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Control of *Solenopsis invicta* with Delayed-action Fluorinated Toxicants[a]

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The fire ant, *Solenopsis invicta* Buren, was accidentally imported into the southern United States and has become a serious medical and agricultural pest of nine south-eastern states and Puerto Rico. For a bait toxicant to be effective in fire ant control it must have delayed activity over a wide range of concentrations. Less than 1% of the 7500 compounds screened since 1958 has had delayed action. This paper describes several classes of fluorinated compounds that exhibit delayed activity. These compounds are used to illustrate the following three approaches to the discovery of delayed-action toxicants: (a) random screening of compounds for delayed action; (b) design of delayed activity through the synthesis of pro-insecticides; and (c) serendipity. As a result of these investigations two new classes of insecticides were discovered. A compound from one of these classes (tetrahydro-5,5-dimethyl-2(1*H*)-pyrimidinone (3-(4-(trifluoromethyl)phenyl)-1-(2-(4-(trifluoromethyl)phenyl)-ethynyl)-2-propenyl-idene) hydrazone, has been commercialised, while one or more compounds from the second new chemical class (fluorinated sulphonamides) are under development. Fluorinated delayed-action insecticides have made significant contributions to current fire ant control and will no doubt play a role in the future.

1. Introduction

1.1. Distribution

Fire ant control with toxic baits has had a long and interesting history because of the need to consider social insect ecology and physiology, as well as the specific peculiarities of these pests. Although there are indigenous fire ant species in the United States, it is the South American species, *Solenopsis invicta* Buren, that poses the greatest medical and agricultural problems. This species was introduced accidentally into the Mobile, Alabama area of the United States from Brazil in the nineteen-thirties. An inordinate rapid spread in the southern United States was attributed to their high reproductive capacity and opportunistic behaviour, which enabled them to stow away in the soil of commercially distributed ornamental plants. Currently, *S. invicta* infests nine states (Alabama, Arkansas, Georgia, Florida, Louisiana, North Carolina, South Carolina and Texas) and the Commonwealth of Puerto Rico.[1,2] Its lack of cold hardiness has limited its spread to the north, whilst westward movement has slowed considerably due to the arid climate and mountains of west Texas and New Mexico. Strict quarantine measures have helped to prevent a West Coast invasion. However, in terms of suitable habitat and climate, further spread of *S. invicta* in the United States could extend along and up the entire West Coast.

1.2. Economic and medical problems

S. invicta presents a two-part problem in the United States. (a) Its highly aggressive behaviour when nests are disturbed can result in painful stings to humans and their pets. Approximately 30% of the

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population in infested areas are stung each year and about 1% of these are hypersensitive to ant venom or require some type of medical care. It has been estimated that 0.6% of the people stung suffer from systemic anaphylaxis, which means that 75 000 persons per year may require the aid of a physician.[3] (b) *S. invicta* adversely affects the yields of several important agricultural crops, such as soya beans, corn, potatoes, eggplant and okra.[3] Recent studies have demonstrated serious fire ant damage to young citrus trees in Florida.[3] However, when fire ants attack plant or animal pests its predatory nature becomes beneficial.[4] Thus, an overall view of fire ants must balance the nature of the ant as a pest versus its potential benefits.

1.3. The bait toxicant concept

Initially, heptachlor and dieldrin were used for long term residual control of fire ants; however, in 1962 a bait formulation of mirex was developed.[5] The bait concept, which incorporated a delayed-action insecticide formulated in a food attractant, is exemplified by mirex and was an important step in eliminating the use of residual insecticides, decreasing the amount of chemical applied to the environment, and targeting the pesticide so that fewer non-target organisms were affected. It also defined the physical properties and toxic characteristics of future fire ant toxicants.

A typical fire ant mound may contain up to 150 000 adults. At any given time only a small proportion of these adults actually leave the subterranean protection of the mound.[1] It is these food gathering workers that first come in contact with the bait-formulated insecticide. They carry it to nest-mates or ingest and store the material in their communal crop. If the insecticide is too fast-acting, the foraging workers die before they have a chance to bring the bait back to the nest.

This behaviour requires that a bait-formulated toxicant exhibits delayed toxicity. In addition, foraging workers regurgitate food and the toxicant to other members of the colony via trophallaxis, so that within several hours the material is passed to the majority of immature and adult ants in the colony, including the queen. During this process the toxicant is diluted; therefore, a bait-formulated toxicant must exhibit delayed action over a wide range of concentrations. Other important factors are that the toxicant must be (a) soluble in the food attractant (most commonly soya bean oil); (b) acceptable to the ants (some toxicants are repellent); and (c) environmentally acceptable.[6]

1.4. Need for new toxicants

There are many compounds that kill fire ants, but very few that have delayed activity over a range of concentrations. Mirex appeared to be the perfect solution. It dissolved readily in soya bean oil, was acceptable to the ants, had excellent delayed toxicity properties, and was inexpensive.[5] However, after 15 years of use and applications to 140 million acres, the United States Environmental Protection Agency determined that mirex presented an environmental hazard and cancelled all registrations in 1978.[7] This eliminated the only effective bait for fire ant control and generated an urgent need for environmentally safe replacement toxicants.

2. Experimental methods

2.1. Primary bioassay

To screen the multitude of possible compounds for delayed toxicity effectively, the following primary bioassay procedure was developed. Test compounds were evaluated under controlled conditions using worker ants isolated in plastic cups. They were fed the candidate toxicant dissolved in the soya bean oil food attractant/solvent. The compounds were initially evaluated at a 1% concentration; if >85% mortality occurred the compound was tested at three concentrations (1.0, 0.1, and 0.01% by wt). To expedite evaluation of primary screening results a classification system was devised: class 1, inactive; class 2, kills too fast and classes 3–5 represent increasing degrees of delayed activity and toxicity.[6]

2.2. Colony and field bioassays

If a compound exhibits delayed toxicity over a 10-fold or greater range of concentrations (classes 4,

5) it was evaluated in a second bioassay against laboratory colonies. In this test the compound was fed on a one-time basis (48–96 h) to queenright colonies in the laboratory. The mortality in the test colonies was monitored until the colony died or recovered. If repeated laboratory tests of the bait killed the ant colonies reproducibly then the material was tested against natural field populations.[6]

Field trials of bait-formulated toxicants were the most labour intensive and expensive part of the evaluation procedure. The toxicant was formulated in soya bean oil, absorbed on to a carrier (corn-cob grits or pregel defatted corn grits), and delivered to the bioassay area at the prescribed rate via a tractor- or aircraft-mounted granular applicator. Effectiveness was determined from comparisons of the number of active fire ant nests before and 6 to 24 weeks after treatment.[6]

3. Results and discussion

Most of the 7500+ chemicals that have been bioassayed were inactive, and less than 1% had satisfactory delayed activity (class 4 and 5). The one class 5 compound was mirex and most of the class 4 compounds were closely related to mirex, including kepone.

3.1. The random screening method

Among the class 4 compounds discovered in the general screening programme was American Cyanamid's trifluoromethyl-substituted amidinohydrazone (**I**, Figure 1), tetrahydro-5,5-dimethyl-2(1*H*) - pyrimidinone(3 - (4 - (trifluoromethyl)phenyl) - 1 - (2 - (4 - (trifluoromethyl)phenyl)ethenyl) -2-propenylidene) hydrazone. This compound is now the lead representative of a new chemical class of insecticides.[8]

Of the many amidinohydrazone derivatives tested compound **I** gave the most consistent delayed activity over a range of concentrations in the primary bioassay (class 4) and in laboratory colony tests **I** was toxic to workers and brood. In all cases the queen died within four weeks.[9] Initial fields trials, however, were discouraging and almost led to the demise of the compound as a fire ant bait toxicant. Subsequently, a great deal of effort was expended in modifying and testing formulations to overcome apparent inadequacies. These included increasing the concentration of **I** in soya bean oil (using oleic acid as a cosolvent) and doubling the amount of soya bean oil absorbed on to the carrier by switching from corn-cob grits to pregel defatted corn grits.[9,10] The end result was a dramatic increase in the control of field populations (from *ca* 34% to 88% mound reduction).[10,11] Compound **I**, which was on the brink of extinction, was until recently the only bait-formulated toxicant registered by the EPA for fire ant control (marketed as 'Amdro' by American Cyanamid).

3.2. Controlled-release systems

There are several potential methods of modifying or formulating fast-acting toxicants into delayed-action toxicants or formulations.[12] Microencapsulated and matrix-bound fast-acting toxicants have

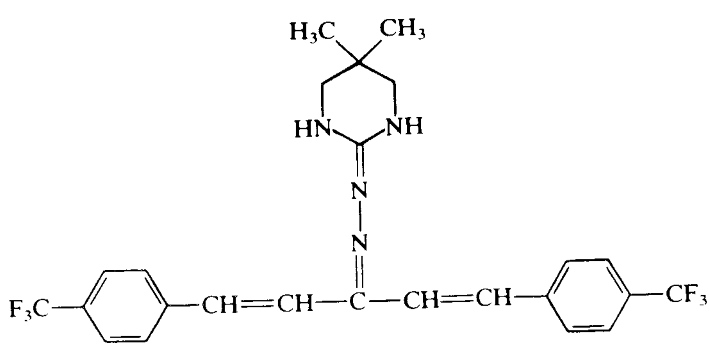

Figure 1. Chemical structure of tetrahydro-5,5-dimethyl-2(1*H*)-pyrimidinone(3-(4-trifluoromethylphenyl)-1-(2-(4-trifluoromethyl(phenyl)ethenyl)-2-propenylidene)hydrazone (**I**) (Amdro).

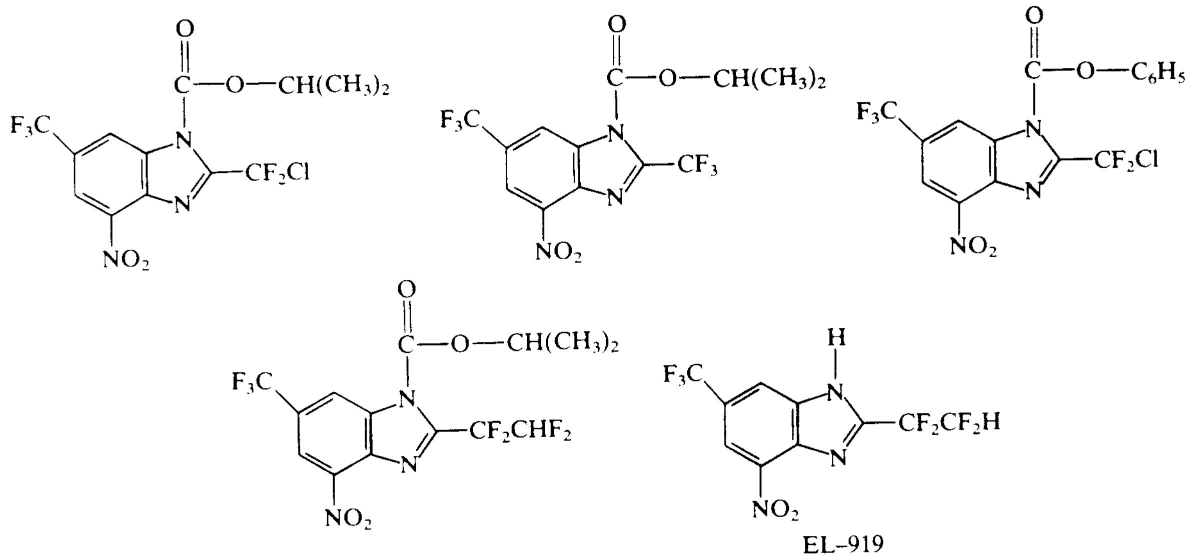

Figure 2. 4-Nitro-6-trifluoromethyl benzimidazole derivatives found to be fast-acting fire ant insecticides.

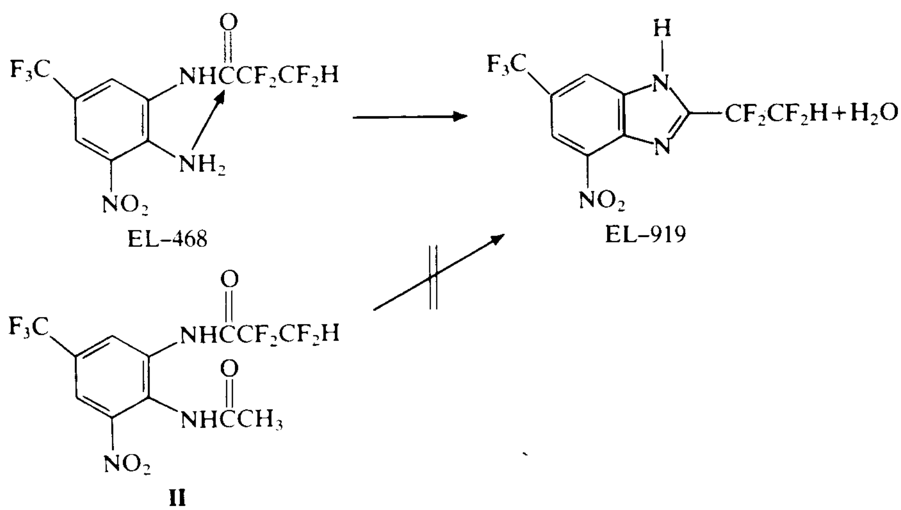

Figure 3. Dehydration of EL-468 to highly toxic EL-919. Compound **II** cannot undergo this dehydration and is non-toxic to fire ants.

been unsuccessful with fire ants, because worker ants injest only liquids (particles >0.9 μm in diameter are filtered out in their pharynx).[13] More pertinent to this discussion is the protoxicant approach, where the material presented to the ants has little toxicity, but is made toxic after ingestion by the natural chemical action of the insect's metabolism.

3.2.1. Pro-insecticides

One example of this approach is exemplified by a series of fluorinated benzimidazole derivatives (Figure 2) prepared by Eli Lilly and Company. All of these compounds had class 2 activity; i.e., they had excellent toxic properties, but no delayed action. EL-919 and most of the others shown in Figure 2 gave 100% mortality after 24 h. The precursor in the synthesis of EL-919 is the phenylenediamine, EL-468 (*N*-(2-amino-3-nitro-5-(trifluoromethyl)phenyl)-2,2,3,3-tetrafluoropropanamide) (Figure 3). The dehydration of EL-468 to EL-919 readily occurs in polar aprotic solvents. Therefore, depending on the inate toxicity of EL-468, this is an ideal situation for the in vivo conversion of a weak or non-toxic compound (EL-468) into a potent toxicant (EL-919).

It was not possible to measure the inate toxicity of a compound as labile as EL-468. However, analogues of EL-468 (**II**, Figure 3) that cannot dehydrate and cyclise to the toxic benzimidazole, were totally non-toxic. This provided indirect evidence of the non-toxic nature of EL-468.

Primary screening results for EL-468 showed excellent class 4 activity, and in laboratory colony tests the compound gave high worker mortality and reproducible whole colony kill. Field test results for EL-468 were also excellent[14] and it appeared that EL-468 would join **I** in the market place for control of the fire ant. Unfortunately, late in the EPA registration process potential teratogenicity problems were detected and Eli Lilly decided to withdraw EL-468 from further EPA and commercial consideration.

3.2.2. Pro-insecticides by design

Kochansky and co-workers[15] designed and synthesised delayed-action toxicants for fire ant control based on the pro-toxicant approach. Their hypothesis was that sodium fluoroacetate, a potent fire-ant toxicant with no delayed action (Table 1) would be inactive when esterified to naturally occurring lipids; however, the ester linkage would be readily hydrolysed within the ant, releasing the highly toxic fluoroacetic acid.

The results (Table 1) illustrate the ameliorating effects of derivatising sodium fluoroacetate with 2 sterols and a fatty alcohol. Cholesteryl fluoroacetate showed good delayed activity at 1% as did sitosteryl fluoroacetate; however, delayed action was not observed at any other concentrations. Octadecyl fluoroacetate gave too fast an action or at lower concentrations not enough activity, although delayed action was suggested by the 0.1% concentration data. These efforts did not result in a commercial product; however, they did illustrate the potential of the pro-insecticide approach to delayed-action toxicants for fire ant control.

3.3. Serendipity

The serendipity approach developed from a collaborative project on pendant toxicants (a variant of pro-toxicants) beween this USDA laboratory and the Southern Research Institute, Birmingham, Alabama.[16] The first major problem was to find an insecticide with suitable function to bond chemically to a polymer backbone. Several years ago, random screening showed up several fluori-

Table 1. Toxicity of fluoroacetyl derivatives when fed in soya bean oil to fire ant workers[15]

Compound	Concn. (%)	Mortality (%) at specified days: 1	2	3	6	8	10	14
Sodium fluoroacetate[a]	0.01	3	8	15	22	22	23	25
	0.1	88	100					
	1.0	100						
Cholesteryl fluoroacetate[b]	0.01	2	3	3	4	4	6	10
	0.1	0	0	0	2	2	3	6
	1.0	3	15	38	84	91	95	100
Sitosteryl fluoroacetate[c]	0.01	2	2	5	9	11	13	22
	0.1	1	2	2	9	13	19	37
	1.0	7	21	42	66	79	87	96
Octadecyl fluoroacetate[d]	0.01	0	1	1	6	10	11	15
	0.1	1	1	2	17	33	44	62
	1.0	98	99	99	99	99	99	99
Mirex (standard)[e]	0.01	0	0	1	10	22	32	61
	0.1	0	1	15	84	91	96	98
	1.0	0	44	84	95	95	100	

[a] Mean of 3 replicates.
[b] Mean of 9–12 replicates.
[c] Mean of 6–9 replicates.
[d] Mean of 9 replicates.
[e] Mean of 15 replicates.

Table 2. Toxicity of fluoroaliphatic sulphonamides to fire ant workers[18]

	$C_8F_{17}SO_2NR_1R_2$			Mortality (%) at specified days[a]								
Compound	R_1	R_2	Concn (%)	1	2	3	6	8	10	14	17	21
III	-H	-H	0.01	0	0	0	3	7	7	10	20	23
			0.1	0	0	0	2	33	77	92	95	98
			1.0	43	85	98	100					
IV	-H	$-CH_3$	0.01	0	0	2	3	7	7	7	23	40
			0.1	0	0	7	88	97	98	100		
			1.0	17	93	100						
V	-H	$-C_2H_5$	0.01	0	0	0	0	2	2	10	22	50
			0.1	0	0	2	80	97	97	98	98	100
			1.0	25	100							
VI	-H	$-C(CH_3)_3$	1.0	0	0	0	0	0	0	5		
VII	$-CH_3$	$-CH{=}CH_2$	0.01	0	0	0	8	8	13	25	37	57
			0.1	0	7	33	77	90	92	100		
			1.0	100								
VIII	-H	$-CH_2CH{=}CH_2$	0.01	2	2	2	2	2	2	12	37	75
			0.1	3	3	3	48	60	78	93	98	100
			1.0	13	53	80	100					
IX	$-CH_3$	$-C_4H_8OH$	0.01	0	0	2	5	8	8	8	10	13
			0.1	0	0	0	2	5	30	75	85	92
			1.0	0	2	10	83	85	95	100		
X		$-CH{=}NCH{=}CH-$	0.01	2	3	3	8	8	8	13	15	18
			0.1	0	2	3	10	10	52	67	80	88
			1.0	0	2	17	92	100				
Mirex (standard)			0.01	0	0	0	0	17	38	58	63	92
			0.1	0	0	50	73	90	98	100		
			1.0	5	83	97	100					

[a] Mean of three replicates.

nated primary alcohols that were active against fire ants.[17] Although not commercially available, these alcohols served as models to test the pendant-toxicant hypothesis. This approach did not come to fruition, partly because of poor product solubility in soya bean oil. Fluorinated surfactants were added to the formulations in an effort to overcome these difficulties. The surfactants were routinely screened for toxicity and, surprisingly, they showed inate delayed toxicity against fire ants. This serendipitous discovery has resulted in a new class of insecticides that have potential for insect control beyond the fire ant.[18]

The surfactants and analogues described here were provided by 3M Company. Most active compounds fit the generalised fluorinated sulphonamide formula, $R_fSO_2NR_1R_2$, where R_f is a fluoroaliphatic radical and R_1 and R_2 are any chemically compatible structures. Primary screening results for components where R_f was held constant (C_8F_{17}-) and the nitrogen substituents were varied, clearly illustrated the flexibility and delayed action of this class of compounds. The chain-length of the alkyl-substituents had little effect on the delayed action of the compounds (**IV** and **V**, Table 2); however, activity was lost if R_1=H and R_2=t-butyl (**VI**, Table 2). The actual cause of this inactivity is unknown. Unsaturated N-substituents had either fast or delayed action depending on the position of the double bond. If the unsaturation was directly attached to the sulphonamide nitrogen, fast action was observed, whereas delayed activity was obtained when the unsaturated group was separated from the nitrogen by methylene (**VII** and **VIII**, Table 2).[18]

With the exception of **IX** (Table 2), the overall activity of the respective compounds diminished when one of the R-groups was a mono-alcohol or polyether. If the R-groups contained two hydroxyls, in any of the possible combinations, activity was lost.[18]

Class 1 activity was observed if one of the R-groups contained an epoxide, amine, amide, or aromatic carboxylic acid. However, a number of other heptadecafluorooctylsulphonamide analogues that are difficult to categorise had excellent delayed activity. Perhaps the most interesting were compounds that incorporated the sulphonamide nitrogen in a heterocyclic ring; e.g., the imidazole, **X**. These compounds should help direct the synthesis of other fluorinated sulphonamide toxicants which may extend the toxic range of this class of compound.[18]

Activity of this class of insecticide is dependent on a perflorinated R_f moiety (6–8 carbon optimum length), as well as the sulphone part of the molecule.[18] Although the amide moiety was not essential for activity, e.g., $C_8F_{17}SO_3H$ was active, it does provide a great deal of flexibility in both structural type and activity.

Several of the best delayed-action analogues were bioassayed against laboratory colonies; all of these compounds gave very good colony mortality. Those giving the most consistent results were tested in the field, and based on the results, **V** was chosen for potential commercial development as a delayed-action toxicant for fire ant control.

A few fast-acting and/or water-soluble fluorinated sulphonamide analogues were tested against other insect pests and gave good results against housefly adults, American and German cockroaches, and mosquitoes. Surprisingly the compounds are especially active against anopheline mosquito larvae.[19] The versatility of this class of compounds relative to their potential water and/or oil solubility and their fast or delayed action portends an interesting future.

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