SESSION 2

NEW COMPOUNDS, FORMULATIONS AND USES

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IMIDACLOPRID, A NOVEL SYSTEMIC NITROMETHYLENE ANALOGUE INSECTICIDE FOR CROP PROTECTION

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ABSTRACT

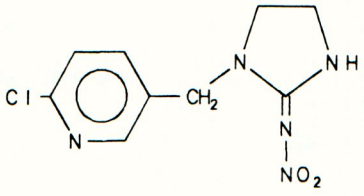
Imidacloprid is a highly effective insecticide with low mammalian toxicity, for control of sucking insects such as aphids, leafhoppers and planthoppers, thrips and white flies including resistant strains. It is also effective against Coleoptera (e.g. Atomaria sp., Leptinotarsa decemlineata, Lissorhoptrus oryzophilus, Lema oryzae), Diptera (e.g. Oscinella frit and Pegomya sp.) and Lepidoptera (e.g. Lithocolletis sp.). No activity was found against nematodes and spider mites. Due to its excellent systemic characteristics, the product is especially appropriate for seed treatment and granular application. Effective early season control with long-lasting protection is achieved in crops such as cereals, maize, rice, potatoes, sugar beet and cotton. Pests attacking later in the season can be controlled by foliar applications in the above mentioned crops, as well as in citrus, deciduous fruits, vegetables and other crops. The product will be marketed under the names Gaucho for seed treatment and Confidor for foliar and soil application (in Japan: Admire). Selected trials on the biological activity of imidacloprid in both laboratory and field are presented and discussed.

INTRODUCTION

Following the discovery of the insecticidal properties of the heterocyclic nitromethylenes by Soloway et al. (1979), Nihon Tokushu Noyaku Seizo K.K. synthesized highly active compounds from this chemical group, leading to the development of imidacloprid.

Nitromethylenes are effectors of the nicotinic acetylcholine receptor (Benson, 1989; Schroeder et al., 1984) and our own preliminary investigations indicate that imidacloprid also acts on this receptor. The present paper describes the technical properties of the compound and its biological activity in laboratory and field studies.

CHEMICAL AND PHYSICAL PROPERTIES

Common name:	Imidacloprid
Chemical name:	1-(6-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylideneamine
Code number:	NTN 33893
Molecular formula:	$C_9H_{10}ClN_5O_2$
Structural formula:	
Molecular weight:	255.7
Physical state:	Crystalline
Vapour pressure:	2.0×10^{-9} mbar at 20° C
Melting point:	143.8° C modification 1 136.4° C modification 2
Solubility (20° C)	0.51 g AI/1000 ml water
Formulations:	70 WS, 350 FS, 5 GR, 2 GR, 1 GR, 200 SL, 10 WP, 0.25 DL, combinations

TOXICOLOGICAL AND ECOBIOLOGICAL PROPERTIES

Acute oral, rat ♂,♀ (LD50):	ca. 450 mg/kg
Acute dermal, rat ♂ ♀ (LD50):	> 5000 mg/kg
Acute inhalations rat (LC50, 4 h) dust:	> 5323 mg/m³
Eye, skin irritation, rabbit:	None
Mutagenicity (in vivo):	Negative
Teratogenicity:	Negative
Sensitization:	No evidence of skin-sensitizing potential
Golden orfe (LC50, 96 h):	237 mg/l
Japanese quail (LD50):	31 mg/kg
Earthworm (LC50):	10.7 mg/kg substrate (dry weight)
Water flea (EC50, 24 and 48 h):	> 32 mg AI/l

BIOLOGICAL PROPERTIES

Laboratory studies

Materials and methods

The biological efficacy of imidacloprid was tested after foliar application. Systemic properties and residual effect against early season pests were determined after homogeneous incorporation of the compound into soil, seed treatment or seedling box application. Evaluations of efficacy were usually made 3 - 4 days after the artificial infestation.

Insecticidal activity and residual effect

Foliar application of imidacloprid proved to be highly active against a number of agriculturally important insect species, in particular sucking insects such as the leafhoppers Nephotettix cincticeps, planthoppers Nilaparvata lugens, Laodelphax striatellus, Sogatella furcifera, aphids Myzus persicae, Aphis gossypii, and thrips Hercinothrips femoralis (Table 1). It was also effective against the white fly Bemisia tabaci, rice stem borer Chilo suppressalis, rice leaf beetle Lema oryzae, and rice water weevil Lissorhoptrus oryzophilus. Imidacloprid is superior both to buprofezin and etofenprox, the standard products in rice, to the aphicide pirimicarb and to cartap, which like imidacloprid, is also an effector of the nicotinic acetylcholine receptor.

TABLE 1 Acute toxicity (LC95, ppm) of imidacloprid and standard products, foliar application

Species			imidacloprid	cartap	buprofezin	etofenprox	pirimicarb
Nephotettix cincticeps	L_3	S	0.32	200	8	8	-
Nilaparvata lugens	L_3	R	0.064	-	8	8	-
Laodelphax striatellus	L_3	R	1.6	-	8	40	-
Sogatella furcifera	L_3	S	1.6	-	1.6	8	-
Myzus persicae	MP		1.6	> 1000	-	-	8
Aphis gossypii	MP		1.6	-	-	-	8
Hercinothrips femoralis	MP		1.6	-	-	-	-
Bemisia tabaci	L_2		8	-	8	-	-
Chilo suppressalis	L_1		8	1.6	-	-	-
Lema oryzae	I	S	8	40	-	40	-
Lissorhoptrus oryzophilus	I		40	> 1000	-	40	-

S susceptible strain
R organophosphorus- and carbamate-resistant strain
L_1, L_2, L_3 1st, 2nd, 3rd larval stage
I Imago, MP mixed population

The outstanding systemic properties of imidacloprid make it excellent for soil and seed treatment against sucking insects (Table 2).

TABLE 2 Residual activity (mortality > 95 % in weeks) of imidacloprid in comparison to standard products against aphids, leafhoppers and planthoppers

APPLICATION/species		dosage	imidacloprid	aldicarb	carbofuran	disulfoton
SOIL APPLICATION		ppm AI	1.25	1.25	1.25	-
Myzus persicae			> 8	3	< 1	-
Aphis fabae			5	-	-	-
SEED TREATMENT		g AI/kg seed	1.0	-	-	-
Aphis fabae			> 5	-	-	-
Aphis gossypii			> 5	-	-	-
SEEDLING BOX APPLICATION		g AI/box	1.0	-	-	5.0
Nephotettix cincticeps	R		> 11	-	-	< 1
Nilaparvata lugens	R		> 11	-	-	< 1
Laodelphax striatellus	S		> 11	-	-	1
Sogatella furcifera	S		> 11	-	-	> 11

Incorporated into the soil at 1.25 ppm, the product provides long-lasting protection against M. persicae in cabbage plants and Aphis fabae in broad beans. Granular treatment with imidacloprid at 1 g AI/seedling box provides excellent control of leafhoppers and planthoppers in rice. Similarly, seed treatment (1 g AI/kg) controls A. fabae on bean and A. gossypii on cotton for at least 5 weeks.

Efficacy against resistant populations

Because its site of action is different from that of organophosphorus, carbamate, and pyrethroid insecticides, imidacloprid is also highly effective against strains of insects such as aphids, leafhoppers, and planthoppers which have become resistant to conventional insecticides (Table 3). Resistant strains (R) of N. cincticeps, N. lugens, and L. striatellus have lost susceptibility to organophosphates and carbamates, M. persicae to organophosphates, carbamates, and pyrethroids. Compared with the susceptible populations (S), none of the resistant strains showed reduced sensitivity to imidacloprid.

TABLE 3 Acute toxicity (LC50, ppm) of imidacloprid against susceptible (S) and resistant (R) strains of leafhoppers, planthoppers and aphids.

Species	leaf dipping			root dipping		
	S	R	R/S	S	R	R/S
Nephotettix cincticeps	0.064	0.064	1	0.0022	0.00099	0.5
Nilaparvata lugens	0.064	0.064	1	2.9	2.3	0.8
Laodelphax striatellus	0.32	0.32-1.6	1-5	16	9.8	0.6
Myzus persicae	1.6	1.6	1	-	-	-

S susceptible strain
R organophosphorus- and carbamate-resistant strains (N. cincticeps, N. lugens, L. striatellus), organophosphorus-, carbamate- and pyrethroid-resistant strain (M. persicae)

Field studies

Imidacloprid has been tested successfully over several years in numerous crops all over the world. Examples below show important areas of use and methods of application. Furthermore the product will be marketed in other important crops such as citrus, deciduous fruits and vegetables.

Rice

Infestation with leafhoppers and planthoppers causes great economic damage, particularly in rice cultivation in Asia. A great expansion of infested areas and the occurrence of resistant strains make these pests one of the greatest plant protection problems in rice. Imidacloprid proved highly effective against all economically important hopper species after granule application to seedling boxes or broadcast, seed treatment, spraying, or dusting. Imidacloprid applied to seedling boxes shortly before transplanting was superior to a standard treatment sequence of seedling box application followed by 3 sprays for the control of smaller brown planthopper, L. striatellus and suppression of infestation with rice stripe virus (Fig. 1). Lasting control of the green leafhopper N. cincticeps was achieved by dust application of imidacloprid at 100 g AI/ha (Fig. 2).

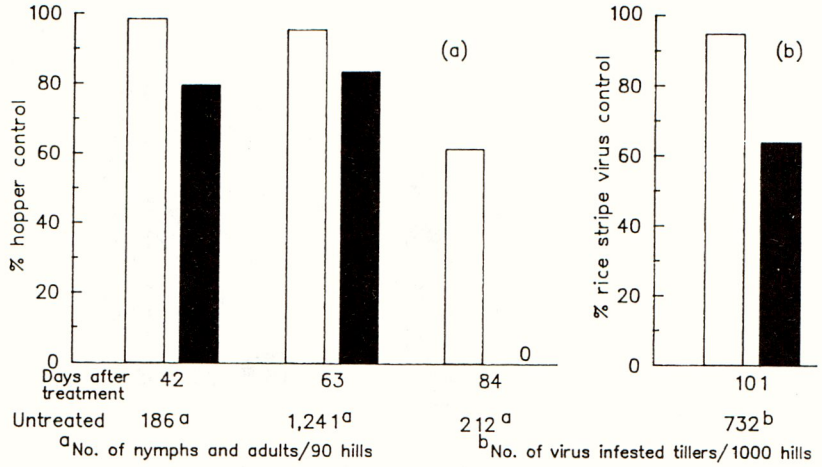


Fig. 1 Control of Laodelphax striatelius (a) and rice stripe virus (b) on paddy rice (Japan 1989) after applying imidacloprid 2 GR (▭) to rice seedling boxes (1 g AI/box), or carbosulfan 5 GR (3.5 g AI/box) followed by 3 sprays (2 week intervals) of etofenprox 200 EC, 0.01 % AI, 1000 l/ha (▬)

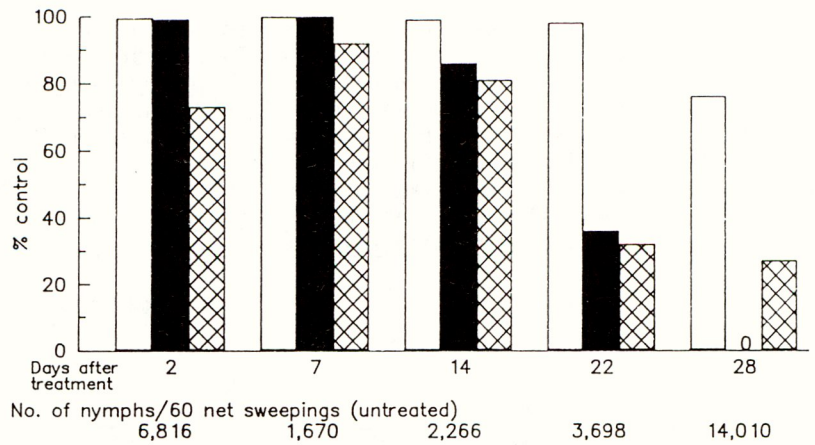


Fig.2 Control of Nephotettix cincticeps on paddy rice (Japan 1989) by dust application of imidacloprid 0.25 DL, 100 g AI/ha (▭), etofenprox 0.5 DL, 200 g AI/ha (▬) or buprofezin 1.0 DL, 400 g AI/ha + BMPC 2.0 DL, 800 g AI/ha (▨)

Potatoes

In potatoes, imidacloprid is outstandingly suited to the control of wireworms, flea beetles, aphids, leafhoppers, Colorado potato beetles and other leaf insects. It is possible to use the active ingredient in soil and seed treatment formulations and for foliar application. Various methods of application (e.g. infurrow spray, infurrow granular application, and tuber dressing) provided excellent protection of potato plants up to 60 days after treatment against a severe infestation of insecticide resistant Colorado potato beetles Leptinotarsa decemlineata in the USA (Fig. 3).

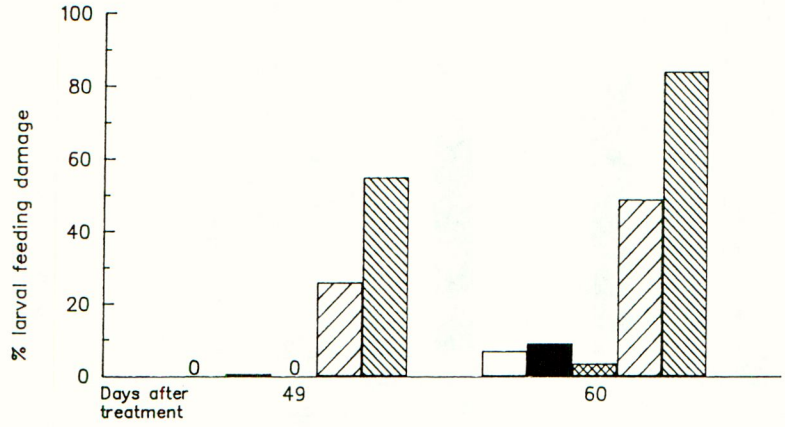


Fig.3 Control of resistant Leptinotarsa decemlineata on potatoes (USA 1989) after treatment with imidacloprid 240 FS, 0.02 g AI/m, infurrow spray (▭), imidacloprid 5 GR, 0.02 g AI/m, infurrow granule (▬), imidacloprid 1.25 DS, 12.5 g AI/100 kg, tuber dressing (▨), aldicarb 15 GR, 0.3 g AI/m, infurrow granule (▨) or untreated (▨)

Cotton

Early season pests cause considerable damage to young plants especially in the first few weeks after emergence. They occur regularly in most cotton-growing countries and are usually controlled by using granules with the seed or spraying after emergence. Imidacloprid can also be applied in these ways; more interesting, however, is its use as a liquid or dry seed treatment. Field studies in Brazil showed that the residual effect of imidacloprid at 350-490 g AI/100 kg of undelintered seed was clearly superior to conventional standards against A. gossypii and a mixed population of Thrips tabaci and Frankliniella sp. (Fig. 4).

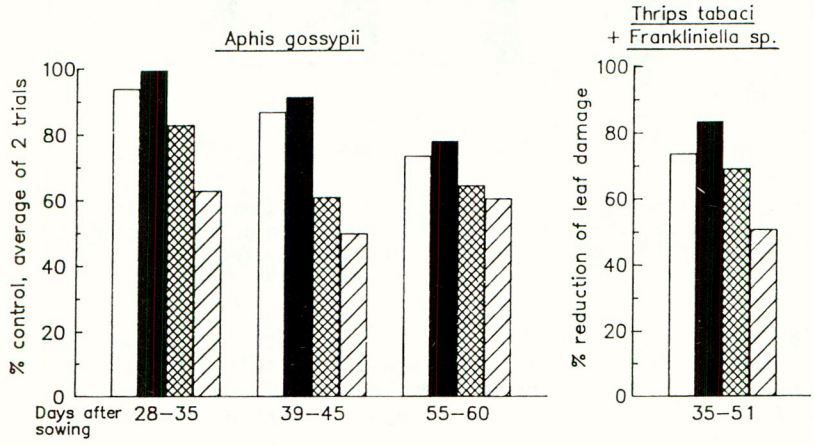


Fig.4 Control of early season pests on cotton (Brazil 1988) with imidacloprid 70 WS, 350 g AI/dt (▭), imidacloprid 70 WS, 490 g AI/dt (▬), disulfoton 50 DS, 1750 g AI/dt (▨) or acephate 75 DS, 750 g AI/dt (▨)

Cereals

One of the most important areas of use for imidacloprid in cereal cultivation is the control of aphid feeding damage and virus vector aphids (Schmeer et al., 1990). Doses of 35-150 g AI/100 kg of seed gave over 95% control of Schizaphis graminum on wheat in Brazil 6 weeks after sowing where there were >100 aphids/plant on untreated areas and 90 % control of aphids more than 9 weeks after sowing. Seed treatment with imidacloprid also controls soil insects in cereals, e.g. wireworms Agriotes sp. or frit flies Oscinella frit.

Maize

In many countries, wireworms are one of the most important pests of maize. The damage consists in a reduced crop density, depressed growth, and yield loss. In France, imidacloprid applied as a seed treatment at a dose of 490 - 700 g AI/100 kg of seed produced outstanding control of a severe infestation with Agriotes sp. (Fig. 5). Imidacloprid also provides very good control of the following important pests in maize: Heteronychus arator, Phyllophaga spp., Protostrophus spp., Astylus atromaculatus, Bupho-nella murina, O. frit, various species of termites, aphids, jassids, and thrips as foliar pests.

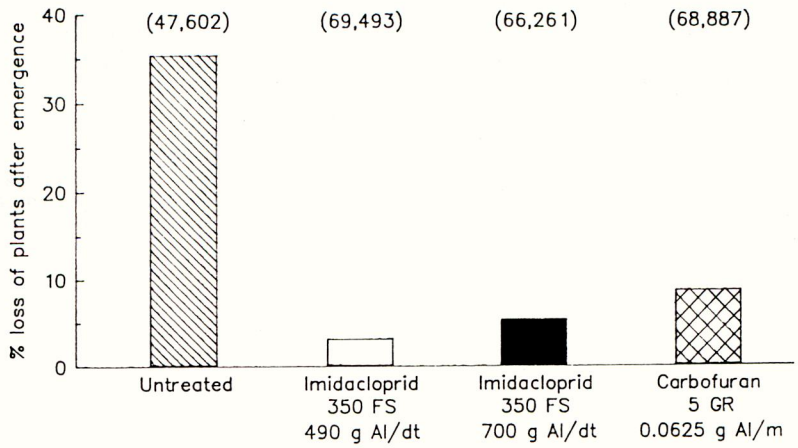


Fig.5 Control of wireworms, Agriotes sp., on maize (France 1990).
Figures in parentheses show the no. of plants/ha 40 days after sowing.

Sugar beet

Imidacloprid is particularly suitable for the treatment of pelleted sugar beet seed to protect against infestation with early season pests. It controls the soil pests pigmy mangold beetle Atomaria linearis, wireworms Agriotes sp., other Coleoptera species, Collembola, and millipedes. Because of its systemic properties imidacloprid also controls foliar pests in sugar beet such as virus vector aphids and beet fly (Schmeer et al., 1990).

CONCLUSION

Imidacloprid is a nitromethylene analogue insecticide with low mammalian toxicity. It controls resistant insects, has a broad spectrum of activity especially against sucking pests, excellent systemic properties and high residual activity. These properties combine to give the product a large number of uses in a very wide variety of crops. In addition to leaf and soil application, seed treatment in particular will be well to the fore. Following many years of extensive field trials all over the world, it is planned that the product will be introduced onto the market from 1991 for use in cereals, maize, rice, potatoes, sugar beet, vegetables, cotton, and other crops.

REFERENCES

Benson, J.A. (1989) Insect Nicotinic Acetylcholine Receptors as Targets for Insecticides. 1989 British Crop Protection Conference Monograph No.43, Progress and Prospects in Insect Control, pp 59 - 70.

Schmeer, H.E; Bluett, D.J.; Meredith, R.; Heatherington, P.J. (1990) Field evaluation of imidacloprid as an insecticidal seed treatment in sugar beet and cereals with particular reference to virus vector control. Proceedings of the 1990 Brighton Crop Protection Conference - Pests and Diseases (In Press).

Schroeder, M.E.; Flattum, R.F. (1984) The Mode of action and Neurotoxic Properties of the Nitromethylene Heterocycle Insecticides. Pesticides Biochemistry and Physiology 22, 148 - 160.

Soloway, S.B.; Henry, A.C.; Kollmeyer, W.D.; Padgett, W.M.; Powell, J.E.; Roman, S.A.; Tieman, C.H.; Corey R.A.; Horne, C.A. (1979) Nitromethylene Insecticides. Advances in Pesticide Science, Fourth International Congress of Pesticide Chemistry Zurich, Switzerland, July 24-28, 1978, Part 2, pp 206-217.

FIELD EVALUATION OF IMIDACLOPRID AS AN INSECTICIDAL SEED TREATMENT IN SUGAR BEET AND CEREALS WITH PARTICULAR REFERENCE TO VIRUS VECTOR CONTROL

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ABSTRACT

Imidacloprid (common name) is a new insecticidal compound. Of particular interest in North-West Europe is its high activity against aphids in cereals and sugar beet when applied as a seed treatment.

The trials conducted with imidacloprid pelleted seed in the UK have shown in 1989 and 1990, with an unusually high aphid pressure and incidence of virus yellows, better and longer lasting control of *Myzus persicae* in sugar beet than the standard aldicarb granule; against *Aphis fabae* imidacloprid was equal to the standard. The reduction of virus yellows infection incidence was at least as good as that achieved by aldicarb. In trials harvested in 1989 on the Continent the control of virus vectors resulted in a significant increase in sugar yields over untreated and the standard seed treatments.

Besides aphids the whole complex of soil pests, and also other leaf pests, are equally or better controlled with imidacloprid than with carbofuran or aldicarb *(Atomaria lineatus, Blaniulus guttulatus, Agriotes lineatus, Pegomya hyoscyami)*.

In high risk areas for barley yellow dwarf virus (BYDV) on the British South and South-West coast, imidacloprid as a seed treatment controlled aphids and BYDV as effectively as a well timed pyrethroid spray.

INTRODUCTION

Imidacloprid, 1-(6-chloro-3-pyridyl-methyl-N-nitroimidazolidin-2-ylideneamine, code number NTN.33893, has a spectrum of activity covering the most economically important pests in the main arable crops in North-West Europe such as aphids in cereals, sugar beet and potatoes as well as soil pests such as pygmy mangold beetle and wireworms (Elbert et al, 1990).

Of particular interest is the ability of imidacloprid to effectively control leaf feeding pests over long periods after emergence, via the seed treatment route. Insecticide-resistant strains of aphids, which are an increasing problem (Smith and Furk, 1989), are effectively controlled by this new compound (Dewar and Read, 1990).

In sugar beet, virus yellows cause a reduction in yield and sugar content as well as an increase in the concentration of impurities (Na, K, amino-N) which reduce the extractibility of the sugar (Smith and Hallsworth, 1990).

Despite the multiple use of aphicidal sprays in sugar beet, the estimated financial loss caused by virus yellows (beet yellows virus, beet mild yellowing virus) was nearly £10 million in the 1989 UK crop (Dewar and Smith, 1990). In 1990 this figure could be even higher, as in earlier virus epidemics (Heathcote, 1978).

Another potential use of imidacloprid is the control of barley yellow dwarf virus (BYDV) vectors in cereals. Yield losses in barley can be as much as 90% in crops infected early at GS 10-12 (Plumb, 1981). In previous years in the UK more than half a million hectares were sprayed in the autumn with pyrethroids against cereal aphids. In the last two mild years a BYDV epidemic has spread throughout North West Europe causing heavy yield reductions in winter barley. In winter wheat yield losses of more than 30% have been observed in some areas like Lower Saxony (Huth, 1990).

MATERIALS AND METHODS

Sugar beet

Two series of trials were carried out in 1989 and 1990 in the United Kingdom with pelleted monogerm seed of the cultivar 'Rex' prepared by Germaines. In the trials described from Belgium, France and West Germany seed prepared by the companies KWS (cv. Gala, Dunja) and Socograines (cv. Aramis) was used.

The analysed seed loadings were within ± 10% of rates as presented in the tables (30, 45, 70, 90 and 110g AI of imidacloprid per unit of pelleted seeds, equivalent to 100,000 seeds). The lower rate of 30g was only tested in 1989 and replaced by 45g in the 1990 season. The 90g rate of the 1989 results with the cultivars Dunja (Belgium, FRG) and Rex (UK) is a corrected figure (the original target rate was 110g AI/unit, but analyses showed an actual loading close to 90g ai/unit). All trials were drilled by precision drills e.g. "Stanhay Webb 5870".

The granule treatments used as a standard were applied by applicators such as the 'Granyl', as a narrow band in the furrow with the seed. To provide the most prolonged control of aphids the highest recommended rate of 5.1g aldicarb 10 GR per 10m (= 1020g AI/ha) was applied.

A randomised block design with 4 replicates was used. The plot size was 4m x 15m and the spacing 50cm between and 20cm within the rows. In the wireworm trials quoted from W.Germany a higher density of seed in the row (25 per m) was used for evaluation purposes.

Winter barley

Five replicated trials were conducted in Britain to evaluate imidacloprid as a seed treatment for the control of aphids transmitting BYDV in winter barley, cv. 'Marinka'.

Formulations used were a dry seed treatment (70 WS) in 1989 and a flowable (350 FS) in 1990. Rates of 70, 105 and 210g AI of imidacloprid/ 100kg seed were tested against a standard pyrethroid spray, in this case cyfluthrin (50 EC), which was applied between GS 21-24. Spray applications were made using pressurised knapsack sprayers with fan nozzles, a water volume of 200 l/ha and pressure of 2 bar.

A randomised block design was used with plots of 1.5m x 15m sown at a seed rate of 160kg/ha using an Oyjord drill. Untreated guard plots of the same size were drilled between every plot.

All assessment data were analysed statistically on transformed data (angular transform for percentages, SQRT (count + 0.5) for counts) and significances are indicated in the tables of results.

RESULTS AND DISCUSSION - SUGAR BEET

Aphid control

Myzus persicae

The main virus vector, *M.persicae*, appeared in the trials during 1989 and 1990 when the plants had between 4 and 14 leaves. Aphid numbers were in all treatments significantly lower than in untreated plots. At three of the six sites the 70g and 90g rates (per unit seed) gave significantly better control than aldicarb granules at 1020g AI/ha (Tab.1). In the other three trials very high levels of control were achieved with imidacloprid at 70g and 90g AI/unit proving at least equivalent to the standard, but with a tendency for better efficacy in two trials especially at the 90g rate. As the mean values indicate, imidacloprid at 70g and in particular at 90g AI/unit seed was superior to aldicarb (1020g AI/ha) in controlling *M.persicae*.

TABLE 1. % Control of *Myzus persicae* in sugar beet by the use of imidacloprid as a seed pelleting compound at six sites in England 1989/90

Year	89	89	89	89	90	90	
Site	Shotley	Bridlington	Thurston	Newent	Caldecote	Thurston	
Area	Suffolk	N Yorks.	Suffolk	Glos.	Beds.	Suffolk	
Days after drilling	47	55	49	36	60	60	
Growth stage (leaves)	10	10-12	6-8	4	6	12-14	MEAN(range)
Untreated (aphids/10 plants)	(291)	(513)	(92.1)	(88)	(15.6)	(25.2)	
imidacloprid 30/45g AI/unit	97.4	80.0	73.2	97	80.8	76.2	87 (73-92)
imidacloprid 70g AI/unit	98.8	84.7 Δ	91.6 Δ	99.6	84.0	93.1	92 (84-100)
imidacloprid 90g AI/unit	99.6	86.7 Δ	97.0 Δ	99.6	96.0	99.0	96 (77-100)
aldicarb 10 GR 1020g AI/ha	94.2	48.7	77.0	99.7	52.0	90	77 (49-100)

All treatments differ sig. from untreated. Δ = sig. different from the standard (p = 0.05%)

Aphis fabae

Aphis fabae tends to arrive late in the season and is only of minor importance as a vector of virus yellows; but infestations can cause direct feeding damage and promote fungal infection.

The control of *A.fabae* by imidacloprid, assessed at four sites in England 9-13 weeks after drilling, was at least as effective as that given by the aldicarb standard (Tab.2).

Because the crops were assessed at a more advanced growth stage (14-23 leaves), both imidacloprid and aldicarb gave a more variable control of *A.fabae* than *M.persicae*. Nevertheless, good control levels were reached with the higher rates of imidacloprid.

TABLE 2. % Control of *Aphis fabae* in sugar beet by the use of imidacloprid as a seed pelleting compound at four sites in England 1989/90

Year	1989	1989	1989	1990	
Site	Bridlington	Shotley	Spalding	Thurston	
Area	Humberside	Suffolk	Lincs.	Suffolk	
Days after drilling	69	62	91	86	
Growth stage (leaves)	16-20	14	17	23	MEAN(range)
Untreated (aphids/10 plants)	(489.3)	(4250)	(2352)	(236.5)	
imidacloprid 30/45g AI/unit	42	96	84.3	36.2°	65(36-96)
imidacloprid 70g AI/unit	82.7 Δ	94.4	77.0	70.1	81(70-94)
imidacloprid 90g AI/unit	83.2 Δ	94.1	87.9	59.5	81(60-94)
aldicarb 10 GR 1020g AI/ha	46.6	97.4	87.9	25.3°	64(25-797)

All treatments gave sig. control except (°). Δ = sig.diff. from the standard (p=0.05%)

Virus yellows

The overall assessment of six sites in England during 1989 and 1990 shows that imidacloprid in seed pellets gave better control of virus yellows infection than aldicarb granules applied at more than 10 times the rate of AI/ha (Tab.3). This was also confirmed by the work by Dewar and Read (1990).

A trial in Belgium with larger plots (5 x 100m) harvested during 1989 shows how the reduction in virus yellows infection is reflected by increases in the sugar yield (Tab.4). Similar yield benefits from the use of imidacloprid over other pelleting materials were obtained for example in Normandy (France) and also in the Cologne Basin (FRG).

TABLE 3. % Control of virus yellows by imidacloprid in pelleted sugar beet seed at six sites in England in 1989 and 1990

Year	1989	1989	1990	1990	1990	1990	
Site	Saxmundham	Newent	Thurston	Caldecote	Newent	Rushock	
Area	Suffolk	Glos.	Suffolk	Beds.	Glos.	Worcs.	
Days after drilling	139	169	79	88	107	87	MEAN(range)
Untreated (% infection)	(8.7)	(37.5)	(5.1)	(16.8)	(4.5)	(17.0)	
imidacloprid 30/45g AI/unit	80.2	84.7	82.6	70.3	77.2	52.9	74(53-85)
imidacloprid 70g AI/unit	91	82.7	95.2	70.6 Δ	77.8	57.4	79(57-95)
imidacloprid 90g AI/unit	44	81.3	74.2	74.7 Δ	94.4	80.9	75(44-94)
aldicarb 10GR 1020g AI/ha	82.2	70.7	86.8	37.6°	66.7	60.3	67(38-86)

All treatments gave sig. control except (°). Δ = Sig. different from the standard (p=0.05%)

TABLE 4. The effect of imidacloprid seed pelleting on the incidence of virus yellows and sugar yields (relative)in Belgium, France and W.Germany in 1989

Area of the sites:	Hainaut/Wallonie		Calvados/Normandy	Cologne Basin
	% Virus Yellows (inf.plants 21.9.89)	Sugar yield (rel.)	Sugar yield (rel.)	Sugar yield (rel.)
Cultivar		Gala	Aramis	Gala
Untreated	97	(9.74 t/ha)	(8.94 t/ha)	(10.15 t/ha)
imidacloprid 30g AI/unit	32	117*	-	-
imidacloprid 60g AI/unit	-	-	116*	-
imidacloprid 70g AI/unit	13* Δ	136* Δ	-	115*
imidacloprid 90g AI/unit	17* Δ	-	119*	112*
carbofuran 30/35g AI/unit	97	102	105	103
tefluthrin 12g AI/unit	96	93	108	-
aldicarb 10 GR 1000-1020g AI/ha		-	112	106*

* = sig.diff. from untreated Δ = sig.diff. from the 30g rate and standards (p=0.05%)

Other leaf pests

Damage caused by larvae of the beet leaf miner, *Pegomya hyoscyami*, on two sites with heavy infestations in 1989 was fully prevented by the two higher imidacloprid rates as well as by the aldicarb standard (Tab.5). The 30g rate was less effective but still gave more than 90% control. In 1990 slightly lower control levels between 80 and 90% were observed for imidacloprid but apparently due to the extreme drought the effectiveness of aldicarb (Tab.5) was greatly reduced.

On two trial sites sugar beet flea beetles, *Chaectocnema concinna*, caused leaf holing. Imidacloprid generally gave better control than aldicarb granules.

Caterpillars of the tortrix moth, *Cnephasia* spp., occur occasionally as a beet pest. At one site in East Anglia imidacloprid appeared to give improved control of this pest relative to aldicarb, although the difference was not statistically significant.

TABLE 5. % Control of leaf miner, flea beetles and tortrix moth larvae in sugar beet by imidacloprid seed treatments, England 1989/90

	Pegomya hyoscyami		*Chaetocnema concinna*	*Cnephasia* spp.
	2 sites	1 site	2 sites	1 site
	Yorks.'89	Suffolk '90	Glos. '89	Suffolk '89
Rate/ha	2-6 leaf stage		4 leaf stage	4 leaf stage
Untreated (% plants inf.)	(69)	(72)	(57)	(13)
imidacloprid 30/45g AI/unit	92 Δ	83 Δ	76	70
imidacloprid 70g AI/unit	100	89 Δ	75	59
imidacloprid 90g AI/unit	100	86 Δ	84	80
aldicarb 10 GR 1020g AI/ha	99	43	54	42

All treatments diff. sig. from untreated. Δ = values diff. sig. from the standard (p=0.05%)

Crop safety

On sites with no infestation pressure from soil pests there was no significant influence on crop stand.

Arthropod soil pests

Atomaria linearis, *Blaniulus guttulatus* and *Agriotes lineatus*

Pygmy mangold beetle, spotted snake millipedes and wireworms can cause serious plant loss through feeding on seedlings. The results so far indicate that imidacloprid gives good protection of the emerging crop in situations where these three pests occur.

Excellent levels of damage reduction were obtained as a result of controlling pygmy mangold beetle (Tab.6).

TABLE 6. % Reduction of severe root attack* by *Atomaria linearis*, 2 trials, England 1990

Cultivar Growth stage (no. of leaves)	Dunja 2-4	Rex 2-4
Untreated (% severely attacked plants)*	(13.5)	(28.1)
imidacloprid 45g AI/unit	92	81.5
imidacloprid 70g AI/unit	92	85.2
imidacloprid 90g AI/unit	100	100
imidacloprid 110g AI/unit	100	100
carbofuran 30g AI/unit	100	-
tefluthrin 6g AI/unit	84.6	-
tefluthrin 12g AI/unit	92.3	-
aldicarb 10 GR 1020g AI/ha	-	85.2

* damage which severely reduces or terminates further plant growth (top 2cm of hypocotyl assessed)

Judging from the reduced plant numbers in untreated the imidacloprid treatments prevented a substantial loss of plants and gave significant improvements in crop stand in the presence of both pygmy mangold beetle and millipedes at each of two sites in England (Tab.7). Similar increases in crop stand were found at three sites in West Germany infested with wireworms.

TABLE 7. Crop stand under heavy infestation pressure of *Atomaria linearis* and *Blaniulus guttulatus* (mean of 2 sites in England 1990 and *Agriotes lineatus* (mean of 3 sites in W.Germany 1989)

	Relative crop stand (untreated = 100)	
	England	W.Germany
Cultivar	Rex	Gala
Untreated (plants/m)	(2)	(11)
imidacloprid 45g AI/unit	145 Δ	173 Δ
imidacloprid 70g AI/unit	143 Δ	178 Δ
imidacloprid 90g AI/unit	144 Δ	176 Δ
aldicarb 10 GR (5.1g/10m)	124	101
carbofuran 5 GR (6g/10m)	120	130

Δ = values sign. diff. from the standards (p=0.05%)

RESULTS AND DISCUSSION - WINTER BARLEY

Crop safety

Imidacloprid treated seed showed no differences in crop stand compared to the untreated over five sites drilled in autumn 1989 and 1990.

Aphid control

Aphid counts *(Rhopalosiphum padi* and *Sitobion avenae)* were made at three sites with noticeable infestations. All imidacloprid treatments were as effective as the pyrethroid standard and gave between 97 and 100% control.

Barley yellow dwarf virus control

Under the extremely high infection pressure in the South and South West of the UK in the last two seasons, imidacloprid gave a rate related response. With 105g AI/100kg seed imidacloprid controlled the spread of BYDV as effectively as the standard pyrethroid spray (Tab.8). The efficacy of the 70g rate is probably underestimated for practical conditions as untreated guard plots on either side of the treatments provided a constant source of infection.

TABLE 8. % Control of BYDV in winter barley, cv Marinka at five sites by imidacloprid seed treatments in England 1989 and 1990

Site	Norwich	Dover	Deal	Kingsbridge	Penmaen	
Area	Norfolk	Kent	Kent	Devon	South Wales	
Drilled on	22.9.88	21.9.88	25.9.89	29.9.89	26.9.89	
Assessed on	11.5.89	19.4.89	10.5.90	4.5.90	19.6.90	
Growth Stage	39	33	55	39	85	Mean
Untreated (% BYDV)	(19)	(98)	(66)	(95)	(70)	
imidacloprid 70g AI/100kg	82.7	84.6	92.4	88.7	91.8 Δ	88.0
imidacloprid 105g AI/100kg	85.3	92.8	92.4	94.4 Δ	97.9	92.5
imidacloprid 210g AI/100kg	90.7	94.6	97.0	96.0 Δ	100.0	95.6
cyfluthrin 12.5g AI/ha	92.0	89.0	97.0	88.8	99.3	93.2
Spray Application dates	11.11.88	18.10.88	1.11.89	31.10.89	13.11.89	

All treatments sig. diff. from untreated. Δ = diff. sig. from the standard (p=0.05%)

The control figures shown above were reflected in the yields obtained in the 1990 harvest (Tab.9).

TABLE 9. Grain yield in t/ha of cv. Marinka, 3 sites 1990

	Mean	Range
Untreated	1.8 t/ha	(0.7 - 2.8)
imidacloprid 70g AI/100kg	4.7	(3.6 - 5.3)
imidacloprid 105g AI/100kg	5.1	(4.1 - 5.8)
imidacloprid 210g AI/100kg	5.4	(4.1 - 6.8)
cyfluthrin 12.5g AI/ha	4.9	(3.8 - 5.4)

All the treatments differed significantly from untreated but not from each other. The yield response of the imidacloprid treatment was rate related, with 70 and 105g AI/100kg achieving levels similar to the pyrethroid spray.

CONCLUSIONS

As imidacloprid offers a new concept for controlling leaf and soil pests in sugar beet and cereals via a seed treatment, it will undoubtedly have an impact on farming practices in the nineties.

The success of aphicidal sprays against virus vectors is very much determined by the correct application timing. This problem will be overcome by the use of imidacloprid as a seed treatment which gives prolonged protection for the seedlings.

In sugar beet, imidacloprid also has the potential to replace insecticidal granules and other insecticidal pelleting materials as it is effective against aphids and the whole complex of pests at crop establishment.

As a cereal seed treatment, particularly in BYDV high risk areas with mild winters, imidacloprid offers a secure and reliable alternative to autumn insecticidal sprays.

Seed treatments are often the best form of targetting a crop protection chemical from the environmental point of view. The described seed application of imidacloprid will surely provide advantages over existing pest control methods such as the use of granules in sugar beet and sprays in cereals.

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REFERENCES

Dewar, A. and Smith (1990) The continuing story of virus yellows. *British Sugar Beet Review*, **58** No.1, 15-19.

Dewar, A. and Read L.A. (1990) Evaluation of an insecticidal seed treatment, imidacloprid, for controlling aphids on sugar beet. *BCPC, Pests and Diseases 1990*, Proceedings (In Press)

Elbert, A. Overbeck, H., Iwaya, K. and Tsuboi, S. (1990) Imidacloprid, a novel nitromethylene systemic insecticide for crop protection. *BCPC, Pests and Diseases 1990*, Proceedings Vol.1.

Heathcote, G.D. (1978) Review of losses caused by Virus yellows in English sugar beet crops and the cost of partial control with insecticides. *Plant Pathology* **27**, 12-17.

Huth, W. von (1990) Barley yellow dwarf-ein permanentes Problem fuer den Getreideanbau in der Bundesrepublik Deutschland? *Nachrichtenbl. Deut. Pflanzenschutzd. (Braunschweig)* **42** (3), 33-39.

Plumb, R. T., (1981) Chemicals in the control of cereal virus diseases. in *Strategies for the Control of Cereal Diseases*. J. F. Jenkyn and R. T. Plumb, p.135-145, Oxford, 1981.

Smith, H.G. and Hallsworth P.B. (1990). The effects of yellowing viruses on yield of sugar beet in field trials, 1985 and 1987. *Ann. Appl. Biol.* (1990), **116**, 503-511.

Smith, S. and Furk, C. (1989) The spread of the resistant aphid. *British Sugar Beet Review* **57** No.2, 4-6.

AC 303,630 - AN INSECTICIDE/ACARICIDE FROM A NOVEL CLASS OF CHEMISTRY

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ABSTRACT

AC 303,630, a member of a novel class of insecticides, is a broad spectrum insecticide/acaricide on many economic crops. It shows insecticidal activity by foliar application as well as by limited systemic uptake through the roots in a hydroponic system. AC 303,630 is highly active by ingestion, possesses contact activity and provides moderate residual activity on plants. It is effective at low application rates. Initial work has shown that the compound is equally effective against resistant and susceptible insects and mites. No phytotoxicity has been observed at field use rates. AC 303,630 is classified as moderately toxic to mammals based on acute toxicity studies and is nonmutagenic in the modified Ames and Chinese hamster ovary tests. AC 303,630 is under full scale global development for the control of insects and mites on cotton, vegetables and fruit. Field work has confirmed the effectiveness seen in laboratory and greenhouse studies.

INTRODUCTION

AC 303,630 is a member of a novel class of insecticides, the pyrroles, which were discovered at the Agricultural Research Division, American Cyanamid Company, Princeton, NJ, USA. The compound combines efficacy against a wide range of insect pests (Miller, et al., 1990) through contact and stomach activity with moderate residual activity on plants. Activity of AC 303,630 is comparable to that of the synthetic pyrethroid flucythrinate against insects. It shows excellent efficacy against piercing-sucking and chewing insects and mites. Limited plant systemic activity has been demonstrated when it is applied around the root zone of plants. On account of its different mode of action, AC 303,630 is equally effective against phosphate- and pyrethroid-resistant and susceptible insects and mites. In some cases the action of AC 303,630 may be somewhat slower than that observed with organophosphates and pyrethroids. AC 303,630 is considered moderately toxic to mammals based on single oral dosages administered to rats and shows low dermal toxicity to rabbits.

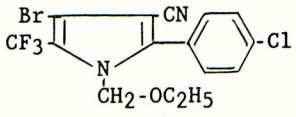
The remarkable insecticidal activity of AC 303,630, its unique structure, and the commercial possibility of its use as an insecticide/acaricide prompted us to report on its chemical, physical and biological characteristics.

CHEMICAL AND PHYSICAL CHARACTERISTICS

Designation: AC 303,630, CL 303,630

Chemical Name: 4-bromo-2-(4-chlorophenyl)-1- ethoxymethyl - 5- trifluoromethyl pyrrole-3-carbonitrile

Structure:



Molecular Formula: $C_{15}H_{11}BrClF_3N_2O$

Molecular Weight: 407.6

Melting Point: 91-92°C.

Color and Physical State: White solid

Solubility: Soluble in acetone, diethyl ether, DMSO, THF, acetonitrile, alcohols. Insoluble in water.

TOXICOLOGY OF TECHNICAL AC 303,630

Single oral dose, female rats: LD_{50} = 459 mg/kg

Single oral dose, male rats: LD_{50} = 223 mg/kg

Single dermal dose, rabbits: LD_{50} = >2000 mg/kg

Rabbit eye irritation: Slightly irritating

Japanese carp: LC_{50} = 0.5 ppm

Nonmutagenic in the modified Ames test and in the Chinese hamster ovary test

INHERENT TOXICITY OF AC 303,630

Test procedures

The test materials were dissolved in 50% acetone-50% water. For tests with Spodoptera eridania larvae and Empoasca abrupta, the primary leaves of lima bean plants (Phaseolus limensis var. Carolina) were dipped for 3-5 s. After the leaves were dry, each was placed in a 9 cm plastic Petri dish with moist Whatman No. 1 filter paper on the bottom, and 10 S. eridania larvae or E. abrupta were added. The tests were kept at 26.5 ± 1° C with 40 ± 10% r.h. Mortality counts were made after 3 d. The LC_{50} values were calculated from the dosage-response data with four concentrations, using the method of Finney (1952).

The same method was used with true leaves from cotton plants (Gossypium sp. var. Stoneville 213) for tests on 1st-instar larvae of Heliothis virescens. For 3rd-instar larvae, each leaf was cut into five sections. Each section was placed in a 20 ml plastic cell containing one 3rd-instar larva and a 2.5 cm length of dental wick saturated with water. The cell was covered with MYLAR* film. To test Tetranychus urticae, approximately 100 mites were transferred to bean leaves 1 h before treatment. Both mites and leaves were dipped for 3-5 s.

For posttreatment infestation tests, test materials were allowed to dry thoroughly before infestation with mites.

Insecticidal activity

In laboratory tests, AC 303,630 was as effective as flucythrinate against 3rd-instar S. eridania larvae and 1st-instar H. virescens larvae, but superior to flucythrinate against the older 3rd-instar H. virescens larvae and adult T. urticae (Table 1). In a laboratory LC_{50} leaf-dip test, AC 303,630 was about 2-fold less active against mixed stages of E. abrupta than flucythrinate.

Table 1 - Inherent insecticidal toxicity of AC 303,630 against Lepidopterous larvae, leafhoppers and mites.

	LC_{50} (ppm)				
Compound	SAW3	TBW1	TBW3	LHmix	Mites
AC 303,630	2.6	2.2	3.2	0.92	1.58
flucythrinate	3.0	1.3	9.4	0.52	>10

SAW3 = Southern armyworm (Spodoptera eridania), 3rd-instar
TBW1 = Tobacco budworm (Heliothis virescens), 1st-instar
TBW3 = Tobacco budworm (H. virescens), 3rd-instar
LHmix = Western potato leafhopper (Empoasca abrupta), mixed stages
Mites = Twospotted spider mite (Tetranychus urticae), adult, OP-resistant strain

Acaricidal activity

AC 303,630 was compared with dicofol and cyhexatin, using infestation both pre- and posttreatment (Table 2). The LC_{50} values for the preinfested plants show that AC 303,630 is 9.0 and 3.5 times more effective than dicofol and cyhexatin, respectively. For the posttreatment infested plants, AC 303,630 is about 14 and 2.5 times more effective than dicofol and cyhexatin, respectively. The results of these studies indicate that AC 303,630 is highly effective against OP-resistant two-spotted spider mites.

Table 2 - Inherent toxicity of AC 303,630 as an acaricide against OP-resistant Tetranychus urticae on bean leaves.

Compound	LC_{50} (PPM) Preinfested	Postinfested
AC 303,630	1.0	1.9
dicofol	9.0	26.9
cyhexatin	3.5	4.7

Phytotoxicity

No phytotoxicity was observed on young cotton and lima bean plants with technical AC 303,630 dissolved in 50% acetone-50% water when tested at rates up to 1000 ppm.

Plant systemic activity

AC 303,630 exhibited excellent root-systemic activity in rice (Oryza sativa) against 1st-instar fall armyworm (Spodoptera frugiperda) in comparison with carbofuran. The bare roots of young rice seedlings were soaked in AC 303,630 or carbofuran in Hoagland's solution containing 1% acetone and 0.1% EMULPHOR* EL620 as an emulsifier. The plants were held in a glasshouse under high intensity discharge lamps. After 7 and 15 days of uptake, the foliage was excised and placed in a 9 cm plastic Petri dish with moist Whatman No. 1 filter paper on the bottom and 10 1st-instar S. frugiperda larvae were added. Mortality was assessed after three days. Under these conditions, AC 303,630 was at least 10 times more effective than carbofuran (Table 3). It also had better systemic residual activity providing 100% control with 3 ppm at 15 DAT compared with 4% control given by carbofuran.

Table 3 - Percent mortality of 1st-instar Spodoptera frugiperda after soaking bare roots of rice plants.

Concentration (mg/l)	% Mortality assessed after 3 Days AC 303,630 Days of Uptake 7	15	carbofuran Days of Uptake 7	15
100	100	100	97	79
30	100	100	69	68
10	100	97	67	32
3	54	100	14	4

FIELD PERFORMANCE

Toxicity to insecticide resistant diamondback moth larvae

Resistance in diamondback moth larvae (Plutella xylostella) to the major categories of insecticides, i.e., chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids, benzoylphenyl ureas and

Bacillus thuringiensis has occurred in many parts of the world (Kao et al. 1989). AC 303,630 was evaluated on cabbage in the field against a strain of diamondback moth larvae in the Philippines that could not be controlled by the available commercial insecticides. At a rate of 0.1 kg AI/ha, AC 303,630 was superior to teflubenzuron + deltamethrin at 0.045 + 0.025 kg AI/ha (TFB+DM) (Table 4). The population of larvae in the TFB+DM plots showed a steady increase in comparison to a decline to less than one larva per plant in all of the AC 303,630 treatments.

Table 4 - Performance of AC 303,630 against resistant Plutella xylostella on cabbage in the Philippines.

Treatment	AI Kg/Ha	Larvae per Plant: Pre-Treatment	6 d after 1st Applic.	3 d After 2nd Applic.
AC 303,630	0.1	2.1	0.7	0.2
	0.2	2.1	0.7	0.1
	0.4	1.6	0.3	0.0
teflubenzuron + deltamethrin	0.045 + 0.025	1.0	1.5	1.9
Untreated	-	1.5	4.6	4.3

Toxicity to lepidopterous larvae on lettuce

The outstanding performance of AC 303,630 in laboratory studies was also confirmed in a field trial against several species of lepidopterous larvae in California, USA. The compound was formulated as a 240 g/l EC and one foliar application was made to lettuce (Lactuca sp.) in a water volume of 375 l/ha using a single-nozzle overhead sprayer. AC 303,630 was superior to the standard cypermethrin at an equivalent rate against beet armyworm (Spodoptera exigua) and tobacco budworm (H. virescens) and equal to cypermethrin against cabbage looper (Trichoplusia ni) (Table 5). The data also demonstrated that AC 303,630 has moderate residual activity by providing control for at least 10 days after treatment.

Table 5 - Performance of AC 303,630 against Lepidopterous larvae on lettuce in California.

Compound	AI Kg/Ha	Larvae/20 Plants 10 DAT: CL	BAW	TBW
AC 303,630	0.1	0	1	2
	0.2	0	0	0
cypermethrin	0.1	0	5	4
Untreated	-	14	7	10

CL = Cabbage looper, Trichoplusia ni
BAW = Beet armyworm, Spodoptera exigua
TBW = Tobacco budworm, Heliothis virescens

CONCLUSIONS

AC 303,630 is a representative of a novel class of compounds showing promise as an insecticide/acaricide In several tests AC 303,630 was more effective than the standard insecticides cypermethrin and flucythrinate and in laboratory studies was more effective as an acaricide than dicofol or cyhexatin.

In summary, AC 303,630 possesses the following characteristics:

(1) A broad spectrum insecticide/acaricide
(2) Effective by both stomach and contact action.
(3) No cross-resistance to other insecticides.
(4) Moderate residual activity on plants.
(5) Selective systemic activity by root uptake in a hydroponic system.
(6) Moderate oral and low dermal toxicity to mammals.
(7) Effective at low application rates.

The unique chemistry and the insecticidal/acaricidal activity demonstrated with AC. 303,630 warrant continued evaluation toward commercialization.

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REFERENCES

Finney, D.J. (1952) Probit Analysis, Edition 2, Cambridge University Press, Cambridge

Kao, C-H.; Hung, C-F.; and Sun, C-N. (1989) Parathion and Methyl Parathion Resistance in Diamondback Moth (Lepidoptera: Plutellidae) Larvae. Journal of Economic Entomology, 82, 1299-1304.

Miller, T.P; Treacy, M.F.; Gard, I.E.; Lovell, J.B.; Wright, D.P. Jr.; Addor, R.W.; Kamhi, V.M. (1990) AC 303,630 - Summary of 1988-89 field trial results. Proceedings 1990 Brighton Crop Protection Conference - Pests and Diseases (In press).

AC 303,630 - SUMMARY OF 1988-89 FIELD TRIAL RESULTS

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ABSTRACT

AC 303,630 demonstrates broad spectrum pesticidal activity and is the lead candidate of a new class of compounds. Two emulsifiable concentrate formulations of AC 303,630 were field-tested at 0.037-0.500 kg AI/ha during 1988-89 on 16 different crops and ornamentals in Europe, North America, South America and the Philippines. Commercially acceptable levels of control were obtained against 35 insect and mite pest species representing the orders Acari, Coleoptera, Diptera, Homoptera, and Lepidoptera.

AC 303,630 (4-bromo-2-(4-chlorophenyl)-1-ethoxymethyl-5-trifluoromethyl-pyrrole-3-carbonitrile), the lead compound in a new class of insecticide chemistry from American Cyanamid, demonstrates broad spectrum insecticidal/acaricidal activity (Lovell et al., 1990). Initial field testing of AC 303,630, under the direction of Insecticide Discovery, was conducted during 1988-89.

Two emulsifiable concentrate (EC) formulations, a 240 g AI/l EC and a 200 g AI/l EC, were field tested. Tests were established in randomized complete block designs with four replications. Foliar applications of AC 303,630 were made at rates ranging from 0.037 to 0.500 kg AI/ha and compared with standard treatments applied at indicated rates and with untreated checks. Initial and any subsequent applications were timed in accordance with label directions and local recommendations for the standards. Data summarized here indicate the rates at which AC 303,630 reduced pest populations or the associated plant damage to levels equal to, or significantly less, than the commercial standards in the respective trials.

Results from mite trials were converted to adjusted percent control (Henderson & Tilton, 1955) and all data recorded as percentiles were normalized prior to statistical analyses using the arcsin square root y transformation (Steel & Torrie, 1980). Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test was used to determine significant mean separations ($P = 0.05$) (SAS Institute, 1985). Comparisons given represent trials in which the standards and/or AC 303,630 were statistically different from the untreated checks, i.e. trials with insignificant pest pressure were not considered. Summaries are presented by pest species within crop group(s). To simplify the summaries, various rates of AC 303,630 tested were combined and are reported in the following kg AI/ha categories; 0.125 = 0.037 - 0.125, 0.250 = 0.200 - 0.250, and 0.500 = 0.400 - 0.500.

Cotton trials (Table 1) were conducted in Brazil and the USA. Abamectin (0.011 kg AI/ha) and propargite (1.08 kg AI/ha) were the comparative standards employed in the tests to determine efficacy against Tetranychus urticae and Polyphagotarsonemus latus. Cypermethrin (0.056-0.067 kg AI/ha), deltamethrin (0.005-0.01 kg AI/ha), cyfluthrin (0.028 kg AI/ha) and esfenvalerate (0.034 kg AI/ha) served as standards against Heliothis virescens, Helicoverpa zea, and Alabama argillacea. Control of Heliothis/Helicoverpa spp., statistically greater than or equal to the standards, required the 0.250 kg AI/ha rate. The 0.125 kg AI/ha rate was sufficient to control the other species.

TABLE 1. Summary of AC 303,630 vs. standard treatments - Comparative results of 1988-89 cotton field trials conducted in Brazil and the USA.

Pest (No. of trials)	AC 303,630 Rate (kg AI/ha) Providing Control >= Standard (*P*=.05)
Tetranychus urticae (5)	0.125
Polyphagotarsonemus latus (2)	0.125
Alabama argillacea (1)	0.125
Heliothis/Helicoverpa spp. (9)	0.250

Abamectin (5 ppm applied in 2000-2500 l/ha) was the comparative standard utilized in the Brazilian orange trials depicted in Table 2. AC 303,630, applied to runoff at the designated concentration, provided excellent control of both Brevipalpus phoenicia and Phyllocoptruta oleivora.

TABLE 2. Summary of AC 303,630 vs. standard treatment - Comparative results of 1988-89 orange field trials conducted in Brazil.

Pest (No. of trials)	AC 303,630 Rate (ppm) Providing Control >= Standard (*P*=.05)
Brevipalpus phoenicia (3)	62.5
Phyllocoptruta oleivora (1)	62.5

Top fruit and grape trials were conducted in France, Italy, and the USA (Table 3). Clofentezine (0.14 kg AI/ha) was the acaricide standard used while azinphos-methyl (0.78 kg AI/ha), methidathion (0.57 kg AI/ha), phosalone (0.6 kg AI/ha) and fenvalerate (0.056 kg AI/ha) were the comparative insecticides. All pest species listed were competitively controlled with the 0.125 kg AI/ha rate of AC 303,630.

TABLE 3. Summary of AC 303,630 vs. standard treatments - Comparative results of 1988-89 apple, plum and grape field trials conducted in France, Italy and the USA.

Pest (All Single Trials)	AC 303,630 Rate (kg AI/ha) Providing Control >= Standard (P = .05)
Archips argyrospila	0.125
Conotrachelus nenuphar	0.125
Lobesia botrana	0.125
Pandemis cerasana	0.125
Phyllonorycter blancardella	0.125
Tetranychus urticae	0.125
Typhlocyba pomaria	0.125

Soybean trials conducted in Argentina and Brazil, with deltamethrin (0.004-0.0075 kg AI/ha) as the standard treatment, are summarized in Table 4. Efficacy statistically greater than or equivalent to that provided by the standard was achieved in all trials with the 0.125 kg AI/ha rate of AC 303,630 tested for control of Anticarsia gemmatalis, Colaspis sp., Pseudoplusia includens, and Cerotoma trifurcata. Equivalent control of Loxostege biffidalis required 0.250 kg AI/ha.

TABLE 4. Summary of AC 303,630 vs. standard treatment - Comparative results of 1988-89 soybean field trials conducted in Argentina and Brazil.

Pest (No. of trials)	AC 303,630 Rate (kg AI/ha) Providing Control >= Standard (P = .05)
Anticarsia gemmatalis (4)	0.125
Colaspis sp. (2)	0.125
Pseudoplusia includens (1)	0.125
Cerotoma trifurcata (1)	0.125
Loxostege biffidalis (1)	0.250

In maize trials in Brazil, France, and the USA, deltamethrin (0.005-0.02 kg AI/ha), carbaryl (1.12 kg AI/ha), and chlorpyrifos (1.12 kg AI/ha) were the comparative standards (Table 5). Agrotis ipsilon control equivalent to the chlorpyrifos standard was obtained at the 0.125 kg AI/ha rate of AC 303,630. AC 303,630 applied at 0.250 kg AI/ha provided H. zea control equal to a carbaryl standard. Control of Ostrinia nubilalis and Spodoptera frugiperda statistically equivalent to the deltamethrin standard required 0.250 and 0.300 kg AI/ha of AC 303,630, respectively.

TABLE 5. Summary of AC 303,630 vs. standard treatments - Comparative results of 1988-89 maize field trials conducted in Brazil, France, and the USA.

Pest (No. of trials)	AC 303,630 Rate (kg AI/ha) Providing Control >= Standard (P = .05)
Agrotis ipsilon (1)	0.125
Helicoverpa zea (1)	0.250
Ostrinia nubilalis (1)	0.250
Spodoptera frugiperda (4)	0.300

Individual comparative standards used in cabbage and lettuce trials in the Philippines and the USA included Bacillus thuringiensis var. Kurstaki (9.9-39.5 x 10^9 IUP/ha), cypermethrin (0.112 kg AI/ha), methomyl (0.5-0.56 kg AI/ha), and bendiocarb (0.5 kg AI/ha). Additionally, tank mixtures of B. t. var. Kurstaki + chlorpyrifos (17.9 x 10^9 IUP/ha + 1.12 kg AI/ha), deltamethrin + teflubenzuron (0.025 + 0.045 kg AI/ha), and methomyl + permethrin (0.5 + 0.112 kg AI/ha) were also used. Control of H. virescens, Trichoplusia ni, Spodoptera exigua, and Plutella xylostella was statistically greater than or equal to the standards in every trial at the 0.125 kg AI/ha rate (Table 6).

TABLE 6. Summary of AC 303,630 vs. standard treatments - Comparative results of 1988-89 cabbage and lettuce field trials conducted in the Philippines and the USA.

Pest (No. of trials)	AC 303,630 Rate (kg AI/ha) Providing Control >= Standard (P = .05)
Trichoplusia ni (7)	0.125
Spodoptera exigua (5)	0.125
Plutella xylostella (4)	0.125
Heliothis virescens (1)	0.125

Vegetable and sugar beet trials were conducted in Brazil, Canada, Italy, the UK, and the USA with permethrin (0.1-0.112 kg AI/ha), deltamethrin (0.008 kg AI/ha), fenvalerate (0.168 kg AI/ha), methomyl (1.12 kg AI/ha), phorate (2.2 kg AI/ha), pirimicarb (0.14 kg AI/ha), and propargite (0.57 kg AI/ha) used as comparative standards. Control of Aphis fabae, S. exigua, T. urticae, H. zea, Liriomyza spp., and Neoleucinodes elegantalis was equal to the standards at the 0.125 kg AI/ha rate, while 0.250 kg AI/ha was required for Keiferia lycopersicella (Table 7). Performance of AC 303,630 against Leptinotarsa decemlineata and Phthorimaea operculella was superior to the standards at all tested rates.

TABLE 7. Summary of AC 303,630 vs. standard treatments - Comparative results of 1988-89 tomato, eggplant, potato, celery and sugar beet field trials conducted in Brazil, Canada, Italy, the UK, and the USA.

Pest (No. of trials)	AC 303,630 Rate (kg AI/ha) Providing Control >= Standard (P = .05)
Aphis fabae (1)	0.125
Helicoverpa zea (1)	0.125
Leptinotarsa decemlineata (1)	0.125
Liriomyza spp. (3)	0.125
Neoleucinodes elegantalis (1)	0.125
Phthorimaea operculella (1)	0.125
Spodoptera exigua (1)	0.125
Tetranychus urticae (1)	0.125
Keiferia lycopersicella (1)	0.250

Comparative standards in rice trials in the Philippines included monocrotophos (0.45-0.75 kg AI/ha) and combination products containing fenobucarb plus azinphos-ethyl (0.333 + 0.267 kg AI/ha) and fenobucarb plus chlorpyrifos (0.15 + 0.3 kg AI/ha). Control of Nephotettix nigropictus and N. virescens was equivalent to the standards at the 0.250 kg AI/ha rate (Table 8). Equivalent control of Hydrellia philippina required the 0.500 kg AI/ha rate. Equivalent or superior control of Cnaphalocrocis medinalis was obtained with the lowest rate tested (0.125 kg AI/ha).

TABLE 8. Summary of AC 303,630 vs. standard treatments - Comparative results of 1988-89 rice field trials conducted in the Philippines.

Pest (No. of trials)	AC 303,630 Rate (kg AI/ha) Providing Control >= Standard (P = .05)
Cnaphalocrocis medinalis (2)	0.125
Nephotettix nigropictus (2)	0.250
Nephotettix virescens (1)	0.250
Hydrellia philippina (2)	0.500

In summary, AC 303,630 EC formulations applied at rates of 0.500 kg AI/ha or less, have demonstrated the ability to provide commercially competitive levels of control against 35 pest species of insects and mites representing 16 families and 5 orders. The majority of these (25 species representing 15 families and 5 orders) were controlled at rates of 0.125 kg AI/ha or less. Full scale development of this broad spectrum novel insecticide/acaricide is underway on a variety of crops and ornamentals.

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REFERENCES

Henderson, C. F.; Tilton, E. W. (1955) Tests with acaricides against brown wheat mite. Journal of Economic Entomology 48, 157-161.

Lovell, J. B.; Wright, D. P., Jr.; Gard, I. E.; Miller, T. P.; Treacy, M. F.; Addor, R. W.; Kamhi, V. M. (1990) AC 303,630 - an insecticide/acaricide from a novel class of chemistry. Proceedings 1990 Brighton Crop Protection Conference - Pests and Diseases (In Press).

SAS Institute. (1985) SAS User's Guide: Statistics. Cary, N. C.: SAS Institute, pp. 113-137.

Steel, R. G. D.; Torrie, J. H. (1980) Principles and Procedures of Statistics, 2nd ed. New York: McGraw-Hill, p. 236.

BIO 1020, A NEW MICROBIAL INSECTICIDE FOR USE IN HORTICULTURAL CROPS

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ABSTRACT

Under the code name BIO 1020, a microbial insecticide has been developed, based on a wild-type strain of the fungus *Metarhizium anisopliae* . The fungus can be produced on a large scale by fermentation. The product consists of dry granules with a shelf life of at least six months. When these 0.5 to 1.0 mm diameter granules are mixed with soil, an intensive conidiation takes place on the granules. The conidia infect a number of important insect pests, in particular *Otiorhynchus sulcatus* (black vine weevil). Trials in the greenhouse and also under practical conditions have shown very good effectiveness against *O. sulcatus* in several ornamental crops, such as *Azalea*, *Rhododendron*, *Taxus*, *Cyclamen*, *Chrysanthemum*, *Fuchsia*, *Geranium*, *Hedera* and *Begonia*. Mixing the granules into the potting soil before planting results in a level of control similar to that given by chemical standards, with even better residual effects. Intensive studies have shown no particular toxicological risks or environmental hazards.

TECHNICAL DATA

Active ingredient:

Metarhizium anisopliae (Metschn.) Sorok. 1883 var. anisopliae; (Domsch et al., 1980), DSM 3884, Hyphomycet, wild-type strain, not genetically altered by any procedure.

Metarhizium anisopliae is one of the most important entomopathogenic fungi with a worldwide distribution. It does not grow in soil but can be cultivated easily on solid and liquid sterile media. The optimum growth temperature is 25°C.

Formulation:

active ingredient:	100%
type:	granules 0.5 - 1.0 mm in diameter
bulk density:	400 g/l
solubility:	not soluble
proportion of dust:	practically dust-free

By a special patented fermentation procedure *M. anisopliae* is grown in fermenters in the form of so-called "pellets". After fermentation these pellets are dried to granules and packed into vacuum plastic bags. Low temperature favours shelf-life.

TOXICOLOGICAL AND ENVIRONMENT-RELATED DATA

Pathogenicity:	*M. anisopliae* is not pathogenic to mammals and does not grow at 37°C
Acute oral LD_{50}:	rat (male and female) > 2000 mg/kg = maximum applicable amount
Acute dermal LD_{50}:	rat (male and female) > 2000 mg/kg = maximum applicable amount
Irritation:	rabbit: not irritating to skin, slight irritation to eyes
Sensibilisation:	no reaction with dry granules
Contamination:	BIO 1020 is free of microorganisms which are hazardous to man and domestic animals
Bird toxicity:	No adverse effects have been observed in an orientating test on quails

As a fungus, which is specialised almost exclusively to insects (some strains may also infect nematodes), *M. anisopliae* shows no toxicological effects on mammals. Like all biological material, BIO 1020 may cause allergic reactions. However, the dust-free granules represent rather a low risk compared with, for example, pollen grains. Beneficial animals are not affected by BIO 1020. In orientating tests, honey bees, earthworms, *Daphnia sp.* and Collembola were not influenced.

APPLICATION AND MECHANISM OF ACTION

BIO 1020 is packed and distributed in plastic bags under vacuum. Mixing the granules with soil, peat or other growing substrates results in water uptake by the granules. At temperatures above 12 to 15°C the fungus starts to develop hyphae immediately followed by conidiation. After 3 to 7 days (depending on the temperature) each granule has produced about 1 million conidia. The conidia are the natural infectious and surviving structures of *M. anisopliae* and can stay alive in the soil for many months. If insects come into contact with spores, the spores adhere, germinate and penetrate into the host. After proliferation in the haemolymph, the insect dies and the fungus produces new spores on the insect cadavers.

Because conidia move very little in soil, the whole volume of the growing substrate should be treated and therefore application has to be protective. 1 g BIO 1020 per litre of substrate (= 1 kg per m^3) intensively mixed usually results in a titre of more than 10^6 conidia per g of soil, which leads to satisfactory control of black vine weevil.

If temperatures at potting time are low, it may be useful to prepare a ten-fold concentrated pre-mixture of granules and potting soil, for example, to keep less volume of substrate under temperatures favourable for conidiation. When the conidia are produced this pre-mixture can be diluted and the potting soil is ready for use.

Because conidia are viable for a long time, a protective application provides a sufficient protection against black vine weevil for at least one vegetation period.

BIOLOGICAL ACTIVITY

Host spectrum and range of biological control

Strains of *M. anisopliae* are usually restricted to a certain range of host insects mainly belonging to the Coleoptera. BIO 1020 behaves similarly in general but is also effective against Lepidoptera and Diptera (TABLE 1). Infectivity under laboratory conditions should be distinguished from practical biological control. In laboratory trials, where insects are intensively inoculated with conidia, insect hosts are infected very rapidly. In greenhouse or open field trials, however, where the host plants, on which the insect pests develop, are included in the experiment sufficient control cannot always be achieved. Onion fly (*Phorbia antiqua*) for example, is susceptible to conidia of BIO 1020. In practice, however, the adult fly lays its eggs directly on to the base of the young onion plant. The hatching larvae immediately penetrate the plant and can no longer be reached by conidia in the soil.

TABLE 1. Pathogenicity of BIO 1020 against different pests and effectiveness

\- no infection or effectiveness
\+ insects regularly infected
+/- low infectivity or inconsistent results

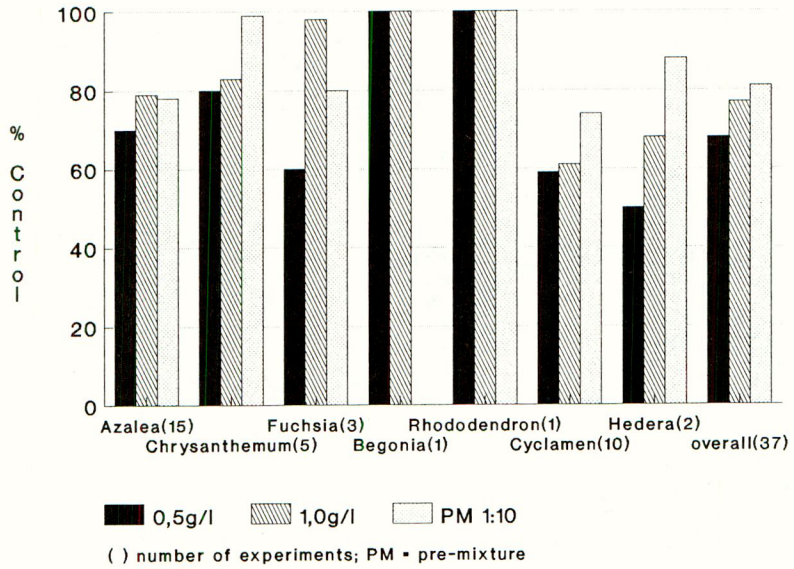
	Infection	Effectiveness
Hemiptera		
Aphids	+/-	-
White Flies	+/-	-
Hymenoptera		
Honey bees	-	-
Ants	+/-	-
Coleoptera		
Black vine weevil *(Otiorhynchus sulcatus)*	+	+
White grubs *(Melolontha sp.)*	+	+
Wireworms *(Agriotes sp.)*	+	+
Wireworm *(Tenebrio molitor)*	+	+
Diabrotica balteata	+	+
Flea beetles *(Phyllotreta sp.)*	+	+
Lepidoptera		
Cutworm *(Agrotis segetum)*	+	+
Swift moth *(Hepialus lupulinus)*	+	+
Diptera		
House Fly *(Musca domestica)*	+	+
Cabbage and onion flies *(Phorbia spp.)*	+	-
Common crane flies *(Tipula sp.)*	+/-	-
Termites	+	+/-
Cockroaches	+	-
Collembola	-	-
Mites	+/-	-
Nematodes	-	-
Snails	-	-

The susceptibility of different insects to BIO 1020 is different. *Tenebrio molitor*, for example, is very sensitive, whereas *O. sulcatus* or *Melolontha sp.* need a higher infection pressure. All stages of susceptible pests are infected (eggs, all larval stages, pupae and adults). Honey bees, both adult and brood, are not affected at all.

Greenhouse tests

BIO 1020 was tested against *O. sulcatus* on different ornamental crops in 37 greenhouse trials (Fig. 1). In these tests BIO 1020 was mixed with the potting substrate (commercially available growing substrate with a high peat content) at an application rate of 0.5 or 1.0 g /l. For comparison a pre-mixture (as described above) was used, at a rate of granules comparable to 1 g /l. The test pots were inoculated with 10 eggs or 3-5 larvae of *O. sulcatus* immediately after potting. The degree of control achieved (Fig. 1) depended on the amount of product applied. 1 g BIO 1020 per litre usually gave better control than 0.5 g/l. The pre-mixture sometimes improved control. Overall, 70 - 80% control was achieved.

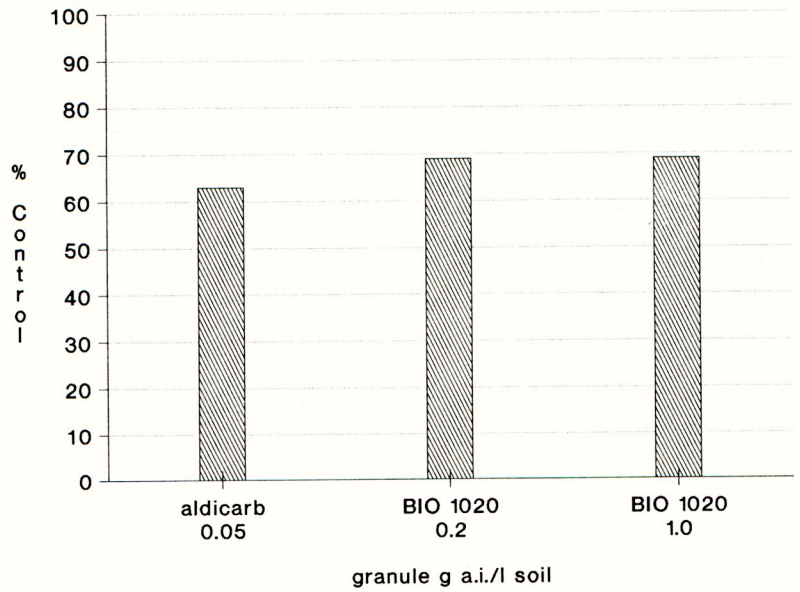
Fig. 1. Effectiveness of BIO 1020 against *Otiorhynchus sulcatus* eggs and larvae in ornamentals under greenhouse conditions 28 days after treatment.



Field trials

Azalea plants were potted into soil treated with BIO 1020 at the rate of 0.2 or 1.0 g /l. For comparison, a granular formulation of aldicarb (0.05 g a.i. ≙ 1 g granules/l soil) was used. The pots were placed outside in a nursery. Forty percent of the untreated plants were infected by *O. sulcatus* when the treatments were evaluated, 156 days after planting. All the treatments gave a similar level of control (> 60%); (Fig. 2).

Fig. 2. Control of *Otiorhynchus sulcatus* in *Azalea* under field conditions in a nursery



BEHAVIOUR OF BIO 1020 IN THE SOIL

The mobility of BIO 1020 or its conidia in soil was investigated in a laboratory test using a bioassay. PVC-columns (diameter 5 cm, length 18 cm) were filled with standard soil (sandy loam). 50 mg of BIO 1020 were applied on top of the columns and slightly mixed with the soil at the surface. Water was applied to the top of the columns at a rate of 5 ml per column per week. Incubation took place at 20°C for 4 or 8 weeks, after which the soil columns were cut into segments of 2 cm. The soil of each segment was incubated with 10 larvae of *Tenebrio molitor* for 10 days. The mortality of the insects was then determined.

TABLE 2. Mobility of *M. anisopliae* conidia in soil

depth of segment (cm)	% mortality of *T. molitor* larvae incubation time	
	4 weeks	8 weeks
0 - 2	100	100
2 - 4	85	84
4 - 6	65	68
6 - 8	51	41
8 -10	30	46
10 -12	26	7
12 -14	21	23
14 -16	13	38
16 -18	5	22

The number of conidia as indicated by the larvae mortality in the bioasssay, declined rapidly the deeper the test segments were taken (TABLE 2). Below 6 cm a practically acceptable control could not achieved. However, *T. molitor* is a very susceptible host of *M. anisopliae* and these results cannot be compared directly with those against *O. sulcatus*. Moreover, the soil in the test columns was not a core of undisturbed soil.

From other trials under practical conditions we knowed that the movement of conidia of BIO 1020 was much lower than in the model test. Consequently, an eradicative treatment of already infested plants would not be successful, because the infective stages of the fungus would not reach their insect hosts. BIO 1020, therefore, has to be applied protectively by mixing it into the whole growing medium. Since the relatively large conidia are not transported downwards contamination of groundwater is unlikely.

LONG-TERM EFFECTS

Ten litres of soil were mixed with BIO 1020 at an application rate of 1 g/l and stored in a container at a temperature of 18°C. From this stock, aliquots of 100 ml were removed every 4 weeks and tested against *Tenebrio molitor*. After 12 weeks of incubation, maximum control was achieved, which remained stable for at least half a year. Additional tests showed that the activity could be enhanced by returning the test samples to the stock container probably because *M. anisopliae* increased its conidia titre on the infected host insects.

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REFERENCES

Domsch, K.H.; Gams, W.; Anderson, T.-H. (1980) Compendium of Soil Fungi. *Academic Press, Vol 1, 2.*