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Chemical Control of Terrestrial Gastropods

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History of Chemical Control of Terrestrial Gastropods

'Snails, earwigs and all other creatures, hurt not the vines, nor the land nor the fruit of the trees, nor the vegetables . . . but depart into the wild mountains . . .'. So prayed the martyr Trypho in the 10th century AD (Taylor, 1894), confirming that terrestrial gastropods have had pest status since antiquity. Psychic control methods then appear to fall into abeyance, although the depredations continue, and, while the Reverend Gilbert White noted, in England in 1777, that slugs 'much injure the green wheat' (White, 1777), he did not invoke divine intervention.

The improved farming methods of the agricultural revolution produced better crops, and an increasing awareness of the need for pest control to secure these increases in output. In 1821, while riding near Newbury, William Cobbett observed 'a piece of wheat with cabbage leaves laid all over it at eight to ten feet from each other. It was to catch the slugs' (Cobbett, 1930). A shrewd commentator on the agricultural scene, he dismissed this early attempt at 'mechanical' control of pestiferous gastropods and stated that 'the only effectual way to destroy them is to sow lime, in dust and not slaked . . . at dusk . . . the slug is wet . . . the smallest dust of hot lime kills him, and a few bushels to the acre are sufficient'.

This early endorsement of chemical control is borne out in the subsequent development of control methods, with chemicals of one sort or another being deployed either as repellent coverings placed around plants, as dusts or sprays broadcast over the soil surface or, more latterly, as poisons incorporated into attractive foodstuffs as baits.

The chemicals thus used have been chosen largely empirically, the better ones being discovered by trial and error with whatever materials were to hand. Writers at the end of the 19th century and beginning of the 20th recommended readily available materials, such as lime, salt, soot

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and ashes (Theobald, 1895), carbolic acid and sawdust (French, 1906) and tobacco dust (Gahan, 1907). Other simple chemicals promoted as molluscicidal dusts and sprays included Bordeaux mixture (Lovett and Black, 1920), essentially a suspension of cupric hydroxide originally developed as a viticulturalists' fungicide, and a range of inorganic metal salts, such as aluminium sulphate (Durham, 1920), copper sulphate (Anderson and Taylor, 1926), potassium aluminium sulphate (Anon., 1930) and iron sulphate (Palombi, 1948). At about this time a variety of simple chemicals were also recommended as repellent barriers around plants, ranging from naphthalene (Massee, 1928) to corrosive sublimate (mercuric chloride) (Miles *et al.*, 1931). None were very effective and all were severely limited in their use by their phytotoxic effects: today's legislators would look askance at their widespread use on environmental grounds.

The use of poison baits seems to have first been suggested by Tyron (1899), who recommended baits containing the early fungicide Paris green (copper acetoarsenate), and many authors subsequently advocated baits containing copper and arsenical compounds. Other inorganic salts, such as barium, calcium and sodium fluorosilicate, were also advanced as stomach poisons (Shropshire and Compton, 1939). Baits incorporating derris root (*Lonchocarpus* Kunth spp.; Fabaceae), which contains the naturally occurring insecticide rotenone, were first suggested by Thompson (1928), marking a move toward baits deploying more complex organic poisons.

The first major advance in chemical control was made with the serendipitous discovery, *c.* 1934 in South Africa, of the molluscicidal properties of metaldehyde, a solid polymer of acetaldehyde, then on sale as a solid fuel for picnic stoves (Gimingham, 1940). In the UK it was first mentioned in the amateur gardening press (Hadden, 1936) and 4 years later was the most popular and generally recommended bait poison for use against terrestrial gastropod pests (Gimingham, 1940). Metaldehyde baits then remained the mainstay of terrestrial gastropod chemical control until the advent of carbamate-based baits in the 1950s.

Although undoubtedly more efficient than the chemicals used up to that time, metaldehyde-containing baits did not always protect crops successfully. The short field life of the baits and the need to synchronize applications with periods of pest activity reduced effectiveness, a problem only partly solved by later developments in carrier formulation. Consequently, the search for contact-acting molluscicides that could be applied as dusts or sprays, as were insecticides, fungicides and herbicides, still continued. Indeed, many of the chemicals so tested were themselves taken from these sources. The herbicide dinitroorthocresol (DNOC) successfully killed *Deroceras reticulatum* (Müller) (Agriolimacidae) in field trials in Belgium, but at an impracticably high rate (van den Bruel and Moens, 1958). Broadcast applications of the nitrogenous fertilizer calcium cyanamide were also shown to reduce damage by gastropods on a field scale, but again at 'fertilizer' rather than pesticide rates (300 kg ha^{-1})

(van den Bruel and Moens, 1956). Its use was further restricted by phytotoxic effects on young plants, and the material was never widely adopted.

The discovery, in Switzerland in the early 1940s, of the insecticidal properties of dichlorodiphenyltrichloroethane (DDT) (Lauger *et al.*, 1944) began the era of synthetic organic pesticides, and many were subsequently evaluated for molluscicidal activity. Often the results of tests for molluscicidal activity were conflicting. Buckhurst (1947) found that DDT gave excellent results in small plot trials, but Frömming (1950) found it ineffective in laboratory tests. Thomas (1949) thought benzene hexachloride (BHC) 'very poisonous' to *Deroceras agrestis* (Linnaeus), but Cleland (1952) found both DDT and BHC ineffective as seed dressings. In the USA, Schread (1958) obtained some control of the agriolimacid *D. reticulatum* and the limacid *Limax maximus* Linnaeus using dieldrin, while Stephenson (1959) claimed a reduction in damage to potatoes (*Solanum tuberosum* Linnaeus; Solanaceae) using aldrin in the UK. Getzin and Cole (1964) tested a number of pesticides against gastropods in laboratory tests and concluded that chlorinated hydrocarbons and organophosphates were poor molluscicides, with the exception of zinophos (*O*,*O*-diethyl-*O*-pyrazinyl phosphorothioate) and phorate (*O*,*O*-diethyl-*S*-ethylthiomethyl phosphorodithioate). The activity of phorate in granular formulation was then confirmed in small plot tests by Judge (1969) and as an emulsion in field trials on maize (*Zea mays* Linnaeus; Gramineae) by Barry (1969). However, despite the number of biologically active new molecules coming forward, no commercially successful molluscicides emerged from either the chlorinated hydrocarbon or the organophosphate classes of pesticides.

The next significant event was the detection of molluscicidal activity in another group of synthetic pesticides, the carbamates. In a screening programme started in 1954 to find a substitute for the arsenical baits used to control the introduced helicid snail *Cantareus aspersus* (Müller) in Californian citrus (*Citrus* Linnaeus spp.; Rutaceae) groves, Pappas and Carman (1955) obtained good results with baits containing isolan (1-isopropyl-3- methyl-5-pyrazolyl dimethylcarbamate). Other carbamates were subsequently shown to be effective against terrestrial gastropods. Ruppel (1959) demonstrated the activity of sevin (1-naphthyl-*N*-methylcarbamate) baits, although it appeared to be less effective against *D. reticulatum* than against the milacid *Milax gagates* (Draparnaud). In comparisons between metaldehyde baits and those containing various organochlorine, organophosphorus and carbamate insecticides, Getzin and Cole (1964) concluded that, while most were poor molluscicides, the carbamates were generally active. They reported good control with baits containing Bayer 37344 (4-methylthio-3,5-xylyl-*N*-methylcarbamate), later 'methiocarb', a synthetic molecule originally of interest because of its insecticidal and acaricidal properties (Unterstenhöfer, 1962). This compound was developed as a 4% a.i. bait formulation (Martin and Forrest, 1969) and under the trade names Draza and Mesurol achieved equal or greater commercial success then the metaldehyde-based baits already available. Other carbamate baits were subsequently developed, such as those incorporating cloethocarb (2-(2-chloro-1-methyloxy-ethoxy)phenyl methylcarbamate) (Harries *et al.*, 1980) and thiodicarb (3,7,9,13-tetramethyl-5,11-dioxa- 2,8,14-2,8,14-trithia-4,7,9,12-tetra-aza-pentadeca, 12-diene-6,10-dione) (Yang and Thurman, 1981). A recent variation on the theme of redeploying insecticides as active ingredients in baits for gastropod control is the product Malice, which contains bensultap (*S*,*S´*-2-dimethylaminotrimethylene di(benzenethiosulphonate)), an insecticide originally derived from a programme of chemical synthesis based on nereistoxin, a poison found in a species of marine annelid worm (Sakai, 1969). A related insecticide, cartap hydrochloride (*S*,*S*-(2-dimethylaminotrimethylene)bis(thiocarbamate)), applied to seeds reduced damage by *D. reticulatum* in wheat (*Triticum aestivum* Linnaeus; Gramineae) fields in southern England (Scott *et al.*, 1984) but was considered too toxic to birds to warrant commercial development.

The development of new, specifically molluscicidal chemicals was favoured by the World Health Organization, interested from the 1960s in controlling freshwater gastropods that act as intermediate hosts for trematodes causing human schistosomiasis. Trifenmorph (*N*-tritylmorpholine) was developed for this market and sold as Frescon (Boyce *et al.*, 1966), but also proved effective against the amphibious species, *Lymnaea truncatula* (Müller) (Lymnaeidae), in pasture and was employed to reduce transmission of the liver fluke, *Fasciola hepatica* Linnaeus (Fasciolidae) to domesticated ungulates (Crossland *et al.*, 1969). Its use, however, was never extended to terrestrial gastropod pests. Another synthetic molecule developed around this time for schistosomiasis control was niclosamide (2,5-dichloro-4-nitrosalicylanilide), subsequently marketed as Bayluscide (Gönnert and Schraufstätter, 1958). In recent years this molluscicide has been applied to the control of species of *Pomacea* Perry (Ampullariidae) (Schnorbach, 1995), which are increasingly important pests of rice (*Oryza sativa*) Linnaeus; Gramineae in the Asian region (Halwart, 1994; Cowie, Chapter 5, this volume).

More than 1000 plant species have been evaluated since 1933 as sources of naturally occurring chemicals to control aquatic gastropods involved in trematode transmission (Marston and Hostettman, 1985), and some 70 natural products with molluscicidal activity have been isolated. In some instances the active components are saponins and, interestingly, a synthetic surfactant, sodium dodecyl sulphate, has recently been found effective against *Pomacea* in rice (Tzeng *et al.*, 1994). While the search for naturally occurring molluscicides has tended to focus on aquatic gastropods as targets, some plant-derived compounds active against terrestrial pest species have also been discovered, although their commercial viability is yet to be established. Hussein *et al.* (1994) isolated a cardenolide, usharin, from an Egyptian desert shrub and found it highly toxic by contact to the helicid *Theba pisana* (Müller). Likewise, Hagin and Bobnick (1991) isolated 6-hydroxy-1,2,3,4-tetrahydro-*b*-

carboline-3-carboxylic acid from the couch grass *Agropyron repens* (Linnaeus) Beauvois (Gramineae). While inactive against three species of freshwater gastropods, it was toxic to *D. reticulatum*, *Deroceras laeve* (Müller) and in the arionid *Arion subfuscus* (Draparnaud) on contact and by ingestion in baits.

The early use of simple metal compounds to poison gastropod pests was unsuccessful, mainly because these materials were quickly dispersed when broadcast applied, or were too distasteful when incorporated in baits. Recently, it has been found that, by combining a metal with an appropriate organic ligand, molluscicides can be produced which are effective in bait formulations. Baits containing iron and aluminium chelates have been shown to be as effective as metaldehyde- and carbamate-based baits under field conditions (Henderson *et al.*, 1989, 1990). While the optimist might detect progress on this aspect of chemical control, the pessimist might feel that, after 170 years or so, it has come full circle.

Historically, therefore, chemical control of terrestrial gastropods began with the ad hoc use of whatever unsophisticated materials were to hand, progressed to the use of chemicals developed for other pest or parasitological problems and has only recently turned to the discovery of materials specifically for the purpose. The main constraint on progress has always been the difficulty of delivering the toxin to the target. To this is now added the increasing cost of developing new molluscicides, particularly environmental safety testing, which must be recovered from a relatively small market. In the immediate future it seems likely that chemical control will continue to depend on pesticidal molecules and formulations whose development and registration costs have already been covered by more extensive markets, or on chemicals of such limited toxicity that rigorous environmental safety testing is not required.

Modes of Action

Since molluscicides are applied either as baits or broadcast in solid or emulsified form, terrestrial gastropods encounter them via food uptake or by dermal contact, and the chemicals act either as stomach or contact poisons. Generally, stomach-poison activity increases with increasing lipophilicity in the molecule because of the lipophilic character of the plasma membranes that have to be penetrated. Another factor influencing the effectiveness of molluscicides delivered by the oral route is their stability under different pH conditions. In the digestive tract conditions range from pH 5.7 in the oesophageal crop to pH 7.9 in the distal intestine (Kelly *et al.*, 1996). For contact-action poisons, the relationship between lipophilicity, acidity and molluscicidal activity is less simple, since the molecules have first to penetrate the thick hydrophilic mucus layer and then to dissolve in the underlying lipophilic plasma membrane of the skin (Briggs and Henderson, 1987).

Ingested molluscicides are only partly transported down the digestive tract in the food mass, since a large proportion is quickly resorbed by the cells of the oesophagus (Triebskorn *et al*., 1990). After being released from these cells into the haemolymph, molluscicidal molecules are distributed in the body by the haemolymph and are quickly transported to peripheral tissues and organs. Using radiolabelled metaldehyde and cloethocarb, it has been shown that, only a few minutes after ingestion by *D. reticulatum*, molluscicides have left the oesophageal-crop cells and entered the cells of the stomach and the digestive gland via the cell bases that abut the haemolymph space (Triebskorn *et al*., 1990). Soon afterwards, metaldehyde could also be detected in the skin, the reproductive tract and the nervous system (R. Triebskorn, unpublished data). The rapid resorption of a metaldehyde metabolite, paraldehyde, by the cells of the digestive tract has also been demonstrated by Booze and Oehme (1985). Clark *et al*. (1995) and Triebskorn *et al*. (1999) have shown that iron chelate molluscicides also pass out of the digestive tract and into the haemolymph soon after ingestion, before finally becoming concentrated in the digestive gland. Unlike individuals treated with a non-toxic chelate, which accumulated large amounts of iron in the digestive gland, animals killed by a toxic chelate characteristically contained less iron in the digestive gland and more in the body wall and the reproductive tract.

Cells in which the molluscicidal chemicals or their metabolites can be localized after ingestion or dermal contact have to be considered as primary targets for such chemicals. Methods used to detect such primary targets include X-ray analysis, autoradiography, energy-filtering transmission electron microscopy (EFTEM), atomic absorption spectroscopy (AAS) (summarized by Triebskorn, 1995; Triebskorn *et al*., 1996, 1999) and inductively coupled plasma atomic emission spectrophotometry analysis (ICPAES) (Bullock *et al*., 1992). However, even in parts of the body that do not come into direct contact with the toxic molecule, pathological effects can be found which are related to the reaction of the animals' metabolism to the toxic conditions. These reactions may be considered as secondary reactions to the toxic input.

After dermal application, the primary targets for molluscicides are the epithelial cells of the skin, including the mucus cells (Triebskorn *et al*., 1998). Henderson (1970) showed that reactions in the skin follow not only after direct contact with metaldehyde itself, but also after exposure to the vapour of its main metabolite, acetaldehyde. The gastropod skin is known to be involved in the acquisition and resorption of various molecules and free ions from the environment (Henderson, 1970; Zylstra, 1972; Machin, 1977; Ryder and Bowen, 1977; Bullock *et al*., 1992). Chemicals passing through the skin reach the haemolymph and are thus transported throughout the body.

The mode of action of molluscicidal chemicals depends on their properties and is best understood for carbamates, such as methiocarb and cloethocarb. These act primarily as cholinesterase inhibitors (e.g. Casida, 1963). Perhaps the simplest observation on the mode of action is the desiccation effect caused by metaldehyde. Two other aspects of the mode of action of molluscicidal chemicals are their influence on metabolic enzymes and energy supply, and the detoxification processes that may limit the effectiveness.

Neurotoxic effects

Terrestrial gastropods poisoned by carbamates, such as methiocarb, soon become immobilized as the muscle tonus is lost (Mallet and Bourgaran, 1971; Godan, 1983). The carbamates behave as reversible, competitive inhibitors of acetylcholinesterase, which allow local accumulation of acetylcholine at organs innervated by cholinergic nerves (Metcalf, 1955; Casida, 1963), usually acting on the anionic and esteric binding sites of cholinesterase (Metcalf and Fukuto, 1965; Eldefrawi and Eldefrawi, 1990).

This interaction of carbamates with cholinesterases is not specific to gastropods as the same mode of action occurs in earthworms (Young and Wilkins, 1989b) and carabid beetles (Buchs *et al.*, 1989). Inhibition of cholinesterases in gastropods can also be caused by phosphoric acid esters (Pessah and Sokolove, 1983; Bakhtawar and Mahendru, 1987) and by bensultap, a sulphonate (Atger *et al.*, 1990).

Symptoms of metaldehyde poisoning in gastropods include increased mucus secretion, convulsions and paralysis (Booze and Oehme, 1986). Details of the mechanism by which metaldehyde or acetaldehyde causes convulsions are still unknown, but Booze and Oehme (1986) have shown that acetaldehyde acts as a releasing agent for noradrenaline and 5-hydroxytryptamine (serotonin) in gastropods. They suggest that metaldehyde may act as a releasing agent for the neurotransmitter GABA, thus acting directly on the central nervous system. This thesis is supported by the work of Homeida and Cooke (1982). Recent electron-microscope studies have revealed that mucus cells in the skin of *D. reticulatum* are innervated by neurons that can be stained with antibodies against serotonin and those binding to amino acid decarboxylase or dopamine-*b*-hydroxylase, two enzymes involved in the synthesis of serotonin and catecholamines. In animals treated with metaldehyde, this immunostaining is much more intense than in controls, suggesting that metaldehyde may not only increase the release of serotonin but also its rate of synthesis and recycling (Triebskorn *et al.*, 1998). Neurotoxic effects may result in alterations of locomotion (Wedgwood and Bailey, 1988) and of feeding behaviour (Wright and Williams, 1980; Wedgwood and Bailey, 1986; Bourne *et al.*, 1988; Bailey, 1989; Bailey *et al.*, 1989), which are of particular significance when attempting to deliver the chemicals in baits. Wright and Williams (1980) suggested that the observed reduction in feeding caused by molluscicidal chemicals is due to a paralysing effect on the digestive-tract wall immediately after ingestion, terminating the feeding response. A direct effect on the nerves controlling the digestive tract may also be involved. Roach (1968) has shown that digestive-tract motility in *Arion ater* (Linnaeus) is largely independent of central control but is 'coordinated' by an extensive nerve plexus in the gut wall. Bailey *et al*. (1989) claimed that both metaldehyde and methiocarb shorten meals by interfering with the neural control of feeding, and that metaldehyde may also disable the muscles involved in feeding. The effects observed by Bailey *et al*. (1989) could also be due to the impact of metaldehyde on neuronal transmission, as demonstrated with *Lymnaea stagnalis* Linnaeus by Mills *et al*. (1990, 1991a,b,c), as metaldehyde induced bursting activity and paroxysmal depolarizing shifts in the motor neurons of the feeding system.

Desiccation effects

Increased mucus production followed by increased mucus secretion is one of the first reactions of gastropods to many kinds of stressors, including mechanical stimuli or chemical irritation caused by molluscicidal chemicals (Godan, 1983; Triebskorn and Ebert, 1989; Triebskorn *et al*., 1998). One effect of the extruded mucus is to form a protective barrier preventing direct contact between the toxin and the epithelia of the skin or digestive tract, so reducing the toxicity of the chemicals (Port and Port, 1986; Triebskorn and Ebert, 1989). At the same time the mucus may also dilute the chemical. In some cases the mucus may even detoxify the chemical, provided that it is pH-sensitive or unstable under certain (usually acid) pH conditions, as are both metaldehyde and cloethocarb. On the other hand, the animals risk desiccation due to the high water content of the extruded mucus, and lose large amounts of ions entailing high energy costs for resynthesis (Triebskorn *et al*., 1996, 1998). Port and Port (1986) claim that dehydration is the most frequent cause of death following metaldehyde poisoning in gastropods, and desiccating effects have also been noted in cases of molluscicide poisoning in vertebrates, such as dogs (*Canis domesticus* Linnaeus) (Canidae) and cats (*Felis catus* Linnaeus) (Felidae) (Maddy, 1975; Booze and Oehme, 1986). Increased mucus secretion following metaldehyde ingestion may also induce additional toxic effects, as the acidic mucus hydrolyses the metaldehyde to acetaldehyde, particularly in the anterior part of the digestive tract (Booze and Oehme, 1985).

Effects on metabolic enzymes and energy metabolism

Carbamates are not only cholinesterase inhibitors but in many cases are general inhibitors of esterases (Casida, 1963). They have been used to differentiate esterases and to elucidate the nature of the enzymatically active sites (Augustinsson, 1960). Tegelstrom and Wahren (1972) have shown that, while certain isoforms of esterases can be inhibited both by eserine and by methiocarb plus eserine, others are inhibited by methiocarb and not by eserine. It has also been shown that both cloethocarb and methiocarb can induce activity in non-specific esterases in the digestive gland of *D. reticulatum* within a few hours of treatment, although in the cells of the digestive tract these enzymes are inhibited (Triebskorn, 1991a). These apparently contradictory results may be due to the fact that non-specific esterases include carboxylesterases, arylesterases and acetylesterases, which may differ in sensitivity to carbamates and may be unevenly distributed in the digestive tract. The high esterase activity in the digestive gland, in molluscicide-treated individuals, may be due to the observed destruction of the (secondary) lysosomal system in digestive cells (Triebskorn and Künast, 1990). It may also arise through the release of enzymes into the cytoplasm and autolysis of the cells, as has been reported by Banna (1980) for aquatic species treated with trifenmorph. However, after metaldehyde ingestion, a strong increase in the activity of non-specific esterases has been found in the resorptive cells of the digestive gland (Triebskorn, 1991a), even though the lysosomal system showed less damage than after carbamate poisoning (Triebskorn, 1989).

A second intracellular digestive enzyme system, the acid phosphatases, which are found in primary lysosomes, has been shown to be influenced by molluscicide treatment in *D. reticulatum.* Enzymes of this group can catalyse the breakdown of ester bonds in orthophosphate esters under acid conditions and are involved in the attack on pyrophosphate bonds. They can also act as transphosphorylases. The activity of these enzymes has been shown to be completely inhibited after cloethocarb treatment, and strongly reduced after methiocarb and metaldehyde treatment (Triebskorn, 1991a). Inhibition of acid phosphatase by trifenmorph has also been demonstrated in *Bulinus truncatus* (Andouin) (Planorbidae) by Banna (1980). Kela and Bowen (1995) describe a reduction, but not a total inhibition, of acid phosphatases in the digestive gland of *L. stagnalis* after treatment with a naturally occurring molluscicide.

Reduction of an enzyme’s activity may not be due to direct inhibition of the enzyme by a chemical, as described, for example, by Dauterman and Hodgson (1990). It might also be related to the cessation of protein synthesis, due to the effect of the toxin on the general metabolism of the animal, or be caused by the destruction of the membrane compartment in which the enzyme is located. On the other hand, activation of an enzyme may not necessarily be related to a toxin-specific induction of the respective enzyme synthesis or to a specifically induced increase in its activity. In many cases changes in enzyme activity levels are related to general metabolic responses accompanying increased mucus production and secretion or to other detoxification mechanisms (see below), which need large amounts of energy (Triebskorn *et al.*, 1996). In achieving acute toxicity, which is the goal for an effective molluscicide, the early phase of metabolic activation is usually followed by a decrease and finally a cessation of enzymatic activity coincident with cell and animal death. In this context, the observed molluscicide-induced alteration of metabolic enzyme activity in terrestrial gastropods can be interpreted, at least in part, as metabolic reactions reflecting different stages of intoxication–detoxication.

Since detoxification processes, including mucus production and extrusion, are energetically expensive, the required energy must be provided by increasing intracellular digestion and activating various enzyme systems. These include the above-mentioned intracellular digestive enzymes, and also the enzymes involved in transport processes, such as the alkaline phosphates or the ATPases. Alkaline phosphatases break down ester compounds of orthophosphate acids under alkaline conditions. A special type of alkaline phosphatase with a pH optimum of 7.5 is capable of catalysing the hydrolytic breakdown of ATP. This is the cell-membrane ATPase, the activity of which depends on the presence of sodium and potassium ions. After treatment with both carbamates and metaldehyde these transport enzymes are initially activated, but are then totally inhibited (Triebskorn, 1991a; Triebskorn *et al.*, 1996). Correlated in many cases with this activation of transport enzymes after exposure to molluscicidal chemicals is the reduction in intracellular energy stores and increased biotransformation (Triebskorn *et al.*, 1996). Kela and Bowen (1995) observed a shift of alkaline phosphatase from the brush border of the digestive gland to the lumen and a marked inhibition of ATPase activity in animals treated with a naturally occurring plant molluscicide.

Effects of detoxification

Detoxification processes are reactions to limit damage by toxins or to excrete them. With regard to the efficacy of molluscicides, detoxification reactions are obstacles to be overcome. Detoxification includes: (i) enzymatic reactions leading to the biotransformation of chemicals by oxidation (phase I reactions); (ii) hydrolysis or conjugation (phase II reactions); and (iii) several other biochemical processes, such as the induction of stress proteins or metallothioneins involved in direct interactions with the toxins and maintenance of intracellular homoeostasis. The general aim of all these processes is either to facilitate excretion of the toxin or its transformation into a non-toxic storage product, which is especially important for metal poisons.

On hydrolysis, the main breakdown product of metaldehyde is acetaldehyde, which in turn may be reduced to ethanol with nicotinamide adenine dinucleotide (NADH), the reaction being catalysed by alcohol dehydrogenase. Although oxidative processes are not involved in this degradation, a significant increase of several mixed-function oxygenases (phase I enzymes, MFO enzymes) has been observed in metaldehyde-treated *D. reticulatum* (Triebskorn, 1991a). This is possibly due to an indirect impact of the molluscicide on oxygenases, which are normally involved in the metabolism of steroid hormones.

Carbamates are normally detoxified or biotransformed by oxidation of the phenol ring and *N*-methyl group by microsomal oxidases, by hydrolysis and by several types of conjugation (formation of glucuronides, sulphates and glucosides) (Wegler, 1970; Wilkinson, 1976). One of the most important metabolic pathways of the *N*-methyl- and the *N*,*N*-dimethylcarbamates is the *N*-methyl hydroxylation of the carbamyl moiety (Wilkinson, 1976). Metcalf and Fukuto (1965) claim that aliesterases may be involved in detoxification of carbamates, while Gordon and Eldefrawi (1960) speak of ‘carbamate esterases’. However, most of our knowledge of carbamate biotransformation is restricted either to vertebrates or to insects, and there is little information on terrestrial gastropods. Photometric measurements of enzyme activities in the digestive gland of *D. reticulatum* treated with methiocarb have revealed the induction of two enzymes involved in the oxidative degradation of carbamates: arylhydrocarbon hydroxylase and NAD phosphate (NADPH)-neotetrazolium reductase. No such induction was found when the animals were treated with cloethocarb, but activation of the enzyme glucose-6-phosphate dehydrogenase, which catalyses the formation of the $NADPH_2$ needed for the above reaction, has been detected (Triebskorn, 1990).

The role of the digestive gland in the biotransformation of molluscicides is very important. In experiments with *D. reticulatum* using radiolabelled cloethocarb and metaldehyde, radioactivity accumulated in the basophilic cells of the digestive gland a short time after application of the chemicals (Triebskorn *et al*., 1996; R. Triebskorn, unpublished data), and was accompanied by increased activity of the phase I detoxification enzymes in the same cells. Ultrastructural changes in the basophilic cells have also been shown to follow exposure to metaldehyde and carbamate (Triebskorn, 1989; Triebskorn and Künast, 1990), and similar changes have been related to an induction of phase I detoxification enzymes in vertebrates by Klaunig *et al*. (1979). The metal residue deriving from isotopically labelled iron chelate molluscicides applied to *D. reticulatum* was found concentrated in the digestive gland irrespective of the chelate used (Clark *et al*., 1995), and its localization in the digestive cells was confirmed using EFTEM (Triebskorn *et al*., 1996, 1999). The observations fit well with the known role of the digestive gland of gastropods in metal detoxification (e.g. Dallinger *et al*., 1989; Marigomez and Dussart, 1996).

Cytological Effects

Cytological effects of chemicals of known molluscicidal activity have been investigated in various tissues by light- and electron-microscope studies (Ryder and Bowen, 1977; Triebskorn, 1989, 1991b, 1995; Kela and Bowen, 1995; Rondelaud and Dreyfuss, 1996; Triebskorn and Köhler, 1996; Triebskorn *et al.*, 1998, 1999). Light-microscope investigations are useful in detecting gross structural effects on target tissues, such as the lesions induced in the oesophageal crop of *D. reticulatum* by cloethocarb (Triebskorn and Florschütz, 1993) or necrosis in the skin after oral or dermal application of metaldehyde (Triebskorn *et al.*, 1998). Other changes demonstrated include alterations in glycogen and lipid content in the oesophageal crop and digestive gland, and qualitative and quantitative differences in mucus induced by molluscicides (Triebskorn *et al.*, 1998).

Transmission electron-microscope studies have shown that cellular damage caused by molluscicidal chemicals is most pronounced in the following cell types: (i) mucous cells in the skin, digestive tract and salivary gland; (ii) digestive cells of the digestive gland; (iii) basophilic cells of the digestive gland; and (iv) epithelial cells of the oesophageal crop (Triebskorn, 1989, 1991b; Triebskorn and Ebert, 1989; Triebskorn and Künast, 1990: Triebskorn *et al.*, 1998). These effects are discussed in more detail below.

At the subcellular level, different organelles exhibit different spectra of reaction (Köhler and Triebskorn, 1998). The endoplasmic reticulum (ER) emerges as a very sensitive organelle in that it reacts to low dose levels of chemicals soon after exposure. Alterations such as dilatation, degranulation and vesiculation of the cisternae and formation of concentrically arranged cisternae occur, probably due to the induction of detoxification enzymes rather than to pathological effects of the chemical. Mitochondria are also sensitive to many stressors and rapidly exhibit gross pathological symptoms, such as swelling, shrinkage, disruption of cristae or the formation of intramitochondrial cristae (Ghadially, 1988; Köhler and Triebskorn, 1998). The observed changes in the mitochondria may be due to interactions with the lipophilic components of the mitochondrial membrane disrupting ion transport.

In gastropods treated with molluscicides the most noticeable effects on the Golgi bodies are seen in the mucocytes: these include dilatation of the cisternae and an increase in their number, increased vesicle formation and the breakdown of the Golgi-system membranes (Triebskorn *et al.*, 1998).

Mucocytes of the digestive tract and skin

One type of mucous cell predominates in the oesophagus (including the crop), intestine and salivary gland of *D. reticulatum*. It is pear-shaped and characterized by a basally located nucleus, large cisternae in a wide, laminar, granular ER, large Golgi complexes and mucous vacuoles, which coalesce in the apical region of the cells. Three types of mucous cells can be distinguished in the skin:

1. Pedal gland cells which are located in the sole. The ultrastructure of this cell type is comparable to that of the mucous cells of the digestive tract and the secretory product varies in appearance from spotted and electron-dense to fluffy and less electron-dense.
2. Mantle gland cells are the most numerous type of mucous cell found in the skin of *D. reticulatum*, occurring in the mantle, dorsal epidermis and foot. They are characterized by their prominent Golgi apparatus, consisting of ten to 20 stacked lamellae, and large mucous vacuoles containing slightly electron-dense material. In mature cells they displace the nucleus and cytoplasm to the periphery.
3. Peripodal gland cells, which are club-shaped cells located in the foot fringes. This cell type is characterized by a basally located nucleus and by the very long cisternae of the rough ER.

In all types of mucous cells in the skin and digestive tract, chemical-specific and dose- and time-dependent responses to molluscicides occur, which reflect either the increased demand for mucus (phase 1) or pathological effects (phase 2) (Triebskorn *et al.*, 1998). Dilatation, vesiculation or degranulation of the ER, dilatation of the Golgi membranes and swelling of mucous vacuoles are all phase 1 reactions to molluscicide exposure. Phase 2 is characterized by the general breakdown of the ER membranes, Golgi systems and vacuoles, and by swelling or bursting of the mitochondria, karyolysis and, in some instances, autolysis of complete cells (Triebskorn *et al.*, 1998).

When cells are compared after similar exposure times, cellular damage is generally less severe after exposure to carbamates than to metaldehyde. Histological preparations of *D. reticulatum* tissues made after carbamate ingestion show that mucocytes can alter the type of mucus produced, as their immediate reaction is to secrete large amounts of acidic mucus into the digestive-tract lumen and then to resynthesize large amounts of neutral mucus within the cells (Triebskorn *et al.*, 1998). Increased secretion into the digestive tract can also be seen immediately following metaldehyde ingestion. Subsequently, however, the destruction of the Golgi apparatus and ER brings mucus production to a halt.

Cells of the digestive gland

Three cell types can be distinguished in the digestive gland of *D. reticulatum*:

1. Columnar digestive cells, which dominate the epithelium in untreated animals.
2. Cone-shaped crypt cells, which have secretory functions and are characterized by having large amounts of granular ER, numerous Golgi bodies and secretory vesicles. These cells increase in number under toxic conditions (Cajaraville *et al.*, 1990).

3. Goblet-like excretory cells, characterized by large vacuoles containing electron-dense material.

Cytological effects induced by carbamates and by metaldehyde have been recorded, particularly in the first two types of cell (Triebskorn, 1989; Triebskorn and Künast, 1990; Triebskorn *et al.*, 1996). In the digestive cells of molluscicide-treated animals, pinocytosis ceased and the microvilli were shortened. This was followed by increased coalescence of vacuoles and their fusion with lysosomes, indicating the activation of intracellular digestive processes. Finally, the cells became dominated by large vacuoles and large secondary lysosomes. Increased vacuolization of digestive cells in *L. stagnalis* following treatment with a naturally occurring molluscicide has been reported by Kela and Bowen (1995), and in *D. reticulatum* exposed to various metals (Triebskorn and Köhler, 1996). Generally, after molluscicide treatment, lysosomes containing acid phosphatases are not found near the apices of the digestive cells, as they are in the cells of untreated animals, and glycogen storage is drastically reduced.

In the basophilic digestive-gland cells of molluscicide-treated *D. reticulatum*, structural modifications of the ER have been observed which appear to be correlated with the induction of enzymes involved in biotransformation and cellular transport. All chemicals showing molluscicidal activity examined so far have significantly reduced the storage products in the basophilic cells. Epithelial necrosis in the freshwater gastropods *Lymnaea glabra* (Müller) and *Bellamya dissimilis* Müller (Viviparidae) has been reported after the application of the molluscicide niclosamide and the pesticides endosulfan, methylparathion, quinalphos and 2,2-dichlorovinyldimethylphosphate (DDVP) (Jonnalagadda and Rao, 1996; Rondelaud and Dreyfuss, 1996).

Epithelial cells of the oesophageal crop

Apart from a few cells that are translucent under electron microscopy, the epithelium of the oesophageal crop is dominated by columnar storage cells, which are characterized by large amounts of lipid and carbohydrate. Shortly after exposure to metaldehyde, to pesticides such as carbamates, pentachlorophenol and lindane or to environmental pollutants such as heavy metals, these storage products are greatly reduced. When the toxic load exceeds a certain limit, epithelial cell damage occurs and pathological symptoms, such as the bursting of mitochondria and the disruption of the ER membranes and the microvillous border, appear (Triebskorn *et al.*, 1996). Morphological damage to the epithelial cells of the oesophageal crop has also been described by Bourne *et al.* (1991), following ingestion of sublethal doses of metaldehyde and methiocarb. These authors demonstrated that endocytosis in the oesophageal crop was also impaired. Manna and Ghose (1972) found that, in

Achatina fulica Bowich (Achatinidae) treated with endrin, cell damage within the digestive tract was most severe in the intestine and rectum, while pronounced shrinkage of muscles occurred in the oesophageal crop.

Techniques for Evaluating Molluscicidal Activity

Many comparative studies of molluscicidal activity have been published. The diversity of methods used indicates the perceived difficulty of evaluating chemicals in a way relevant to their ultimate deployment in the field. Evaluation methods can be conveniently thought of as falling into three categories, namely laboratory experiments, terrarium trials and field trials. Laboratory experiments under controlled conditions establish whether a candidate compound has any useful activity and indicate its likely mode of action so that an appropriate formulation for delivery can be chosen. This is often followed by tests of the chosen formulation in enclosed terraria designed to resemble field conditions, but still confining the animals to allow accurate assessments of mortality and detailed observation of behaviour. The definitive assessment of a molluscicide is its performance in the relevant cropping situation. Gastropod numbers and associated crop damage are compared on treated and untreated areas in a field trial. The size of plots is determined by the post-treatment assessments planned and the design is replicated to facilitate statistical analysis (Anon., 1986).

Laboratory experiments

The first methodical examination of a molluscicidal chemical was probably that of Cragg and Vincent (1952), in which the effect of metaldehyde on *D. reticulatum* was investigated. The animals were confined with metaldehyde, but not in contact with it, to check for fumigant activity. The animals were also held on filter-paper soaked in an aqueous solution and dusted with the powdered compound to test for contact activity. Stomach-poison activity was assayed by injecting the compound into the lumen of the anterior oesophageal crop in solid form and in aqueous solution. These tests showed that metaldehyde was toxic by dermal contact and by ingestion but did not allow for accurate determination of the lethal dose. Methods for measuring the median lethal dose (LD_{50}) were subsequently developed (Henderson, 1968, 1969, 1970) and have been used to compare the molluscicidal activity of various compounds as stomach poisons, including several carbamate pesticides (Hunter and Johnston, 1970).

Forcibly injecting chemicals into the digestive tract allows accurate assessment of the lethal dose but obviously gives no indication of its acceptability when incorporated into bait. Various voluntary ingestion methods have been used to discover whether a candidate molluscicide is suitable for use in bait and to determine the optimum concentration to use. Quantified measurements of bait ingestion by *D. reticulatum* and *Tandonia budapestensis* (Hazay) (Milacidae) were obtained by Wright and Williams (1980) when these species were confined with wheat-flour baits containing an inert tracer, chromic oxide. Wedgwood and Bailey (1986) devised a method of analysing the feeding responses of *Arion hortensis* de Férussac by attaching molluscicidal baits to a record-player stylus, thus picking up and recording the bite frequency electronically. The results obtained by both methods unfortunately support the general conclusion that the addition of molluscicidal chemicals reduces ingestion of bait by the gastropods and compromises the ingestion of a lethal dose. With a view to improving bait performance, voluntary ingestion methods have also been used to identify different carriers, with which the molluscicidal chemical is mixed, that increase ingestion (e.g. Frain and Newell, 1982; Henderson *et al.*, 1992; Clark *et al.*, 1997). This topic is reviewed in depth by Bailey (Chapter 2, this volume).

Getzin and Cole (1964) screened proprietary pesticides against *Prophysaon andersoni* (Cooper) (Arionidae) by spraying animals directly in a Potter spray tower. However, Getzin and Cole (1964) observed that most pesticides applied in this way were ineffective because they were quickly removed by mucus secretion. The increased mucus secretion in response to noxious compounds made it uncertain how much of the topically applied dose penetrated the test animals. Subsequently, Young and Wilkins (1989a) devised a technique in which mucus secretion was delayed by anaesthetizing agriolimacids and applying measured amounts of test formulations to an area of the dorsal epidermis that had been wiped clear of mucus. This method was used to compare the toxicity of six compounds, including three metal salts, and methiocarb as the molluscicidal standard. Another method, appropriate only to shelled species, is that of Hussein *et al.* (1994), in which known amounts of test compounds are applied in solution or emulsion to the body surface of individual animals with a micropipette. On animal contraction, the applied materials are contained within the shell and in intimate contact with the animal's tissues.

Since terrestrial gastropods are most likely to acquire contact poisons by crawling on treated surfaces, many laboratory evaluation methods involve exposure of animals to compounds deposited on inert surfaces, rather than treating the animals directly. When *D. reticulatum* were held on glass plates coated with a range of phenolic and other chemicals, the relative toxicities differed from those obtained when the same chemicals were injected into the oesophageal-crop lumen (Briggs and Henderson, 1987). When comparing the activity of a range of iron and aluminium compounds as contact poisons against this species, substitution of a wet-soil substrate for a dry-glass one reduced activity generally, although the attenuation was less with chelated compounds than with simple metal salts (Henderson and Martin, 1990).

More recently, techniques have been developed for assessing the antifeedant or deterrent properties of secondary plant metabolites and other compounds for use as topically applied plant protectants. Barratt *et al.* (1993), for example, dipped cabbage (*Brassica oleracea* Linnaeus var. *capitata* Linnaeus) (Brassicaceae) leaf discs in extracts of over 50 plants and fungi and compared the fresh-weight loss after exposure to *D. reticulatum*: reductions in feeding of up to 87.5% were recorded. Dawson *et al.* (1996) applied test materials to glass plates and to leaf surfaces by electrostatic spraying and monitored the proportion of time spent on the treated and untreated surfaces using time-lapse video recording. At the standard test concentration of 50 $\mu g\ cm^{-2}$, common salt was only slightly repellent, while several proprietary surfactants were effective repellents. However, these surfactants were ineffective in the field because they were rapidly lost from plant surfaces under wet conditions.

Terrarium trials

Trials in enclosed areas of soil, with or without plants, are often used to predict how test formulations will perform under field conditions. Judge (1969) followed laboratory experiments with 74 candidate molluscicides by evaluating 23 of the most active ones under such simulated field conditions. Groups of *D. laeve* and *D. reticulatum* were confined in wooden trays containing pea (*Pisum sativum* Linnaeus) (Fabaceae) seedlings growing in potting compost. Trays were treated with test formulations, either sprayed on to the plants or applied as granules, and kept in a glasshouse. After 7 days, mortality in the gastropods and damage to the plants were recorded. Of the 23 compounds found active in laboratory tests, only four were effective in the terrarium trials, including the organophosphate phorate and the oxime carbamate aldicarb, which were ‘spectacularly’ molluscicidal under these conditions. In subsequent field trials with granular formulations, ‘none of these treatments achieved conspicuous success in slug control’ (Judge and Kuhr, 1972).

Field trials

While molluscicide efficacy can be expressed in terms of its effect on gastropod numbers, the inherent difficulty of accurately assessing field populations in certain cropping situations means that this approach is less reliable than quantifying the effect on the crop itself. Population estimates based on numbers found in soil-surface refuge traps vary with the level of activity, which is weather-dependent. Estimates based on numbers found using soil-sampling methods, such as that of South (1964), are more accurate but are generally regarded as too laborious for extensive use. A third method, which appears to offer accuracy comparable to soil sampling but which is less laborious, has been described by Ferguson *et al*. (1989). In the 'defined area trap' all gastropods at the surface within a metal cylinder driven into the soil are collected over a period until the area is exhausted, thus providing an estimate of gastropod numbers per unit area.

Post-treatment counts of the number of gastropods killed by a test material have long been used as an evaluation procedure, most recently by Meredith (1996). While dead gastropods are reassuring proof of toxicity, the real measure of efficacy is the size of the residual population. Ultimately, because of the difficulty of getting accurate population estimates, evaluations based on numbers at best give an indication of the effectiveness of new treatments in comparison with existing products. Field tests of baits against *C. aspersus* in citrus orchards were less difficult for Pappas and Carman (1955): test baits were broadcast into individual trees and dead and live *C. aspersus* in and beneath each tree counted 10 days later. Unfortunately, few gastropod pest situations are as experimenter-friendly.

Reduction of crop damage is the most unequivocal measurement of a molluscicide's efficacy. When present in large numbers, terrestrial gastropods can reduce the gross yield of crops by damaging the parent plants. Even in small numbers they may greatly reduce the market value of the harvested produce, either by causing blemishes or merely by being present in the end-product. The type of damage inflicted therefore dictates the field assessment method and how accurate it must be.

When assessing treatments applied to wheat seed to control damage by *D. reticulatum*, Scott *et al*. (1984) counted damaged seeds in soil samples taken after germination and also compared grain yields from whole plots at harvest. Results of the two methods were in general agreement: the most effective teatment reduced the proportion of seeds initially damaged from 44% to 7% and an increase in grain yield of 50% was subsequently recorded. Glen and Orsman (1986) used an indirect method to compare the effectiveness of two bait formulations with a broadcast application of aluminium sulphate applied to bare-soil plots. Glasshouse-grown seedlings of Chinese cabbage (*Brassica chinensis* Linnaeus) were transplanted into the plots 2 days after treatments were applied and damage to the leaves of the indicator plants was visually assessed over the following 12 days. Treatments prevented damage to the indicator plants for 4 days, implying that protection was due to a brief depression of feeding activity rather than a reduction in the pest population. In Brussels sprouts (*Brassica oleracea* Linnaeus convar. *oleracea* var. *gemmifera*), where mild surface grazing of the outer leaves of the sprouts renders the crop unsaleable, Dawson *et al*. (1996) evaluated sprays repellent to *D. reticulatum* in field plots by removing individual sprouts from sample plants and visually scoring for damage.

Field-trial evaluation techniques are therefore dictated by the cropping situation involved and with such polyphagous animals a range of methods is required. In a group of pests whose activity levels are notoriously unpredictable, reliable field assessment demands a degree of replication that is often difficult to satisfy.

Application Technologies

Most pesticides are applied in spray, dust or granular formulations but only occasionally as baits. In contrast, molluscicides directed against terrestrial gastropods are only occasionally delivered as sprays or dusts but are more usually deployed in baits. For this reason, application technology is largely concerned with the composition of baits and how, where and when to apply them.

The first effective use of a molluscicide in a bait was probably with metaldehyde, in 1936, when recipes for making formulations using loose bran were published in the UK gardening press (Hadden, 1936). Thereafter, in efforts to extend the post-application life of such baits, various additives were tested. Thomas (1948) increased the effectiveness of metaldehyde/bran baits with a casein glue binder and the majority of commercial baits are now produced in compressed pellet form to delay disintegration. Such pellets are usually, though not invariably, grain-offal-based with the toxicant dispersed throughout the carrier medium. One departure from this generality is a formulation recently developed in New Zealand in which the active ingredient (metaldehyde) is incorporated into an edible matrix which is then applied as a coating over an inert granular core (Barker *et al.*, 1991). This formulation is claimed to be more easily dispensed from application machinery and to give a higher density of bait particles per unit area.

Because most commercial baits are similar in size to fertilizer granules, they can be broadcast using existing tractor-mounted machinery. They can also be applied by purpose-built distributors, usually involving a gravity-fed hopper discharging on to a horizontally spinning disc. These distributors can also be mounted on low-ground-pressure vehicles, such as ‘quad’ bikes, which can operate in the wet soil conditions that favour pest activity, or on vehicles conducting other operations, such as fungicide spraying, to reduce crop disturbance.

In crops susceptible to gastropod damage at or before germination, such as cereals, baits can be mixed with the seed and sown with it. This may give better control in very coarse seed-beds but in general soil-surface applications made prior to sowing are more effective (Green *et al.*, 1992).

Deployment of molluscicides against terrestrial gastropods in formulations other than baits is less common. Aqueous sprays of copper sulphate were included in UK recommendations for controlling gastropods in arable crops as recently as 1984 (Anon., 1984), but in practice the use of copper and other metal salts as dusts or sprays has been largely superseded. Metaldehyde has also been applied in emulsified form as a spray, if its introduction into an overhead irrigation system can be so regarded. Howitt (1961) reduced damage to cocksfoot (*Dactylis glomerata* Linnaeus; Gramineae)/clover (*Trifolium repens* Linnaeus; Fabaceae) pasture in the western USA in this way, although it is not clear whether the molluscicide was toxic by contact or after ingestion of treated foliage. Electrostatic spraying has been used experimentally to give high, localized deposition on growing plants (Dawson *et al.*, 1996), although the materials tested were repellents rather than molluscicidal.

Molluscicides are routinely sprayed into watercourses for the control of aquatic species that act as hosts for trematodes causing human and animal schistosomaisis, but since there are few aquatic molluscan crop pests this method of application is rarely applicable. A notable exception is the so-called golden apple snail (*Pomacea* sp.), which attacks paddy rice in Asia: molluscicides for the control of this pest can be delivered as aqueous emulsions in sprays (Schnorbach, 1995; Palis *et al.*, 1996).

Molluscicides are also deployed as seed dressings: Scott *et al.* (1984) successfully treated wheat seeds to prevent grain hollowing of wheat in the UK. At treatment rates high enough to be effective in laboratory tests many of the candidate compounds were too phytotoxic, but in field trials good protection culminating in increases in grain yield were given by a methiocarb seed dressing. This was applied to the seed as a slurry in a Rotostat seed-treatment machine using a methylcellulose sticker. This technique has been further developed in the Netherlands by Ester *et al.* (1996) using polymer 'film-coating' techniques, which allow higher seed loadings without phytotoxic effects.

Transfer of pesticides to their targets is an inefficient process. The proportion of the amount of insecticide applied that actually effects control has been estimated at between 0.02% and 0.03% (Graham-Bryce, 1975). Delivery of molluscicides to aquatic species is helped by continuing accumulation from the surrounding water. Using radiolabelled 4´-chloronicotinanilide Duncan *et al.* (1977) found that, after 2 h , the rate of uptake by *Biomphalaria glabrata* (Say) (Planorbidae) from flowing water remained constant. With terrestrial species the situation is more difficult. They are relatively large in comparison with most insect pests and they are only intermittently active in the crop or on the soil surface. Exposed surfaces are protected by a disposable layer of aqueous mucus and shelled species have additional protection. These factors severely constrain the choice of biocides suitable for chemical control, which have to be delivered in a relatively large amount, and reduce the options open for their delivery.

Although application of molluscicides in bait formulation is relatively inefficient it remains the best option available under many circumstances. Thus far, application techniques have developed to optimize the usage of relatively ineffective chemicals and are only likely to change significantly following the emergence of new molluscicides and better formulations.

Conclusions

The importance of terrestrial gastropods as crop pests has greatly increased over the past 30 years, and in the process demands for effective controls have outstripped the development of chemical control measures.

The increased pest status has been brought about by a number of contributory factors. In temperate regions, changes in husbandry practices, such as autumn rather than spring sowing of cereal crops, the expansion of dense-canopy crops, such as oilseed rape (*Brassica napus* Linnaeus var. *oleifera* Linnaeus), and the adoption of minimum-tillage regimes, which conserve soil moisture, have all favoured the survival and reproduction of these moisture-dependent animals. In tropical and subtropical areas the most noticeable developments have been the introduction and spread of exotic species, which have become serious pests, the most dramatic examples being the giant African snail, *A. fulica* (Mead, 1961; Raut and Barker, Chapter 3, this volume), and more recently the golden apple snails, *Pomacea* spp. (Halwart, 1994; Cowie, Chapter 5, this volume), and the bean slug, *Sarasinula plebeia* (Fischer) (Vaginulidae) (Rueda *et al.*, Chapter 4, this volume). Such changes, occurring against a background of increasing demand in food quantity and quality, have combined to make terrestrial gastropods more important crop pests than ever before.

The development of chemical control measures has not kept pace for a number of reasons – some technical, others economic. Perhaps crucial has been the fragmented nature of the market for molluscicidal products, which made commercial research investment relatively unattractive. Primary screens for new molecules did not normally contain a test gastropod, although compounds that demonstrated activity against major pest groups, such as insects, might subsequently be assessed for molluscicidal activity. It is noticeable that even today terrestrial gastropod control still relies heavily on metaldehyde, a chance amateur discovery, and on carbamate pesticides originally developed as acaricides and insecticides.

The technical problems surrounding effective delivery to terrestrial gastropods stem largely from the conflicting requirements for adequate water solubility to penetrate a mucous barrier and adequate persistence in wet environments to match the intermittent activity of the pests. Failure to solve this conundrum has resulted in the near-universal adoption of bait carriers. While these offer some advantages in terms of low rates of active ingredient and a degree of target specificity, they still compare poorly with control measures for other pests, notably insects, themselves a fairly inefficient process. The proportion of the applied dose of insecticide that is transferred to the target has been estimated at 0.02–0.03% (Graham-Bryce, 1975). When the comparative toxicity of the materials used is low the process becomes even less efficient: the LD_{50} of the synthetic pyrethroid deltamethrin to *Musca domestica* Linnaeus (Muscidae) is equivalent to 0.02 mg kg^{-1}, while the equivalent value for metaldehyde to *D. reticulatum* is 1.0 g kg^{-1} (Henderson and Parker, 1986).

Improved formulation of existing molluscicides has probably achieved as much as it can and innovation is required to make significant advances in chemical control of terrestrial gastropods. The recent research interest in modes of action of molluscicides provides a more rational approach to the search for better materials, while the extension of screening to naturally occurring compounds has produced a chemically diverse range of active materials (e.g. Adewunmi and Monache, 1989; Airey *et al.*, 1989; Abdel-Aziz *et al.*, 1990; Watkins *et al.*, 1996). Two compounds, one isolated from a grass (Hagin and Bobnick, 1991) and one from a desert shrub (Hussein *et al.*, 1994), have been found to be more active than some synthetic pesticides. Fundamental studies on the biosynthesis of gastropod mucus (Cotterell *et al.*, 1993, 1994) raise the prospect of novel, target-specific control agents, while investigations into the chemosensory mechanisms of *D. reticulatum* hold out the promise of plant protection based on behaviour-modifying chemicals that do not present a toxic hazard (Dodds *et al.*, 1996).

Whether acting as poisons, as feeding deterrents or in other behaviour-modifying ways and whether deployed as baits, sprays or seed dressings, it is hard to escape the conclusion that what is required is a number of new, better, active ingredients. If the impetus of current research can be maintained they may well be found.

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