Is combining different grain protectants a solution to problems caused by resistant populations of stored-product insects?

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Abstract

Insect control during storage of grains can utilize grain protectants. Decades of continuous use of contact insecticides, particularly organophosphates and pyrethroids, have led to recurring instances of resistance of various populations of stored-product insects to those insecticides, which is now one of the most limiting factors for their continued use in the future. The search for a solution to the problem of resistance relies on its timely detection, regular monitoring and holding at an acceptable level, and on the use of highly effective alternative control measures. A way to manage the problem of resistance is to introduce new insecticides with novel mechanisms of activity, but it is a costly and long process, so that only diatomaceous earth (DE) and spinosad have proved over the past two decades to be adequate alternatives to contact insecticides. However, some of their deficiencies have prevented their widespread use. The current anti-resistance tactics therefore based on the available grain protectants as their combined application may be a solution against resistant populations. Besides synergistic effects, combined application may also alleviate the negative effects of some substances and so meet the increasingly restricting criteria for food safety and protection of human health and the environment. The present paper focuses primarily on the results of a research into the efficacy of different combinations of contact insecticides, DE, botanicals and some additional measures against susceptible and resistant populations of stored-product insects.

Keywords: stored-product insects, resistance, grain protectants, combined use

1. Contact insecticides/grain protectants and resistance of stored-product insects

Despite of recurring consideration of biorational approaches to the management of stored-product insects intending to avoid chemical insecticides (Phillips and Throne, 2010), contact insecticides as grain protectants are still the most effective alternative to the fumigant methyl bromide (Zettler and Arthur, 2000; Fields and White, 2002; Arthur, 2012). Since the introduction of a number of compounds in the late 1960s, their selection has changed with the deepening knowledge and new standards of application for different insecticides in storage ecosystems. Organophosphates (OP) and pyrethroids (PY), as well as insect growth regulators (IGR) to some extent, are the most intensively used chemicals on a global scale, and the choice of registered products based on different active ingredients depends on the needs of any particular country. White and Leesch (1996), Couteux and Lejeune (2005), Collins (2006), Whitehead (2007) and MacBean (2012) have shown that malathion, dichlorvos, chlorpyrifos-methyl, pirimiphos-methyl and fenitrothion

are the leading organophosphate insecticides, while metacriphos and etrimfos are less frequent; regarding PYs, the most frequent compounds include deltamethrin and cyfluthrin, and the synergist piperonyl butoxide, while pyrethrins, permethrin, resmethrin, bioresmethrin, cypermethrin and fenvalerate are less popular. Considering IGRs, only methoprene is currently used as a grain protectant (Athanassiou et al., 2011).

The repeated use of organophosphates, malathion in particular, and pyrethroid based insecticides has resulted to changes to the susceptibility (i.e., resistance) of some populations of stored-product insects. Apart from selection pressure, the biological properties of various insect species are also improtant factors causing resistance, coupled with various environmental factors, such as primarily the temperature (Busvine, 1971; Subramanyam and Hagstrum, 1996; Kljajić and Perić, 2006, 2007). Temperature has a significant influence on insect activity (e.g. reproduction, mobility, metabolism), insecticide toxicity (e.g. their reduced or increased efficacy), and residual insecticide activity (Tyler and Binns, 1982; Kljajić et al., 2009, 2014). Resistance to a single insecticide has been found in most tested populations of stored-product insects, and the resistance of red flour beetle *Tribolium castaneum* (Herbst) to malathion became a major issue after reports have shown its widespread occurrence (Champ and Dyte, 1976; Subramanyam and Hagstrum, 1996; Boyer et al., 2012). Cross- and multi-resistance, the even more threatening forms of resistance, have been detected primarily in warm climatic regions and in populations of maize weevil *Sitophilus zeamais* (Motsch.) (Brasil and Mexico), but also in populations of lesser grain borer *Rhyzopertha dominica* (F.), sawtoothed grain beetle *Oryzaephilus surinamensis* (L.) and *T. castaneum* (Australia). However, reports have also been made in continental regions (Serbia) for populations of grain weevil *Sitophilus granarius* (L.) (Collins, 1990; Guedes et al., 1995; Perez-Mendoza, 1999; Ribeiro et al., 2003; Kljajić and Perić, 2006; Daglish, 2008).

Pesticide resistance management (PRM), which is a component of Integrated pest management (IPM) programmes, focuses on preventing the onset and evolution of resistance in pest populations (Metcalf, 1989; Croft, 1990). Overcoming resistance in stored-product insects basically relies on timely detection of such issues at their very beginning and on their monitoring (i.e., recording their level and change over time) (Champ and Dyte, 1976; Busvine, 1980; Tabashnik, 1990; Kljajić and Perić, 2006; Boyer et al., 2012), and on organizing resistance management by applying highly effective alternative measures, such as the use of new grain protectants or combined use of available grain protectants (Collins, 1990; Subramanyam and Hagstrum, 1996; Daglish, 1998).

2. New grain protectants and protectants that may potentially be used for control of stored-product insects

A way to manage the problem of resistance is to introduce new insecticides with novel modes of action, which is a costly and long process. New comounds need to meet the increasingly restricting criteria regarding their toxicity to mammals, selectivity for beneficial insects and safety for the environment. A crucial problem is that new insecticides are expected to fulfil the requirement of fast degradation, leaving no residues in food, while their principal purpose is to ensure months-long protection from pests in stored products. All these facts have contributed to the status that diatomaceous earths (DEs) and spinosad have had over the past 20 years as the most promising alternatives to conventional grain protectants..

DEs are naturally occurring insecticides with negligible toxicity to mammals, and they can be applied using a technology similar to residual insecticides. DEs cause mortality of stored-product

insects by damaging their cuticles and causing water loss, rather than by provoking some metabolic change (Ebeling, 1971; Korunić, 1998; Fields and Korunić, 2000). DEs are therefore considered fit to control populations of stored-product insects that are resistant to contact insecticides. The risk of insects developing resistance to DEs is minimal in practice (Korunić, 1998), although such a possibility would be realistic after many years of continued use, as Vayias et al. (2008) have shown in a warning laboratory report. Several DE-based products have been registered worldwide and their efficacy is high at the application rates of 0.5-1.5 g/kg. However, despite the advantages of DEs over tradicional grain protectants, they have not been widely accepted by large-scale storage systems. The main deficiency of DEs that prevents their more intensive use in large storages is their impact on the physical properties of grain, including flow rate and test weight (Korunić, 1998; Subramanyam and Roesli, 2000).

Spinosad is a broad-spectrum insecticide based on metabolites of the actinomycete *Saccharopolyspora spinosa* Mertz and Yao. Many research reports have shown that spinosad is highly effective against stored-product insects (Daglish and Nayak, 2006; Subramanyam et al., 2007; Athanassiou et al., 2008; Vayias et al., 2010). Even though the insecticide has been approved as a grain protectant by the US Environmental Protection Agency (EPA) at a rate of 1 mg/kg (Hertlein et al., 2011) it is still not used commercially. Compared to conventional grain protectants, spinosad has a number of advantages: low toxicity to mammals, high efficacy at rates several times lower than OPs, and particularly its high efficacy against *R. dominica* populations resistant to conventional grain protectants (Nayak et al., 2005). Even though it is not yet used commercially, several studies have detected significant differences in the susceptibilities of various field populations to spinosad (Fangeng et al., 2004; Athanassiou et al., 2008; Sehgal et al., 2013a), including populations of stored-product insects resistant to contact insecticides (Nayak et al., 2005). At the start of its commercial use, caution is therefore required, as well as close monitoring of the susceptibility of stored-product insects to spinosad in order to extend the period of its effective use as much as possible. One of possible solutions would be to combine it with other grain protectants (Daglish, 2008).

Spinetoram is a new member of the spinosyns family and it could be used in the future as a grain protectant after the most recent research has shown its high efficacy against stored-product insects at rates lower than those of spinosad (Vassilakos et al., 2012; Vassilakos and Athanassiou, 2013, Athanassiou and Kavallieratos, 2014).

Abamectin is the most popular insecticide of the avermectin family which belongs to an earlier generation of natural insecticides synthesized from fermenting products of the actinomycete *Streptomyces avermitilis* Kim and Godfellow, and it is used to protect many crops from insect and mite pests. Kavallieratos et al. (2009) showed that abamectin is highly effective against the rice weevil *Sitophilus oryzae* (L.), *R. dominica* and confused flour beetle *Tribolium confusum* Jacquelin du Val at the rate of 1 mg a.i/kg and that it could be use in practice as a grains protectant. Toxicological properties of abamectin, and insuficient data on its persistence and degradation in grains, may prove its deficiency and a limiting factor for its widespread use.

The introduction of neonicotinoids (e.g. thiamethoxam, imidacloprid, acetamiprid) in the late 1990s made the control of crop pests resistant to OPs, PYs and carbamates considerably easier due to their different mechanism of activity. Many neonicotinoid-based products have been used to treat cerial grains, while Arthur et al. (2004) showed that the neonicotinoid thiamethoxam was highly effective against *S. zeamais*, *S oryzae*, *R. dominica* and *O. surinamensis* at the rate of 4 ppm a.i/kg wheat and maize grain. Later research has shown that the residual efficacy of

thiamethoxam significantly decreases as early as 60 days after treatment, and that field populations of *R. dominica* have demonstrated significant differences in their susceptibility to that insecticide (Wakil et al., 2012, 2013). Apart from thiamethoxam, imidacloprid could also be used as a grain protectant in the future. Nayak and Daglish (2006) found imidacloprid highly effective in controlling resistant populations of psocids at the rate of 10 mg/kg, noting in particular that none of the products registered in Australia was sufficiently effective to control those populations. However, the same rate of imidacloprid has not been found sufficiently effective against *S. oryzae* and *T. castaneum* populations resistant to tradicional grain protectantns (Daglish and Nayak, 2012). Further research into the stability of neonicotinoid residues, as well as definition of their permissible maximums in grains and treatment costs, are required in order to understand the full potential of neonicotinoids as grain protectants.

The pyrazole insecticides fipronil and ethiprole are interesting as potential grain protectants as they are already used for the control of cockroaches and other urban pests that may be found in storages and mills (Silverman and Liang, 1999). Arthur (2002) found the ethiprole application rate of 10 mg ai/kg wheat or maize to be highly effective against *S. oryzae* and *S. zeamais* adults as long as six months after treatment. On the other hand, Kavallieratos et al., (2010) found fipronil applied to wheat, maize, barley and paddy rice at 1 mg ai/kg under different temperatures and relative humidity to be highly effective against *S. oryzae*, *R dominica*, *T. confusum* and the larger grain borer *Prostephanus truncatus* (Horn). Although fipronil was highly effective at low rates, the authors called for more research into the persistence and potential harmfulness of fipronil residues in grains to protect the consumers.

3. Combined use of contact insecticides

Various insect species can be found in storage facilities and it is not possible for a single insecticide to be highly effective against all of them (Collins, 2006). One explanatatory reason is that stored-product insects have different degrees of intrinsic tolerance of contact insecticides. For example, *Sitophilus* species are significantly more susceptible to OP insecticides than to PYs. On the other hand, *R. dominica* is very susceptible to PYs, while less so to OPs, particularly to pirimiphos-methyl. Also, *T. castaneum* is generally more susceptible to PYs and less susceptible to OP insecticides, especially to malathion, so that the species quickly develops resistance to that compound (Kljajić and Perić, 2006; Andrić et al., 2010; Rumbos et al., 2013). It is therefore mostly OPs and PYs that are combined due to their synergism and enhanced efficacy, while resistance is harder to develop because of their different mechanisms of activity (Subramanyam and Hagstrum, 1996). In combining OPs and PYs, their synergism is strongest immediately after treatment, while residual activity carries no significant benefits, or antagonism (Daglish et al., 1996; Daglish, 1998). Nevertheless, additive effect does not need to be evident always in combinations of insecticides, as in the case of the combination of spinosad with methoprene (Athanassiou et al., 2011) or spinosad with spinetoram (Athanassiou and Kavallieratos, 2014). Mixtures of OPs and PYs, such as fenitrothion + cyfluthrin, fenitrothion + fenvalerate, pirimiphos-methyl + deltamethrin and pirimiphos-methyl + permethrin, are already being used to control stored-product insects (Obeng-Ofori, 2010). Such mixtures have become a necessity in some countries after OPs were put under increasing restrictions, so that, for example, chlorpyrifos-methyl may now only be used in the U.S. in a mixture with deltamethrin at a far lower rate than in the past (Sehgal et al., 2013a).

Mixtures of contact insecticides are also available as a method to control resistant populations but, just like some other methods used for resistance management, they also have their advantages and flaws. Summing up the current knowledge on the subject, Subramanyam and Hagstrum (1996) pointed at the special importance of the ratio of insecticides in mixtures as heterozygous insects may survive insecticide treatments at lower (sublethal) rates and trigger resistance to them. Insecticide mixtures should therefore be used when resistance is weak, while insecticide rotation is a better solution when strong resistence is detected through monitoring. However, the potentials of insecticide mixtures may be most clearly understood by testing them on field and resistant populations. A recent study has shown that a combination of chlorpyrfos-methyl (3 mg/kg) and deltamethrin (0.5 mg/kg) caused 100 % mortality and offspring reduction in field populations of *R. dominica*, *T. castaneum* and *O. surinamensis*, while spinosad (1 mg/kg) achieved high efficacy only against field populations of *R. dominica* (Sehgal et al., 2013a). Treatments of concrete surfaces with mixtures of chlorpyrfos-methyl and deltamethrin have also demonstrated high efficacy against field populations of *R. dominica*, *T. castaneum* and *O. surinamensis*, while β-cyfluthrin has been highly effective only against *R. dominica* populations (Sehgal et al., 2013b). Testing the efficacy of binary combinations of chlorpyrifos-methyl (5 and 10 mg/kg), spinosad (0.6 mg/kg) and s-methoprene (0.6 mg/kg) against five species of stored-grain beetles resistant to OPs, PYs and methoprene, Daglish (2008) found the combination of chlorpyrifos-methyl (10 mg/kg) and spinosad (1 mg/kg) to be the most effective against all populations except the OP-resistant *O. surinamensis*, and the combination of chlorpyrifos-methyl (10 mg/kg) and s-methoprene (0.6 mg/kg) against all except the methoprene-resistant *R. dominica*. The author noted in conclusion that it was very difficult to find a perfect combination of insecticides to control all resistant populations. The available data indicate that country-wide monitoring of the levels of susceptibility of stored-product insect populations is crucial, and a basis for a adequate choice of insecticides and doses in mixtures.

4. Combination of DEs and contact insecticides

DEs offer a promising alternative to conventional grain protectants but they are still not widely used. The main reason is the fact that, depending on the type of DE formulation, as well as target species, commodity and environmental factors (temperature and relative humidity), high efficacy against storage insect pests requires rates exceeding 1,000 ppm, which have a significant impact on the physical qualities of grains. As a result of this fact and a trend to intensify the use of DEs, their potential has been frequently examined in combinations with other grain protectants, mostly at low doses of insecticides. DE doses are significantly reduced in mixtures, and consequently the negative effect on grains. An advantage is that mixtures combine at least two different modes of action, desiccation and chemical toxicity. Several studies have shown a significant potential and synergism of combinations of DE and insecticides. Arthur (2004a) found the formulation F2 (active ingredients: 0.03% deltamethrin, 0.37% piperonyl butoxide, and 0.95% chlorpyriphos-methyl, plus 10% mineral oil and 88.0% diatomaceous earth Protect-It) to be extremely effective in wheat, maize and paddy rice at the rate of 100 ppm/kg against *S. oryzae*, *S. zeamis*, *R. dominica* and *T. castaneum*. In that study, the combination of *s*-methoprene and DE produced an additive effect and reduced the concentrations of both components required to suppress the progeny of *R. dominica*, compared to each insecticide individually (Arthur, 2004b). A significant synergism was found in a combination of DE (250 ppm) and beta-cyfluthrin (0.125 or 0.25 ppm) for control of *T. castaneum* and especially of *S. oryzae* (Athanassiou, 2006). Two diatomaceous earth (DE) formulations enhanced with abamectin are highly effective against storage coleopterans at the

rates of 75-125 ppm (Athanassiou and Korunic, 2007; Athanassiou et al., 2006, 2007). Korunic et al. (2010) found a formulation based on DE and deltamethrin and applied at 100 ppm (100 ppm contains 0.1 ppm of deltamethrin and 90 ppm of DE) to have a high residual efficacy against *S. oryzae*, *R. dominica* and *T. castaneum* even 12 months after treatment.

All these studies used laboratory populations of stored-product insects, a fact which raises the question of efficacy of the ratios of DE and insecticides in different formulations in practice and against field or resistant populations of stored-product insects. Research results have shown so far that such formulations have strong initial efficacy but the rate of insecticide degradation and the effect of DE on degradation are still not clear enough. Fast degradation is a problem as it would decrease its residual efficacy and cause resistance to develop through insect exposure to sublethal doses of insecticides (Subramanyam and Hagstrum, 1996). Formulations based on DE and insecticides are assumed to be able to control all resistant populations of insects in storages except those resistant to the insecticide used in the mixture or some related compound. Field populations and those from different parts of the world show different levels of susceptibility to DE (Rigaux et al., 2001; Kavallieratos et al., 2007; Rojht et al., 2010; Athanassiou et al., 2011, 2014) and insecticides (Kljajić and Perić, 2006; Athanassiou et al., 2008), which makes it especially important to determine the optimal ratio of DE and insecticides in any mixture formulation or in combined use. This has also been confirmed by studies of the efficacy of DE + spinosad combinations against European populations of *T. confusum* (Vayias et al., 2009).

5. Combination of DEs and botanicals

Many plant derivates, such as essential oils, have a potential to control stored-product insects, but they are sometimes unacceptible because of their instability and high doses. The pyrethrins from *Chrysanthemum cinerariifolium* and azadirachtin from *Azadirachta indica* have become prominent for being highly effective in storages at low rates (Weaver and Subramanyam, 2000; Athanassiou et al., 2005; Kavallieratos et al., 2007). Several studies have shown that some botanicals readily improve the efficacy of DEs, which makes DE more acceptible. A combination of essential oil of *Allium sativum* and DE has shown a strong synergistic effect and high initial efficacy against *S. oryzae* (150 ppm oil + 250 ppm DE) and *T. castaneum* (20 ppm oil + 250 ppm DE) (Yang et al., 2010). Monoterpenoids as components in many plant essential oils are also able to increase DE efficacy significantly. The chemical cinnamaldehyde, an active constituent of *Cinnamonum verum*, and eugenol originating from *Syzygium aromaticum*, in combination with DEs significantly increase the DE toxicity to *S. oryzae* and the cowpea weevil *Callosobruchus maculatus* (F.) (Islam et al., 2009). A DE formulation modified with bitterbarkomycin (BBM) from the root of the plant species *Celastrus angulatus* has demonstrated a high efficacy against several storage pests at very low doses (less than 150 ppm) (Athanassiou et al., 2008, 2009). Current research results are encouraging and giving hope that successful formulations of DEs and botanicals for control of storage pests may be expected in the near future. An advantage of such combinations is that both components are naturally occurring, but further research into the toxicity and acceptability of botanicals to be used is still needed.

6. Conclusions

Solving the problem of resistance of stored-product insects to conventional grain protectants is a complex task. Monitoring the susceptibility of insects in storages to the insecticides used in practice, as well as the levels of susceptibility, and choosing the effective alternatives are the most important segments of anti-resistant tactics. The fact that only two DE insecticides and

spinosad have been recognized over the past 20 years as promising alternatives to conventional grain protectants adds to the difficulty of the current situation. Combined use of grain protectants has therefore become a realistic option for resistance management which, as many other approaches, has its advantages and faults, while a perfect combination remains an unattained ideal. Besides the existing combinations of insecticides, many studies have shown that combining DE with low doses of insecticides or botanicals has realistic potentials in storage facilities. It is important to note that several studies have warned of considerable differences in the susceptibility of field populations to insecticides that are still used in storages, which points at a need to focus on field or resistant populations of stored-product insects in research so as to be able to show the potential of insecticides and their combinations. Choosing the most adequate grain protectants and their doses is a crucial issue in creating mixtures and it requires an integrated approach of the science, chemical industry and grain industry in order to find out the best solution.

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