Chapter 3 Insect Pest Management in Stored Grain

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Introduction

Once cereal grain is harvested and put into storage, it provides a resource for a range of insect pests of stored grain. With the exceptions of Sitophilus zeamais Motschulsky (the maize weevil) (Giles and Ashman [1971\)](#page-15-0), *Prostephanus trunctatus* (Horn) (the larger grain borer) (Tigar et al. [1994\)](#page-18-0), Sitotroga cerealella (Olivier) (Lepidoptera: Gelechiidae) (the Angoumois grain moth) (Trematerra [2015\)](#page-18-0), these insects rarely attack grain in the field before harvest, but once grain is in storage there is a degree of inevitability that insect infestation will occur. Insects can be carried into storage via infested harvesters or other machinery (Sinclair and White [1980\)](#page-17-0), and infested grain can be moved from storage to storage during the postharvest

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handling of grain (Perez-Mendoza et al. [2004](#page-17-0)). Even without human help, field studies have demonstrated the importance of flight in some species, and so flying insects represent another source of infestation (e.g. Mahroof et al. [2010](#page-16-0); Ridley et al. [2011a](#page-17-0), [b\)](#page-17-0). This means that methods are needed to either disinfest grain or to protect it from infestation during storage. The aim of this chapter is to review recent advances in insect pest management in stored grain, focussing primarily on research published in the last 10 years on chemical and non-chemical methods, ranging from methods that are well established to those that are still being evaluated. As many papers have been published on this broad topic, sometimes we cite only a few papers from the many published to illustrate particular recent advances.

Disinfestation

Insecticides

For many years, the dichlorvos was available for grain disinfestation, but this organophosphate (OP) is being phased out because of concerns about its chronic health effects. Dichlorvos had the advantage over other OPs used on stored grain of having considerable vapour action (Desmarchelier and Banks [1977\)](#page-14-0), so it was a useful treatment in situations where insufficient gas tightness was achievable for fumigation to be effective, though resistance in Rhyzopertha dominica (F.) (the lesser grain borer) has long been a concern (Zettler and Cuperus [1990](#page-18-0)). Dichlorvos served a valuable purpose, and the loss of this insecticide has created a need for a rapid disinfestant for situations where fumigation is not feasible because of poor gas tightness or resistance to phosphine.

Fumigation

Phosphine and methyl bromide continue to be the main fumigants used in stored products after many decades of use and despite issues facing each. For phosphine, there has been a continued development of resistance in a range of species, and much of the recent research on phosphine has been about understanding the impact of resistance and providing a basis for improved fumigation ensuring control of resistant types. In the case of methyl bromide, the restricted access to this fumigant has stimulated the search for alternatives (Bell [2000](#page-14-0)), some of which have been registered for use on stored grain.

Although phosphine resistance was detected in many countries around the world in the 1970s, it was relatively uncommon at that time (Champ and Dyte [1976](#page-14-0)), but studies since then have shown that the problem is increasing. This is demonstrated by the fact that populations with resistant individuals can be common (e.g. Opit

et al. [2012](#page-17-0); Daglish et al. [2014,](#page-14-0) [2015](#page-14-0)), and that newer or stronger types of resistance are emerging (e.g. Lorini et al. [2007](#page-16-0); Pimentel et al. [2009;](#page-17-0) Jagadeesan et al. [2012;](#page-15-0) Opit et al. [2012](#page-17-0); Nayak et al. [2013](#page-17-0)). Obviously, the detection of phosphine resistance raises concerns about its impact on fumigations, so, many recent papers have investigated the practical significance of phosphine resistance. Daglish et al. [\(2002](#page-14-0)) fumigated mixed-age populations of Sitophilus oyzae (L.) (the rice weevil) to determine times to population extinction at constant concentrations. An important finding was that the expression of resistance (i.e. time to population extinction) depended on the concentration used. They tested a susceptible strain, a weakly resistant strain and a strongly resistant strain, and found that differences between strains were greatest at low concentrations and negligible at high concentrations such as 1.5 mg L^{-1} (\approx 1000 ppm). For at least one species, therefore, resistant insects would be phenotypically susceptible in short fumigations with sufficiently high concentrations. To a lesser extent, this was also the case with mixed-age populations of susceptible, weakly resistant and strongly resistant R. dominica (Lorini et al. [2007\)](#page-16-0). In recent years, researchers have also investigated times to population extinction in psocids of the species Liposcelis bostrychophila Badonnel (Nayak and Collins [2008\)](#page-16-0) and Cryptolestes ferrugineus (Stephens) (the rusty grain beetle) (Kaur and Nayak [2015](#page-15-0)). By far the greatest threat is strongly phosphine resistant C. ferrugineus, with an estimated time to population extinction of 10 days at 2 mg L⁻¹ (\approx 1400 ppm) and 25 °C. Nayak et al. [\(2003\)](#page-16-0) demonstrated the unique ability of the eggs of the psocid Liposcelis bostrychophila Badonnel to delay its development under phosphine fumigation as a way to survive a fumigation and emerge to adults, several days after the end of fumigation. These authors have recommended that to control these psocids using phosphine, a relatively low concentration of phosphine should be applied for extended exposure times (e.g. 0.05 mg L^{-1} for 16 days) that should allow all eggs to hatch to the much less tolerant nymph stage.

Several studies have been published recently on phosphine fumigation trials and these have had a focus on controlling resistant insects. Rajendran and Muralidharan [\(2001](#page-17-0)) carried out trials on bagged paddy rice stored outdoors and inside warehouses in southern India. There was considerable loss of phosphine from the sheeted bag stacks, despite using new sheets and ensuring that sheets were weighed down with sand snakes, and this was particularly so for the outdoor stacks which had lost phosphine at about twice the rate that indoor stacks did. The authors attributed the loss of phosphine to several factors including sorption by the paddy rice, and permeation and leakage through the fumigation covers, and they suggested that the registered application rate of 3 g t⁻¹ of phosphine (released from aluminium phosphide) was probably insufficient to control resistant populations. In another paper, Wang et al. [\(2006](#page-18-0)) reported the results of a Chinese study on phosphine fumigation of paddy rice in sheeted indoor bag stacks. The application rate was 1.67 g m⁻³ of phosphine (released from aluminium phosphide) based on the enclosed bag stack volume but the mean maximum concentration was about 50% lower, and the authors attributed this mainly to sorption. Although all natural and caged insects were dead after 21 days of fumigation, the authors raised the

possibility that resistant pupae might have survived had they been tested. Recently, Ridley et al. [\(2011a,](#page-17-0) [b\)](#page-17-0) reported Australian trials of phosphine fumigation of wheat stored in silo bags fumigated with an application rate of 2.1 g t⁻¹ of phosphine (released from aluminium phosphide). The fumigations were judged to be effective in controlling strongly phosphine resistant R. dominica based on examination of cages containing all developmental stages after the 17-day fumigation period. Carpaneto et al. [\(2016\)](#page-14-0) conducted silo bag trials in Argentina showing that silo bags were often insufficiently gas-tight for phosphine fumigation and made a recommendation for improving gas retention.

Phosphine sorption by grain has been suggested to play a role in the loss of phosphine observed during fumigation field studies (e.g. Rajendran and Muralidharan [2001](#page-17-0); Wang et al. [2006](#page-18-0); Ridley et al. [2011a](#page-17-0), [b](#page-17-0)), and several recent laboratory studies provide some insights into the practical impact of this process. By fumigating a range of cereal grains, Reddy et al. [\(2007](#page-17-0)) showed that sorption reduced the likelihood of target terminal concentrations being achieved. Daglish and Pavic [\(2008](#page-14-0)) investigated effect of dose (based on the volumetric capacity of fumigation vessel) on sorption in wheat. They found that the daily percentage decline in gaseous phosphine was negatively correlated with dose, and that re-fumigated wheat was less sorptive. In a subsequent study, they showed that wheat was less sorptive the longer it was stored before being fumigated for the first time (Daglish and Pavic [2009](#page-14-0)). The sorption was faster at 25 °C than 15 °C, and the decline in sorptive capacity was greater when grain was stored at 25 $^{\circ}$ C than at 15 °C. They confirmed that re-fumigated wheat tended to be less sorptive, but they concluded that re-fumigated wheat was less sorptive because it had been in storage longer. The practical implications of these studies are that the impact of sorption on phosphine fumigations will be greatest in low dose fumigations or fumigations of freshly harvested grain.

Methyl bromide has received very little research attention in recent years, and this may be partly because of the focus on reducing its use because of its status as an ozone-depleting substance. One exception is the work of Athanassiou et al. [\(2015](#page-13-0)) investigating methyl bromide efficacy under laboratory conditions against five psocid species: Liposcelis paeta Pearman, L. entomophila (Enderlein), L. decolor (Pearman), L. bostrychophila and Lepinotus reticulatus Enderlein. They showed that eggs were more tolerant than mobile stages and identified concentrations of methyl bromide needed to control psocids in 48 h fumigations in the absence of significant amounts of grain. Further laboratory or field investigation of methyl bromide efficacy in the presence of grain would be valuable because sorption by grain can greatly reduce the fumigant concentration (e.g. Cherif et al. [1985\)](#page-14-0).

Sulfuryl fluoride, although initially used for fumigating mills and other structures to control stored product insects, is now being used to fumigate grain. Recent research has focused on control of insects and sorption in laboratory studies. Although some laboratory efficacy studies were conducted with structural fumigation in mind, the results are useful with regard to sulfuryl fluoride fumigation of grain. Bell and Savvidou ([1999\)](#page-14-0) estimated combinations of concentration and

exposure period needed to control eggs of Ephestia kuehniella Zeller (the Mediterranean flour moth) and Baltaci et al. [\(2009](#page-13-0)) did the same for eggs and other stages of E. elutella (Hübner) (the warehouse moth). They found that tolerance to sulfuryl fluoride varied greatly with egg age and that sulfuryl fluoride was less effective at lower temperatures. Athanassiou et al. ([2012\)](#page-13-0) investigated sulfuryl fluoride efficacy against five psocids (L. paeta, L. entomophila, L. decolor, L. bostrychophila and L. reticulatus) and showed that eggs were more tolerant than nymphs or adults. Jagadeesan et al. [\(2015\)](#page-15-0) compared the responses of susceptible and strongly phosphine resistant strains of Tribolium castaneum (Herbst) (the red flour beetle) to sulfuryl fluoride. They found that eggs were the most tolerant stage and there was no cross-resistance between phosphine and sulfuryl fluoride, indicating the potential for the latter in managing phosphine resistance. Studies like these help build a picture of sulfuryl fluoride efficacy against stored grain insects but carefully monitored field trials are needed. Although many field trials have been conducted evaluating the efficacy structural fumigations (e.g. Campbell et al. [2010](#page-14-0)) they do not provide data helpful to understanding sulfuryl fluoride efficacy against insects in grain fumigations. Opit et al. ([2016\)](#page-17-0) conducted trials in the USA in small metal bins containing wheat and assessed efficacy against R. dominica and T. castaneum in the wheat or in muslin bags in the wheat. The target dose was a concentration x time product of 1500 mg-h L^{-1} and the fumigations lasted up to 1 day. High levels of control were achieved against both species but sulfuryl fluoride was more effective against R. dominica (complete control) than T. castaneum (some survival). Nayak et al. ([2016\)](#page-17-0) conducted trials in Australia in bunkers (sheeted grain piles) and a concrete silo, and assessed efficacy against natural infestations and caged containing mixed-age populations. The target dose was a concentration x time product of 1500 mg-h L^{-1} and the fumigations lasted up to 14 days. Complete control was achieved of natural infestations and caged populations. The species controlled included R. dominica, T. castaneum, C. ferrugineus and S. oryzae; although not all species were present in all fumigation trials.

Two recent studies investigated the potential role of sulfuryl fluoride sorption by grain. Sriranjini and Rajendran [\(2008](#page-17-0)) fumigated a range of grain types with sulfuryl fluoride in the laboratory and showed that sorption reduced the amount of fumigant gas in all cases. Subsequently, experimentation on wheat showed that sulfuryl fluoride is sorbed faster than phosphine but slower than methyl bromide, and temperature is a major factor affecting sorption with faster sorption occurring at higher temperature (Hwaidi et al. [2015\)](#page-15-0). These studies show that, as with other fumigants, sorption by grain can be expected to contribute to gas loss during commercial fumigations.

Two other fumigants that have progressed from research to registration for grain are ethyl formate and carbonyl sulphide. In the case of ethyl formate, recent research comprised Australian research on ethyl formate applied as a liquid and ethyl formate applied as a vapour with carbon dioxide. Caged insects were inserted into the grain to assess fumigant efficacy. Trials in farm bins showed that ethyl formate applied as a liquid had the potential for use on stored grain (Ren and Mahon [2006](#page-17-0)). In that study, ethyl formate was applied in two stages to avoid the

problem of rapid sorption reducing exposure of insect to lethal concentrations. Complete control of key pests such as Sitophilus and Tribolium species was achieved. Laboratory research was also conducted on combining ethyl formate with carbon dioxide to improve penetration through the grain mass, reduce sorption, increase efficacy and reduce flammability (Haritos et al. [2006](#page-15-0); Damcevski et al. [2009;](#page-14-0) Dojchinov et al. [2009](#page-15-0)). Ren et al. [\(2008](#page-17-0)) demonstrated the efficacy of carbonyl sulphide applied as a liquid to wheat in a large concrete silo in Australia, with complete control possible of caged of mixed-age populations of the key pests R. dominica, T. castaneum and S. oryzae. Research on ethyl formate and carbonyl sulphide has led to the registration of cylinderised formulations of carbonyl sulphide and ethyl formate + carbon dioxide. To our knowledge, however, only the latter is available in the market.

Several recent field studies have focussed on determining the strengths and weakness of fumigating bulk grain with ozone gas. The major challenge in using ozone is to optimise its application so as to maximise its penetration through the grain bulk so that high levels of mortality can be achieved before it degrades into oxygen. A pilot study using recirculation showed the rapid decay of ozone as it passed through bulk wheat highlighting a practical challenge (Hardin et al. [2010\)](#page-15-0). Kells et al. ([2001\)](#page-15-0) evaluated ozone against caged adults in maize at doses of up to 50 ppm and 5 days of exposure. They found that efficacy varied across species and complete mortality was difficult to achieve. Tribolium castaneum, for example, was harder to control than *S. zeamais*. Similarly, Bonjour et al. ([2011\)](#page-14-0) evaluated ozone against bags of insects in wheat at doses of up to 70 ppm and 4 days of exposure. They found that efficacy varied across species. Rhyzopertha dominica, C. ferrugineus and O. surinamensis, for example, were harder to control than S. oryzae. These studies show that ozone has potential but that complete control of all species may not be possible.

Other Methods

Considerable research has been undertaken on heat disinfestation of stored products. The concept of the application of heat treatment is simple: temperature is increased until it reaches a lethal level for insects; this level is considered to be 50 °C (Mahroof et al. [2003a](#page-16-0), [b;](#page-16-0) Yu et al. [2011\)](#page-18-0). Nevertheless, reaching this lethal threshold is not always easy, as different structures have different physicochemical properties that are expected to negatively influence the overall efficacy of the application (Yu et al. [2011\)](#page-18-0). Quantification of the responses of different species and developmental stages within species to flameless catalytic infrared radiation has been the focus of several recent studies (Khamis et al. [2010](#page-15-0), [2011a](#page-15-0), [b\)](#page-15-0), and another study suggests that there are no negative effects of this treatment on grain (Khamis et al. [2010,](#page-15-0) [2011c\)](#page-16-0). Elevated temperatures have been also used tested successfully in combination with other methods, such as diatomaceous earths (Dowdy and Fields [2002\)](#page-15-0), contact insecticides (Kljajic et al. [2009](#page-16-0)) or nitrogen (Athanassiou et al. [2016a\)](#page-13-0).

Although there are numerous techniques to implement controlled or modified atmospheres, nitrogen seems to be the most promising agent for disinfestation of stored grain. Given that approximately 80% of the atmosphere contains nitrogen, usually there is no need to use gas in cylinders, but only nitrogen generators or pumps that can take the nitrogen from the air and introduce the gas in the area that is to be treated (Navarro et al. [2012a](#page-16-0), [b\)](#page-16-0). The basis of this application is to reduce oxygen, usually to less than 1% (Adler et al. [2000;](#page-12-0) Athanassiou et al. [2016b](#page-13-0)). In practice, nitrogen can be applied either on commodities, in designated areas that are made for this purpose, i.e. nitrogen chambers, or on target facilities, e.g. a silo or a warehouse, either empty or with products (Navarro [2006](#page-16-0), [2012](#page-16-0); Navarro et al. [2012a](#page-16-0)). For some commodities, it is now known that the application of nitrogen does not affect some key organoleptic properties, while, under certain circumstances, can also reduce microbial load (Navarro [2012](#page-16-0); Navarro et al. [2012b;](#page-16-0) Athanassiou et al. [2016b](#page-13-0)).

There are numerous other methods that have been utilised for disinfestation. One method that gains in importance is the use of pheromones for moth suppression through mating disruption. This concept has been successfully tested in storage and processing facilities in various types of commodities and facilities in Europe, and found effective in suppressing populations of Ephestia spp. or Plodia interpunctella (Hübner) (the Indian meal moth) (Pyralidae). Currently, there are several formulations that are commercially available for moths (Trematerra et al. [2011](#page-18-0), [2013;](#page-18-0) Athanassiou et al. [2016c\)](#page-13-0), while there are recent efforts to develop the first formulation for beetles, using *Lasioderma serricorne* (F.) (the cigarette beetle) (Anobiidae) as a target species (Mahroof and Phillips [2014\)](#page-16-0).

Apart from heat treatment, there is a renewed interest for the use of 'cold treatments'. Insects are generally tolerant to cold, so care should be taken to calculate the target temperature and the required exposure interval that is needed to obtain complete control, without affecting the commodity (Fields [1992;](#page-15-0) Flinn et al. [2015\)](#page-15-0). Generally, the most difficult to control life stage is the egg (Fields [1992;](#page-15-0) Johnson and Valero [2003\)](#page-15-0).

Many papers have been published on the potential of essential oils extracted from a wide range of plant species, and the vast majority of these report preliminary screening. Rajendran and Sriranjini ([2008\)](#page-17-0) reviewed the literature on essential oils, highlighting their potential but also a number of constraints, including lack of data on sorption, tainting and residues in food. Research on allicin, one the components of garlic essential oil, is an example of research that that has gone beyond preliminary screening. Lu et al. [\(2013](#page-16-0)) investigated fumigant toxicity of allicin in the laboratory against different developmental stages of three insect species, showing that the presence of wheat reduced efficacy because of sorption. Laboratory studies like this one provide more practical information than preliminary screening studies, but studies on bulk grain stored under realistic are also needed.

Other disinfestation treatments such as carbon dioxide, changes in pressure, and micro-biocontrol agents, are briefly discussed in other chapters of this book.

Protection

Cooling

Temperature management is an important component for insect pest management in stored bulk grains, particularly in temperate regions of the world. Aeration is described as using ambient air to cool the grain mass to temperatures that will limit insect population growth. A common threshold is 15 \degree C, which is the lower limit of development for most stored product insects (Howe [1965](#page-15-0); Fields [1992\)](#page-15-0). Typical airflow rates used are $0.0515-0.31$ m³/min/metric tonne (t), depending on the specific commodity and harvest date (Reed and Arthur [2000](#page-17-0); Navarro et al. [2012c\)](#page-16-0). Aeration is not to be confused with grain drying, which uses airflow rates several orders of magnitude higher than those used for aeration, with the emphasis on drying grain to moisture contents required for long-term storage (Reed and Arthur [2000\)](#page-17-0). Aeration does not necessarily eliminate an existing infestation, and the most optimal use of aeration involves usage with other components in a management plan.

There are several established methodologies for utilising aeration, and most involve using some type of controller to activate aeration fans thresholds (Arthur and Casada [2010\)](#page-12-0). In Australia, aeration controllers were developed that used web-bulb temperatures for precise activation and optimization of moisture management (Wilson and Desmarchelier [1994](#page-18-0)). In the USA, more simple controllers are used that activate fans when temperatures fall below a specified threshold (Ranalli et al. [2002](#page-17-0); Arthur et al. [2008](#page-13-0); Arthur and Casada [2005](#page-12-0), [2010\)](#page-12-0). There are several recent papers that give a detailed review of aeration strategies, one, in particular, is Navarro et al. [\(2012c\)](#page-16-0), which is a chapter in the book "Stored Product Management", available from the Kansas State University ([www.k-state.edu\)](http://www.k-state.edu). Other publications can be found that also give a more detailed review of aeration, along with numerous references, that readers can consult for more information (Navarro et al. [2012c](#page-16-0)).

There are several recent advances in utilising aeration for insect pest management, including historical weather data to predict hours available for cooling stored grains in different geographic regions (Arthur and Johnson [1995](#page-12-0); Arthur and Siebenmorgen [2005](#page-12-0)). These studies examined the various hours necessary for cooling grains at different airflow rates at specified temperature thresholds, and predicted hours needed for cooling. Analysis was done by using a q-Basic code that used daily high and low temperatures, and sunrise and sunset data, to predict temperature each hour (Arthur and Johnson [1995](#page-12-0)), and was subsequently utilised for a number of successive studies based on the same procedures. These procedures can be adapted for any site in the world that has similar temperature and sunset data, and is an excelled method for examining the feasibility of aeration.

Analysis of weather data as described above can be incorporated into insect pest population models to predict the impact of aeration on population development (Arthur et al. [1998,](#page-13-0) [2001](#page-13-0), [2011a](#page-13-0), [b;](#page-13-0) Arthur and Flinn [2000](#page-12-0)). It can also be used to

predict a number of insect generations that could occur in a given time period (Arthur et al. [2003](#page-13-0)). Results of these model simulation studies show the benefits of using aeration, particularly in warm regions where winter temperatures are not normally low enough to cool the grain mass. In the USA, a web-based management system was developed for stored rough rice (also known as paddy rice) that includes aeration along with other management options (Arthur et al. [2011a,](#page-13-0) [b\)](#page-13-0). Although the recent modelling efforts have mainly been done in the USA, historical weather data are available worldwide, and can be utilised in a similar manner to incorporate aeration into management plans for bulk grains.

Another recent advance in aeration is using defined cycles to cool a grain mass, and incorporating an initial cooling cycle in advance of what can be accomplished in waiting for temperatures to cool so that the specified threshold of 15 \degree C can be achieved. Arthur and Casada ([2005\)](#page-12-0) conducted studies in experimental grain bins and showed that using a summer cooling cycle at 22° C resulted in lower pest populations in stored wheat compared to waiting to use the standard first autumn cooling cycle of 15 °C. Another aspect for consideration is using suction aeration, which pulls the cool air down through the grain mass, rather than the standard pressure aeration, which pushes the cool air upward through the grain mass (Arthur and Casada [2010](#page-12-0), [2017](#page-12-0)). Cooling the top portion resulted in lower temperatures, which in turn led to lower insect populations, as assessed through the use of probe traps (Arthur and Casada [2010](#page-12-0), [2017\)](#page-12-0). However, in the Arthur and Casada [\(2017](#page-12-0)) study, high infestation rates during the third year of a 3-year study mitigated the positive effects of aeration. One possible reason for those high infestations was the fact that the grain could have been infested when it was loaded into the bins for the third year of the study. Thus, aeration should be considered as a component of integrated management plans, not as a direct killing agent such as fumigation. It is unlikely that aeration alone will eliminate an existing infestation.

Grain Protectant Insecticides

Grain protectants continue to play a major role in protecting grain during storage. The scientific literature on grain protectants is extensive and can only be reviewed very selectively here. As with the fumigant phosphine, the continued development of resistance in a range of species has provided much of the impetus for research on protectants. Malathion resistance was already of concern in the 1970s (Champ and Dyte [1976](#page-14-0)) and other resistances have developed since then. Pyrethroid resistance has developed in R. *dominica* (Lorini and Galley [1999](#page-16-0)), T. *castaneum* (Collins [1990\)](#page-14-0) and S. zeamais (Guedes et al. [1995\)](#page-15-0); and methoprene resistance has developed in R. dominica (Daglish et al. [2013](#page-14-0)).

Despite extensive research on potential new grain protectants in recent years (see below), only one new grain protectant from a new chemical group has been registered and adopted. Spinosad was developed from the fermentation products of a bacterium found in soil (Salgado [1998\)](#page-17-0), before being evaluated as a grain protectant. Since 2002, many papers have been published on the susceptibility of a wide range of stored grain insects to grain treated with spinosad, including strains that were resistant to other grain protectants (e.g. Fang et al. [2002a,](#page-15-0) [b;](#page-15-0) Nayak et al. [2005;](#page-17-0) Vayias et al. [2009\)](#page-18-0). These studies showed that R. dominica is much more susceptible than other major pest species, including strains of R. dominica that are resistant to other grain protectants. Other laboratory studies showed that spinosad remains active against this species for long periods of storage (Fang and Subramanyam [2006;](#page-15-0) Daglish and Nayak [2006](#page-14-0)). Long-term efficacy of spinosad on bulk-stored wheat was demonstrated in silo-scale studies in the USA and Australia, with wheat treated at an application rate of 1 mg kg^{-1} which ultimately became the registered rate for this protectant (Fang et al. [2002a,](#page-15-0) [b](#page-15-0); Flinn et al. [2004;](#page-15-0) Subramanyam et al. [2007;](#page-17-0) Daglish et al. [2008](#page-14-0)). Spinosad residues were shown to be relatively stable on stored wheat in field studies in the USA and Australia (Fang et al. [2002a,](#page-15-0) [b](#page-15-0); Subramanyam et al. [2007](#page-17-0); Daglish et al. [2008](#page-14-0)), which matched the results from the laboratory (Daglish and Nayak [2006](#page-14-0)). Due to its widespread use by the Australian grain industry, a discriminatory dose of 1 mg kg⁻¹ has been established to monitor for potential resistance development in R. dominica, based on an investigation of the base-line susceptibly of field populations of this species (Nayak and Daglish [2017\)](#page-16-0).

A major and long-standing challenge with grain protectants is finding a single protectant or combination of protectants that will provide protection against the pest species of concern. One reason is that there can be large interspecific differences in susceptibility to grain protectants, and another is the development of resistance. This problem can be illustrated by a laboratory study from Australia looking at the efficacy of three grain protectants applied alone or in binary combinations against resistant strains of five pest species (Daglish [2008](#page-14-0)). The five species were R. dominica, T. castaneum, C. ferrugineus, S. oryzae and Oryzaephilus surinamensis (L.) (the saw-toothed grain beetle). The three grain protectants were chlorpyrifos-methyl (an organophosphorus compound), s-methoprene (a juvenile hormone analogue) and spinosad (a biopesticide). At the time of the study, chlorpyrifos-methyl and s-methoprene were registered in Australia and spinosad was under consideration. The most effective combinations were spinosad (1 mg kg^{-1}) + chlorpyrifos-methyl (10 mg kg^{-1}) which controlled all strains except for OP-resistant O. surinamensis, and chlorpyrifos-methyl (10 mg kg⁻¹) + s-methoprene (0.6 mg kg^{-1}) which controlled all strains except for methoprene-resistant R. dominica. The result is that spinosad is usually applied in Australia in combination with chlorpyrifos-methyl and s-methoprene. In another study, Nayak and Daglish ([2007\)](#page-16-0) have shown the advantage combined treatment of spinosad and chlorpyrifos-methyl to control four *Liposcelis* psocid species that have shown variable levels of resistance to a range of currently registered grain protectants in Australia.

There are several commercial formulations available that combine more than one substance. One of the oldest ones in the market is Storicide II, which contains the OP chlorpyrifos-methyl with the pyrethroid deltamethrin. This combination has been found to be effective against stored-product insects (e.g. poscids) that are tolerant to other substances (Athanassiou et al. [2009a](#page-13-0)). In that study, the authors noted that psocids were susceptible to this combination, and hypothesised that this was mostly due to the presence of chlorpyrifos-methyl in the mixture, as psocids were found also susceptible to pirimiphos-methyl.

There have been many studies published on potential new grain protectants from a range of chemical groups, with most of these studies undertaken in the laboratory. Spinetoram, a spinosyn-based insecticide, has been evaluated in both short-term and long-term laboratory studies (Vassilakos et al. [2012,](#page-18-0) [2015\)](#page-18-0). Based on experience against insect pest of field crops, spinetoram was expected to have greater potency than spinosad but these studies show that spinetoram efficacy against stored grain beetles is broadly similar to that of spinosad. Despite the promising results for spinetoram, evaluation has not progressed to field trials. Other compounds that have been evaluated in the laboratory include ethiprole (a phenyl-pyrazole) (Arthur [2002\)](#page-12-0), imidacloprid (a neonicotinoid) (Daglish and Nayak [2012\)](#page-14-0), thiamethoxam (a neonicotinoid) (Arthur et al. [2004](#page-13-0)) and indoxacarb (an oxadiazine) (Daglish and Nayak [2012\)](#page-14-0). In general, all tested compounds show potential in the laboratory at varying doses depending on the species tested, but none have progressed to field trials.

There are several combinations of two or more active ingredients that can be used as grain protectants. For example, a combination of diatomaceous earth with natural pyrethrum and piperonyl butoxide was found more effective and with higher 'speed of kill' than other formulations that were based on diatomaceous earth alone (Athanassiou et al. [2004](#page-13-0); Athanassiou and Kavallieratos [2005\)](#page-13-0). Tucker et al. [\(2015](#page-18-0)) found that the insect growth regulator methoprene could be used successfully with synergized pyrethrin aerosols for the control T . *castaneum*. Arthur [\(2002](#page-12-0)) tested different combinations of ethiprole with other insecticides with satisfactory results for wheat and maize. Liu et al. [\(2016](#page-16-0)) showed the feasibility of using aeration with methoprene against a wide range of insect species. In general, there are numerous studies that showed that certain combinations may have some benefits in comparison with the application of a single active ingredient, as the range of species that can be controlled can be expanded (Daglish [2008](#page-14-0); Arthur [2012](#page-12-0); Athanassiou et al. [2009a](#page-13-0)). In this context, currently, there are several commercial formulations available for admixture with the grains, which combine two active ingredients with different modes of action, such as one OP, either pirimiphos-methyl or chlorpyrifos-methyl, and another substance, usually a pyrethroid (e.g. deltamethrin or cypermethrin) or a neonicotinoid (thiamethoxam). These combinations can also be used in order to mitigate insect resistance to certain insecticides, as these populations will be exposed to substances that have different modes of action (Daglish [2008\)](#page-14-0).

Field studies were completed in Australia on bifenthrin (a pyrethroid) (Daglish et al. [2003](#page-14-0)) and diflubenzuron (a chitin synthesis inhibitor) (Daglish and Wallbank [2005\)](#page-14-0) providing data on efficacy against key pest species and stability of residues. Bifenthrin which targeted R. dominica was tested in combination with chlorpyrifos-methyl so that the combination treatment would give broad spectrum control. Bifenthrin was seen as a potential replacement for bioresmethrin which had been withdrawn from the Australian market, but the results showed that bifenthrin failed to control pyrethroid-resistant R. dominica. Diflubenzuron targeted S. oryzae and proved to be effective against this species. Surprisingly, the diflubenzuron + s-methroprene resistant combination controlled methoprene-resistant R. dominica. Neither bifenthrin nor diflubenzuron progressed to registration, despite the promising results.

Plant products have been widely evaluated for stored product protection, but registered products for this use are extremely few. Detailed lists of the plant species that have been evaluated so far are given by Prakash and Rao ([1997\)](#page-17-0) and Weaver and Subramanyam ([2000\)](#page-18-0). There are different ways that these compounds can be used, but the most common evaluations refer to their repulsive activity and their insecticidal effect. For example, Arthur et al. $(2011a, b)$ $(2011a, b)$ $(2011a, b)$ $(2011a, b)$ reported that catmint oil could be utilised further as a repellent of T. castaneum and T. confusum. Neem oil, which is produced by the neem tree, *Azadirachta indica*, has also some repulsive activity to insects (Athanassiou et al. [2014](#page-13-0)) but most of the studies available are about its insecticidal value. The major active ingredient of neem, azadirachtin, is now registered as an insecticide on various crops. Athanassiou et al. ([2005\)](#page-13-0) found that azadirachtin was effective for the control of S. oryzae, R. dominica and T. confusum, but at dose rates that were much higher than the currently used grain protectants, which constitutes such an application unrealistic. Nevertheless, the future of plant extracts for stored product protection, especially for use as grain protectants, remains uncertain and should be regarded on the basis of additional research that goes far beyond their insecticidal value (Athanassiou et al. [2015\)](#page-13-0). Hence, even if a plant extract is proved effective as an insecticide, there will be always additional requirements that are needed for registration purposes, such as toxicological and ecotoxicological data. In this effort, the economics of the development of such plant product-based formulations, i.e. its cost-effectiveness, should always be examined in more detail.

One other parameter that should be taken into account when grain protectants are applied is their uneven distribution. In fact, uneven distribution may lead to the occurrence of zones within the grain bulks that are under-dosed or even untreated areas, which allow insect colonisation and progeny production. Daglish and Nayak (2010) (2010) demonstrated that the efficacy of s-methoprene against R. dominica was negatively influenced by an uneven application on wheat. Moreover, in that study, the authors suggested that this phenomenon may be related with selection of resistance. Similar results for the uneven distribution have also been reported in the case of spinosad (Athanassiou et al. [2009b](#page-13-0)) and spinetoram (Vassilakos and Athanassiou [2012](#page-18-0)).

Conclusions

The aim of this chapter was to review recent advances in insect pest management in stored grain, ranging from methods that are well established to those that are still being evaluated. This topic has been the subject of considerable laboratory and field research as evidenced by the large and growing body of published studies. Resistance to phosphine and various insecticides, as well as the phase out of methyl bromide as an ozone-depleting substance, continue to be major drivers for research on management of insects in stored grain. Other research has focussed on improving basic understanding of various technologies or ways of improving methods currently in use. Despite extensive research on a wide variety of chemical and non-chemical treatments, very few have been commercialised. Two examples are spinosad which has been registered as a grain protectant and sulfuryl fluoride which is now available as a grain fumigant. The interest in non-chemical treatments, especially aeration cooling, is encouraging. In general, integrated pest management is seen as the goal of entomologists, requiring the strategic integration of multiple methods to provide maximum effect, with minimal health and environmental risks. Some of the chemical and non-chemical treatments reviewed in this chapter have great potential to be used as part of an integrated approach.

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