Developing a new grain protectant-efficacy testing in Europe

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Abstract

The system of grain storage varies widely across Europe but basic similarities in climate; the types of cereal and international trading have resulted in a relatively small group of pests becoming universally distributed. An effective grain protectant must control these pests and must satisfy national test methods with regard to efficacy and persistence.

Work done in Belgium, France, Spain and the UK to assess a potential new chemical, ProStore, is reported here as an example of the development process. Initial testing in the UK showed the value of using a mixture of two chemicals to extend the range of pests controlled without high application rates. The mixture, consisting of the synthetic pyrethroid, bifenthrin, and the organophosphorus, malathion, was tested according to national registration requirements of Belgium, France and UK. Two formulations of ProStore were developed: an emulsifiable concentrate and a low volume, ready-to-use liquid.

The testing involved a variety of techniques and species of insects, and assessment periods of up to a year. In most cases, formulated pesticide was applied to grain which was then stored under controlled conditions. Samples were withdrawn at intervals and bioassayed with various species of stored grain pests. Some different tests were done against mites and the immature forms of grain weevils.

A field trial was also done in Spain using the ready-to-use formulation to assess performance under practical conditions in a warm climate.

Introduction

Europe produces about 200 million tonnes of grain each year with France and Germany being the largest individual producers, followed by the UK, Spain and Italy. The exact details of storage vary widely between the different countries but infestation problems during storage occur widely throughout the European Community. The variation in the type and size of storage, coupled with different approaches to store management, means that there is no unified system of pest control. However, there is some control of quality standards of grain and grain products and these standards usually include a requirement that grain is free from infestation. This standardisation of certain aspects of quality has occurred because of rules set by the Common Agricultural Policy which demand that certain requirements are met before subsidy payments are made and the influence of trading standards set by the export trade.

Freedom from live pests is one common quality standard that appears to be applied throughout all EU Member States and the detection of a single insect at point of sale can have serious financial consequences for the seller. Therefore, farmers and storekeepers expend substantial resources on pest prevention and control. The methods used will vary between countries and the farmer or storekeeper will usually make the final choice. A wide range of options has been developed, including integrated pest management strategies that use a combination of physical and chemical methods. However, contact insecticides remain an import option because of their ease of use and low risk to operators.

The wide variability in approaches to control, particularly with regard to chemical control measures, makes the introduction of Europe-wide techniques rather difficult. However, efforts are being made to rationalise the registration process throughout the EU. This paper maps the development processes with regard to efficacy testing of contact pesticides in several EU Member States and uses one pesticide to illustrate the systems.

Approaches to Storage and Pest Problem

Belgium

Grain storage and production

Belgium is a relatively small producer of grain although agriculture is an important component of the economy. However, Belgium provides an important transit corridor for grain exports and imports. Storage is divided between farms and commercial stores with farmers often holding stocks...
principally for feeding their own stock. Storage follows a conventional pattern using both a range of bins and flat stores. The principal cereals are wheat, barley, rye and a small amount of oats (Nutelet and Leblon, 1986).

Infestation

Some survey work of infestation in grain and grain stores has been done over a 3 year period. In general commercial stores were more heavily infested than farm stores but the average level of infested stores was greater than 50%. Sitophilus granarius was the most commonly found pest with Oryzaephilus surinamensis a close second. Tribolium spp. was also very common with S oryzae, Cryptolestes ferrugineus and Rhyzopertha dominica also occurring in more than 10% of the stores. The level of infestation remained largely constant over the three years of the survey (Letelher et al., 1994).

Control measures

Pesticides applied directly to the grain are the most commonly used control method, particularly at commercial stores. Both emulsifiable concentrates and ready-to-use formulations are used. Fumigation with phosphine is also widely used to deal with established populations.

The Ministry of Agriculture must approve any pesticide before they can be registered and given a label. There is a formal testing programme, which must be done via an official testing station such as the University at Gembloux. These tests must be done in mini-silos and are usually run over a 12-month period. All major pests found in Belgian grain are included in the bioassay schedule.

France

Grain storage and production

France produces between 50 and 60 million tonnes of grain per year, mostly wheat, maize and barley. Much of this grain is used for animal feed processing, or is fed directly to cattle. However, a significant amount is used for milling into flour, or for malting. There is also an exportable surplus ranging from 9 to 16 million tonnes per year. At harvest, most grain is delivered to co-ops or private storage companies (called 'storage organisations') and less than 20% of the harvest remains on the farms at the site of production. The type of storage varies widely but on-floor bulk stores have become more important in the past fifteen years. These are mainly large in size with a holding capacity of 2000 to more than 50000 tonnes.

The wheat and barley harvest in France occurs during July and August and there is no need for artificial drying because the moisture content is below 15% (wet basis) in most cases. By contrast, the harvest of maize occurs from the end of September to November and there is an absolute need for speedy drying before storage. Consequently, most grain co-ops and commercial storage organisms have high-throughput maize dryers (these are also used for sunflower seed and sorghum grain drying).

Infestation

The last general survey of insect infestation of cereal grain in France (Fleurat-Lessard 1980; Provost 1980) showed that pest insects were regularly found in stores located in the cereal growing areas. After harvest and during the following winter or spring, secondary species such as O surinamensis, T. castaneum, A. avenae, T. confusum were dominant. A more recent survey, limited to the Western France (Vidal et al. 1984), showed that, whilst secondary species were dominant in country stores located close to the production sites, primary pests such as Sitophilus granarius, S oryzae, and R. dominica, were dominant by the time grain reached the port silos. On delivery to these end-destination silos, the percentage of infested deliveries was less than 10% and this residual infestation is dealt with by treating the grain with dichlorvos as it enters the silos and sometimes also as it is loaded onto the ship.

Even though France is a major user of contact insecticides for pest control in stored grain protection, no attempt has been done recently to detect the occurrence of resistance in insect pest strain.

Control measures

Residual insecticide application remains the most popular means for insect control in stored grain in France. Several active substances have been registered for direct application on grain. When grain is for sale, dichlorvos or a mixture of dichlorvos with pirimiphos-methyl or chlorpyrifos-methyl is preferred for a rapid and complete killing effect on visible infestations. For grain to be stored, residual insecticides with long-term protection such as pirimiphos methyl, chlorpyrifos-methyl, deltamethrin or malathion are most frequently used. Fumigation with phosphine is the other main method of pest control but is only used as a curative treatment in order to eradicate infestation in bulk grain when other treatments are uneconomic. Preventive fumigation with phosphine is used for less than 2% of bulk stored grain in France.

Cooling with aeration has recently become very popular as a method of grain protection that is insecticide-free. Theoretically all commercial grain stores equipped with temperature probes in each grain bin and with aeration facilities can use this technique as recommended by the ITCF advisors (Berhaut and Lasseran 1985). In practice, about 30% of commercial grain stores regularly use aeration immediately after harvest (Lasseran 1994).

Efficacy testing of contact pesticides

Before the Registration Committee of the Ministry of Agriculture will allow the provisional use of a new residual pesticide in France, the candidate must be proved to be at least equally effective as a reference product that is already registered and in use. The efficacy data must be obtained.
through bioassays following official procedures. All the testing procedures for plant protection pesticides are produced by the French National Organisation for Plant Protection (ANPP) through the BioAssays Commission (CEB). For grain protectants, the candidate must be compared to a 'standard' product, both for acute insecticidal efficacy and for persistence. The target pest species are three major pests commonly found on grain in France: *S. granarius*, *T. castaneum* and *R. dominica* (1995 edition of Protocol 106). Testing is done on small batches of grain, 50 or 100 kg, after the insecticide has been applied under conditions than mimic full-scale treatments. Data from full-scale testing is not required.

**United Kingdom**

**Grain storage and production**

The UK produces between 20 and 24 million tonnes of grain per year, comprising mostly of wheat and barley with a little oats. The grain is used for animal feed, milling into flour, maltting and distilling, and there is an exportable surplus of 5 – 6 million tonnes per year. The majority of grain storage is on farm at the site of production. It is estimated that the total farm storage capacity is between 18 and 21 million tonnes (Prickett, 1988). There are also a growing number of commercial storage facilities. The type of storage varies and there is no one standard system. However, one common feature of grain storage in the UK is the trend towards on-floor bulk stores. These will vary from 100 – 500 tonne bulks on farms to commercial stores holding more than 50,000 tonnes.

The UK grain harvest occurs during July to September with the more northerly areas being later. Depending on the weather, much of the harvest may need artificial drying before the grain can be stored with safety. Therefore, many farms have some form of drying facility.

**Infestations**

Several surveys of infestation in UK-stored grain have been done. The most recent of these (Prickett, 1989), showed that primary pest insects were found at 10% of farm grain stores and at 27% of commercial stores. The level of infestation in the grain was always lower but the detection of insects at a site must be considered to represent a threat that will require action on the part of the farmer or storekeeper to prevent the spread of insects into the grain (Prickett, 1992). The most common pest species were *Oryzaephilus surinamensis*, *Sitophilus granarius*, *S. oryzae* and *Cryptolestes ferrugineus*. Other insects such as *Rhyzopertha dominica* and *Tribolium castaneum* were found occasionally.

Some of the above survey work included testing insects collected from farm and commercial grain stores for resistance to some pesticides. Resistance to organophosphorus insecticides was detected in some strains of *O. surinamensis*, *S. granarius*, *S. oryzae* and *C. ferrugineus*. However, the testing was based on the FAO discriminating dose method and the levels of resistance were generally very low (Prickett & Muggleton, 1991).

**Control measures**

Physical control methods, such as a combination of cooling and drying, are widely used. Many commercial standards require grain moisture contents of below 14.5% so, that if the grain is above this level at harvest, it is dried before or during the first weeks of storage. Ambient, night-time air is used to cool grain during storage and the UK climate offers the option of cooling to below 10°C in most years.

Despite the use of physical measures, infestations still develop and a range of chemical options may be employed. Fumigation with phosphine is widely used but UK law prohibits the use of fumigants other than by accredited servicing companies. Therefore, the use of contact insecticides, both to treat empty stores and to admix with the grain, are also widely used. From the farmer and storekeeper's points of view, contact insecticides have the advantage that they can be used directly without the need to involve a servicing company.

Pesticide use was surveyed by the Ministry of Agriculture, Fisheries and Food in 1995 and the data collected suggested that about 5 million tonnes of grain was treated by admixture and far less was fumigated with phosphine.

**Efficacy testing of contact pesticides**

In the UK part of the data requirements for registration of a new pesticide include production of efficacy data. This must include results for all pests that are mentioned on the label and all label claims must be supported by efficacy data. This information is defined in Guidelines issued by the Pesticides Safety Directorate of the Ministry of Agriculture, Fisheries and Food (ANON, 1986). The testing procedure is not specified so that the choice of method is left open but full details of all methodology must be given. For a grain protectant, fully replicated trials are required in which the test product is compared to a 'standard' product. Where appropriate, these tests should include both laboratory and field assessments. A report covering test results must be submitted in the form of an Efficacy Overview in which all the testing is summarised.

It is now recognised that because of the similarities in storage pests throughout Europe that data generated outside the UK can be used to support a UK registration. However, any such data must be shown to be relevant to farm and commercial grain stores in this country.

**Development of a new Grain Protectant**

In order to illustrate the procedures outlined above, testing
done on one candidate grain protectant and the results
generated are given in the following sections The
insecticide use as an example is ProStore™ (ProStore is a
trademark of FMC Corporation, Philadelphia, USA), a
mixture of the synthetic pyrethroid bifenthrin and the
organophosphorus malathion ProStore is being developed
and marketed by FMC, Europe NV

Methods

Initial tests in UK

Bifenthrin was tested under laboratory conditions against
a range of pests of stored grain (Prostephanus truncatus,
R dominica, O surinamensis, S oryzae and T castaneum) at doses of between 0.2mg and 1.0mg/kg. It
showed good activity against most of these pests and the
activity was maintained over a 3-month period

Further testing was then done using a mixture of
bifenthrin and malathion applied to grain. All treatments
were done by spraying pesticide onto 25kg-batches of grain
tumbling in a cement mixer This technique offers a
convenient way of mimicking practical treatments and should
deposit the majority of the applied dose evenly over the
grain After treatment, the grain was held in plastic or
metal containers All treatments were replicated and, in the
case of efficacy work, an appropriate standard pesticide was
included in the trials The treated grain was stored either at
ambient temperature or at 20°C

Samples of grain were removed for assessment at a range
of intervals extending up to 6 months after treatment
Bioassays were carried out using standard laboratory practice
and batches of between 25 and 50 insects were exposed to
the treated grain for 1 or seven days at 25°C Either 3 or 5
replicates were used and insects were exposed on untreated
grain as controls. Some additional tests were done at low
temperatures to examine the action of the mixture under
such conditions and with these the methodology was changed
to take account of the slower action of pesticides at low
temperatures This work was reported at a previous

Trials in Belgium

Four pests of stored grain were used for mini-silo
experiments: S granarius, S oryzae, R dominica, O
surinamensis These insects came from laboratory
cultures that had been maintained at Gembloux for at least 7
years in absence of exposure to any pesticides. When used
for tests, the insects were between 0 and 15 days of age

Wheat, variety Camp Remy, grown in the locality of
Gembloux and harvested in 1992 was used as the substrate
for the tests This grain did not receive any treatment with
a post-harvest pesticide before being used in the
experiment Moisture content of the wheat, determined
with a Cereal Tester moisture meter, was about 14%

The grain was held in metal bins of 425-dm³ capacity

(300kg wheat), 75 diameter and 90 cm deep cylinder with a
40 cm deep cone at the base. Twenty-four holes in the side
of each silo allowed access for temperature and humidity
measuring.

Grain treatment

The doses of bifenthrin/malathion, rates of dilution of the
formulations, amounts of diluted formulation applied were in
accordance with the manufacturer’s recommendations (0.3
+ 6.0mg/kg bifenthin + malathion) The only exception
was that the UL formulation was diluted further in kerosene
to facilitate the small-scale treatment

The pesticide was applied to 50kg batches whilst it was
being mixed in a mechanical mixer. The grain was first
moistened with 100ml of distilled water, added with a small
hand-held spray gun. Mixing was continued for 5 minutes
and then the pesticide solution was sprayed onto the grain
using a small, hand-held spray gun for EC formulations and a
compressed-air spray gun for ULV formulations. The grain
was mixed for a further 15 minutes before being transferred
to the mini-silos for storage. This process was repeated five
times with each formulation to produce a total of 250 kg
wheat/silo Each treatment was replicated twice and
another silo was set up containing untreated control wheat.
The silos containing the treated and control grain were held
in a room where the temperature and the relative humidity
was not controlled accurately but were monitored each time
a sample was collected

Sampling system

Five samples weighing about 200g were collected from
each silo by means of a grain sampler, at intervals after
 treatment of 1 day, 1, 3, 6 and 12 months. After mixing,
the 1kg samples were then divided into two parts: one part
being used for residue analysis and the other for biological
assessment Normally, biological testing was done
immediately after treatment but samples for chemical
assessment were stored in a deep freeze at -18°C until
analysed. All samples were given unique identifier numbers

Biological assessments

The grain was bioassayed by exposing 50 insects on a 100g
sub-sample of wheat in wide-necked, open-topped jar. The
neck of each jar was treated with a PTFE solution to prevent
the insects escaping. Four replicates were used and insects
were exposed on untreated grain as controls. After adding
insects The jars were stored at 27°C and 70% RH and the
mortality of the insects was assessed after 1, 7, 14 or 21
days. Insects were considered to be unaffected if they could
walk normally and dead if they showed no response to
stimuli

Tests in France using official protocol

Bioassays with insects

The bifenthin-malathion treated grain was tested using
the official procedure (ANPP/CEB protocol ref. 106) which
aimed to demonstrate that the two formulations, e.g., ProStore 420 EC™ and ProStore 157 UL™, were at least equivalent to reference products against *S. granarius* and *R. dominica* adults. The tests covered both prevention and curative effects (the latter only tested on adults of *S. granarius*). The dose of ProStore™ applied on grain was 0.3 + 0.6 mg kg⁻¹ bifenthrin + malathion respectively. The reference products were ProPirgan SLD™ (primaiphos-methyl) at 4 mg kg⁻¹ and DDVP (dichlorvos) at 10 mg kg⁻¹ used for preventative and curative effect respectively.

Curative effect is the ability of the insecticide to eliminate an established infestation of *S. granarius* in batches of wheat with a population density of more than 100 adults per kg. Preventive effect is considered as the ability of a treated grain to kill the adults coming into contact with this treated grain at a range of intervals after the treatment.

The curative effect of ProStore™ was assessed on samples withdrawn from 50 kg batches of infested grain 1 day and 6 weeks after they had been treated (corresponding to two consecutive generations of *S. granarius*). The preventive effect was assessed on samples of sound, treated grain taken at regular intervals of time after treatment which were then bioassayed. These tests were done 42, 90 and 180 d after the treatment. Each sample of about 500 g of wheat was bioassayed with 200 adults of the two test species *S. granarius* and *R. dominica*.

**Test on grain mites**

Efficacy was tested 1 day and month after grain had been treated by bioassays with two mite species, *Acarus siro* (L.) and *Tyrophagus putrescentiae* (Schrank). The reference product was Nuvan bital™ which is registered for grain protection against storage mites (5 and 0.25 mg kg⁻¹ of dichlorvos and chlorpyrifos-methyl respectively). ProStore™ 420 EC was compared at two different doses corresponding to (0.3 + 6) and (0.4 + 8) mg kg⁻¹ of bifenthrin + malathion respectively.

**Field trial in Spain**

In order to assess the efficacy of ProStore under practical conditions and to assess the effects of Southern European climate, a treatment was done at a farm at Tordillos near Salamanca, Spain.

The grain used was barley, variety Biannche, from the 1997 harvest that had been held on the farm from harvest and had not been treated with a post-harvest pesticide before the trial. About 70 tonnes of barley were stored in a heap on the concrete floor of a solid, weatherproof barn. A mixed infestation of several species of stored grain insect pests was well established and the farmer commented that insects were a perennial problem during storage at the farm. The moisture content of several representative samples of grain from the heap were checked with an electrical moisture meter and found to have a mean moisture content of 13.5%.

To facilitate treatment, the grain was moved from one side of the barn to the other using a screw-auger powered by hydraulic pressure from a tractor. ProStore 157 UL was applied with a commercial air-assisted applicator. The nozzle was secured just under the discharge spout of the auger so that the atomised ProStore was directed at right angles to the stream of falling grain. A metal plate was situated behind the grain stream to reflect back any ProStore droplets that penetrated the grain stream.

Before treatment, a batch of grain was moved into a trailer using the auger. The trailer was filled for 17 minutes, weighed and then tipped into another similar storage barn. The empty trailer was then weighted so the rate of grain flow could be calculated. The angle of the auger was not changed so that this conveying rate was assumed to represent the rate at which grain was moved during the treatment. The untreated grain moved during the calibration was retained as an untreated control and tipped in an adjacent barn.

The applicator was adjusted so that 40 ml of ProStore 157 UL was applied per tonne of grain, giving a theoretical dose of 0.3 mg/kg bifenthrin + 6.0 mg/kg malathion. A total of about 54 tonnes of barley was treated and measurements of the amount of ProStore used at the end of the trial showed that 54.3 ml/tonne had been applied.

Following the application of ProStore, the treated grain formed a discrete heap in the barn and this was used in subsequent assessments. Two forms of post-treatment assessment were done: bioassays and checks on the established population. Samples were withdrawn from both treated and control grain 1, 2, 4, 8, 16 and 24 weeks after treatment using a multi-compartment sampling spear (1.5 m long, with 10 ports). Samples were extracted from three points in the treated grain and a single point in the control. Two sets of samples were collected. One of these was sieved on the spot and numbers of live and dead insects were recorded. Pitfall traps were also used to monitor the endemic population. A second set of sample was sent away for laboratory bioassay using adult *O. surinamensis*, *S. granarius*, *R. dominica* and *T. castaneum*. The bioassay followed the procedures laid down in the Official French Test Methods as detailed earlier in this paper.

The treatment was completed without problems and the circumstances of storage will ensured that the barley treated during this trial remained a discrete and readily identified batch of grain. The approach to and method of treatment were typical of those that might be used to treated grain on a small/medium-sized farm. The quality of the grain was also typical of farm-stored grain that it had not been pre-cleaned and its moisture content was unchanged from the time of harvest. The grain was also infested with a range of insect pests.
Results

Initial tests in UK

Initial testing of bifenthrin-treated grain demonstrated that this compound had a wide spectrum of efficacy against some stored grain pests. However, a dose of at least 1mg/kg of bifenthrin would be needed to allow the compound to be used alone. The results are summarised in Table 1.

A compilation of results from grain bioassayed at intervals over a 6-month period at two laboratories, is given in Table 2. Assessments made during this work also showed that bifenthrin/malathion at 0.3 + 6.0mg/kg gave 100% knockdown of O. surinamensis, S. oryzae, S. granarius and R. dominica after 24h exposure, with freshly treated grain. Additional testing confirmed that the dose of 0.3 + 6.0mg/kg bifenthrin/malathion gave complete control of A. avenae, C. ferrugineus and a UK resistant strain of O. surinamensis with both freshly treated grain and after it had been aged for 3 months. Complete control of T. castaneum was obtained with freshly treated grain but this was reduced slightly to 95% when the grain was aged for 3 months.

Table 1. The percentage mortality of four species of stored grain beetle bioassayed on grain treated with bifenthrin, pirimiphos-methyl or deltamethrin

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Dose (mg/kg)</th>
<th>O. sur</th>
<th>S. ory</th>
<th>R. dom.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>0.2</td>
<td>100</td>
<td>99</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>100</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>100</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>Pirimiphos-methyl</td>
<td>0.2</td>
<td>96</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>0.2</td>
<td>92</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>100</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>100</td>
<td>100</td>
<td>4</td>
</tr>
</tbody>
</table>

O. sur = Oryzaephilus surinamensis
S. ory = Sitophilus oryzae
R. dom. = Rhyzopertha dominica

Table 2. The percentage mortality of 3 species of stored grain beetles bioassayed, over a 6-month period, on grain treated with bifenthrin/malathion or pirimiphos-methyl

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Dose (mg/kg)</th>
<th>O. sur</th>
<th>S. gran</th>
<th>R. dom.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Bifenthrin + malathion</td>
<td>0.3/6.0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Pirimiphos-methyl</td>
<td>4.0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

O. sur = Oryzaephilus surinamensis
S. gran = Sitophilus granarius
R. dom. = Rhyzopertha dominica

Table 3. The percentage mortality of two species of grain beetle exposed at 5 and 10°C to grain treated with bifenthrin/malathion or pirimiphos-methyl. Insects were exposed to the treated grain for 5 days.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Dose (mg/kg)</th>
<th>Temperature of exposure (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O. sur</td>
</tr>
<tr>
<td>Bifenthrin + malathion</td>
<td>0.3/6 0</td>
<td>100</td>
</tr>
<tr>
<td>Pirimiphos-methyl</td>
<td>4.0</td>
<td>100</td>
</tr>
</tbody>
</table>

O. sur = Oryzaephilus surinamensis
S. ory = Sitophilus oryzae

885
The bifenthrin/malathion mixture was very active at temperatures of 5 and 10°C against *O. surinamensis* and *S. oryzae*, and the results are summarised in Table 3. These temperatures are normal during the treatment of grain during the winter in temperate climates and are also common during the disinfestation of export cargoes.

**Testing in France**

The results are summarised in the Tables 4, 5, 6 and 7.

### Table 4. Curative effect: Percent kill of a *S. granarius* population infesting wheat grain stored at two temperatures after a treatment with ProStore™ insecticide, at two formulations, and in comparison with registered insecticides (reference) and untreated control.

<table>
<thead>
<tr>
<th>Storage Temperature</th>
<th>Time elapsed after the treatment</th>
<th>Control (untreated)</th>
<th>DDVP 10 ppm</th>
<th>Pirgram SLD 4 ppm</th>
<th>ProStore 420 EC (0.3 + 6) ppm</th>
<th>ProStore 157 UL (0.3 + 6)(0.15 + 3) ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>20°C</td>
<td>1 d</td>
<td>3.3</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>42 d</td>
<td>4.6</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>25°C</td>
<td>3 d</td>
<td>4.3</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>42 d</td>
<td>1.6</td>
<td>64.7</td>
<td>97.9</td>
<td>83.9</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 5. Preventative efficacy of ProStore™ insecticide on *R. dominica* placed on treated wheat at different time intervals after grain treatment (% mortality of 200 adults in two replicates).

<table>
<thead>
<tr>
<th>Storage time</th>
<th>Untreated control</th>
<th>Reference Pirgram™</th>
<th>ProStore™ 420 EC</th>
<th>ProStore™ 157 UL</th>
</tr>
</thead>
<tbody>
<tr>
<td>42 d</td>
<td>0.5</td>
<td>0.8</td>
<td>4.5</td>
<td>23.5</td>
</tr>
<tr>
<td>90 d</td>
<td>0.5</td>
<td>0.8</td>
<td>6.0</td>
<td>8.5</td>
</tr>
<tr>
<td>180 d</td>
<td>0.3</td>
<td>0.3</td>
<td>4.8</td>
<td>13.8</td>
</tr>
</tbody>
</table>

The preventive efficacy of ProStore™ on *S. granarius* was equivalent to the efficacy of Pirgram™ reference, except in one case: 6 months of storage of grain Against mites, the immediate efficacy of ProStore™ at the normal dose was equivalent to the efficacy observed with Nuvan bital™ reference. The reinforced (highest) dose had at least the same level of efficacy than the reference. One month after the treatment, only the reinforced dose had a comparable effect to the reference, but the mean percentage of kill was low for all the products, even for the reference (≤ to 51 % of kill, maximum level) (Table 6).

Grain treated with ProStore™ 157 UL killed only 85% of re-infesting insects on grain stored at 20°C (Table 5). Nevertheless, for *R. dominica*, the Pirgram™ reference failed to control this species meanwhile ProStore™ had a remarkable efficacy on a 3 month period at least.

The results of the bioassay with *R. dominica* (Table 7) showed that ProStore™ has a remarkable efficacy against *R. dominica* which is not controlled by the reference Pirgram™. This is not a surprising result because *R. dominica* is insensitive to OP's at regular dosage. The protective effect of ProStore™ residues lasted at least six months for the target species *R. dominica*.
### Table 6

Percentage of kill of two species of mites in wheat grain treated by ProStore™ at two different doses in comparison to Nuvan bitotal™ treated wheat and untreated control (normal dose = 0.3 + 6 mg kg⁻¹ of bifenthrin + malathion; reinforced dose = 0.4 + 8 mg kg⁻¹ bifenthrin + malathion).

<table>
<thead>
<tr>
<th>Storage temperature</th>
<th>Insect species</th>
<th>Control 1 (untreated)</th>
<th>Control 2 (untreated)</th>
<th>ProStore™ SLD 1</th>
<th>ProStore™ SLD 2</th>
<th>ProStore 1 420 EC</th>
<th>ProStore 2 157 UL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acarus siro</td>
<td>Time after treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control delay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 h</td>
<td></td>
<td>24 h</td>
<td>24 h</td>
<td>24 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 month</td>
<td></td>
<td>1 month</td>
<td>1 month</td>
<td>1 month</td>
<td>1 month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48 h</td>
<td>7d</td>
<td>48 h</td>
<td>7d</td>
<td>97.5</td>
<td>97.5</td>
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<td>47.5</td>
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<td>99.5</td>
<td>99.5</td>
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<td></td>
<td></td>
<td></td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Tyrophagus putres.</td>
<td>48 h</td>
<td>7d</td>
<td>48 h</td>
<td>7d</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>97</td>
<td>97</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 7

Preventative efficacy (% kill) of two different formulations of ProStore™ applied at the same dose on sound gram, tested at various intervals of time after the treatment with adults of S. granarius and R. dominica (mortality assessed 7d after deposition on treated grain samples).

<table>
<thead>
<tr>
<th>Storage duration (m days)</th>
<th>Species</th>
<th>1</th>
<th>30</th>
<th>90</th>
<th>180</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. granarius</td>
<td>100 (1)</td>
<td>100 (1)</td>
<td>100 (2)</td>
<td>97.4 (1)</td>
<td>16.3 (1)</td>
</tr>
<tr>
<td></td>
<td>O. surinamensis</td>
<td>100 (1)</td>
<td>100 (2)</td>
<td>100 (3)</td>
<td>100 (2)</td>
<td>95.0 (1)</td>
</tr>
</tbody>
</table>

### Testing in Belgium

No problems were experienced when applying either of the formulations to grain. There was no strong smell either during or after the treatment and the operator who diluted and applied the insecticide, reported no discomfort or other problems.

### Biological assessments

The bioassay results are given in Tables 8, 9 and 10.

Heat freshly treated with the ULV formulation gave 100% mortality of all species 1 day after treatment. This level of kill was maintained for between 1 and 3 months depending on species. Complete control of all species was obtained after 7 days exposure to the treated grain for the first 3 months of the experiment and complete control of O. surinamensis and R. dominica was obtained throughout the 12 month period. S. granarius and S. oryzae were the most tolerant species but even with these insects more than 80% mortality was obtained 6 months after the grain was treated (Table 9). Very similar results were obtained with wheat treated with the EC formulation except that the period of complete control of S. granarius and S. oryzae was extended more than 6 months (99% mortality with one Silo) and > 80% mortality was obtained 12 months after treatment (Table 8).

### Table 8

Mortality with ProStore EC formulation (percentage of mortality for the control).
Table 9. Mortality with ProStore ULV formulation (percentage of mortality for the control).

<table>
<thead>
<tr>
<th>Species</th>
<th>Exposure (days)</th>
<th>Storage duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 30 90 180 360</td>
<td></td>
</tr>
<tr>
<td><em>O. surinamensis</em></td>
<td>1 100 (0)</td>
<td>100 (0) 100 (0) 38 (0)</td>
</tr>
<tr>
<td></td>
<td>7 100 (0)</td>
<td>100 (1) 100 (1) 100 (0)</td>
</tr>
<tr>
<td><em>R. dominica</em></td>
<td>1 100 (1)</td>
<td>100 (0) 100 (2) 18 (0)</td>
</tr>
<tr>
<td></td>
<td>7 100 (1)</td>
<td>100 (0) 100 (2) 100 (0)</td>
</tr>
<tr>
<td><em>S. granarius</em></td>
<td>14 100 (1)</td>
<td>100 (0) 100 (2) 73 (1)</td>
</tr>
<tr>
<td></td>
<td>21 100 (1)</td>
<td>100 (2) 100 (3) 80 (1)</td>
</tr>
<tr>
<td><em>S. oryzae</em></td>
<td>14 100 (1)</td>
<td>100 (1) 100 (0) 87 (3)</td>
</tr>
<tr>
<td></td>
<td>21 100 (2)</td>
<td>100 (1) 100 (2) 92 (4)</td>
</tr>
</tbody>
</table>

Table 10. Mortality with EC formulation (percentage of mortality for the control).

<table>
<thead>
<tr>
<th>Species</th>
<th>Exposure (days)</th>
<th>Storage duration (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 30 90 180 360</td>
<td></td>
</tr>
<tr>
<td><em>O. surinamensis</em></td>
<td>1 100(0)</td>
<td>100(0) 100(0) 96(0) 97(2)</td>
</tr>
<tr>
<td></td>
<td>7 100(0)</td>
<td>100(1) 100(1) 100(0) 100(3)</td>
</tr>
<tr>
<td><em>R. dominica</em></td>
<td>1 100(1)</td>
<td>100(0) 100(2) 100(0) 100(0)</td>
</tr>
<tr>
<td></td>
<td>7 100(1)</td>
<td>100(0) 100(2) 100(0) 100(0)</td>
</tr>
<tr>
<td><em>S. granarius</em></td>
<td>7 100(0)</td>
<td>100(0) 100(1) 99(1) 68(0)</td>
</tr>
<tr>
<td></td>
<td>14 100(1)</td>
<td>100(0) 100(2) 100(1) 100(1)</td>
</tr>
<tr>
<td><em>S. oryzae</em></td>
<td>1 100(0)</td>
<td>100(1) 86(0) 61(1) 2(1)</td>
</tr>
<tr>
<td></td>
<td>7 100(1)</td>
<td>100(1) 100(0) 100(2) 84(2)</td>
</tr>
</tbody>
</table>

A limited number of tests using *S. granarius* and *S. oryzae* were done with grain treated with both formulations, in which the exposure period was increased (Tables 8 and 9). Counts done after 14 and 21 days exposure always gave an increase in mortality and, with the EC formulation, 14 days exposure gave 100% mortality 12 months after treatment. The increase in mortality with the ULV formulation was significant but did not reach 100% even after 21 days exposure. After 8 months, only 6% mortality was obtained for *S. granarius* and 95% for *O. surinamensis* (Table 10).

Spanish field trial

Bioassay results

Samples of grain arrived at the testing laboratory within 2 days of collection and were immediately set up for bioassay. The four species of insects were exposed to the samples for 7 and 14 days at 25°C. The results for the 7-day exposures are given in Table 11. Extending the exposure to 14 days had little effect on the mortality and control mortality at 7 days never exceeded 2.5% for any species.

Table 11. Mortalities of insects exposed to samples of grain treated with ProStore 157 UL at an intended rate of 4L/100tonnes during a field trial in Salamanca, Spain.

<table>
<thead>
<tr>
<th>Species</th>
<th>Time after treatment in weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 4 8 16 24</td>
</tr>
<tr>
<td><em>S. granarius</em></td>
<td>100 100 100 100 100 1.5</td>
</tr>
<tr>
<td><em>R. dominica</em></td>
<td>100 100 100 100 100 100</td>
</tr>
<tr>
<td><em>O. surinamensis</em></td>
<td>100 100 100 100 96 4</td>
</tr>
<tr>
<td><em>T. castaneum</em></td>
<td>100 100 100 100 100 100</td>
</tr>
</tbody>
</table>
Assessment of grain in store

Pre-treatment assessments of the endemic infestation showed that the following species were present: Stegobium panicum, Tribolium sp., Cryptolestes ferrugineus, Carphophorus sp., R. dominica, and O. surinamensis, with the first four species being the most numerous.

Seven days after treatment no live insects were found in the treated grain or in traps in the treated grain, although live S. panicum, Tribolium sp. and C. ferrugineus were present is samples removed from the control. Observations were continued for 24 weeks after treatment and no live, unaffected insects were detected in traps placed in the treated grain during that period. Three moribund Tribolium sp. were found in traps.

The temperature of both the treated and control grain was about 22°C at the start of the trial. However, the temperature fell to around 10°C after 10 weeks. The infestation in the control grain declined in line with the fall in temperature and no live insects were detected between weeks 10 and 22. At the final observation 24 weeks after treatment, 2 live S. panicum were found in the control.

The moisture content of the grain started at about 14%, rose to 15.5% at week 16, and then fell to about 13.5% by week 24.

Discussion and Conclusions

There is considerable diversity between the storage systems used in Belgium, France and the UK for grain. More importantly, the control of the storage system is in the hands of professional storekeepers in France, whilst in the UK and Belgium much of the storage is in the hands of farmers. There will also be climatic differences between Northern UK and Southern France but it is likely that the artificial environment of a grain store will reduce the impact of these differences on conditions in the grain.

The single European market has had a major influence on grain quality. Firstly, the European Commission has imposed some rules on food quality. This body has also set minimum standards that must be met before certain grain payments are released. However, the marketing opportunities offered by the single market have probably had the greatest impact. A trader can sell grain anywhere in the Community provided he has a market and can meet quality standards. The effects of this have been to raise all internal standards towards those of the most fastidious market.

Despite differences in storage practice and climate, the spectrum of grain pests found across Europe appears broadly similar, as do the basic concerns over their control. Pests in grain are as unacceptable in Gent in Belgium as they are in Bordeaux in France. The admixture of a residual pesticide with grain is regarded as a cost-effective approach to control and is the most widely used method in Belgium, France and the UK.

The registration of a new pesticide now falls under pan-European regulations with regard to safety and environmental factors. However, efficacy remains an area where individual Member States retain some control and have their own approaches to efficacy testing.

ProStore insecticide was developed with the aim of offering a new grain protectant that would control pests of grain storage in as much of Europe as possible. To meet this requirement the product had to control all the major insect pests, provide a sufficient period of protection from a single application and satisfy the various nation's efficacy requirements. The product, of course, also had to meet all safety requirements but those items are not covered in this paper. The use of a mixture of two active ingredients is unusual but not unique. However, the combination of organophosphorus and synthetic pyrethroid compounds allowed the extension of the spectrum of activity without application rates at or above current Maximum Residue Limits.

Despite differences between national requirements, ProStore insecticide meets the efficacy requirements of several, if not all, European countries. It will control all major pests and will give at least 3 months protection from a single application. These findings resulted from an extensive testing laboratory programme in three countries and were confirmed by field testing in Spain.

Acknowledgements

Laboratory testing in Belgium was done by Université of Gembloux. The testing in France was done at Laboratory TEC, Anglet and INRA Laboratory, Bordeaux. The testing in England was done by the Central Science Laboratory and Agrisearch UK. The field trial in Spain was organised by Aplicaciones Bioquimicas of Salamanca.

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