernels with holes			
Source	df	*F*	*P*
Maize hybrid	4	1.6	0.19
DE	4	27.3	<0.01
Maize hybrid x DE	16	0.5	0.93
Number of holes per kernel			
Source	df	*F*	*P*
Maize hybrid	4	5.3	<0.01
DE	4	22.6	<0.01
Maize hybrid x DE	16	3.2	<0.01

of grains, and there is a high level of safety when instructions of use are followed properly (Korunić 2013). The present study is the first attempt to report on the simultaneous evaluation of the efficacy of DEs applied as grain protectants on several maize hybrids against *P. truncatus*. DEs exhibit reduced effect as maize protectants. For example Athanassiou et al. (2003) showed that the DE SilicoSec was considerably less effective on maize than on barley or rice, against adults of *S. oryzae*. Also, additional studies clearly indicate that DEs are generally less effective on maize than on other grains (Vayias et al. 2006; Vayias and Stephou 2009; Kavallieratos et al. 2015). Apart from DEs, there are studies showing that some grain protectants are less effective on maize, as compared with other grains, i.e., chlorfenapyr against *R. dominica* and *S. oryzae* (Kavallieratos et al. 2011), novel pyrrole derivatives against the Mediterranean flour moth, *Ephestia kuehniella* Zeller and *T. confusum* (Boukouvala et al. 2017), spinosad against *S. oryzae* (Chintzoglou et al. 2008), and spinetoram against *S. oryzae* (Vassilakos et al. 2015). Nevertheless, the factors that are responsible for this reduced efficacy of DEs on maize are poorly understood. In an earlier study, Athanassiou and Kavallieratos (2005), by using PyriSec, found that the adherence of this DE was significantly lower on maize than on barley, oats, rice, rye, triticale, and wheat, which means that the retention of DE particles is less on the external part of maize kernels than on the external part of the kernels of other grains. In this regard, we assume that there are specific interactions of DE particles with the external part of the maize kernel, which do not exist in the case of other grains. For spinosad, Chintzoglou et al. (2008), by using high-performance liquid chromatography/mass spectrometry method (LC/MS), noted that dissipation of the insecticide was higher on maize than on barley and wheat.

Our study shows that DE application can be very effective for the control of *P. truncatus* in several maize hybrids. In light of our findings, DEA-P was highly effective at low doses, if

Table 5 Number of kernels (mean per vial ± SE) without holes of five maize hybrids treated with three DEs 60 days after the insertion of the parental *P. truncatus* adults

DE	Control	DEA-P		Protect-It	PyriSec		
Dose (ppm)	0	75	150	500	500		
Maize hybrid						*F*	*P*
Calaria	72.2 ± 15.9 B	116.0 ± 1.3 Ac	118.0 ± 2.2 Ac	66.9 ± 13.7 B	69.8 ± 16.5 B	4.7	<0.01
Doxa	93.7 ± 18.2 B	148.2 ± 2.7 Aa	154.2 ± 2.1 Aa	97.8 ± 16.4 B	88.2 ± 2.1 B	8.3	<0.01
Rio Grande	59.3 ± 13.7 C	114.8 ± 1.5 Ac	115.8 ± 1.3 Ac	77.7 ± 12.3 BC	80.2 ± 9.8 BC	7.0	<0.01
Sisco	102.1 ± 14.4 B	152.8 ± 1.9 Aa	154.9 ± 0.8 Aa	96.8 ± 19.5 B	85.6 ± 2.7 B	5.4	<0.01
Studio	86.4 ± 12.6 B	126.2 ± 1.7Ab	127.0 ± 2.2Ab	89.3 ± 12.1 B	96.8 ± 9.2 B	5.0	<0.01
F	1.3	91.7	113.8	0.8	1.1		
P	0.29	<0.01	<0.01	0.55	0.38		

Within each row, means followed by the same uppercase letter are not significantly different. Within each column, means followed by the same lowercase letter are not significantly different; in all cases df = 4, 40; Tukey-Kramer (HSD) test at *P* = 0.05. Where no letters were provided, no significant differences were recorded

Table 6 Weight (g) of kernels (mean per vial ± SE) without holes of five maize hybrids treated with three DEs 60 days after the insertion of the parental *P. truncatus* adults

DE	Control	DEA-P		Protect-It	PyriSec		
Dose (ppm)	0	75	150	500	500		
Maize hybrid						*F*	*P*
Calaria	26.8 ± 5.6 B	46.1 ± 0.5 Abc	46.1 ± 0.4 Ab	26.9 ± 5.3 B	27.6 ± 6.4 B	5.4	<0.01
Doxa	27.7 ± 5.1 B	45.5 ± 0.5 Abc	45.8 ± 0.7 Ab	29.2 ± 4.7 B	37.5 ± 2.4 B	6.8	<0.01
Rio Grande	23.0 ± 5.0 B	46.8 ± 0.3 Aab	47.0 ± 0.5 Aab	31.3 ± 4.7 B	32.5 ± 3.6 B	9.2	<0.01
Sisco	28.4 ± 5.0 B	48.7 ± 0.4 Aa	49.0 ± 0.4 Aa	26.9 ± 6.3 B	33.2 ± 4.7 B	6.8	<0.01
Studio	29.9 ± 4.1 B	44.7 ± 0.7 Ac	45.5 ± 0.9 Ab	31.4 ± 4.0 B	34.0 ± 2.9 B	6.5	<0.01
F	0.3	9.8	5.5	0.2	0.7		
P	0.90	<0.01	<0.01	0.94	0.60		

Within each row, means followed by the same uppercase letter are not significantly different. Within each column, means followed by the same lowercase letter are not significantly different; in all cases df = 4, 40; Tukey-Kramer (HSD) test at $P = 0.05$. Where no letters were provided, no significant differences were recorded

compared to those currently used for other DEs. For example, a considerable proportion of the exposed *P. truncatus* adults was still alive after 14 days of exposure on all maize hybrids treated with 500 ppm of Protect-It, even though it was 3.3 times higher than the maximal application tested dose of DEA-P. Similar results have been also found for PyriSec; this DE contains natural pyrethrum and has been proved more effective than many of the commonly used commercial DEs against different stored-product pests (Kavallieratos et al. 2007). On the other hand, DEA-P was very effective at dose rates as low as 75 ppm, given that mortality was 98% or higher, after 7 days of exposure, regardless of the hybrid tested. In fact, the increase of DEA-P dose to 150 ppm did not increase mortality further, which clearly suggests that 75 ppm can be used with success for the control of *P. truncatus*. These results support previous findings documenting that the new enhanced DEA-P was highly effective for the control of this species (Athanassiou et al. 2007). Apart from parental mortality, DEA-P was able to reduce *P. truncatus* progeny production in all hybrids. In fact, in most of the cases tested, progeny production was completely suppressed (100%), while in the cases where progeny was recorded, its numbers did not exceed 0.2 adults per vial. In contrast, for the other two DEs, progeny production was extremely high and did not differ than that in the control vials in almost all cases. This high suppression of progeny production for DEA-P is related with the increased parental mortality. Apparently, the high level of efficacy of DEA-P is mainly due to the presence of abamectin in the formulation. Abamectin, a neurotoxic insecticide that acts as an agonist to GABA receptors (White et al. 1997) has been found very effective for the control of stored-product insects. In an earlier study, Kavallieratos et al. (2009) noted that for *R. dominica,* abamectin caused 100% parental adult mortality on maize treated with 1 ppm at 20 and 30 °C. Abamectin,

Table 7 Number of kernels (mean per vial ± SE) with holes per vial of five maize hybrids treated with three DEs 60 days after the insertion of the parental *P. truncatus* adults

DE	Control	DEA-P		Protect-It	PyriSec		
Dose (ppm)	0	75	150	500	500		
Maize hybrid						*F*	*P*
Calaria	34.0 ± 9.5 A	0.3 ± 0.2 Bab	0.3 ± 0.2 Bab	40.4 ± 10.6 A	29.3 ± 11.0 A	5.7	<0.01
Doxa	48.3 ± 13.0 A	0.3 ± 0.2 Bab	0.9 ± 0.3 Ba	42.7 ± 10.3 A	28.3 ± 8.2 A	7.5	<0.01
Rio Grande	52.8 ± 11.0 A	0.0 ± 0.0 Bb	0.0 ± 0.0 Bb	38.1 ± 11.7 A	33.6 ± 8.8 A	8.4	<0.01
Sisco	53.0 ± 12.1 A	0.9 ± 0.3 Ba	0.8 ± 0.3 Bab	47.8 ± 16.0 A	43.3 ± 11.0 A	6.5	<0.01
Studio	36.0 ± 10.2 A	0.3 ± 0.2 Bab	0.1 ± 0.1 Bab	32.3 ± 9.7 A	28.1 ± 8.1 A	5.9	<0.01
F	0.7	2.9	3.7	0.2	0.5		
P	0.62	0.04	0.01	0.92	0.77		

Within each row, means followed by the same uppercase letter are not significantly different. Within each column, means followed by the same lowercase letter are not significantly different; in all cases df = 4, 40; Tukey-Kramer (HSD) test at $P = 0.05$. Where no letters were provided, no significant differences were recorded

Table 8 Weight (g) of kernels with holes per vial of five maize hybrids treated with three DEs 60 days after the insertion of the parental *P. truncatus* adults

DE	Control	DEA-P		Protect-It	PyriSec		
Dose (ppm)	0	75	150	500	500		
Maize hybrid						*F*	*P*
Calaria	4.8 ± 1.6 A	0.1 ± 0.1 B	0.1 ± 0.1 B	7.8 ± 2.1 A	5.6 ± 2.6 A	4.3	<0.01
Doxa	7.9 ± 2.2 A	0.1 ± 0.1 B	0.2 ± 0.1 B	7.8 ± 2.1 A	5.0 ± 1.5 A	6.7	<0.01
Rio Grande	11.0 ± 2.5 A	0.0 ± 0.0 B	0.0 ± 0.0 B	9.2 ± 2.9 A	8.0 ± 2.1 A	7.4	<0.01
Sisco	9.3 ± 2.2 A	0.2 ± 0.1 B	0.2 ± 0.1 B	12.6 ± 4.3 A	7.8 ± 1.9 A	5.8	<0.01
Studio	8.2 ± 2.9 A	0.1 ± 0.1 B	0.0 ± 0.0 B	6.5 ± 2.0 A	5.7 ± 1.6 A	5.0	<0.01
F	1.0	1.0	2.7	0.7	0.5		
P	0.43	0.43	0.04	0.60	0.75		

Within each row, means followed by the same uppercase letter are not significantly different. Within each column, means followed by the same lowercase letter are not significantly different; in all cases df = 4, 40; Tukey-Kramer (HSD) test at $P = 0.05$. Where no letters were provided, no significant differences were recorded

however, is not registered so far for direct application on grains, but it is formulated and registered against agricultural pests (i.e., leafminers, psyllids, thrips, and mites). Probably its use could be assessed in combination with other active ingredients. The same holds in the case of thiamethoxam, which is not registered as a grain protectant in Europe, but it is registered as a mixture with pirimiphos-methyl in Africa (Chigoverah et al. 2014).

Based on our results, given that the adherence ratios were always higher for DEA-P than Protect-It or PyriSec to all maize hybrids and that mortality levels along with progeny production were always by far higher and lower respectively than Protect-It or PyriSec on all maize hybrids, it can be concluded that adherence is probably a factor that determines the differences in the efficacy of DEA-P. Similar results have been found for the DE Insecto when it was applied on two wheat varieties (Athos and Pontos) against *R. dominica*, *S. oryzae*, and *T. confusum* (Kavallieratos et al. 2010). On the other hand, grain damage indices could be used as reliable indicators of insect infestation patterns and also varietal resistance (Throne et al. 2000). The number of maize kernels without holes showed that the presence of DEs reduced the infestation rates in most of the cases examined and also that DEA-P had the highest numbers of kernels (or weight of kernels) without holes. At the same time, the increase of the dose rate of DEA-P did not result in further decrease of infestation patterns. The number and weight of kernels with holes or the number of holes per kernel, illustrate more clearly the differences among DEs but also among hybrids. In this context, Doxa and Sisco performed better than Calaria, Rio Grande, or Studio based on the significant differences found concerning the numbers of kernels without holes at both doses of DEA-P tested. This trend was also evident when maize hybrids remained untreated or were treated with Protect-It,

Table 9 Number of holes per kernel (mean per vial ± SE) of five maize hybrids treated with three DEs 60 days after the insertion of the parental *P. truncatus* adults

DE	Control	DEA-P		Protect-It	PyriSec		
Dose (ppm)	0	75	150	500	500		
Maize hybrid						*F*	*P*
Calaria	10.3 ± 1.6 Aab	0.3 ± 0.2 B	0.3 ± 0.2 B	25.8 ± 9.2 Aa	8.2 ± 2.0 A	6.0	<0.01
Doxa	8.2 ± 1.2 Aab	0.7 ± 0.4 B	0.5 ± 0.2 B	12.3 ± 3.7 Aab	7.2 ± 1.3 A	7.6	<0.01
Rio Grande	11.6 ± 1.6 Aa	0.0 ± 0.0 B	0.0 ± 0.0 B	8.2 ± 2.2 Aab	11.3 ± 2.7 A	11.7	<0.01
Sisco	5.9 ± 1.1 Ab	0.7 ± 0.3 B	0.6 ± 0.2 B	4.1 ± 1.2 Ab	4.9 ± 1.1 A	8.1	<0.01
Studio	8.0 ± 1.2 Aab	0.6 ± 0.3 C	0.3 ± 0.3 C	3.7 ± 1.0 Bab	4.8 ± 1.3 AB	11.2	<0.01
F	2.7	1.2	1.1	3.9	2.3		
P	0.04	0.35	0.33	<0.01	0.08		

Within each row, means followed by the same uppercase letter are not significantly different. Within each column, means followed by the same lowercase letter are not significantly different; in all cases df = 4, 40; Tukey-Kramer (HSD) test at $P = 0.05$. Where no letters were provided, no significant differences were recorded

Table 10 Mean adherence (% ± SE) of three DEs at five maize hybrids

DE	DEA-P	Protect-It	PyriSec	*F*	*P*
Maize hybrid					
Calaria	84.0 ± 3.8 b	80.9 ± 2.1 bc	74.6 ± 3.3 c	2.3	0.14
Doxa	92.7 ± 0.8 Aab	75.3 ± 1.9 Bc	73.4 ± 2.2 Bc	30.2	<0.01
Rio Grande	95.0 ± 1.1 Aa	87.9 ± 1.2 Bab	90.7 ± 1.2 Ba	9.9	<0.01
Sisco	90.3 ± 2.4 ab	81.8 ± 4.0 abc	80.9 ± 2.0 bc	3.0	0.08
Studio	93.7 ± 2.1 a	91.0 ± 1.3 a	90.3 ± 1.6 ab	1.1	0.35
F	3.6	7.0	13.2		
P	0.02	<0.01	<0.01		

Within the same row, means followed by the same uppercase letter are not significantly different; df = 4, 25. Within the same column means followed by the same lowercase letter are not significantly different; df = 2, 15; Tukey-Kramer (HSD) test at $P = 0.05$. Where no letters were provided, no significant differences were recorded

although no significant differences were detected. An additional indication about the adequacy of 75 ppm for the management of *P. truncatus* is provided by the fact that no significant differences were found in the number of kernels without holes with the 150 ppm treatment of any maize hybrid. For PyriSec and Protect-It, the numbers of kernels with holes and the weight of kernels with holes were comparable with those in the control vials, which further consists the reduced insecticidal effect of these DEs against *P. truncatus*.

Variant susceptibility of different varieties or hybrids of grains to stored-product insects, in conjunction with the application of contact insecticides, has not been examined in detail. In an earlier study, Kavallieratos et al. (2010) compared three wheat varieties, Athos, Pontos, and Sifnos regarding the insecticidal effect of three DEs and spinosad dust and found that all four insecticides were less effective when applied on Pontos for the control of *R. dominica*, *S. oryzae*, and *T. confusum*. Similarly, Fang et al. (2002) found that the insecticidal effect of liquid spinosad differed remarkably among different classes of wheat. These variations are manifested at two levels. Some stored-product insects are more prone to thrive in certain commodities. In this regard, population growth can be elevated in certain commodities. For example, Opit and Throne (2008) found that population growth of *Lepinotus reticulatus* Enderlein (Psocoptera: Trogiidae) was higher on oats, rice, barley, milo, and wheat than on maize. For some commodities, there are specific interactions with certain insecticides, which, indirectly, affect their insecticidal effect (Chintzoglou et al. 2008). Nevertheless, it is still unclear whether variations in insect mortality are correlated with some physical and chemical characteristics of the commodity. For DEs and spinosad, Kavallieratos et al. (2010) found that among three wheat varieties, survival of *R. dominica*, *S. oryzae*, and *T. confusum* was greater on the one that had a highest gluten index and kernel size. However, in that study, the authors did not found any relation between insecticidal effect and other grain properties, such as protein content. Similarly, Fang et al. (2002) found that there was no correlation between the insecticidal effects of spinosad against the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), the Indian mealmoth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), *R. dominica*, *S. oryzae*, and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) with some of wheat characteristics, such as kernel diameter, kernel hardness, kernel weight, protein content, dockage, and fiber. McGaughey et al. (1990) noted that kernel hardness negatively affected the oviposition of *S. oryzae* but not that of *R. dominica*. Still, the results for the effect of kernel characteristics on population growth of stored-product insects are not always consistent, and they are often controversial (Bhatia and Gupta 1969, Amos et al. 1986, Sinha et al. 1988, Throne et al. 2000, Toews et al. 2000). Our data indicate not only that the tested DEs had generally similar performance among maize hybrids but also that any differences observed in mortality, and progeny production could be mainly attributed to the characteristics of the formulations per se.

Overall, the findings of the present study show that DEA-P is very effective for the control of *P. truncatus* in a wide range of maize hybrids. At the same time, *P. truncatus* population growth was practically completely suppressed on the treated grains. The fact that DEA-P is effective at dose rates that are notably lower than other DEs means that the use of DEA-P may affect only minimally the test weight of the treated grains, which is the main disadvantage of DEs in "real world" applications by degrading their commercial value (Korunić 2016). In this regard, DEA-P and abamectin should be more thoroughly examined on the basis of potential registration at the post-harvest stages of agricultural commodities.

Acknowledgements This work was partially supported by the project "Control of stored products insects" (Hellenic Ministry of Rural Development and Food, Directorate of Plant Protection).

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