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Sustaining the Effectiveness of Pesticides and Host Plant Resistance

Chairman and Session Organiser	Dr I Denholm
Papers	8A-1 to 8A-4

SUSTAINING THE EFFICACY OF DICOFOL AGAINST CITRUS RUST MITE (PHYLLOCOPTRUTA OLEIVORA): A CASE-HISTORY OF INDUSTRIAL AND ACADEMIC COLLABORATION

T. J. DENNEHY

Arthropod Resistance Management Laboratory, Department of Entomology, University of Arizona Cooperative Extension, Tucson, Arizona. 85721

C. OMOTO

Departamento de Entomologia, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Av. Padua Dias, 11, 13418-900 Piracicaba-SP BRAZIL

ABSTRACT

Summarized is a four-year effort to assemble a resistance management program for citrus rust mite (Phyllocoptruta oleivora) and the acaricide dicofol. Methods were developed for culturing, selecting with dicofol and bioassaying citrus rust mite in the laboratory. After characterization of an 8.8-fold resistance, a discriminating concentration was identified and used in subsequent surveys of resistance conducted throughout the major regions of citrus production in Florida. Field populations varied widely in the frequency of resistance. Laboratory and field studies illustrated that dicofol resistance declined at a substantial rate in the absence of selection. Where field failures with dicofol were evident, susceptibility to dicofol could be regained by suspending use for 3-4 years. Large-plot field evaluations of different dicofol use regimes were conducted at two locations in Florida over the course of four years. These trials yielded strong concordance between laboratory estimates of resistance and efficacy of dicofol in the field. By using rotations of different classes of acaricides and limiting use of dicofol to once per year, citrus rust mite susceptibility to dicofol was sustained.

INTRODUCTION

The acaricide dicofol has been in use for many years and continues to be an important tool in integrated pest management (IPM) programs throughout the world. It is highly effective in controlling many species of phytophagous mites and has low toxicity to some key beneficial organisms. The citrus rust mite, Phyllocoptruta oleivora, is one of the most important pests of citrus in Florida (McCoy and Albrigo, 1975). This minute, eriophyid mite, measuring ca. 0.15 mm, damages citrus fruit and leaves as a result of its feeding, and proliferates under conditions of high humidity.

Dicofol has been used successfully for over thirty years to control both citrus rust mites and tetranychid spider mites in citrus. However, in recent years, repeated use of this chemical has been associated with problems in controlling citrus rust mite. Such reports motivated the producer of the Kelthane® formulations of dicofol, Rohm & Haas Co., to launch a unique collaboration between Florida citrus growers and researchers at the University of Florida and Cornell University, aimed at building a resistance management program for dicofol in Florida citrus. Dicofol resistance in Florida citrus was a promising candidate for management, owing to characteristics of the chemical and the citrus system. Resistance to dicofol has been shown to be unstable in many, though not all, systems around the world in which it has been studied (e.g., Dennehy & Granett, 1984; Dennehy *et al.*, 1990; Mable & Pree, 1992). Acaricide use in Florida citrus, though significant, is comparatively moderate: two to four applications are required per year (Knapp, 1993). Significantly, there is a diversity of acaricides registered in

Florida. The products used most often are: abamectin, dicofol, ethion, and fenbutatin oxide. Therefore, rotations of chemical classes is feasible with acaricides used in Florida citrus.

Integrated management of citrus rust mite has been promoted extensively over the past two decades by the University of Florida (Anonymous, 1989). They have validated sampling methods and thresholds for this pest, as well as other recommendations for minimizing the use of chemicals and promoting biological control in the citrus system. Therefore, Florida citrus provided a reasonably good candidate for sustaining the efficacy of pesticides through resistance management. Herein, we provide an overview of the four-year project that succeeded in identifying and validating a sustainable use recommendation for dicofol in Florida citrus.

ASSEMBLING THE RESISTANCE MANAGEMENT PROGRAM

As is often the case with newly established resistance management projects, many essential biological details regarding the maintaining, handling and testing of citrus rust mite had to be resolved as the starting point of our efforts (Denholm & Rowland, 1992). From the beginning, we had our practical end point clearly in mind: to determine whether sustainable use patterns could be identified for dicofol in Florida citrus. To reach this end, the following questions had to be answered:

- Can we maintain citrus rust mite in isolated culture at Cornell University (i.e., in New York)?
- Can we identify a reliable bioassay method, one that sufficiently models the practical pest-pesticide interface such that resistance will be expressed in the laboratory in manner correlated with the field?
- Will we be able to isolate dicofol-resistant citrus rust mite from Florida groves?
- What will be the intensity of the resistance to dicofol and the frequency of resistant individuals in the major citrus production regions of Florida?
- Will citrus rust mite resistance to dicofol be stable or unstable in Florida groves?

Culturing and bioassay methods

We reared citrus rust mite populations in cages on 'Sunburst' mandarin seedlings. These cages were built of Plexiglas® and were connected to humidifiers, controlled by humidistats, that maintained high relative humidity within the cages (Omoto *et al.*, 1994a). This cage design made it possible to isolate numerous mite populations from different citrus groves and to sustain them throughout the year for investigations.

We bioassayed susceptibility of citrus rust mite to dicofol using a leaf-dip, residual method. Leaves of 'Sunburst' mandarin seedlings were immersed in solutions of different concentrations of dicofol (Kelthane® 1.6 EC, Rohm and Haas Company, Philadelphia, PA), and placed on wet cotton pads in open Petri dishes. A small arena of Tanglefoot® was then formed on the treated leaf surface and citrus rust mites were transferred, one at a time, into the arenas, using a single eyelash. Mortality was assessed 24 hours after treatment (Omoto *et al.*, 1994a).

Isolation and characterization of resistance

To isolate resistance from Florida populations, we collected mite samples from groves where dicofol had been used intensively in previous years. In the laboratory, these caged populations were sprayed repeatedly with dicofol, to promote homogeneity of resistance. They were contrasted with a reference susceptible population, collected from a research grove belonging to the University of Florida, in which no pesticide had been used since its planting in 1987.

The responses to dicofol of S and R populations are shown in Figure 1. The intensity of citrus rust mite resistance to dicofol was not great, i.e. we found only an 8.8-fold difference between S and R populations. This was dramatically different from what had been reported for dicofol resistance in other pests. For example, in tetranychid spider mites, the intensity of resistance to dicofol is commonly as high as 100 to 1000-fold or greater (e.g., Dennehy & Granett, 1984).

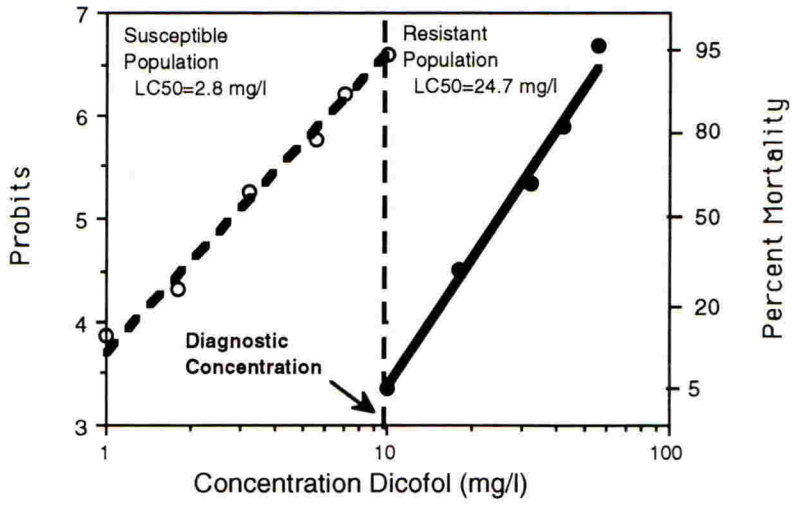

Figure 1. Expression of citrus rust mite resistance to dicofol, relative to a susceptible population, in leaf-dip residual bioassays . The noted diagnostic concentration of 10 mg/l was used to monitor resistance in subsequent field and laboratory trials.

Frequency of resistance in Florida citrus groves

Based on the responses of S and R populations (Figure 1), a concentration of 10 mg/l dicofol was chosen to monitor the frequency of dicofol-resistant mites in subsequent field and laboratory studies. In bioassays using this discriminating concentration, most susceptible citrus rust mites died (>95% mortality) yet most resistant mites survived (<5% mortality). In 1991, we used the discriminating concentration bioassays to survey the susceptibility to dicofol of mite populations from 19 different commercial citrus groves selected in the three major citrus production regions in Florida: the Central Ridge (8 groves), Indian River (7 groves), and the Flatwoods (4 groves). We found significant differences in the frequency of resistant citrus rust mite from one grove to another. The least susceptible populations had less than 50% mortality in discriminating concentration bioassays (Figure 2). The most susceptible populations responded similarly to the susceptible reference population.

By contrasting bioassay results with observations of field performance of dicofol, we gained confidence that sites with high frequencies of survivors of our discriminating concentration (e.g., >50% survivorship, Figure 2) were very likely to have problems with dicofol performance. Similarly, those with high mortality were very likely to obtain satisfactory efficacy from dicofol. To refine further the 'critical frequency' of dicofol resistance, throughout the 4-year course of the project we correlated laboratory bioassay results with growers experience, as well as with our own field trial results (see below). Field performance of dicofol was observed to be acceptable at groves with $\leq$ 20% survivors of discriminating concentration bioassays.

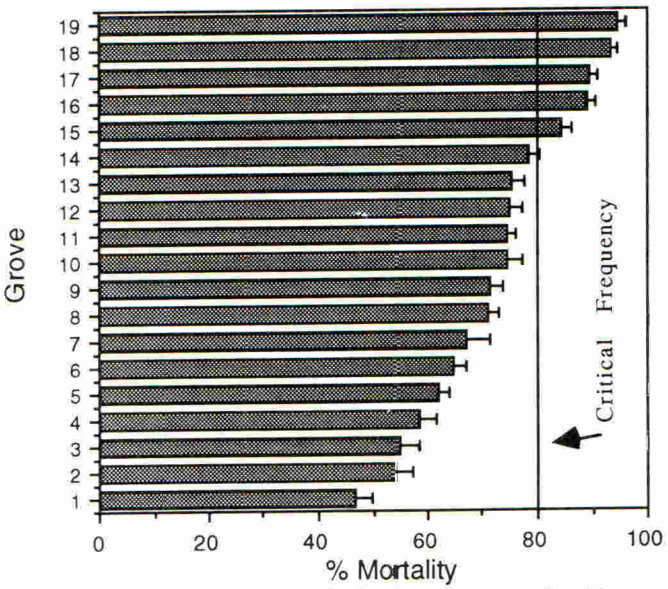

Figure 2. Mean (±SEM) mortality in discriminating concentration bioassays of 19 different populations of citrus rust mite from Florida citrus groves. Shown also is the provisional critical frequency.

Reliability of the monitoring data

To determine the reliability of our monitoring data, we evaluated several sources of variability that could affect the precision of our estimates of susceptibility to dicofol (Omoto *et al.*, 1994b). Testing of field collected mites was found to result in underestimating slightly the true frequency of resistant individuals in populations, presumably due to stresses that caused field-collected resistant individuals to be less tolerant of dicofol than were their offspring. Based on these findings, we made standard the practice of culturing populations for 3-4 weeks (3-4 generations) at 27 ± 2 °C before testing their susceptibility to dicofol. Bioassays conducted on three successive days, of both field-collected and laboratory-reared populations produced very similar susceptibility estimates, therein strengthening our confidence in the bioassay procedure. Also tested was tree-to-tree variability in susceptibility of mites and differences in susceptibility between mites collected from fruit versus foliage. Between-tree variability was not significant within the groves evaluated. Also, populations originating from leaves and fruit responded similarly (Omoto *et al.*, 1994b) in bioassays. We concluded that our sampling and bioassay methodology was generating highly reproducible estimates of the susceptibility to dicofol.

Stability of resistance

We evaluated the dynamics of citrus rust mite resistance to dicofol under cage and field conditions, in order to understand how resistance increased in response to selection, and if and how it decreased in the absence of selection. Laboratory studies of three populations, possessing different frequencies of resistance to dicofol, revealed striking reductions in resistance over a six month period (Omoto, 1994). Complementary field studies were conducted over the course of three years in commercial groves located at Fort Meade and Davenport. These studies confirmed that the laboratory results reflected field realities--in the absence of dicofol selection, populations increased markedly in susceptibility (Figure 3).

These findings indicated strongly that the instability of dicofol resistance could be exploited to sustain susceptibility of citrus rust mite to dicofol.

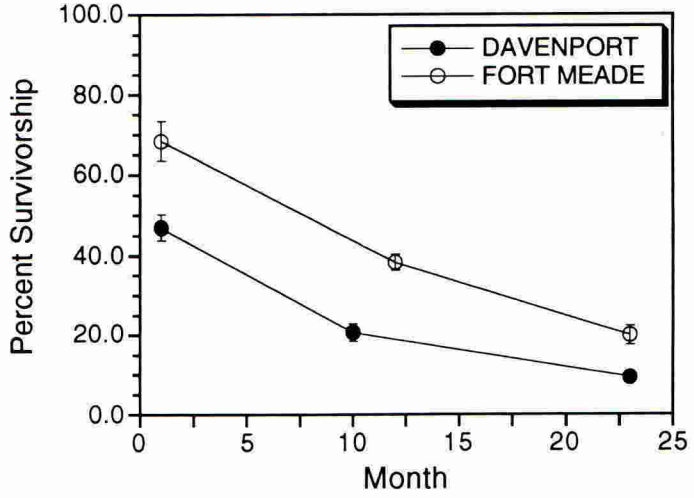

Figure 3. Decline in the frequency of dicofol-resistant citrus rust mites over the course of two years, when the acaricide was not used, at the Davenport and Fort Meade citrus groves. Shown is the mean (±SEM) survivorship in diagnostic concentration (10 mg/l), leaf-dip residual bioassay.

IDENTIFYING A SUSTAINABLE USE PATTERN FOR DICOFOL

Resistance frequencies in large-plot trials

Since the laboratory and field studies had shown that resistance to dicofol was unstable, the issue then was one of determining how often this acaricide could be used in Florida citrus without resulting in a net increase in resistance from one season to the next. To answer this question different treatment regimes of dicofol were evaluated, under commercial application conditions, in commercial citrus groves located at Frostproof and La Belle, Florida. These trials were initiated in 1990 and continued through 1992 (Table 1). At each site, plots of two to four acres each were established in which dicofol was used: (a) twice per year, (b) once per year, (c) no dicofol in 1990 and 1991, followed by the use of dicofol in 1992, and (d) no dicofol during the course of the test. Treatments were applied with a conventional airblast sprayer (FMC diesel) and a volume of 250 gallons per acre. Mite population density was monitored per University of Florida procedures (see Omoto, 1994). When additional acaricide treatments were required during the experiment, beyond the predetermined dicofol treatments, we rotated use of acaricides of different chemical groups, such as ethion, abamectin (applied with petroleum oil), and fenbutatin oxide (Table 1). Resistance was monitored in each treatment before the first acaricide application each year. Additionally, the efficacy of dicofol was documented in each experimental block using standard procedures for monitoring mite population density (detailed in Omoto, 1994).

Bioassays revealed a clear positive relationship between the number of dicofol applications per year and the frequency of dicofol-resistant mites (Figure 4). Over the three seasons that the trials were conducted, it became clear that using dicofol twice per year was not sustainable; it resulted in a progressive increase in the frequency of resistant mites from one year to the next. However, using dicofol once per year was found to be sustainable. Resistance frequencies even declined somewhat under the once-per-year regime.

TABLE 1. Treatment regimes of dicofol (Kelthane®) evaluated in large-plot trials conducted at two commercial citrus groves in Florida.

Treatments*	1990			1991			1992		
	SP	SU	FA	SP	SU	FA	SP	SU	FA
1. KEL 2X per yr	KEL	AGR	KEL		KEL	KEL		KEL	KEL
2. KEL 1X per yr	KEL	AGR	VEN		KEL	VEN		KEL	ETH
3. No KEL for 2 yr	ETH	AGR	VEN		ETH	VEN		KEL	ETH
4. Control									

* KEL = Kelthane (dicofol), AGR = Agri-mek (abamectin) plus petroleum oil, VEN = Vendex (fenbutatin oxide) and ETH= ethion

Application timing: SP = Spring, SU = Summer and FA = Fall

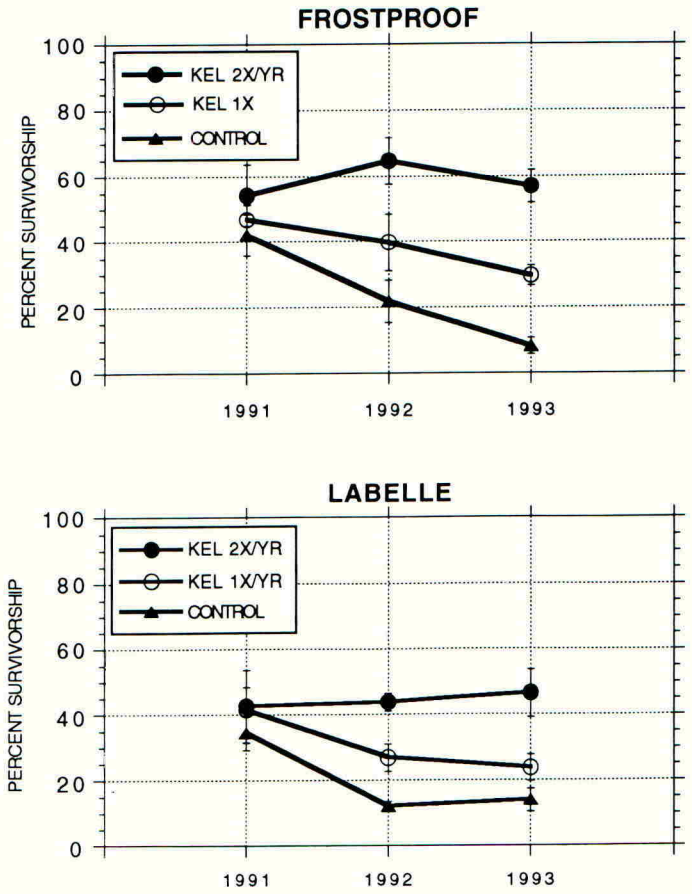

Figure 4. Changes in the frequency of dicofol-resistant citrus rust mite in large-plot trials in which dicofol was used twice per year versus once per year. Shown is the mean (±SEM) survivorship in diagnostic concentration (10 mg/l dicofol), leaf-dip, residual bioassays.

Linking laboratory and field results

Our resistance bioassays showed that using dicofol once per year would not result in increased resistance over time. However, could we trust our bioassay to be providing a realistic reflection of dicofol performance? To address this question we contrasted bioassay results and results of dicofol efficacy trials from all the test plots in 1992. The results demonstrated a strong concordance between the laboratory and the field (Figure 5). The more often dicofol was used each season, the higher the frequency of resistance (in bioassays), and the less mite suppression was in the field trials. Also, the performance of dicofol was significantly improved when this acaricide was not used for two years, owing to a sharp decline in resistance.

LABORATORY BIOASSAY

FIELD PERFORMANCE

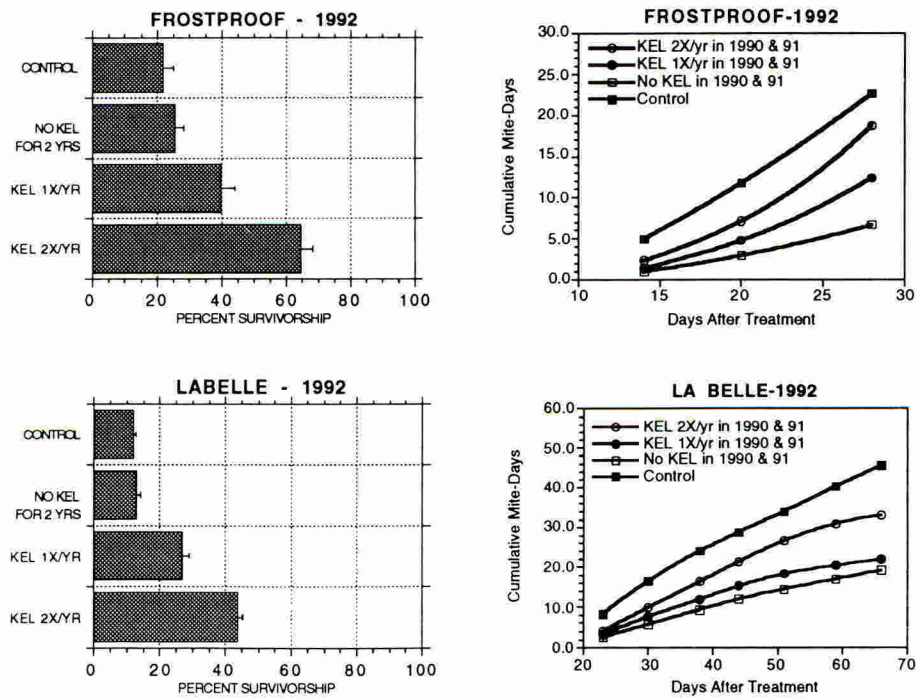

Figure 5. Contrasts of bioassays results (frequency of dicofol-resistant mites) and efficacy of dicofol in 1992 evaluations of chemical use regimes.

CONCLUSION--SUSTAINABLE EFFICACY

In this project we identified and described dicofol resistance in citrus rust mite populations in Florida. We showed that this resistance was unstable and, most importantly, we defined and validated in field experiments, a sustainable use pattern. It is now critical that similar studies be conducted for the other acaricides used for controlling citrus rust mite in Florida citrus, so that the suite of acaricides employed in citrus can be managed in a mutually beneficial fashion. The sustainable resistance management recommendations for dicofol in Florida citrus are as follows: 1) limit dicofol use to once per year; 2) at locations were field failures with dicofol are evident, suspend use for 3 to 4 years; 3) rotate acaricides of different chemical

classes; 4) keep acaricide use to the lowest practical level by following the IPM monitoring guidelines and thresholds set by the University of Florida.

This project represented a highly productive collaboration between University, Industry and agricultural producers. Key elements of the success of the project were: 1) a strong emphasis on linking laboratory and field resistance investigations, 2) a multi-year commitment by Industry for monetary and personnel resources (4 years), irrespective of findings; 3) commitments by University personnel (University of Florida and Cornell University) to work closely with Industry, and to jointly design, execute and analyze trials; 4) commitments from Florida citrus growers to support large plot field trials in their groves for four years. It is our hope that undertakings of this nature will become more common, as interest in practical resistance management programs increases in agriculture.

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PHYLLOXERA (DAKTULOSPHAIRA VITIFOLIAE) ADAPTATION TO SOME ROOTSTOCKS OF THE GRAPEVINE

D. BOUBALS

Chaire de Viticulture, Ecole Nationale Superieure Agronomique, 34060 Montpellier Cedex 1, France

ABSTRACT

In Europe and North America, the grape phylloxera (Daktulosphaira vitifoliae) has demonstrated a capacity to adapt to grapevine rootstocks formerly considered resistant to damage by this species. These are interspecific hybrids between American resistant species of Vitis and V. vinifera, the susceptible species. Novel biotypes of D. vitifoliae exhibit a faster life-cycle and higher fecundity on hybrid rootstocks, leading to the weakening of vines and a major decrease in production.

HISTORICAL BACKGROUND

Grape phylloxera (Daktulosphaira vitifoliae Fitch) (Homoptera: Phylloxeridae) is now a serious pest of viticulture in many parts of the world. Although native to the eastern North America, it was reported in France as early as 1865, following accidental introduction on American vines. It subsequently spread throughout Europe, and is currently invading Turkey. However, D. vitifoliae is apparently still absent from several wine-producing countries including Iran, Afghanistan, India, China, Chile and the main part of Australia.

Symptoms of phylloxera damage to grapevines are galls on the leaves or roots. Leaf galls are very scarce on Vitis vinifera, but more frequent on American species and hybrids, which occasionally need to sprayed with insecticides in the spring to combat heavy infestations. Root galls are of two kinds. Nodosities, or abnormalities of growing rootlets, occur on all species of Vitis, whether susceptible or resistant to phylloxera, but seldom depress the growth of vines. In contrast, tuberosities leading to tissue rot in mature roots can severely depress the growth and yield of susceptible species.

The primary approach to preventing such damage has been to graft V. vinifera varieties yielding high quality wine and berries onto resistant rootstocks derived from wild species of Vitis found in the eastern USA. These rootstocks exhibit nodosities but not tuberosities. The first rootstocks selected and employed in Europe were V. riparia and V. rupestris. Using these it was possible to replant the bulk of European vineyards, except those in areas (eg. Charentes and Champagne) with a high content of lime in the soil. Searches of calcareous areas in the southern USA yielded other species including V. Berlandieri, which is lime-tolerant but unfortunately doesn't provide rooted cuttings.

Several crosses between wild American species of Vitis, and between these and V. vinifera, were made by breeders. Results of field tests for their phylloxera root resistance are summarised in Table 1. Of the interspecific crosses involving V. vinifera, only

Berlandieri x Vinifera showed sufficient resistance to be of practical value. These are also the most resistant to lime, by virtue of their Berlandieri parentage.

TABLE 1. Expression of phylloxera resistance in crosses between Vitis species. (R = resistance of practical value, S = insufficient resistance).

	V. vinifera	V. rupestris	V. riparia	V. Berlandieri
V. vinifera	S			
V. rupestris	S	R		
V. riparia	S	R	R	
V. Berlandieri	R	R	R	R

In crosses with V. vinifera, resistance in V. Berlandieri was partially dominant and under polygenic control. Resistance in V. riparia and V. rupestris in crosses with V. vinifera was also polygenic, but with partial dominance of susceptibility (Boubals 1966a, b).

No interesting rootstocks were obtained from the Riparia x Vinifera crosses. In the Rupestris x Vinifera crosses, rootstocks with low-level resistance to radicolous phylloxera were obtained. One of these, Aramon x Rupestris Ganzin n°1 (AXR1), was the first employed in Europe (Ganzin, 1887). However, Ravaz (1897) observed tuberosities on its roots. In vineyards, failures of AXR1 were reported in Sicily in 1908 (Degrully, 1909; Grimaldi, 1909; Richter, 1909) and in Spain in 1915 (Feytaud, 1920). Following these reports, AXR1 was rapidly abandoned in Europe.

In South Africa, Perold (1927) also observed the failure of AXR1 and was the first to propose the occurrence of a new, more agressive biotype of D. vitifoliae. Also in South Africa, DeKlerk (1979) proposed that the existence of different biotypes might explain differences in the aggressiveness of the insect on varieties 143 B (Riparia x Vinifera) and 101-14 MG+ (Riparia x Rupestris).

Despite these developments, AXR1 rootstocks became widely used in California in the 1980s. 75% of plantings in Napa and Sonoma counties employed AXR1 on the recommendation of researchers at the University of California (Davis). Around 1987 it was observed that vines grafted onto AXR1 were weakening in growth and production. As a consequence, it is now necessary to uproot all vineyards established on this rootstock and replant on more resistant varieties. Further information on the incidence and pest status of D. vitifoliae, and on attempts to manage this through the development of resistant cultivars, is provided by Granett et al. (in press).

DEMONSTRATION OF D. VITIFOLIAE ADAPTATION

Throughout the last century, many workers alluded to differences in the aggressiveness of phylloxera populations. Variation in leaf galling on interspecific hybrids was described in Ontario by Stevenson (1970) and Williams and Shambaugh (1988), while Riley (1870), Borner (1941) and Maillet (1957) reported differences in root damage. None

of these, however, were able to provide a clear demonstration or explanation of this phenomenon.

J. Granett and colleagues in California were the first to show conclusively that differences in aggressiveness reflect adaptive changes in phylloxera populations. Based on work with AXR1, two biotypes of D. vitifoliae were reported: an 'A' biotype causing no tuberosities on AXR1, and a 'B' biotype causing severe damage to roots and consequent weakening of vines grafted onto AXR1. In Granett's view, such biotypes are best regarded as host races, each better adapted to a specific host than other races.

Coinciding with Californian infestations of phylloxera in the 1980s, Granett et al. (1985) demonstrated through life-table studies that insects collected from affected vines on AXR1 showed enhanced growth on roots in laboratory culture. In contrast, insects removed from vigorous vines of AXR1 performed poorly in the laboratory. These populations were designated biotypes B and A respectively. This difference in aggressiveness was observed only on AXR1 and not on other resistant rootstocks in current use in viticulture.

Song and Granett (1993) investigated the weakening of rootstock 41B (Berlandieri x Vinifera) in shallow soils in Charentes, France. This appeared to be another example of phylloxera adaptation to a rootstock with V. vinifera parentage. This variant has not been compared directly with biotypes differing in aggressiveness to AXR1, although in laboratory tests the A and B biotypes proved equally susceptible to 41B. Hence the new variant appears to be genetically distinct from both of those recognised previously.

Similar work in France by Martinez-Peniche (1993) demonstrated phylloxera adaptation to other rootstocks with similar parentage to 41B: AXRn°9 and Fercal. DNA analyses exploiting RAPDs indicated the presence of three biotypes closely associated with particular host varieties. At present, this adaptation is of practical importance in vineyards employing 41B and Fercal rootstocks. More extensive surveys of insects on different rootstocks have disclosed marked differences in the behaviour of phylloxera; their biological significance is being investigated further. King and Rilling (1985) found a German biotype of phylloxera to cause greater damage to AXR1 than one from New Zealand. Again, the relationship between these variants and ones described from California is unclear at present.

CONCLUSIONS

Reports of adaptations causing the loss of phylloxera resistance have so far been confined to rootstocks with V. vinifera in their genetic composition. As a result, it is strongly advised to avoid these in practical viticulture. Rootstocks of V. riparia and V. rupestris, and their hybrids with V. Berlandieri, retain such resistance and are therefore considered best-suited for use on a worldwide basis. Since these now support the bulk of world viticulture, the possiblility of further adaptation by phylloxera extending to American species and hybrids is of major concern.

D. vitifoliae demonstrates very clearly the capacity for pests to evolve and adapt to supposedly resistant crop cultivars. It highlights the danger of complacency and the need for careful surveillance whenever host plant resistance is exploited as a crop protection tactic on a large geographical scale.

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CAN PLANT RESISTANCE GENES BE USED TO ENGINEER BROAD-SPECTRUM DISEASE CONTROL?

J. JONES

The Sainsbury Laboratory, Norwich, Norfolk

-- No abstract or paper submitted --

RESISTANCE MANAGEMENT - MAKING IT HAPPEN

P. K. LEONARD

Cyanamid International, Chaussée de Tirlemont, 105, B-5030 Gembloux, Belgium

R. M. PERRIN

Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY

ABSTRACT

Since its formation in 1984, GIFAP's Insecticide Resistance Action Committee has been the focus of the Agrochemical Industry's efforts to manage insecticide and acaricide resistance. IRAC has been instrumental alongside other groups in surveying product failures due to resistance, developing practical monitoring methods, publishing management guidelines and sponsoring fundamental and applied research in several countries. Various examples are given in this paper. IRAC is responding to the increased need for resistance management as a vital component of sustainable agriculture and sustainable pest control in public and animal health by focusing its resources on those activities which have the greatest probability of reducing selection pressure in the field. These activities include local implementation of strategies by growers, establishing the relationship between monitoring data and the level of control achieved in the field, and particularly communication of its messages and education of all those involved in crop protection. There is optimism that through a co-operative attitude, a wide range of cost-effective products can be preserved for long-term use in IPM.

INTRODUCTION

The Insecticide Resistance Action Committee (IRAC) of GIFAP was founded in 1984 to provide a co-ordinated Industry response to the global development of resistance in insect and mite pests (Jackson, 1986). Resistance is defined here as a reduction in the sensitivity of a population which is reflected in repeated failures of a product to achieve the expected level of control, when used according to the label recommendations for that pest species, and where problems of product storage, application and unusual climatic or environmental conditions can be eliminated.

Insecticides and acaricides are essential components of modern approaches to IPM and sustainable agriculture and sustainable pest control in public and animal health. Attempts by IRAC to preserve their efficacy have been based on a four-phase plan:

1. Surveying resistance worldwide and ranking the priority cases for IRAC's attention,
2. Developing and publishing practical monitoring methods for all major pest species,
3. Establishing sound management guidelines, based on a knowledge of physical, genetic and biochemical mechanisms, to reduce the incidence of field failures

caused by resistance,

4. Encouraging the implementation of management strategies at a local level.

Throughout this process, IRAC has been keen to work in collaboration with officials, academics and other organisations dedicated to managing resistance in line with FAO's code of conduct (Anonymous, 1990a.) This paper serves as a timely progress report on IRAC's achievements to date, and more importantly, summarises our future goals and tactics in the continued struggle against resistance.

ACHIEVEMENTS

IRAC's achievements in the past decade can be highlighted under the headings of the four-phase plan:

Surveys

One of its first successes was to survey resistance, based uniquely on member companies' documented experiences of product failures in field use. This comprehensive record of known or suspected cases around the world was first documented in 1985 (Voss, 1987, 1988). IRAC was thus able to identify the most urgent threats to sustained agricultural production and to encourage the use of appropriate monitoring methods to define the extent of each problem. An updated summary of this survey, classified by insecticide product groups, is due to appear in the next edition of *The Pesticide Manual*. A new, comprehensive survey amongst member companies is scheduled for 1995, prompted partly by the need for reliable information to meet re-registration guidelines in Europe (EC Directive 93/71/EEC, Annex III) and the USA.

Perhaps the most valuable aspect of IRAC surveys has been their contribution to a more balanced appraisal of the true impact of resistance on agriculture. There has been an occasional tendency to sensationalise the threat and geographic spread of the phenomenon, which in some situations, has prompted unnecessary and uneconomic reactions amongst policy-makers or growers.

Monitoring Methods

Reliable data on resistance rather than rumours or assumptions, is the cornerstone of successful management. To help achieve this, the product and crop related working groups of IRAC published a range of field monitoring methods, for use under a wide variety of circumstances, in 1990 (Anononymous, 1990b). These methods, chosen with the potential to be reproducible under field usage, and simple and easy to perform using a minimum of resources, prompted valuable discussion and refinement by resistance workers. Several have been the basis of implemented (e.g. Pakistan, Belgium) or proposed (e.g. China, Poland) large-scale monitoring programmes. IRAC Method No. 7, for leaf-feeding Lepidoptera and Coleoptera, has been validated in the laboratory (Perrin and Löwer, 1994) and under field conditions in Taiwan on *Plutella xylostella* and in Pakistan on *Helicoverpa armigera* (unpublished IRAC reports, 1993-94), proving to be reliable and versatile across a range of crops, pests and stomach- and contact-acting insecticides. The IRAC Cotton working group and IRAC China recently organised a training session on monitoring in Nanjing, China, which

it is hoped will lead to full-scale monitoring in the cotton-growing provinces.

Resistance Mechanisms and Management Guidelines

One of the most lively debates amongst resistance workers has surrounded the question of rotation or alternation of single product groups versus pre-formulated or tank mixtures of products with different modes of action. IRAC has been instrumental in advocating the *principle* of rotations by insect generation, based on exclusive resistance mechanisms, not just mode of action. Fundamental research to elucidate field mechanisms, on which product sequences and other management guidelines can be rationally based, has been supported in several countries.

A good example of the shift in emphasis from 'mode of action' to 'resistance mechanism' is the apple leafminer, *Leucoptera scitella*, in northern Italy. Reports of resistance to diflubenzuron in *L. scitella* in 1986 resulted in a rapid response from the Fruit Crops working group. A novel monitoring technique, IRAC Method No. 9, was developed and used by member companies under laboratory and field conditions. Results showed that resistance to diflubenzuron was indeed well established, but it did not extend to all acylurea insecticides, even though they share a common mode of action. From such a knowledge base, more sensible and less economically-damaging recommendations to growers were made than would have arisen with total avoidance of acylureas.

Implementation of Strategies

The Central Committee of IRAC (currently with 14 members from 11 companies) believes that prevention or containment of resistance is hindered more by inadequate implementation of effective product use patterns by growers and advisors than by limited understanding of pest biology and genetics. Our resources will be increasingly focused on validation and implementation of locally-adapted strategies, in ways outlined later in this paper.

There has been a relatively small number of partially successful campaigns to combat resistance (for example, Australian cotton and West European spider mites). However, the private and public sectors have much to accomplish if sensible IRM and IPM are to prevail in all major markets, prompted preferably by common long term goals and not by immediate crop protection crises.

Whilst *Heliothis* and spider mite strategies have been relatively well publicised, IRAC has played a vital role in promoting resistance management through product labels and use definitions within member companies. IRAC was also instrumental in advising the European Crop Protection Association (ECPA) during consultations with the EC Directorate on resistance guidelines for Annex III of the harmonised EU regulations.

FINANCIAL SUPPORT

Since 1990, member companies have contributed annually to a central fund, managed by GIFAP/ECPA in Brussels. This fund has supported a wide range of resistance-related activities, of either a fundamental or applied nature. The broad allocation of money has been

as follows:

Research	US	$250,000
Communications		$89,000
Travel		$19,000
TOTAL		$358,000

Additional funds have been raised by some national working groups, and the Bt Management working group is self-financing.

A prime example of our commitment to research is the IRAC-sponsored work of Alan McCaffery, at the University of Reading, and James Ottea, at Louisiana State University, on pyrethroid resistance mechanisms and their expression in *Heliothis virescens*. Their studies over many years have contributed to a better understanding of how mechanisms evolve in relation to different selection pressures, and form the basis on which the Beltwide management strategy could be modified in the future (unpublished IRAC report).

On numerous occasions, IRAC has lent its urgent financial support to various research and communication initiatives. Examples include i) the ambitious resistance monitoring and management programme in Andhra Pradesh, India, in collaboration with the Natural Resources Institute (NRI) in the UK and the International Organisation for Resistant Pest Management (IOPRM) (Armes et al, 1994); ii) recent provision of funds to the Plant Protection Institute in Poznan, Poland, to enable IRAC Method No. 7 to be validated for Colorado potato beetle, and iii) assistance with the continued publication of the *Resistant Pest Management Newsletter* by the Pesticide Research Center of Michigan State University.

THE FUTURE

As the title implies, IRAC's main objective must be to "make it happen". Despite the past efforts outlined above, there has never been a more pressing need for action to preserve pesticide efficacy, particularly in developing countries where crop failure often represents a social and economic disaster.

The national and international working groups therefore wish to encourage:

i) the validation and optimisation of management strategies in the field,
ii) a better understanding of the relationship between monitoring data from bioassays and actual levels of pest control achieved by growers,
iii) attempts to educate and train advisers, dealers and growers in successful and sustainable crop protection practices.

To this end, IRAC will rank projects and requests for funding or technical advice according to likely impact on field use patterns and selection pressure for resistance.

IRAC has already strengthened its Communications working group, and has demonstrated its intention to fund practical studies, such as the proposed field evaluation of a rotation strategy for anopheline mosquito control in Mexico. The Field Crops working

group is actively promoting its guidelines for management of acylurea resistance in *Plutella xylostella*, and the Bt Management group has been particularly innovative in delivering its well-founded messages to a wide audience of end-users, environmental pressure groups and the public, with the expert assistance of a public relations firm, Fleishman-Hilliard.

A further example of the desire for "action" is the formation of a 'new acaricide sub-group' (N.A.S.G.) of the Fruit Crops Working Group, to address the threat of resistance developing in Europe to the mitochondrial electron transport inhibitor acaricides (tebufenpyrad, fenazaquin, fenpyroximate and pyridaben). Having established the fact that all these compounds share the same mode of action (Hollingworth et al, 1992, 1994: Motoba *et al* 1992, Anonymous 1993), member companies responsible for these compounds in Europe are collaborating with Rothamsted Experimental Station, with the assistance of IRAC funding, to devise a harmonised monitoring programme. The ultimate aim is to reduce the risk of cross-resistance developing, through a common approach, **before** field failures arise, thus avoiding the severe mite control problems that have plagued the top fruit industry in the past (Leonard, 1992; Sterk and Highwood, 1992).

CONCLUSIONS

IRAC is proud of its contribution, alongside other organisations and individuals, to improved awareness and management of resistance issues. There is no room whatsoever for complacency, and IRAC will continue to seek reliable information and strive to implement sensible insecticide and acaricide use wherever possible. This requires not only the co-operation of manufacturers, regulators, extension services, consultants, sellers and users, but also effective communication and compromise between the technical and commercial departments of every company marketing crop protection agents. Introducing sensible use patterns when a new product is first launched, through appropriate labelling, promotional literature and training is one of the challenges to which Industry is now responding.

We do not pretend that the motivation of short-term sales and profit, and the growers' desires for a 'quick fix', are easy obstacles to overcome, but we remain confident that agrochemicals, both old and new, will continue to play a major role in the cost effective and environmentally-sound production of food and fibre.

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The authors thank all the past and current members of IRAC who have contributed to the successes described in this paper which we dedicate to the fond memory of our former Central Committee chairman, Jean-Jacques Hervé.

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Session 8B

Advances in Pesticide Technology

Chairman	Mr P Nixon
Session Organiser	Mr A Spicer
Papers	8B-1 to 8B-5

NOVEL FORMULATIONS FOR LOCUST AND GRASSHOPPER CONTROL

C. BATES

Collag Corporation, Unit 1 Millhouse Business Centre, Hazel Road, Woolston, Southampton, SO19 7GB (correspondence address)

and

Department of Biology, School of Biological Sciences, Southampton University, Biosciences Building, Bassett Crescent East, Southampton, SO9 3TU

ABSTRACT

A novel oil based microcapsule formulation of malathion has been developed specifically for locust control, promising both extended persistence and increased application efficiency when employing ULV methods. Since use of organochlorine insecticides for locust control was halted a dependence has developed on organophosphorous insecticides, such as malathion and fenitrothion, which suffer from short persistence and the need to target locust hoppers and adults directly. Malathion capsule suspensions raise the possibility of reinstating barrier spraying methods.

INTRODUCTION

Locusts still represent a significant international threat to crops and pasture world-wide (Skaf, Popov & Roffey, 1990). Control still revolves around the large-scale use of insecticides to control outbreaks (Symmons, 1992). Large quantities of organophosphorous insecticides such as malathion and fenitrothion are currently used (MaCuaig, 1983) although, historically, effective control was achieved by strip-spraying persistent organochlorines such as dieldrin (Bennett & Symmons, 1972; Courshee, 1959; Courshee, 1990). Use of organochlorine insecticides is prohibited because of their adverse environmental effects (MacCuaig,1979) and the organophosphorous products in use have short persistence and must be sprayed directly onto locust infestations.

One aim of this project is to develop microcapsule formulations of malathion that possess extended persistence and permit a degree of prophyllaxis. Microencapsulation may also reduce environmental impact and increase selectivity to locusts. In the past, microcapsule formulations have consisted of an encapsulated oil phase liquid pesticide or solution, suspended in a water phase. Such formulations are not suitable for ULV spraying in the tropics because the water phase evaporates immediately upon atomisation and the deposition characteristics of the dry microcapsules are

unpredictable. This paper reports the first developments of an oil suspension of polyurethane microcapsules containing malathion technical material.

The process of microencapsulation entails containing an active ingredient (normally liquid) inside a polymeric shell, thus controlling the rate at which the chemical is released and protecting the bulk of the material from degradation (Wilkins, 1990). Microencapsulation offers a number of other advantages (Phillips, 1968; Tsuji, 1987; Marrs & Scher, 1990; Ainsworth, 1988) including reduced toxicity to vertebrates and reduced evaporative losses during spray application in arid habitats. The disadvantages include higher production costs compared with traditional formulations, restriction of formulation active ingredient levels to approximately 50% and delayed effects because of the lack of rapidly available active ingredient.

From the wide number of microencapsulation techniques available, interfacial polycondensation was chosen because it resulted in liquid sprayable formulations. No further processing is required and in theory, release characteristics can be varied by altering polymer type, monomer identity, polymer cross-linking, wall thickness and microcapsule particle size.

An experimental method was developed for measuring residual toxicity to II instar Desert Locust *(Schistocerca gregaria)* nymphs, with the aim of comparing the efficacy of malathion technical-grade and a malathion microcapsule formulation. The formulations were applied to glass surfaces using a Potter laboratory spray tower, and stored under low light conditions at room temperature until exposure to locusts. The initial biological test results are reported.

MATERIALS AND METHODS

The treatments prepared were malathion technical, diluted to 5% ai in aromatic hydrocarbon, aromatic hydrocarbon alone, polyurethane walled malathion microcapsules with a 5:1 core to wall ratio diluted to 5% ai in paraffin oil, paraffin oil alone and unsprayed blank plates. Ten replicate plates of each treatment were prepared by spraying the preparations onto 10 x 10 cm glass plates using a Potter tower. All treatments were sprayed with an air pressure of approximately 24 kPa and the quantities of spray liquid were adjusted to account for differences in formulation density. A deposit mass of 180 $ugcm^{-2}$ was required to simulate a usage rate equivalent to 900 g ai/ha.

After storage for the specified length of time the plates were placed on raised flat surfaces and single II instar *S. gregaria* trapped over the treated surfaces with clear plastic containers pierced with ventilation holes. Each of the locusts were forced to initially contact the treated surface but were then free to climb the container walls. Exposure of locusts to respective control and active treatments was performed in pairs in an attempt to minimise differences in length of exposure. The hoppers were allowed to feed until shortly before use but no food was provided during the experiment. All treated surfaces were allowed to dry before exposure of locusts: fresh deposits of Solvesso 200 were harmful to the locusts.

The condition of the locusts was visually assessed at a range of times after treatment: locusts climbing the container walls were scored as alive and stationary insects were gently stimulated by movement or soft tapping. The experiment was terminated after 24 h in all cases.

The storage conditions for the sprayed glass plates (ambient laboratory conditions within a fume cupboard) were considerably less harsh with respect to temperature, humidity and ambient light than found under typical field conditions, it is expected that persistence in the field would be shorter.

RESULTS

No locust mortality was found for any of the aromatic solvent and paraffin oil treated plates or on the blank, untreated plates. Mortalities for the remaining are given in table 1. The data indicates more rapid mortality of locust hoppers exposed to malathion technical compared with microcapsules on the day after preparation. All the insects were dead after 24h.

Storage of the glass plates for fifteen days after spraying, resulted in no major changes in mortality rate for the malathion microcapsule treatment but a delay in effects for the malathion technical.

After 36 days storage, the malathion technical treated plates failed to cause any locust mortality, even after 24 h. The malathion microcapsule treatment still killed all the exposed hoppers over 24h.

TABLE 1. Mortality of II instar desert locusts (total of ten replicates) after various exposure times to treated glass plates. The plates had been stored under low light conditions at room temperature for various lengths of time after treatment.

Treatment (storage in days)	Times after intyroduction of locusts				Average deposit /μgcm^{-2}
	2 h	3 - 4 h	7h	24 h	
Malathion Technical (1)	7	10	--	10	180 ($\pm$11)
Malathion Microcapsules (1)	0	7	--	10	190 ($\pm$11)
Malathion Technical (15)	0	0	--	10	190 ($\pm$9)
Malathion Microcapsules (15)	1	7	--	10	190 ($\pm$17)
Malathion Technical (37)	0	--	0	0	200 ($\pm$12)
Malathion Microcapsules (36)	1	--	9	10	190 ($\pm$13)

CONCLUSIONS

The delay in lethal effects in the microcapsule treatments may result from the requirement for the locust hoppers to 'trample' and burst the microcapsules before they are exposed to the active ingredient.

In conclusion encapsulation of malathion offers significant increase in persistence when compared to malathion technical with only a slight penalty of reduction in rapid initial mortality. This may allow a return to barrier spraying, depositing lines of insecticide rapidly by air when upsurge is predicted and dismissing the need for intensive scouting and direct attack.

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NEW FORMULATION TECHNOLOGY APPLIED TO DIAZINON

S. UK, A. SUTTON, H.W. HASSLIN, F. BOURGEOIS, L. SCHWAGER
Ciba Crop Protection Division, Basle, Switzerland

ABSTRACT

In the product improvement process, the latest formulation technology is used to produce a diazinon 600 EW. Diazinon has been formulated in the form of emulsifiable concentrates (in the presence of traces of water, diazinon can produce the toxic degradate O,S-TEPP. In the emulsifiable concentrates, this reaction has been prevented by the addition of a "water scavenger". Ciba has now developed a new technology (patent pending) to formulate diazinon as an oil-in-water emulsion (EW). The excess water in this product causes the rapid breakdown of any O,S-TEPP that may be generated to a harmless product. As a result, a water scavenger is no longer needed in the EW formulation. The EW formulation contains no organic solvent that may have the risk of fire hazards or harmful effect to the users; the main carrier is water. In many field trials the 600 EW showed efficacy equal to the well known 600 EC formulation on all the pests tested. As expected from a water- based formulation, the 600 EW caused no phytotoxicity in most crops except in some grapes varieties during the early stage of the fruit formation.

INTRODUCTION

The most common Ciba formulations of diazinon for use in crop protection are the emulsifiable concentrates as examplified by BASUDIN 600 EC. In the presence of traces of water, diazinon active ingredient can produce the toxic degradate O,S-TEPP (Margot and Gysin 1957). This reaction has been prevented by the addition of a water scavenger to the EC formulations.

The oil-in-water emulsion formulation system that omits the use of volatile organic solvents which are nowadays regarded as undesirable for transport and storage. Water is used as the main carrier. The excess water in the formulation will cause rapid breakdown of any O,S-TEPP that may be generated into a harmless product. Water scavenger is no longer needed.

This paper presents the physico-chemical properties a diazinon 600 EW and results of biological tests in comparison with traditional 600 EC formulatin.

PHYSICO-CHEMICAL PROPERTIES

The 600 EW has a moderate viscosity of about 1000 mPa.s., an emulsion droplet size in the range of 2-5 µm and a flash point of ≥ 100 ° C.

It mixes readily with water and is compatible with the majority of tank-mix partners, these being wither water based products (SC, EWs), emulsifiable concentrates (ECs) or wettable powders (Wps). The storage stability is comparable to other water based products with a shelf-life of at least 3 years under moderate and at least 2 years under tropical conditions.

The 600 EW formulation is a true emulsion of diazinon in water. It contains no organic solvent; therefore, it has no fire hazard and no risk of corrosion to rubber and plastic parts of the spray equipment.

The difference in constituents between the 600 EW and 600 EC formulations is presented in Table 1.

TABLE 1. Typical comparative composition (% w/v) of a diazinon 600 EW and 600 EC formulations

Component	600EW	600 EC
Active ingredient	60	60
Organic solvent	0	30
Water	33	0
Surfactant	2	10
Inert materials	5	0

PHYTOTOXICITY EVALUATION

Greenhouse tests

Tomato (cv. Montfavette, hight 15 cm) and zucchini (cv. Fruebusch, 3-leaf stage) plants grown in pots in a greenhouse were treated in a spray chamber at a rate of 400 litres of spray mixture per hectare. Phytotoxicity evaluation was done 14 days after application by estimating the leaf damage area. The results are shown in Table 2. On tomato both the 600 EW and the 600 EC caused only traces of leaf necrosis even at the high concentration (7.5. g per litre). The zucchini cultivar tested was more susceptible to the high concentration of the 600 EC than the tomato. It should be noted that plants grown in a greenhouse are usually sensitive to sprays. Under normal practice in the field no symptoms of phytotoxicity were observed.

TABLE 2. Comparative phytotoxicity tests on tomato and zucchini in a greenhouse between the 600 EW and 600 EC

Crop	Concentration in g ai/litre (applied at 400 litres/ha)	Mean % leaf damage 600 EW	600 EC
Tomato	2.50	2 (traces)	2 (traces)
Tomato	7.50	2 (traces)	2 (traces)
Zucchini	2.50	2 (traces)	10 (weeks)
Zucchini	7.50	4 (negligible)	35 (moderate)

Field Evaluation

Field trials were conducted at the Ciba Lombang Research Station in Indonesia. Tomatoes (cvs. TW and Gondol Hijau) and cucumbers (cvs. LV and Cisarua) planted in small plots of (5 m^2) were sprayed eight times during the growing period starting at 23 days after planting. Plots of Chinese cabbages (cvs. Nagaoka and Hybrida) were treated in a similar way starting 12 days after planting. The concentration for all sprays was 80 g active ingredient per 100 litres of water applied at 1000 litres per hectare. Observations were made through the crop cycle for any sign of leaf damage. There was no observable sign of leaf damage nor reduced plant growth amongst all the plant culivars tested.

Although safe in most crops, some table grapes varieties (Almeria, Cardinale, Flame, Ribier, Tokay) in Chile showed severe russeting when the diazinon 600 EW was applied during early fruiting (4-5 mm fruit diameter). However, the same varieties showed no sign of russeting when treated at 15-mm fruit size. Other varieties such as Flame, Superior and Thompson seedless never showed any sign of damage. Young fruits of many varieties are normally highly sensitive to a number of insecticide active ingredients.

INSECTICIDAL ACTIVITY

Field trials were conducted on different crop/pest complexes in different parts of the world to compare the efficacy of the 600 EW and 600 EC. The results are presented in Table 3. They indicate that the efficacy of the diazinon 600 EW is the same as the traditional 600 EC. At the recommended rates of 500 - 600 g active ingredient per ha, the 600 EW showed good to excellent control of a wide range of pests on different crops.

TABLE 3. Efficacy of Diazinon 600 EW and Basudin 600 EC on some imprtant pests

Pest (No. of trials)	Crop	Country	Rate gai/ha	% control ± SD 600EW	600EC
Aphis fabae(1)	Beans	Switzerland	600	99	99
Aphis gossypii (1)	Tomato	Spain	600	89	90
Aphis pomi	Apple	Switzerland	500	87±10	90±1
Brevicoryne brassicae(2)	Crucifers	Egypt	500	99	99
Ceroplastes floridensis(3)	Citrus	Egypt	500	97±1	98±1
Cydia pomonella(2)	Apple	Italy	500	81±16	83±11
Dysaphis plantaginea (2)	Apple	Switzerland	500	88±16	99±0.8
Empoasca lybica (2)	Cotton	Egypt	500	78	79
Gargaphia solani (1)	Eggplant	Thailand	500	97	97
Lepidosaphes beckii (3)	Citrus	Egypt	500	92±6.5	98±0.8
Leptocorisa spp. (1)	Rice	Indonesia	500	94	94
Prays citri (2)	Citrus	Spain	600	68±11	77±33
Quadraspidiotus perniciosus (1)	Citrus	Spain	600	95	95

CONCLUSION

BASUDIN 600EW, a water based formulation, is the best choice in product enhancement for diazinon. It eliminates the use of undesirable volatile organic components and prevents the formation of the toxic degradate O,S-TEPP. During storage it is physically and chemically stable for at least three years under moderate conditions. Formulating as a 600EW rejuvenates diazinon by further improving the safety aspects for the spray operators while maintaining its high efficacy on a wide range of major pests.

ACKNOWLEDGEMENTS

We express our sincere appreciation to all Ciba R&D colleagues in Chile, Egypt, Indonesia, Italy, Spain, Switzerland and Thailand for their valuable contributions in conducting the field trials.

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LINKPAK - A REFILLABLE PACKAGING SYSTEM FOR CROP PROTECTION PRODUCTS

T.H. ROBINSON, J.P. COWLAND

Ciba Agriculture, Whittlesford, Cambridge CB2 4QT

ABSTRACT

Refillable pesticide packaging for liquid pesticide formulations has long been requested by European farmers. It is seen as an attractive method of overcoming the physical and environmental problems of packaging waste disposal.

The paper reviews the development of a novel 10 litre refillable container designed to meet the needs of the farmer, while setting new standards for operator safety, ease of measuring part packs and speed of transfer from the container to the sprayer.

BACKGROUND

Draft legislation on packaging waste disposal means that pesticide manufacturers should be pro-active in their approach to formulations and packaging in order to minimise the volume of used packaging entering the waste stream (EEC Draft Proposal, 1991). It is important that when legislation is imposed, it be workable, and targets for reusing, recycling, and recovery be realistic. In practice this means that the outlets for the waste packaging must be in situ, before the legislation is introduced. If not, the legislation is unenforceable, and may have detrimental repercussions on neighbouring countries (White, 1991).

Refillable containers have great potential for reducing the amount of plastic packaging entering the waste stream. They form a significant proportion of the total pesticide packaging in Canada where a surcharge of one dollar is placed on all containers to contribute to the cost of their disposal. This surcharge adds greatly to the fact that over 60% of pesticides sales in Canada are in refillable containers.

In Europe, national packaging legislation including a surcharge on containers has been introduced in Germany, Austria and Belgium, but as yet it has had little effect on the agricultural community which has been exempted from the severest aspects.

A further issue facing the chemical manufacturer is the reduction of operator contamination by improved methods of packaging and transfer of product from the container to the sprayer. The Control of Substances Hazardous to Health (C.O.S.H.H.)

Legislation, when applied to pesticide usage, aims to reduce risks on farm by first assessing the hazards and risks of the products and their usage. Following a hierarchy of control measures the risk must be both minimised and reduced to an acceptable level. The principle of C.O.S.H.H. is that Personal Protective Equipment (P.P.E.) should be the last line of defence against a hazard. Elimination, substitution, engineering control, and operational solutions are preferable.

Applying this logic to pesticide container design, the greatest risk to the operator is incurred when product is transferred from the container to the sprayer. The four main opportunities for contamination of a competent operator are:

1. Removing the cap
2. Removing the secondary seal
3. Pouring the product
4. Rinsing the container

A closed transfer refillable system, whereby the farmer returns the container unrinsed for refilling avoids exposure during steps 2, 3 and 4 greatly reducing the opportunity for operator contamination.

Speed of operation is an important factor in optimising the biological performance of pesticides. Over the past two decades the designers of sprayers have subconsciously vied with chemical manufacturers and vice versa for improving work rates. In the early to mid 1980 s discontent with pouring rate and glugging associated with 45 mm neck sizes led to the widescale introduction of containers with 63 mm necks in 1987. During the last 7 years however, the size of the largest sprayers has virtually doubled, while spray volumes continue to decline.

Operators find that with even the best conventional packs the time take to fill the sprayer with water is generally less than half the time required to fill the sprayer with chemical.

Ciba's intention was to develop a small volume refillable container that would satisfy most of the needs of future packaging legislation. Reducing the potential for operator contamination, and be sufficiently attractive to the end user to be introduced commercially in advance of legislation putting a penalty premium on the use of single trip containers.

DEVELOPMENT PROGRAMME

Two candidate containers were tested in farm trials in 1992 for user acceptability. These were:

i) 20 litre stainless steel small volume refillable (SVR) keg. This required a separate metering device to transfer the product from the container to the sprayer.

ii) The Ciba-Link (CL), a 10 litre prototype pesticide container produced by Link Racing in the US, similar to a closed transfer system used for filling racing cars. The Ciba-Link required a small adaptor to be fitted to the lid of the induction hopper in order to transfer the product to the sprayer.

The 1992 trial also included an operator exposure study using 10 operators to transfer 2 litres of product from 2 x 1 litre conventional HDPE containers, and 2 litres of product from the SVR and the Ciba-Link. Treatments were replicated twice by each operator.

Operators were dressed in Tyvek overalls, Tyvek boot covers, Kimguard disposable gloves, and 3M, 8710E particulate respirator face masks. Scott Pulman paper towels were used as Swabs. A simulated pesticide formulation was made by mixing 5 g of sodium fluorescein per litre of water.

After each transfer of product, the operator was undressed and his clothes cut into sections before being stored in the dark pending analysis by a Perkin Elmer LS30 spectrophotometer. Splash and spillage contamination of the sprayer was cleaned up with the swabs, which were stored and analysed under similar conditions.

RESULTS

TABLE 1. Average operator contamination during tank filling -- μlitre contamination

SAMPLE	HDPE CONTAINER	CIBA-LINK	SVR
Hood	0.08	0.000	0.004
Trunk	2.52	0.004	0.004
Right Arm	0.18	0.006	0.002
Left Arm	0.10	0.008	0.002
Right Leg	0.09	0.000	0.030
Left Leg	0.21	0.000	0.020
Mask	0.04	0.006	0.001
Gloves	9.30	0.100	0.006
TOTAL	12.52	0.124	0.069

TABLE 2. Summary of operator, boots, packaging and sprayer contamination -- μlitre contamination

SAMPLE	HDPE BOTTLE	CIBA-LINK	SVR
Operator (total)	12.52	0.124	0.069
Boots/Ground	0.91	0.110	6.220
Packaging & Sprayer	1619.18	58.690	539.710
TOTAL Area Contamination per Operator	1632.61	58.924	545.999

Farm Trials

The farm usage trials showed a preference for the Ciba-Link system over SVR. The advantages cited were:

Cleanliness	No incidents of accidental spillage
Speed of Filling	10 l of product transferred in <20 secs
Ease of Measurement	More accurate than measuring into a jug
Visual Assessment of Contents	Not possible with opaque containers
Cheap to Fit	Approximately £50
Compact Transfer Valve	

FURTHER DEVELOPMENTS & TESTING

To further improve the ease of use on farm, developments were made to the prototype such that it was operated by a twist action rather than a push action. This modification made the output more controllable and the shut-off more definite. For the operator it was easier to measure out small quantities. This development container which is now the commercial pack is known as the LinkPak.

Further trials were carried out with the commercial pack to ascertain accuracy of measuring out part packs, speed of emptying, and residue on the coupling when emptying.

1. Measurement of Residue on Valve (Anon, 1994)

Full packs of a 250EC propiconazole formulation were coupled to the adaptor and allowed to drain by leaving standing for one minute. After uncoupling, the surface of the coupler was dried with pre-weighed swabs, which were immediately weighed to assess the residue on the valve. The test was repeated 5 times.

2. Measurement of Part Packs

Full packs of a 250EC propiconazole formulation were emptied into a pre-weighed receptable in increments of 2 litres, until empty.

RESULTS

1. Residue

Residue (g) on valve (mean of 5 operations) = 0.42 g

2. Accuracy

Accuracy of Measuring Part Packs (mean of 5 operations)

Indicated Output (litres)	Actual Output (litres)
0-2	2.08
2-4	2.00
4-6	1.99
6-8	1.94

CONCLUSIONS

The original intention of developing a small volume refillable container in advance of legislation has led to the production of a container that offers significant advantages to the end user in terms of safety, work rate and accuracy of measurement.

The LinkPak has been proven in the laboratory and during extensive farm trials in Europe and North America. It has also gained approval from the UK Registration Authorities for a number of products.

The future for the pack lies with its use for a range of products from many manufacturers.

ACKNOWLEDGEMENTS

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INTERMEDIATE BULK CONTAINERS FOR AGROCHEMICALS: EXPERIENCES AND LESSONS FROM PILOT STUDIES IN EUROPE

J M OGILVY

ZENECA Agrochemicals, Fernhurst, Haslemere, Surrey, GU27 3JE

K L TYLER

ZENECA Crop Protection, Fernhurst, Haslemere, Surrey, GU27 3JE

ABSTRACT

ZENECA Agrochemicals has evaluated a range of intermediate bulk containers for use with pesticide products in several European countries in response to user demand for larger units and problems of pack disposal. Types have included simple 120 litre non-returnable HDPE barrels through to purpose designed 400 litre returnable/refillable mini-bulks now used commercially for seed treatments and herbicides. The rationale for introduction, range of tests and pilot field studies carried out are outlined, together with the principal lessons from the work to date. These include design factors, user acceptability, logistics, cleaning and the cost and business case considerations in the context of a changing legislative climate and farming structure.

INTRODUCTION

Interest in the use of larger containers for agrochemicals in Europe has increased significantly in recent years. This has been prompted by a number of factors, especially concerns and problems with disposal of large numbers of smaller containers, but also to afford increased handling efficiency by larger scale users.

Intermediate Bulk Container (IBC) is a term normally taken to apply to portable containers in the range of 400-1000 litres, where some mechanised form of handling is essential. The 400-500 litre refillable mini-bulks now very widely used in the USA are typical of this type of container in agriculture. However, since drums of 100-200 litres also require a degree of mechanised handling these have been included within the term IBC for this paper. Such containers, especially where used as single trip units can provide an important cheaper alternative to purpose built specialised refillable bulk containers.

As a generalisation, IBC's have a limited fit in European agriculture because of diversity of cropping and relatively small farm structure (Moll 1991). Small volume refillable containers (SVR's) in the 10-20 litre range are likely to have a better fit for a wider range of agrochemical use situations, especially in West Europe. However, significant exceptions are developing, notably in seed treatment and in the large scale agriculture of certain former Eastern bloc countries. In response to customer demand and the changing legislative scene, ZENECA Agrochemicals has recently evaluated a range of different IBC options in several European countries.

PILOT FIELD STUDIES

Trials on a semi-commercial base were carried out between 1991 and 1994 with five different bulk containers in three different countries

	Container Type *		
Country	Size (litres)	Manufacturer	Product/Formulation
Germany	120	Mauser	Glyphosate-trimesium SL
	100 & 400	Snyder	Diquat SL
UK	400	Bonar Rotaform	Flutriafol/ethirimol/ thiabendazole FS
Hungary	400	Snyder	Diquat SL
	400	Snyder	Butylate EC

* All in high density polyethylene (HDPE)

The container used for glyphosate-trimesium in Germany was a standard 120 litre drum designed for single trip use, with emptying by means of a simple tap. The units used for diquat in Germany and Hungary were purpose built refillable containers from the USA known as 'EZ Handlers'. These were supplied with specialised electrical pumps and flow meter units. The 400 litre unit used in the UK was a design especially for seed treatments and fitted with sealed couplings to link into existing pumps and metering equipment provided by the seed treatment mills. The 120 litre drums for glyphosate-trimesium and the 400 litre containers for seed treatments are now used on a full commercial scale.

RESULTS

Germany

All three different containers were well accepted by the end users, with a preference for the smaller 100 or 120 litre units. However, under the present circumstances in Germany the main interest in bulk containers for on-farm use is associated with an expectation of lower unit cost. The advantages of easier faster dispensing, improved operator safety and potential reduction in container disposal problems associated with the refillable units were generally of relatively low interest. This is exemplified by the ready acceptance and subsequent successful commercial use of the simple tap emptied 120 litre single trip drum, which is now used for a range of agrochemicals.

Hungary

Interim results from the ongoing trials indicate the 400 litre mini-bulk containers fit well into the large scale sunflower and maize farming sectors. The main advantage was in the speed of handling large quantities of product in a situation where extensive areas have to be treated during relatively short periods. This is especially true of the sunflower desiccation aerial spraying sector using diquat. The main technical problem encountered was in cleaning the containers in order to use the same unit for two different products in any one season. The American mini-bulks are designed for dedicated use and are top opening for safety reasons, which does not facilitate easy cleaning.

UK

The main benefit of the refillable IBC's for seed treatment in the UK has been in avoiding the increasingly difficult and expensive problem of single trip container disposal. This is especially applicable to seed treatment products. In addition, the improved safety in handling associated with the use of closed transfer sealed coupling systems has been recognised as a significant advantage.

The main drawback encountered with refillable IBC's for seed treatment is in cleaning costs. Although used as dedicated containers, the nature of seed treatment formulations does necessitate thorough cleaning before containers are refilled. The bottom opening design does facilitate cleaning, but this is still an expensive time-consuming operation.

COST CONSIDERATIONS

The costs of each purpose built refillable mini-bulk container is of the order of several hundred pounds with considerable extra cost where specialised pump/metering units are also supplied. Although lasting up to 5 years (for polyethylene units) the costs per litre of product are high, particularly because in practice under European conditions it has proved difficult to obtain more than one journey cycle/year. Apart from initial capital costs in hardware there are also very considerable expenses in setting up the infrastructure logistics and in the management of such systems. In comparison, the simple 120 litre drums at £10-12 each are extremely inexpensive in countries where there are currently no major disposal costs or problems.

DISCUSSION/CONCLUSIONS

Experience to date in Europe confirms a demand and acceptance for bulk containers in the range of 100-500 litres for certain niche markets, but the type of container which is most appropriate will differ considerably according to specific conditions and in particular the legislative position on container disposal. In a few market sectors such as the UK seed treatment business, the high cost of using purpose built refillable container systems can be justified by the high value of the products and the value customers attach to avoiding disposal problems and improving operator safety. At the other end of the spectrum, with lower value products and where there is less concern with container disposal and closed transfer is perceived to be of less benefit, single trip containers of the 100-200 litre drum type may be appropriate. This is particularly likely to be the case where the cost per litre of product is the overriding consideration.

ZENECA's experience indicates there is a need for intermediate technology bulk containers which fill a gap between the expensive specialised returnable mini-bulks and the simple 100-200 litre drums. Such containers would incorporate many of the salient features of the returnable mini-bulks but would be less expensive to construct and operate. Encouraging developments have recently been seen in this direction with some IBC manufacturers offering a refurbishment pooling service with free collection of used containers. This is based on the use of a modular design facilitating the dismantling and potential re-use of at least some parts of the used container (Anon 1993). Another development is the availability of close dispense systems for standard 100-200 litre drums which can considerably improve the safety and ease of emptying at relatively little extra cost.

There is little doubt that legislative pressures resulting in higher costs of pack disposal and greater value being attached to reduced operator exposure to concentrate product will increase in Europe. These factors, together with a trend towards larger farm structures and more specialist contractor applicators, are all likely to favour more use of purpose designed refillable or at least returnable bulk packs in some sectors of European agriculture.

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THE USE OF THE NORTH AMERICAN (PHED) AND UNITED KINGDOM (POEM) WORKER EXPOSURE MODELS IN PESTICIDE REGISTRATION

CURT LUNCHICK

Jellinek, Schwartz & Connolly, Inc. 1525 Wilson Blvd, Suite 600, Arlington, VA 22209, USA

PAUL HAMEY

Pesticides Safety Directorate, Mallard House, King's Pool, 3 Peasholme Green, York, Y01 2PX

SESH IYENGAR

ZENECA Agro, Jones Road, P.O. Box 9910, Stoney Creek, ON L8G 3Z1, Canada.

ABSTRACT

The requirement to quantify user exposure to pesticides has become an integral part of the pesticide risk assessment and registration process in Canada, the European Union, and the United States. A tiered approach to estimating worker exposure, described below, provides a powerful tool not only for pesticide registration, but for product development. The first tier of the assessment process involves the use of computerized generic exposure data such as the Pesticide Handlers Exposure Database (PHED) developed in North America and the Predictive Operator Exposure Model (POEM) developed in the United Kingdom. POEM provides an estimate of worker exposure based on a mathematical model empirically developed from exposure study data. The POEM estimate is based on the 75th percentile estimate of exposure. PHED provides an estimate of worker exposure based on mathematical calculations involving exposure study data points. The PHED estimate is a "best-fit" measure of central tendency; however, the 75th percentile of exposure can be obtained from PHED. The daily exposure estimates for orchard use from both models were developed in an exposure assessment for a synthetic pyrethroid submitted to the United States Environmental Protection Agency (EPA). This exercise showed that, with proper definition of the models' variables and the use information, the two models can provide compatible and complimentary first-tier estimates of exposure.

INTRODUCTION

Pesticides are important tools in modern agriculture. The use of these chemicals, however, produces a potential degree of risk. The risk assessment process provides regulatory agencies and the agrochemical industry with a method for estimating the potential for adverse health effects associated with the use of a pesticide. This estimation provides governments and industry with information for the decision-making process concerning pesticide registration and development.

Risk is a function of both exposure and toxicity. The term exposure can have various meanings. For the purpose of this paper, exposure implies the quantity of pesticide available for inhalation absorption, dermal absorption, or oral absorption. Exposure is external compared to the absorbed dose which is internal. The distinction between exposure and the absorbed dose is important to the principle underlying the use of the generic exposure data bases.

The use of generic exposure data and the development of worker exposure databases were proposed and discussed at the 187th meeting of the American Chemical Society in April 1984. Three important papers were presented (Hackathorn and Eberhart, 1985; Reinert and Severn, 1985; and Honeycutt, 1985), which discussed the foundations of the use of generic exposure data in the risk-assessment process. Inherent in the use of generic exposure data was the understanding that exposure is determined by factors such as the method of application, the amount of pesticide handled, the formulation, the patterns of use, the weather conditions, and the individual work practices more than the individual properties specific to any chemical. The absorption of the pesticide after exposure, however, is chemical-specific.

THE TIERED APPROACH

A tiered approach to applicator exposure assessment is an iterative process involving three steps (Carmichael, 1993 and Krieger, 1993). The first tier involves the use of generic exposure data and conservative assumptions for unknowns such as the chemical-specific dermal absorption or the protective value of clothing, personal protective equipment, or possible engineering controls. The power in the first-tier assessment is the ability to identify critical data gaps and then develop these data for the second and third tiers. The first tier also allows a company to conduct a preliminary risk assessment early in a product's development. The second tier involves the replacement of conservative assumptions with actual data for clothing penetration, dermal absorption, and exposure mitigation methods. The third tier involves the measurement of absorbed dose through a biological monitoring study. When adequate generic exposure data are available, the first tier may be the only tier necessary if the first tier assessment demonstrates acceptable risk.

A first tier assessment can be very useful in product development. Early in the product development phase and after development of the acute toxicity, subchronic toxicity, and developmental toxicity, a first tier exposure assessment can be developed for the proposed uses of the new product. Using the existing exposure models permits an early estimation of the potential exposure and risks. The first tier assessment identifies additional data that may be necessary to support registration (dermal absorption, dermal toxicity, or biological monitoring studies); the possible need for exposure mitigation (additional personal protective equipment, closed-loading systems, or enclosed-cab vehicles); and monetary commitments necessary to develop these data to support the registration. The exposure models permit this first-tier assessment with new products before even the first field trials.

PESTICIDE HANDLERS EXPOSURE DATABASE

The Pesticide Handlers Exposure Database was jointly developed by EPA, Health Canada, and the National Agriculture Chemicals Association (NACA) following the proposals presented at the 187th meeting of the American Chemical Society. PHED contains exposure and related data describing the study methods for a large number of workers engaged in mixing/loading and pesticide application (Nielsen et al. 1993).

PHED was initially released (Version 1.0) in May 1992 and contains 253 mixer/loader, 282 applicator, 224 combined mixer/loader/applicator, and 42 flagger data replicates. (Pesticide Handlers Exposure Database Operating Guidance, 1993). The exposure data are analyzed by individual body areas such as hands, chest, or forearms, and the exposure estimates are presented by body area as a median, arithmetic mean, and geometric mean. A total dermal exposure estimate is also presented, which is the sum of the most appropriate measure of central tendency for each body area[1]. Because the individual exposure studies entered in PHED monitored exposure to different body areas, the number of observations for each body area varies.

PHED also classifies the exposure data according to analytical quality assurance procedures and results. Data from laboratory, field, and storage resources are used to classify the exposure data from grade A, the highest, to grade E. EPA and Health Canada generally require grade A or B data from PHED assessments that are used to support registration actions. All grades of data can be used for product development exposure estimates or for designing exposure studies.

Statistical outputs from PHED include the primary exposure summary report and the statistical analysis of univariate, regression, and correlation statistics. The univariate analysis provides exposure estimates at various percentiles. Health Canada and EPA typically use the "best-fit" for central tendency for regulatory purposes, but the 75th percentile of exposure is also available.

A critical step in developing a PHED exposure estimate involves defining the file subset for the pesticide of interest. Definitions available to the user for defining the subset include, for example, formulation type, methods of application, engineering controls, application rate, spray rate, and container size. The subset must be representative of the pesticide use conditions being examined in order for the PHED exposure estimate to be useful.

The current version of PHED does not meet the full potential of the system. PHED Version 1.0 contains limited data for certain use scenarios, such as hand-held sprayers; formulations, such as granules; and clothing scenarios, such as coveralls over a shirt and pants. Also, the number of high-quality grade data required for regulatory decisions is limited. A new version of PHED (Version 1.1) is currently scheduled for release in November 1994. It may contain approximately 1200 additional data records, including data from studies conducted in Europe and Asia.

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[1] PHED uses the geometric mean for lognormal distributions, arithmetic mean for normal distributions, and the median when the distribution is neither lognormal or normal for estimating the total dermal exposure.

PREDICTIVE OPERATOR EXPOSURE MODEL

The Predictive Operator Exposure Model was created in the UK. following a decision in May 1985 by the Joint Medical Panel of the Scientific Subcommittee on Pesticides and the British Agrochemical Association Toxicology Committee to review existing pesticide worker exposure data for the feasibility of developing a generic exposure model (Martin 1986).

POEM is a semi-quantitative model empirically based on pesticide worker exposure data. Variables defining the use conditions of the pesticide of interest are used in POEM to predict the daily exposure. Key use conditions entered into POEM include formulation type, formulation concentration, container size and pouring characteristics (defined as contamination/operation), hectares treated, and spray volume. POEM estimates exposure for vehicle-drawn spray equipment and controlled droplet (CDA) equipment, low-level hand-held outdoor sprayers, low- and high-level hand-held CDA sprayers, and very low-, low-, and high-volume orchard sprayers.

The POEM exposure estimate is based on the 75th percentile of exposure developed from mathematical modelling of exposure data evaluated by the Working Party on Pesticide Operator Exposure. POEM has been reviewed in the UK. and has been accepted by the Scientific Subcommittee and the Advisory Committee on Pesticides and is used for regulatory purposes by the Pesticides Safety Directorate. As with PHED, defining the use conditions is critical, and the user must be familiar with pesticide exposure study methodology to interpret the exposure estimates and recognize their limitations.

COMPARATIVE CASE STUDY

POEM and PHED were recently used in a submission to EPA of a synthetic pyrethroid. Both models were used for airblast application because by itself, PHED did not contain sufficient grade A or B airblast applicator replicates to support reregistration. Currently, 15 grade A or B replicates for each body area are generally considered necessary for regulatory purposes by EPA and Health Canada.

Both models require proper definition of the use conditions to provide meaningful prediction of exposure. The use information used in both models is provided in Table 1. The use information is representative of how the product is used in the United States because the resultant exposure estimates were submitted to EPA. The metric equivalents entered into POEM, such as container size, are not reflective of typical European container sizes. The flexibility of the models is an advantage that supports their use in Europe and North America.

POEM predictions were run for the orchard high-volume and orchard low-volume application methods. Table 2 presents the POEM printout for the low volume spray. For a grower wearing long pants and a shirt during mixing/loading and application and also wearing protective gloves during mixing/loading only, the daily exposure estimate was 1.0 mg/kg bw/day (see item F on Table 2). The daily exposure estimate for the high volume spray was 0.49 mg/kg bw/day. The two exposure estimates provide the estimated range of exposure expected based on the label recommended spray rates of 30 gal/acre to 300 gal/acre (300 l/ha to 2850 l/ha).

TABLE 1. Summary of use information

Use Pattern[1]	POEM	PHED
Formulation	EC	EC, solution, aqueous suspension
Concentration	380 mg/ml	3.2 lb AI/gallon (US)
Container size	4 litres	1 gallon (US)
Container neck characteristics	Narrow neck	Not applicable
Application dose	0.42 litres product/ha	0.14 lb AI/acre
Spray rate-high volume	2850 l/ha	300 gallons (US)/acre
Spray rate-low volume	300 l/ha	30 gallons (US)/acre
Work rate	28 ha/day	70 acres/day
Duration of exposure	6 h	Not applicable

[1] The metric and American units of measurement are approximately equivalent. PHED currently does not take metric input.

To obtain a daily exposure estimate from PHED, the mixer/loader and applicator files were both utilized. The mixer/loader subset was defined as EC, aqueous suspension, or solution formulations manually poured (open poured) into spray tanks of 100 gallon capacity or greater. Container sizes were defined as between 1 and 10 gallons. PHED estimates exposure on a per lb AI (or hourly), rather than daily basis. To maximize the number of observations per body area the mixer/loader was assumed to wear only gloves. Based on grade A or B data, the dermal exposure to the mixer/loader was 0.018 mg/lb AI.

The application file subset was defined as airblast application from open-cab vehicles in which dust and granular formulations were excluded. The crop grapes was also excluded because the grape study in PHED included a lateral mist blower, which is not used for this pesticide. Airblast applicator exposure at rates less than 100 gal/acre were not statistically different ($p>0.05$) from exposure at rates greater than 100 gal/acre. (Note that the POEM estimates for high and low volume are also similar.) Based on grade A or B data, the dermal exposure to an applicator wearing long pants and a shirt was 1.0 mg/lb AI As with POEM, the inhalation exposure was negligible (less than 1% of the dermal exposure) compared to dermal exposure.

The combined PHED estimate of mixer/loader and applicator exposure is 1.0 mg/lb AI To estimate daily exposure, the use data of a 0.14 lb AI/acre application rate and work rate of 70 acres/day (Table 1) are used. A 60-kg worker applying 0.14 lb AI/acre to 70 acres/day handles 9.8 lb AI/day. (POEM assumes a 60-kg body weight.) The daily exposure is calculated as follows:

Daily Exposure = 1.0 mg/lb AI x 9.8 lb AI/day ÷ 60 kg bw = 0.16 mg/kg bw/day

The PHED estimate is 7.5-fold lower than the POEM low volume estimate and 3-fold lower than the POEM high volume estimate. These differences are less than the variability between replicates that is often encountered in individual pesticide worker exposure studies. The other important difference is that the PHED estimate is based on the best-fit measure of central tendency, and the POEM estimate is based on the 75th percentile of exposure.

TABLE 2. POEM summary of low volume orchard sprayer exposure

PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

A. PRODUCT DATA					
1. Product name	SYN PRYETH				
2a. Active ingredient					
2b. Concentration	380	mg/ml			
3. Formulation type	EC				
4a. Main solvent	Aromatic				
4b. Concentration of solvent	NA				
5. Maximum in-use ai concentration	0.532	mg/ml			
B. EXPOSURE DURING MIXING AND LOADING					
1a. Container size	4	litres			
1b. Hand contamination/operation	0.2	ml			
2. Application dose	0.42	litres product/ha			
3. Work rate	28	ha/day			
4. Number of operations	3	/day			
5. Hand contamination	0.6	ml/day			
6. Protective clothing	gloves				
7. Transmission to skin	10	%			
8. Dermal exposure to formulation	0.06	ml/day			
9. Concentration of ai	380	mg/ml			
10. Dermal exposure to ai/person	22.800	mg/day			
11. Dermal exposure to ai/kg bw	0.38	mg/kg bw/day			
C. EXPOSURE DURING SPRAY APPLICATION					
1. Application technique - upward air-blast no cab low volume					
2. Application volume	300	spray/ha			
3. Volume of surface contamination	50	ml/h			
4. Distribution	Hands		Trunk	Legs	
	10		65	25	%
5. Clothing	none		permeable	permeable	
6. Penetration	100		15	20	%
7. Dermal exposure	5		4.875	2.5	ml/h
8. Duration of exposure	6	h			
9. Total dermal exposure to spray	74.25	ml/day			
10. Concentration of ai	0.532	mg/ml			
3. Dermal exposure to ai/person	39.501	mg/day			
11. Dermal exposure to ai/kg bw	0.658	mg/kg bw/day			
E. INHALED EXPOSURE DURING SPRAY APPLICATION					
1. Inhalation exposure	0.02	ml/h			
2. Duration of exposure	6	h			
3. Concentration of ai	0.532	mg/ml			
4. Inhalational exposure to ai	0.064	mg/day			
5. Percent absorbed	100	%			
6. Absorbed dose	0.001	mg/kg bw/day			
F. PREDICTED EXPOSURE					
1. Inhalation	0.001	mg/kg bw/day			
2. Dermal exposure	1.038	mg/kg bw/day			
3. Total potential exposure	1.039	mg/kg bw/day			

It was not necessary for the EPA submission to have PHED estimate exposure based on the 75th percentile. However, for the comparative purposes of this paper, the PHED-based 75th percentile of exposure was estimated for the airblast mixer/loader and applicator. Table 3 presents a comparison of the PHED estimates based on central tendency and the 75th percentile.

TABLE 3. Comparison of central tendency and 75th percentile PHED exposure estimates

Job Function	Central Tendency	75th percentile
Mixer/Loader	0.018 mg/lb AI	0.3 3 mg/lb AI
Airblast Applicator	1.0 mg/lb AI	7.0 mg/lb AI
Combined	1.0 mg/lb AI	7.3 mg/lb AI

If the 7.3 mg/lb AI 75th percentile exposure estimate from PHED is used to estimate the daily exposure to a 60-kg individual handling 9.8 lb AI/day, the daily exposure estimate is as follows:

Daily Exposure = 7.3 mg/lb AI x 9.8 lb AI/day ÷ 60 kg bw = 1.2 mg/kg bw/day

The PHED exposure estimate based on the 75th percentile of exposure, 1.2 mg/kg bw/day, is essentially the same as the low volume orchard spray POEM estimate of 1.0 mg/kg bw/day, based on the 75th percentile of exposure. Both estimates are similar to the POEM high volume exposure estimate of 0.49 mg/kg bw/day.

The above POEM estimates were based on the product not being restricted to supply in a "wide-necked" pack which reflects US container variability. If the product was packaged only in the "wide-necked" packs such as a 5 litre container with an ECPA 63 mm standard neck, the exposure during mixing/loading is predicted by POEM to be 0.019 mg/kg bw/day. PHED currently does not contain "wide-necked" container mixer/loader exposure data, although the use of such containers is increasing in the US and Canada.

SUMMARY

The use of worker exposure models to predict worker exposure is becoming an important step in the risk assessment process and therefore, has importance in product development, regulation, and product stewardship. Using these generic models in the first tier assessment provides the user with a powerful tool in estimating worker exposure and resultant risk without the large capital investment necessary to conduct a worker exposure study. The first tier assessment also identifies specific data gaps that may need to be filled. This permits the commitment of resources to specific identified needs.

PHED in North America, and POEM in the UK, have been developed as worker exposure models based on data obtained from worker exposure studies. Careful selection of the pesticide use information is critical to both models, and the users must be knowledgeable with pesticide worker exposure studies. Limited comparisons of exposure estimates, developed from both models, demonstrate acceptable compatibility. Concurrent use of both PHED and POEM is encouraged for product development purposes and for consideration by

regulatory agencies in Canada, Europe, and the United States. Use of both systems allows users greater flexibility, permits a more informed judgement on exposure, and augments data gaps in each system such as PHED's lack of "wide-necked" mixer/loader exposure data.

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Session 8C

Advances in the Control of Public Health Pests

Chairman	Dr N R Price
Session Organiser	Professor J P Edwards
Papers	8C-1 to 8C-5

DEVELOPMENT OF THE FIRST CAT FLEA BIOLOGICAL CONTROL PRODUCT EMPLOYING THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA CARPOCAPSAE*

S.A. MANWEILER

biosys, 1057 East Meadow Circle, Palo Alto, CA 94303 USA

ABSTRACT

The demonstration that larvae and pupae of the cat flea, *Ctenocephalides felis*, are susceptible to infection by the nematode *Steinernema* (=*Neoaplectana*) *carpocapsae*, the availability of many products based upon this nematode for controlling soil insects, significant outdoor cat flea infestations plaguing many parts of the United States (and other warm countries), pressure to reduce reliance upon chemical control products, and tests by various University researchers led to the development of *S. carpocapsae* as the first product for controlling *C. felis* biologically. Product development focused upon defining which aspects of cat flea infestations were compatible with nematode-based control and how to demonstrate efficacy. The resulting products work very well as the outdoor segment of an effective control program also involving on-animal and indoor control measures. The ability of *S. carpocapsae* to readily penetrate the flea cocoon and kill the pupa within is a major advantage over chemical pesticides.

INTRODUCTION

Nematodes of the genus *Steinernema* parasitize a wide range of insect species (Poinar, 1975, 1979) including immature fleas (Silverman *et. al.*, 1982). Insect hosts are parasitized by a third stage infective juvenile (IJ) nematode that locates the insect, either by following CO_2 or temperature gradients or waiting for the host to pass by. *Steinernema* species enter the insect body through natural openings (i.e., spiracles, anus, mouth). Once inside the host's haemocoel, the IJ releases a symbiotic bacterium (*Xenorhabdus* sp.) that multiplies, kills the host, and renders the host interior conducive to nematode reproduction. The nematodes feed upon the *Xenorhabdus* and develop into fourth stage juveniles and adult males or females that mate and give rise to a second generation. Several generations can develop in this manner, the number dependent upon the size of the insect host. When host resources are nearly depleted the nematodes simultaneously become IJs that are resistant to the environment outside the host. Only this stage (IJ) can survive outside an insect host in nature (Georgis, 1992).

Products containing steinernematid nematodes have only recently become commercially available. The cost of production has been reduced enough to compete with many chemical pesticides through the development of reliable *in vitro* production methods. Several available formulations, stable at ambient temperatures (25°C) for up to 5 months,

permit commercial distribution. Most available products are labeled for use against agricultural or ornamental plant pests (Georgis, 1990, 1992).

Demonstrating efficacy against a chosen insect target involves the development of assays to quantify nematode-induced insect mortality, a thorough knowledge of the target insect biology, and many tests under different environmental conditions to optimize application strategies (Georgis and Gaugler, 1991).

PRODUCT DEVELOPMENT STRATEGY

Nematode-based control and cat flea developmental biology

Steinernematid nematodes are most effective against insects that inhabit soil because the soil offers protection from environmental extremes (i.e., desiccation, UV light) detrimental to nematodes and facilitates location of hosts (Begley, 1990; Klein, 1990). Flea control typically involves treatments of animals and their indoor and outdoor habitats with chemical pesticides (Silverman *et. al.*, 1982). Larval and pupal fleas developing outdoors in soil are ideal candidates for control with nematodes.

Assays to quantify nematode-induced mortality

Most consumers see only the adult fleas, which they find on themselves or their pets. Unfortunately, few widely accepted ways to accurately quantify adult flea densities in field situations are available, meaning that more indirect methods have had to be employed (Table 1). These began with laboratory-based tests of nematodes against all stages of fleas developing in soil. Initially direct mortality was observed. Subsequent tests examined adult flea emergence. After nematode-induced mortality was demonstrated, substrate (e.g., soil, sand, gravel, turfgrass, bark) and nematode dosage effects were explored. Only economically feasible application volumes and dosages were evaluated.

Assay designs then diverged in two directions. One involved large plastic tubs filled with soil or other substrates in which fleas commonly develop outdoors. The idea was to create a test environment closer to the "real world" while still controlling variation enough to allow statistical differentiation between significant effects. The other involved pairing soil assays with pre- and post treatment questionnaires to link a quantified measure of flea mortality with opinions of people whose properties were treated with nematodes. Consumer acceptance is ultimately required for the commercial success of any product. Expert urban entomologists at Texas A&M University, Louisiana State University, and North Carolina State University helped develop these assays and used them to measure nematode efficacy.

Soil assays: small containers

An assay developed at Texas A&M University to measure outdoor insecticide persistence (Palma and Meola, 1990) was modified to measure mortality of flea larvae placed onto soil previously sprayed with nematodes (B&G sprayer, 200 KPa, 500,000 nematodes per m^2, 2 litres per 10 m^2). In this assay small soil samples (c. 30 cm^3) collected from a site

where nematodes had been applied previously were placed in glass vials. A set number of fleas of the same developmental stage were placed onto the soil in each vial. Mortality was tallied 24 and 72 hours afterward (Table 1).

This assay was modified by L. Foil and G. Henderson (Louisiana State). Flea eggs were introduced into small glass vials containing either sand, potting soil, or gravel. Nematodes were applied (pipette, 500,000 nematodes per m^2, 4 litres per 10 m^2) to subgroups of these vials after the fleas had been allowed to develop into young larvae (instar 1-2), older larvae (instar 3-4), or pupae. Adult emergence from vials treated only with water and nematode-treated vials was compared to evaluate nematode effects.

Table 1. Assays developed to quantify efficacy of nematodes against immature cat fleas.

Research Group	Assay Unit/Procedure	Goals
Texas A&M	Nematodes applied to soil in field. Small soil samples retrieved and placed in small glass vials. Fleas placed on soil sample surface.	Demonstrate that nematodes can kill different developmental stages within 24 to 72 hours.
Louisiana State	Soil (or other substrate) samples placed in vials. Flea eggs allowed to develop in soil. Nematodes applied when fleas are at a desired developmental stage.	Demonstrate ability of nematodes applied to immature fleas to reduce adult emergence. Examine substrate effects.
Louisiana State	Pupae (naked or in cocoons) in glass vials exposed to nematodes.	Demonstrate ability of nematodes to penetrate the flea cocoon and prevent adult emergence.
biosys	Nematodes applied to soil in field. Small soil samples retrieved and placed in small glass vials. Fleas placed on soil sample surface.	Compare different nematode application dosages. Include a standard insecticide as a reference.
North Carolina State	Soil (or other substrates) placed in larger (diameter 34 cm, 13 cm high) tubs. Flea larvae added followed by nematodes.	Demonstrate ability of nematodes applied to immature fleas to reduce adult emergence. Examine substrate effects. Get closer to "real world."
Texas A&M	Pre- and post treatment questionnaires along with pre- and post treatment soil samples.	Evaluate how well client satisfaction and soil assay results are related.

This test permitted examination of nematode efficacy against different developmental stages of fleas inhabiting three substrates similar to those where fleas commonly develop outdoors. Other tests involved comparisons of mortality caused by different nematode dosages and a standard insecticide (Table 1).

The ability of *S. carpocapsae* to penetrate the flea cocoon was demonstrated by exposing single cocoons or naked pupae placed individually in small tubes to water or three dosages of nematodes (1, 25 or 100 nematodes per pupa). Cocoons spun in silk or sand were included as separate groups to examine substrate effects. Adult emergence 10 days after exposure to nematodes was the measure of efficacy (Table 1).

Soil assays: larger containers

J. Arends and R. Brandenburg (North Carolina State) expanded the small container assay by employing larger tubs containing soil, sand or turfgrass. Fifty lab-reared third instar flea larvae were placed in each tub and allowed to acclimate for 24 hours before nematodes were applied with a standard CO_2 backpack sprayer with a single 8003 flat fan nozzle at 200 KPa. Sprayer output was 0.4 litres per 10 m^2 with a dosage of 250,000 nematodes per m^2. An additional 12 litres of water per 10 m^2 was applied immediately before the nematodes. Adult emergence from tubs treated with water only was compared with nematode-treated tubs (Table 1).

Outdoor assays: questionnaires and soil samples

Scientists from Texas A&M together with a professional pest controller (K. Kestenbaum) applied nematodes to the yards of 3 persons who requested non-chemical flea control. Client satisfaction was measured with questionnaires completed before and one month after the nematode application (2 litres per 10 m^2, 500,000 nematodes per m^2). The questionnaire included detailed determinations of what the client considered to be a bad flea infestation (i.e., client aversion to fleas), how many fleas were present before and after the treatment, how the client searched for fleas, the client's opinion of how well the nematodes controlled their flea problem, the client's opinion of the safety of chemical insecticides, the relative importance of safety, price, and efficacy in determining which control agent the client would use, the client's reasons for trying nematode-based flea control, the number and kind of pets, where the pets are allowed to enter, who the client relies upon for flea control (i.e., themselves, professional) and past experiences with flea control attempts. Small soil samples (c. 30 cm^3) were retrieved from each yard immediately after treatment from areas to which only water (control) was applied and others sprayed with nematodes. These samples were placed in small vials in the lab followed by 20 flea larvae (instar 2-3) per vial. Adult emergence from vials containing nematode and control samples was compared as a measure of nematode efficacy (Table 1).

RESULTS

Soil assays: small containers

Cat flea larvae and pupae were very susceptible to *S. carpocapsae* in the initial direct mortality assay; flea eggs were not killed (Table 2).

Table 2. Results of various tests of nematodes against immature cat fleas.

Research Group	Procedure	Results
Texas A&M	Nematodes applied to soil in field. Samples challenged in the lab. 500,000 nematodes per m^2 2 litres per 10 m^2	Egg: No mortality[a] Larva: 100% mortality Pupa: 95% mortality
Louisiana State	Nematodes applied to soil in lab. 500,000 nematodes per m^2 4 litres per 10 m^2	Egg: 70-100% adult reduction Larva (1-2): 87-100% adult reduction Larva (3-4): 87-100% adult reduction Pupa: 70-97% adult reduction
Louisiana State	Pupae exposed in lab. Nematode dosages: 0, 1, 25, 100 nematodes per pupa.	Naked Pupae: 70-85% adult reduction[b] Cocoon (sand): 95-100% adult reduction Cocoon (silk): 95% adult reduction
biosys	Nematodes applied to soil in field. Samples challenged in the lab. 250,000 or 500,000 nematodes or 25 g Diazinon G (2%) per m^2 3 litres per 10 m^2 (nematodes)	Nematodes (dosage per m^2): 250,000 : 90.3% larval mortality 500,000 : 97.6% larval mortality Diazinon: 90.3% larval mortality
North Carolina State	Nematodes applied to substrate in lab. 250,000 nematodes per m^2 0.4 litres per 10 m^2	Sand: 100% adult reduction Bermudagrass: 94.8% adult reduction Tall Fescue: 95.5% adult reduction Pine Straw: 95.8% adult reduction
North Carolina State	Nematodes applied to soil in lab. 250,000 nematodes per m^2 0.4 litres per 10 m^2	Pupae (1-7 days old) 97.5% adult reduction Pupae (7-13 days old) 87.2% adult reduction
Texas A&M	Nematodes applied to soil in field. Samples challenged in the lab. 500,000 nematodes per m^2 2 litres per 10 m^2 Pre- and post treatment questionnaires.	Larva: 67% adult reduction Questionnaires indicated that the clients were satisfied with the level of control achieved. None requested retreatment.

[a] 72 hours after nematodes were applied. [b] 25 and 100 nematodes per cocoon.

S. carpocapsae readily penetrated flea cocoons spun in silk or sand and suppressed adult emergence by 95-100% at dosages roughly equivalent to 250,000-500,000 nematodes per m^2. Application rates of 250,000 and 500,000 nematodes per m^2 both resulted in larval mortality above 90%, mortality levels comparable to that achieved by Diazinon G, an insecticide commonly used to control immature fleas outdoors in the USA. Between 70-100% suppression of adult flea emergence was observed in the tests at Louisiana State, the degree of suppression increasing as nematodes were applied earlier in the flea developmental cycle. Suppression was similar in soil, sand, and gravel. These results strongly suggest that *S. carpocapsae* can effectively control immature cat fleas developing outdoors.

Soil assays: larger containers

Adult suppression in larger tub assays (North Carolina State) was very high in turfgrass (Tall Fescue, Bermudagrass), a mulch commonly used in landscaping (pine straw), and sand (Table 2). Younger (1-7 days) and older (7-13 days) pupae were readily killed. Both tests further support the validity of the initial laboratory assays and the conclusion that *S. carpocapsae* can effectively control immature cat fleas outdoors.

Outdoor assays: questionnaires and soil samples

All three clients were pleased with the results of cat flea control achieved by comprehensive (indoor with IGR, on-animal with flea shampoo, outdoor with *S. carpocapsae*) measures. None requested retreatment, and all reported a significant decrease in adult flea levels on themselves and their pets. All were in favor of avoiding chemical insecticides, but all would use chemicals if non-chemical methods did not control fleas. All had tried to control fleas themselves and had hired professionals. All had worked with the professional pest controller before, were confident that she knew how to control fleas, and reliably followed her instructions. Significant larval flea mortality was observed in the soil sample assay although mortality (67%) was lower than that observed in comparable assays (90.3-100%)(Table 2).

CONCLUSIONS AND DISCUSSION

Flea test results

The initial lab assays demonstrated that flea larvae and pupae are readily killed by *S. carpocapsae*. This nematode can effectively penetrate the flea cocoon. Flea eggs are not directly killed by *S. carpocapsae*. However, the 70-100% reduction of adult emergence in tests where the fleas were eggs when treated with *S. carpocapsae* demonstrated that larvae hatching from these eggs are killed. Similar larval mortality caused by *S. carpocapsae* (250,000 nematodes per m^2) and Diazinon G (25 g per m^2) strongly suggested that *S. carpocapsae* is as effective as a commonly employed chemical insecticide at economically feasible dosages. Effectiveness of *S. carpocapsae* was similar in sand, soil, and gravel.

The larger tub assays verified the results of the initial lab assays. *S. carpocapsae* effectively suppressed adult flea emergence when applied to larval or pupal cat fleas.

Efficacy in turfgrass and mulch equaled that in sand, soil, and gravel, further expanding the range of substrates examined. All of these substrates are currently treated with chemical insecticides to control fleas in the USA.

The questionnaires indicated that consumers were convinced that *S. carpocapsae* significantly reduced their flea infestations. Consumer satisfaction with nematode products for controlling insect pests of home lawns and gardens is obviously due to the perceived safety of nematodes as well as perceived efficacy. High satisfaction combined with a significant flea mortality in the soil assay suggests that results of both are indicative of effective flea control. Variation in the results of soil samples from client yards and lab tests demonstrates that consumer satisfaction (i.e., perceived successful flea control) can occur in situations where soil assays seem to predict lower efficacy.

Product development and introduction

Results of the efficacy tests justified a limited market introduction to learn more about the attractiveness and efficacy of nematode-based flea control products. In 1993 two products containing *S. carpocapsae* were assembled, one for homeowners (retail) and another for professional pest controllers. The retail version (BioFlea®) was patterned after nematode products designed for home use against insect pests of gardens and lawns. The professional version (Vector®) was designed for use by pest controllers (PCO) offering flea control services. These products were positioned as part of an overall flea control program rather than being identified as a replacement for chemical insecticides (Table 3).

Table 3. Role of nematode products in a flea control program including outdoor, indoor, and on-animal measures. For best results indoor, outdoor, and on-animal control measures must be applied simultaneously.

Segment	Control Options
Indoor	Choose from many available adulticides for "knock down" treatments. Borate carpet treatments or IGR applications may be used to prevent future indoor infestations. Frequent vacuuming of carpets can also help.
On-animal	Use a flea dip or shampoo to eliminate adult fleas and thereby prevent reinfestation of areas (indoor and outdoor) where fleas are developing. IGR preparations for application directly to animals may also be helpful.
Outdoor	Apply *S. carpocapsae* to areas where fleas are developing to eliminate outdoor sources of reinfestation. Low persistence adulticides can be tank mixed with *S. carpocapsae* to control all stages of fleas outdoors simultaneously.

The ability of *S. carpocapsae* to actively locate flea larvae and cocoons and effectively penetrate the cocoons were emphasized as advantages over chemicals. The safety issue was also mentioned although not emphasised. *S. carpocapsae* can be tank mixed with many biorational and chemical insecticides (Georgis, 1992) adding to its flexibility as a flea control agent. For example, *S. carpocapsae* and a low persistence adulticide may be applied together to eliminate immature and adult fleas (those waiting in cocoons for a host to pass by) simultaneously, a recommended strategy to rapidly control some high density flea infestations. Public response has been very positive. PCOs who were taught how to use *S. carpocapsae* effectively reported few callbacks for retreatment. In many cases, nematodes were able to control fleas in situations where chemicals had proven ineffective. Indeed, these types of successes have significantly increased interest in and sales of flea control products containing *S. carpocapsae*. In 1994, a new more flexible nematode formulation became available. A large company agreed to distribute products containing *S. carpocapsae* for sale in pet stores and Veterinarian offices. The PCO product switched to the new formulation. Cat flea control was added to the labels of products for lawn care professionals, homeowners and golf courses. Repeat purchases by those who have achieved effective flea control with *S. carpocapsae* sufficiently large to justify the proliferation of nematode-based flea control products is one of the best measures of success of these products. Many opportunities for expansion exist, and tests to further refine and validate product efficacy are ongoing.

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EFFECT OF THE CHITIN SYNTHESIS INHIBITOR LUFENURON ON THE GERMAN COCKROACH, *BLATTELLA GERMANICA* (L.)

R. SCHENKER, E.W. MOYSES

ciba Animal Health, Ciba-Geigy Ltd, CH-4002 Basel, Switzerland

ABSTRACT

The benzoylphenyl urea lufenuron is a potent chitin synthesis inhibitor. Laboratory tests with *Blattella germanica* were used to demonstrate the effect of residual deposits on nymphs and adults, and to establish the application rate. A methodological comparison relevant to testing IGR's was made between test units containing *Blattella germanica* nymphs of all ages, and units containing nymphs of uniform age. Exposure to this chemical disrupted the moulting process of each stadium. The Minimal Effective Concentration was between 1 and 5 mg per m^2. Mortalities were mostly synchronous with the moults in the untreated units. Facultative (incremental) long term exposure of adult *Blattella germanica* led to complete suppression of ootheca hatch.

INTRODUCTION

The availability of IGR s for the control of public health pests is increasingly appreciated by the pest control industry. Juvenile hormone analogues (JHAs) are used for the control of mosquitoes, ants, flies, fleas and cockroaches. Chitin synthesis inhibitors (CSIs) are presently entering the market for cockroach and flea control. IGR s are expected to become even more prominent in future to control urban insect pests (Edwards, 1993).

Reviews of the available data relating to the biochemical and biological modes of action of benzoylphenyl ureas (BPU s) have been reported in Wright & Retnakaran (1987). The benzoylphenyl urea lufenuron (N-[2,5-dichloro-4(1,1,2,3,3,3-hexafluoropropoxy)-phenylaminocarbonyl]-2,6-difluorobenzamide) is a potent chitin synthesis inhibitor discovered and developed by Ciba for use in crop protection, for systemic flea control in dogs and cats and for public health use. BPU s show large differences in their toxicity against the immature stages of insects (Neumann & Guyer, 1987). Lufenuron is highly active against the German cockroach *Blattella germanica*.

This paper describes a number of tests made with the objective of investigating the effect of lufenuron on nymphs and adults of *Blattella germanica,* to determine the factors affecting efficacy, and to decide on the label concentration.

MATERIALS AND METHODS

Lufenuron was formulated as 10% WP, 10% water dispersible granules (WG), and as effervescent tablets (TB) containing 200 mg a.i. Application was made to hardboard panels in all experiments. The products were applied in aqueous suspension using a 1.0 mm nozzle and low pressure. Application was made on a conveyor belt for which spraying system and

belt speed were adjusted to give 50 ml of spray per m^2. The panels were stored horizontally in racks. The storage room was maintained in darkness at 25°C and 55% r.h.

Insects of the standard susceptible Geigy strain were used. Adult females carrying ootheca were moved each week to a new container, leaving the young nymphs behind. This gave groups of nymphs the age of which was known to within one week.

'Box' and petri dish units were used for the bioassays. A box unit consisted of a 13.5 x 18 x 6 cm transparent plastic box with tightly fitting lid. Two 2.5 cm diameter holes in the lids (covered with metal gauze) allowed aeration. A petri dish unit consisted of a polystyrol dish (220 x 30 mm) with a tightly fitting lid with similar, 1 cm diameter, aeration holes. In both units food (ground dog biscuit) was supplied in small petri dishes, a water bottle with a cotton wool wick was given, and a piece of egg tray was added as a refuge. The units were kept at 25°C and 55% r.h.

Treated hardboard panels for facultative (incremental) contact tests were placed into the box units and the water bottle was stood on the treated surface so that the insects had to cross the deposit each time they went to drink. For the forced contact tests the nymphs were anaesthetized with carbon dioxide. Twenty nymphs were then counted onto each treated surface and confined under petri dish lids. A piece of waxed paper prevented direct contact of the anaesthetized nymphs with the deposits. After the roaches had fully recovered the waxed paper was removed, and the insects were exposed to the deposit. Talc or vaseline was applied to prevent the nymphs climbing onto the lid. The insects were thereby forced to stay on the deposits. Subsequently, the waxed paper was replaced, the insects re-anaesthetized and transferred to the petri dish units.

Experiments comparing timing of moult with mortality were started with early L_1 nymphs aged 1 to 2 days; late L_1 nymphs, just before moult, aged 6 to 7 days; and L_3 nymphs 4 to 5 weeks of age. Nymphs were immobilized and marked with colour (MODEL MASTER enamel paint, Calderara, Italy) to keep track of moulting.

RESULTS

Methodology

The nymphs of *Blattella germanica* were reared in age groups each spanning one week. In order to establish the optimal composition of the nymph population for the facultative contact tests two population structures were compared. Insects were taken from each of the first six age groups i.e. from 0-1 up to 5-6 weeks of age for the mixed age bioassay. For the separate age bioassay the insects were also taken from each of the first six units but exposed as separate age groups.

Many nymphs disappeared in the mixed culture during the 3 week bioassay period in the units treated with an experimental WP formulation but hardly any in the untreated units. Table 1 shows the difference between the two systems used in terms of missing insects.

The missing insects seem to have been eaten by the other insects. Direct observations in a supplementary experiment confirmed cannibalism of affected insects.

Holding insects in separate age groups prevents cannibalism of affected insects. This system has also the advantage that the effectiveness against different ages of immature *Blattella germanica* instars can be judged more accurately than in the mixed age test.

Table 1. Total **number** / *percentage* of missing insects over the 21 day bioassay period from 600 nymphs (4sets of 150) for each concentration.

	Concentration [mg a.i. per m^{-2}]			
	20	10	5	Control
Mixed Age	**157**/*26*	**201**/*33*	**211**/*35*	**10**/*2*
Separate age	**19**/*3*	**31**/*5*	**32**/*5*	**19**/*3*

Facultative exposure (MEC)

Bioassays of six weeks facultative (incremental) exposure allowed the observation of a 'final' mortality. Table 2 pools the data for three nymphal ages, two deposit ages and four replications of 25 nymphs. The MEC (minimal effective concentration) was 5 mg a.i. per m^2 in this experiment.

Table 2 Percentage mortality of *Blattella germanica* exposed to deposits with several concentrations of lufenuron aged for 3day and 3 months over a 42 day bioassay period from 600 nymphs used for each concentration.

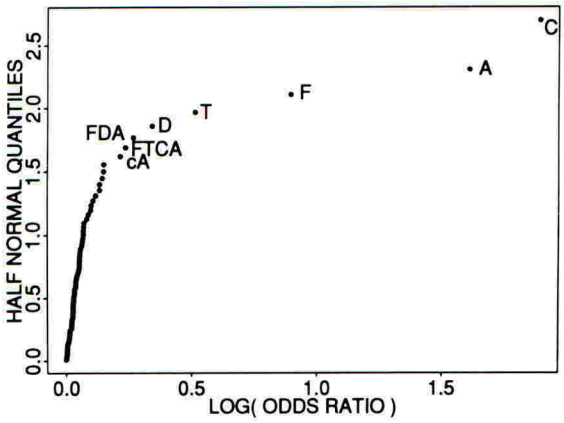
	Formulation							
	10		WP 10		TB1		TB2	
	3 d	3 m	3 d	3 m	3 d	3 m	3 d	3 m
20	100	100	100	100	-	-	-	-
5	100	100	99	100	100	100	100	100
1	96	90	93	80	99	99	86	89
0.2	28	35	32	36	-	-	-	-
0	25	9	25	9	9	23	9	23

Factors influencing efficacy

Two effervescent tablet formulations TB3 and TB4 with different particle sizes (volume median diameter of 5.3 and 9.9 µm respectively) were compared. Three nymphal ages (1, 3 and 6 weeks of age) two ages of deposit (2 and 90 days) and three concentrations (0.25, 0.5 and 1 mg per m^2) were used to determine insect mortalities after 21 and 42 days in sets of 4 x 25 insects.

The factors formulation (F), age of deposit (D), observation time (T), concentration (C) and nymphal age (A) were analysed by logistic regression including all linear, quadratic and interaction terms. The result is graphically displayed in Figure 1 showing the effect of the different factors on the survival probability (odds ratio) of the insects.

Figure 1 Effect of different factors on odds ratio for survival



The concentration C and the nymphal age A are clearly the most important factors. Six week old nymphs were about one fifth as susceptible to lufenuron as the first instar nymphs. They are followed by formulation F and observation time T. The age of the deposit D as well as some few interactions (FDA, FTCA, cA) had a very small but measurable effect, which is however biologically irrelevant.

Moult / mortality correlations

During intermoult of larval instars all BPU s inhibit the deposition of post-ecdysial lamellae in the procuticle (Degheele, 1990). Therefore the primary effect of lufenuron on *Blattella germanica* was expected to be the disruption of the moulting process. To demonstrate this effect, four experiments were set up to compare mortality in treated units with moulting in untreated units. Treatment involved facultative exposure of nymphs of varying age to deposits of the 10 WP formulation of 1mg lufenuron per m^2. The actual time points for the comparison were the time at which 50% of the insects moulted in the control group, and the time at which 50% of the insects died in the treated group.

If moulting and mortality are linked one would expect that the characteristic moulting time to be near the characteristic mortality time. Figure 2 shows the relationship between mortality during facultative exposure and moulting time in untreated units. A nonparametric method (smoothing splines, Hastie & Tibshirani 1990) was used to model the curves over time. In table 2 the 50% mortality and moulting times are given with approximate 95% confidence limits.

Most of the 1 to 2 day old nymphs died at the first moult from L_1 to L_2 (Figure 2a). Eighty seven per cent of the six to seven day old (L1) nymphs were able to moult normally at the first moult and therefore survived. Most died synchronous with the second moult from L_2 to L_3 (Figure 2b).

At the start of the experiment some of the 4 to 5 week old L_3 nymphs were approaching a moult. Others were however killed at moult and so moult and mortality were not synchronous (Figure 2c). Many of the survivors died at the following moult from L_4 to L_5 (Figure 2d).

Figure 2 Relationship of mortality of treated insects (observed: •, smoothed curve: -) and moulting of untreated insects (observed: o, smoothed curve: x) with 50 % mortality and 50 % moulting time points.

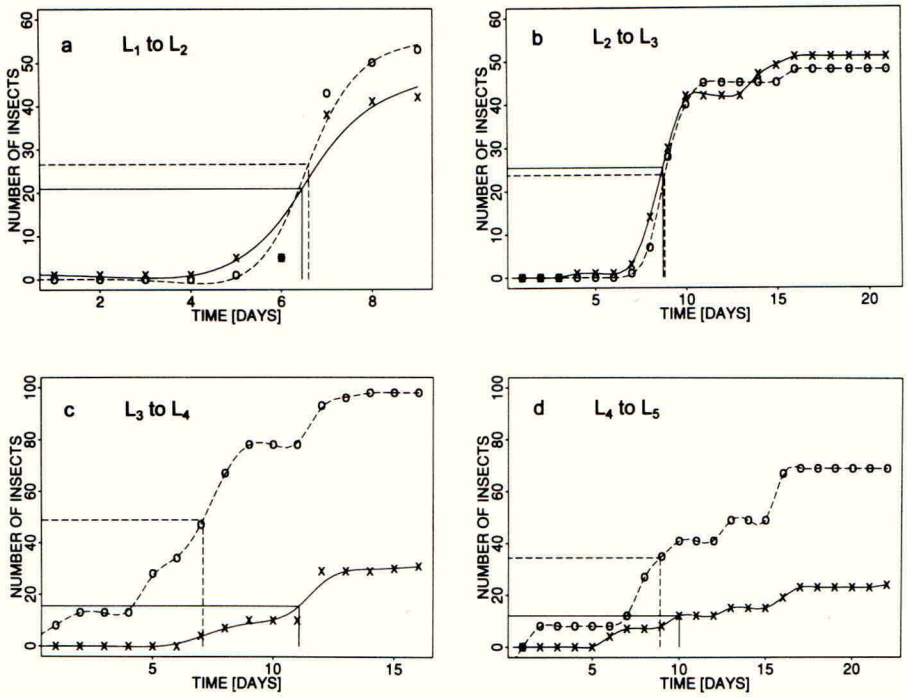


Table 3 Time in days to reach 50 % mortality and moulting with approximately 95 % confidence limits.

	Moult interval			
	L1 - L2	L2 - L3	L3 - L4	L4 - L5
Est. Mortality time	6.45	8.71	11.07	10.01
Lower/upper limits	5.41/7.42	8.58/8.85	10.04/11.62	9.60/12.53
Est. Moulting time	6.60	8.82	7.09	8.91
Lower/upper limits	6.03/7.31	8.67/8.99	6.57/7.46	8.04/12.09

Further development of survivors

An experiment with aged deposits and low concentrations using the effervescent tablet formulation TB4 demonstrated that many surviving insects developed into adults which could produce offspring (Table 4). However, no offspring were produced at 1 mg a.i. per m^2 in this experiment.

Table 4 Numbers of living adult males and females developed from 4 x 25 nymphs and numbers of F1 nymphs produced by these adults. Age of deposit was 3 months.

mg a.i. m^{-2}	age*	females	males	F1 nymphs	nymphs/fem.
1	1 week	0	0	0	0
	3 weeks	0	0	0	0
	6 weeks	1	1	0	0
0.5	1 week	0	0	0	0
	3 weeks	0	0	0	0
	6 weeks	12	11	241	20
0.25	1 week	13	5	181	13.9
	3 weeks	24	17	469	20.5
	6 weeks	45	39	820	18.2
Control	1 week	38	51	770	20.3
	3 weeks	41	48	664	16.2
	6 weeks	40	53	820	20.5

* Age of nymphs at start of the experiment.

Effect on adults

Various possible scenarios for adult exposure were studied to see if invading adults might be affected in their ability to reproduce following exposure to spray deposits. Four series of experiments were made modeling the situations where virgin males, virgin females and gravid females invaded a treated area for one hour and where virgin males and virgin females had facultative contact with a treated area for an indefinite period of time. Detailed counts were made of mortalities and reproductive success in terms of mortality of untreated adults, ootheca production, deformation of ootheca, abortion of non-fertile ootheca, number of fertile ootheca, numbers of F_1 nymphs, mortality of F_1 nymphs and moult to next instar.

In no instance did the exposure of adult *Blattella germanica* lead to premature deaths of treated males or females and no obvious deformities were observed. The effects on reproduction are summarized in Table 5.

Females subjected to long term facultative exposure and mating with males subjected to the same conditions produced no offspring. Deformed ootheca were produced after facultative exposure of virgin males and females and after treatment of virgin males. Facultative long term exposure of young males and females led to complete suppression of the F_1 generation, whereas the one hour forced exposure of virgin males and/or females led to a 20 - 40 % reduction of offspring. The short exposure of virgin females led however to a further 50% mortality of F_1 offspring seven weeks later. Some ootheca turned black and remained attached to the female and later even to the following ootheca. Production of nymphs was only slightly affected following one hour forced exposure of gravid females.

Table 5 Reproductive success of gravid females, virgin females or virgin males after forced exposure for 1 h to lufenuron and then mated with normal males and females respectively and of virgin males and virgin females mating during facultative exposure to lufenuron (number/*percentage*).

Treatment	Concentr.	Ootheca*				Nymphs			
	mg a.i. m^{-2}	Total O.	Fertile	Deformed	Aborted	Total N.	per f	% moulted	% control
Gravid f	50	39	36/*92*	0	3/*8*	1321	34	*86*	*1*
1h exp.	10	39	33/*85*	0	6/*15*	1226	31	*92*	*8*
	0	40	36/*90*	0	4/*10*	1332	33	*95*	-
Virgin f	50	39	24/*62*	1/*3*	15/*38*	835	35	*39*	*40*
1h exp.	10	37	32/*86*	0	5/*14*	1110	35	*61*	*20*
	0	38	37/*97*	0	1/*3*	1387	27	*85*	-
Virgin f/m	50	39	0	22/*56*	39/*44*	0	0	**	*100*
fac. exp.	0	40	38/*95*	0	2/*5*	1380	37	**	-
Virgin m	50	40	22/*55*	19/*48*	18/*45*	962	44	*97*	*27*
1h exp.	10	40	23/*58*	16/*40*	16/*40*	967	42	*98*	*27*
	0	39	34/*87*	15/*15*	5/*13*	1324	39	*97*	-

* Fertile, deformed and aborted ootheca can be in more than one category

** Eperiment terminated

DISCUSSION

Testing IGR s is more complex than testing conventional insecticides because of the delayed onset of activity. During this long holding period a number of interactions may influence the test results. Cannibalism has caused a loss of 35 % of insects in a nymph population of mixed age whereas in a test system with nymphs of the same age this loss was 5 % at the most. *Blattella germanica* is susceptible to cannibalism at moult (Cornwell 1975). As the identification and separation of intermediate instars is difficult, the use of nymphs of the same age from synchronized rearing units is a valid alternative. Various formulations showed similar activity: MEC about 5 mg lufenuron per m^2.

The most important factor influencing efficacy is application rate. A decrease of the application rate from 1 mg per m^2 to 0.25 mg per m^2 increases the odds ratio for survival by a factor of 30 to 60. Similarly, by increasing the age of nymphs at start from 1 week to 6 weeks, the odds ratio for survival increases by a factor of about 25. Therefore the age specific sensitivity is the second most important factor. Using formulation TB3 instead of TB4, the odds ratio for survival increases by a factor of about 5. The better efficacy of TB4 is explained by the larger particle size (median of TB3 is 5.3 µm, of TB4 9.9 µm). Reid *et al.* (1992) showed that larger particles of flufenoxuron (volume median diameter 12.2 µm) are more active than smaller ones (7.7 or 2.8 µm). The other factors have a clearly smaller effect. For example, a drastically shorter deposit storage time increases the odds ratio for survival merely by a factor of about 2 indicating the good residual activity of the deposit.

Exposure of nymphs to lufenuron led to mortalities synchronous with moult. The effect of lufenuron on *Blattella germanica* nymphs was also directly observed to be moulting disruption. Insects exposed from just before moult can however survive the moult to die at the next moult.

Further observations of nymphs exposed to very low concentrations of TB4 (e.g. 0.25 mg per m^2, Table 4) show that survivors are fertile. But whereas DeMark (1989) found that adults surviving exposure to a number of BPU s during the fifth instar were often deformed and weak but mostly able to reproduce normally, no obviously deformed adults were observed after exposure to lufenuron. The MEC for this formualtion was 1 mg per m^2.

Adults exposed to a much higher concentration (50 mg per m^2) produced no offspring. Fertility of the ootheca was inhibited. Once the ootheca are formed, however, the effect is small but early exposure of adult males and females decreases reproductive success. DeMark (1990) showed that timing of exposure related to maturity of the ootheca is also of critical importance when BPU s are fed to adult *Blattella germanica.*

The exposure of a cockroach population to a lufenuron deposit will therefore first lead to moult inhibition of the small nymphs. Nymphs shortly before moult will undergo moult but die at the next moult. Gravid females will have normal offspring which will die at the first moult. Emerging adults will have a reduced fertility leading to further population reduction. These multiple effects of lufenuron on a *Blattella germanica* population will lead to a relatively rapid reduction of the population when compared to JHA s which disrupt the last moult and sterilize the F_1 generation.

The results obtained led to a recommended trial application rate of 10 mg of lufenuron per m^2 for the control of *Blattella germanica* populations.

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THE EFFECTIVENESS OF PRALLETHRIN AGAINST PUBLIC HEALTH PESTS

Y. ABE, S. SENBO, Y. TAKADA, H. KAWADA AND T. ITO

Agricultural Chemicals Research Laboratory, Sumitomo Chemical Co., Ltd.,
4-2-1, Takatsukasa, Takarazuka, Hyogo, 665, Japan

ABSTRACT

Prallethrin is a pyrethroid insecticide, which has extremely rapid knockdown activity in various flying insects and remarkably high lethal activity in various insects, as well as low toxicity to mammals. Furthermore, it shows a wide pesticidal spectrum against various public health pests, *e.g.* houseflies, mosquitoes, cockroaches, wasps, hornets, poisonous snakes and scorpions. It is also suggested that prallethrin can effectively kill the *kdr*-type houseflies.

INTRODUCTION

Prallethrin (ETOC™) is a high performance pyrethroid developed by Sumitomo Chemical Co., Ltd. for public health pest control. The compound has one asymmetric carbon atom in the alcohol moiety in addition to two asymmetric carbon atoms in the acid moiety, so that there are eight possible stereo-(or optical and geometrical) isomers. Prallethrin is the most potent of these isomers.

Racemic prallethrin was synthesized and tested for the first time by Gersdorff *et al.* (1961). However, further investigation of these isomers has not been carried out due to the lack of a convenient method of synthesis. Recently Sumitomo scientists have succeeded in the synthesis of individual stereoisomers of the compound by applying chemico-enzymatic reactions and investigated the biological activity of these stereoisomers in detail (Matsunaga *et al.*, 1987; Umemura *et al.*, 1993) (Table 1).

TABLE 1. Lethal activity of prallethrin isomers against houseflies (*Musca domestica*) by topical application.

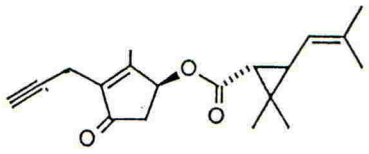
Isomer		LD_{50} (μ g/♀)	Relative activity
Alcohol	Acid		
S	*(1R)-trans*	0.043	656 (17)
S	*(1R)-cis*	0.11	256
R	*(1R)-trans*	0.25	113
R	*(1R)-cis*	0.32	88
S	*(1S)-trans*	3.30	8.5
S	*(1S)-cis*	10.8	2.6
R	*(1S)-trans*	18.5	1.5
R	*(1S)-cis*	28.2	1.0
(Ref.)Pyrethrins		0.73	(1.0)

CHEMICAL AND PHYSICAL PROPERTIES

Prallethrin is a yellow to yellow-brown liquid, soluble in the most of organic solvents. Vapor pressure of prallethrin is similar to that of *d*-allethrin (Pynamin™Forte). Differential thermal analysis revealed that prallethrin was relatively stable to heat and began to evaporate around 130°C. These suggest that prallethrin is suitable for uses with heat-evaporation type fumigations, such as mosquito coils, mosquito mats and vaporizer liquids, in addition to spray type formulations.

Chemical name: *(S)*-2-Methyl-4-oxo-3-(2-propynyl)cyclopent-2-enyl *(1R)-cis,trans*-chrysanthemate

Chemical structure:



Molecular formula: $C_{19}H_{24}O_3$
Molecular weight: 300.40
Specific gravity: d_4^{20}1.03
Viscosity: 1056 cP (20℃), 550 cP (25℃)
Vapor pressure: 3.5 x 10^{-5}mmHg (20℃), 1.0 x 10^{-4}mmHg (30℃)
Solubility: Miscible with most aromatic and aliphatic hydrocarbons, chlorinated hydrocarbons and other organic solvents. Low solubility in water (8.51ppm/25℃)

BIOLOGICAL PERFORMANCES

Houseflies and mosquitoes

The knockdown activity of prallethrin was extremely rapid on houseflies (*Musca domestica*), showing the shortest knockdown-time-50 (KT_{50}) among 13 insecticides tested,

TABLE 2. Insecticidal activity of oil-based (OBA) and water-based (WBA) aerosols.
Test method: CSMA aerosol test method for flying insects.
Test insect: Housefly (*Musca domestica*, CSMA strain, male and female adults)
Mosquito (*Culex pipiens pallens*, female adults)

Active ingredient in aerosol	%(w/w)	Type	KT_{50}(min.) - Mortality(%)	
			Housefly	Mosquito
Prallethrin	0.075	OBA	9.3- 30	3.3- 37
Prallethrin	0.15	OBA	6.1- 62	2.6- 69
Prallethrin/*d*-Phenothrin/PBO	0.075/0.075/0.3	OBA	7.5- 82	3.2- 99
Prallethrin/Cyphenothrin	0.1/0.3	OBA	7.4- 92	2.7-100
Prallethrin	0.075	WBA	6.9- 68	4.0- 99
Prallethrin	0.15	WBA	4.8- 82	3.2-100
Prallethrin/*d*-Phenothrin/PBO	0.075/0.075/0.3	WBA	5.7-100	4.0-100
Prallethrin/Cyphenothrin	0.1/0.3	WBA	5.3-100	3.5-100
Pyrethrins/PBO (OTA)*	0.2/1.6	OBA	8.5- 93	9.8-100

*: OTA, Official Test Aerosol, PBO: Piperonyl butoxide

including ten pyrethroids and three organophosphorus and carbamate insecticides (Abe, 1993). The rapid knockdown effect is very important if immediate results are required, *e.g.* wasps attacking humans, flies infesting a dining area, or scorpions striking humans.

The insecticidal activity of oil-based and water-based aerosol was evaluated against houseflies and mosquitoes (Table 2). Prallethrin alone gave very quick knockdown at the concentrations of 0.075% and 0.15%. The combination of prallethrin and *d*-phenothrin (Sumithrin™) or cyphenothrin (Gokilaht™) gave increased mortality, giving better efficacy than the official test aerosol (OTA). The results revealed that oil-based and water-based aerosols provide good insecticide formulations for control of houseflies and mosquitoes.

Mosquitoes

The insecticidal evaluation of mosquito mat formulations containing prallethrin indicated that prallethrin-10mg mat maintained good knockdown for 8 hours. It showed higher insecticidal activity than the *d*-allethrin-40mg mat and the *S*-bioallethrin-20mg mat (Abe, 1992).

The mosquito mat formulations are now acceptable to consumers for mosquito control worldwide. A mosquito mat keeps its effectiveness for 8-12 hours, after which another mat must be placed on the electric heating device for the next night. Mosquito vaporizer liquids do away with this inconvenience by retaining their effectiveness for 30 days or more (at 12 hours use/day). Since the product was commercially launched in the Japanese market in 1983, it is gaining a greater proportion in the market.

Prallethrin vaporizer liquid showed a stable and uniform insecticidal efficacy, keeping 1.6-2.4 minutes of KT_{50} and 100% mortality during 20 days (at 12 hours use/day). It was confirmed that a vaporizer liquid containing 0.30g of prallethrin in a 45ml-bottle (0.667%,w/v) maintained remarkably high effectiveness for 30 days (Table 3).

TABLE 3. Insecticidal efficacy of a mosquito vaporizer liquid against mosquitoes (*Culex pipiens pallens*, female adults).
Test method: The Japanese guidelines for the registration of insecticides by using glass cylinder (20 cm in diameter, 80 cm in height).

Active ingredient	Day	%Knockdown at indicated time (min.) 1	2	3	4	5	6	10	15	20	KT_{50} (min.)	Mortality (%)
Prallethrin	1st	0	53	90	95	100	100	100	100	100	1.9	100
0.667 %(w/v)*	10th	0	30	75	100	100	100	100	100	100	2.4	100
	20th	0	78	98	100	100	100	100	100	100	1.6	100

*: *n*-Paraffin solution in 45 ml-bottle.

Cockroaches

Prallethrin had the highest flushing activity among the insecticides tested, including ten pyrethroids and three organophosphorus and carbamate insecticides. It showed approximately 2.3 times more flushing activity than pyrethrins to German cockroaches (Abe, 1993).

Test results of aerosol formulations exhibit that prallethrin has not only a high knockdown activity, but also a strong killing activity. In order to increase the effectiveness of prallethrin, the combined formulation of prallethrin with another killing agent, such as *d*-

phenothrin or cyphenothrin appeared extremely effective. This efficacy is present in oil-based as well as water-based aerosols (Table 4).

TABLE 4. Insecticidal efficacy of oil-based (OBA) and water-based (WBA) aerosols against German cockroaches (*Blattella germanica*, male and female adults). Test method: CSMA direct spray method for cockroaches.

Active ingredient in aerosol	%(w/w)	Type	KT_{50} (min.)	Mortality (%)
Prallethrin	0.075	OBA	3.8	30
Prallethrin	0.15	OBA	1.9	64
Prallethrin/*d*-Phenothrin/PBO	0.075/0.075/0.3	OBA	3.4	60
Prallethrin/Cyphenothrin	0.1/0.3	OBA	2.0	100
Prallethrin	0.075	WBA	3.9	65
Prallethrin	0.15	WBA	2.8	93
Prallethrin/*d*-Phenothrin/PBO	0.075/0.075/0.3	WBA	3.8	90
Prallethrin/Cyphenothrin	0.1/0.3	WBA	3.4	100
Pyrethrins/PBO (OTA)*	0.2/1.6	OBA	7.0	72

*: OTA, Official Test Aerosol, PBO: Piperonyl butoxide

Wasps and Hornets

Japanese Ministry of Health and Welfare statistics indicate that *ca.* 50 people are killed by wasp or hornet stings each year. According to the data, numerous people have died of anaphylaxis within one hour of being stung by a wasp or hornet. There are less than 10 people a year who are killed by poisonous snakes (southern islands) or wild bears (northern islands), both of which are regarded as extremely dangerous animals in Japan. However, in Japan today it is clear that wasps and hornets are potentially at least as dangerous as snakes and bears.

TABLE 5. Efficacy of aerosols against paper wasps (*Polistes rothneyi*).

Active ingredient in aerosol (%)		Spray time (min.)	% Knockdown at indicated time (sec.) 10	20	40	60	120	240	300	360	Mortality (%)
Prallethrin	0.3	0.2	55.6	100							100
S-421	0.3										
d-Resmethrin	0.06	0.2	0	0	22.2	55.6	100				100
d-Tetramethrin	0.45										
Propoxur	2.0	2.0	0	0	0	0	0	0	66.7	100	100
DDVP	0.5										

Test method: Three paper wasps (*Polistes rothneyi*) were freed in a cage (8 cm in diameter, 14 cm in height). Aerosol was sprayed to the cage from 30 cm distance for an

indicated time (spray dose: *ca.* 2.3-3.1 g) and knocked-down insects were counted. Mortality was observed after one day. The test was replicated three times.

Results: Quick action is the most important for a containment offensive against wasps and hornets. Prallethrin aerosols fully satisfy this requirement (Table 5). By using this excellent property, two types of aerosols were developed to control wasps and hornets: a large type for attacking nests of wasps or hornets; and a small type for protecting humans from attack by wasps or hornets.

Poisonous snakes

Test method: Oil-based pressurized sprays containing prallethrin were sprayed onto a venomous snake from *ca.* 2 m distance. Spraying time was one second for Mamushi (*Agkistrodon blomhoffii*), and five seconds for Habu (*Trimeresurus flavoviridis*), and the discharge rate of the spray was *ca.* 50-80 g/sec. The test was replicated five times.

Results: Immediately after treatment with a pressurized spray, the snakes moved slowly and did not appear to be outwardly harmed. Ten to 20 minutes later, usually they stopped moving, and they shook or vibrated their heads. Subsequently they became excited and tried to bite surrounding air repeatedly, and eventually died about 4 hours after treatment. Although prallethrin spray alone was remarkably effective, a combination of prallethrin and a synergist (S-421) was even more effective, showing 100% mortality after four hours on both Mamushi and Habu (Table 6).

TABLE 6. Lethal effect of pressurized sprays against two poisonous snakes, Mamushi (*Agkistrodon blomhoffii brevicandus*) and Habu (*Trimeresurus flavoviridis*).

Active ingredient in spray	(%)	Mortality (%)			
		Mamushi		Habu	
		4 hr.	8 hr.	4 hr.	8 hr.
Prallethrin	0.3	100	100	80	100
Prallethrin/S-421	0.3/0.9	100	100	100	100
S-421	0.9	0	20	0	40
Pyrethrins	0.3	60	80	60	60
Control*	-	0	0	0	0

*: Spray only solvent (kerosene) without active ingredient.
S-421: 1,1'-Oxybis(2,3,3,3-tetrachloropropane).

The hazard posed by the Habu is serious in Amami and Okinawa Islands, southern islands in Japan. Approximately 300 to 350 Habu snake bites are reported each year. Usually the Habu will stretch out and strike at humans in a 1.5 m radius. The highly pressurized sprays tested can discharge prallethrin solution over a distance of at least 3 m. Therefore the use of the prallethrin spray would be very useful for protecting people from the bite of venomous snakes.

The behavior of the snakes treated with prallethrin spray was similar to that of insects such as cockroaches, which become uncoordinated and excited before dying. Accordingly the nervous system of the snakes might be affected by prallethrin (Toriba *et al.*, 1992). Prallethrin was also observed to have a flushing action on venomous snakes (Habu). This effect would

be useful in practice in removing snakes from their hiding places.

Scorpions

Test method: A scorpion (*Heterometrus sp., ca.* 10-13 cm long), caught in Thailand, was placed in a polyethylene cup (15 cm in diameter, 15 cm in height). A pressurized spray containing prallethrin was sprayed on the scorpion from *ca.* 70 cm distance for 0.5 second, and the discharge rate of the spray was *ca.* 50-80 g/sec. After spraying, the scorpion was transferred into a clean polyethylene cup, and then knockdown and mortality were observed. The test was replicated six times.

Results: A prallethrin spray showed 17% knockdown in 10 minutes and 100% mortality after 24 hours. The synergistic effect of S-421 was not significant. Pyrethrins and permethrin exhibited slower knockdown activity than prallethrin although both showed 100% mortality after 24 hours (Table 7).

Scorpions have four pairs of functional legs and a pair of palpi modified to form grasping or seizing organs. The posterior segments of the abdomen terminate in a stinger equipped with poison glands. In order to protect humans from striking attacks with the scorpion venom apparatus, the rapidity with which prallethrin acts is a more valuable attribute than the mortality which this compound eventually caused (Table 7).

TABLE 7. Efficacy of pressurized sprays against scorpions (*Heterometrus sp.*).

Active ingredient in spray	(%)	% Knockdown in indicated minutes				% Mortality	
		3	10	30	60	60 min.	24 hr.
Prallethrin	0.3	0	17	33	83	50	100
Prallethrin/S-421	0.3/0.3	0	17	33	100	50	100
Pyrethrins	0.3	0	0	17	67	17	100
Permethrin	0.3	0	0	0	0	0	100
Control*	-	0	0	0	0	0	0

*: Spray only solvent (kerosene) without active ingredient.

Insecticidal activity to the *kdr*-type houseflies

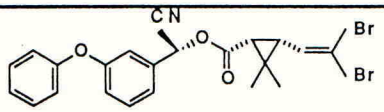
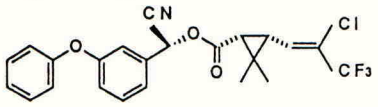
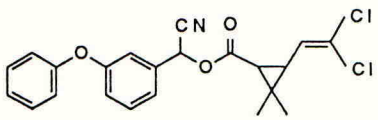
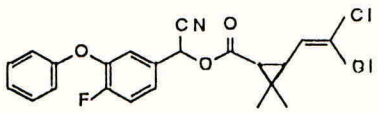
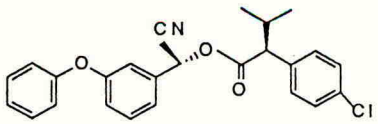
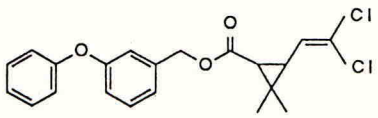
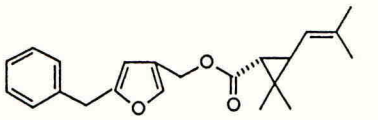
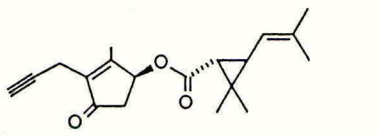
Insecticide resistance is becoming increasingly an urgent worldwide problem. The pyrethroid resistance due to lowered nervous sensitivity is called *kdr*-type resistance, and it has several characteristics:

1. The *kdr* gene causes lower sensitivity toward DDT and pyrethroids in nerves,
2. The gene confers resistance on insects to all pyrethroids known up to this time,
3. It can give high resistance,
4. It is recessive.

The Akagi colony of houseflies (*Musca domestica*), collected in Akagi (Japan), was used in this study. The Akagi colony was selected with permethrin for 15 generations to obtain Akagi PP15. It has been established by electro-physiological and genetic studies that the Akagi PP15 strain has a major recessive knockdown resistance (*kdr*) factor on the third chromosome. This factor is associated with neuronal insensitivity to permethrin. The susceptible Bx^2 strain has one dominant marker on the third chromosome, and its phenotype shows that the wing is snipped from various directions. The homozygote of this gene is lethal.

The contribution of the *kdr* factor on the third chromosome to resistance to pyrethroids was investigated by the backcross method using F_1 males. The males of Bx^2 strain ($+/Bx^2$) were crossed with the females of the Akagi PP15 strain in mass, and then F_1 males ($+/Bx^2$)

TABLE 8. Susceptibility of backcrossed progeny of housefly (Akagi PP15 strain ♀ × F_1 progeny $+/Bx^2$ ♂) to pyrethroids.

Compound	Structure	LD50 (μg/female fly) +/+	LD50 (μg/female fly) $+/Bx^2$	C_{kdr}
deltamethrin		>40	0.032	>1300
λ-cyhalothrin		>40	0.068	>590
cypermethrin		>40	0.096	>420
cyfluthrin		>40	0.11	>360
esfenvalerate		>40	0.11	>360
permethrin		>40	0.19	>210
d-resmethrin		8.6	0.078	110
prallethrin		18	0.80	23

were backcrossed to the females of the Akagi PP15 strain in mass. By using the topical application, the toxicity of some pyrethroids was evaluated to the backcrossed progeny (Akagi PP15 strain × F_1 progeny) (Table 8). The contribution of the *kdr* factor (C_{kdr}) was calculated by the equation below:

$$C_{kdr} = \frac{LD_{50} \text{ of backcrossed progeny } (+/+)}{LD_{50} \text{ of backcrossed progeny } (+/Bx^2)}$$

In the case of the pyrethroids with 3-phenoxybenzyl alcohol moiety, a remarkable difference between the LD_{50} values of the two genotypes was apparent, showing between 110 and 1,300-fold or greater values of C_{kdr}. On the other hand, the difference between the activities of prallethrin in the two genotypes was much less, showing 23 of C_{kdr}.

The contribution of the *kdr* factor to pyrethroid resistance was dependent on the structure of the pyrethroids (Takada *et al.*, 1992). Resistance to the pyrethroids with 3-phenoxybenzyl alcohol moiety was closely associated with the *kdr* factor, while the contribution of the *kdr* factor to the pyrethroids with aliphatic or cyclopentenolone alcohol was less than that of the pyrethroids described above. In contrast, the contribution of the *kdr* factor was not largely related to acid moiety of the pyrethroids tested. The structure of prallethrin (which has the cyclopentenolone alcohol) suggests that this molecule will be less effected by the *kdr* factor. Therefore, prallethrin might be expected to be particularly effective against pests having the *kdr* mechanism.

Application of emulsified prallethrin to some pyrethroid resistant houseflies (including Akagi PP15) has shown that prallethrin can be effective against the *kdr*-type resistant houseflies in combination with a synergist (*eg:* piperonyl butoxide) or an organophosphorus insecticide (*eg:* fenitrothion). Whereas phenoxybenzyl-type pyrethroids were much less effective, even in combination with a synergist (Kawada *et al.*, 1994).

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A NEW APPROACH FOR THE CONTROL OF INSECTS IN INDUSTRIAL PREMISES

J.M. GROOME, R. MARTIN

Roussel UCLAF Environmental Health Ltd., McIntyre House, High Street, Berkhamsted, Herts., HP4 2DY

D. LESIEWICZ

Roussel UCLAF Corporation, 95 Chestnut Ridge Road, Montvale, NJ 07645.

ABSTRACT

Turbocide GOLD® is an acronym for a Gas Operated Liquid Dispensing system that produces a fine insecticidal aerosol to control pests in industrial premises. It is based on the variable inflow mixing of separate sources of carbon dioxide and insecticide concentrate using a patented ejector system. Washout of the concentrate container ready for re-filling is an integral part of the system. Production of the spray involves no handling of insecticide and is controlled from outside the area to be treated, so that operator exposure is completely avoided. A trial is described in which a 70,000 m^3 tobacco warehouse in North Carolina was treated with a concentrate containing pyrethrins, piperonyl butoxide and hydrocarbon solvent. Aerial concentration of insecticide, droplet size and distribution of the aerosol within the warehouse were monitored. Excellent control of the indicator species, German Cockroach, Red Flour Beetle, Confused Flour Beetle and Cigarette Beetle was achieved.

INTRODUCTION

Conventional treatments of industrial premises to control insect pest populations have often involved an operator, heavily clad in safety equipment, carrying or pushing a sprayer around a warehouse for possibly hours at a time. If we also take into account any diluting, mixing and transferral of formulation into the spraying machine that may be necessary, then the potential for operator exposure to the insecticide is high.

In order to carry out the pest control the premises would have been evacuated of all personnel and any machinery turned off. Extended closedowns can be costly in terms of lost production and need to be kept to a minimum. The operator may also be left with the problem of disposing of empty insecticide containers. This is an issue with which the United States Environmental Protection Agency in particular has become increasingly concerned (Fitz, 1992).

One of the objectives in the development of Turbocide GOLD® was to address the problems of operator exposure, the time taken to treat a large warehouse and the disposal of empty pesticide containers. This paper will describe how this new spray system addresses, and provides solutions to these problems.

SYSTEM DESCRIPTION

The system is illustrated in Fig.1. It consists of four main components:

(i) A concentrate cylinder fitted with a patented connector and a dip tube. The cylinder is returnable/refillable, sealed and tamper indicating. The seal is broken as it is attached to the system.

(ii) A cylinder of liquid carbon dioxide fitted with a liquid offtake (dip) tube. These are widely available from a number of suppliers.
A non-return valve is fitted to the cylinder outlet.

(iii) An adjustable ejector device (Armitage & Peacock, 1992; Tice & Eitner, 1992) which uses the pressure and flow of liquid carbon dioxide (CO_2) to remove the concentrate from the concentrate container and mix it with the CO_2 stream.

(iv) A network of tubing and nozzles installed near the ceiling of the warehouse. The concentrate/CO_2 mix flows from the ejector, through the tubing and is atomised at the nozzle blocks to produce a fine insecticidal aerosol. Each nozzle block usually contains four nozzles.

SYSTEM OPERATION

Operation of the system is straightforward. The transit cap is removed from the concentrate container and the ejector is screwed on top. This breaks the seal. The CO_2 cylinder is then connected and turned on. Spraying is started by turning the on/off valve in the control box. The concentrate is dispensed within 5 minutes, but liquid CO_2 continues to flow for several minutes after this, cleaning out the concentrate container, the tubing and nozzles. When the CO_2 cylinder is emptied both it and the concentrate cylinder are detached and returned for re-filling.

TRIAL

Site and conditions

A 70,000 m^3 tobacco warehouse near Aberdeen, North Carolina, was used for all tests. It was of an entirely steel construction with a concrete floor. Ambient conditions during the trial were 19 $\pm$ 2 ^{o}C and 40 $\pm$ 4 % relative humidity.

Spray system

A 64 nozzle spray system with associated tubing was installed near the ceiling of the warehouse. One representative quarter of this system was selected for physical and biological monitoring of the sprays produced and is represented in Fig.2. The cylinders and control box were located outside of the area to be treated. Approximately 6 kg of formulation and 32 kg of CO_2 were in the concentrate cylinder and liquid carbon dioxide cylinder respectively for each treatment.

FIGURE 1. System diagram.

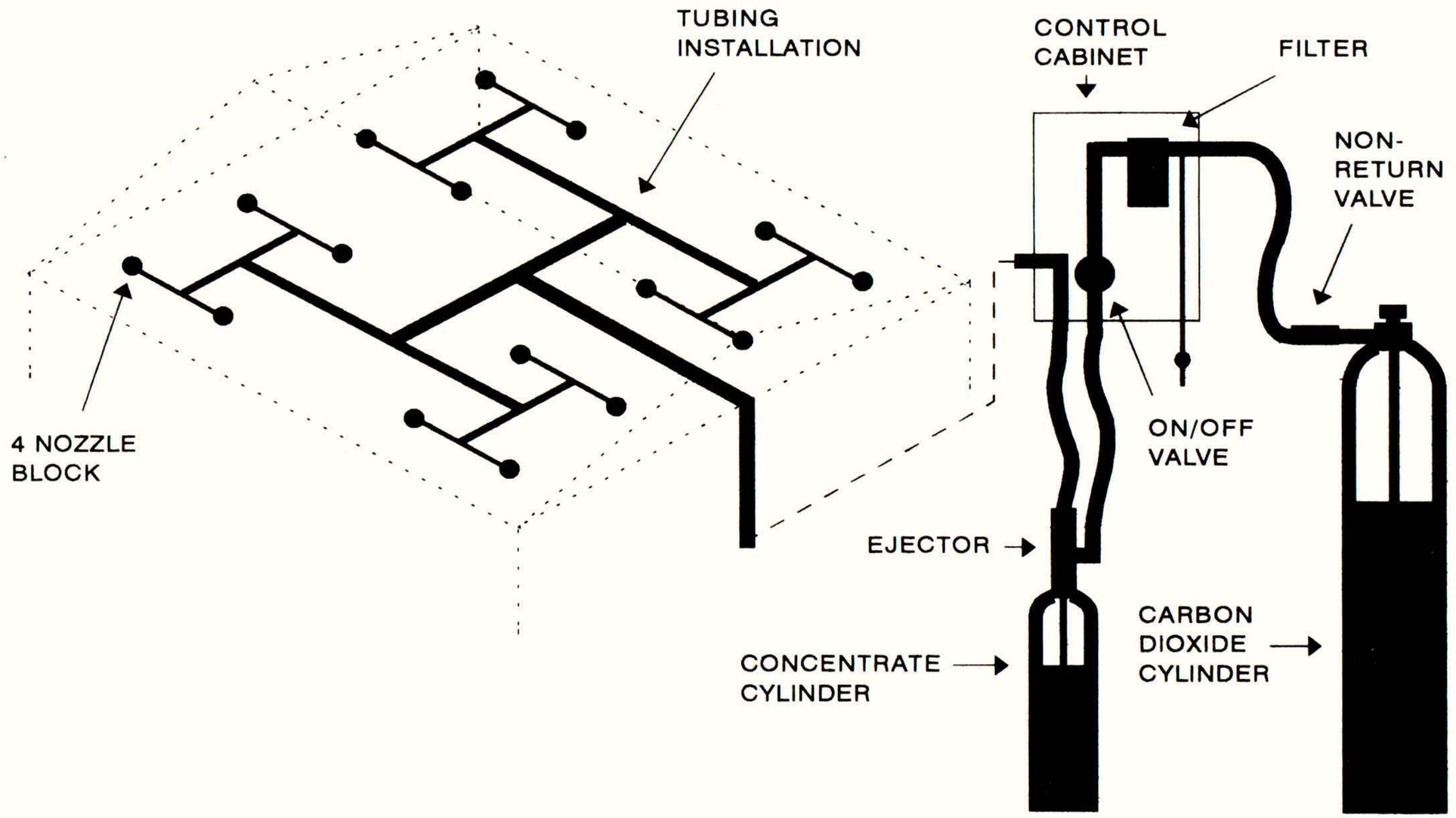
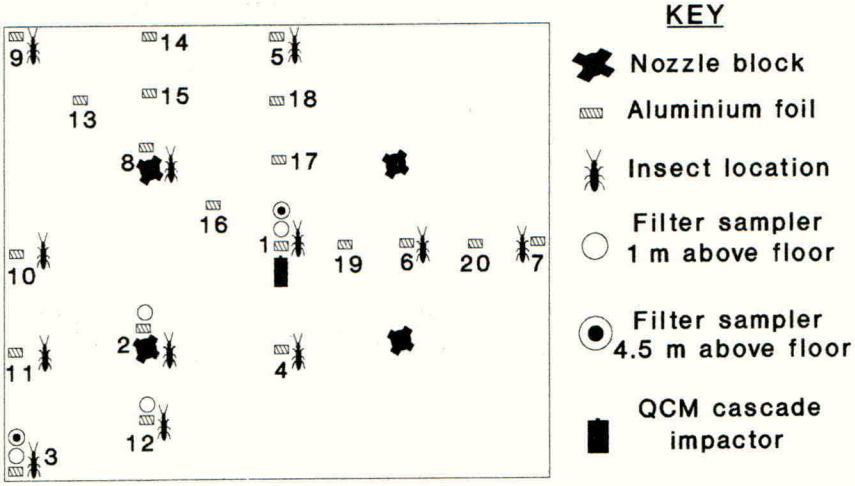


FIGURE 2. Location of sampling positions



Formulation and dose levels

The formulation in the concentrate cylinder was:

	% w/w
Pyrethrins	4
Piperonyl butoxide	32
Hydrocarbon solvent	64

When discharged into the treatment volume this gave a pyrethrins dose level of 3.5 mg m^{-3}.

Aerial concentration

Levels of pyrethrins airborne were measured using filters attached to vacuum pumps. Casella AFC 123 personal air sampler pumps were used to draw air at 2.0 l min^{-1} through 37 mm diameter cellulose nitrate membrane filters of pore size 0.8 μm. Six sampling positions were used as shown in Fig.2. Filters were changed at intervals throughout the sampling period.

Droplet size

Droplet size distributions were measured using a Berkeley Controls Quartz Crystal Microbalance Model C1000A 10-stage Cascade Impactor (QCM). Crystal frequencies corresponding to the mass of droplets collected on each of the 10 stages were recorded. These were used to construct a size distribution for the sampled aerosol, from which the 10 %, 50 % (Mass Median Diameter, MMD) and 90 % by mass undersize points were determined. The effective cut-off diameters ranged from 0.06 μm for stage 10 to 35.92 μm for stage 1.

Deposit levels

Deposition of pyrethrins onto the floor was assessed using 15 cm x 6.5 cm pieces of aluminium foil stapled to white cards and positioned around the warehouse as shown in Fig.2. After exposure, the foils were detached from the backing card, folded and placed into glass vials for extraction and chemical analysis.

Spray time

The time taken for all of the concentrate to be discharged was assessed by direct observation of the spray leaving the nozzles.

Concentrate container washout

After each treatment the concentrate cylinder was detached and the ejector coupling unscrewed. 100 ml of hexane were poured into the cylinder which was then rolled around on it's side so that the hexane washed the whole of the interior of the cylinder. A sample of the hexane was taken and analysed to determine the quantity of concentrate left behind after discharge.

Ambient conditions

Temperature and relative humidity were measured at a height of 1 m above the warehouse floor using a Grant Instruments Squirrel meter/logger type SQ8-2U/2L fitted with two Vaisala temperature and humidity probes.

Biological monitoring

The following species were used:

(i) German cockroach (Blattella germanica)
(ii) Red flour beetle (Tribolium castaneum)
(iii) Confused flour beetle (Tribolium confusum)
(iv) Cigarette beetle (Lasioderma serricorne)

For cockroaches only adult males were used. For the other species mixed adults were tested.

Ten insects of each species were placed at each of locations 1-12 shown in Fig.2. The sites were selected such that they were in direct-line and out-of-line with the spray nozzles and represented maximum and minimum distances from the nozzles.

Cockroaches and cigarette beetles were held in gauze-covered pint paper cups during exposure, whilst the red flour beetles and confused flour beetles were held in petri dishes. After exposure for 6 hours the test insects were removed to a clean area and held under ambient conditions for mortality assessments at 24, 48 and 72 hours post treatment.

Results

Under the conditions of this trial the time taken to spray out the whole of the 6 kg of concentrate varied between 5 min 47 s and 5 min 53 s. Due to the nozzles being regularly spaced out near the ceiling, the aerosol was dispersed evenly throughout the upper portion of the warehouse within 10 minutes of turning the on/off valve. The droplets then descended and diffused to fill the whole of the warehouse with a fine insecticidal aerosol.

The droplet size distribution of the aerosol varied with time as shown in Fig.3. The MMD decreased steadily over the first hour due to evaporation of the hydrocarbon solvent and sedimentation of the larger droplets in the distribution. Droplet size decreased more slowly thereafter.

FIGURE 3: Variation of droplet size with time after treatment

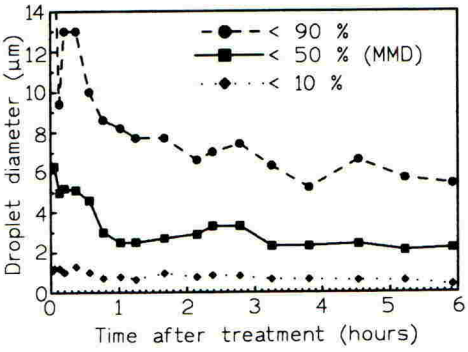
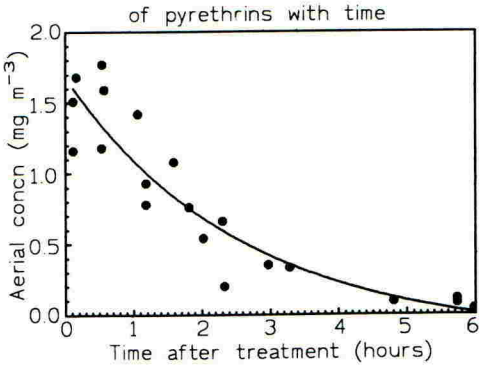


FIGURE 4: Variation of aerial concentration of pyrethrins with time



The fine droplet size meant that the aerosol was able to penetrate all the recesses of the warehouse via diffusion and any small air currents within the closed building.

The aerial concentration of pyrethrins, Fig.4, declined in an exponential fashion from a peak of approximately 1.8 mg m^{-3}. The nominal concentration of 3.5 mg m^{-3} was not reached due to the need to run the filter samplers for long enough to collect sufficient material for chemical analysis. The maximum aerial concentration occurred right after the completion of spraying. This emphasises the speed with which the system can treat a large volume.

The combination of an evenly spaced nozzle system and a fine aerosol meant that the warehouse was treated evenly with insecticide. Typical deposit levels across the floor of the warehouse are presented in Table 1.

TABLE 1. Deposit levels of pyrethrins at various points across the warehouse floor

Position number	Pyrethrins deposit level (mg m^{-2})	Position number	Pyrethrins deposit level (mg m^{-2})
1	13.2	11	8.4
2	23.8	12	8.3
3	4.7	13	11.8
4	8.0	14	8.6
5	8.4	15	12.6
6	17.2	16	10.0
7	22.4	17	11.2
8	24.8	18	5.9
9	7.7	19	15.8
10	8.8	20	11.2
	Mean = 12.1	Standard deviation = 5.7	

Deposition was generally even across the whole floor, although not surprisingly there was a tendency towards higher deposition adjacent to the nozzle blocks where all of the droplets were generated.

This even distribution was reflected by the biological data. For the purposes of this comparison, sampling positions 1,2,4,6,8 and 12 on Fig.2 were classified as interior and positions 3,5,7,9,10 and 11 as at the perimeter of the warehouse. The mortality data are summarised in Table 2 and show that there was little difference in performance between the interior and perimeter positions.

TABLE 2. Summary of insect mortality data

Species	% mortality 48 h after treatment	
	Interior	Perimeter
German cockroach	99.4	98.9
Cigarette beetle	100.0	100.0
Red flour beetle	96.1	81.1
Confused flour beetle	100.0	95.0

Container washout data are presented in Table 3. The mean percentage of concentrate discharged was 99.9749 %. The concentrate container was therefore considered clean and safe for return and refilling with more concentrate. The tubing and nozzle system was left similarly clean and safe.

TABLE 3. Container washout data

Treatment number	Mass of concentrate loaded (kg)	Concentrate discharged (% m/m)
1	6.0	99.9783
2	6.0	99.9744
3	6.0	99.9721

CONCLUSIONS

Turbocide GOLD® represents a new approach to the control of insects in industrial premises by providing solutions to a number of problems: first, the insecticide is supplied in a returnable/re-fillable, sealed, tamper indicating container. Washout of the container is an integral part of the system design, leaving the container clean and ready for return. Container and residue disposal problems are eliminated. Second, operator exposure is completely avoided. No mixing or dilution of insecticide is required and treatment is carried out remotely from a control panel outside of the treatment area.

Third, treatment is very rapid and almost independent of building size. This minimises the time that the warehouse needs to be closed down for pest control. Fourth, a fine aerosol is produced that remains airborne for 2 to 6 hours. It penetrates all areas and distributes evenly throughout the treatment volume, and the aerosols produced are highly efficacious against a range of insect pests. In addition, the system is cost effective due to low labour requirements, reduced closedown times and no waste disposal expenses, and finally the separation of the propellant from the concentrate allows considerable flexibility in the range of formulations and active ingredients that may be used. This may allow applications of the system outside of the Public Health pest control field.

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ENHANCING RODENTICIDE PERFORMANCE BY UNDERSTANDING RODENT BEHAVIOUR

D.P. Cowan, D. S. Bull, I.R. Inglis, R.J. Quy and P. Smith

Central Science Laboratory, Ministry of Agriculture Fisheries and Food, London Road, Slough Berkshire SL3 7HJ

ABSTRACT

Rodents continue to pose problems despite effective rodenticides being available. This suggests that adequate bait consumption is not always achieved. An improved understanding of the way rodent behaviour constrains bait uptake should offer ways of enhancing rodenticide performance. Studies are reported concerning rat behaviour in the laboratory, under semi-natural controlled conditions and in the field. Delaying the onset of symptoms by microencapsulation and understanding why some but not all rats show cautious sampling behaviour towards novel food should reduce learned aversions towards non-anticoagulant rodenticides. Furthermore, cautious, "neophobic" behaviour towards novel containers by rats may be a major constraint on effectiveness. Hence, the characteristics that elicit these responses need to be identified and eliminated from bait container designs.

INTRODUCTION

Population management of commensal rodent species, such as the Norway rat (*Rattus norvegicus*), relies mainly on the use of rodenticide baits. The main advances in the effectiveness of such methods over the past forty years have been in the development of novel active materials. The anticoagulants have led the way beginning with the first-generation materials such as warfarin, through to the arrival of a series of more potent second-generation materials since the mid-1970s. These developments have been reviewed recently by Buckle (1994) and, in general, very effective rodenticides are now available for most circumstances. Despite this availability, however, commensal rodent problems persist. For instance, a recent survey suggests that rodent infestations are increasing in some situations (IEHO, 1994). There is thus room for improvement in control practice. One means of achieving this is through a better understanding of feeding behaviour in relation to rodenticide baits. Two particular characteristics of rat behaviour constrain bait consumption. Firstly, rats are generally "neophobic", that is cautious regarding both novel foods, such as rodenticide baits, and novel objects, such as bait containers (e.g. Mitchell, 1976). Secondly, rats are readily able to associate illness with recently consumed novel food, subsequently becoming "bait shy" or, more formally, developing learned or conditioned aversions to the novel food (e.g. Rozin, 1968). Indeed, the delay in the onset of symptoms after exposure to anticoagulants and the consequently reduced capacity of animals to develop learned aversions, is generally accepted as the main reason for the particular success of these materials. These behaviours have traditionally been studied in the laboratory. This paper explores how such observations in the laboratory can be related to those made under semi-natural but relatively controlled settings and those made in the field.

METHODS

Laboratory study

One hundred and fifty-five rats born in the laboratory from parents caught on farms in the county of Sussex were housed, at weaning, in wire mesh cages. They were provided with rat and mouse No. 1 pelleted diet (SDS Ltd., Witham, Essex, UK) from a hopper at the rear of the cage and water *ad libitum*. The animals were maintained on a 12:12 hour light:dark cycle. At between three and six months of age animals were offered approximately 100g of pinhead oatmeal (Killgerm Ltd.) containing 5% w/w corn oil and 1% sodium saccharin presented in a galvanised metal feeding bowl measuring 90mm x 75mm. The bowl was placed at the left side of the front of the cage and anchored with a retaining clip. The animals had no prior experience of either the diet or the feeding bowl. The bowl was presented within half an hour of the beginning of the dark phase of the light cycle to minimise disturbance. The novel bowl and diet were presented to each rat for four consecutive days during each of three consecutive weeks. Thus the total exposure to the novel stimuli was 12 days. The amounts of novel and laboratory diet eaten by each animal were recorded for each of these days.

Arena study

Eight colonies of wild rats were studied. Each was derived from a male and female trapped on farms in the counties of Sussex and Hampshire in southern England and housed in a large arena measuring 10m by 5m (see Shepherd and Inglis, 1987 for details). At one end of each arena was a stack of hay bales and, at the opposite end, a water font situated between two feeding sites. Each feeding site consisted of a food pot containing ground laboratory diet on a raised platform. Two experimental procedures are reported here for colonies that had been acclimatised to the arenas for at least five weeks. The first involved presenting a novel food in a familiar container. Here, the food available at one of the feeding sites was changed to a mixture of pin-head oatmeal, 5% corn oil and 1% sodium saccharin. The food at the other site remained unchanged. The amounts of the novel and familiar foods consumed were recorded daily for five days. At the end of the five day period the novel food was replaced with the familiar diet The colonies were then left undisturbed for at least two weeks before initiating the second experimental procedure which consisted of familiar food, i.e. ground laboratory diet, placed in a novel container. Here the food pot at the preferred feeding site (i.e. the one most visited during the previous two weeks) was replaced with a novel container resembling the metal drinking font. The other feeding site remained unchanged with familiar food in the familiar feeding pot. The novel container was presented for 10 rather than five consecutive days as little food was consumed during the first five days. The amounts of food consumed from the novel and familiar containers were recorded each day during this period.

Field study

Seven field trials were carried out on farms in the county of Sussex between March and May 1994. Each site was surveyed for signs of rat infestation in and around the farm buildings. A pre-treatment census of the size of the population in the infested area on each farm was carried out by the tracking plate method (Quy *et al.*, 1993). Rodenticide bait, consisting of either 0.05% bromadiolone or 0.05% difenacoum in a variety of grain bases, was then laid in wooden bait boxes throughout the infested area for three consecutive weeks. Each bait box

measured 360mm long x 255mm wide x 125mm high, had an entrance at either end and bait was placed behind a 10mm high baffle to one side of the box. Each box had a galvanised metal lid and initially contained 100g of bait. Between 9 and 32 bait boxes were used at each site. The amounts of bait removed from each bait box were recorded every Wednesday, Friday, and Monday. If all bait was removed from a bait box the amount laid there was doubled. Conversely, if no bait was removed for two consecutive days the amount of bait laid was reduced by 50% to a minimum of 25g. After three consecutive weeks of baiting all bait was removed and a further census undertaken. In the week following the census approximately 50g of rodenticide bait were placed down all rat burrows in the infested area. The number of burrows baited was approximately twice the number of bait boxes used at each site. Baited burrows were again visited every Wednesday, Friday and Monday but for only two rather than three consecutive weeks. Any burrows from which bait had apparently been completely removed were re-baited with 50g of material at each visit. A final census was undertaken during the week after burrow baiting had been completed.

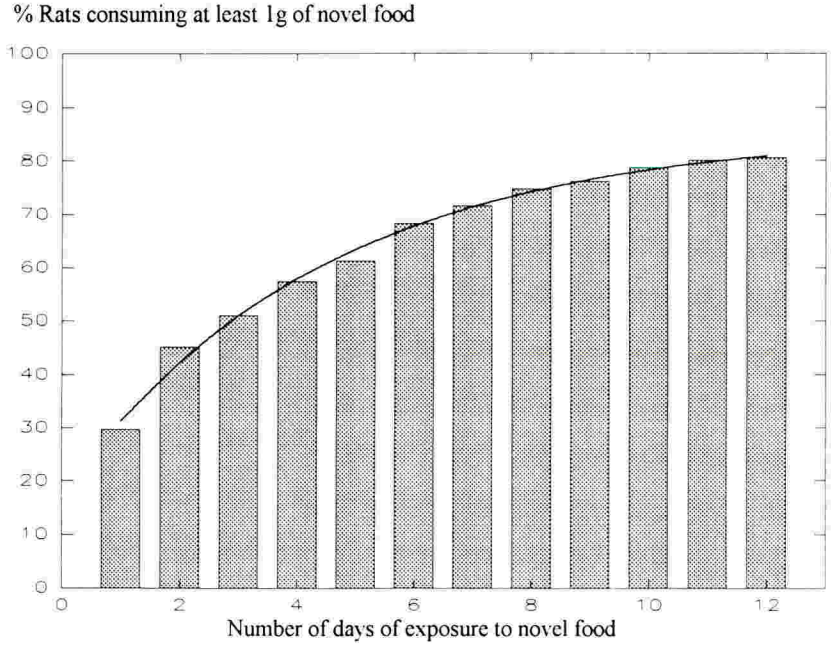

Figure 1. Proportion of wild rats housed in the laboratory eating at least 1g of novel food from a novel container over 12 days of exposure to this food (Exponential curve fitted to the equation $y = 96.7/[1+(x/11.32)^{2.37}]+110.4$; $R^2 = 0.98$).

RESULTS

Laboratory Study

The time taken to first consumption of novel food is shown in Figure 1. There was considerable variation amongst individuals with 29.7% of animals consuming novel food on the first day of exposure contrasting with 19.4% which failed to consume any novel food

during the entire 12 days of exposure. In general, once rats began to consume novel food, their consumption rapidly increased. Thus, novel food formed 47% of the diet on the first day of consumption (for the 125 rats who took at least 1g during the trial), rising to 72% on the second day. Furthermore, novel food formed 81% of the diet by the twelfth day of consumption (for the 45 rats which consumed at least 1g on the first day of exposure). Figure 2 shows the amounts of novel food consumed during the first day of consumption. Again considerable variation is apparent with some rats immediately consuming large quantities of the novel food whilst others adopted a more cautious sampling strategy by consuming relatively small initial amounts.

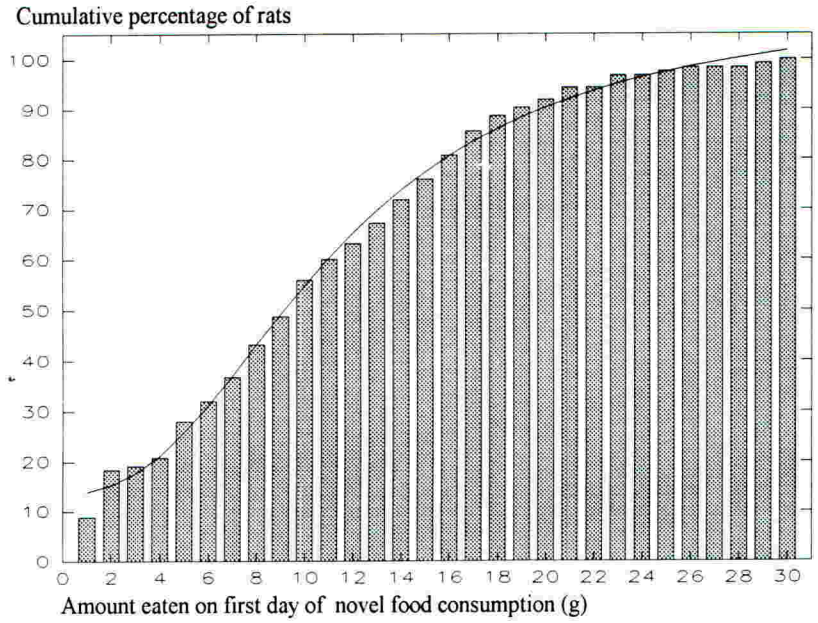

Figure 2. Amounts of novel food eaten from a novel container by 125 wild rats housed in the laboratory during the first 24 hours each individual consumed novel food, presented as the cumulative % of rats eating the given amount or less (Logistic curve fitted for the equation $y = 67.8[1-\exp(-0.23x)]+17.56$; $R^2 = 0.98$).

Arena study

Figure 3 shows that novel food placed in a familiar container was rapidly adopted into the diets of colony rats with only a small increase in the amounts consumed over the five day trial. In contrast, the introduction of familiar food in a novel container led to a substantial delay in consumption such that, even after 10 days of exposure to the novel container, the rats were still obtaining substantially less than 50% of their diet from it.

Field study

Although the estimated number of rats alive on the farms was lower after three weeks of baiting in boxes than in the pre-treatment censuses (Table 1) this difference was not significant

(Wilcoxon matched-pairs Z = 1.52, P = 0.128). The population was apparently eliminated on only one of the seven farms by this time. In contrast, two subsequent weeks of baiting down burrows caused a substantial reduction in mean population size which was significantly lower than that left after baiting in boxes (Wilcoxon matched-pairs Z = 2.20, P = 0.028). Indeed, the population was apparently eliminated for a further five farms, with rats present on only one farm at the end of the trial.

Table 1. Mean estimated numbers of rats present on seven farms before treatments, after three weeks of baiting using bait boxes and after a further two weeks of baiting down burrows (±SE).

Mean number of rats present pre-treatment	Mean number of rats after 3 weeks baiting in boxes	Mean number of rats after 2 weeks of burrow baiting
65.7±12.6	41.7±15.5	1.0±1.0

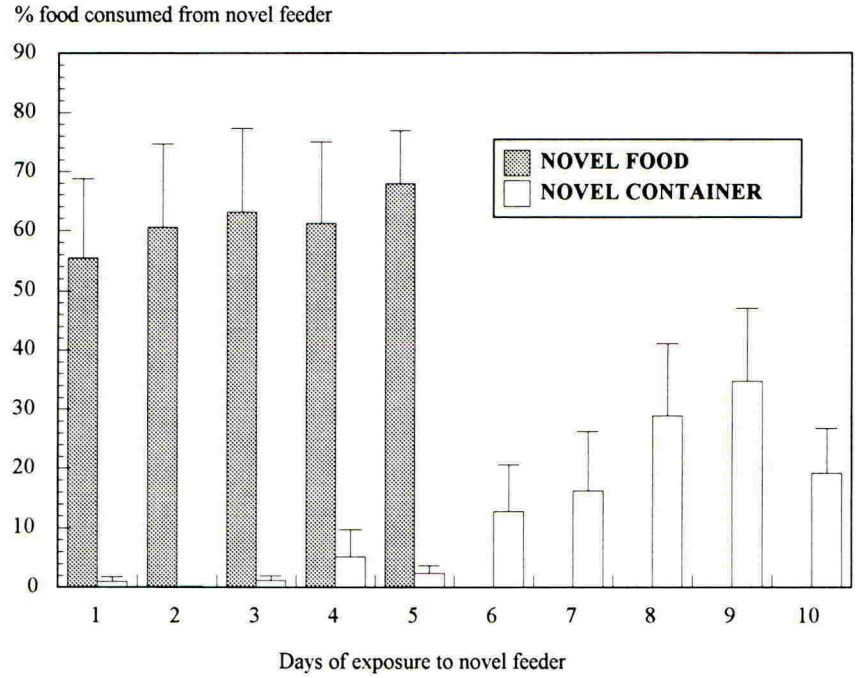

Figure 3. Daily amounts of food consumed from the novel feeder, expressed as a percentage of total food consumption (± SE), when the novel feeder consisted of either novel food in a familiar container (filled bars) or familiar food in a novel container (open bars).

DISCUSSION

The way rats adopt a novel food into their diets can be viewed as a two stage process. Firstly, the time taken for them to begin to sample the food and secondly, the subsequent

change in the amount consumed. The laboratory studies reveal substantial individual variation in both these processes which have implications for practical rodent control. The amounts eaten during the first day of consumption in the laboratory provide insight into the potential for animals to develop learned aversions. In order for an animal to learn to avoid a toxic novel food it has to avoid consuming a lethal dose before the onset of symptoms. The general expectation is thus for sampling small amounts of the novel food and then "monitoring" the consequences (Nott and Sibly, 1993). Clearly many of the rats did not adopt this strategy, consuming large amounts of novel food on the first day any was taken. Such animals would not have the opportunity to develop an aversion that would save them from lethal exposure to rodenticide bait. Other animals, however, were more conservative, eating relatively small initial amounts. These would be more likely to develop an aversion. This individual variation is only recently becoming evident (Nott and Sibly, 1993) being at odds with the conventional view that rats generally adopt a sampling strategy (e.g. Rozin, 1968). If the characteristics of animals displaying different strategies could be understood then baiting strategies might be developed that reduce the likelihood of animals being able to develop aversions prior to consuming lethal doses of rodenticide.

The potential for individuals to develop an aversion will depend on their initial rate of consumption of the novel food, the toxicity of the rodenticide concerned and speed of onset of symptoms. The data can be used to make a prediction for calciferol, a so-called "sub-acute" rodenticide that produces physiological symptoms within 24 hours of consumption and which generates aversions in the laboratory amongst rats exposed to 7.5 mg per kg body weight (Prescott *et al.* 1992). This dose is equivalent to a 250g rat consuming 1.9g of bait containing 0.1% of active material. The LD_{50} for calciferol is in the region of 50mg per kg body weight (Meehan, 1984). This is equivalent to 12.5g of bait consumed by a 250g rat. Using the logistic model fitted to the data in Figure 2 it is apparent that 52% of rats consumed amounts of novel food that would have the potential to generate aversions but less than that required for the LD_{50}. This potential is clearly not realised in practice where calciferol is generally effective (Rennison, 1974). This may partly be due to aversions not being complete, so that animals still consume lethal doses despite reduced calciferol consumption once symptoms onset. Alternatively, the novelty represented by a novel food in a novel container to an animal housed in highly stable laboratory conditions may represent an extreme setting, whereby cautious sampling behaviour is favoured. The natural habitat of the rat is generally much less stable and thus cautious sampling is perhaps less prevalent in the real world. Nevertheless, calciferol is not always successful and some field trials yield poor results that could be interpreted in terms of learned aversion (Rennison, 1974; Brunton *et al.*, 1993). Perhaps the stability of the environment on some farms, which constrains the effectiveness of anticoagulants (Quy *et al.*, 1992), also poses a further problem for materials with the potential to generate aversions, due to initial cautious sampling against a background of stability. Undermining that stability may thus be a particularly important means of enhancing the effectiveness of such materials.

Overcoming aversions to "fast acting" rodenticides, such as zinc phosphide which generates long-lasting aversions after minimal exposure (Shepherd and Inglis, 1993), will require a finer view of feeding behaviour than gross 24 hour consumption rates. Critical information concerns the way "meals" are made up from a number of visits to a food source (e.g. Shepherd and Inglis, 1987; Sibly *et al.*, 1990; Berdoy, 1993) and how these meals are distributed in time (Berdoy and MacDonald, 1991). It is anticipated, however, that delaying the onset of symptoms for a number of hours by using microencapsulation, would substantially

reduce the potential for aversions to develop (e.g. Nadian *et al.*, in press). This approach might usefully be extended to sub-acute rodenticides such as calciferol, particularly if in addition to delaying the onset of symptoms, microencapsulation masked the taste of rodenticide thereby restricting the cues available for aversions to develop.

The common theme running through the laboratory, arena and field studies was neophobia. In the laboratory, some rats had failed to sample novel food from a novel container despite 12 days of effectively continuous exposure. However, here responses to a novel container were not separated from those towards a novel food. That it is the container rather than the food that elicits the strongest expression of neophobia is supported by both the arena and field studies. It could be argued, for the arena study, that because the food in the novel container was familiar there was little incentive for rats to sample it, whilst the novel food, being ultimately more palatable than the familiar food, as demonstrated by both the arena and laboratory studies, offered a greater reward. However, the novel container was placed at the previously preferred feeding site and, since both foods were identical, at least 50% of the food should have been taken from the novel container, which clearly was not the case. Hence, a conservative conclusion is that container neophobia is at least as important as neophobia towards novel food. This view is supported by the field evidence where significant control was generally only achieved after bait was placed down burrows rather than in bait boxes. Perhaps the wider dispersion of baits with burrow baiting and baiting for an additional two weeks contributed to this effect. However, the magnitude of the population reduction once burrows were baited suggests that bait boxes of the type used here constrain effectiveness and, at the very least, lead to longer treatments. There are increasing pressures to use bait containers to reduce putative environmental risks from rodenticide use (Jacobs, 1990). However, those features of bait container design that elicit neophobia are not understood and studies of design factors influencing effectiveness are limited (Kaukeinen, 1987). That the use of bait stations may delay uptake of bait by rodents is acknowledged (Kaukeinen, 1994). However, the extent of container neophobia found here is more than anything previously considered. If such neophobia significantly lengthens treatments then the potential for exposure of non-target species will be increased, perhaps offsetting any enhancement of environmental safety derived from bait container use. Thus, research is urgently required to understand the characteristics of bait containers that generate neophobia and, where possible, eliminate these features from container designs.

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