Session 5A

New Compounds, Formulations and Uses - Fungicides

Chairman	Dr J Gilmour
Session Organiser	Dr P Gladders
Papers	5A-1 to 5A-7

CGA 219417 - A NOVEL BROAD-SPECTRUM FUNGICIDE

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ABSTRACT

CGA 219417 is a novel broad-spectrum fungicide of the class of pyrimidinamines. It is currently being developed by Ciba as a foliar fungicide for cereals, grapes, fruit, vegetable and field crops and as a seed dressing on barley.

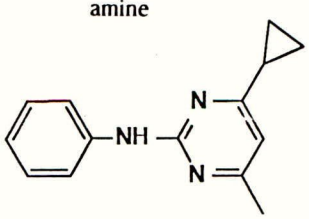
On cereals, CGA 219417 exhibits a broad fungicidal spectrum against a wide range of pathogens such as *Pseudocercosporella herpotrichoides, Erysiphe graminis, Pyrenophora teres, Rhynchosporium secalis* and *Leptosphaeria nodorum*. The strength of CGA 219417 on grapes, vegetables, field crops and strawberries is its excellent activity against *Botrytis* spp. In addition, CGA 219417 shows good activity against *Alternaria* spp. on vegetables and field crops. On deciduous fruit, the key target pathogens are *Venturia, Alternaria* and *Monilinia* spp.

Its new mode of action (inhibition of methionine biosynthesis) and its excellent safety for users, consumers and environment makes CGA 219417 a promising product for flexible use in integrated disease control practices.

INTRODUCTION

The broad-spectrum pyrimidinamine fungicide CGA 219417 was discovered in 1987 by Ciba and introduced into the cereal market in 1994 in France and Switzerland. This paper is a review of its performance against key target pathogens in cereals, grapes, fruit, vegetable and field crops.

CHEMICAL AND PHYSICAL PROPERTIES

Common Name (BSI proposed name):	cyprodinil
Chemical Class:	pyrimidinamine
Chemical Name (IUPAC):	*N*-(4-cyclopropyl-6-methyl-pyrimidin-2-yl)-aniline
Chemical Name (CA):	4-cyclopropyl-6-methyl-*N*-phenyl-2-pyrimidin-amine
Structural Formula:	
Molecular Formula:	$C_{14}H_{15}N_3$
Molecular Weight:	225.3

PRODUCT SAFETY

Mammalian toxicity

Acute oral LD50	Rat:	> 2000 mg/kg
Acute dermal LD50	Rat:	> 2000 mg/kg
Acute inhalation LC50	Rat:	> 1200 mg/m^3 ①
Eye irritation	Rabbit:	non-irritant
Dermal irritation	Rabbit:	non-irritant
Dermal sensitization	Guinea pig:	non-sensitizing
Mutagenicity	5 tests	no mutagenic potential
Teratogenicity	Rat, Rabbit	no teratogenic potential
Chronic toxicity / Oncogenicity	Rat, Dog, Mouse	no oncogenic potential
Reproduction	Rat	no adverse effects
Metabolism	Rat	rapid absorption and elimination; no accumulation

Toxicity to wildlife

Birds (2 species)	LD50	> 2000 mg/kg practically non toxic
Fish (4 species)	LC50	0.98-1.17 mg/l toxic ②
Bees (oral)	LD50	316 ug/bee practically non toxic

Environmental behaviour

Hydrolysis:		stable
Photolysis:	water	$T_{0.5}$=0.4-13.5 days degradable
	soil	$T_{0.5}$=28-33 days
Mobility		RMF=0.1 non-leaching
Soil degradation		DT_{50}=24-59 days readily degradable

① maximum attainable concentration
② under laboratory conditions in clean water; pratical relevance under evaluation

FORMULATION

For foliar uses CGA 219417 used alone or formulated in mixtures with other fungicides will mainly be commercialised as a water dispersible granule. In 1994, the WG 75 was launched in France and Switzerland in cereals. For seed treatment, emulsion formulations are in development.

BIOLOGICAL KEY FEATURES

A key characteristic of CGA 219417 is its new mode of action. Studies on *B. cinerea* indicate an inhibition of methionine biosynthesis (Masner, Muster and Schmid, in press). The most important consequence of this new mode of action is the lack of cross-resistance potential with products of the triazole, imidazole, morpholine, dicarboximide and phenylpyrrole chemistry.

CGA 219417 is a systemic product, which shows a good uptake into plants after foliar application. Inside the plants CGA 219417 is transported throughout the tissue and acropetally in the xylem. Site of action studies on key target pathogens such as *P. herpotrichoides*, *B. cinerea* and *V. inaequalis*, have shown CGA 219417 to be effective both by inhibiting their penetration and their mycelial growth inside and on the leaf surface.

FIELD EXPERIMENTS

Material and methods

All trials were conducted using a randomized plot design with 3-4 replications.
In cereals, plot sizes ranged from 12.5 - 36 m^2. Treatments for eyespot were applied at GS 29-32, those used against foliar diseases at first signs of disease attack and those for ear diseases on wheat at GS 55-69. Spray volumes were 200 - 500 l/ha. Severity of diseases was assessed on stems, leaves and ears.
In grapes, top fruit and vegetables/strawberries plot sizes ranged from 5 - 20 plants, 3 - 4 trees and 5 - 20 m^2, respectively. Spray volumes were 500 - 1000 l/ha in grapes, 800 - 2000l/ha (Japan: 2000 - 4000 l/ha) in top fruit, 1000 - 2000 l/ha in vegetables and 1000 l/ha in strawberries. Treatments were applied either on a routine base or at specific growth stages, depending on the pathogen. Severity (grapes, vegetables) or incidence (fruit, vegetables, strawberries) were assessed on twigs, leaves and fruit.
In field crops, plot sizes were 12.5 - 36 m^2 and spray volumes ranged from 200 - 500 l/ha in field peas and beans and from 500 - 600 l/ha in potatoes. Treatments were applied on a routine basis. Severity of diseases was evaluated on leaves and pods.

Results

Foliar and stem base diseases of cereals

In cereals, CGA 219417 (600 g AI/ha) gives excellent control of *P. herpotrichoides* better or at least equal to current standards (Table 1). By combining eyespot activity with good powdery mildew control, the product is optimally suited for early season applications especially in wheat. Unix 75 WG was this year already a great success against these 2 key pathogens in France and Switzerland. On wheat, CGA 219417 in addition gives good control of *S. nodorum* on ears.

On barley, besides being an outstanding eyespot product, CGA 219417 (500 g ai/ha) gives excellent control of *P. teres* superior to best standards and good control of *E. graminis* and *R. secalis* (Leadbeater *et al.*, 1994; Heye *et al.*, 1994; Bocquet *et al.*, 1994).

TABLE 1. Control of key target pathogens on wheat and barley by CGA 219417.

Target Pathogen	Activity on Wheat (600 g AI/ha)	Barley (500g AI/ha)
Pseudocercosporella herpotrichoides	****①	****
Erysiphe graminis	***	***
Leptosphaeria nodorum ears	***	NA
Pyrenophora teres	NA	****
Rhynchosporium secalis	NA	**(*)

**** = superior to standard *** = equal to standard ** = useful activity
NA = not applicable
① equal to standard prochloraz at 500 g AI/ha in UK and Germany

Seed Treatment for cereals

As a seed treatment, CGA 219417 used at the low rate of 5 g AI/100 kg seed gave excellent control of *Pyrenophora graminea* and seedborne *Pyrenophora teres* on barley (Leadbitter *et al.*, 1994) and is therefore seen as an alternative to currently used seed treatment products.

Grapes

On grapes, CGA 219417 is an excellent product for control of *Botrytis*. At 375 to 500 g AI/ha 2-4 applications provided over many years of testing a consistently high level of control superior to dicarboximide standards (Table 2). Besides this outstanding *Botrytis* activity, it shows good activity against ripe rot (*Glomerella cingulata*) and sour rot (*Aspergillus* sp., *Rhizopus* sp., *Cladosporium* sp.). No negative effects on fermentation and wine quality were observed.

TABLE 2. Control of *Botrytis cinerea* on grapes by CGA 219417, 1989-93 trials.

Treatment	Rate g AI/ha	% infected bunch surface at harvest				
		France	Switzerland	Italy	Germany	Spain
Untreated		38.6	44.3	39.0	25.8	42.4
Standard		20.5	17.2	29.0	10.6	23.8
CGA 219417	375	8.0	10.4	8.8	4.9	16.6
CGA 219417	500	4.5	5.8	3.5	2.8	11.0
Number of trials		9	7	2	2	2

2-4 applications between late flowering and 4 weeks before harvest, according to local practice

Standards: F: vinclozolin 750 or vinclozolin+thiram 500+3200 g AI/ha
CH: folpet 1600/diethofencarb+carbendazim 625+625/vinclozolin 1000 g AI/ha
I, E: vinclozolin 750 g AI/ha
D: vinclozolin 500 g AI/ha

The risk of resistance, which threatens the performance of current market botryticides, accentuates the need for an anti-resistance concept immediately at the market introduction of a new product. The key solution for *Botrytis* control is a ready mixture of CGA 219417 with fludioxonil (3:2), which gives excellent control even under high disease pressure situations (Table 3). By recommending 1 - 2 applications of CGA 219417 + fludioxonil in alternation with botryticides of a different chemistry, we hand over to the farmer a concept, with which he can meet the high disease management standards of today and tomorrow.

TABLE 3. Control of *B. cinerea* on grapes by CGA 219417+fludioxonil (1993 trials).

Treatment	Rate g AI/ha	% diseased bunch surface at harvest France	Switzerland	Italy
Untreated		59.3	41.6	33.8
Standard		26.1	16.0	19.9
CGA 219417 + fludioxonil	375+250	2.2	5.7	3.6
CGA 219417 + fludioxonil	450+300	1.8	3.3	ND*
Number of trials		3	3	4
Spray schedule		ABCD	ABCD	BD

Application timings: A=end flowering; B=before bunch closure; C=begin of berry ripening; D=4 weeks before harvest.

Standards: F: vinclozolin 750 or vinclozolin+thiram 500+3200 g AI/ha ;
CH: folpet 1600 (A) / diethofencarb+carbendazim 625+625 (B,C) / vinclozolin 1000 g AI/ha (D)
I: vinclozolin 750 g AI/ha (B, D) *ND = no data

Vegetables, Strawberries and Field Crops

The strength of CGA 219417 on vegetables, strawberries and field crops is its excellent activity against *Botrytis* spp. As for grapes the key solution for vegetables (tomato, eggplants, cucumber, *Phaseolus* beans) and strawberries is the mixture CGA 219417 + fludioxonil. At 37.5 + 25 g AI/hl the performance with 2 - 3 applications in strawberries and 3 - 4 in vegetable crops was excellent even under high disease pressure and superior to dicarboximide standards (Table 4).

TABLE 4.Control of *B.cinerea* on vegetables and strawberries by CGA 219417+fludioxonil.

Treatment	Rate g AI/hl	% infected fruits tomato/cucumber	strawberries
Untreated		26.2	29.8
Standard		12.9	10.3
CGA 219417+fludioxonil	37.5+25	1.4	6.6
Countries		Spain	Italy, France, Spain, Switzerland
Number of trials		5	9

Standards: tomato/cucumber vinclozolin 75 g AI/hl
strawberries: procymidone 50 g AI/hl; vinclozolin 75 g AI/hl; alternation dichlofluanide 200 g AI/hl / vinclozolin 100 g AI/hl

Against *Botrytis* spp. on field peas and beans, CGA 219417 at 500 g AI/ha gave a good control superior to residual products and equal to dicarboximide compounds. For these crops, mixtures are also under evaluation.

The second major group of target pathogens for CGA 219417 on vegetable and field crops are *Alternaria* leaf spot diseases. At 250 g AI/ha, CGA 219417 controls *A. solani* on tomatoes and potatoes equally well as difenoconazole and clearly superior to dicarboximide standards and protectant products like chlorothalonil, respectively (Table 5). On carrots 500 g AI/ha are better than standards.

With CGA 219417 + fludioxonil, the farmer derives additional benefit from the good powdery mildew control on tomatoes and the activity against *Sclerotinia* on *Phaseolus* beans and lettuce.

TABLE 5. Control of *Alternaria* early blight on tomatoes and potatoes (1990-94 trials).

Treatment	Rate g AI/ha	% infected leaf surface	
		tomatoes	potatoes
Untreated		68.6	78.4
Standard		15.0	28.7
CGA 219417	250	13.9	7.7
Countries		S.Africa, Brazil	S. Africa, Brazil
Number of trials		6	16

Standards: tomatoes: difenoconazole 75-150 g AI/ha
potatoes: iprodione 750 g AI/ha or chlorothalonil 1300 g AI/ha

Deciduous fruit

CGA 219417, with its excellent activity against *Venturia, Alternaria* and *Monilinia* spp. is set to become a key product for disease control practices in pomefruit and stonefruit. Since 1989, CGA 219417 has been tested against the economically most important fruit disease *V. inaequalis* on apples. At 15 g AI/hl, it outperformed protectant standards on leaves and matches them on fruit (Table 6).

Against *Alternaria* leaf spot diseases, which are of major importance in the Far East, CGA 219417 was shown to be a good product. Particular emphasis was given to test CGA 219417 against *A. mali* on apples (Table 6). At 25 g AI/hl, CGA 219417 was clearly better than the local protectant standard (oxine-copper + captan).

TABLE 6. Control of key target pathogens on apples by CGA 219417 (1989-1994 trials).

Treatment	Rate g AI/hl	% diseased plant part		
		Venturia inaequalis		*Alternaria mali*
		leaves	fruit①	leaves
Untreated		64.6	93.1	54.8
Protectant standard		9.4	17.5	22.5
CGA 219417	15	2.1	16.7	ND*
CGA 219417	25	ND*	ND*	12.7
Countries		Italy, France, Switzerland, Germany		Japan
Number of trials		17	11	6

Standards: *Venturia*: captan (100-150 g AI/hl) or dithianon (37.5-75 g AI/hl)
Alternaria: oxine-copper+captan (100 g AI/hl)
① 1989 - 1993 trials only
*ND = no data

For scab control, CGA 219417 solo or in mixture with captan (1:3) will be commerzialized. According to the strength of CGA 219417 against leaf scab, both products will be positioned in the early season with 3 - 4 applications until the end of flowering. Outstanding leaf scab control can be obtained even under extremely high disease pressure (Table 7). This excellent performance, combined with a systemic activity allows to implement flexible use strategies with CGA 219417-based products. Furthermore, CGA 219417 has no negative effect on beneficials and is therefore well suited for use in integrated disease control practices.

TABLE 7.Use strategy trials of CGA 219417 or CGA 219417+captan for leaf scab control (1993/94 trials).

Treatment	Rate g AI/hl	% leaves affected
Untreated		91.2
Protectant standard		20.0
CGA 219417	15	2.9
CGA 219417 + captan (1:3)	15+45	1.1
Countries		Italy, France, Germany
Number of trials		8

Standard: captan (100-150 g AI/hl) or dithianon (75 g AI/hl)

The strength of CGA 219417 on stonefruit is its good control of *Monilinia* blossom and twig blight and *Monilinia* fruit rot comparable to dicarboximide standards with 2 - 3 applications at 15 and 25 g AI/hl, respectively. On apples *Monilinia* blossom blight control at 50 g AI/hl is equal to competitor products.

CONCLUSIONS

CGA 219417 is a novel broad-spectrum fungicide, which gives high level control of economically important diseases in cereals, grapes, fruit, vegetable and field crops. With its new mode of action and its favourable safety profile for users, consumers and environment, it offers the features for flexible integrated disease control practices and a partner for implementation of anti-resistance strategies in many key crop-pathosystems.

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ICIA5504 : A NOVEL BROAD-SPECTRUM SYSTEMIC FUNGICIDE FOR USE ON FRUIT, NUT AND HORTICULTURAL CROPS

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ABSTRACT

ICIA5504 is a novel systemic fungicide from the β-methoxyacrylate group which shows an outstanding spectrum of disease control on a diverse range of dicotyledonous crops. The compound is particularly effective on Cucurbitaceae providing unique control of both downy and powdery mildew. On tomato outstanding control of early blight is combined with good control of late blight. On peanut control of major soil-borne diseases (white mold and peg rot) and foliar leaf spots is associated with an excellent yield response. ICIA5504 can control pathogens from each of the four major fungal groups and demonstrates good systemic, curative and residual activity in dicotyledonous crops.

INTRODUCTION

ICIA5504 is a novel systemic fungicide from the β-methoxyacrylate group. The chemical and biological properties of ICIA5504 were first described by Godwin *et al.* (1992).

In this paper we demonstrate the excellent broad spectrum activity of ICIA5504, which is expressed through its systemic properties in a range of dicotyledonous crops. These properties confer efficient redistribution of ICIA5504 in the foliage; curative action, and persistent disease control.

The value of an outstanding spectrum of disease control has been shown not only by the diversity of dicotyledonous food crops which benefit from treatment with ICIA5504, but also the range of pathogens controlled on any single crop. Examples are presented here to show the ability of ICIA5504 to control fungal pathogens from the ascomycetes, basidiomycetes, deuteromycetes and oomycetes. This is an important feature of disease control in many dicotyledonous crops where representatives from one or more of these groups may attack the crop in the same growing season.

SYSTEMIC ACTIVITY

Methodology

Tests were carried out in the glasshouse (Tables 1 and 2) or in a polythene tunnel (Table 3). Young cucumber plants were sprayed to run off with chemical treatments. Data were arc sine transformed for analysis of variance. Treatment means within data columns followed by different letters indicate significant difference at the 5% level.

Root systemicity

ICIA5504 demonstrated a high level of systemicity when applied as a root drench to young cucumber plants to control *Sphaerotheca fuliginea* (cucumber powdery mildew) (Table 1). Plants were inoculated 14 days after chemical application. Disease control was demonstrated on all leaves, including those not present at the time of application (leaf 3 and leaf 4). Dose responses were similar for all leaves, ranging from no disease control at 6.25ppm to 100% control at 50ppm (Table 1).

TABLE 1. Systemic activity of ICIA5504 by root uptake on cucumber against *Sphaerotheca fuliginea* - Glasshouse studies - UK, 1993.

Rate (mg AI/l)	% Disease control[1]			
	Leaf 1[2]	Leaf 2	Leaf 3	Leaf 4
Untreated	(95)[3]	(91)	(96)	(94)
6.25	0	0	0	0
12.5	3	43	38	27
25	74	92	64	63
50	100	100	100	100

[1] Assessments were made 22 days after chemical application.
[2] Leaf 1 was the oldest true leaf.
[3] ( ) % leaf area showing sporulating disease.

Foliar systemicity and curative action

ICIA5504 showed efficient translaminar action on cucumber leaves, moving from the treated area on the abaxial surface to the adaxial surface to control *S. fuliginea* (Table 2). Application of treatments one day after inoculation with *S. fuliginea* also demonstrated the curative action of ICIA5504 in this experiment. Different dose response curves were seen for ICIA5504 and hexaconazole;

ICIA5504 demonstrated a more gradual response with markedly higher levels of disease control at the lowest two rates (Table 2).

Residual action

ICIA5504 showed good control of *S. fuliginea*, in a trial conducted in a polythene tunnel in the UK (Table 3). Disease control at 250mg AI/l was equivalent to the commercial standard when applied either 7 days or 14 days before inoculation.

TABLE 2. Translaminar and curative activity of ICIA5504 by foliar application on cucumber against *Sphaerotheca fuliginea* - Glasshouse studies - UK, 1993.

Rate (mg AI/l)	% Disease control[1]	
	ICIA5504	Hexaconazole
Untreated	(95)[2]	(95)
0.4	33	0
2	79	0
10	83	75
50	93	100

[1] Assessments were made 11 days after chemical application.
[2] ( ) % leaf area showing sporulating disease.

TABLE 3. Control of *Sphaerotheca fuliginea* by foliar spray application on cucumber with 7 or 14 day protectant spray interval - Polythene tunnel - UK, 1993.

Treatment	Rate (mg AI/l)	% Disease control	
		7 day interval (8DAA6)[1]	14 day interval (15DAA3)
Untreated	-	[2](94) A	(94) A
ICIA5504	125	94 B	82 B
ICIA5504	250	98 B	89 BC
Fenarimol	24	98 B	91 C

[1](8DAA6) 8 days after the sixth application, etc.
[2]( ) % leaf area infected.

SPECTRUM OF DISEASE CONTROL

Methodology

The spectrum of disease control provided by ICIA5504 is exemplified using data from field experiments. Trials chosen are representative of several trials for each pathogen. Unless otherwise indicated, data for different pathogens on each crop are taken from separate trials. Data in Tables 4 to 9 are presented as mean values derived from 3 to 6 replicates in a randomised block design. The trial against pecan scab was unreplicated and was consequently not statistically analysed (Table 10). Application volumes ranged from 300 l/ha to 2000 l/ha and were appropriate to obtain good spray coverage and commensurate with local practice.

Sweet melon

ICIA5504 demonstrated its unique broad spectrum of action in a trial conducted on sweet melon in Taiwan (Table 4). The crop was under severe disease pressure from three different pathogens: *Pseudoperonospora cubensis* (downy mildew); *Sphaerotheca fuliginea* (powdery mildew), and *Didymella bryoniae* (gummy stem blight). Only ICIA5504 effectively protected the crop from all three pathogens. Applications were made on a 14 day schedule, showing the persistence of effect from ICIA5504.

TABLE 4. Control of *Pseudoperonospora cubensis* (PSPECU), *Sphaerotheca fuliginea* (SPHRFU) and *Didymella bryoniae* (DIDYBR) on sweet melon by foliar spray application in Taiwan, 1993.

Treatment	Rate (mg AI/l)	% Disease control		
		PSPECU (6DAA3)	SPHRFU (9DAA3)	DIDYBR (9DAA3)
Untreated	-	[1](81) A	[1](87) A	[2](71) A
ICIA5504	100	83 C	100 D	96 D
ICIA5504	200	91 C	100 D	96 D
Metalaxyl + mancozeb	250 + 1200	53 B	13 B	33 B
Vinclozolin	500	31 B	13 B	79 C
Oxythioquinox	100	31 B	45 C	26 B

[1] ( ) % leaf area infected.
[2] ( ) % stem area infected.

Peanut

Two applications of ICIA5504 showed excellent control of *Sclerotium rolfsii* (white mould) (Table 5). Yield responses associated with control of *S. rolfsii* and *Rhizoctonia solani* (peg rot) have frequently exceeded 50% by comparison with untreated plots. ICIA5504 also demonstrated useful activity against foliar leaf spot pathogens of peanut, *Mycosphaerella arachidis* (early leaf spot) and *Mycosphaerella berkeleyii* (late leaf spot) on a 14 day schedule.

TABLE 5. Control of *Mycosphaerella arachidis* (MYCOAR), *Sclerotium rolfsii* (SCLERO) and *Rhizoctonia solani* (RHIZSO), on peanut by foliar spray application in the USA, 1993.

Treatment	Rate (g AI/ha)	% Disease control		% Yield increase	
		[1]MYCOAR (19DAA7)	[1]SCLERO (60DAA2)	[2]SCLERO	[1]RHIZSO
Untreated	-	[3](69) A	[4](33) A	[5](2330) A	[5](5920) A
ICIA5504	100	55 B	-	-	-
ICIA5504	400	-	85 B	58 C	15 B
Chlorothalonil	1000	78 B	-	-	-
Flutolanil[6]	1020	-	79 B	35 B	8 AB

[1] Artificial inoculation was used.
[2] Average yield increase over 5 trials.
[3] ( ) % infected leaflets.
[4] ( ) % killed stems.
[5] ( ) Yield in kg/ha.
[6] One application of flutolanil was made.

Phaseolus bean

Applications of ICIA5504 on a 14 day schedule gave excellent control of *Uromyces phaseoli* (bean rust) and *Isariopsis griseola* (angular leaf spot) on the leaf and *Glomerella cingulata* (anthracnose) on the pod (Table 6). The spectrum of disease control shown by ICIA5504 was superior to that of the commercial standards.

TABLE 6. Control of *Uromyces phaseoli* (UROMAP), *Glomerella cingulata* (GLOMCI) and *Isariopsis griseola (PHAIGR)* on long bean by foliar spray application in Malaysia and Brazil, 1992 and 1993.

Treatment	Rate (mg AI/l)	% Disease control		
		Malaysia	Brazil	
		UROMAP (14DAA3)	GLOMCI (14DAA4)	PHAIGR (14DAA4)
Untreated	-	[1](66) A	[2](20) A	[3](46) A
ICIA5504	200	84 C	85 C	78 C
Chlorothalonil	3750[4]	62 B	70 C	80 C
Triadimefon	125	47 B	-	-
Carbendazim	1250	46 B	30 B	9 A

[1] ( ) % leaf area infected in the middle and lower portion of the plant.
[2] ( ) % pod area infected.
[3] ( ) % leaf area infected on the whole plant.
[4] 1500 mg AI/l applied in the Malaysian trial.

Tomato

ICIA5504 demonstrated excellent activity against *Alternaria solani* (early blight) on tomato in Brazil. Disease control exceeded that provided by the commercial standards on a 14 day schedule. ICIA5504 also showed good control of *Phytophthora infestans* (late blight) on a 10 day schedule (Table 7).

TABLE 7. Control of *Alternaria solani* (ALTESO) and *Phytophthora infestans* (PHYTIN) on tomato by foliar spray application in Brazil, 1993.

Treatment	Rate (mg AI/l)	% Disease control	
		ALTESO (8DAA5)	PHYTIN (5DAA4)
Untreated	-	[1](92) A	(66) A
ICIA5504	200[2]	99 C	83 B
Chlorothalonil	2000	78 B	82 B
Difenoconazole	125	65 B	-

[1] ( ) % leaf area infected.
[2] Rate of application in the ALTESO trial was 187 mg AI/l.

Citrus

Two applications of ICIA5504 showed good control of *Elsinöe australis* (citrus scab) on lemon in Brazil. Disease control was equivalent to that achieved with the commercial standard (Table 8).

TABLE 8. Control of *Elsinöe australis* on citrus by foliar spray application in Brazil, 1993.

Treatment	Rate (mg AI/l)	% Marketable fruit[1, 2]
Untreated	-	44 A
ICIA5504	200	62 B
Benomyl	250	61 B

[1] 0 to 0.5% fruit surface infected.
[2] Assessments were made at harvest, 14 weeks after the 2nd chemical application.

Peach

In two separate trials in the USA, ICIA5504 showed control of *Monilinia fructicola* (brown rot) equivalent to that of the commercial standard and control of *Venturia carpophila* (scab) surpassing that of the commercial standard on growers' schedules (Table 9).

TABLE 9. Control of *Monilinia fructicola* (MONIFC) and *Venturia carpophila* (VENTCA) on peach by foliar spray application in the USA, 1993 (MONIFC) and 1991 (VENTCA).

Treatment	Rate (mg AI/l)	% Disease control			
		MONIFC (63DAA3)		VENTCA (9DAA6)	
Untreated	-	[1](55.6)	A	[2](80.4)	A
ICIA5504	200	-	-	98	C
ICIA5504	267	93	B	-	-
Vinclozolin	1200	91	B	-	-
Captan + benomyl[3]	1200 + 1200	90	B	73	B

[1] ( ) % fruit infected.
[2] ( ) % fruit surface infected.
[3] In VENTCA trial, benomyl rate was 300 mg AI/l.

Pecan

ICIA5504 was very effective in controlling *Cladosporium caryigenum* (scab) on leaves and on the nuts on a grower schedule (Table 10).

TABLE 10. Control of *Cladosporium caryigenum* on pecan by foliar spray application in the USA, 1993.

Treatment	Rate (mg AI/l)	% Disease control	
		Leaf (15DAA5)	Nuts (6DAA7)
Untreated	-	[1](92.2)	[2](100)
ICIA5504	141	98	95
Triphenyltin-hydroxide	169	71	93

[1] ( ) % lesions/leaf.
[2] ( ) % nuts infected.

Crop Safety

ICIA5504 demonstrates good crop safety at appropriate rates on a wide variety of dicotyledonous crop species and cultivars.

CONCLUSIONS

ICIA5504 is a broad spectrum systemic fungicide with excellent potential for use on dicotyledonous crops (and also on monocotyledonous crops; see Godwin *et al.*, 1992). When applied as a foliar spray on a diverse selection of crops against fungal pathogens representing all four major fungal groups, ICIA5504 provides levels of disease control equivalent to or better than those of current commercial standards. Furthermore, it affords a spectrum of disease control unavailable in any other systemic or non-systemic fungicide. Application intervals are consistent with a compound showing good persistence of effect, and effective application rates provide good margins for crop safety in all but exceptional circumstances.

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Godwin, J.R.; Anthony, V.M.; Clough, J.M.; Godfrey, C.R.A. (1992) ICIA5504 : A novel, broad spectrum, systemic β-methoxyacrylate fungicide. *Proceedings of the 1992 Brighton Crop Protection Conference - Pests and Diseases,* **1**, 435-442.

KTU 3616: A NOVEL FUNGICIDE FOR RICE BLAST CONTROL

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ABSTRACT

KTU 3616, a cyclopropanecarboxamide, is a novel fungicide for rice with a unique structure, discovered and developed jointly by Nihon Bayer Agrochem K.K. and Bayer AG. It is a safe compound with low mammalian and fish toxicity. It has systemic properties and controls both rice leaf and panicle blast infection by granular or foliar treatment. It is characterized by long lasting effectiveness after application to rice seedling boxes before transplanting. The primary mode of action appears to be the inhibition of dehydration reaction from scytalone to 1,3,8-trihydroxynaphthalene in the melanin biosynthesis pathway of *Pyricularia oryzae*. KTU 3616 has excellent selectivity during all stages of the rice crop.

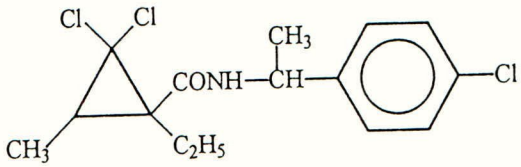
INTRODUCTION

KTU 3616 is a new fungicide with the chemical structure of cyclopropyl carboxamide. It was invented, patented and developed jointly by Nihon Bayer Agrochem K.K. and Bayer AG for its marketing as a rice blast fungicide. This paper describes the chemical properties of KTU 3616 and its biological activities on rice blast in different application methods under field conditions.

CHEMICAL AND PHYSICAL PROPERTIES

Chemical name : (1R,3S/1S,3R)-2,2-dichloro-N-[(R)-1-(4-chlorophenyl)ethyl]-1-ethyl-3-methylcyclopropanecarboxamide (CA)

Structural formula :



Molecular formula : $C_{15}H_{18}Cl_3NO$

Molecular weight : 334.67

Appearance : colourless powder

Melting point : 145 - 149 °C

Vapor pressure : $2.70 \cdot 10^{-07}$ Pa (20°C)

Water solubility : 1.7 mg/l:(1R,3S)isomer
1.9 mg/l:(1S,3R)isomer (pH 7/20°C)

Partition coefficient: log Pow 4.23:(1R,3S)isomer
log Pow 4.28:(1S,3R)isomer (22 °C)

TOXICOLOGY

Acute oral rat	:	male and female	(LD50)	>5000 mg/kg
Acute oral mice	:	male and female	(LD50)	>5000 mg/kg
Acute dermal rat	:	male and female	(LD50)	>2000 mg/kg
Acute inhalation rat	:	male and female	(LC50)	>5000 mg/m^3

Eye irritation rabbit: no irritation
Skin irritation rabbit: no irritation

Skin sensitization guinea pig: no sensitization

Mutagenicity : no mutagenic effects in procaryotic and eucaryotic test systems

Aquatic, carp	:	LC50 (48 h)	5.6 mg/l
water flea	:	LC50 (3 h)	>20.0 mg/l

FORMULATIONS

As a single compound KTU 3616 will be available as a suspended concentration formulation (150 SC), a dust formulation (0.5 DP) for foliar application and as a granule formulation (4 GR) for seedling box application.

BIOLOGICAL PROPERTIES

Test methods

The antifungal activity was tested in *in vitro* experiment, using many plant pathogens. The mycelial growth on agar medium amended with KTU 3616 was measured.

The inhibition of melanin biosynthesis of plant pathogens was checked by evaluating the colony colour change on agar medium amended with KTU 3616.

Field trials were performed with 3-4 replicates in randomized block design. Plot sizes varied between 20 and 60 m^2. The foliar applications against rice leaf blast were made during the tillering stage of rice. The first application was done at the first outbreak of leaf blast and the second followed one week later. Against panicle blast, rice plants were treated twice at the booting and heading stage. The granule application into seedling boxes was made just before the transplanting of the rice seedlings. The granule application to paddy water was carried out approximately one week before the outbreak of leaf blast and at the end of the tillering stage for the control of panicle blast.

Disease severity indexes were calculated as follows:

Disease severity index for leaf blast = (5A + 4B + 3C + 2D + E + 0.5F) ÷ (5 x No. of assessed hills) x 100

(A: No. of infected hills of which % infection area was more than 50 %, B: between 25 and 50 %, C: between 10 and 25 %, D: between 5 and 10 %, E: between 1 and 5 % and F: less than 1 %)

Disease severity index for panicle blast = (3A + 2B + C) ÷ (3 x No. of assessed panicles) x 100

(A: No. of panicles where the whole panicle was damaged, B: more than 1/3 of a panicle damaged, C: less than 1/3 of a panicle damaged)

RESULTS AND DISCUSSION

In vitro activity of KTU 3616

As shown in Table 1 KTU 3616 had virtually no direct effect on the mycelial growth of a wide range of fungi on agar medium. However, it was shown that KTU 3616 had a strong effect on the colour of the following fungi colonies: *Botryosphaeria berengeriana*, *Valsa ceratosperma* and *Pyricularia oryzae*. The pot and field tests have indicated that KTU 3616 was effective only against *Pyricularia oryzae*, *in vivo*.

Mode of action

Chromatographic analysis of the blast culture media amended with KTU 3616 by thin-layer chromatography has shown an impressive accumulation of scytalone, an intermediate substance in melanin biosynthesis. This suggests that KTU 3616 might inhibit the dehydration of scytalone to yield 1,3,8-

trihydroxynaphthalene in the fungal melanin biosynthesis pathway (Wheeler, 1982).

TABLE 1. Mycelial growth and fungal melanin biosynthesis inhibition by KTU 3616.

Pathogen	Radial growth inhibition (%)		Colony colour change
	100 mg/l	200 mg/l	100 mg/l
Phytophthora nicotianae var. *parasitica*	78	80	No test
Pythium sp.	49	46	No test
Botryosphaeria berengeriana f.sp. *piricola*	27	41	+++
Cochliobolus miyabeanus	1	10	(+)
Diaporthe citri	23	9	+(+)
Gibberella fujikuroi	14	14	-
Sclerotinia sclerotiorum	26	33	-
Valsa ceratosperma	12	17	+++
Thanatephorus cucumeris	17	24	+
Alternaria brassicicola	5	5	+(+)
Aspergillus niger	15	22	-
Botrytis cinerea	20	21	-
Colletotrichum lagenarium	29	43	++
Fusarium oxysporum f.sp. *lycopersici*	10	3	-
Penicillium digitatum	0	0	-
Pyricularia oryzae	17	15	+++

+++: Very strong, ++: Strong, +(+): Medium, +: Weak, (+): Very weak, -: No change

Field trials

Foliar treatment with SC-formulation

KTU 3616 was tested with an SC-formulation against rice leaf and panicle blast, especially with respect to its persistence. KTU 3616 provided good control of both leaf and panicle blast at 100 and 200 g AI/ha after spraying at the standard interval. At the prolonged interval KTU 3616 was inferior to the standard treatments. The foliar treatment against blast disease with KTU 3616 was very effective, although it required the normal treatment frequency.

TABLE 2. Control of rice leaf and panicle blast by foliar application of SC-formulation, 1989 (Japan).

Treatment	Rate (g AI/ha)	Spray timing A*	B*	C*	D*	% disease control: Leaf blast	Panicle blast
Untreated (Disease severity index)						0 (43)	0 (41)
KTU 3616	100	x	-	x	-	51	55
	100	x	x	x	x	89	83
	200	x	-	x	-	70	68
	200	x	x	x	x	93	92
Tricyclazole	200	x	-	x	-	36	69
	200	x	x	x	x	62	89
Edifenphos +fthalide	150 200	x	x	x	x	90	83
Number of trials						5	5

*: A for tillering stage, B for one week after A, C for booting stage, D for one week after C
x: Treated, -: Not treated

The SC-formulation of KTU 3616 was tested in Japan, Taiwan, Columbia and Brazil for the control of rice leaf and panicle blast from 1990 to 1992. Very high performance was achieved at 100 g AI/ha. The efficacy against leaf and panicle blast was equal to tricyclazole at 200 g AI/ha and to the mixture of edifenphos at 150 g AI/ha + fthalide 200 g AI/ha.

TABLE 3. Control of rice leaf and panicle blast by foliar application of SC-formulation, 1990-1992 (Japan and Taiwan).

Treatment	Rate (g AI/ha)	% disease control: Leaf blast (Japan)	(Japan)	(Taiwan)	Panicle blast (Japan)	(Japan)	(Taiwan)
Untreated (Disease severity index)		0 (38)	0 (41)	0	0 (39)	0 (34)	0 (34)
KTU 3616	100	75	81	84	83	89	86
Tricyclazole	200	58	61	65	86	89	76
Edifenphos +fthalide	150 200	77	-	-	78	-	-
Number of trials		7	11	3	6	10	2

Foliar treatment with DP-formulation

KTU 3616 is also being developed as a dust formulation. It was formulated as 0.5 % dust and tested on rice leaf and panicle blast at 200 g AI/ha in comparison with tricyclazole 1.0 % DP and edifenphos 2.0 + fthalide 1.5 % DP. KTU 3616 was very effective both on leaf and panicle blast and it had efficacy comparable to the standards.

TABLE 4. Control of rice leaf and panicle blast by foliar application of DP-formulation, 1990-1992 (Japan).

Treatment	Rate (g AI/ha)	% disease control: Leaf blast (1990-1991)	(1990-1992)	Panicle blast (1990-1991)	(1990-1992)
Untreated (Disease severity index)		0 (37)	0 (35)	0 (24)	0 (22)
KTU 3616	200	64	70	84	87
Tricyclazole	400	48	-	88	-
Edifenphos +fthalide	800 600	61	62	85	86
Number of trials		7	11	6	10

Granule application

KTU 3616 is unique in its lasting efficacy when applied as granule formulation into seedling box. Rice leaf blast can be controlled with existing granule products which are applied either into seedling box or to the paddy water surface. KTU 3616 was tested for both application methods. It was shown that the dosage rate needed for the seedling box application was far less than that applied to the paddy surface water.

TABLE 5. Control of rice leaf blast by seedling box or paddy water surface application with GR-formulation, 1989 (Japan).

Treatment	Rate (g AI/box)	(g AI/ha)	% disease control Leaf blast
Untreated (Disease severity index)			0 (48)
KTU 3616	4.0 (0 DAT)	-	88
	-	2000 (5 DAT)	88
	-	2000 (15 DAT)	94
	-	2000 (30 DAT)	95
Tricyclazole	4.0 (0 DAT)	-	46
Pyroquilon	-	2000 (30 DAT)	86
Probenazole	-	3200 (30 DAT)	84
Number of trials			2

DAT: Days after transplanting
Usually 200 boxes are used for the transplanting in 1 ha. 4.0 g AI/box is approximately equivalent to 800 g AI/ha.

With the existing granule fungicides, leaf blast and panicle blast should be treated separately using sequential treatments. KTU 3616, however, can effectively control both leaf and panicle blast after a single seedling box application prior to transplanting.

TABLE 6. Control of rice leaf and panicle blast by seedling box or water surface application with GR-formulation, 1990 (Japan).

Treatment	Rate (g AI/box)	(g AI/ha) (Leaf)	(g AI/ha) (Panicle)	% disease control Leaf blast	Panicle blast
Untreated (Disease severity index)				0 (32)	0 (22)
KTU 3616	2.0	-	-	94	85
	-	2000	2000	86	89
Tricyclazole	3.2	-	-	50	63
Pyroquilon	-	2000	2000	84	84
Probenazole	-	3200	3200	89	59
Number of trials				4	4

Usually 200 boxes are used for the transplanting in 1 ha. 2.0 g AI/box is approximately equivalent to 400 g AI/ha.

KTU 3616 4 % GR has been tested since 1991 in experiments with seedling box application by the Japanese government institutes. The compound provided excellent performance against rice leaf and panicle blast.

CONCLUSIONS

KTU 3616 is a novel fungicide with a unique mode of action and effectiveness only on rice blast. It is applicable in the formulations such as SC, DP and GR. KTU 3616 is outstanding in its lasting efficacy, controlling both leaf and panicle blast with a single seedling box application prior to the transplanting.

REFERENCE

Wheeler, M.H. (1982) Melanin biosynthesis in *Verticillium dahliae*: Dehydration and reduction reactions in cell-free homogenates. *Experimental Mycology* **6**, 171-179.

ACTIVITY OF PYRIMETHANIL ON *VENTURIA INAEQUALIS*

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ABSTRACT

Pyrimethanil is a new fungicide for the control of *Venturia* and *Botrytis* spp. It has a novel mode of action, inhibiting secretion of cell wall degrading enzymes in *Botrytis*, thus acting as a pathogenesis inhibitor. Although the excellent protectant activity of pyrimethanil is most widely exploited, it has the additional benefit of good curative properties. The precise activity of pyrimethanil on the infection process of *Venturia* on apple seedling leaves has been established by microscopic techniques. Using sprays timed to coincide with key differentiation stages in early pathogenesis, pyrimethanil has been shown to arrest *Venturia* development at the point at which the pathogen starts to undergo rapid growth extension from a discrete primary subcuticular reserve. This mode of action also confers upon pyrimethanil antisporulant activity when applied pre-symptomatically, and subsequently prevents lesion development.

INTRODUCTION

Apple scab, caused by *Venturia inaequalis*, is a widespread disease in commercial orchards, and is the primary target for pesticide applications on apples. In recent years, there has been a move towards improving fungicide use to minimise unneccesary applications. This has been achieved by the implementation of integrated pest management (IPM) strategies aimed at eradicating incipient infections and reducing primary ascospore inoculum. This requires efficient control of apple scab, attained not only by the use of a fungicide with good selective toxicity to *V. inaequalis*, but more importantly by using precisely timed fungicide applications. This demands a thorough understanding of the activity of a compound on key stages in the life cycle of the fungus, as well as of its mode of action. Effects on pre-symptomatic stages in early pathogenesis can be assessed by microscopic analysis. A similar approach has also been adopted in the study of the effect of sterol biosynthesis inhibitors on *V. inaequalis* (Kelley & Jones, 1981, Siebels & Mendgen, 1994). Data obtained in this way can be used to direct complementary biochemical mode of action studies, ultimately leading to a more complete understanding of the product so that usage can be optimised.

This paper reports the results of microscopic analyses of the effect of timed curative applications of pyrimethanil on key developmental stages in the infection of apple seedling

leaves by *Venturia inaequalis*. Field trial data are also presented.

MATERIALS AND METHODS

Controlled environment studies

Fungal culture

Spore suspensions were prepared from air-dried and frozen infected leaves by brief thawing in chilled sterile distilled water (SDW), vortex mixing for 20s to dislodge spores and filtration through two layers of muslin to remove debris. After centrifugation for 5min at 1500 x **g**, pelleted spores were resuspended in SDW and gently rolled on a bench rotator for 2h at room temperature. Spore suspensions were enumerated and adjusted to 1×10^5 ml^{-1} in SDW.

Plant growth and inoculation

Chitted apple seeds were grown to the four leaf stage in John Innes No.3 compost in individual $7cm^2$ Jiffy pots, maintained in a controlled environment room (18h photoperiod, 220µM m^{-2} s^{-1}, day temperature 19 ± 2°C, night temperature 15 ± 2°C). Leaves 1-4 were inoculated with 20µl droplets of spore suspension as two rows of three on each half of the leaf. Batches of plants were placed in 50 x 50 x 40cm lidded perspex humidity chambers on a 4cm high perforated mesh base, which was flooded with hot water prior to sealing and covering with black polythene. This maintained high humidity and reduced transpiration during the initial infection period of 48h, after which time light was admitted. Lids were removed from humidity chambers after 5days. Visible symptoms were apparent from 8-12d post-inoculation (PI).

Fungicide application

A 40% SC formulation was used, with a non-fungicide formulation as a control. Solutions (300mg AI/l) were made up in SDW immediately prior to use and sprayed onto the plants to run-off with a fine-nozzle mist sprayer at various times after inoculation. Plants were subsequently returned to the growth conditions described above. Batches of pyrimethanil-treated plants were segregated from untreated ones, due to the high vapour activity of the compound.

Light microscopy

Infected leaves were boiled for approximately 2 min in lactophenol-ethanol solution (20% (w/v) phenol, 40% (v/v) glycerol, 40% (v/v) ethanol) containing 0.02% (w/v) trypan blue. After rinsing in water, leaves were carefully transferred to a saturated solution of chloral hydrate (ca. 250% (w/v)) and left for 11-48h until chlorophyll was fully extracted. After rinsing in water, leaves were stored and mounted in 70% (v/v) glycerol. This technique stained conidiophores and spores, which had ruptured the cuticle, dark blue. Subcuticular mycelium which was not stained by this technique, was revealed by an adaptation of the method of Preece (1962). Fresh infected leaf discs were punched out with a 5mm cork borer and placed upper epidermis down in freshly prepared 2% (w/v) pectinase in McIlvaines' sodium phosphate - citrate buffer (pH4) to which 0.001% (w/v) sodium azide was added as a bacterial inhibitor. Using a water-pump vacuum dessicator, leaf discs were infiltrated with solution for 10min, and after rapid air admission discs were transferred to a 37°C water bath for 16-20h. After this time, cuticles were sufficiently 'loosened' to permit ingress of a 0.01% (w/v) aqueous solution of Blankophor. In many cases, the lower epidermis was completely removed, which

also facilitated stain penetration through to the subcuticular mycelium on the upper leaf surface. Discs were stained by dark immersion in a 0.01%(w/v) aqueous solution of Diethanol for 10min, followed by rinsing in water and immediate viewing under epifluorescence UV illumination. Germlings, appressoria, runner hyphae and conidiophores stained turquoise, primary stromata stained yellow. Conidia were weakly stained. Quantitative data on the effect of pyrimethanil on various infection structures was achieved by enumeration and/or direct measurements using an calibrated eyepiece graticule. In each case 100 structures were assessed from three randomly selected inoculum droplets and replicated in the same number of randomly selected leaves. A similar randomised assessment system was adopted for measurements made from LTSEM preparations (see below).

Low temperature scanning electron microscopy (LTSEM)

Infected leaf areas beneath inoculum droplets were dissected and processed as described previously (Daniels *et al.*, 1991). This technique, in which specimens were instantly vitrified in sub-cooled liquid nitrogen, results in preservation of the fungus-host interaction in a natural state.

Orchard studies

Fungicide application in field trials

Using the same formulation described previously, apple trees at trial sites at the Research Station of Gorsem, Belgium, were treated at a dose rate of 30g AI/hl pyrimethanil (formulated as a 400g AI/l suspension concentrate, SCALA®) using a knapsack sprayer delivering 300l water /ha. Since fungicide dose rates were calculated on the basis of a water volume of 1500l/ ha, desired rates were multiplied by 5 to compensate. Various spray protocols were adopted to investigate preventative and curative activity, optimum timing, incorporation into commercially adopted spray schemes and spray efficacy in response to scab warning systems. Preventative treatments depicted herein were performed according to registration requirements at 7-10d intervals throughout the season.

Visible symptom assessment

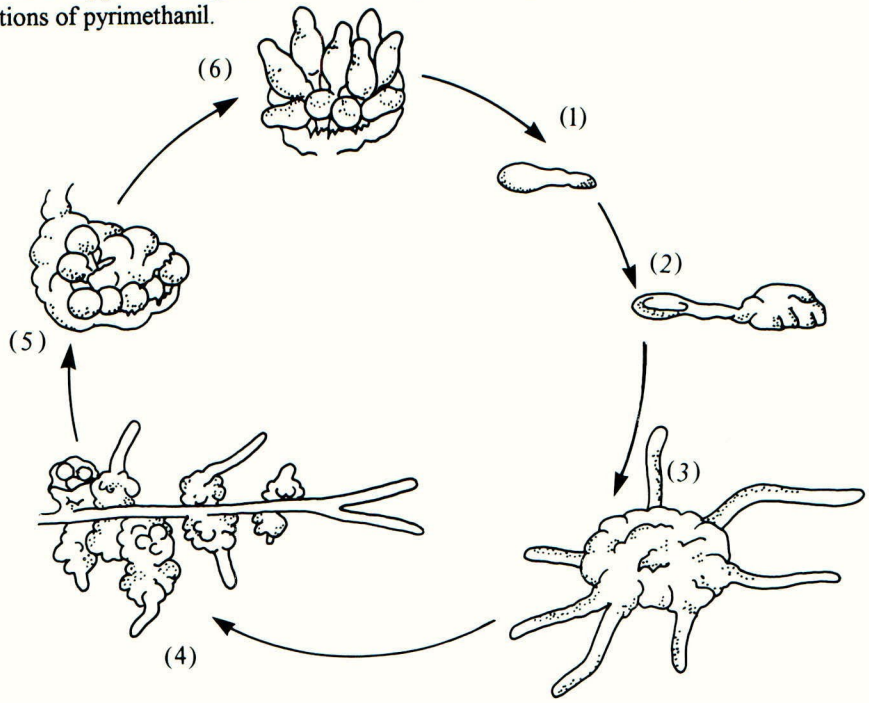
Infection of 100 leaves and fruit was recorded in four replicate plots on a scale of 0 - 3, where 0 = symptomless, 1 = one to two scab lesions, 2 = three to five lesions, 3 = full infection. Degree of infection was calculated by the Townsend-Heuberger formula, and efficacy was determined according to Abbot (1925).

RESULTS & DISCUSSION

The natural infection process

Considering the economic importance of apple scab, there is little published information on the early pathogenesis of the disease, particularly those stages occuring between formation of the subcuticular infection hypha and eventual conidiophore differentiation (reviewed by Becker, 1993). Key stages in the differentiation of *V. inaequalis* conidia on apple seedling leaves were therefore identified by daily observation of the infection process using the techniques and growth conditions described previously. Results are summarised in Fig. 1 overleaf.

FIGURE 1. Diagrammatic summary of key stages in the natural infection process of *Venturia inaequalis* on apple seedling leaves identified by microscopic techniques as targets for timed applications of pyrimethanil.



From this data, six key developmental stages were clearly defined, against which pyrimethanil treatments could be targetted. The timing of these key stages between three standard *V. inaequalis* isolates was slightly variable, but was reproducible for any given isolate. The stages were: **(1)** spore germination and appressorium formation (24-48h), **(2)** cuticular penetration by infection hyphae and formation of primary stromata (48-72h), **(3)** differentiation of runner hyphae from primary stromata (3-5d), **(4)** rapid extension growth of runner hyphae & formation of secondary stromata, conidiophores and secondary runner hyphae (5-7d), **(5)** spore maturation and cuticle rupture (7-9d) and **(6)** profuse surface sporulation with visible symptoms (8-15d). Spray applications were therefore numbered according to this system.

Effect of timed applications of pyrimethanil in controlled environment studies

Spray 1

Pyrimethanil had no effect on spore germination and appressorium formation when applied as a 300mg AI/l spray.

Spray 2

At this stage, infection hyphae penetrate the host cuticle, and differentiate into a discrete sub-cuticular cell aggregate composed of close-packed inflated cells one layer thick known as a primary stroma (Fig. 2). The cuticle stretches to accommodate expansion of the primary stroma which stops growing once a critical diameter is achieved. Although pyrimethanil does not prevent formation of primary stromata, it inhibits their further differentiation (Fig. 3). These arrested cells then die.

FIGURE 2. Primary stroma (ps) of *V. inaequalis* formed by subcuticular differentiation of an infection hypha formed from the appressorium (a). c = conidium, arrow indicates stretched cuticle. Scale bar = 10µm.

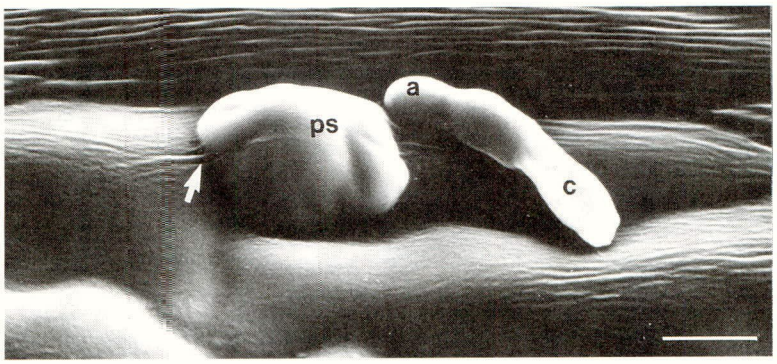
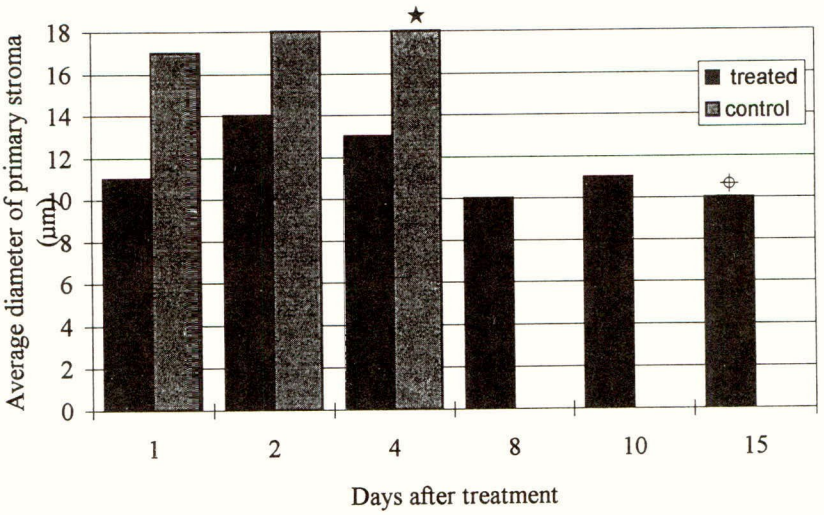


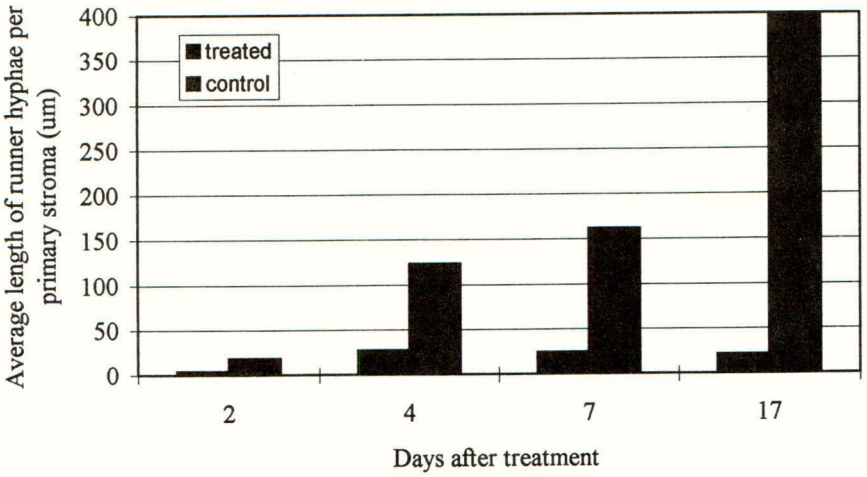
FIGURE 3. Histogram showing the effect of a 24h post-inoculation pyrimethanil spray at 300mg AI/l on the subsequent development of *V. inaequalis* primary stromata on apple seedling leaves. ★ = start of runner hypha differentiation, with associated inability to discern the primary stroma. ⊕ = 98% of primary stroma dead.



Spray 3

Once primary stromata have reached optimum size, peripheral cells differentiate to form rapidly extending adventitious or "runner" hyphae. Preliminary data suggest that this stage requires an exogenous host-derived nutrient source, possibly provided by partial degredation of the underlying host epidermal cell wall. This stage is the primary target of pyrimethanil, which inhibits runner hyphae formation (Fig. 4).

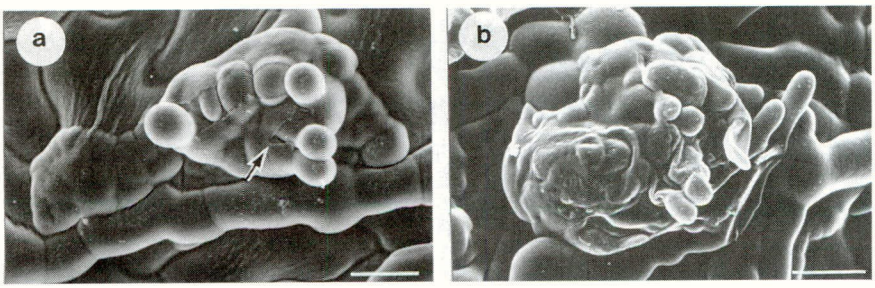
FIGURE 4. Histogram showing the effect of a 300mg AI/l pyrimethanil spray on the differentiation of runner hyphae from primary stromata of *V. inaequalis* on apple seedling leaves.



Spray 4

Following rapid extension growth of runner hyphae, lateral hyphae are produced which differentiate into secondary stromata. These either produce secondary runner hyphae, and/or differentiate conidiophores (Fig. 5a). Sprays timed to coincide with this stage inhibit formation of secondary runner hyphae in the manner described above, and also inhibit the normal development of conidiophores, causing conidial abortion prior to cuticle rupture (Fig. 5b). In addition extension growth of "'mother" primary runner hyphae is inhibited.

FIGURE 5: (A) Normal conidiophore formed on an untreated leaf, showing cuticle rupture (arrowed) as the conidiophore tip cells expand to form conidia. Scale marker = 10μm. (B): Abnormal conidiophore showing conidial abortion, formed following a 300mg AI/l pyrimethanil spray at stage **(4)**. Scale marker = 10μm.



Spray 5

Although pyrimethanil applications timed to coincide with conidial maturation at the time of cuticle rupture have no discernible effect on the subsequent liberation of mature spores, conidiophores at earlier developmental stages respond to pyrimethanil by failing to undergo tip cell expansion, resulting in formation of abnormal conidia and/or abortion of conidia. Such sprays significantly reduce sporulation, and may prevent lesion development.

Spray 6

Sprays made on actively sporulating lesions also significantly reduce lesion development in a persistent manner. This is a function of the combined activity of pyrimethanil on the various differentiation stages described previously. Average lesion diameters determined by the clearing and staining technique described in the methods section were reduced by 40%, 2 weeks after treatment of actively sporulating lesions.

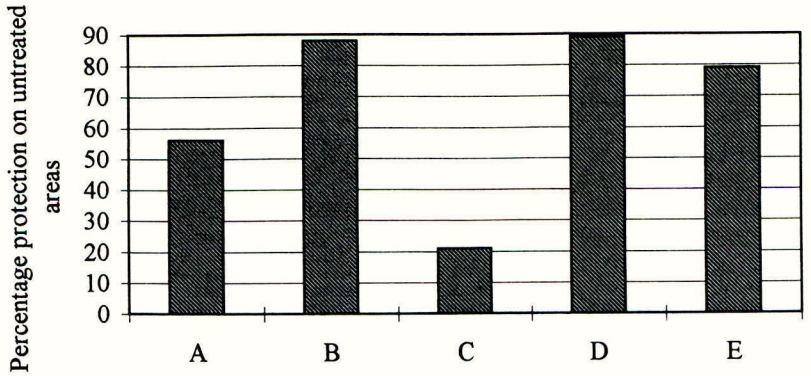
Field trial data

At a rate of 30g AI/hl and spray intervals of 7-10d pyrimethanil shows excellent field efficacy against *V. inaequalis*. Performance is comparable to a typical reference contact scab fungicide (Table 1). The high field efficacy of pyrimethanil, particularly against leaf scab, is enhanced by its efficient vapour activity combined with its translaminarity and ability to redistribute within the leaf (Fig. 6), without being transported throughout the whole plant.

TABLE 1. Percentage efficacy of pyrimethanil on *V. inaequalis* as a full season spray in comparison to a standard reference contact scab fungicide. #1 = cv. Golden Delicious (11 sprays at 7- 10d intervals). #2 = cv. Jonagold (17 sprays at 7-10d intervals).

	Pyrimethanil	Captan	
Leaves	97.8	94.8	#1
Fruit	96.7	96.5	
Leaves	97.9	92.3	#2
Fruit	96.5	94.9	

FIGURE 6. Translaminarity and lateral systemicity of pyrimethanil against *V. inaequalis* in apple leaves, demonstrated in controlled environment studies. Spore suspensions were spray inoculated. Key: A = abaxial surface treated, adaxial assessed; B = adaxial surface treated, abaxial assessed; C = tip treated, base assessed; D = base treated, tip assessed; E = left half treated, right half assessed.



Orchard results obtained elsewhere (J. Tromas, AgrEvo France, pers. comm.) for the curative activity of pyrimethanil corroborate data obtained from the mode of action studies described in this paper. Both lead to the recommendation that pyrimethanil should be used from budburst to flowering either preventatively at 7-10d intervals, or curatively up to 3d after scab warnings. Thereafter as an anti-resistance strategy, pyrimethanil should only be used in partnership with a scab compound having a different mode of action, and for example additional powdery mildew activity.

DISCUSSION

Detailed microscopic analysis of the infection of apple seedling leaves by *V. inaequalis* indicate that pyrimethanil inhibits critical early stages in pathogenesis, preventing lesion development and inhibiting sporulation. Other studies of the effect of pyrimethanil on the natural infection process of *Botrytis fabae* on broad bean leaves have shown that the compound suppresses the normal lytic function of hyphae which results in host cell necrosis during lesion formation (Daniels & Lucas, unpublished data, Milling *et* al., 1994). Biochemical analysis subsequently revealed this pyrimethanil-induced inhibition of pathogenesis to be due to perturbed secretion of extracellular hydrolytic enzymes known to degrade the host cell wall (Milling & Richardson, unpublished data). It thus seems likely that pyrimethanil has a similar mode of action on *V. inaequalis*, and work is currently in progress to confirm this.

In summary, pyrimethanil is highly suitable for incorporation into IPM systems due to its flexible and efficient control of both pre- and early post-symptom infections, and suitability as a partner in mixed formulations used mid-season. Pyrimethanil also has the advantage of excellent crop safety and environmental profiles.

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A FAMILY OF NEW, NON-ETU GENERATING DITHIOCARBAMATE MICROBICIDES

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ABSTRACT

The antimicrobial preparations containing new dithiocarbamate compounds of the general formula **Z·D·Q·Y**, control in the greenhouse and in the field populations and propagules of phytopathogenic microorganisms (bacteria and fungi) at rates of 100-750 g AI/ha, and on propagation materials 100-1000 g AI/t. In the general formula **Q** and **D** are metal atom and dodecyl guanidine base, while **Z** and **Y** represent dithiocarbamate and acid moieties. Deterioration of these compounds in the environment does not lead to the formation ethylenethiourea. They have low toxicity to plants and other non-target species and possess wide spectrum of action and high efficacy, comparable to those of mancozeb and other ethylenethiourea-generating dithiocarbamates. The antifungal activity of the new compounds depends on the quality of metal atom and generally increases in the order Fe < Cu < Zn < Mn. Mixtures of derivatives with **Q** = Zn+Mn or Fe+Cu exhibit significant synergetic joint actions in controlling a range of fungal diseases. Mancozeb in marketed mixtures with fungicides of various modes of action can be replaced with the new compounds.

INTRODUCTION

The Zn- and Mn-containing derivatives of ethylenebisdithiocarbamate (zineb, maneb, and mancozeb) alone or in combination with other pesticides are extensively used in agricultural practice to combat losses caused by microbes invading cultivated plants. They have several advantageous properties, such as good crop tolerance, broad activity spectrum, oligo-site mode of action, and low price (Sijpesteijn *et al.*, 1977). The future of these compounds is uncertain because of their possible degradation to the toxic metabolite ethylenethiourea (ETU). Therefore, there has been a search for replacements of ETU-generating antimicrobial compounds. The new compounds should be at least equally effective, and should be suitable for a range of different applications. The main difficulty of finding such replacement compounds is caused by differences in biology, biochemistry and metabolic activity of target microbes. Therefore, only oligo-site inhibitors can be considered. Dialkyldithiocarbamates are suitable candidates, but their use is limited by somewhat lower efficacy and a tendency to decompose during storage. In an attempt to improve their potency, mixed ligand complexes of ziram with dodine base were prepared (GE Pat. No. 2 144 123).

The present paper introduces new preparations based on dialkyldithiocarbamates proposed for replacement of ETU-generating compounds, and reports on their biological activity in the laboratory and in the field.

CHEMICAL AND PHYSICAL PROPERTIES

During syntheses of different preparations containing metal dithiocarbamates and alkylguanidine compounds, we observed the occurrence of a quantitative chemical reaction between metal dithiocarbamates and salts of alkylguanidines in water, the products of which possess high antimicrobial potency. Synthesis of a related compound of the GE 2 144 123 patent was carried out in methanol, with the exclusion of carbon dioxide.

Properties of a representative substance, code number NKI-42650 (Fig. 1), of the family (I) of molecular formula $(C_{21}H_{45}N_5O_2S_4)_{x+y}Zn_xMn_y$ are:

Molecular weight (x=1 y=0): 542;
Melting point: 184-189 °C;
Physical state: solid, white, waxy powder;
Solubility (20 °C): < 0.1 g/1000 ml water;
Acute oral toxicity (rat, LD50): > 2000 mg/kg;
Mutagenicity: Prokaryote - none, Eukaryote - none

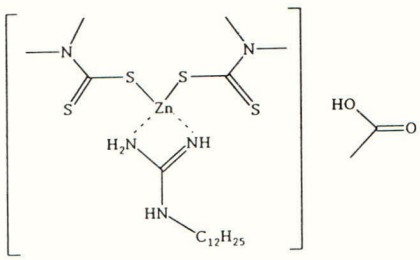

Figure 1. Chemical structure of NKI-42650.

BIOLOGICAL ACTIVITY

Materials and methods

The biological activity of the new compounds was tested in vitro in amended agar media against axenic cultures of phytopathogenic bacteria and fungi (Tables 1 and 2). Their in vivo efficacy was studied by seed treatments and leaf sprays. Evaluations were usually made 3-4 days after the appearance of first symptoms, measuring the intensity of infection by a 5 step scale or evaluating the yield, and inhibition calculated as related to the untreated control. The methods used by Bánki (1978) and Oros & Virányi (1986) were followed. The significance of effects was tested by *t* or *F* probes. The character of joint action was evaluated according to Horsfall & Dimmond (1941).

Test on mutagenicity

Frequency of resistant to agrocin *Agrobacterium tumefaciens* C-54 mutants to compounds in axenic cultures was determined as follows: after incubation of 5 ml of standard cell suspension of bacterium (10^6 cell per ml) with 5 µg of compound for 12 hours at 22-26°C, 5 ml of sterile distilled water was added and aliquots (1 ml) mixed with 4 ml of agar (10 g Difco Agar No. 3 in 1 litre distilled water) at 45°C and immediately poured over the surface of agar plate (15 ml in 90 mm Petri dish) containing agrocine produced on Kerr's medium (Kerr, 1980). The plates were incubated for 48 hours and the number of colonies tolerant to agrocine was counted.

The effect of chemicals on the frequency of benomyl sensitive revertants of *Mycosphaerella tulasnei* was determined as follows: the compounds were added to potato-dextrose agar (PDA) at a final concentration of 5 mg AI/l before pouring in 90 mm Petri dishes (20 ml each). The plates were inoculated with 10^5 conidia in 5 ml of 1% agar-agar layering on the surface. After 5 days the formed conidia were washed off and 10^5 conidia in 5 ml of 1% agar-agar was poured on the surface of 20 ml PDA containing 10 mg AI/l of diethofencarb in 90 mm Petri dish. The number of colonies was determined after 96 hours incubation at 20-22 °C. Their sensitivity to benomyl was checked randomly.

RESULTS

The efficacy of NKI-42650 in vitro against bacteria was equivalent to that of ziram and dodine, and in most of cases similar to streptomycin (Table 1). Compared with known substances, differences in the spectrum of antibacterial activity were demonstrated. The tolerance of non-phytopathogenic *Agrobacterium radiobacter*, *Erwinia uredovora* and *Pseudomonas fluorescens* was significantly higher to this new compound than to ziram and dodine.

TABLE 1. Antibacterial effect of dithiocarbamate derivatives and dodine.

Species	Origin	MIC values (mg AI/l)			
		NKI-42650	Dodin	Ziram	Strept.
Agrobacterium radiobacter	soil	25-50	>250	6-13	250-500
Agrobacterium tumefaciens	grape	25-50	125-250	6-13	>1000
Bradyrhizobium japonicum	soya	25-50	>250	13-25	>1000
Erwinia herbicola	chestnut	25-50	50-100	25-50	13-25
Erwinia chrysanthemi	banana	3-6	13-25	13-25	3-6
Erwinia uredovora	rust	25-50	>250	6-13	3-6
Pseudomonas fluorescens	soil	>250	>100	50-100	250-500
Pseudomonas lachrymans	cucumber	25-50	>250	13-25	3-6
Pseudomonas phaseolicola	bean	25-50	>250	6-13	3-6
Xanthomonas malvacearum	cotton	3-6	13-25	3-6	13-25
Xanthomonas phaseoli	bean	3-6	25-50	3-6	13-25
Xanthomonas vesicatoria	tomato	6-13	25-50	6-13	13-25
Corynebacterium michiganense	tomato	6-13	50-100	6-13	1-3
Curtobacterium flaccumfaciens	bean	6-13	50-100	6-13	1-3

Minimal inhibitory concentration (MIC) values were determined in vitro according to Oros, Cserháti & Szőgyi (1983). Strept. = Streptomycin (SERVA).

The spectrum of antifungal activity of NKI-42650 is broader and it is more active than dodine and ziram (Table 2), in particular against *Ascochyta pisi*, *Fusarium graminearum*, *Colletotrichum lindemutianum*, *Botrytis alli*, *Phytophthora parasitica* and *Rhizoctonia solani*.

The frequency of spontaneous mutants of *Agrobacterium* or *Mycosphaerella* tolerant to agrocine or diethofencarb, respectively, was increased only when these microbes were exposed to sublethal doses of mancozeb.

TABLE 2. Antifungal activity of dithiocarbamate derivatives and dodine.

Species	Origin	LD50 (mg AI/l)		
		NKI-64250	Dodin	Ziram
Mucor racemosus	air	341	1196	4255
Rhizopus nigricans	pepper	34	981	154
Penicillum sp.	grape	307	11381	1283
Alternaria solani	potato	89	686	428
Nectria cinnabarina	currant	44	655	180
Didymella applanata	raspberry	108	53	287
Fusarium graminearum	wheat	51	2410	234
F. oxysporum	tomato	62	440	261
Phoma betae	sugar beet	93	440	247
Cladosporium cucumerinum	cucumber	138	207	347
Colletotrichum lindemutianum	bean	138	9318	1349
Helminthosporium carbonum	maize	79	325	367
Ascohyta pisi	pea	506	1390	1283
Botrytis alli	onion	138	888	1383
Botryodiplodia theobromae	coconut	251	2665	668
Macrophomina phaseolina	sunflower	46	343	194
Thielaviopsis basicola	tobacco	138	163	448
Rhizoctonia solani	potato	560	2801	701
Trametes versicolor	peach	56	163	1233

LD50 values were determined by plotting on log/probit paper the radial growth inhibition data measured on PDA medium.

TABLE 3. Alterations in frequency of tolerant mutants due to exposure of microbes to sublethal doses of pesticides.

No.	Compounds	Frequency of tolerant mutants[a]	
		A. tumefaciens	*M. tulasnei*
1.	Control	6-12	1-7
2.	Mancozeb	5-34	18-27
3.	Ziram	4-9	5-9
4.	NKI-42650	7-13	2-9
5.	NKI-94109	3-11	3-8

[a]Minimum and maximum number of colonies in ten parallels. NKI-94109 is a compound of molecular formula I where x=2, y=1.

Seed treatments and foliar applications of NKI-42650 and related compounds proved to be highly active against a number of agriculturally important fungal species, in particular *Fusarium, Colletotrichum, Venturia* and *Plasmopara*.

The influence of modifications in the structure of acid moiety (**Y**) of the compound was studied using tomato powdery mildew and bacterial leaf

spot of tomato. The variations in chain length (C_2-C_{18}) of alkylcarboxylic acids did not modify the efficacy of preparations (**Y**=acetic acid, **Q**=zinc) against these two pathogens. In preparations where the acid moiety alone is biologically active a significant increase in effectiveness in the expected direction was demonstrated. In addition, these compounds are characterized by increased therapeutic indices. Their building blocks alone can not be used because of phytotoxicity (nalidixic acid, 2-(1-methylheptyl)-4,6-dinitrophenol), lack of chemical stability (dimethyldithiocarbamic acid), or low efficacy (3-[1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazole-1-yl)-but-2-yl]-p-(2-carboxy-ethyl)-p-(methyl-phosphinic acid)). These effects were also demonstrated in sunflower downy mildew and wheat powdery mildew systems.

The influence of the quality of metal atom (**Q**) on the effectiveness of the new compounds (**Y**=acetic acid) was studied <u>in vitro</u> as well as in the forms of seed treatments and foliar sprays. The order of efficacy observed <u>in vitro</u> is different from that <u>in vivo</u>. The Horsfall's order Cu > Zn > Mn > Fe (Rich, 1960) was verified <u>in vitro</u> (for example ED50 values found for *Pythium ultimum* were 1.8, 2.9, 6.1 and >100 mg AI/l, respectively), while data from studies <u>in vivo</u> (Fig. 2) show that the order of efficacy is determined by the host-parasite system and the average order of activity of preparations containing different metals is Mn > Zn > Cu > Fe. Efficacy of the new compounds surpasses those of ziram or mancozeb.

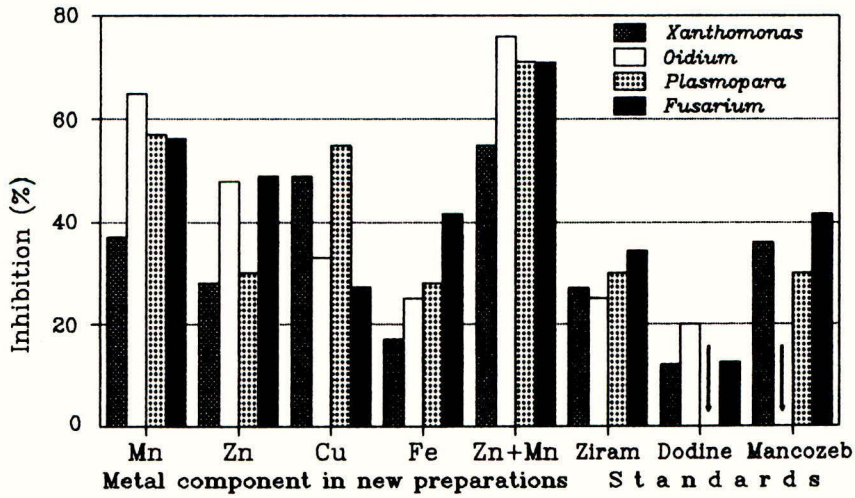

Figure 2. The influence of the **Q** metal atom on the effectiveness of compounds in host-parasite systems. Acetic acid represented in each case the acid moiety (**Y**), and the preparations were applied as leaf sprays (500 mg AI/l) against tomato pathogens, and as seed treatments against *Fusarium* (300 g AI/t) and *Plasmopara halstedii* (50 mg AI/l). The reference compounds were used in comparable doses.

When new compounds containing different **Q** atoms were used in mixtures their activity significantly increased against both *X. vesicatoria* and *P. halstedii* (Fig. 3). Optimum Zn:Mn ratio was found in the range of (4:1)-(2:3) ($P < 0.001$). Similar interactions were observed when Fe and Cu were used as **Q** atoms.

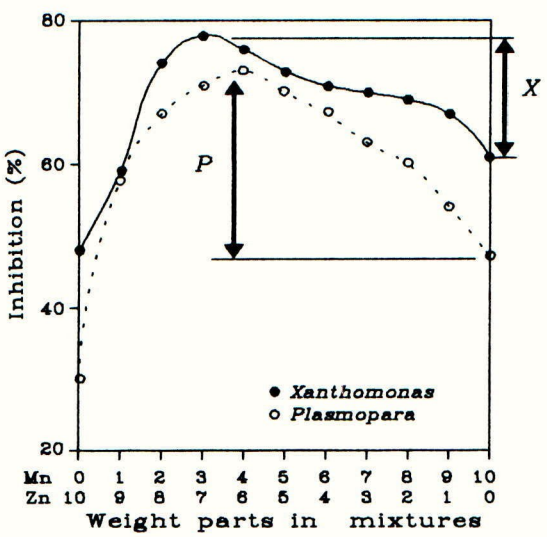

Figure 3. Joint action of NKI-42650 and NKI-43724 in control of bacterial spot of tomato (500 mg AI/l leaf spray) and sunflower downy mildew (125 mg AI/l seed treatment).

Tomato plants and sunflower germlings were treated before and after infection, respectively. The NKI-43724 is a compound of molecular formula I where X=0, y=1. The marked differences between maximum response values and most potent treatments are those demanded for "synergism" according to Worley (1969).

When evaluating activity of NKI-42650 and its mixtures with metalaxyl against soil-, seed- and airborne oomycetes (Table 4) in greenhouse efficacy comparable to commercial preparations (Ridomil and Ridomil Zineb) was demonstrated.

TABLE 4. Joint action of NKI-42650 and metalaxyl in control of pea damp off, sunflower downy mildew and early blight on tomato.

Treatments No.	Compounds	Effectiveness (%) against *Pythium ultimum*	*Plasmopara halstedii*	*Phytophthora infestans*
1.	NKI-42650	68b	20a	62a
2.	Zineb	41a	9a	58a
3.	Metalaxyl	86d	58b	86d
4.	1+3 (4:1)	84cd	76c	82cd
5.	2+3 (62:13)	76c	43b	76bcd

Healthy seedlings of pea (cv. Rhone Dwarf) and sunflower (cv. GK-70) were counted, and diseased leaf-area of tomato plants (cv. K548) measured using a 5-step scale of evaluation, and disease inhibition was calculated. Pea seeds were treated with 300 g AI/t two days before sowing. Sunflower germlings and tomato plants were treated after and before infection with 20 and 10 mg AI/l solutions, respectively.

In a heavily infested field the new compounds in combination with benomyl significantly reduced the *Fusarium* contamination of wheat seeds in repeated applications, as well as the evolution of powdery mildew infection (Table 4). Especially significant beneficial effect was demonstrated when the emergence of seeds of treated ears was studied. The high rate of *Fusarium* contamination in itself did not lead to a fatal diminishing of emergence when benomyl was applied in combination.

TABLE 5. Effect of fungicides on mildew severity, *Fusarium* contamination of seeds and emergence of wheat.

Treatments No.	Compounds	Dose g AI/ha	*Erysiphe* Diseae Intensity	*Fusarium* contamined seeds %	Plant Emergence %
1.	Control	---	3.38a	94.8ab	24.0a
2.	NKI-42650	1000	2.46b	85.8bc	62.8c
3.	NKI-43724	1000	2.47b	89.8b	62.3a
4.	NKI-94109	1000	2.21bc	81.5cd	63.7b
5.	Mancozeb	1000	3.29a	98.0a	28.0b
6.	Benomyl	350	2.00c	78.0cd	71.3bc
7.	4+6 (1:4)	1000	1.96c	74.3d	74.8c
8.	5+6 (1:4)	1000	2.33b	76.5cd	67.5bc

The applications were carried out 6 days before and after artificial infection of flowers with *F. graminearum* spores (10^5 cell/ml).
Table contains data on *Erysiphe* disease intensity as evaluated by a 5 step scale, rate (%) of *Fusarium* contamination established, and percentage of germinated seeds sown in sterile soil from treated plots.

The advantageous interaction arose even in cases when a new compound was included into the control programme and applied alone not simultaneously with others (Tables 6 and 7).

TABLE 6. Effect of fungicides on plant survival and production of French beans.

Treatments	Leaf spray	NKI-94109			
	Dose	0%		0.1%	
Seed dressing	g AI/t	A	B	A	B
Untreated	0	40	363	55	318
Pencycuron	125	50	410	75	693
LSD (P=0.05)		10	140	10	140

A=Number of plants which survived/plot, B=pods/100 plant. French beans (cv. Lingua di fuco) sensitive to *Rhizoctonia* and *Colletotrichum* were sprayed after development of primary leaves.

The manifestation of synergetic interaction was evident (Table 6) particularly influencing the formation of pods on French beans.

When NKI-42650 treatments were included only twice in the control programme, against apple scab, the degree of control increased dramatically (Table 7). Evaluating the efficacy of treatments one should take into consideration, that the population of *Venturia* in the orchard concerned exhibited tolerance to benzimidazoles and dodine due to the repeated application of these fungices previously. Powdery mildew, Monilia and minor pathogens (*Botrytis, Alternaria, Phomopsis*, etc.) were fully controlled throughout the experiment.

TABLE 7. Control of apple scab disease.

No.	Treatment	% Leaf scab	Efficacy	
			%	%increase
1.	Untreated	65.8	---	---
2.	Standard[a]	30.1	54.2	---
3.	NKI-42650[b]	19.5	69.9	15.7
4.	NKI-42650+benomyl[c]	11.5	82.5	29.3

[a]= Standard treatment: Fenarimol + triadimefon, tank-mixture (1:1 w/w of active substances). [b]= In the 7th, 8th and 10th, 11th treatments fenarimol+triadimefon mixture was replaced with NKI-42650. [c]= In the 7th, 8th and 10th, 11th treatments fenarimol+triadimefon mixture was replaced with a combined preparation of NKI-42650 and benomyl (7:3 w/w of active substances). The trees (cv. Golden Delicious) were sprayed in the usual manner (75 g AI/ 100 l) 14 times before assessment.

The superiority of the new compounds as compared to mancozeb and zineb against range of pathogens used alone or in combination with benomyl and metalaxyl is evident independently on the method of application ($P<0.05$).

ACKNOWLEDGEMENT

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