A comparison of two methods to determine the susceptibility of sawtoothed grain beetle (*Oryzaephilus surinamensis*) populations to pirimiphos-methyl from Canterbury, New Zealand

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Abstract Resistance of sawtoothed grain beetle (*Oryzaephilus surinamensis*) to organophosphate insecticides is documented internationally. There are anecdotal reports of reduced efficacy in New Zealand but to date no empirical assessments have been made. Two-laboratory-based test methods using either a dust (Actellic® Dust) or liquid (Actellic® 50EC) formulations of pirimiphos-methyl, were compared to determine the response of five Canterbury sawtoothed grain beetle populations. A mini-silo method employed grain treated with the recommended application rate (200 g a.i./tonne seed) of pirimiphos-methyl dust. A Petri-dish method treated internal surfaces of 50-mm diameter Petri dishes with liquid pirimiphos-methyl at concentrations from 0–0.1 g a.i./L to determine an estimated LC₅₀ (lethal concentration for 50% mortality) for each population. The rank order of mortality (highest to lowest) in the mini-silo test was similar to the Petri-dish LC₅₀ rankings for the five populations tested. The results illustrate variation in responses to pirimiphos-methyl concentrations by sawtoothed grain beetle populations, indicating both methods are potential options for future resistance testing and will aid the development of management strategies for control of stored insect pests.

Keywords bioassay, insecticide resistance, mini silo, Petri dish, pirimiphos-methyl, sawtoothed grain beetle, *Oryzaephilus surinamensis*, cereal grain

INTRODUCTION

Oryzaephilus surinamensis (L.) (sawtoothed grain beetle) is one of the most important secondary pests of stored grain products worldwide (Rees 2004). The species is established and widespread in New Zealand (Archibald & Chalmers 1983) and has been recorded in surveys by French (1971), Somerford (1971), (Ferguson 1979) and Chapman et al. (2016). Prior to the 1970s, there was no information available on the incidence of pests in farm-stored grain in New Zealand (French 1971). Storage hygiene is the most important management practice to reduce

storage losses by insects (Bengston 1978, Grains Research and Development Corporation 2013) but Ferguson and Waller (1982) found full advantage of storage hygiene is rarely utilised and consequently chemical control measures are often used. Chemical control of sawtoothed grain beetles in New Zealand has relied heavily on the use of the organophosphate insecticide pirimiphos-methyl (Chapman et al. 2016), a grain protectant designed to provide up to nine months' protection of uninfested grain (Collins 2017). Introduced in the late 1970s – early 1980s (Ferguson & Waller 1982) pirimiphos-

methyl has become integral to the control of insect pests. Pirimiphos-methyl can be used as a structural treatment, where the internal surfaces of a grain store are treated with a dust, smoke or liquid formulation prior to grain fill. Liquid and dust formulations of pirimiphos-methyl can also be used to treat grain post-harvest as it is augered into a grain store (AgriMedia 2019). The Foundation for Arable Research (2017) reported 90% of the surveyed Canterbury grain growers used a structural insecticide treatment, while 79% treated grain with insecticide as it entered storage. Reliance on pirimiphos-methyl has been driven by the limited availability of alternative insecticidal products in New Zealand. Of eight registered grain protectant insecticides in New Zealand, seven contain an organophosphate and, of these, six contain pirimiphos-methyl (AgriMedia 2019). Growers are further limited in the types of storage structure available. In Australia, use of fumigants such as phosphine for control of insect pests is widespread (Collins 2017); however, effective fumigation relies on gas-tight silos and none of the grain silos currently on farm and marketed for sale in New Zealand meet sealed silo standards (Grains Research and Development Corporation 2014).

Resistance of sawtoothed grain beetle to pirimiphos-methyl has been documented internationally (Boyer et al. 2012; Arthropod Pesticide Resistance Database 2019), and confirmed cases have been recorded in Queensland and New South Wales, Australia in the 1980s and 1990s where resistance factors of 2.2–55 fold were recorded (Attia & Frecker 1984; Collins 1985, Herron 1990; Wallbank 1996). Sawtoothed grain beetle resistance to pirimiphos-methyl has not been recorded in New Zealand. Chapman et al. (2016) commented on anecdotal reports that pirimiphos-methyl appeared less effective against stored grain pests in New Zealand than in the past but it has not been determined whether reduced efficacy was the result of resistance development, poor application practice or other factors. Growers will face potential market access constraints for both animal feed and milling grain should resistance

to pirimiphos-methyl develop, due to typically poor storage hygiene practices and the limited chemical control options otherwise available. In addition, the long-term use of organophosphates is under review in New Zealand and overseas (Chapman et al. 2016). To date, no empirical assessments have been made of the status regarding the response of sawtoothed grain beetle to pirimiphos-methyl in New Zealand (Chapman et al. 2016).

Internationally, the impregnated filter paper FAO Method No. 15 is used to test for resistance to pirimiphos-methyl (Champ & Dyte 1980). This method relies on the use of technical-grade liquid pirimiphos-methyl which is not representative of how grain is treated on-farm. The mini-silo method in this study was developed as a practical bioassay method that could be readily used on-farm to test the label rate of pirimiphos-methyl dust on treated grain. The Petri-dish method (Kabir & Chapman 1997) was used to describe a population's response more fully and was chosen to ensure uniform treatment of the interior surfaces of the Petri dish. Application by this method provides no opportunity for highly mobile sawtoothed grain beetles to escape exposure to residues in the test arena. In time, a discriminating concentration could be determined that would differentiate between susceptible and less susceptible populations.

The objective of this research was to develop bioassay methods to determine variation in the susceptibility to pirimiphos-methyl by Canterbury populations of sawtoothed grain beetles. Testing additional populations from throughout New Zealand as they become available with the bioassay methods described in this paper could provide a more comprehensive assessment of the status of pirimiphos-methyl resistance in sawtoothed grain beetle.

MATERIALS AND METHODS

Test beetle populations

Five sawtoothed grain beetle populations were collected from on-farm locations in Canterbury. Canterbury populations were collected because the region is the major cereal grain growing

area in New Zealand. The history of insecticide use during grain storage was recorded for each population (Table 1). Population 1 was used as a reference even though it had been treated previously with insecticides. It was the first population with sufficient numbers to test and was reference susceptible. All populations were reared in isolation at 25°C at 65% relative humidity to multiply sufficient numbers for insecticide bioassays. Daylight was not controlled. Insect colonies were housed in 2-L plastic containers with ventilated screw-top lids and were reared on a diet of rolled oats with yeast. Generations 3–5 were tested at each bioassay, although this was difficult to determine as populations were established with mixed-age adults.

Resistance assessment using a mini-silo method

Insects were contained in 400-mL ventilated plastic containers filled with ca. 300 g wheat grain previously treated with a pirimiphos-methyl dust formulation (Actellic® Dust 20 g pirimiphos-methyl/kg) at the recommended label rate (200 g/1000 kg seed). Dust was applied to the grain and thoroughly mixed. The control treatment

contained untreated seed. The treated grain was compared to the control for each population. Each population was tested against population 1 as a reference population. Forty mixed age and sex adult insects from each of the five populations (estimated generation 3–5) were assigned treatments and placed in separate containers. There were five replicates of each treatment. Treatments were assigned randomly within blocks, and that arrangement of containers was maintained in an incubator. The closed containers were held in an incubator for 48 hours at 25°C under the natural day:night photoperiod during spring i.e. 12 h light:12 h dark. After this time, beetles were separated from the grain and were classified as alive, moribund or dead. Dead insects had no obvious sign of appendage movement, moribund insects had signs of appendage movement but were unable to walk, and alive insects were able to walk more than one body length. Percentage mortality (moribund+dead beetles) in the control treatment was used to correct the percentage mortality in the insecticide treatments using Abbott's formula (Abbott 1925). Data was a normal approximation to a binomial distribution and was arcsine transformed. There

Table 1 Origin and insecticide exposure during storage of sawtoothed grain beetle (*Oryzaephilus surinamensis*) populations from Canterbury tested by the mini-silo and Petri-dish concentration-mortality methods.

Population	Collection area	Previous structural* insecticide treatment	Previous insecticide treatment at silo filling
1	Ellesmere district	pirimiphos-methyl (Actellic® bomb)	pirimiphos-methyl (Actellic® dust)
2	Ellesmere district	Not treated	pirimiphos-methyl (Actellic® dust)
3	Pendarves district	Not treated	maldison, magnesium phosphide (*Magtoxin pellets) - post filling
4	Methven district	pirimiphos-methyl (Actellic® bomb)	pirimiphos-methyl (Actellic® dust)
5	Temuka district	pirimiphos-methyl (Actellic® bomb)	pirimiphos-methyl (Silo® EC)

*A structural treatment is used to coat the inside of an empty grain store prior to filling with grain.

were no differences between transformed and untransformed data, so untransformed data has been reported. Statistical analysis of percentage mortality was completed with Genstat® (19th edition VSN International Ltd, UK), using split plot analysis of variance (ANOVA).

Resistance assessment using a Petri-dish concentration mortality method

Small plastic Petri dishes (50-mm diameter) with tight-fitting lids were used as the test arena on which to expose selected insects to insecticide (Kabir & Chapman 1997). The internal surfaces of the Petri dishes (lid and base) were sprayed with insecticide suspensions using a Potter Precision Spraying Tower (Potter 1952). A range of six concentrations with a two-fold dilution (0, 0.00625, 0.0125, 0.025, 0.05 and 0.1 g a.i./L) of pirimiphos-methyl (Actellic® 50 EC) were tested. Two millilitres of suspension was applied to each lid/base pair in the Potter tower operated at 68 kPa. Each lid/base pair was left for ten seconds to allow the insecticide spray droplets to settle. The insecticide residue on lids and bases was dried for ca. thirty minutes at 25°C after which thirty mixed age and sex adult sawtoothed grain beetles were introduced to each Petri dish. There were four replicates of each treatment. Batches of beetles from the same populations as the mini silo method were assigned randomly to the Petri dishes. A water-only control was also prepared in the same manner. The Petri dishes were held for 24 hours at 25°C under the natural day:night photoperiod during spring i.e. 12 h light: 12 h dark. Beetles were assessed as being alive, moribund or dead as described in the mini-silo method above. The lethal concentration required to cause 50% mortality (LC_{50}) was determined by probit analysis using Genstat® software (19th edition VSN International Ltd, UK).

RESULTS

Mini-silo trial

The designated 'susceptible' population (no. 1) was tested with each of the other four population and resulted in 85.1–99.5% mortality on all occasions. Insect mortality was >90% for, 3 and

5 so they were also considered susceptible to pirimiphos-methyl applied at the recommended label rate (Table 2). These populations were not significantly different to population 1 with $P=0.26$ for population 3 and $P=0.08$ for population 5 respectively (Table 2). Population 4 had 66.5% mortality so was less susceptible than population 1 ($P=0.006$) and population 2 only showed 10.4% mortality showed so had the lowest susceptibility to pirimiphos-methyl. Population 2 was significantly different to population 1 ($P<0.001$).

Petri-dish trial

The rank order from the most, to the least susceptible population was 1, 3, 5, 4 and 2 (Table 3). A comparison of the resistance factors of populations 2–5 with population 1 showed 5.4-, 1.5-, 4.2- and 2.7-fold differences between populations respectively.

The responses of insect populations to pirimiphos-methyl by mini-silo and the Petri-dish methods followed a similar rank order in that population 1 sustained the highest mortality in the mini-silo method (average 96.1%) and had the lowest LC_{50} value (0.014 g pirimiphos-methyl/L). In contrast, for both methods, population 2 sustained the lowest mortality in the mini-silo method (10.4%) and had the highest LC_{50} in the Petri-dish method (0.075 g pirimiphos-methyl/L). The remaining three populations were intermediate between populations 1 and 2.

DISCUSSION

Surveys conducted by Chapman et al. (2016) highlighted the reliance on pirimiphos-methyl for the control of stored insect pests in New Zealand and indicated anecdotal reports of reduced product efficacy. However, that survey found it was not possible to conclude whether insecticide use was effective in managing pest populations in storage facilities, and whether or not pest resistance contributed to pest-control outcomes (Chapman et al. 2016).

As noted, sawtoothed grain beetle resistance to pirimiphos-methyl has been documented internationally (Boyer et al. 2012; Arthropod

Table 2 Percent corrected mortality of five sawtoothed grain beetle populations when exposed to 200 g product/tonne (Actellic® dust) of grain using the mini-silo method.

	Percent mortality (%)	
	Untreated	Treated with pirimiphos-methyl
Test 1		
Population 1	4.8	85.1
Population 2	5.0	10.4
F.pr population		<0.001
F.pr treatment		<0.001
LSD (0.05)		7.0
Test 2		
Population 1	3.2	99.5
Population 3	5.9	91.5
F.pr population		0.26
F.pr treatment		<0.001
LSD (0.05)		5.6
Test 3		
Population 1	1.0	98.1
Population 4	2.8	66.5
F.pr population		0.006
F.pr treatment		<0.001
LSD (0.05)		9.2
Test 4		
Population 1	0.9	91.2
Population 5	0.9	95.9
F.pr population		0.08
F.pr treatment		<0.001
LSD (0.05)		4.0

Table 3 LC₅₀ and resistance factor of five sawtoothed grain beetle populations from Canterbury to the liquid formulation of pirimiphos-methyl using the Petri-dish concentration mortality method.

Population	LC ₅₀ (g a.i./L)	95% C.I. LC ₅₀	Slope	± SE of slope	Resistance factor*
1	0.014	0.012-0.015	4.04	0.35	-
2	0.075	0.064-0.087	4.84	0.56	5.4
3	0.021	0.017-0.023	2.98	0.43	1.5
4	0.059	0.051-0.068	5.63	0.70	4.2
5	0.038	0.034-0.042	5.07	0.55	2.7

*Resistance factor = multiple by which a population is more resistant (LC₅₀) to pirimiphos-methyl than the most susceptible population.

Pesticide Resistance Database 2019). Given the limited number of registered grain protectants, most of which contain pirimiphos-methyl, there is high potential for resistance development in New Zealand as wide use of persistent insecticides against insect populations, with little or no immigration of susceptible insects are ideal for the development of resistance (Taylor & Georgiou 1982). The majority of stored grain beetles do not infest standing crops, most are long lived and can survive on very small amounts of food, such as residual grain in an incompletely cleaned grain stores or equipment such as ducting, augers and bins (Grains Research and Development Corporation 2017). Sawtoothed grain beetles are highly mobile and are capable of walking long distances, such as across a yard between grain stores (Rees 2004, Grains Research and Development Corporation 2017). Typically, infestations come from existing populations either within the grain store or from another grain store nearby (Grains Research and Development Corporation 2017).

Internationally, the FAO Method No. 15 using impregnated filter paper is used to test for resistance to the liquid formulation of pirimiphos-methyl (Champ & Dyte 1980). The mini-silo method in this study tested the dust formulation of pirimiphos-methyl commonly used by Canterbury growers and assessed if the recommended label rate was achieving effective control. The Petri-dish method more precisely defined the population responses to determine whether there were any detectable differences between local populations and whether results from one method aligned with another. This method also ensured uniform coverage of internal surfaces and that insects remained in continuous contact with the insecticide residues.

As a practical tool, the mini-silo method could be used by growers' on-farm as it does not require specialist equipment. The Petri-dish method, while giving more valuable information about the population's response, requires specialist equipment and is a more research-orientated method. However, there is potential to derive a test kit using a discriminating concentration for

the Petri-dish method. All tested populations had been previously exposed to organophosphates.

Results showed agreement between both testing methods. The rank order of mortality (highest to lowest) in the mini silo (96.1 and 10.4% for populations 1 and 2) was similar to the LC_{50} ranking from most to least susceptible (0.014 and 0.075 g pirimiphos-methyl/L for populations 1 and 2 respectively). If this correlation can be confirmed by testing additional populations, a rapid method using the Petri-dish method could be used to confirm the pirimiphos-methyl susceptibility of sawtoothed grain beetle populations collected directly from the field.

Attia and Frecker (1984) reported a <40-fold resistance factor for sawtoothed grain beetles in Queensland, Australia, while Herron (1990) reported a resistance factor range of 2.2–44.2, with 70% of sawtoothed grain beetle populations resistant to pirimiphos-methyl in New South Wales, Australia. Wallbank (1996), also in New South Wales, detected 95% resistance to pirimiphos-methyl in sawtoothed grain beetles with a maximum resistance factor of 55 compared to the susceptible strain. These studies utilised the impregnated filter paper technique FAO Method No. 15 (Champ & Dyte 1980). The resistance factors calculated from the present results (1.5–5.4) are lower than those made elsewhere. It is possible that due to the test method used in the current study, resistance factors were different to those generated by the impregnated filter paper method. Chapman and Penman (1984) stated that low resistance factors, such as those reported in this paper, are often argued as falling within the range of natural variation for the species. There are no data defining the susceptibility of sawtoothed grain beetle populations from New Zealand so it is not possible to conclude from the results presented that the response of the populations to pirimiphos-methyl represents the original susceptible status of the species in New Zealand. The results do show, however, there is variation in the response to pirimiphos-methyl among sawtoothed grain beetle populations collected from Canterbury. Given confirmed cases of resistance internationally and that

sawtoothed grain beetle populations have been subjected to similar chemical selection pressures in New Zealand, there is a high risk for resistance development in New Zealand. Further testing of populations is needed to confirm whether the results to date adequately define the extent of variation in response to pirimiphos-methyl and, more importantly, whether some populations are sufficiently tolerant/susceptible to result in control failures in the field. This information will aid in the development of best management practices including storage hygiene to control stored insect pests in a future where chemical control may no longer be effective or acceptable.

CONCLUSIONS

Two laboratory-based methods have been developed to determine the response of sawtoothed grain beetle populations to pirimiphos-methyl. The rank order of mortality (highest to lowest) in the mini-silo method was similar to the LC_{50} ranking from most to least susceptible. The results illustrate there is variation between sawtoothed grain beetle populations but it is not possible to determine whether significant resistance to pirimiphos-methyl occurs in the field. Ongoing testing of additional populations is planned.

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