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Interests in the Mixture Ethyl Formate/Allyl Isothiocyanate for the Fumigation of Infested Wheat by the Rice Weevil: *Sitophilus oryzae* L. and the Granary Weevil: *Sitophilus granarius* L.

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Abstract: Since several post – harvest insecticides like dichlorvos or methyl bromide are being phased out in the European Union, new alternatives have to be found. The volatile liquid mixture ethyl formate/allyl isothiocyanate (AITC) 95%/5% (w/w), was recently tested successfully and patented by the Australian Stored Grain Research Laboratory (SGRL). We have carried out trials with an application rate of 60 g/m^3 and an exposure time of 24 and 48 hours, and with an application rate of 120 g/m^3 and an exposure time of 24 hours against the two species: *Sitophilus oryzae* and *Sitophilus granarius*. Ethyl formate concentrations were measured by GC, FID. These trials were carried out with samples of 12 kilograms of infested wheat in 30 – litre gas – tight drums at about 20 C. Applications were made by introducing the mixture with a syringe through a septum on the grain in movement. With an application rate of 60 g/m^3, the mortality rate of granary weevil adults is about 42 % after 24 and 48 h, and respectively 80 and 92 % for the mortality rate of rice weevil adults after 24 and 48 h. The pupae and/or aged larvae emergence reduction is very low: between 0 to 40 % depending on the conditions. However, results of treatments with an application rate of 120 g/m^3 show respectively a control of 96.6 and 99.3 % of granary weevil and rice weevil adults. Hidden stages of granary weevils and rice weevils show respectively a emergence reduction of 97.4 and 92.5 % for pupae and aged larvae, 98.9 % for larvae of both species, 100 and 99.8 % for eggs. This efficacy is encouraging, but grain treatment with this application rate of 120 g/m^3 is higher than the lower explosive limit (85 g/m^3), so new ways of application must be found.

Key words: fumigation, *sitophilus oryzae*, *sitophilus granarius*, ethyl formate, allyl isothiocyanate

Introduction

In European Union, several grain insecticides were recently phased out methyl bromide, dichlorvos, malathion.. Besides some contact insecticides like chlopiriphos methyl pirimiphos methyl, deltamethrin, and phosphine, there is a need for new ways for grain disinfestations. It is the case with the mixture ethyl formate (EF)/ allyl isothiocyanate (AITC) which can be potentially a promising alternative. EF is an old fumigant which presents an interesting insecticide effect tested at the beginning of 20th century [1]. Lateron, many trials have been carried out on stored grain pests with success [2]. Currently, pure ethyl formate is used on dried fruits in Australia, and more recently it registered in mixture with CO_2 under the trade name "VAPORMATE". The Australian Stored Grain Research Laboratory (SGRL) have carried out several trials on wheat with pure EF [3] and with a mixture composed of 95% of EF and 5% (w/w) of a synergist: MITC (methyl isothiocyanate), an isothiocyanic ester similar to AITC[4]. These isothiocyanic esters are extracted from the plant family Cruciferae[5] and enhance the efficacy of EF. The main advantages of the mixture EF/AITC are that the insecticide efficacy is very quick compared with Phosphine, the fumigant registered worldwide and the EF residues decline after fumigation to natural levels without forced aeration[6]. In Australia, the MRL fixed for dried fruits is 1 mg/kg and the natural levels of EF are sometimes (few months after harvest) higher than just after fumigation[7]. Moreover, EF can be applied safely because its Threshold Limit Value (TLV) is 100 ppm and its toxicological classification Xn. The main inconvenient of EF is its very low flash point (–22℃) and a low flammability level at 85 g/m^3. The application rate should be below this limit [8] or 92.5 g/m^3[4] in accordance with temperature rate. The internal stages of *Sitophilus sp.* are difficult to control with a successful of 100%, and to obtain this result an application rate above the flammable level is necessary [3]. That's why AITC is involved in the reduction of concentration in EF necessary

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to control all stages of *Sitophilus sp.*

These trials were carried out to investigate the efficacy of the mixture with an application rate of 60 g/m^3 and a double dosage 120 g/m^3 on the rice weevil: *Sitophilus oryzae* and the granary weevil: *Sitophilus granarius* in wheat. The double dosage was tried to find a better efficacy but in practice the flammability problem will have to be solved. In the same time, this study allowed the observation of sorption of the mixture in infested wheat.

Materials and Methods

Rearing Technique

The insects used in these trials were reared on wheat in a rearing room at 25℃ (±1℃) and 60% r. h. (±5%). Two species were used in this study, the granary weevil: *Sitophilus granarius* L. and the rice weevil: *Sitophilus oryzae* L. When first adults emerged three kilograms of infested wheat were mixed with nine kilograms of non-infested wheat to avoid a too high infestation rate in 30 liters gastight drums and therefore anoxia during trials. The final infestation rate was checked by an X - Ray machine and was between 0.5% to 2% of the grains.

Pre-fumigation Procedure

To begin, samples of 12 kg of infested wheat were put into the drums. A temperature sensor (Captsystemes) was put in each drum and it was programmed to take a measure every 10 minutes. Every drum was equipped with a septum and two pipes, in the aim to respectively inject liquid mixture of EF/AITC and measure gas concentrations during the fumigation.

In the first series, the trials were carried out with the two species of weevils and with two exposure times, 24 and 48 hours. So, eight drums (four treated drums and four control drums) were placed in the same conditions of temperature: about 20℃. The application rate of the mixture was 60 g/m^3.

In the second series, a double dosage was applies: 120 g/m^3. The exposure time was just 24 hours and the sensibility of the two species was tested in the same conditions than in the first series.

Fumigation

The injection of the EF/AITC mixture was made with a syringe through a septum on the grain in movement. Drums were turned 5 minutes after injection in order to homogenize the insecticide. Drums were placed at about 20℃ during the fumigation. Ethyl formate concentrations were measured by Gas Chromatograph (GC), with Flame Ionization Detector (FID) (Varian Star, 3400 CX).

Post - fumigation Procedure

At the end of the exposure time, a measure of oxygen concentration was taken to be sure that insects in gas-tight drums were not in anoxia. The material used was an oxygenmeter HM16N (±1% error). The drums were opened just after the last gas concentration measurement. After fumigation and aeration, the moisture content of the grain was taken with a moisture meter (Chopin, Wile 55) in each drum.

Insect Efficacy of the EF/AITC Mixture

Just after the fumigation the wheat was sifted and all adult insects were observed to see if they were dead, alive or dying. For the controls a sample of only 2 kilograms was sifted. All dying adults were placed in the rearing room and 24 hours later they were observed once again and identified like dead or alive.

To determine the efficacy on hidden stages, a sample of 1 kilogram's of each drum was placed, after the first sifting and without adults, in the rearing room. Eleven days later, all samples were sifted compared with the controls in order to obtain the emergence reduction. This period corresponds to the emergence of pupae and aged larvae at the time of fumigation [9]. Thirty - two days after the end of fumigation, another sifting was made in the order to obtain emergence reduction of larvae (except the oldest larvae). To finish, a last sifting was made thirty - eight days after fumigation to obtain emergence reduction of eggs.

Results and Discussion

Fumigation with An Application Rate of 60 g/m^3

The first results of this study reveal that EF concentrations in drums declined very quickly after injection (Fig. 1). This graph shows that after 24 hours concentrations are below 1 g/m^3 in the two drums shown in this figure. So, ethyl formate was quickly sorbed after the beginning of the fumigation. In fact, one hour and thirty minutes after injection, concentrations are already reduced by half (between 28 and 29 g/m^3). In a previous study, AITC concentrations were measured by GC (TSD) and show the same rate of sorption [10]

Before aeration, oxygen concentration meas urements show that the concentrations in the headspace atmosphere of the drums were slightly lower than normal atmosphere (less than 3%).

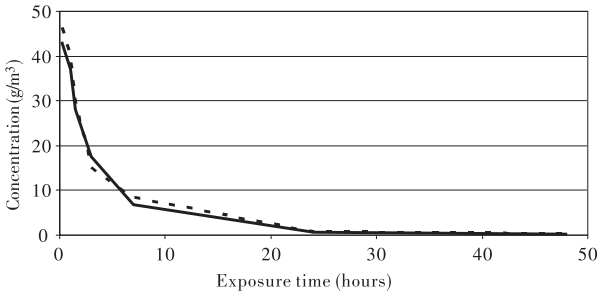

Fig. 1 Evolution of ethyl formate concentration measured by GC (FID) in the drums of *Sitophilus granarius* (solid line) and *Sitophilus oryzae* (broken line) fumigated at 60 g/m^3 with an exposure time of 48 hours.

But in every case, the oxygen percentage was lower in the control drum than in the treated drum (Table 1), as a result of more live insects in the controls. The grain moisture is between 14.3 and 15.2%.

Table 1. Oxygen concentrations in every drum at the end of fumigation

Species	Exposure time (hours)	O_2 concentration in treated drums (%)	O_2 concentration in control drum (%)
S. oryzae	24	20.2	19.5
S. granarius	24	20.3	19.7
S. oryzae	48	19.7	18.3
S. granarius	48	20.0	18.5

The first sifting after fumigation (Fig. 2) shows that less than half of granary weevil adults were dead (42%) and there were no differences between the two exposure times tested. On the other hand between 92% of rice weevil adults were killed in 24 hours fumigation and 80% after 48 hours fumigation. In the control, the mortality rate was between 0.5 and 1.5%, except in the control of the drum with granary weevils with an exposure time of 48 hours, the mortality rate of this control was about 7%. The fumigation seems to be more efficient on the rice weevil adults than on the granary weevil adults. So, after these results it's obvious that the exposure time of the fumigation didn't give better results with a period longer than 24 hours. After 24 hours, gas concentrations are very low, less than 1 g/m^3 and practically 0 g/m^3 after 48 hours. That confirms that the second day of fumigation is useless because it remains no gas and then it do not enhance the efficacy of the treatment.

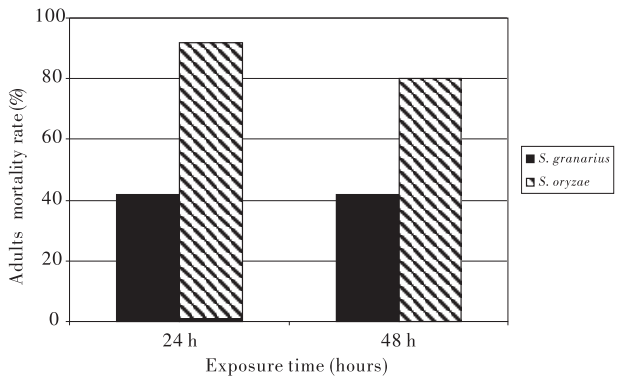

Fig. 2 Mortality rate of *S. granarius* and *S. oryzae* adults after fumigation with the mixture EF/AITC with exposure times of 24 and 48 hours and an application rate of 60 g/m^3

The results of emergence reduction (Table 2), corresponding to pupae and aged larvae during the fumigation, confirm the lake of efficacy of the treatment with this application rate. Indeed, there were not emergence reductions in the samples of rice weevils, very few for the granary weevil samples: 21% for the 24 h fumigation and less than 40% for the 48 h fumigation. These results show the ineffectiveness of EF/AITC mixture on hidden stages of *Sitophilus. spp*. That's why, after this ineffectiveness, the siftings were stopped and the trials with this application rate were considered uninteresting since the mixture was not efficient on the most resistant stages of these species, eggs and pupae[11].

Table 2. Emergence reduction of pupae and aged larvae of *Sitophilus granarius* and *Sitophilus oryzae* after fumigation with the mixture EF/AITC with an application rate of 60 g/m^3

Species	Exposure time (hours)	Number of insects emerged/kilogram's of wheat in treated samples	Number of insects emerged/kilogram's of wheat in control samples	Emergence reduction (%)
S. oryzae	24	47	37	0
S. granarius	24	23	29	20.7
S. oryzae	48	48	48	0
S. granarius	48	20	33	39.4

Fumigation with an Application Rate of 120 g/m^3

The concentration of EF in drums, after an injection of the mixture EF/AITC, decreases very quickly (Fig. 2) and after six hours of fumigation it remained less than 20 g/m^3 in the two drums. After an exposure time of 24 h, concentrations were between 1 and 2 g/m^3 of EF.

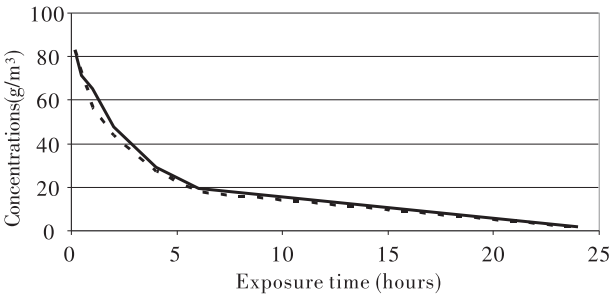

Fig. 3 Evolution of ethyl formate concentration measured by GC (FID) in the drums of *Sitophilus granarius* (solid line) and *Sitophilus oryzae* (broken line) fumigated at 120 g/m^3 with an exposure time of 24 hours.

The grain moisture was measured just after aeration and it was between 15.3% and 15.7%.

After fumigation, the first sifting (Fig. 4.) reveals that 99.3% of granary weevil adults were killed and 99.6% of rice weevil adults and less than 3% in the control (respectively 1.3% and 2.8%). So, when the dosage is twice more important, the efficacy is much better but all insect adults are not killed.

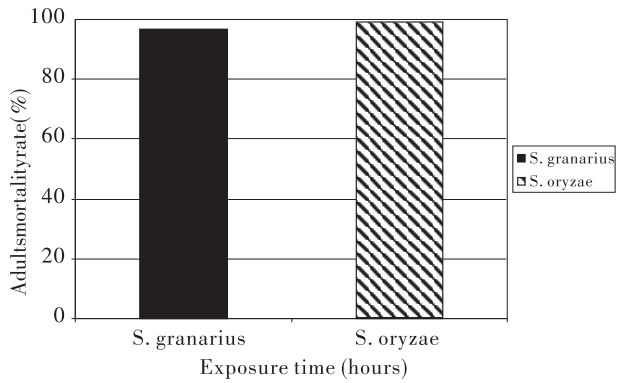

Fig. 4 Mortality rate of adults after fumigation with the mixture EF/AITC with an exposure time of 24 hours and an application rate of 120 g/m^3

The second sifting, eleven days after the end of fumigation, shows that the emergence reduction of pupae and aged larvae is better than in the first trials with an application rate of 60 g/m^3 (Table 3).

Table 3. Emergence reduction of pupae and aged larvae of *Sitophilus granarius* and *Sitophilus oryzae* after fumigation with the mixture EF/AITC with an application rate of 120 g/m^3

Species	Number of insects emerged/kilogram's of infested wheat in treated samples	Number of insects emerged/kilogram's of infested wheat in control samples	Emergence reduction (%)
S. oryzae	4	53	92.5
S. granarius	2	77	97.4

The third sifting, thirty – two days after the end of the fumigation, reveals that the emergence reduction of larvae was very important with 98.9% for the two species. But the total efficacy is not yet reached. Larvae stages are the most sensible hidden stages but the fumigation did not reach a 100% efficacy.

The fourth and last sifting, 38 days after the end of the fumigation shows that the emergence reduction of eggs is complete for the granary weevil sample with 100% of emergence reduction (Table 4). For the rice weevil sample, just three insects emerged and the total emergence reduction is not reached even if 99.8% of eggs were killed during the fumigation.

Table 4. Emergence reduction of eggs of *Sitophilus granarius* and *Sitophilus oryzae* after fumigation with the mixture EF/AITC with an application rate of 120 g/m^3

Species	Number of insects emerged/kilogram's of infested wheat in treated samples	Number of insects emerged/kilogram's of infested wheat in control samples	Emergence reduction (%)
S. oryzae	3	1564	99.8
S. granarius	0	1008	100

Conclusion

For grain disinfestations, the volatile liquid mixture ethyl formate/allyl isothiocyanate (AITC) 95 %/5 % (w/w), was tried with an application rate of 60 g/m^3 and an exposure time

of 24 and 48 hours, and with an application rate of 120 g/m^3 and an exposure time of 24 hours against the two species: *Sitophilus oryzae* and *Sitophilus granarius*. There are big differences of efficacy between the two fumigations with the two concentrations tested (60 and 120 g/m^3). However the control of the two stored products insects *Sitophilus granarius* and *Sitophilus oryzae* is not reached except for the eggs of *Sitophilus granarius*. It is then possible to think that a higher dosage could kill all stages of these two species of weevils. If the gas exposure time is longer, the efficacy of the fumigation will certainly not increase since the sorption of EF by wheat is very quick and after 24 hours the gas concentration is very low, below 2 g/m^3 of EF. But the main problem with this mixture Ethyl formate/Allyl isothiocyanate (95%/5%) is the flammable level. Fumigations with an application rate of 120 g/m^3 is already higher than this flammable level, and to be fully efficient the dosage should be still increased. We are far beyond the flammability level of 85g/m^3. The main factor which could help to maintain a high level of gas, would be to decrease the sorption. To face this problem, the technique of application or the formulation of the mixture should be adapted.

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