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Developments in phosphine resistance in China and possible implications for Australia

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Abstract. Resistance to phosphine was characterised in strains of rice weevil, *Sitophilus oryzae*, and the psocids *Liposcelis entomophila* and *L. decolor* from China and Australia. Mixed-age cultures (containing all life stages) of insects were tested using a flow-through apparatus. The criterion of response was 'time to population extinction' defined as the exposure period, in days, at which 100% mortality of adults and no live progeny were achieved. Chinese *S. oryzae* took 11 and 7 days for population extinction at 200 and 700 ppm phosphine, respectively, compared with the Australian strain, which was controlled in 7 and 5 days, respectively. Similarly, the Chinese strains of *L. entomophila* and *L. decolor* were generally more difficult to control than the corresponding Australian strains. The Chinese strains of *L. decolor* showed resistance levels stronger than any grain storage insect pest species so far detected in Australia. This research allows us to evaluate the likely significance of potential new resistance to the Australian grain industry and to prepare effective fumigation dosages and resistance management strategies to combat new strong resistances before they emerge here.

Introduction

Phosphine has been the leading grain disinfestant for decades in Australia and Asian countries such as China, India, Indonesia, the Philippines and Vietnam, where up to 80% of the harvested grain is treated with this fumigant. Due to the combined advantages of low cost, ease of use and acceptance as a residue-free treatment, it is likely that most countries will continue to rely on phosphine as the major tool used to manage insect pests of stored grain for the foreseeable future (Collins et al. 2001). In recent years, however, the sustained viability of phosphine has been challenged by the development of resistance in several pest species in the Australian/Asian region (Pike 1994; Sayaboc and Gibe 1997; Collins 1998; Daglish and Bengston 1998; Bengston et al. 1999; Cao et al. 1999b, 2003; Rajendran 1999; Collins et al. 2001; Nayak et al. 2002). Some Asian insect strains, specifically those from China, have developed stronger levels of resistance to phosphine than resistant strains detected in Australia (Ren et al. 1994; Daglish and Bengston 1998; Bengston et al. 1999; Zeng 1999). Collaborative research, supported by the Australian Centre for International Agricultural Research, between the Queensland Department of Primary Industries (QDPI) and partner organisations in China, has focused on several aspects of phosphine resistance and its management in stored grain pests common to both countries (Daglish and Bengston 1998; Bengston et al. 1999; Cao et al. 1999a,b,c; Daglish et al. 2002). We are interested in working with China because the Chinese have been using phosphine intensively for much longer than we have in Australia. Examining the development of phosphine resistance in China is like looking into the future at what is likely to happen in Australia. In this paper, we compare resistance levels detected in the rice weevil, *Sitophilus oryzae* (L.), and two psocid pests, *Liposcelis entomophila* (Enderlein) and *L. decolor* (Pearman), from Australia with respective resistant strains from China. We also suggest fumigation protocols to control them. The rice weevil is already a major pest of stored grain in Australia, particularly in the north-east of the grain belt. The two psocid species are a serious problem for the central storage system in many areas of Australia, particularly at export terminals. We have imported under quarantine a rice weevil strain from China, which Chinese scientists claim has very high resistance to phosphine. In this paper, we present a preliminary evaluation of this resistance. In addition, we compare results of assays of phosphine-resistant psocids done in China with results of tests of resistance undertaken on Australian strains.

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Materials and methods

Experiments on *S. oryzae* and Australian psocids were carried out at the QDPI laboratories. Experiments on the resistant Chinese psocids were conducted at the laboratories of School of Grain Oil and Food, Zhengzhou Institute of Technology, Zhengzhou, PR China.

Test insects

The Australian phosphine-resistant rice weevil (strain QSO335) was collected in 1990 from Millmerran, south-eastern Queensland and underwent selection to promote homozygosity (P.J. Collins, unpublished data). The Chinese phosphine-resistant rice weevil (strain Santai) was collected in 1998 from Sichuan Province in China and is now under selection with phosphine at the quarantine facility at the QDPI laboratories. The phosphine-resistant Australian psocids, *L. entomophila* (strain QLE-R) and *L. decolor* (strain NLD-R), were collected from Warwick, Queensland and Gunnedah, NSW, respectively, and have not undergone any deliberate selection. The Chinese phosphine-resistant *L. entomophila* (strain SCHYLe1) and *L. decolor* (strain GDMMLd1) were collected from Sichuan and Guangdong provinces, respectively, and have not undergone any selection. Rice weevils were cultured on whole wheat at 25°C, 55% relative humidity (RH) and 12:12 light:dark (L:D), whereas the psocid cultures were maintained on a wheat-based diet (whole wheat, kibbled wheat, whole wheat flour and brewers' yeast in the proportion of 10:10:10:0.1) at 30°C, 70% RH and 12:12 L:D (Nayak and Collins 2001).

Fumigation of mixed-age cultures

Insect cultures were specially prepared for this set of experiments so that all life stages would be exposed to phosphine. The fumigation procedure was essentially the same as described by Winks and Hyne (1997) and Daglish et al. (2002) where the mixed-age cultures of insects, held in plastic containers, were placed in stainless steel chambers. Phosphine and air were allowed to flow in and out in one direction through these chambers, controlled separately by mass flow controllers. Experiments were conducted at 25°C and 55% RH. With *S. oryzae*, adults were removed at the end of the fumigation and mortality was assessed after 2 weeks. A second assessment was done after 8 weeks from the end of fumigation to allow time for eggs, larvae and pupae to emerge to adults (Daglish et al. 2002). *Time to population extinction* was defined as the exposure period (in days) at which 100% mortality of adults and no live progeny were achieved. Containers without adult progeny were re-checked after another 8 weeks to confirm the population extinction. With psocids, *Time to population extinction* was assessed 3 weeks from the end of each fumigation period by looking at the treated media for any live insects. This assessment provided data on time taken, in days, required to completely control all life stages at each fumigation rate (Nayak et al. 2002).

Results and discussion

Time to population extinction of the Santai strain of *S. oryzae* was 4 and 2 days longer at phosphine concentrations of 200 and 700 ppm, respectively, compared with the resistant Australian strain (Table 1). Moreover, the Santai strain had higher resistance than most the phosphine-resistant strain of lesser grain borer *(Rhyzopertha dominica* (F.)) reported in Australia (strain QRD569) (Collins et al. 2001). The latter is the most phosphine-resistant strain known of any grain storage insect pest.

Table 1. Time to population extinction (in days) of resistant strains of the rice weevil (*Sitophilus oryzae*) from Australia and China at fixed concentrations of phosphine, 25°C and 55% relative humidity.

Phosphine mg/L (ppm)	Australian strain[a] (QSO335)	Chinese strain (Santai)
0.3 (200)	7	11
1 (700)	5	7

[a] G.J. Daglish and H. Pavic, Queensland Department of Primary Industries, unpublished data.

At concentrations of 100 and 200 ppm, the Chinese *L. entomophila* (strain SCHYLe1) was more resistant to phosphine than the Australian strain (QLE-R) (Table 2). However, time to population extinction was the same at 700 ppm of phosphine (Table 2). The Chinese *L. decolor* (strain GDMMLd1), however, possessed a much stronger level of resistance than the Australian *L. decolor* (strain NLD-R) (Table 2).

Table 2. Time to population extinction (in days) of resistant strains of psocids from Australia and China at fixed concentrations of phosphine, 25°C and 55% relative humidity.

Phosphine mg/L (ppm)	*Liposcelis entomophila*		*Liposcelis decolor*	
	Australian strain (QLE-R)	Chinese strain (SCHYLe1)	Australian strain (NLD-R)	Chinese strain (GDMMLd1)
0.15 (100)	8	12	<6	13
0.3 (200)	7	10	<6	11
1 (700)	3	3	<1	5

At all concentrations tested, the time required to achieve population extinction was much greater in the Chinese strain than that required for the Australian strain. In addition, the resistance level of the Chinese *L. decolor* was greater than resistance levels observed in either the Australian or Chinese strains of *L. entomophila* (QLE-R and SCHYLe1), and equal to the levels recorded in the most resistant strains of rice weevil and lesser grain borer from Australia (Collins et al. 2001).

The rice weevil and the two psocid species studied are important pests in the grain storage systems of both Australia and China. Due to the lack of suitable alternatives, phosphine will continue to be extensively used in Australia. Therefore, it is possible that resistance in Australia may be selected to the levels now found in Chinese strains. Recent research on resistance management suggests, however, that irrespective of the development of resistance in several pests, the viability of phosphine can be sustained because it is possible to develop suitable protocols to control strongly resistant insects (Collins et al. 2001). Our current results suggest that with proper resistance monitoring and further characterisation of the strong resistance, we would be able to manage the stronger resistance in rice weevil and psocids in Australia if it occurs.

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