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Potential of flavesone as a grain protectant: Long-term efficacy and residues for controlling the lesser grain borer, *Rhyzopertha dominica* (F.), in stored wheat

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ABSTRACT

A study was undertaken to evaluate the synthetic biopesticide, flavesone, as a potential grain protectant for the lesser grain borer, *Rhyzopertha dominica* (F.), in wheat. The search for new grain protectants is critical because of the propensity of *R. dominica* to develop genetic resistance to various insecticides such as organophosphates (OPs), carbamates, synthetic pyrethroids and juvenile hormone analogues. Wheat was treated with 0, 60, 90 or 120 ppm of flavesone as it passed through an auger into large (1 tonne) storage bags. The treated wheat was stored for 13 months under sheltered ambient conditions in southeast Queensland, and samples were also collected 1 week after treatment and stored for 13 months in the laboratory at 30 °C and 55% RH for comparative purposes. Bioassays of wheat stored under laboratory and ambient conditions, showed that an application rate of 60 ppm provided protection for at least 13 months from a susceptible strain, based on high levels of suppression of the F_1 generation. Untreated wheat stored under ambient conditions became heavily infested with *R. dominica*, while none were detected in any of the treatments, thus further confirming the potential of flavesone as an effective grain protectant. However, bioassays showed that the 60-ppm rate only provided 3 months protection from a strain that was resistant to OPs and pyrethroids, indicating potential non-target site resistance present in this strain. Higher application rates of 90 and 120 ppm were required for 13 months protection. As reported in some other grain protectant studies, initial flavesone residues were lower than the targeted rates, suggesting potential for improving formulation and application methods. Flavesone residues remained relatively stable throughout the study period. We believe that the data generated through this study will provide foundation in establishing field application rate for this new molecule and its possible registration for use by industry to mitigate resistance problems.

1. Introduction

Grain protectants have played a major role in managing the threat of insect infestation in stored grain since the 1960s (Arthur, 1996). They are applied to grain as it is loaded into storage facilities to provide long-term protection against invading insects. Residue levels of treated grain must, however, not exceed the national or international maximum residue limits (MRLs) (Arthur, 1996). One of the most widespread insect pests of stored grain is the lesser grain borer, *Rhyzopertha dominica* (F.), which infests a wide range of cereal grains, and causes considerable damage through the feeding of larval and adult stages (Edde, 2012). This species is present in all major grain growing regions in Australia (McCulloch et al., 2022). Grain protectants have been used for many decades to protect grain from *R. dominica* infestations, leading to this species developing resistance to a range of insecticides of different chemical groups. For example, field-derived resistance to organophosphorus insecticides including chlorpyrifos-methyl, fenitrothion, pirimiphos-methyl, and malathion has been reported from several countries including Australia (Collins, 2006; Daglish, 1998; Guedes et al., 1996, 1997; Navarro et al., 1986; Zettler and Cuperus, 1990).

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Moreover, field-derived resistance has developed in Australia and other countries to synthetic pyrethroids including bioresmethrin and deltamethrin (Chen and Chen, 2013; Collins et al., 1993; Daglish et al., 2003; Daglish and Nayak, 2018; Lorini and Galley, 1999, 2001; Ortega et al., 2021), and in Australian to the insect growth regulator methoprene (Daglish et al., 2013).

Due to the development of resistance in *R. dominica* to multiple grain protectants during the 1990s and early 2000s, the industry was in desperate need of new insecticides with unique modes of action. Several previous laboratory and field scale research experiments on a new biopesticide, spinosad, showed its excellent potential to control *R. dominica*, including resistant populations (Fang et al., 2002a, 2002b; Nayak et al., 2005, Subramanyam et al., 2007; Daglish et al., 2008), leading to the registration of this compound for use as a grain protectant in Australia. However, a recent study involving artificial selections in the laboratory indicated that resistance to spinosad could develop in field populations of *R. dominica* (Wang et al., 2018), highlighting the necessity to seek alternative grain protectants to address emerging challenges and ensure long term effective control strategies for the future.

Flavesone is a beta-triketone compound that occurs in nature in many plant species especially the family Myrtaceae (Spooner-Hart, 2013). This molecule can also be produced synthetically by a chemical process. Research on the biological activity of flavesone has confirmed that it is efficacious against a range of insect pests (Spooner-Hart, 2013). Other research has shown activity against insect strains that are resistant to conventional insecticides (May, 2016). This could be related to the unique insecticidal mode of action of flavesone which is significantly different from any of the currently used insecticides. Considering the potential of flavesone and the existence of insecticide-resistant populations of *R. dominica* infesting stored grains, the current study in collaboration with Bio-Gene Technology Limited explored the long-term effectiveness of flavesone in stored wheat in the field against *R. dominica*. Since flavesone is new to the grain industry and not registered as a protectant in Australia, the research evaluation trial involved establishing laboratory and field efficacy and residue data in treated wheat, a prerequisite for the registration of new grain protectant molecules in Australia (Daglish et al., 2008). Therefore, a detailed long-term residual efficacy study was carried out, with the following objectives: (1) Evaluate the efficacy of three different dose rates of flavesone, 60, 90 and 120 ppm applied to wheat which was then stored in the laboratory and ambient conditions, (2) Determine the residues of flavesone concentration in treated wheat, starting from the initial application until the end of the storage period (13 months), and (3) Establish the natural insect infestation profile of grain insect pests that were encountered in the field storage. This comprehensive study on efficacy and residue data for flavesone would not only pave the way to register this novel insecticide compound as a grain protectant in Australia but also facilitate developing integrated control measures for the grain industry.

2. Materials and methods

2.1. Study design

Based on a preliminary laboratory test, we estimated that 60 ppm of flavesone would kill all *R. dominica* adults and prevent the production of adult progeny. To account for potential loss of efficacy over time we included additional treatments at 90 and 120 ppm. In the current study, wheat was treated with four flavesone application rates including a control (0, 60, 90, and 120 ppm) following the standard procedure used by Australian farmers for applying grain protectants to wheat as it is loaded into storage. After treatment, the wheat was divided into four 1-tonne bags and stored in a shed under ambient conditions for the duration of the trial. To measure temperature and humidity two *i*-buttons (Thermochron, Castle Hill, NSW, Australia) were placed in the 60-ppm treated wheat: one on the surface and one at a depth of 30 cm, each being equidistant from the sides of the bag. After treatment grain samples from each bag were sent to a laboratory in Brisbane where they were stored at 30 °C and 55% rh. Efficacy and residue levels in the treated wheat were assessed after various storage periods (1, 2, 3, 6, 9, and 13 months) under both ambient (field) and controlled laboratory conditions.

2.2. Grain treatment

The wheat used in the study was classified as Australian Prime Hard and was sourced from a local farmer at Yangan in southeast Queensland several weeks prior to the trial, which began on December 11, 2018. An experimental oil-in-water formulation of flavesone, (Flavocide™500EW) containing 500 g/L of the active ingredient was utilised. Three 1-tonne lots of wheat were treated during transfer from a truck to 1-tonne bags using an auger equipped with two spray nozzles. Three different aqueous solutions containing Flavocide™ were prepared, each of 1L volume representing three application rates of flavesone, 60, 90, and 120 ppm (mg/kg), and each applied to 1-tonne of wheat. An additional tonne of wheat received 1 L of water only and served as the untreated control.

2.3. Efficacy and residue levels in wheat stored under laboratory conditions

One week after treatment (0.25 months), 1 kg samples of wheat were sampled from each treatment group (0, 60, 90, and 120 ppm), and transferred to the laboratory, where they were stored at 30 °C and 55% rh. The wheat samples were scooped from various locations on the surface of each bulk bag to a depth of about 30 cm, with each 1-kg lot being placed into 5L plastic containers. Upon arrival at the laboratory, three lots of 80 g of wheat were taken from each treatment and were used for bioassays with *R. dominica*, while 500 g portions were sampled from each treatment and frozen at −20 °C for residue testing. The remaining wheat was returned to storage and subsequent samples taken after 1, 2, 3, 6, 9, and 13 months for long-term efficacy bioassays and residue testing.

Two laboratory strains of *R. dominica* were used in the bioassays: a longstanding susceptible strain (QRD14) and a strain resistant to organophosphates and pyrethroids (QRD1440). The resistant strain began as a suspect resistant population collected in April 2007 from southern Queensland, which was then subjected to a series of selections with deltamethrin to eliminate susceptible genotypes (Daglish and Nayak, 2018). The same bioassay method was applied to wheat samples stored in the laboratory and ambient conditions. For each 80 g replicate, 50 unsexed adults (1–3 weeks post-emergence) were added to a 250 mL jar, which was then covered with a perforated plastic lid with filter paper for air exchange. Jars containing immature insects were held for an additional 6 weeks at 30 °C and 55% RH to allow them to mature into adults (F_1). Three bioassay results were considered for evaluating the residual efficacy of flavesone during storage: (i) percentage adult mortality, (ii) number of live F_1 adults, and (iii) percentage progeny reduction. As the number of F_1 adults produced can vary across generations, calculating percentage progeny reduction is useful to quantify the impact of a grain protectant on population growth. Percentage progeny reduction was calculated using the mean number of live F_1 progeny produced in Flavocide treated grain and in the corresponding untreated control:

Progeny reduction (%) = (1 – (number in treated wheat)/(number in untreated wheat))*100

The mortality data for the susceptible strain were analysed using two-way ANOVA with storage period and flavesone dose as factors, after arcsine-transformation of mortality. The mortality data for the resistant strain were analysed in the same way. Two-way ANOVA was also used to analyse the progeny data with storage period and flavesone dose as factors, after log-transformation (loge(N+1)) of the number of live F_1

progeny. The data for the bioassays of wheat stored under laboratory and field conditions were analysed separately. Within each strain, Pearson Correlation Coefficient was used to compare adult mortality at each combination of dose and storage period between the two storage conditions. All analyses were done using GenStat Software (GenStat, 2022).

Flavesone residue testing was conducted by a commercial laboratory using HPLC-MS/MS following the method developed by Eurofins Agroscience Testing Pty Ltd (2019). Residue levels in wheat samples collected after 0.25, 1, 2, 3, 9, and 13 months of storage, either in the laboratory or under ambient conditions, were analysed. To assess the potential decline in flavesone residues in wheat stored in the laboratory or under ambient conditions, each result was expressed as a percentage of the target application rate for each treatment (60, 90 and 120 ppm).

Efficacy and residue levels in wheat stored under ambient conditions and monitoring of natural infestations.

Samples were collected from four 1-tonne bags at intervals of 0.25 (1 week), 1, 2, 3, 6, 9, and 13 months of storage under ambient conditions. The samples were taken to monitor natural infestations, in the bags, conduct bioassays with *R. dominica,* and test for flavesone residues. To collect the samples, spear-sampling was performed at several locations within each bag until approx. 2 kg of wheat was collected. From this, a 500-g sample was collected and stored at −20 °C for residue testing as previously described. Additionally, three 1-kg batches from each treatment were used to monitor natural infestations. These batches were placed in 2-L plastic jars, each covered with muslin cloth and a perforated plastic lid. Upon arrival at the laboratory, the wheat was sieved and screened for live beetles of three target species: *R. dominica*, *Sitophilus oryzae* (L.) (rice weevil) and *Tribolium castaneum* (Herbst) (red flour beetle). The beetle counts were summed across the three 1-kg batches to calculate the beetle density (beetles/kg). Bioassays using *R. dominica* were conducted as described earlier using three 80-g replicate wheat samples collected at 1, 2, 3, 6, 9 and 13 months of storage.

3. Results

3.1. Temperature and humidity in wheat stored under ambient conditions

Table 1 presents the mean monthly temperature and relative humidity data recorded from the i-buttons placed on the wheat surface and at a depth of 30 cm from the 60-ppm treated bag. The mean monthly temperatures were almost identical at the surface and at 30 cm depth; however, the recorded monthly temperature range was greater at the surface of the wheat. Over the 13-month trial period, temperatures fluctuated between 13 and 28 °C, reflecting seasonal changes from the start of summer (December 2018) to the end of summer (January 2019) during storage. Mean monthly relative humidity ranged from 46 to 66% on the surface and from 42 to 51% at 30 cm depth. As with temperature, the range of relative humidity was greater at the surface compared to the 30 cm depth.

Table 1
Monthly (mean and range) temperature and relative humidity were measured at 0 and 30 cm depth in wheat treated with flavesone at 60 ppm and stored under ambient conditions in a 1-tonne bag.

Month & Year	Temperature (°C)		Relative humidity (%)	
	0 cm	30 cm	0 cm	30 cm
December 2018	26 (18-35)	26 (25-28)	55 (38-74)	42 (42-44)
January 2019	28 (20-36)	27 (26-29)	53 (31-75)	43 (42-45)
February 2019	26 (19-36)	27 (24-28)	53 (23-67)	44 (43-46)
March 2019	25 (18-33)	25 (24-27)	57 (35-69)	45 (44-47)
April 2019	21 (17-25)	21 (20-24)	60 (49-67)	46 (45-48)
May 2019	17 (8-22)	17 (14-20)	58 (48-67)	47 (45-49)
June 2019	13 (6-21)	14 (12-16)	66 (48-88)	47 (46-49)
July 2019	13 (3-19)	13 (11-15)	61 (43-85)	49 (47-51)
August 2019	14 (5-22)	14 (13-16)	53 (38-79)	50 (49-52)
September 2019	18 (8-27)	17 (15-20)	49 (33-68)	51 (49-52)
October 2019	21 (14-31)	21 (19-23)	50 (34-67)	50 (49-52)
November 2019	25 (15-33)	24 (21-28)	46 (33-68)	50 (48-51)
December 2019	27 (19-34)	27 (25-29)	48 (32-64)	49 (48-51)
January 2020	28 (22-34)	28 (26-29)	50 (36-59)	49 (48-51)

3.2. Efficacy of flavesone-treated wheat stored under ambient and laboratory conditions

Table 2 presents the results of efficacy bioassays using the susceptible *R. dominica* strain. Both the application rate ($F_{3,48} = 976.7$, $P < 0.001$) and storage period ($F_{5,48} = 109.7$, $P < 0.001$) significantly influenced 14-d adult mortality in bioassays of wheat stored in the laboratory, and there was a significant interaction between these two factors ($F_{15,48} = 26.6$, $P < 0.001$). The results were similar for bioassays of wheat stored under ambient conditions. Both the application rate ($F_{3,48} = 3272.9$, $P < 0.001$) and storage period ($F_{5,48} = 406.5$, $P < 0.001$) significantly influenced 14-d adult mortality, and there was a significant interaction between these two factors ($F_{15,48} = 80.4$, $P < 0.001$). Mortality rates declined over the storage period, with the smallest decrease observed in the 120-ppm treatment and the largest in the 60-ppm treatment. Despite this decline, all three treatments prevented the production of live F_1 adult progeny throughout the 13-month storage period (Table 2).

Bioassay results show also indicated that the OP/SP-resistant strain of *R. dominica* was less susceptible to flavesone compared to the susceptible strain, in terms of adult mortality and progeny production (Table 3). Both the application rate ($F_{3,56} = 686.2$, $P < 0.001$) and storage period ($F_{6,56} = 270.9$, $P < 0.001$) significantly influenced 14-d adult mortality in bioassays of wheat stored in the laboratory, and there was a significant interaction between these two factors ($F_{18,56} = 44.8$, $P < 0.001$). Similarly for bioassays of wheat stored under ambient conditions, both the application rate ($F_{3,56} = 6881.2$, $P < 0.001$) and storage period ($F_{6,56} = 251.6$, $P < 0.001$) significantly influenced 14-d adult mortality, and there was a significant interaction between these two factors ($F_{18,56} = 36.0$, $P < 0.001$). Both application rate and storage duration impacted 14-d adult mortality in bioassays of wheat stored in the laboratory and under ambient conditions, irrespective of the strains tested. Specifically, mortality declined over time, with the smallest reduction seen in the 120-ppm treatment and the largest in the 60-ppm treatment.

Both the application rate ($F_{3,56} = 716.4$, $P < 0.001$) and storage period ($F_{6,56} = 16.2$, $P < 0.001$) significantly influenced production of live F_1 adults in bioassays of wheat stored in the laboratory, and there was a significant interaction between these two factors ($F_{18,56} = 7.9$, $P < 0.001$). Similarly for bioassays of wheat stored under ambient conditions, both the application rate ($F_{3,56} = 747.6$, $P < 0.001$) and storage period ($F_{6,56} = 13.0$, $P < 0.001$) significantly influenced progeny production, and there was a significant interaction between these two factors ($F_{18,56} = 7.5$, $P < 0.001$). The 90- and 120-ppm treatments achieved a 99–100% reduction in progeny across all bioassays up to 13 months of storage (Table 3). Although the 60-ppm treatment was less effective than the 90- and 120-ppm treatments, it consistently resulted in fewer live adult progeny (0.3–33.7) compared to the untreated controls (121.7–365.0) (Table 3).

Mortality results for each strain, were very similar under laboratory and ambient conditions. For the susceptible strain, mortality at each application rate and storage period was significantly correlated between the two storage conditions (Pearson Correlation Coefficient: $r = 0.988$, $P < 0.001$). Similarly, a significant correlation was observed for the OP/SP-resistant strain between laboratory and ambient conditions ($r = 0.968$, $P < 0.001$).

3.3. Flavesone residues in wheat stored under ambient and laboratory conditions

Table 4 presents the flavesone residue levels in wheat samples taken during storage. For wheat stored under laboratory conditions (30 °C,

Table 2
Results of bioassays of susceptible *Rhyzopertha dominica* in wheat treated with flavesone initiated after various periods of storage in the laboratory at 30 °C and 55% rh and field storage at ambient conditions.

Month[a]	Storage in the laboratory				Storage in the field			
	Control	60 ppm	90 ppm	120 ppm	Control	60 ppm	90 ppm	120 ppm
Adult mortality (%) (mean & SE)								
0.25	0.7 ± 0.7a	100f	100f	100f	0.7 ± 0.7a	100e	100e	100e
1	1.3 ± 0.7a	100f	100f	100f	0.0 ± 0.0a	100e	100e	100e
2	1.3 ± 1.3a	99.3 ± 0.7ef	100f	100f	0.7 ± 0.7a	100e	100e	100e
3	0.0 ± 0.0a	90.1 ± 2.2cd	100f	100f	1.3 ± 0.7a	95.3 ± 1.8d	99.3 ± 0.7e	100e
6	1.3 ± 0.7a	74.7 ± 1.8bc	91.3 ± 2.4d	100f	0.7 ± 0.7a	78.7 ± 2.9c	99.3 ± 0.7e	100e
13	2.0 ± 1.2a	7.3 ± 1.8a	51.3 ± 17.5b	94.0 ± 1.2de	3.3 ± 1.3a	11.3 ± 2.4b	55.3 ± 4.4b	83.3 ± 2.4c
Live F_1 adults (mean ± SE) [a]								
0.25	203.3 ± 12.0	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	203.3 ± 12.0	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)
1	172.7 ± 33.9	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	176.7 ± 50.6	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)
2	180.3 ± 31.3	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	112.7 ± 27.0	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)
3	147.3 ± 28.0	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	168.3 ± 9.3	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)
6	219.7 ± 43.9	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	305.3 ± 58.5	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)
13	380.7 ± 78.2	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	486.0 ± 244.9	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)

**Percentage reduction in adult progeny shown in brackets. Means within type of storage (laboratory or field) followed by different lower-case letters are significantly different ($P < 0.05$) based on analysis of transformed data.

[a] The 9th-month bioassay could not be carried out due to the unavailability of an adequate number of healthy adults of *R. dominica* strain for the bioassays.

Table 3
Results of bioassays of organophosphorus and synthetic pyrethroid-resistant *Rhyzopertha dominica* in wheat treated with flavesone initiated after various periods of storage in the laboratory at 30 °C and 55% rh and field storage at ambient conditions.

Month	Storage in the laboratory				Storage in the field			
	Control	60 ppm	90 ppm	120 ppm	Control	60 ppm	90 ppm	120 ppm
Adult mortality (%) (mean & SE)								
0.25	0.0 ± 0.0a	99.3 ± 0.7f	100f	100f	0.0 ± 0.0a	99.3 ± 0.7ij	100j	100j
1	2.7 ± 1.8a	90.0 ± 2.3de	99.3 ± 0.7f	99.3 ± 0.7f	1.3 ± 0.7a	87.9 ± 0.1gh	99.3 ± 0.7ij	100 j
2	0.0 ± 0.0a	49.3 ± 2.9b	87.3 ± 5.8de	99.3 ± 0.7f	0.7 ± 0.7a	64.0 ± 4.0def	93.9 ± 4.1hi	99.3 ± 0.7ij
3	2.7 ± 1.8a	17.3 ± 2.4a	59.3 ± 3.7bc	96.7 ± 2.4ef	0.7 ± 0.7a	25.3 ± 5.3abc	71.8 ± 1.0efg	93.3 ± 2.4hi
6	2.0 ± 1.2a	6.0 ± 1.2a	19.3 ± 4.7a	78.0 ± 2.0cd	0.0 ± 0.0a	4.0 ± 2.3a	49.3 ± 3.5cde	82.7 ± 4.1fgh
9	4.0 ± 2.0a	1.3 ± 0.7a	5.3 ± 1.8a	54.7 ± 1.3b	2.0 ± 0.0a	2.7 ± 0.7a	22.7 ± 5.5 ab	45.3 ± 4.7bcd
13	2.0 ± 1.2a	3.3 ± 1.8a	20.7 ± 1.8a	64.7 ± 2.9bc	0.7 ± 0.7a	4.7 ± 1.6a	24.7 ± 5.2abc	58.0 ± 9.2de
Live F_1 adults (mean ± SE) [a]								
0.25	204.7 ± 110.9d	0.0 ± 0.0a (100)	0.0 ± 0.0a (100)	0.0 ± 0.0a (100)	204.7 ± 110.9f	0.0 ± 0.0a (100)	0.0 ± 0.0a (100)	0.0 ± 0.0a (100)
1	124.3 ± 40.1d	0.0 ± 0.0a (100)	0.0 ± 0.0a (100)	0.0 ± 0.0a (100)	121.7 ± 15.3ef	0.7 ± 0.7a (99.4)	0.0 ± 0.0a (100)	0.0 ± 0.0a (100)
2	139.3 ± 6.7d	0.3 ± 0.3a (99.8)	0.0 ± 0.0a (100)	0.0 ± 0.0a (100)	142.0 ± 9.6f	1.0 ± 1.0a (99.3)	0.0 ± 0.0a (100)	0.0 ± 0.0a (100)
3	196.3 ± 44.2d	2.0 ± 1.0 ab (99.0)	0.3 ± 0.3a (99.8	0.0 ± 0.0a (100)	127.7 ± 17.8ef	1.0 ± 0.6 ab (99.2)	0.0 ± 0.0a (100)	0.0 ± 0.0a (100)
6	215.7 ± 24.6d	8.0 ± 3.5bc (96.3)	1.0 ± 0.0a (99.5)	0.0 ± 0.0a (100)	365.7 ± 38.6f	12.3 ± 5.0cd (96.6)	0.0 ± 0.0a (100)	0.0 ± 0.0a (100)
9	161.3 ± 16.1d	30.0 ± 7.2c (81.4)	1.3 ± 1.3a (99.2)	0.0 ± 0.0a (100)	141.7 ± 31.0f	33.7 ± 6.1de (76.2)	0.7 ± 0.3a (99.5)	0.0 ± 0.0a (100)
13	204.7 ± 49.6d	18.7 ± 6.2c (90.9)	1.2 ± 0.7 ab (99.4)	0.0 ± 0.0a (100)	226.3 ± 36.2f	7.7 ± 4.3bc (96.6)	0.7 ± 0.3a (99.7)	0.0 ± 0.0a (100)

[a] Percentage reduction in adult progeny shown in brackets. Means within type of storage (laboratory or field) and assessment type (adult mortality or F_1 adults) followed by different lower-case letters are significantly different ($P < 0.05$) based on analysis of transformed data.

55% rh), the estimated flavesone residues at 0.25-months were 44.6% (on average) of the target application rates, decreasing to 31.5% by 13-months. This represents a reduction of approximately 29.4% over the storage period. Residue levels exhibited some variability, but the overall trend indicates a notable decline. For wheat stored under ambient conditions the results are broadly similar (Table 4). Here the estimated residues at 0.25 months were 58.2% of their target rates, falling to 29.7%, by 13 months, indicating a 50.0% decrease over the same period.

3.4. Natural infestations in wheat stored under ambient conditions

Live adults of *R. dominica*, *S. oryzae*, and *T. castaneum* were detected in untreated wheat stored under ambient conditions. In the control wheat, the density of *R. dominica* adults was 0.42 6.25, 4.58 and 40.00 (beetles/kg), after 3, 6, 9 and 13 months, respectively. In contrast no *R. dominica* infestations were detected in the 60-, 90- or 120-ppm treatments. Live adults of *S. oryzae* were detected in the untreated

Table 4
Estimated flavesone residues in treated wheat grains over different storage periods in the laboratory and ambient field storage conditions.

Month[a]	60 ppm	90 ppm	120 ppm	Percentage of target (mean & SE)
Laboratory conditions				
0.25	25.5	42.6	52.6	44.6 (1.4)
1	22.9	34.3	49.7	39.2 (1.1)
2	18.2	27.0	46.5	33.0 (2.9)
3	18.2	26.3	42.2	31.6 (1.8)
6	15.7	23.0	46.3	30.1 (4.2)
9	13.7	20.5	44.5	27.6 (4.8)
13	18.6, 13.5	35.5, 27.2	42.1, 36.7	31.5 (2.3)
Ambient conditions				
0.25	31.1	49.2	81.6	58.2 (5.5)
1	27.5	47.3	55.1	48.1 (2.2)
2	22.1	37.6	50.4	40.2 (1.7)
3	18.6	33.1	51.3	36.8 (3.4)
6	19.8	28.0	42.5	33.2 (1.2)
9	14.7	27.9	31.0	27.1 (2.0)
13	20.8, 15.6	28.2, 16.0	47.1, 36.9	29.7 (3.0)

[a] Wheat samples were stored frozen until screening. Samples collected after 0.25, 1, 2, 3, 6 and 9 months were screened on one occasion. The 13-month samples were screened later, on two separate occasions.

control wheat at 2, 6, and 13 months (0.42, 3.33 and 9.58 adults/kg, respectively). They were also detected in the 60-ppm treatment at 6 and 13 months (1.25 and 1.25 adults/kg, respectively). Live *T. castaneum* adults were detected in the untreated control wheat after 13 months (3.75 adults/kg), and in the 60-ppm treatment at 6 and 13 months (0.42 and 1.25 adults/kg, respectively), as well as in the 90-ppm treatment after 13 months (1.67 adults/kg). These results confirm that flavesone treatments at 60, 90 and 120 ppm effectively protected wheat from *R. dominica* infestations for 13 months. However, flavesone was less effective against *S. oryzae* and *T. castaneum* compared to *R. dominica*.

4. Discussion

This study is the first to evaluate the efficacy of flavesone, a new synthetic beta-triketone compound, applied to wheat following current industry practices for the use of grain protectants. We assessed its efficacy against *R. dominica* during long-term storage both in the field and under controlled laboratory conditions (30 °C, 55% rh). Complete suppression of progeny is a key criterion for evaluating the success of a grain treatment, as it is expected that surviving parental adults would eventually die out leading to population extinction (Daglish, 1998, 2008; Nayak et al., 2005). Our results indicated that flavesone was highly effective against the insecticide susceptible strain of *R. dominica* achieving no live F_1 adults over 13 months of storage at all tested rates (60, 90 and 120 ppm). Despite a gradual decline in parental adult mortality over time (100%–64.7%), flavesone at 120 ppm provided 100% progeny reduction throughout the test period. In contrast, flavesone at 60 ppm was less effective against the organophosphate (OP) and synthetic pyrethroid (SP) resistant - *R. dominica* strain, with live F_1 progeny evident in the grain from the 2nd month onwards. For the 60- and 90-pm treatments, progeny reduction varied with storage conditions and periods, with 60 ppm effective for up to 1 month in the laboratory and 0.25 months in the field, and 90 ppm effective for up to 2 months in the laboratory and 6 months in the field.

Flavesone residue analysis revealed an uneven distribution of flavesone residues in the wheat. Residue levels were initially about half of the target application rates with some variation between the laboratory and ambient conditions. This discrepancy could be due to the different sampling and testing procedures. For instance, testing of wheat stored under ambient conditions reflects the repeated sampling of the 1-tonne bags over a 13-month period. However, testing of wheat stored under laboratory conditions reflects repeated sampling of smaller sample lots that were initially taken from the bags and subsequently transferred to the laboratory 1-week post-treatment. If the treatment of wheat resulted in a higher uniformity of residues, then the expectation was that the initial results from the two sources of wheat would match closely. The 13-month residue samples were also measured on two separate occasions, and thus highlights another potential source of variation in the data. It should be noted that residue data from the second analytical test was consistently lower than the first test, indicating a variation in results for identical samples that underwent residue screening on different dates. Bengston et al. (1987) reported that the initial residues estimated from wheat grain treated with cyfluthrin and cypermethrin in large-scale concrete silos were 55–90% of the target application rate. The authors discussed the possible reasons could be the variation between storage sites and the laboratories that processed the samples. There may also be some degree of uncertainty (Omeroglu et al., 2012) in the steps of quantifying flavesone residues in the laboratory. It appears that greater replication in future trials is likely to give a clearer picture of both the initial residue level and residue decay during storage.

Lower than expected initial residues can occur in grain protectant trials, but sometimes overdosing can also occur (Daglish et al., 1995, 2003, 2008; Daglish and Wallbank, 2005). Field application of grain protectants would be expected to result in considerable variation. When grain is sprayed with a grain protectant as it is loaded into storage, it is unlikely all individual grain kernels will be treated. Factors such as uneven application, spray drift or delivery method (nozzles, auger moving rate) could contribute to this variation. To our knowledge, only data on the effect of uneven application and underdosing on efficacy are available in the literature. Laboratory and field studies show that adequate control of targeted stored grain beetles is possible despite uneven application of various compounds such as malathion, spinosad, s-methoprene, spinetoram and thiamethoxam (Minnet and Williams, 1971,1976; Daglish and Nayak, 2010; Vassilakos and Athanassiou, 2012; Subramanyam et al., 2014; Tsaganou et al., 2021). Field studies also show that adequate control of target stored grain beetles is possible if there is a degree of underdosing of various compounds such as chlorpyrifos-methyl, methoprene, spinosad and various synergised pyrethroids (phenothrin, cyfluthrin and cypermethrin) (e.g. Bengston et al., 1987; Daglish et al., 1995, 2003; Daglish et al., 2008).

The residual efficacy results obtained in this study, despite the lower than targeted application rates, did generally follow a similar pattern of that observed for other grain protectants as indicated above. We suggest the residue estimation procedure could be improved by better sample representation and homogenization. Given these considerations, these results are likely conservative estimates of flavesone treatment efficacy. The results also suggest an initial loss of flavesone residues early during storage but that residues were otherwise relatively stable throughout the study period.

Natural infestations of *R. dominica* were detected in untreated stored under ambient conditions, but none were found in the 60-, 90- or 120-ppm treatments confirming the efficacy of flavesone against this species. Conversely, *S. oryzae* and *T. castaneum* were detected in wheat treated with 60 ppm and *T. castaneum* in wheat treated with 90 ppm, highlighting reduced efficacy against these species compared to *R. dominica*. The presence of these pests in the control wheat stored is not surprising because all three species are present in this region (McCulloch et al., 2022; Toon et al., 2024).

Grain protectants aim to provide long-term protection of grain from invading insects. Therefore, our bioassays simulated real world scenarios of *R. dominica* adult infestation of flavesone treated wheat over time. We focused on progeny production rather than just adult mortality, as complete 100% progeny reduction (i.e. zero live progeny) is the ideal control outcome. This is because the survival of adults would be acceptable if they did not produce adult progeny. Mortality rates declined faster than progeny reduction, indicating that assessing

flavesone efficacy based solely on adult mortality would underestimate how long the treatments were effective against *R. dominica*. Published studies also show that residual efficacy can be underestimated if only adult *R. dominica* mortality is considered (Desmarchelier, 1977; Daglish, 1998). Reduced progeny production may result from various lethal and sublethal effects on adults or immature stages (eggs, larvae or pupae). The observed toxicity effects observed with immature insect life stages could be due to the unique mode of action of the flavesone, thus future investigations should explore these effects in detail to benefit the industry.

A significant finding was that the OP/SP-resistant *R. dominica* strain was harder to control than the susceptible strain. *Rhyzopertha dominica* is known for developing resistance to various grain protectants from different chemical groups (Zettler and Cuperus, 1990; Guedes et al., 1996; Lorini and Galley, 1999, 2001; Chen and Chen, 2013; Daglish et al., 2013). Although Australian populations of *R. dominica* have never been exposed to flavesone, existing resistance mechanisms other than target site resistance could reduce its efficacy. Despite this, the dose required to control the resistant strain (120 ppm) was only 2-fold higher than the lowest dose tested.

Interestingly, flavesone's efficacy declined similarly regardless of whether the wheat was stored under laboratory (30 °C, 55% RH), or under ambient conditions. This suggests that temperature and relative humidity did not significantly affect the efficacy decline of flavesone over time, contrasting with other grain protectants like chlorpyrifos-methyl (an OP), whose efficacy decline (when applied at 6 ppm against *Sitophilus zeamais* Motschulsky was greater at higher temperatures and moistures (Arthur et al., 1991).

The development of resistance to various registered grain protectants, and stricter regulatory requirements, have limited the available options for the industry. In Australia, while spinosad is effective against resistant *R. dominica* populations, it requires combination with an organophosphate (e.g. chlorpyrifos-methyl) and an insect growth regulator (e.g. s-methoprene) for comprehensive control of resistant populations of four other key pest species *T. castaneum*, *C. ferrugineus*, *S. oryzae* and *O. surinamensis* (L.) (sawtoothed grain beetle) (Corteva AgriscienceTM, 2024). Despite the heavy reliance on fumigants such as phosphine and sulfuryl fluoride, protectants remain crucial for effective pest management, particularly in storages where grain is stored for 9–12 months (Daglish et al., 2008), and where phosphine resistance is prevalent (Nayak et al., 2020). Our research highlights the potential of flavesone as a future grain protectant, though further studies are needed prior to its registration for use by industry. Future research should explore flavesone's efficacy in combination with other grain protectants (i.e. OPs, pyrethroids and insect growth regulators) against *R. dominica* and other key pest species, validation of laboratory data in field conditions, and more comprehensive laboratory and field evaluation of flavesone residues during storage.

CRediT authorship contribution statement

Gregory J. Daglish: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rajeswaran Jagadeesan:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Philip R. Burrill:** Writing – original draft, Methodology, Investigation, Conceptualization. **Peter D. May:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Alexander J. Wade:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Manoj K. Nayak:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Manoj K. Nayak reports financial support was provided by Bio-Gene Technology Limited, Melbourne, Australia. An editor of this journal (Manoj K Nayak), an editorial board member of this journal (Rajeswaran Jagadeesan), a former editor and editorial board member of this journal (Gregory J. Daglish). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data available upon reasonable request.

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