MTF 651: A NEW SOIL-APPLIED FUNGICIDE FOR THE CONTROL OF PLASMODIAL FUNGI

G. R. DIXON, M. A. CRAIG

Department of Horticulture, SAC / University of Strathclyde, SAC - Auchincruive, Ayr, KA6 5HW

P. J. BURGESS

Department of Crop Biology, SAC - Aberdeen, 581 King Street, Aberdeen, AB9 1UD

J. THOMAS

Department of Plant Pathology, National Institute of Agricultural Botany, Huntingdon Road, Cambridge, CB3 0LE

ABSTRACT

Dust and suspension concentrate formulations of flusulfamide (MTF651) have been evaluated by field experimentation for the control of *Plasmodiophora brassicae*, the causal agent of clubroot disease of brassicas and *Spongospora subterranea* var. *subterranea*, the causal agent of powdery scab in potatoes. Trials were sited at Auchincruive, Cambridge and Trawsgoed for clubroot and Aberdeen and Rothienorman for powdery scab. Applications of MTF651 at 0.6kg AI /ha, to the soil prior to planting significantly reduced the incidence of clubroot on cabbage and cauliflower. Soil treatment using 1.8kg AI /ha MTF651 was most effective in reducing the incidence of powdery scab when this developed as a result of soil-borne inoculum while spray treatment of seed tubers prior to planting at a rate of 1000 mg AI /l was most effective in controlling powdery scab which developed as a result of tuber-borne infection.

INTRODUCTION

The order Plasmodiophorales contains about 35 species of soil-borne organisms. These are characterised by the production of multinucleate plasmodia which may develop into a group of zoosporangia or cysts and the formation of anteriorly biflagellate, heterocont primary and secondary zoospores from the cysts and sporangia. Within the order are two important plant pathogens: *Plasmodiophora brassicae,* the causal agent of clubroot disease of members of the Brassicaceae and *Spongospora subterranea* var. *subterranea,* the causal agent of powdery scab disease

which principally affects species, varieties and cultivars of *Solanum* but has also been recorded on a wider host range not restricted solely to the Solanaceae (Karling,1968).

The invasion of host roots by *P. brassicae* incites massive disruption of the host growth regulator metabolism leading to the formation of extensive galls. The galls impede water metabolism preventing translocation to the foliage which wilts and frequently accumulates anthocyanin pigments. Photosynthates are re-directed to the root system, yields are reduced substantially and frequently the host plant dies.

Spongospora subterranea var. *subterranea* causes a range of symptoms on the roots, stolons and tubers of the potato plant. Tuber infection results in the formation of unsightly scabs containing masses of brown powdery spore balls which erupt through the periderm. Under some conditions, cankerous forms of the disease develop. Although powdery scab infection has not been correlated with direct yield losses, the appearance of infected tubers is such that the quality and therefore financial value are greatly diminished. This fungus also acts as the vector of potato mop top virus (PMTV), thereby further increasing crop damage associated with it.

Control of both pathogens is largely restricted to the application of cultural methods and disease avoidance techniques, despite the world wide economic importance of the host crops, few resistant cultivars have been bred and only a very limited range of agrochemicals possess the requisite fungicidal activity. Currently only cresylic acid is approved for use against *P. brassicae* and a mixture of maneb and zinc oxide against *S. subterranea* var. *subterranea* in the UK (Ivens,1994). Reliance on such a narrow spectrum of agrochemicals is very unsatisfactory, consequently there is substantial interest in new formulations which may possess activity against either or both fungi. This paper reports field experimentation in 1993, at several sites where dust and suspension concentrate formulations of flusulfamide (2', 4 - dichloro - α, α, α, - trifluoro 4' - nitro - *m* - toluenesulfonanilide) [MTF 651] have been tested for the control of each pathogen. MTF 651 is a new soil-applied fungicide discovered by Mitsui Toatsu Chemicals Incorporated, Japan, and currently under development in Europe through Euro-Japan International Ltd., on behalf of the discoverers.

MATERIALS AND METHODS

Chemical and physical properties of MTF 651

Chemical name : 4-chloro-N-(2-chloro-4-nitrophenyl)-α, α, α, -trifluoro-*m*-toluene sulphonamide, empirical formula: $C_{13}H_7O_4C1F_3N_2S$, molar mass: 415.18, melting point,171.5 - 172.5°C, vapour pressure: 2.69 x 10 9 mm Hg/20°C, specific gravity: 1.739 / 23.0°C (solid), solubility: acetone 31.4, xylene 1.4, ethyl acetate 12.5, chloroform 1.7, methanol 2.4, hexane 0.05, g/100g solvent 25 C, distilled water 2.9 mg/l.

Evaluation for clubroot control

Field trials were established at SAC-Auchincruive and NIAB-Trawsgoed and -Cambridge using modular raised transplants of cabbage (*Brassica oleracea* var. *capitata)* cv. Castello and cauliflower *(B. oleracea* var.*botryis)* cv. White Rock. The land at Auchincruive and Trawsgoed was naturally infested with *P. brassicae* while that in Cambridge had been artificially infected. Details of transplant and plot husbandry are given in Table 1. MTF 651 was applied at 1.2, 0.9 and 0.6 kg AI /ha; dust formulation was applied to the soil surface following mixture with 2kg/plot dry horticultural grade sand (Auchincruive) and directly from a plastic dispencer (Trawsgoed and Cambridge), in the former the plot surface was raked to a depth of 3cm and in the latter cultivated mechanically to a depth of 10cm. The suspension concentrate was applied in 400l/ha water using an Oxford Sprayer (Model CO2). In each trial there were two control treatments viz: no chemical applied and treatment with thiophanate - methyl (Mildothane, 500g AI /l SC), applied as a root drench after transplanting at Auchincruive and prior to transplanting at Trawsgoed and Cambridge. This material is no longer cleared for use on vegetable brassica crops for clubroot control but was used under 'Experimental Permit Conditions' as the only recently approved chemical with an efficacy claim against this pathogen which was available in sufficient quantities. Experiments at all sites were designed as randomised blocks and composed of 6 replicates at Auchincruive and Trawsgoed and 4 at Cambridge. Cabbage plants at Auchincruive were grown in five row plots with 300mm centres at a density of 20 plants per row; at Trawsgoed the cauliflowers were also grown in 5 row plots with a population of 25 plants per row; at Cambridge the plots were of 2 rows with 20 plants per row with 0.5m between and 0.4m within row spacings. Transplanting was accomplished mechanically at Auchincruive and manually at Trawsgoed and Cambridge.

Disease symptoms were assessed visually using at Auchincruive a 0-3 scale, where 0 = nil symptoms, 1 = <10 percent of root diseased, 2 = < 50 percent of root diseased, 3 = >50 percent of root diseased and at Trawsgoed and Cambridge 0-5 scale, where 0 = nil symptoms, 1 = <20, 2 = <40, 3 = < 60, 4 = < 80 and 5 = > 80 per cent of roots clubbed. Appropriate yield and quality characteristics were recorded at each site.

Evaluation for powdery scab control

Two field experiments were established in 1993 by SAC-Aberdeen, in the first (Rothienorman) mostly uninfected tubers were planted into soil which was heavily infested with the pathogen and in the second (Aberdeen) infected tubers were planted into soil with a high inoculum potential; thereby representing the two main sources of host infection. Each experiment contained 6 treatments: untreated control, soil treatments with 1.2 and 1.8 kg AI /ha MTF 651, seed treatments prior to planting with 400 and 1000 mg AI /l MTF 651 and a mixture of maneb and zinc oxide (Mazin) applied as a dust formulation to the tubers before planting. The health status of tubers was evaluated prior to planting and shown in Table 2. Seed tuber

treatments with MTF 651 were applied, when dormancy had broken but the sprouts were <3mm, as an hydraulic spray (2 l/ha) to tubers passing over a roller table; treated tubers were allowed to dry before returning to the cold store. At both sites, soil treatments were applied with an Azo sprayer (2.5 bar, 196 l/ha) and incorporated mechanically immediately afterwards. Plots at Rothienorman were 5m (20 tubers) and at Aberdeen 6m (25 tubers) long and 4 drills wide with the central 2 rows used for assessment. Each trial was designed as a randomised block with 4 replicates at Rothienorman and 5 in Aberdeen. At the termination of each experiment the incidence (percentage of diseased tubers) and severity (mean surface area of tubers infected) were assessed on a sample of 100 washed tubers. Details of crop husbandry are shown in Table 3.

TABLE 1. Husbandry procedures for clubroot experiments.

	Site		
Procedure	Auchincruive	Trawsgoed	Cambridge
Plant rising	modular under protection using peat based compost		
Foliar feed	3 times weekly at week 5	twice weekly at week 4	
Cabbage root fly control	chlorfenvinphos granules incorporated into compost	fonofos drench prior to transplanting	
Cultivations	primary ploughing and secondary harrowing to planting tilth		
Fertiliser (NPK)	260:100:135 kg/ha	260:120:120 kg/ha	
Planting date	10 June 1993	10 June 1993	1 June 1993
Post-planting herbicides	chlorthal-dimethyl + propachlor	tebutam + propachlor	nil*
Post-planting insecticides	chlorfenvinphos deltamethrim methiocarb	chlorfenvinphos thiometon methiocarb	nil nil methiocarb
Foliar feed	nil	calcium + boron, molybdenum	

All chemicals applied in accordance with 'Label Recomendations'
* = At Cambridge polyethylene covers were used to suppress weed growth and consequently no herbicides were required

TABLE 2. Incidence and severity of powdery scab on seed tubers prior to planting.

Site	*Spongospora subterranea var. subterranea*	
Rothienorman	16	1.7
Aberdeen	57	18.6

* % incidence = percentage of tubers with pathogen
** % severity = mean surface area of tubers infected

TABLE 3. Husbandry procedures for powdery scab experiments.

Procedure	Site	
	Rothienorman	Aberdeen
Main disease source	soil	seed
Cultivation	sprayed (paraquat), disced, harrowed, de-stoned	winter ploughed, disced, rotaspiked
Fertiliser (NPK)	100:200:85 kg/ha	135:135:170 kg/ha
Cultivar	Estima	Estima
Date of planting	11 May 1993	4 May 1993
Planting method	Packman cup planter	manual
Drill width	76 cm	76 cm
Herbicides	linuron	monolinuron + paraquat, cycloxydim
Fungicide and insecticide	cymoxanil + mancozeb + oxadixyl; cyoxonil + mancozeb; fentin acetate + maneb deltamethrin + heptenophos	cymoxanil + mancozeb + oxadixyl, mancozeb, fentin hydroxide
Dessiccation	diquat + fentin hydroxide	diquat
Date of lifting	22 October 1993	20 October 1993

All chemicals applied in accordance with 'Label Recommendations'

RESULTS

Results obtained with MTF 651 for the control of clubroot are shown in Table 4, and those obtained for the control of powdery scab are shown in Table 5. In the Auchincruive experiment, MTF 651 significantly reduced clubroot symptoms compared with both the untreated control and applications of thiophanate - methyl; indeed there was no significant difference between the two control treatments. There was no significant difference in the level of control achieved between the various rates of MTF 651, but the lowest rate appeared most effective. Applications of the suspension concentrate formulation were more effective in reducing clubroot symptoms in both the Trawsgoed and Cambridge experiments compared with dusts. Again there was no significant difference between the control treatments. The most efficaceous treatments were: 0.6 kg AI /ha SC and 1.2kg AI /ha Dust at Trawsgoed and 0.9 kg AI /ha SC and 1.2 kg AI /ha Dust at Cambridge.

Soil applications (1.8 kg AI /ha) of the SC formulation of MTF 651 significantly reduced both the incidence and severity of powdery scab at each site. At Rothienorman where disease pressure was particularly high this treatment resulted in a significantly reduced incidence of disease when compared with all other treatments. Seed treatment was ineffective at this site, where the main source of inoculum was

soil-borne. At the Aberdeen site, however, where disease was seed tuber-borne application of MTF 651 at 1000 mg AI /l appeared to be an effective treatment.

TABLE 4. Results of field experiments testing MTF 651 for control of *Plasmodiophora brassicae* (clubroot).

Treatment	Dose (kg AI/ha)	Site Auchincruive*	Trawsgoed**	Cambridge**
Control (untreated)		2.3	43.8	25.0
Thiophanate-methyl		2.3	31.8	25.2
Dust formulations				
MTF 651	0.6	1.7	25.6	10.5
	0.9	1.9	29.6	6.3
	1.2	1.8	21.2	4.3
SC formulation				
MTF 651	0.6	NA	16.1	4.8
	0.9	NA	16.6	3.5
	1.2	NA	20.8	7.0
LSD (P = 0.05)		0.55	14.02	9.40

NA = not applied, * = 0-3 scale, ** = Disease Index (%) calculated as weighted means from 0-5 scale, scales defined in the text

TABLE 5. Effects of soil and seed treatments with MTF 651 on powdery scab.

Treatment	Dose Rate	Site Rothiernorman Incidence*	Severity**	Aberdeen Incidence*	Severity**
Control (untreated)		98.2	21.9	28.8	5.6
Maneb + zinc oxide		67.0	17.0	10.6	1.4
MTF651 soil	1.2+	54.7	12.9	10.8	2.1
	1.8	38.0	3.9	6.0	1.0
MTF651 soil	400 ++	85.5	18.1	11.0	1.8
	1000	87.0	25.3	6.0	0.8
LSD (P = 0.05)		14.83	9.05	5.38	1.64

* incidence= % diseased tubers, **severity= surface area of tuber infected
+ = kg AI/ha, ++ = mg AI/ha

Other characteristics were recorded particularly in relation to the growth, yield and quality of each host (cabbage, cauliflower and potato) but no significant differences were observed between treatments.

DISCUSSION

The efficacy of MTF 651 (flusulfamide) for the control of clubroot in brassica vegetables and powdery scab in potatoes using dust and suspension concentrate formulations, has been established by field experimentation distributed widely throughout Great Britain. It appears that the suspension concentrate formulation is superior to dust and that the lower rates of use may be most cost-beneficial. Records were made of yield and product quality characteristics, the data indicated that use of MTF 651 resulted in financial gains; indeed in the Auchincruive experiment cabbage yields were increased by 25% and at Trawsgoed the cauliflowers showed improved foliar growth and curd quality.

The need for additional fungicides for the control of both *P. brassicae* and *S. subterranea* var. *subterranea* is amply justified by the paucity of current systems of control. Very few cultivars of vegetable brassicas possess resistance to *P. brassicae* (Dixon & Robinson, 1986), while only one chemical currently possess a 'Label Recommendation' (cresylic acid) and this requires to be applied with very high volumes of water. Research has identified that applications of boron (Webster & Dixon, 1991; Craig & Dixon, 1993), specific surfactants (Humpherson-Jones, 1993) and calcium cyanamide (Humpherson-Jones, *et al* 1992) will significantly reduce clubroot disease but no general recommendations for effective use are yet available. Similarly there is a lack of potato cultivars with resistance to powdery scab and only a single fungicide combination (maneb and zinc oxide) cleared for use. The control of powdery scab by maneb and zinc oxide is highly variable and particularly poor when subjected to high disease pressures (Burgess *et al*, 1992). Such conditions occurred at the Rothienorman site in this trial series where the degree of control offered by MTF 651 soil treatment was significantly greater than maneb and zinc oxide. Further development work with MTF651 is therefore, much needed to establish the most efficient application rates and timings associated with formulations having greatest fungicidal effect.

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TOWARDS THE RATIONAL USE OF TRIAZOLE MIXTURES FOR CEREAL DISEASE CONTROL

SHEILA J. KENDALL, D.W. HOLLOMON

Department of Agricultural Sciences, University of Bristol, Institute of Arable Crops Research, Long Ashton Research Station, Bristol BS18 9AF

D.A. STORMONTH

Brown Butlin Group Ltd. Brook House, Manor Street, Ruskington, Lincs., NG34 9EP

ABSTRACT

The diversity of triazole chemistry is matched by considerable variation in biological activity. Despite an underlying common mode of action, differences in disease control spectrum, uptake, mobility, persistence and cross-resistance patterns all contribute to extensive biological diversity of triazoles, and might provide a basis for improving performance through use of triazole mixtures. In some of these mixtures, synergistic action may well enhance overall performance still further. The importance of these factors in underpinning the use of triazole mixtures is discussed against a background of limited field performance data. By combining a rapidly translocated triazole with one that moves more slowly and is evenly distributed, improved control of late season wheat diseases may be achieved with a combined dose rate equivalent to each product used alone. However, a much extended series of field experiments is needed to confirm any underlying scientific basis for improved disease control using triazole mixtures.

INTRODUCTION

The first systemic triazole fungicide, triadimefon was introduced into agriculture almost twenty years ago. Its broad spectrum and strength of activity, coupled with good systemic movement, set new standards for cereal disease control, and triadimefon was soon accepted by growers especially for mildew and rust control. This heralded an intensive period of research which not only identified their mode of action as inhibitors of fungal sterol biosynthesis (at the 14α-demethylase step; DMIs, Köller, 1991), but also examined their metabolism and mobility within plants. Extensive development work optimised both timing and disease control and contributed to the widespread use of triazoles as both seed treatments and foliar sprays. The appearance of triazole resistance in cereal mildews (*Erysiphe graminis*) (Fletcher & Wolfe, 1981), added another dimension to this research as efforts were made to evolve strategies to minimize the resistance problem. All these research activities complemented programmes of chemical synthesis and screening in many companies, which still provide new triazoles for evaluation and development. As a result, some 25 different triazoles are now used in agriculture, and a similar number are available as antifungals in medicine.

FIELD PERFORMANCE OF TRIAZOLE AZOLE MIXTURES

TABLE 1. Field performance of triazole mixtures against late season diseases of wheat.

a. Control of brown rust (*Puccinia recondita*) on cv. Riband, Lincolnshire 1993

Treatment at GS55	Rate g AI/ha	% control 28 DAT	Yield t/ha	Margin over input cost Δ £/ha
Flutriafol	125	69b+	9.1b	108
Flutriafol + tebuconazole	94 + 62.5	88c	10.1a	206
Flutriafol + tebuconazole	62.5 + 125	91c	10.7a	265
Tebuconazole	250	91c	10.4a	232
Untreated		(97)a	7.8c	-

b. Control of septoria diseases: *Septoria* (*Leptosphaeria*) *nodorum* and *Septoria* (*Mycosphaerella*) *tritici* on leaf 2 and leaf 3 on cv. Riband, Lincolnshire 1993

Treatment at GS39	Rate g AI/ha	% control L3 28DAT	% control L2 42DAT	Yield+ t/ha	Margin over input cost Δ £/ha
Trial 1					
Flutriafol	125	74bc	93b	11.0a	188
Flutriafol + tebuconazole	62.5 + 125	79c	98b	11.5a	235
Tebuconazole	250	69bc	99b	11.4a	222
Untreated		(48)a	(28)a	8.9b	-
Trial 2					
Flutriafol	125	65bc	55bc	6.20b	
Flutriafol + tebuconazole	62.5 + 125	89c	77c	7.15c	
Flutriafol + cyproconazole	62.5 + 32	54b	47b	6.40b	
Untreated		(6%)a	(15%)a	5.60a	

Δ Margin Over Input Cost = Benefit less fungicide treatment cost
Figures in parenthesis are % leaf area infected in untreated control

\+ Figures in the same columns followed by the same letter are not significantly different at P = 0.05

Source: Stormonth (unpublished results)

The success of the agrochemical industry in developing new triazoles has certainly offered improvements in disease control, but even early triazoles still offer value for money. Differences in mobility, disease control spectrum, curative and eradicant action provide opportunities to complement these properties in mixtures to improve field performance. In this review we explore some of the scientific issues that might underpin the rational use of triazole mixtures.

Glasshouse experiments with triadimenol(Bayfidan)/tebuconazole (Folicur) mixtures showed benefits for wheat mildew control, over what could be achieved using either triazole alone (Kaspers, *et al.*, 1987). Subsequent work extended these findings to field performance, and led to the introduction of a co-formulation (Silvacur; Matador), in which both triazoles are used. More recent field studies have examined other triazole mixtures, which not only improve control of specific diseases, but also enhance yield and quality (Table 1). In mixtures where the dose of each partner was reduced, so that overall the mixture rate was equivalent to the field rate of either partner when used alone, control of both brown rust and Septoria diseases was as good, and sometimes better, than when a single azole was used. Although yield increases were not always significant, these trials indicate that by using cheaper fungicide mixtures, the level of disease control can match that achieved with a single product, but at less cost. Many uncontrolled environmental factors can influence field results, but with similar findings obtained at a number of sites (Du Rieu *et al.*, 1994), there may be underlying scientific reasons for the benefits obtained from using triazole mixtures.

Disease control spectrum

Seldom is a single disease the sole cause of crop damage. A feature of triazole fungicides, and indeed a major factor driving development of new ones, is that none provided sufficient activity against all the important cereal diseases. Nevertheless, scope to combine triazoles to complement weaknesses in their disease control spectra, is rather limited. Triazoles have a wide range of activity against cereal powdery mildews, but where mildew is a factor, a morpholine would be the choice for a mixture partner with a DMI. For *Rhynchosporium secalis* in barley, either benzimidazoles or morpholines are the best mixture partners, especially as a strategy to combat triazole resistance (Kendall *et al.*, 1993). Net blotch (*Pyrenophora teres*) is another barley disease where triazoles show a range of activities, but triazole mixtures seem not to have been explored as a way to improve control. Laboratory and greenhouse tests show wide differences in activity of triazoles against eyespot (*Pseudocercosporella herpotrichoides*), but so far only flusilazole (Sanction) has any practical value for eyespot control in the field although the imidazole, prochloraz is widely used also. Differences between triazoles in their control of rusts and Septorias can be rather small (Table 2), so that whereas triazole mixtures seem to offer benefits for control of late season wheat diseases, differences in their disease control spectrum may not be a major factor. Epoxiconazole (Opus) can provide excellent Septoria control (Figure 1) but like many other triazoles, where timing is not optimum differences may emerge and control of *S. nodorum* may be better than that of *S. tritici*. This emphasizes that the curative and eradicative properties of triazoles can be important in controlling the two Septoria diseases.

The underlying biochemical basis for any differences in disease control between

triazoles is not always clear. Sterol 14α-demethylase (14DM) is a major target site for all triazoles (Kwok & Loeffler, 1993), and is an essential enzyme in the sterol biosynthesis pathway in all pathogenic fungi. But triazoles probably have other target sites, including other steps in sterol biosynthesis. In cereal powdery mildew, the effect of flutriafol (Pointer) on the introduction of the Δ^{5-6} double bond is just as great as its effect on 14DM (Senior, 1991). In *R. secalis*, propiconazole (Tilt) inhibits both sterol Δ^{22} desaturase and 14DM (Girling *et al.*, 1988). Any benefits of triazole mixtures may well derive, at least in part, through complementation of these different modes of action.

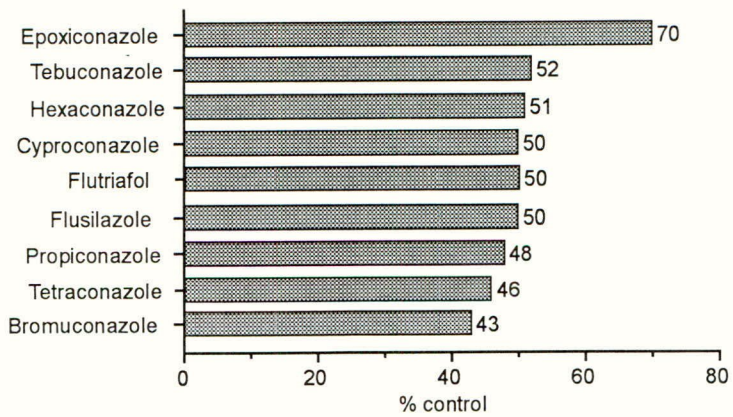
TABLE 2. Efficacy of triazole fungicides in controlling foliar diseases of wheat.

Fungicide	Partition coefficient octanol/water (log P)	Disease		
		Puccinia recondita	*Septoria tritici*	*Septoria nodorum*
Triadimenol	3.2	**	*	*
Tebuconazole	3.7	***	***	**
Propiconazole	3.8	**	**	**
Flusilazole	3.75	**	**	***
Cyproconazole	2.9	***	***	**
Flutriafol	2.3	**	**	**
Epoxiconazole	3.4	***	***	***

*** consistently good ** good * some activity

This table is based on the authors' experiences, including work with immunodiagnostics to identify Septoria species.

FIGURE 1. Triazoles and Septoria species control in France



Source: Seven ITCF trials 1992 (C. Maumené, personal communication 1994)

Mobility and metabolism

Fungicide mobility is related to lipophilicity (Shephard, 1985) and is a function of the logarithm of the concentration ratio between two phases, usually octanol-water (Log P). In fact, all commonly used triazole fungicides penetrate through the cuticle reasonably quickly, and it is their subsequent systemic movement in the translocation stream which varies. Log P ranges from 2.3 for flutriafol (Table 2) which is very mobile and accumulates at leaf margins, to propiconazole (3.9) which moves slowly once inside the leaf (Table 3). Tebuconazole (3.7) and flusilazole (3.75) accumulate only slightly at leaf tips, and achieves a very even distribution within leaf tissue (Kuck and Thielert, 1987; Smith *et al*, 1992). Difenoconazole (Plover) penetrates leaf tissue quickly, and has good translaminar activity, but moves only slowly in the transpiration stream (Leadbeater, personal communication, 1994). The impact of mobility differences on disease control is shown in Table 3, and highlights the fact that triazoles translocated slowly within plant tissue may make good mixture partners with faster moving triazoles.

Metabolism of triazoles by fungi to inactive derivatives is very limited and, apart from the possible conversion of triadimefon to the more active triadimenol (Clark *et al.*, 1978), metabolism does not seem a major factor governing any improved performance of triazole mixtures. Differential rates of degradation within plants may be important, but we have been unable to find much published data on this aspect of performance.

TABLE 3. The translocation of flutriafol and propiconazole in barley as measured by control of barley powdery mildew (*E. graminis* f.sp. *hordei*)

0.5 mg/l droplets applied to section A	Leaf Section A (base)	B	C	D (tip)	overall mean A - D
	mean % disease				
Flutriafol	69	41	0	0	27.5
Propiconazole	3.8	0	37	80	30.2

From: Shephard, 1985

Synergism and antagonism

Triazole fungicides interact with both the haem and the sterol substrate binding site of the target 14DM. They not only vary in their ability to block the oxygen function of the haem, but also in their ability to bind in place of the natural sterol substrate (normally, 24-methylene dihydrolanosterol). Triazoles also possess at least one asymetric carbon or silicon atom, and complementation with mixtures of the effects of different stereoisomers may well enhance disease control. The formulation used for one fungicide of a mixture may well enhance the uptake and movement of a second triazole. These subtle differences in the action of triazoles may well account for the synergistic interaction observed in laboratory and greenhouse studies. A weight ratio between 1:1 and 1:4 produced the maximum synergistic action for mixtures of cyproconazole (Alto) with another triazole (Gisi, 1994). Synergism was most pronounced when mixture components were applied together, but less clear when foliar applications were split. We have explored the interaction between tebuconazole and propiconazole or triadimenol, and measured the growth of both *Neurospora crassa* and *Cladosporium cucumerineum*, *in vitro*, at all combinations of the two fungicides used in five-fold dilution series. We

then calculated the ED_{50} value for each fungicide at a single concentration of the other. The basis of our analysis was non-interaction between the mixture partners, and any deviations away from the ED_{50} value in the total absence of the mixture partner, indicated either synergy or antagonism. Five experiments were carried out and all produced similar results. Data for one experiment with *N. crassa* are shown in Figure 2, in which growth at each propiconazole concentration in the absence of tebuconazole has been normalized to 100 for the purposes of comparison. Not only did we observe synergism with some triazole mixtures, but at other combinations we observed antagonism. The significance of synergy and antagonism identified under defined laboratory and greenhouse conditions has yet to be explored seriously with triazole mixtures in the field.

Resistance

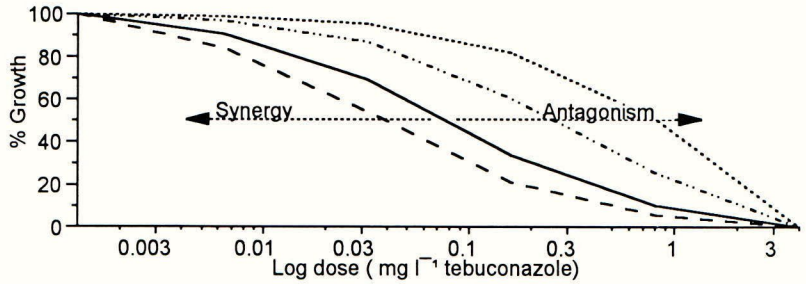
Resistance is a problem associated with DMIs in several important cereal diseases. Cross-resistance is generally the case, but the level of resistance can differ substantially between triazoles, and often in an unpredictable manner (Kendall, 1986). However, resistance factors for tebuconazole were consistently lower than for triadimenol in strains of different fungi (Table 4). Where resistance to triadimenol is already a problem in a pathogen population, mixtures containing tebuconazole are likely to give improved disease control, especially where the mixture partner provides better control of other diseases than triadimenol. Nevertheless, where two triazoles are selecting for different resistance factors, mixtures may well generate populations resistant to both triazole partners.

TABLE 4. Cross-resistance patterns between triadimenol and tebuconazole in different cereal pathogens.

Disease	No. strains	Activity = ED50 mg/ml	
		Triadimenol	Tebuconazole
E. graminis f.sp. *hordei*			
Resistant	14	1.25 ± 0.08	0.115 ± 0.008
Sensitive	10	0.019 ± 0.002	0.014 ± 0.002
Resistance factor		66	8
E. graminis f.sp. *tritici*			
Resistant	8	1.50 ± 0.112	0.29 ± 0.042
Sensitive	18	0.087 ± 0.006	1.01 ± 0.01
Resistance factor		17	3
R. secalis*			
Resistant	48	51.2	10
Sensitive	53	0.8	0.4
Resistance factor		64	25

*Activity = Minimum inhibitory concentration mg/ml
Data from Hollomon and Butters, 1991; De la Pena,1990

FIGURE 2. Sensitivity of *Neurospora crassa* to tebuconazole in the presence of different propiconazole concentrations. (Kendall and Hollomon, unpublished results)



	Propiconazole (mg l⁻¹)			
Tebuconazole	0	0.0064	0.032	0.16
ED_{50} (mg l⁻¹)	0.077	0.038	0.252	0.824
Se ± (P=0.05)	0.007	0.008	0.016	0.042

CONCLUSIONS

Despite some underlying similarities between triazoles in their mode of action, they in fact represent a diverse group of fungicides in many other respects. Although there are indications of some scientific basis for improving cereal disease control through use of triazole mixtures, the significance of these factors has yet to be established through field experiments. The interesting results based on a few trials need to be extended to cover a much wider range of field conditions, different cultivars and mixture combinations, before firm conclusions can be drawn. Scope for simply enlarging the cereal disease control spectrum by using triazole mixtures is rather limited, with possible benefits directed towards control of the late season foliar wheat diseases, rusts and Septorias. More significantly perhaps, disease control may be improved by mixing a fast moving triazole with one that remains evenly distributed within the leaf over a long period, and which has good curative action, especially against Septorias. Other complementary properties of triazoles such as vapour phase activity and redistribution over leaf surfaces emphasise the likely complexity underlying any enhanced disease control observed with some mixtures compared to an individual product used alone. Synergy of triazole mixtures may also play a part in improved disease control, although synergistic interactions observed in laboratory and greenhouse experiments need confirming under different field conditions. Moreover, if triazole mixtures do improve disease control we need to establish whether dose rates should be lowered to maintain disease control and selection pressure at a constant level, or whether rates can be maintained and biological performance improved. Despite this caution, maximizing the properties of triazoles through mixtures in order to cheapen disease control is a worthwhile aim, and should be evaluated further through a more systematic series of field experiments.

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Session 5B

The Biology and Control of Mites

Chairman	Dr D V Alford
Session Organiser	Mr M A Easterbrook
Papers	5B-1 to 5B-4

CONTROL OF THE FRUIT TREE RED SPIDER MITE *(Panonychus ulmi)* AND THE APPLE RUST MITE *(Aculus schlechtendali)* IN APPLE ORCHARDS

G. STERK

Dep. Zoology, Gorsem Research Station, B-3800 Sint-Truiden
Present Address: BIOBEST N.V., Ilse Velden 18 B-2260 Westerlo, Belgium

ABSTRACT

The fruit tree red spider mite, *Panonychus ulmi,* and the apple rust mite, *Aculus schlechtendali,* are important pests in apple orchards in most European countries. Both species have developed resistance against several compounds of different chemical groups. Some new acaricides are now coming onto the market. Their effects on both species are discussed. Because of the importance of the predatory mite, *Typhlodromus pyri,* against fruit tree red spider mites and rust mites in apple orchards in most west European countries, the side-effects of different new acaricides or insecticides with acaricidal activity on this important antagonist are also discussed.

INTRODUCTION

Panonychus ulmi, the fruit tree red spider mite, is a widespread and important pest of apple and other fruit trees, including plum, cherry and pear. During the 1960s and 1970s, *Panonychus ulmi* became resistant against several compounds like omethoate, dimethoate and some acaricides. At the moment, there are difficulties in several European countries with the ovolarvicidal acaricides, hexythiazox and clofentezine (Sterk and Highwood, 1992).

Another important mite is the small apple rust mite, *Aculus schlechtendali.* This species was unknown to most fruit growers, being controlled with each treatment with organophosphates, pyrethroids, chlorinated hydrocarbons or carbamates against different pests. In 1985 however, serious problems started to occur in apple orchards in Belgium and The Netherlands, leading to severe economic damage. A number of trials showed clearly resistance to synthetic pyrethroids, even those with good acaricidal activity, carbamates, chlorinated hydrocarbons and organophosphates (Sterk and Highwood, 1992).

In contrast to other pests which became resistant, like the pear sucker, *Psylla pyri*, a number of compounds are still very effective against fruit tree red spider mites and/or rust mites, making these problems for the present less urgent. This is one of the main reasons that integrated pest management in apple orchards with the introduction of the predatory mite, *Typhlodromus pyri*, is less prevalent in some countries. Some new acaricides may also fit into IPM in apple orchards, and are therefore not only tested for their activity against the fruit tree red spider mite and the apple rust mite, but also their toxicity to the predatory mite is evaluated in field trials.

EFFECTS ON NOXIOUS MITES

Materials and methods

This is a revieuw of the trials, made in Belgian orchards, between 1988 and 1993.

Table 1 . Compounds

Active ingredient	Formulation	Company	Code
pyridaben	150 EC	Nissan	NCI 129
tebufenpyrad	20 WP	Mitsubishi, Cyanamid	AC 801757 MK-239
fenpyroximate	050 SC	Nihon Nohyaku	NNI 850 R 104430
fenazaquin	200 SC	DowElanco	EL 436
abamectin	018 EC	Merck, Sharpe & Dohme	LX1225-01
brofenprox	100 EC	Mitsui	MTI 732
flufenoxuron	100 WDC	Cyanamid	WL 115110
flucycloxuron	25 L	Mitsui, Duphar	PH 70-29

Pyridaben, fenpyrad, fenpyroximate and fenazaquin are acaricides, acting on mitochondrial respiration. In some cases, they also have an activity against Homoptera like the white fly, *Trialeurodes vaporariorum*, or the pear sucker, *Psylla pyri*.

Abamectin, based on *Streptomyces avermitilis*, is, in addition to being a very good acaricide, also very effective against leafminers, pear suckers and the western flower thrips, *Frankliniella occidentalis*.

The activity of brofenprox is not yet fully known, but it is also a very active acaricide.

Flufenoxuron and flucycloxuron are insect growth regulators, ot the chitine-synthese inhibitor type, with a very good activity on some insect species like codling moth, *Cydia pomonella*, and winter moth, *Operophtera brumata*, but which normally will be brought into the market as acaricides. Both of them are also known as acaricidal insect growth regulators.

Fruit tree red spider mite

The trials were carried out in orchards with a homogeneous population of fruit tree red spider mites. Trials were carried out on winter eggs, hatching larvae, at 50 % hatch of the winter eggs and on mixed stage populations in summertime.

Treatments were made by using a knapsack sprayer, type Stihl. The amount of water was dependent on the height of the trees and the plant system, with 1000 litres per Ha being the standard, according to a standard orchard of average age and height, and planted in a one row system. Standard compounds were clofentezine (Apollo 480 SC) and hexythiazox (Nissorun 10 WP) for the trials on winter eggs and hatching larvae, and azocyclotin (Peropal 25 WP) for the trials on larvae, adults or mixed summer populations.

The assessment was made when mobile stages were abundant. Twenty five or 50 leaves, depending on the level of infestation, were collected, brushed and the living larvae and adults were counted under a binocular microscope.

The results were calculated by using the Abbott-formula.

Apple rust mite

These trials were carried out in orchards with a homogeneous population of apple rust mites. Trials were carried out before blossom and on mixed populations in summertime. Treatments were made as for *Panonychus ulmi*. Negative standard compounds were clofentezine (Apollo 480 SC) and hexythiazox (Nissorun 10 WP) for the trials with flufenoxuron (Cascade) and flucycloxuron (Andalin), and azocyclotin (Peropal 25 WP) was used as a positive standard in the trials on mixed summer populations.

The assessment was made when mobile stages were abundant. Twenty five or 50 leaves, depending on the rate of infestation, were collected, brushed and the living larvae and adults were counted under a binocular microscope.

The results were calculated by using the Abbott-formula.

Results

The results are given as % mortality. The number in brackets indicates the year when the trials were carried out.

Table 2 . Fruit tree red spider mite : ovolarvicidal acaricides and acaricidal growth regulators

Product % formulated compound	Winter-eggs	Start hatching	50 % hatching	Mixed population
flufenoxuron 0.050	89 (6)		100 (2) 100 (3) 100 (5) 95 (6)	86 (2) 76 (3)
flucycloxuron 0.050			95 (1) 100 (5) 98 (6)	84 (3)
clofentezine 0.030	98 (2) 96 (3) 89 (4) 100 (6)	97 (1) 94 (2) 92 (4) 99 (5)	92 (1) 96 (3) 99 (5) 98 (6)	
hexythiazox 0.030	95 (2) 76 (3) 100 (6)	96 (2) 77 (4) 98 (5)	98 (3) 100 (5) 99 (6)	

(1) = 1988, (2) = 1989, (3) = 1990, (4) = 1991, (5) = 1992, (6) = 1993

Table 3. Fruit tree red spider mite : acaricides active on mobile stages of *P. ulmi*

Product % form. compound	Winter-eggs	Start of hatching	50 % hatch	Mixed population
pyridaben 0.050			92 (2) 99 (3) 98 (5) 99 (6)	96 (1) 97 (2) 98 (3) 99 (4) 100 (5)
pyridaben 0.100		65 (1)	100 (1)	100 (1)
tebufenpyrad 0.050	4 (3) 23 (4)		100 (4) 100 (6)	100 (2) 100 (4) 100 (5)
fenpyroximate 0.080	26 (3)		92 (2) 99 (5) 100 (6)	95 (2) 100 (3) 100 (4) 99 (5)
fenpyroximate 0.100			100 (5) 100 (6)	100 (4)
fenazaquin 0.070	0 (3)		98 (2) 93 (3) 100 (6)	100 (2) 100 (3)
fenazaquin 0.100			96 (3) 100 (5)	99 (2) 100 (3)
brofenprox 0.035	39 (4)		91 (5)	100 (4) 100 (5)
brofenprox 0.050			98 (6)	
brofenprox 0.070			99 (5)	99 (3) 99 (4)
abamectin 0.100	13 (4)	46 (4)	99 (3) 99 (6)	
azocyclotin 0.100			98 (1) 84 (2) 99 (3) 98 (5) 99 (6)	70 (1) 67 (2) 85 (3) 72 (4) 97 (5)

(1) = 1988, (2) = 1989, (3) = 1990, (4) = 1991, (5) = 1992, (6) = 1993

Table 4. Apple rust mite : ovolarvicidal acaricides and acaricidal growth regulators

Product % formulated compound	Preblossom	Mixed summerpopulation
flufenoxuron 0.050	98 (6)	98 (2), 88 (5)
flucycloxuron 0.050	92 (6)	93 (5)
clofentezine 0.030	52 (3), 43 (4), 72 (6)	63 (6)
hexythiazox 0.030	15 (3), 8 (4), 0 (6)	

Table 5. Apple rust mite : acaricides active on mobile stages of *A. schlechtendali*

Product % formulated compound	Preblossom	Mixed summerpopulation
pryidaben 0.050	94 (6)	98 (1), 98 (2) 89 (5), 94 (6)
pyridaben 0.100		98 (1)
tebufenpyrad 0.050	38 (4), 60 (6)	58 (3), 59 (5)
fenpyroximate 0.080	74 (6)	61 (5)
fenpyroximate 0.100	74 (4), 74 (6)	93 (5)
fenazaquin 0.070		87 (2)
fenazaquin 0.100	7 (4), 50 (6)	
brofenprox 0.035	48 (6)	98 (3), 96 (5) 96 (6)
brofenprox 0.050	56 (4), 58 (6)	94 (6)
brofenprox 0.070		95 (3), 96 (5)
abamectin 0.100	100 (3), 98 (6)	
azocyclotin 0.100	91 (2), 98 (3), 88 (6)	85 (1), 99 (2), 81 (3), 92 (5)

(1) = 1988, (2) = 1989, (3) = 1990, (4) = 1991, (5) = 1992, (6) = 1993

Discussion

Flufenoxuron and flucycloxuron are very active on hatching larvae of the fruit tree red spider mite, and are also effective against rust mites. The standards clofentezine and hexythiazox are weak or show no effect at all on this important pest. This means that a treatment during the blossom period with flufenoxuron or flucycloxuron would have the maximum effect on mites and other pests, considering the fact that they also show activity on caterpillars like the winter - moth, *Operophtera brumata*.

The new acaricides with activity on mobile stages are clearly very effective from the moment that 50 % of the winter eggs have hatched. On winter eggs they show only weak activity and their persistence is not long enough to improve this performance.

All of them are certainly very effective in summertime on mixed populations of the fruit tree red spider mite, but the activity on rust mites is different. Only abamectin and pyridaben are comparable with the standard azocyclotin early in the season. Besides these compounds, brofenprox is also effective in the summer on mixed populations. The activity of the other compounds on apple rust mites is rather insufficient.

EFFECTS ON PREDATORY MITES

Material and methods

The trials were done on trees with a sufficient number of predatory mites. A mean value of at least 1 predatory mite per 2 leaves is required. Relatively young bush trees are very suitable for such trials as they are usually left untreated by the growers. It is best to choose orchards where the predatory mite has been introduced in the last year. Because of the abundance of spider mites and rust mites the densities of the predatory mite will be much higher in these recently infested orchards than in stabilised plots. Trees with a sufficient population of predatory mites are selected and the number of predatory mites is precounted on 50 leaves. The numbers of possible prey ; in this case *P. ulmi, Tetranychus urticae, Tydeus spec.* and eriophids, mainly the rust mite, *A. schlechtendali,* are also recorded, to avoid host population density influences.

The trees are sprayed with the test compound by means of a knapsack mist blower until run-off. The control plot is treated with water. Standard compounds are fenbutatin oxide (Torque L) as a non-toxic and bifenthrin (Talstar 100 EC) as a toxic reference.

After +/- 1 and/or 2 weeks after the treatment the remaining mobile stages of predatory mites and possible prey are counted again on 50 leaves.

The results are calculated with the Henderson-Tilton formula and divided into four categories corresponding to the set standard for field methods of the IOBC Working group "Pesticides and Beneficial Organisms".

$$\text{Results : Henderson - Tilton} \quad WG = 100\ \% \left(1 - \left(\frac{K1}{K2} \times \frac{R2}{R1} \right) \right)$$

K1 = Total number of target species before treatment in the control plot
K2 = Total number of target species after treatment in the control plot
R1 = Total number of target species before treatment in the test plot
R2 = Total number of target species after treatment in the test plot

Four evaluation categories are used. 1 = harmless (< 25 %), 2 = slightly harmful (25 - 50 %), 3 = moderately harmful (50- 75 %) and 4 = harmful (> 75 %).

Compounds, dose rates and results

Table 6. Ovolarvicidal acaricides and acaricidal growth regulators

Active ingredient	Formulation	Dose % formulated compound	Category
clofentezine	480 SC	0.030	1
hexythiazox	10 WP	0.030	1
flufenoxuron	100 WDC	0.050	1
flucycloxuron	25 L	0.050	1

Table 7. Acaricides active on mobile stages of phytophagous mites

Active ingredient	Formulation	Dose % formulated compound	Category
tebufenpyrad	20 WP	0.025	2/3
		0.050	4
fenpyroximate	050 SC	0.050	1
		0.080	1/2
		0.160	4
fenazaquin	200 SC	0.075	3
		0.100	4
pyridaben	150 EC	0.050	4
brofenprox	100 EC	0.010	4
		0.020	4
fenbutatin oxide	055 SC	0.045	1/2
bifenthrin	100 EC	0.030	4

Discussion

The insect growth regulators, even the strong acaricides flufenoxuron and flucycloxuron have no toxic effect at all on this predatory mite.

From the new group of acaricides, only fenpyroximate shows a good selectivity for *Typhlodromus pyri*, like the standard fenbutatin oxide. Tebufenpyrad, fenazaquin and brofenprox were all rather harmfull in these trials, like the toxic standard bifenthrin. Abamectin was not applied in these trials, but is also known to be harmless.

CONCLUSION

The new acaricides, now reaching the market, are more effective against *Panonychus ulmi* than the classical miticides, reducing the number of treatments in one season. Some of them are also very effective against rust mites. Depending on their cross-resistance the anti-resistance strategy against the fruit tree red spider mite, based on the ideas of IRAC, can now be improved.

Unfortunately, not all of them fit into IPM-schemes where predatory mites are introduced. Beside fenpyroximate and abamectine, both insect growth regulators, flufenoxuron and flucycloxuron are promising for IPM apple orchards. However, the fact that a compound is not selective for certain important antagonists should never be a reason to withdrawn the product for IPM. It is the task of the local specialists and advisory people to decide if, when and where a compound has a place in an IPM spraying program.

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THE DEVELOPMENT OF CONTROL STRATEGIES FOR *VARROA JACOBSONI* IN COLONIES OF *APIS MELLIFERA*

B.V. BALL

Institute of Arable Crops Research, Department of Entomology and Nematology, Rothamsted Experimental Station, Harpenden, Herts., AL5 2JQ

ABSTRACT

The transfer of the honey bee parasitic mite *Varroa jacobsoni* from its natural host, *Apis cerana*, to *Apis mellifera* and its spread to almost every continent has caused great concern. The loss of many thousands of honey bee colonies has been attributed to mite infestation. Current controls are based on the use of a range of chemical acaricides but these are applied without detailed knowledge of mite population levels in colonies or damage thresholds. A better understanding of the reproductive biology and population dynamics of *V. jacobsoni* and host-parasite interactions will contribute to the development of effective control strategies which minimize chemical inputs.

INTRODUCTION

The honey bee parasitic mite *Varroa jacobsoni* was first described by Oudemans in 1904 on its natural host, *Apis cerana*, in Java and it is present in all regions where this species of bee is indigenous. Reports of *V. jacobsoni* in colonies of *Apis mellifera*, the European honey bee, in Japan, the Philippines and eastern Russia occurred about the middle of this century (De Jong, *et al.*, 1982b). Since that time the importation of stocks and migratory beekeeping have distributed the parasite widely and it is now established in colonies of *A. mellifera* on almost every continent. The spread of the mite across the European mainland was accompanied by reports of devastating colony losses and *V. jacobsoni* is considered a serious threat to world beekeeping, although its effect on colonies is unpredictable and is still poorly understood.

The discovery of the mite in central Europe in the 1970's prompted a period of intensive scientific investigation. However, the largest proportion of published work in the last 20 years reports the efficacy of a range of chemicals for mite control. This may have been justified as an initial response to the establishment of an exotic and damaging pest but this approach must be viewed as a short term solution. In all fields of insect pest control there are numerous examples of the problems that have arisen from placing undue reliance on chemical treatments. Successful long term control strategies which minimize chemical inputs depend upon a detailed knowledge of the biology and behaviour of the target pest. In these respects our knowledge of *V. jacobsoni* and the nature of its association with *A. mellifera* is incomplete. The aims of this presentation are therefore to review briefly existing control measures and, against a background of current information on the reproductive biology of *V. jacobsoni* and host-parasite interactions, to identify areas for increased research effort which will contribute to the

development of effective strategies for the future.

MITE REPRODUCTION

Adult female *V. jacobsoni* feed on the haemolymph of adult bees by piercing the thin membraneous areas between the abdominal sclerites. Mature female mites leave the adult bees and enter honey bee brood cells to reproduce at the stage when the last larval instar is about to be sealed in its cell. The first egg is produced about 60 hr after the cell is sealed and this develops into a male. Subsequent offspring are produced at about 30 hr intervals and all are female. The adult female and female progeny feed on the haemolymph of the developing honey bee pupa and emerge from the cell with the young bee. Male mites live only a short time and their sole purpose is to fertilize sister mites within the same cell.

Mite reproduction on its natural host is almost exclusively limited to the drone brood which is present in colonies only during certain periods of the year and which represents a relatively small proportion of the total bee population. In addition, *A. cerana* has pronounced behavioural traits which enable worker bees to detect and eject mites from sealed brood cells and adult bees actively groom each other to remove mites from the colony. These factors effectively limit the growth of the mite population and limit any damaging effect of infestation.

The mite has a much greater potential for population increase on its new host, *A. mellifera,* as it can successfully reproduce in the worker brood cells. Recent research on mite ontogenesis in worker brood cells in honey bee colonies in this country (Martin, 1994) has shown that although four offspring (one male and three females) can complete their development before the bee emerges, on average only 1.45 female offspring will be produced. This is explained by a significant difference between the mortality of successive female offspring which increases from less than 10% for the first female to 50% for the second and approximately 80% for the third and fourth. In addition, this study also determined that approximately 35% of mites invading brood cells failed to produce viable offspring by the time the bee emerged.

There are significant differences between subspecies of *A. mellifera* in the proportion of non-reproducing female mites in worker brood and in the development periods of worker pupae, which limit the number of mite offspring that are produced or that mature before the bees emerges. Small differences in the reproductive rate can lead to enormous differences between the number of mites produced in a few generations and previous calculations based on theoretical maxima have probably over-estimated mite population development. Nevertheless, untreated colonies are said to die about three or four years after the mites have been detected, although reliable, quantitative data on mite population development over this period are not available.

DAMAGING EFFECTS OF INFESTATION

Newly emerged bees from infested brood cells show a marked reduction in weight in comparison with individuals of the same colony emerging from uninfested cells (De

Jong, *et al.*, 1982a). The weight loss is correlated with the number of mites feeding on the developing honey bee pupa and may be more than 20% when five or more mites are present in the cell. The effect is more pronounced in parasitized worker brood than in drone brood, presumably because protein depletion and reduction in total haemolymph volume is proportionately much greater in the smaller worker pupae. The loss of haemolymph and consequent reduction in hydrostatic pressure has been suggested as a cause of the wing deformity seen in young bees emerging from infested cells. There is some evidence that wing abnormality is correlated with increasing numbers of mites but this effect may be transient in colonies and is often at a low incidence even when infestation is severe. This suggests that factors other than physical damage may be involved.

Qualitative and quantitative changes may be induced in the protein composition of the haemolymph of parasitized bees. Reduction in the total protein concentration is correlated with increasing numbers of mites; the low molecular weight protein fractions in particular, being depleted (Weinberg & Madel, 1985). Changes in the number and type of haemocytes and in the antigenic components of haemolymph are also induced.

The preceding studies suggest that parasitization of the developing honey bee pupa results in the emergence of severely weakened adult bees. This is apparently confirmed by studies on the longevity of infested individuals. The mean lifespan of infested, newly emerged bees shows the greatest reduction of 40-50% during late summer (Kovac & Crailsheim, 1988), which coincides with the dramatic decline in both the adult bee and brood population observed in naturally infested colonies (Ritter, *et al.*, 1984). However, no correlation could be demonstrated between honey bee weight or protein content at emergence and mean lifespan; parameters that might be expected to influence longevity. Moreover, groups of severely infested bees sometimes showed long mean lifespans and no differences in the start of foraging, frequency or duration of flight were observed between infested and non-infested bees of the same colony. The fact that infested bees can have long lifespans and that low rates of infestation can cause severe reductions in longevity indicates that *V. jacobsoni* infestation alone is not the only, perhaps not the primary, cause of mortality.

Recent research has provided new information on the causes of mortality in infested colonies and the role of *V. jacobsoni* as a vector of disease organisms. In severely infested colonies in Germany acute paralysis virus (APV) was found to be a major cause of both adult bee and brood mortality in late summer (Ball & Allen, 1988). In Britain, the virus normally persists as an inapparent, sub-lethal infection in adult bees and has not previously been found to be responsible for mortality in nature. *V. jacobsoni* appears to activate APV to multiply to lethal levels and once infection becomes systemic the mite can then act as a virus vector, transmitting APV to other adult bees or pupae (Ball, 1989).

The factors causing activation of APV replication are at present unknown but damage to tissues during mite feeding may release virus from cells in which it is normally harmlessly contained. Alternatively, the mite may in some way affect the inhibitory mechanism which usually suppresses APV replication. Virus replication may be induced experimentally in adult bees by the injection of foreign proteins and digestive enzymes secreted by the mite may have a similar effect in nature. There is now good evidence

from both field and laboratory studies that *V. jacobsoni* can transmit a range of unrelated honey bee viruses. The variability in the effect of mite infestation in different areas of the world may therefore partly depend upon the natural prevalence of these infections.

CURRENT CONTROL MEASURES AND THEIR LIMITATIONS

Two factors have influenced the development of current control measures. Firstly, mites within sealed honey bee brood cells were unaffected by the first generation of chemical treatments. Secondly, honey and bees wax are natural products of which the consumer makes special demands and chemical residues initially posed significant problems. Current approaches to the control of mite populations may be divided into three categories.

Manipulative methods

These methods exploit the biological necessity of *V. jacobsoni* to enter honey bee brood cells to reproduce. Mites have a strong preference for drone brood and a proportion of the mite population may be removed from infested colonies by the systematic rearing of drone brood that is destroyed after the cells are sealed. Experiments in Germany have demonstrated that the growth of mite populations may be significantly reduced in this way (Rosenkranz & Engels, 1985), but the method is most effective when the level of infestation is low. An alternative method is to confine the queen on a single comb of empty worker brood cells for three successive periods of 8-9 days. Each comb is removed after the cells are sealed and may be either destroyed or returned to the colony for the bees to emerge after the mites have been killed by treatment with formic acid (Fries, 1991). The technique is laborious and because of its demand for adherence to a strict timetable is not practical for all beekeepers, especially those with large numbers of colonies. Moreover, the loss of all young bees over a period of three weeks may severely weaken the colony.

"Soft" chemicals

The use of formic and lactic acids and volatile essential oils from plant extracts has been approved for mite control in some countries. These naturally occurring products are viewed as preferable alternatives to synthetic chemical acaricides but they are not without hazards. Formic acid allowed to evaporate from a plate of pulp board placed beneath the brood nest of the colony killed most mites in tests in Germany, including those in sealed brood cells (Hoppe, *et al.*, 1989), however, the necessary conditions for optimum efficacy are limited in range and difficult to quantify and manage. Treatment may cause the death of the queen in 5-10% of colonies and emerging bees and the oldest stages of brood can also be damaged. In addition, formic acid in the concentration employed (60%) is very caustic and adequate protection is required during handling to avoid direct contact with the skin.

A 15% solution of lactic acid sprayed onto adult bees on every comb in the colony will kill most mites (Kraus, 1991), except those within the sealed brood cells. However, the small difference between the toxicity of lactic acid to bees and mites and the imprecise method of application may result in bee losses or inadequate control of the

mite.

Thymol, eucalyptus oil, menthol and wintergreen oil, applied by various methods to infested colonies, have been shown to exert some control of mite populations, but the efficacy and consistency of results need confirmation. These plant extracts are less hazardous for beekeepers to use but they may impart undesirable flavours or scents to honey.

Treatments using these acids and essential oils need to be repeated several times at intervals of a few days and are therefore impractical for beekeepers with large numbers of colonies. It is also recommended that honey is harvested before treatment to avoid the accumulation of residues.

Proprietary acaricides

Over the past 20 years a wide variety of chemicals have been tested for toxicity to *V. jacobsoni*, many of which are based on agrochemicals used in pest control. However, some products have now been withdrawn and others are registered for use in only a few countries. There are currently five acaricides widely available on the European mainland but only single products, based on the synthetic pyrethroids fluvalinate and flumethrin, are approved for use in the USA and Britain respectively. These products have been favoured by beekeepers because they are simple and quick to apply as the active ingredient is coated onto a plastic carrier which is simply hung between the combs of the colony. The bees pick up the substance adhering to the surface of the plastic strip by body contact and distribute it in this way throughout the colony. As the effectiveness of the pyrethroids persists during the recommended treatment time of several weeks, mites emerging from the brood cells are also killed and 90% of the mite population may be eliminated.

Fumigant strips impregnated with bromopropylate are ignited and allowed to smoulder inside the colony, producing smoke which is toxic to the mites on adult bees. As the method is ineffective against mites within sealed brood cells, treatment is recommended just before the bees form a compact winter cluster. The temperature outside the hive must not fall below 8°C during the evening of treatment and the smoke may cause considerable disturbance to the bees.

The systemic acaricides based on coumaphos and cymiazol are trickled onto bees and distributed within the colony by grooming and food exchange. Treatment is confined to broodless periods and the conditions required for optimum effect are limited in range. These substances may also be fed in sugar syrup to bees but the time of application is critical and there is an increased risk of residues in honey.

If properly applied these acaricides are very effective in reducing mite populations but several have an affinity for wax and improper use or repeated applications may result in the accumulation of residues in the combs. Although the risk of subsequent contamination of honey is slight, persistence of low levels of acaricide in the colony may favour the development of pesticide resistance in mites.

FUTURE DIRECTIONS

The application of commercially available acaricides to control mite populations is likely to remain a favoured option for most beekeepers in the near future. However, their use could be minimized if a simple, reliable means of estimating mite populations in infested colonies could be established and if a correlation between mite population levels and damage thresholds could be demonstrated. At present natural mite mortality is used as an indicator of population levels but this varies widely depending on the time of year, the size of the bee population and the amount of honey bee brood present. This makes estimation of surviving mite populations difficult. Identification of the factors affecting the natural mortality of female mites would not only contribute to more accurate estimates of mite populations but may also provide options for other means of control.

The development of a model of mite population dynamics has been hampered by the lack of data on some aspects of the reproductive biology of *V. jacobsoni* and by the lack of uniformity in the manner in which other information has been gathered. In order to estimate the rate at which the mite population increases in a honey bee colony the number of female offspring produced during the lifetime of a mite and the rate at which they are produced, need to be determined. This fundamental knowledge would contribute to simulations of mite population dynamics which could prove a useful tool in assessing the effectiveness of various treatment regimes and the influence of environmental factors on mite populations.

Some mites produce no offspring, some produce only males and a small proportion reproduce too late to produce viable offspring by the time the bee emerges. Infertility is higher at some times of the year than others and varies considerably with the race of *A. mellifera*. The reason for this is not known but differences in the juvenile hormone titre of developing honey bee pupae are no longer thought to be responsible (Rosenkranz *et al.*, 1993). A more detailed knowledge of the factors affecting the infertility of mites offers the prospect of disrupting mite reproduction and may provide a novel means of limiting mite populations.

Laboratory tests have shown that migrating mites are repelled by geraniol (Hoppe & Ritter, 1988), which is the main component of the Nasanov gland secretion of foraging bees. This may explain the preference of mites for young nurse bees, which keeps them in the honey bee colony near the brood. Disorientation of mites in colonies by the application of geraniol, which would seem harmless to bees, is a possible way of slowing their spread. Suitably arranged sources of geraniol and of the attractants identified by Le Conte *et al.*, (1989) in extracts of drone larvae, might form the basis for efficient traps for mites in bee colonies.

The mortality of infested honey bee colonies has been linked to secondary infection by honey bee pathogens. If disease outbreaks are correlated with the degree of infestation it may be possible to establish a threshold level for recommending some form of intervention to reduce the mite population. Studies on bee immunology, in particular the host response to parasitism, and an understanding of the mechanism of virus activation are needed to identify any differences in host susceptibility and thus identify colonies most likely to suffer damage. Such knowledge could contribute to

honey bee selection programmes aimed at increasing the tolerance of *A. mellifera* to *V. jacobsoni*. However, bee breeding programmes to enhance physiological or behavioural traits which limit mite population development have many difficulties and the relative importance of individual characteristics remains to be determined.

An enzyme-linked immunosorbent assay has been used to detect and quantify APV in individual mites (Allen, *et al.*, 1986). The early detection of secondary infections by sensitive serological tests could help to limit damage to colonies by more effective timing of acaricidal treatments. A better understanding of the factors affecting the persistence and spread of honey bee virus infections in both honey bee and mite populations would also contribute to management strategies designed to reduce their incidence.

There are still immense gaps in our knowledge of the nature of the association between *V. jacobsoni* and *A. mellifera* and there are numerous factors which will affect the outcome of infestation in individual colonies. Investigation of these elements requires a co-ordinated approach drawing on expertise in diverse areas, but the knowledge gained will contribute greatly to our understanding of the problems which is essential for devising effective strategies for the future.

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