Mortality and suppression of progeny production of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) in seven different grains treated with an enhanced diatomaceous earth formulation

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Abstract

DEBBM, an enhanced diatomaceous earth (DE) formulation consisting of a mixture of DE and the plant extract bitterbarkomycin, was applied to seven different grains (wheat, barley, oats, rye, triticale, paddy rice and maize) at two dose rates 50 ppm and 150 ppm. Unsexed, 7d old adults of *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae) were exposed to the DEBBM treated commodities and their mortality was assessed after 7d and 14d of exposure at 25 ^{o}C and 65% r.h. Furthermore, progeny production of the tested species per treated commodity was also assessed. *Sitophilus oryzae* appeared to be more susceptible than *T. confusum* to DEBBM. Performance of DEBBM was better in barley, wheat and oats compared to the remainder of the tested commodities. DEBBM performed better in rye and triticale than in paddy rice against both species although in many cases, significant differences among these grains were not recorded. Despite that DEBBM reached its highest efficacy levels on barley, wheat, and oats it did not suppress progeny production of the treated species in any of the grains. A significant reduction in progeny production of the treated species was recorded in the DEBBM treated grains in comparison with the untreated ones. This reduction in progeny production was expressed more vigorously to *S. oryzae* rather than *T. confusum*. In commodities with high DEBBM performance such as barley, oats or wheat, > 9-fold less progeny of *S. oryzae* were recorded at 150 ppm of DEBBM than in the untreated commodities. Although significantly less progeny of *T. confusum* were recorded in DEBBM treated grains than untreated grains, progeny suppression of this species was neither dose nor commodity dependant.

Keywords: Diatomaceous earth, Bitterbarkomycin, *Tribolium, Sitophilus*, Mortality, Commodity

1. Introduction

The use of diatomaceous earth (DE) for the control of stored product pests is one of the promising alternatives to the conventional insecticides and fumigants that are used in stored product protection. DE has low mammalian toxicity (Quarles, 1992) and is effective against a wide range of stored product insects (Korunic, 1998, Subramanyam and Roesli, 2000; Mewis and Urlichs, 2001; Athanassiou et al., 2005; 2006). Nevertheless, high DE application rates (400-1000 ppm) are required for a satisfactory control of stored product insects (Korunic, 1998; Athanassiou et al., 2005; 2006). At these application rates DEs negatively affect some of the physical properties of grains, which has limited the wide use of DE as grain protectant (Korunic, 1998). According to Arthur (2003) the combination of reduced rates of DE with other insecticides could alleviate some of the effects on physical properties.

Athanassiou et al., (2008a) evaluated a mixture of DE with the plant extract bitterbarkomycine (BBM) against adults of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). This enhanced DE formulation was very effective at application rates less than 150 ppm. BBM, known also as angulatin A, is a sesquiterpene polyol ester, extracted from the roots of the plant *Celastrus angulatus* Max. (Wang et al., 1991). In initial tests, Wang et al. (1991) found that BBM at low doses (25-50 ppm), had strong insecticidal and antifeedant effects against several insect species, especially aphids, while in a latter study Athanassiou et al. (2009) found that low doses of BBM (0.0375–0.0875 ppm) proved very effective against several stored product insect pests including *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae).

The objective of the present study was to evaluate DEBBM as grain protectant against two major stored product insects *Sitophilus oryzae* (L.) (Coleopetra: Curculionidae) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) as applied to wheat, maize, barley, rice, oats, rye and triticale. In addition, progeny production of the above species after exposure on the treated with DEBBM grains was also assessed.

2. Materials and methods

The tested insects were obtained from laboratory cultures. *Sitophilus oryzae* was reared on whole wheat kernels while *T. confusum* was reared on wheat flour plus 5% brewers yeast. Both species were reared at 27±1^{o}C, 65 ± 5% r.h. in continuous darkness. All insects used in the tests were adults < 3 wk old. The DEBBM formulation that was used in the experiments contained 90% DE and 0.05% BBM. The examined commodities were hard wheat (variety Mexa), whole (raw) barley (variety Persephone), oats (variety Cassandra), rye (variety Danko), triticale (variety Vronti), paddy rice (variety Thaibonnet) and maize (variety Dias). All commodities used in the experiments were clean, untreated and infestation free. The moisture contents of the tested commodities, as determined prior to the experiments by a Dickey–John moisture meter (Dickey-JohnMultigrain CAC II, Dickey-John Co, USA), ranged between 10.9 and 11.5%. One kilogram lots were obtained from each commodity and each lot was separately treated with DEBBM at two dose levels (50 ppm and 150 ppm). In addition, 1 kg-lots from each commodity remained untreated and served as controls. Six samples of 60 g each were obtained from each treated or untreated commodity, placed inside small glass vials, and infested with 30 adults of *S. oryzae*. Mortality of adults was assessed 7 d and 14 d post exposure of the weevils on the treated or untreated commodities at 25^{o}C and 65% r.h. After the 14 d bioassays, all dead or alive weevils were discarded and the vials were placed again at the above conditions for an additional period of 50 d. Next, the vials were opened and live adult progeny were counted. The same procedure described above was followed also for *T. confusum* by preparing new commodity lots. Control mortality in any of the exposure did not exceed 3%, and no correction for mortality values was done. Mortality data were subjected to one way ANOVA separately for each species and exposure interval, while the main effects were dose and commodity type. Prior to analysis of progeny production data, a Dunnnet's test for each species and commodity combination revealed that progeny production of the tested species was always significantly higher in untreated commodities than the respective treated ones. Hence, progeny production in the control groups was not included in the analysis of progeny production data. Similarly to mortality data, progeny data were subjected to one-way ANOVA separately for each species, with dose and commodity type the main effects. For the comparison of means for either mortality or progeny production data the Tukey and Kramer's HSD test at P< 0.05 was used (Sokal and Rholf, 1995).

3. Results

3.1. Sitophilus oryzae

Application rate and commodity type significantly affected mortality of S. oryzae adults on DEBBM treated commodities at both of the tested exposure intervals (7d exposure: application rate: $F_{2,125}$=51.1, P<0.001; commodity: $F_{6,125}$=13.6, P<0.001; 14 d exposure: $F_{2,125}$=30.1, P<0.001; commodity: $F_{6,125}$=17.0, P<0.001). After 7d or 14 d of exposure, efficacy of 50 ppm DEBBM did not exceed 17% in treated maize or rice and was always significantly lower than that recorded in the remainder of the treated grains (Fig.1). Weevil mortality was significantly increased following an increase of DEBBM dose to 150 ppm at both exposure intervals. Thus, after 7d of exposure of weevils on the DEBBM-treated grains, efficacy of 150 ppm increased to 88% in treated barley, wheat or oats while the same dose killed less than 50% of the exposed weevils in treated maize or rice (Fig.1). Seven days later, 93.6, 92.8 and 90.2% of the exposed weevils were dead on oats, barley and wheat treated with 150 ppm respectively, while mortality in treated rice or maize was 50.4 or 46.8% respectively (Fig. 1). As a result, significant differences in efficacy of 150 ppm of DEBBM against rice weevil after 7d or 14d of exposure were not recorded among barley, wheat or oats as well as between maize and rice, although a remarkable variation in performance of DEBBM between these two groups of grains (barley, wheat or oats and maize or rice) was always recorded (Fig. 1). Application rate and commodity type also significantly affected progeny production of *S. oryzae* on DEBBM grains (application rate: $F_{2,125}$= 23.7; P<0.001; commodity: $F_{6,125}$= 2.6; P=0.01). Nevertheless, significant differences in weevil progeny between the tested DEBBM doses were recorded only in the case of barley or oats, while in the remainder of the grains, progeny production that was observed on DEBBM treated grains was dose independent (Fig. 2). Progeny production of rice weevil on untreated commodities was 36.5 ± 1.6, 18.9 ± 1.6, 29.5 ± 2.6, 16.3 ± 1.6, 23.7 ± 2.9, 22.3 ± 4.1 and 21.0 ± 1.8 weevils per 60g of barley, maize, oats, rice, rye, triticale and wheat, respectively. Even though the highest values of rice weevil progeny production were recorded in barley (17.3 weevils/60g) or maize (13.7 weevils/ 60g) treated with 50 ppm of DEBBM, progeny production on grains treated with 150 ppm of DEBBM ranged 4.3 - 6.1 weevils per 60 g of commodity and as a result, significant differences between the tested grains were not recorded (Fig. 2).

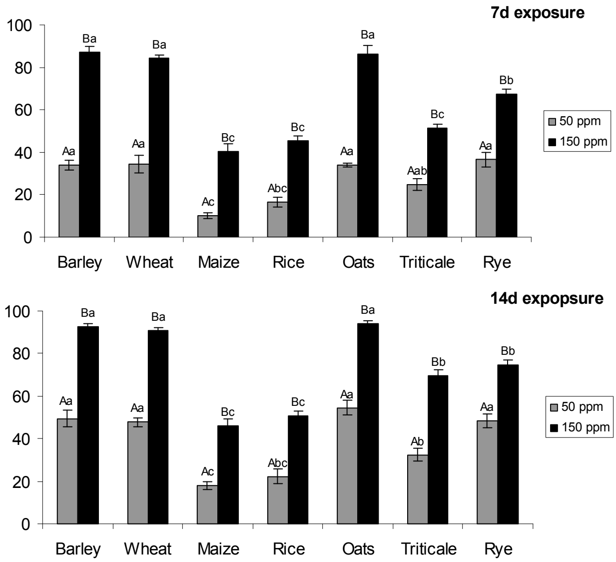

Figure 1 Mean (± SE) mortality of *S. oryzae* adults after 7 d and 14 d exposure on seven grains treated with two doses of DEBBM at 25°C and 65% r.h. Within each exposure interval and application rate means followed by the same lowercase letter are not significantly different. Within each exposure interval and grain, means followed by the same uppercase letter are not significantly different. (Uppercase letters for dose; Lowercase letters for grain. For the comparison of doses *df*=1, 11; For the comparison of grains *df*=6, 35; Tukey and Kramer HSD test at *P*<0.05).

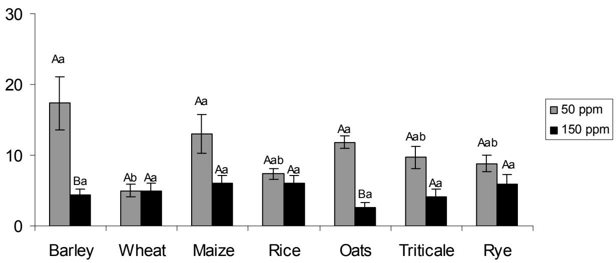

Figure 2 Mean progeny production (number of live weevils ± SE per 60 g of grain) of *S. oryzae* in grains treated with two doses of DEBBM at 25°C and 65% r.h. Within each dose means followed by the same lowercase letter are not significantly different. Within each grain, means followed by the same uppercase letter are not significantly different. (Uppercase letters for dose; Lowercase letters for grain. For the comparison of doses *df*=1, 11; For the comparison of grains *df*=6, 35; Tukey and Kramer HSD test at *P*<0.05).

3.2. *Tribolium confusum*

Mortality of *T. confusum* adults after exposure on DEBBM-treated grains was significantly for dose (7d exposure: $F_{2,125}$=47.2, P<0.001; 14 d exposure: $F_{2,125}$=11.5, P<0.001) and commodity type (7 d exposure: $F_{6,125}$=21.8, P<0.001; 14 d exposure: $F_{6,125}$=22.6, P<0.001) at both of the tested exposure intervals. After 7d of exposure, the maximum performance of 50 ppm of DEBBM was observed in treated oats (30%) and barley (22.2%) although 19.4% of exposed adults were killed in treated wheat (Fig.3). Although this DEBBM dose performed equally well on wheat and barley, significantly more beetles were killed in treated oats than treated wheat. A further increase of dose to 150 ppm resulted in a significant increase in DEBBM efficacy, which increased to 57.8 and 53.3% in treated barley and oats respectively (Fig. 3). The lowest DEBBM performance at 50 ppm was observed in maize or rice where it did not exceed 5%, while in the case of 150 ppm *T. confusum* appeared to be most susceptible in treated maize, rice and rye since mortality on these grains was lower than 35% (Fig. 3). Similar trends in DEBBM performance were observed 7 d later, although efficacy of both DEBBM doses was increased with the increase of exposure. Thus, the best DEBBM performance was observed in 150 ppm treated oats, barley, or wheat where DEBBM efficacy reached 80%, while approximately half of the exposed adults were killed on triticale treated with the same dose (Fig. 3). Even 14 d of exposure on 50 pmm treated maize, rice or oats, was not adequate to control *T. confusum* adults since very low (<12%) mortality was recorded in these grains, though mortalitiy did increase as the dose increased to 150 ppm (Fig.3). Progeny production of confused flour beetle on untreated commodities was 5.5 ± 0.8, 3.2 ± 0.6, 3.4 ± 0.6, 2.2 ± 0.4, 5.3 ± 0.7, 3.5 ± 0.9 and 5.8 ± 1.0 beetles per 60 g of barley, maize, oats, rice, rye, triticale and wheat respectively. In the case of treated commodities, the number of emerged beetles did not exceed 2.7 beetles per 60 g of treated commodity and thus, unlike mortality of this species, application rate ($F_{2,125}$= 1.7; P=0.091) or commodity type ($F_{6,125}$= 1.3; P=0.262) had not a significant impact on progeny production of this species on the DEBBM treated grains that were examined here (Fig. 4).

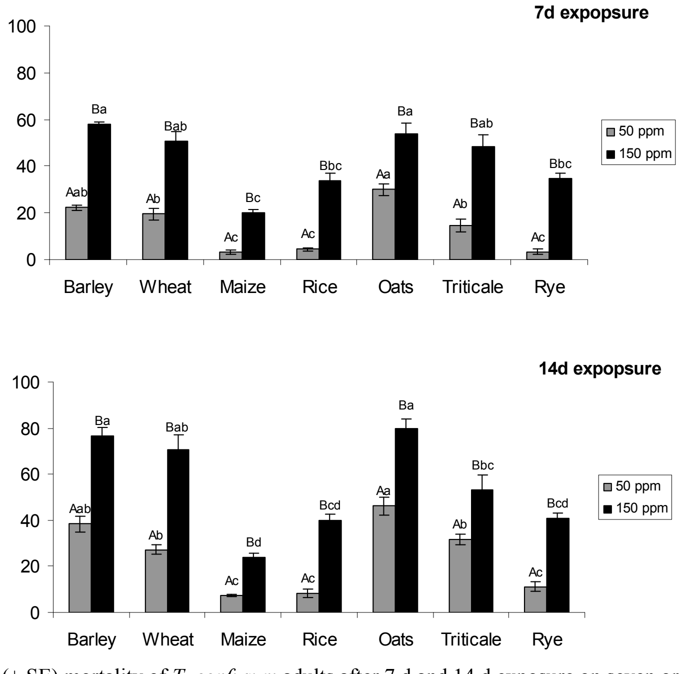

Figure 3 Mean (± SE) mortality of *T. confusum* adults after 7 d and 14 d exposure on seven grains treated with two doses of DEBBM at 25°C and 65% r.h. Within each exposure interval and application rate means followed by the same lowercase letter are not significantly different. Within each exposure interval and grain, means followed by the same uppercase letter are not significantly different. (Uppercase letters for dose; Lowercase letters for grain. For the comparison of doses *df*=1, 11; For the comparison of grains *df*=6, 35; Tukey and Kramer HSD test at P<0.05).

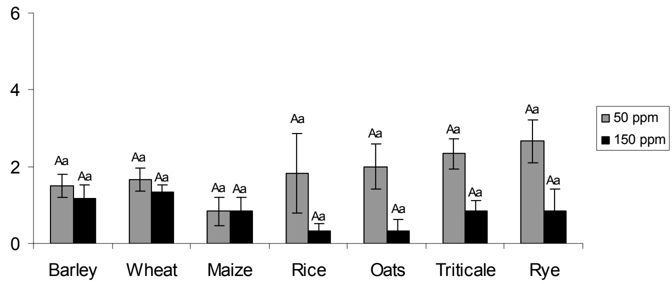

Figure 4 Mean progeny production (number of live beetles ± SE per 60 g of grain) of *T. confusum* in grains treated with two doses of DEBBM at 25°C and 65% r.h. Within each dose means followed by the same lowercase letter are not significantly different. Within each grain, means followed by the same uppercase letter are not significantly different. (Uppercase letters for dose; Lowercase letters for grain. For the comparison of doses *df*=1, 11; For the comparison of grains *df*=6, 35; Tukey and Kramer HSD test at *P*<0.05).

4. Discussion

Tribolium confusum is considered as one of the most tolerant species to DE (Korunic, 1998; Athanassiou et al., 2005) and the present study, this DE formulation appeared to be less efficacious against this species than *S. oryzae*. However, differential performance of DE does not occur only among target species but it can also occur among different commodities. Athanassiou et al. (2003) examined Silicosec, a commercially available pure DE formulation, in a variety of grain commodities and found high efficacy on some commodities but moderate or low effectiveness in others. This differential performance of DE among various commodities was observed also in the present study with the mixture of DE with BBM. Based on our results, the tested grains can be divided into three groups according to the descending order of DEBBM performance. The first group includes barley, wheat and oats, where DEBBM at the tested doses exhibited its highest efficacy levels, the second includes rice and maize where performance of DEBBM was low, while the third group includes triticale and rye (in some cases) where DEBBM exhibited intermediate efficacy levels. The fact that DE exhibits low performance in maize compared to other grains such as wheat or barley has been also reported by other researchers (Athanassiou et al., 2003; Kavallieratos et al., 2005). Athanassiou et al. (2003) found that effectiveness of Silicosec against *S. oryzae* was much lower in maize than in barley while this was also confirmed by a latter study of Kavallieratos et al. (2005) against *R. dominica* F. (Coleoptera: Bostrychidae). Kavallieratos et al. (2005) examined Silicosec and Insecto, which is also a pure DE formulation, in different grain commodities and found higher efficacy levels of these DEs in wheat or triticale than in barley against *R. dominica*. However, this was not the case with our results since DEBBM was either of equal or higher effectiveness compared to wheat against both *S. oryzae* and *T. confusum*. Although this contradictory finding requires further attention, the fact that different species were used in our study compared to Kavallieratos et al. (2005) may be considered as a possible explanation, since diet-preferences that vary among insect species (Baker, 1988; McGauchey et al., 1990) often determine their fitness and consequently their susceptibility to control methods (Athanassiou et al., 2008b).

Differences in morphological traits among kernels of different grains can often influence DE efficacy. The decreased performance of DEBBM in maize may be partially attributed to the wide spaces between the maize seeds that allow insects to crawl through them and thus avoid areas where DEBBM concentration was high (Athanassiou et al., 2003). Furthermore, differences in physicochemical properties of seed perikarp that exist among various grains may affect the retention of DE on kernel surface and thus lead to differential DE efficacy. Athanassiou and Kavalieratos (2005), reported that retention rate of DE on wheat, barley or rice was much higher than maize. The above result can explain the fact that DEBBM was not effective in maize but it can not explain the low performance of this formulation in rice. Although we did not examine retention rate of DEBBM to the different grains tested, it seems that in the case of rice kernels retention rate can not be used to explain efficacy of DEs. Although our findings in rice require further investigation the fact that DEs become gradually inactivated since they absorb oils from the perikarp of the rice kernels McGaughey (1972) could be considered as a possible explanation for the decreased performance of DEBBM in this commodity. Contrary to maize or rice, DEBBM was shown to be effective in barley, wheat or oats and the fact that this high level of protection was achieved with low dose rates is very encouraging to also confirm the efficacy of this DE formulation under field conditions.

DEBBM was not able to suppress the progeny production of the tested species. Since DEs are slow acting insecticides they require a sufficient period of time to act (Subramanyam and Roesli, 2000; Vayias and Athanassiou, 2004; Athanassiou et al., 2005). It seems that during that lag period, treated parentals can mate or oviposit before they eventually die. However, despite that progeny production was not completely suppressed it was more than 9-fold lower in the treated grains than the untreated ones. Hence, we can estimate that DE and/or BBM negatively affected mating or oviposition of the parental individuals.

On conclusion, DEBBM applied at 150 ppm can provide satisfactory control against *S. oryzae* in wheat, barley or oats. However, in the case of *T. confusum* or in the case of rye, triticale, maize and rice, higher than 150 ppm application rates were required for a satisfactory performance of DEBBM. Thus, the labeled rate of such an enhanced DE formulation should be target species and commodity sensitive. In order for DEBBM to be effective, insects should come in contact with its particles. Hence, these low application rates of DEBBM should be also confirmed in field studies given that a significant proportion of grain mass, which remain untreated as long as DE formulations are applied at low levels, may provide shelter to insects that would have previously avoided the DEBBM particles and by this means application of DEBBM at low doses may be rendered ineffective.

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