RESPONDING TO POTENTIAL AND PERCEIVED CASES OF RESISTANCE IN *BLATTELLA GERMANICA* (L.)

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Abstract—German cockroach (*Blattella germanica*) control treatment history has rarely been accompanied by resistance monitoring in the field. Consequently, while cockroach "resistance", receives much publicity, it is a fact that, to date, only a handful of complaints that are due to pyrethroid resistance, have been received. As a result, few sites have been studied for long since any form of management was initiated, so the virtues of theoretically sound resistance management strategies remain unproven, in practice.

Where resistance is suspected, or reported, the first step is to confirm the diagnosis. A series of questionnaires and flow charts have been prepared to address the possibility of operational factors, such as poor application, being responsible for control failure. Test kits are then used to quantify the resistance. If confirmed as product failure, the best "fire-fighting" advice is to switch to an alternative class of active ingredient (AI).

Monitoring any changes in the susceptibility profile of *B. germanica*, in the field, is an essential component of any resistance management strategy. Test kits can provide a measure of phenotypic, physiological tolerance, however the mechanisms that underly resistance, and the possible influence of cockroach behaviour upon the likelihood, frequency and duration of contact with control agents are also extremely important, although they may be more difficult to quantify.

At present, the jury has insufficient data to decide in favour of either rotation or mixture, the two most popular approaches to resistance management, as the "best strategy" for cockroach control. Instead, the Pest control operator (PCO) is best served by having a range of modes of action (chemical, physical and biological) and presentations (eg. residuals, baits) of control agents at his disposal. The key to success lies in implementing pre-emptive management strategies, instead of waiting until control failure occurs. It is necessary to provide the customer with the combination of a varied range of products with technical assistance and advice for their use. This advice extends to non-chemical measures such as general hygiene and the use of monitoring traps to help target treatments in space and time.

INTRODUCTION

There are many "definitions" of resistance, including reference to genetic changes and more practically oriented descriptions where the recommended rate of a product fails to control a population. For the purposes of this paper, the main objective is to address ways and means whereby cockroach control treatments will continue to provide the customer with the desired effect. In doing so, it will be essential to put insecticide resistance among cockroaches into perspective, in terms of the extent of the problem, the potential for it to develop further and the options for minimising its impact.

Tolerance and resistance

It is useful to consider four grades of tolerance along the path to resistance (Figure 1). At the top of the scale are the laboratory reference strains that have been safely closeted away with food, water, harbourages and mates. In the absence of insecticides, immigrants, diseases or any stress that might occur with limited resources, these strains are probably not very streetwise. Out in the field, some strains that have not been exposed to insecticides may still be more tolerant than the laboratory strains simply by virtue of being a little tougher or fitter in a very general sense. Differences in body weight, or changes in nutritional status, for example, may alter insecticide tolerance (Kramer *et al*, 1990).

Once exposed to the presence of insecticides, we assume that natural selection resulted in populations becoming more tolerant through the gradual increase in the frequency of genes that conferred some level of resistance, although the frequency of resistant homozygotes probably remained at a very low level for some time, depending upon such factors as application frequency, chemicals used and immigration rates. Nevertheless, the population remained controllable. Eventually, biological factors such as resistance gene frequency and/or operational factors such as

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K.B. Wildey and Wm H.Robinson (editors). 1993

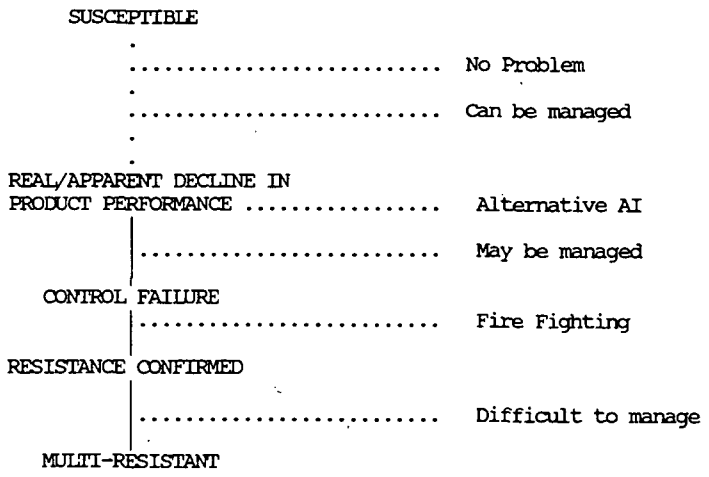

Figure 1. The progressive towards resistance

poor application, resulted in a perceived decline in product performance. Subsequently, the control problem could be tackled in a "fire-fighting" sense, with perhaps a change of product or more frequent applications until the population was no longer controllable due, almost certainly, to multi-resistance. At some point in that progression, the problem passes from readily manageable to almost unmanageable. Clearly, by recognising the likelihood of resistance developing against a background of known treatment history, we should aim to plan control measures to maintain tolerance within the manageable range, and pre-empt product failure.

Resistance mechanisms

Rational resistance management is based upon a knowledge of either the underlying mechanisms or the likelihood of specific mechanisms being present. By examining the journey of an insecticide from spray tank to target site (Figure 2), the potential for resistance can be identified on the basis that a selective advantage will accompany anything that favours survival to reproduce.

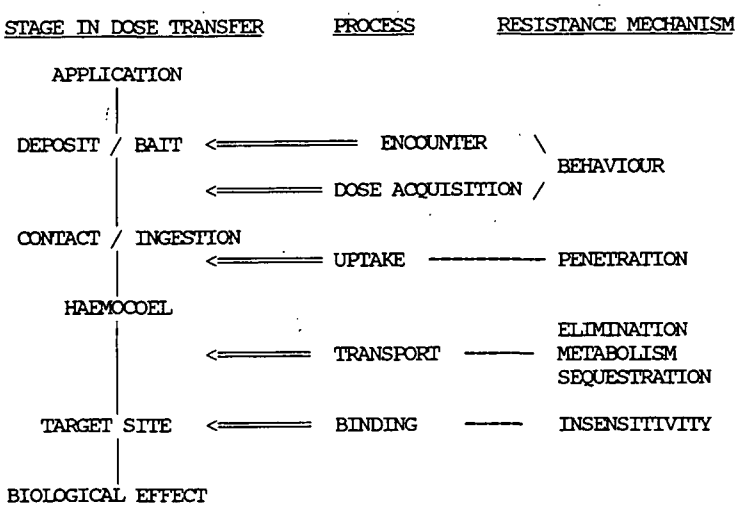

Figure 2. Possible Resistance Mechanism

First, dose acquisition could be minimised by changes in behaviour. Shorter periods of foraging and increased sensitivity to insecticides (culminating in avoidance or repellency) could reduce the exposure period and increase the time between successive exposures such that detoxification via metabolism could occur. Some elements of insecticide formulations are known to affect cockroach behaviour (Ross, 1992; Ross and Cochran, 1992).

In some cases, insecticide vapours may be toxic (Schal, 1992), however, the dose is usually acquired via tarsal contact with residual deposits, or ingestion of baits. In addition to behavioural factors, anything that reduces the penetration rate will be advantageous since the insect will effectively buy more time to metabolise, eliminate or sequester the toxicant.

Having entered the body, the insecticide molecules are subjected to an array of metabolic defences. Consequently, enhanced activities of enzymes such as oxidases and esterases, or the capacity to increase their production, will be favoured. Similarly, there could be selection in favour of the loss of enzymatic activation of insecticides (eg. epoxidation of aldrin, and desulphuration of parathion and diazinon).

Finally, modifications of the target site may reduce its sensitivity to insecticide molecules via, for example, qualitative or quantitative changes in the sodium channel (pyrethroid resistance, "kdr"), or altered acetylcholinesterase (AChE; carbamate and organophosphate resistance).

It is comparatively quick and easy to quantify the tolerance of cockroaches to insecticides using methods such as topical application, or forced exposure to deposits. Determining the underlying mechanisms is possible, but can be very time consuming and inconclusive. Establishing the influence of behavioural factors is often overlooked. Similarly, biological consequences of exposure to insecticides, other than physiological tolerance, are rarely investigated. However, the significance of any changes in the biological fitness of a population, due to insecticide use, should not be underestimated when resistance management strategies are being proposed.

Extent of resistance

Recent reports of pyrethroid resistance at some sites follow previous reports of resistance to other classes of active ingredient (AI). The Village Green strain, from Florida, exhibited factors of resistance (FOR) of x29 - x337 to a range of 10 pyrethroids when field failure of cypermethrin (after several years of continuous use) was reported in 1987 (Atkinson *et al*, 1991). The authors noted that "...increased doses failed to suppress the population." and it is pertinent to mention that the strategy of "if it fails, try a little more" has a very limited future. A similar case history was reported by Robinson and Zhai (1990) at apartments in Roanoke, Virginia, when 4 years exclusive use of cypermethrin culminated in field failure in 1989-90, and a return to chlorpyrifos soon suffered the same fate.

Cockroach control failures in California have been monitored at UC Riverside, in parallel with studies on cockroaches collected from restaurants without regard to any history of control failure. Reierson *et al* (1988) concluded that an FOR of x10 was likely to cause control failure. All 45 strains from control failure sites, as well as over half of those from randomly selected sites had an FOR x10 to chlorpyrifos (Rust and Reierson, 1991). Another survey of strains from across the USA concluded that resistance was apparent to a wide range of insecticides, but that it was possible to choose an effective product for each strain (Cochran, 1989).

Resistance surveys are less extensive outside the USA. It is debateable whether this is due to the comparative paucity of control failures, or the lack of resources to study them. In Australia, for example, a recent report of a *B. germanica* population with 20-fold resistance to deltamethrin was only the second confirmed case of resistance to any insecticide in that country (Horwood *et al.*).

Further evidence that pyrethroid failures are currently the exception, and not the rule, is evident from our records over the last 5 years: only 5 cases of resistance, with an FOR to a pyrethroid of x10, have been demonstrated among thousands of treatments.

Monitoring

Test kits can be used to evaluate and compare tolerance to insecticides in the field. Theoretically, these kits could be used to help choose a product that would control the infestation. In practice,

Control failure is identified by numbers of cockroaches still alive several days after treatment or rapid build up of numbers after treatment.

If possible quantify problem: What are cockroach numbers pre and post-treatments. Use sticky traps to assess if the problem is real or perceived

FIRST ACTIONS: Check product visually, appearance, mixing, suspends OK?

Shelf life OK?

Check application technique

Product mixed and diluted according to label recommendations?

SECOND ACTIONS: **If all OK proceed**

Re-treat premises under trained staff/territory staff supervision ensuring dilution and application is correct

Successful: do staff need more training or supervison

Control failure continues

Send sample of product for check to RUEH, Berkhamsted

Product meets specification

Give the following details:

* History of previous treatments,
* Degree of failure,
* Situation of premises and surroundings,(immigration potentional),
* Control problem isolated or widespread.
* Species present.

Product out of specification

If within storage life:

How has it been stored etc?

RUEH will supply advice and/or arrange Resistance Kit testing

Tests conducted and results sent to RUEH for comment.

Resistance proved
Strategy advice given

No resistance
Advice given if product problems exist

Figure 3. Cockroach control, failure questionnaire

they are most likely to be used retrospectively, as part of an enquiry to decide whether poor control was due to resistance, or operational factors. Roussel Uclaf Environmental Health (RUEH) has developed test kits for representative pyrethroid, organophosphate and carbamate insecticides. These kits are simple to use, and can be sent by air mail to assist the investigations of local staff as a component of a troubleshooting questionnaire (Figure 3). A sample of insects is captured (baited jars or Roatel traps), exposed to the deposit for 10 minutes, and mortality assessed the next day. If resistance is confirmed (FOR x10 is the rule of thumb, based on the studies of Reierson et al., (1988) although there is little information available upon which to judge pyrethroid failures), then the recommendation is to use a different class of AI, preferably one that has not been used previously to control that population. An integral part of the procedure is to advise and make recommendations on building maintenance and sanitation, and not to rely entirely on chemical control measures. Wherever possible, the insecticide use history of the infestation is recorded - unfortunately, this tends to be anecdotal in many areas and it is one key area where more conscientious documentation of chemical use is likely to become invaluable if the best, informed recommendations are going to be tailored to specific sites.

An alternative test kit, based on a glue toxin sticky trap, has been developed by Moss *et al* (1992). As with normal sticky traps, they can be placed in the infested area for a short period (one night) and mortality of captured cockroaches will give a measure of tolerance within 2 days by including a discriminating dose of toxin in the glue.

Management strategies

"Use [organophosphates] continuously, and you could create a monster. In most populations, some cockroaches are already resistant to [organophosphates]. With each [organophosphate] treatment, susceptible cockroaches are eliminated, and resistant cockroaches breed and pass on resistance to their offspring. After many [organophosphate] treatments, more and more resistant cockroaches survive. Control failures are bound to happen. ..... Pyrethroids are a valuable tool in cockroach control." That is the preamble to one management strategy, although I have taken the liberty of substituting organophosphate for pyrethroid, and it could be carbamate or cyclodiene for that matter. But the message is the same: continuous use of anything is likely to result in control failure sooner or later, and insecticides are weapons that are best used in the right way, in the right place at the right time.

There has been considerable discussion of two main strategies for cockroach resistance management: rotation (= alternation) involves a planned sequence of applying one product and then another that has a different mode of action; and mixtures where two or more AIs are applied at the same time. Amid an abundance of papers and reports on the theoretical merits of different strategies, there is a dearth of hard evidence to demonstrate that any of them succeed in the field. It is also unfortunate that management strategies tend to be directed towards sites where resistance is already a problem, rather than where its appearance could be delayed, or prevented.

As the basis for a brief summary of the subject area, I have chosen a recent review by Denholm and Rowland (1992). In essence, a rotation strategy aims to delay resistance, or check its progress, by "...restricting the period of exposure to each selecting agent...on the assumption that frequencies of resistance to each compound decline in the absence of the selector because of dilution by immigration of susceptible homozygotes, decreased fitness of resistant insects, or both." Mixtures have a distinct advantage over rotations, at least in theory, under certain conditions, unless the fitness costs are very large. However, these conditions are very stringent: the insecticides should be equally persistent, resistance genes must be rare, there must not be a common resistance mechanism, the insecticides must be applied at a heterozygote killing rate (in effect the full label rate for each product) and immigration from untreated areas should be possible. An additional virtue of mixtures is the potential to control either different life stages of the same pest, or different species in a pest complex, with a single application. Alternatively, a mixture that contained one persistent and one non-persistent component could offer the logistical advantage of a single spray with a minimal temporal overlap of residues (i.e. almost a rotation). Where these conditions are not met, neither strategy is likely to have much advantage over the other since genes conferring some form of metabolic resistance may act against a range of chemical classes (albeit at different levels of

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K.B. Wildey and Wm H.Robinson (editors). 1993

effectiveness), which is contrary to the mixture strategy. Also, the fitness costs may be small, so rotation may have little practical benefit in terms of "restoring" susceptibility to a resisted compound. However, since 1983, a rotation strategy has successfully delayed the development of pyrethroid resistance in *Heliothis armigera* on cotton in Australia as a result of an area-wide cooperation (Forrester and Fitt, 1992).

Populations of *B. germanica* are contagiously distributed (Appel and Reid, 1992) and mark-release-recapture studies in apartment blocks suggest that movement rates between adjacent apartments may be quite low, even in the presence of common plumbing and ducting (Runstrom and Bennett, 1990). Thus, in many cases, infestations may be quite isolated, with little possible immigration of susceptible individuals. Clearly, this would reduce the likelihood of diluting the frequency of resistant genes, and further emphasises the need to consider each infestation on its own merits and in the light of a specific treatment history.

Ideally, resistance management starts before control failure although, in practice, there are many logistical hurdles to overcome such as the benefits of buying in bulk and the understandable tendency to stick with a product of proven efficacy. Nevertheless, an examination of the characteristics of resistance may help to pre-empt the problem in other populations. These characteristics may be broadly divided into two categories: fitness and mechanisms.

Biological fitness

The comparative tolerance of *B. germanica* strains to insecticides has rarely been accompanied by detailed information on the biological consequence of resistance. Recent studies on two strains with high levels of resistance to pyrethrins and allethrins have provided some evidence of substantial fitness costs associated with resistance, including fewer adult progeny from successive oothecae, longer nymphal development and fewer viable oothecae, but a third strain showed similar life history characteristics to the susceptible strains (Ross, 1991). In the absence of selection pressure, any reduction in fitness would be disadvantageous, so that resistant genes would be diluted over subsequent generations. The immigration of susceptible individuals would accelerate this process.

Unfortunately, reports of "slow developing" field strains, or declining resistance factors have rarely been monitored in any detail, and neither has the correlation, if any, with the treatment history or the prevailing resistance mechanisms. However, there are reports that resistance does not diminish markedly when field strains are reared in the laboratory for several generations (eg. Atkinson *et al,* 1991). Similarly, a laboratory selected (with pyrethroids) strain, Ectiban-R (kdr is the only resistance mechanism) is reported to retain its resistance in the absence of selection pressure, while the parental strain, VPIDLS (selected using DDT, and also exhibiting kdr-type resistance) reverts to susceptibility in the absence of selection pressure. A strain in culture in our laboratories shows pyrethroid resistance that had not declined after 9 generations. Table 1 shows that DDT was cross-resisted, but not other classes of AI, and metabolic inhibitors had little effect, suggesting that a kdr-type mechanism was primarily responsible for the tolerance of this strain. No biological fitness costs were apparent. Conversely, in another strain, a higher FOR to pyrethroids was present upon collection from the field, but this declined to around x5 in 3-5 generations. But, we were unable to identify the resistance spectrum or mechanisms, due to problems rearing those insects, until the FOR was already low.

Resistance mechanisms

Potential resistance mechanisms were addressed in Figure 2. To date, behavioural resistance has received most attention with respect to the performance of baits where the ingestion of sublethal doses and/or the changing palatability of the bait in time were suggested as possible explanations when a decline in performance was noted (Reierson and Rust, 1992). Altering the composition of the bait matrix can restore efficacy (Silverman, unpublished), although physiological resistance to sulfluramid has been reported (Schal, 1992).

Penetration studies have shown no difference between resistant and susceptible strains in the rate of penetration of DDT (Hooper, 1969), carbaryl and malathion (Bull *et al,* 1989) or chlorpyrifos (Siegfried *et al,* 1990). However, reduced cuticular penetration was demonstrated in a pyrethroid

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Table 1. Comparative tolerance of susceptible and resistant *B. germanica* strains to different insecticides with and without synergists.

Insecticide	LD50 (ng/male +/- 95% fiducial limits) RUEH-S strain	RUEH-R	FOR[a]
DDT	18,800. (11 - 32,000)	>400,000.	>20
Chlorpyrifos ethyl	680. (610 - 770)	680. (510 - 1040)	1
Bendiocarb	390. (340 - 450)	970. (780 - 1240)	2.5
Deltamethrin	16. (12 - 21)	148. (105 - 208)	9
DTM + PBO[b]	1.7 (1.2 - 2.4)	27. (21 - 36)	16
DTM + DEF[c]	>12.5	78. (54 - 111)	-
Nymphs/ootheca	43	37	
Hatch - adult (days)	40	45	

[a] Factor of resistance (LD50 RUEH-R / RUEH-S).
[b] 75ug PBO/male
[c] 30ug DEF/male

resistant strain, although nerve insensitivity was considered to be the most important mechanism (Bull and Patterson, 1993).

Topical application of the synergists (metabolic inhibitors) piperonyl butoxide (PBO, a microsomal monooxygenase inhibitor) and s,s,s-tributyl phosphorothioate (DEF, an esterase inhibitor) was associated with around a two-fold reduction in the FOR to permethrin and cypermethrin of a pyrethroid-resistant strain (Atkinson *et al,* 1991). However, the FOR values remained at x30 - x40, demonstrating that there were other significant contributions to the resistance of that strain. In another study, synergists enhanced the activity of bendiocarb in several bendiocarb-resistant strains (but did not eliminate resistance), but resistance to pyrethrins was totally overcome by two oxidase inhibitors (Cochran, 1987). The latter case is an exception to the general picture of multifactorial (but rarely fully characterised) resistance.

Examination of a pyrethroid resistant strain showed no synergistic effect of PBO, but NIA 16388 (an oxidase and esterase inhibitor) reduced the FOR to permethrin from x60 to x8. Electrophysiology studies also demonstrated nerve insensitivity (kdr), possibly via modification of the sodium channels (Umeda et al, 1988). Similarly, Dong and Scott (1991) concluded that the kdr-type resistance in the Ectiban-R strain was due to a qualitative change in the sodium channels.

Extensive studies on the mechanisms responsible for organophosphate and carbamate resistance have been conducted by Scott and co-workers. They concluded that insensitivity of AChE was not an important factor (Siegfried and Scott, 1990). Instead, a combination of increased oxidative and hydrolytic metabolism was implicated, and there were heritable differences in the mechanisms responsible for resistance to propoxur and chlorpyrifos (Siegfried *et al,* 1990; Siegfried and Scott, 1992).

In summary, there is no evidence of penetration resistance in *B. germanica*. Target site insensitivity (kdr) has been implicated in the resistance profile of some pyrethroid-resistant strains, but metabolism may also contribute. Metabolic resistance is usually multifactorial, with subtle differences between the precise nature of the mechanisms underlying tolerance to different compounds. The addition of PBO to formulations, or via a tank mix, is the only commercially available option for using a synergist to overcome resistance, while continuing to use the tolerated compound. This has been very successful, linked with a rotation strategy, in Australia where the progress of pyrethroid resistance in cotton bollworms has been slowed over the past decade, and where the predominant resistance mechanism is mediated by mixed function oxidases (Forrester and Fitt, 1991). However, if different metabolic defences (eg. esterases, hydrolases) or kdr are responsible for resistance, then PBO will be ineffective.

Attempting to prevent and manage resistance

There is a tendency to focus management strategies on rotating or mixing two residual insecticide products when control failure is present or imminent. Although these are convenient options, both

in terms of the application equipment and methods required, they fail to exploit the multi-dimensional array of cockroach control products that are available. Figure 4 shows that changing chemical class is just one of a host of options for controlling cockroaches. The probability of encounter with an AI can be altered by changing application method from broadcast to crack and crevice. Further dimensions can be added by using baits that not only confine the AI to concentrated patches, but require an alternative method of dose acquisition and become depleted, rather than decaying in the manner of a residual deposit. Inorganic compounds such as silica dusts are effective by physical rather than chemical action, and act on the cuticle instead of inside the body. Biological agents will become subject to totally different defence mechanisms (eg. haemocytes) than the metabolic degradation of chemicals.

Figure 4. Multi-dimensional aspects of cockroach control products

Distribution	Dose Transfer	Chemical Class	Presentation	Persistence	Decline
Continuous	Contact	Pyrethroid	Broadcast	>90 Days	
		Pyrethrins			Decay
		Carbamate	Crack		
		OP	and		
	Ingest	Cyclodiene	Crevice	30–90 Days	
		IGR			Degrade
		Sulfluramid	Bait		
		Hydramethylnon		7–30 Days	
	Inhale	Abamectin	Space		
		Biological	Spray		Deplete
Patchy		Inorganic		<7 Days	

CONCLUSIONS

There is little information available on the relationships between treatment history, evolution of resistance mechanisms, and the consequent resistance factors/spectrum. These critical gaps in our knowledge mean that advice is, inevitably, based upon educated guesses, unproven theory, product cost, ease of applicability or vested interests. However, there is little doubt that instigating insecticide use strategies before control failure occurs is advisable. Since continuous use of any product is the fastest route to resistance, it follows that a pre-emptive, planned approach to varying the mode of action and presentation of control agents is a sound investment.

Once the control tactic varies from repeated use of one product until real or apparent failure, there is the potential to utilise a varied approach, whereby a range of modes of action, presentations, and availability in space and time can be exploited. The RUEH cockroach control product range matches this need for breadth and variety, and is supported by technical advice and guidance to assist the customer to plan and project the use of the control agents. We believe that the planned use of a multidimensional approach will keep resistance on the run before it causes control failure.

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