SESSION 2A

NEW COMPOUNDS, NEW CONCEPTS AND NEW USES

Chairman:	Professor Phil Russell *Consultant, Cambridge, UK*
Session Organiser:	Dr Leonard G Copping *LGC Consultants, Saffron Walden, UK*
Papers:	2A-1 to 2A-10

A novel formulation of clomazone for use in rice

D T Schulteis
Wilbur-Ellis Company, 2903 S. Cedar Avenue, Fresno, CA 93715, USA
Email: dschulte@wecon.com.usa

J Heier
Wilbur-Ellis Company, 841West Elkhorn Blvd, Rio Linda, CA 95673, USA

ABSTRACT

The activity of an extruded 5 MEG granular formulation of clomazone, 2-(2-chlorophenyl) methyl-4, 4-dimethyl-3-isoxazolidinone, which has demonstrated excellent control of *Echinochloa* spp. (resistant/non-resistant) and *Leptochloa fascicularis* in California water-seeded rice is described. The physical and chemical properties of the 5 MEG formulation allow aerial applications to rice fields that are in close proximity to sensitive crops and environmental areas. Primary and secondary drift often associated with aerial applications of pesticide products are dramatically reduced with this product.

INTRODUCTION

The standard cultural practice for growing rice in California is water-seeding with continuous flooding of the fields that consequentially require the use of aerial applications of seed, fertilizer and pesticides. The region where rice is grown in California includes many sensitive crops and environmental areas that are susceptible to drift from aerial applications of pesticides. The limited number of registered herbicide products for use on rice in California has contributed greatly to the development of resistance of weeds to these products that have similar chemistries and modes of action.

The US EPA issued registration for the 3 ME formulation of clomazone for use on rice to control weeds. However, this product was not registered for use in California. The potential for drift from aerial and ground applications of this formulation onto sensitive crops and into environmental areas was too great. In California, there are several species of watergrass: *E. oryzoides* (early watergrass), *E. crus-galli* (barnyard grass) and *E. phyllopogon* (late watergrass). Of significant note however, in University of California-Davis research studies, clomazone demonstrated very good early season control of all *Echinochloa* spp. including the resistant species, *E. phyllopogon*, as compared to the standard registered products (Fischer, *et al.*, 2000). As a result, a formulation of clomazone, Cerano 5 MEG, was developed that would alleviate the potential risk of primary and secondary drift yet maintain efficacy against the target weeds and fulfill all USA EPA and California registration requirements.

MATERIALS AND METHODS

The active ingredient in the 5 MEG formulation is clomazone, manufactured by FMC Corporation. The original 4 EC formulation that was developed for pre-plant incorporation to cotton and soybean fields was very efficacious; however, volatilization of active ingredient from moist soil surfaces did occur. A patented, micro-encapsulated (ME) formulation of clomazone, Command 3 ME, was developed by FMC to reduce the rate of volatilization, especially under moist conditions. Although the volatility issue was reduced the product still required dilution with water in order for ground and/or air applications and the risk of primary drift remained. The capsule size of the 3 ME formulation varies from 5 to 15 microns with an average size of 9 microns. Therefore, it is possible that several of these capsules may reside inside a water droplet of 104 microns. A water droplet of 104 microns will drift a considerable distance when released from an airplane; therefore, the possibility of primary drift to non-target crops or environmentally sensitive areas, remained a concern even with encapsulation.

In order to alleviate primary drift, it was decided to develop a solid delivery system that was comprised of particles containing active ingredient that had adequate size and density to drop to the target with little if any drift or generation of fines during application. The particles would have to sink immediately and disintegrate rapidly in order to release the active ingredient, clomazone.

Secondary drift potential was determined using a laboratory method developed to determine volatility from the various experimental granules as compared to the ME product (Keifer, 1998). The method consisted of spreading the granules on a soil surface, mixing the treated soil, splitting it into 4 replicates and placing it into glass tubes. Air was allowed to flow through these tubes and carry any released clomazone out into polyurethane collection devices. After an 18-hour collection period, the amount of clomazone was assessed with an immunoassay specific for clomazone. A diagram of the volatility test unit is illustrated in Figure 1.

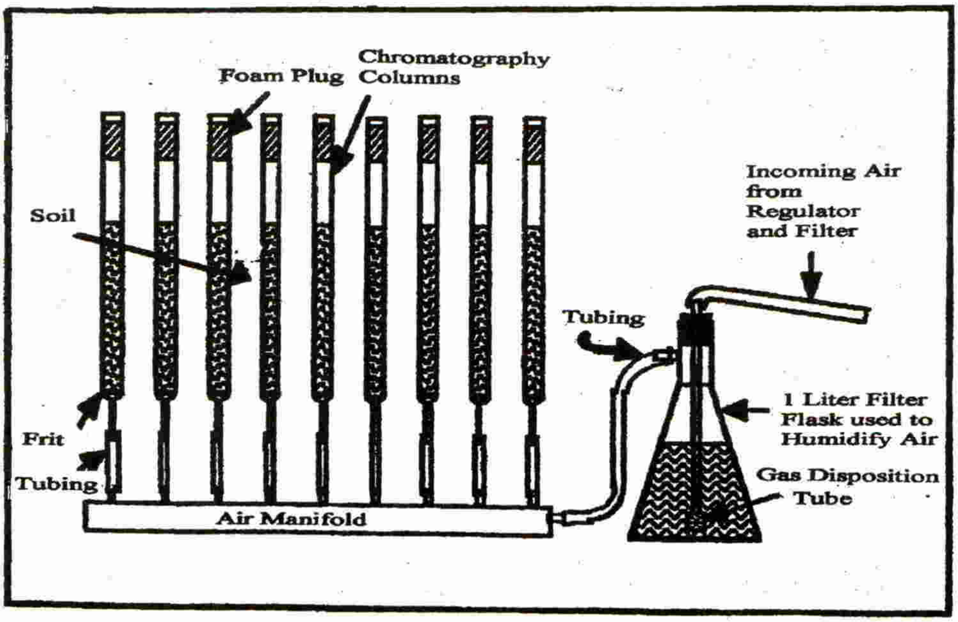

Figure 1. Method used to determine volatility of clomazone from various formulations.

A formulation is considered acceptable if it satisfied the physical and chemical properties of size, density, stability, attrition, disintegration and release of active ingredient upon application into water. It also had to exhibit volatility reduction, efficacy and crop safety equal to or better than the ME formulation of clomazone.

After screening a considerable number of formulations one of them, the 5 MEG, was selected and approved for a large scale experimental use program approved by the US EPA and the California Department of Pesticide Regulation. The EUP program consisted of 55 different field sites with a sum total area of 876 hectares treated with the new product. The application rate of 672.2 g a.i./ha was selected which is equivalent to 13.44 kg/ha of end use product that allowed accurate application and uniform coverage. The application timing varied from 3 days prior to planting up to the one (1) leaf stage of the target *Echinochloa* spp. This time frame allows the application to occur during the safest growth stage of rice to clomazone and the most susceptible stage of target weed growth.

The objective of the EUP program was to verify that this formulation would reflect on a commercial scale the favorable results generated from small, replicated field and laboratory experiments. The parameters studied in the EUP program were: efficacy, crop safety, drift/off-target movement and volatilization. Air monitoring studies were conducted at three sites to determine volatilization potential. High volume air samplers equipped with polyurethane foam collectors were used and positioned around the field as shown in Figure 2.

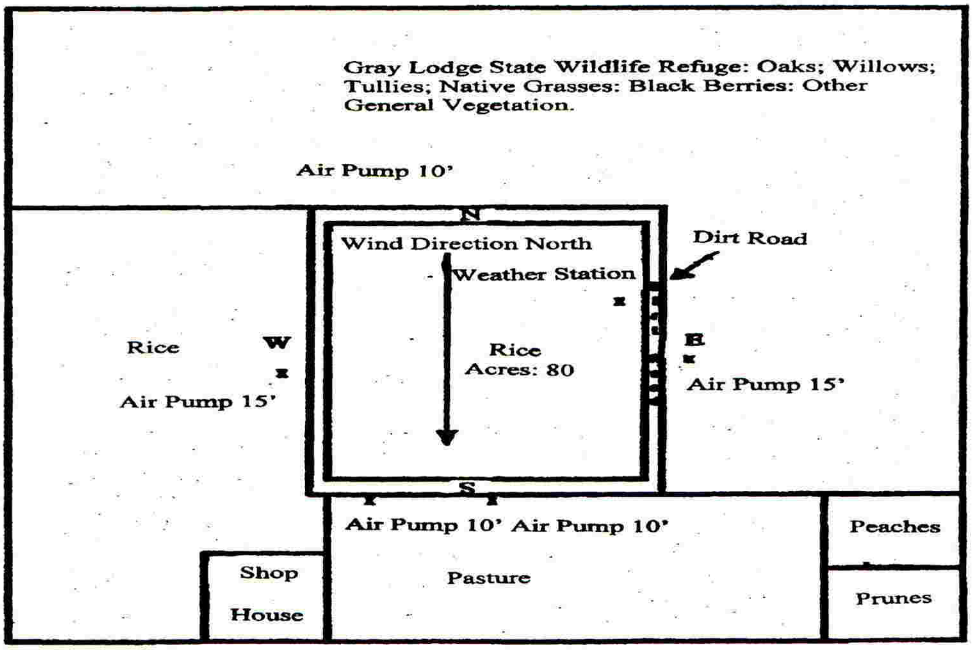

Figure 2. Location of high volume air samples around treatment sites in relation to wind direction. Fenn Site.

The results from each of the parameters studied are described below.

RESULTS

It was very apparent from the many formulations tested that simply applying the active ingredient to a solid carrier with or without a coating would not satisfy the product specifications. Volatility reduction was the most important specification, therefore, it was the deciding factor in the evaluation process. Table 1 lists several of the clomazone formulation candidates and the volatility results. The 5 MEG formulation was the most effective formulation in reducing volatility.

Table 1. Volatility of clomazone from various granular formulations after 18 hours of collection

Test Substance	Description	As % 4 EC
WECO 0001	Microencapsulated	17
WECO 0012	Sand Core	113
WECO 0016	Clay, size 24/48	80
WECO 0017	Clay, size 24/48, coated	234
WECO 0023	Carbohydrate Matrix	136
WECO 0034	Organic Dustless	126
5 MEG	Microencapsulated, Clay	1

The results from the physical and chemical studies on the 5 MEG formulation are listed in Table 2. Upon successfully satisfying the product chemistry and volatilization specifications this formulation was selected as the product of choice for the EUP commercial trials.

Table 2. Physical and chemical properties of the 5 MEG granule rice herbicide

Property	Result
Size	2 mm (w) x 4 mm (l)
Density	1.16
Attrition	< 1.0 %, dustless
Stability	> 2 years
Disintegration Rate	Complete within 15 minutes
Water Sinking Rate	Immediate, no floating

Efficacy

At each field site efficacy evaluations were made at 30 and 60 days after treatment. The 30 day evaluation determined the level of control from only the 5 MEG treatment. The 60 day evaluation determined the level of grass control obtained from this and other herbicides applied to the site. Over all sites and after 30 days the 5 MEG formulation demonstrated complete control of *L. fascicularis*, 98% control of all non-resistant *Echinochloa* spp., 98% control of early *E. phyllopogon* and 88% of late *E. phyllopogon*. Over all sites and after 60 days with the application of other herbicides for broadleaf and grass control the level of control was 100% of *L. fascicularis*, 98% control of all non-resistant *Echinochloa* spp., 99% control of early *E. phyllopogon* and 98% control of late *E. phyllopogon*. Efficacy was as good if not better than the presently registered rice herbicides in California. It introduces a new mode of action that can be utilized in resistant management programs and it complements the water management practices of California rice growers.

Crop Safety

Clomazone is a plant pigment synthesis inhibitor and when absorbed into susceptible plants they turn white and die. Under certain environmental conditions some rice varieties will become bleached and turn white. However, after a short time new vegetative growth will reflect its normal green color and exhibit no significant yield reduction at final harvest. Certain varieties of rice, especially Japanese varieties at the 2 X rate, will exhibit bleaching (Fisher *et al.*, 2000). There was no correlation between the amount of bleaching observed in the 58 sites and crops yield and stand reduction (Figure 3).

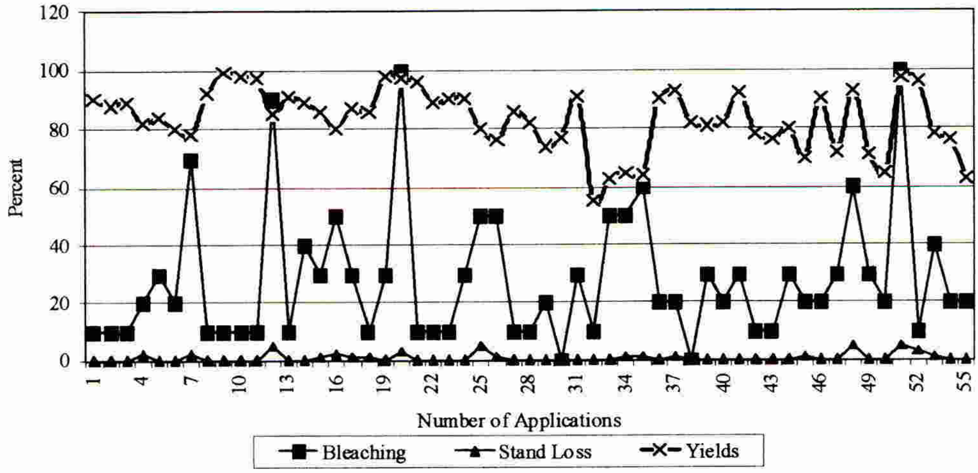

Figure 3. The relationship between bleaching, stand loss and final yield from rice fields treated with the 5 MEG formulation.

Drift/Air Monitoring

There were 54 applications by 18 different aerial applicators to rice fields adjacent to 17 sensitive crops, domestic and environmentally sites. There were no incidents of primary or secondary drift from the target site to these sensitive areas (Figure 4). In addition to the visual evaluations for drift determinations, an air monitoring study was conducted at three sites. High volume air samplers were positioned on each side of the site. In addition, another co-sampler was positioned next to the one positioned on the down wind side of the field. The results from all three sites showed that there was no significant amount of clomazone volatilizing off-site during the three-day collection period following application.

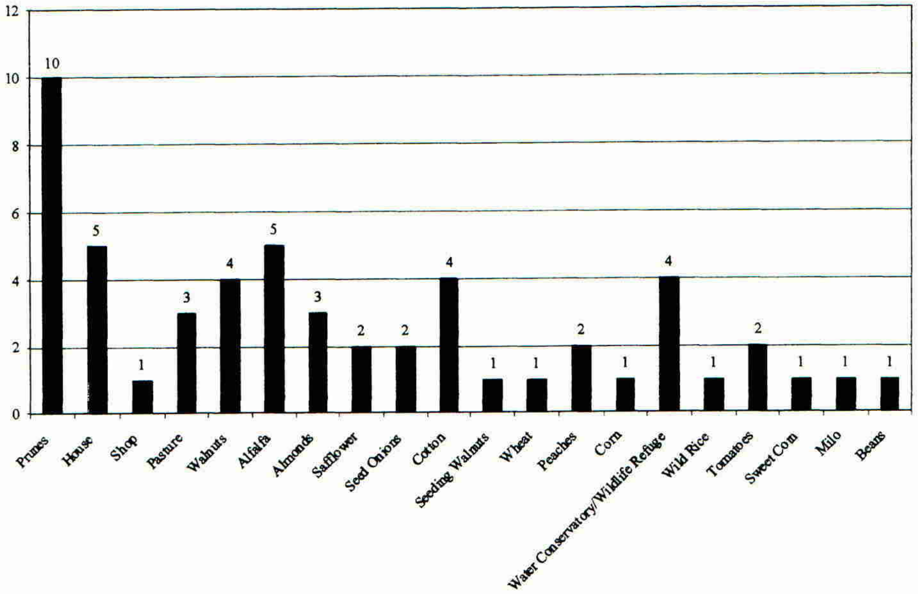

Figure 4. Number of 5 MEG formulation applications to adjacent sensitive crops and environmental areas, with no crop injury.

Based upon the performance of the 5 MEG formulation and the fact that it can be applied safely to fields adjacent to sensitive crops and environmental areas, the product has been registered for use on water-seeded rice by the US EPA and the California Department of Pesticide Regulation.

ACKNOWLEDGEMENTS

We thank the California Department of Pesticide Regulation, the California Rice Commission, the California County Ag Commissioners and FMC Corporation for their assistance in the EUP program.

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Efficacy of a pyrethroid and systemic neonicotinoid to manage an insect and pathogen complex

J D Bradshaw, M E Rice
Department of Entomology, Iowa State University, 110 Insectary, Ames, Iowa, US
Email: bradshaw@iastate.edu

J H Hill
Department of Plant Pathology, Iowa State University, 351 Bessey Hall, Ames, Iowa, US

ABSTRACT

Bean pod mottle virus causes qualitative and quantitative damage to soybeans, *Glycine max*, and is transmitted most efficiently by the bean leaf beetle, *Ceratoma trifurcata* Forster (Chrysomelidae). This pest complex can not be managed with crop rotation and there are no known commercially available resistant cultivars. During the summers of 2002 and 2003, potential strategies for managing bean leaf beetles and *bean pod mottle virus* were evaluated in Iowa, US. The current management strategy in Iowa recommends one early and one mid-season pyrethroid (lambda-cyhalothrin) application. Our study determined the efficacy of applying a systemic neonicotinoid insecticide (thiamethoxam) as a seed treatment, either alone or in combination with foliar applications of lambda-cyhalothrin. Seven management strategies were evaluated: 1) thiamethoxam seed treatment, 2) thiamethoxam seed treatment plus lambda-cyhalothrin foliar application in late May, 3) lambda-cyhalothrin foliar application in late May, 4) lambda-cyhalothrin foliar application in early July, 5) thiamethoxam seed treatment with a lambda-cyhalothrin foliar application in early July, 6) lambda-cyhalothrin foliar application in late May and early July, and 7) an untreated control. The effectiveness of these strategies was measured by comparisons of beetle density, virus incidence, seed quality, and seed weight.

INTRODUCTION

Bean leaf beetles, *Cerotoma trifurcata* Forster, cause economic damage, as adults, to soybeans by feeding on leaves, stems, and pods (Smelser & Pedigo 1992a,b, Pedigo 1994) and, in Iowa, have three populations a year (Smelser & Pedigo 1991). Additionally, bean leaf beetles transmit bean pod mottle virus, a quantitatively- (Hopkins & Mueller 1984, Windham & Ross 1985) and qualitatively-damaging (Yang 1999) soybean disease. The economic importance of this pest complex in the Northcentral United States is the result of an increasing population of bean leaf beetles (Bradshaw & Rice 2003). Short-term management strategies have been developed, finding two applications of lambda-cyhalothrine (approximately one in May and one in July) to be most efficient (Krell 2002). However, wet springs in this region make pesticide applications in April and May difficult. We are studying the efficacy seed-applied thiamethoxam (a systemic neonicotinoid) and foliar-applied lambda-cyhalothrin (a pyrethroid) in soybean to manage bean leaf beetles and bean pod mottle virus.

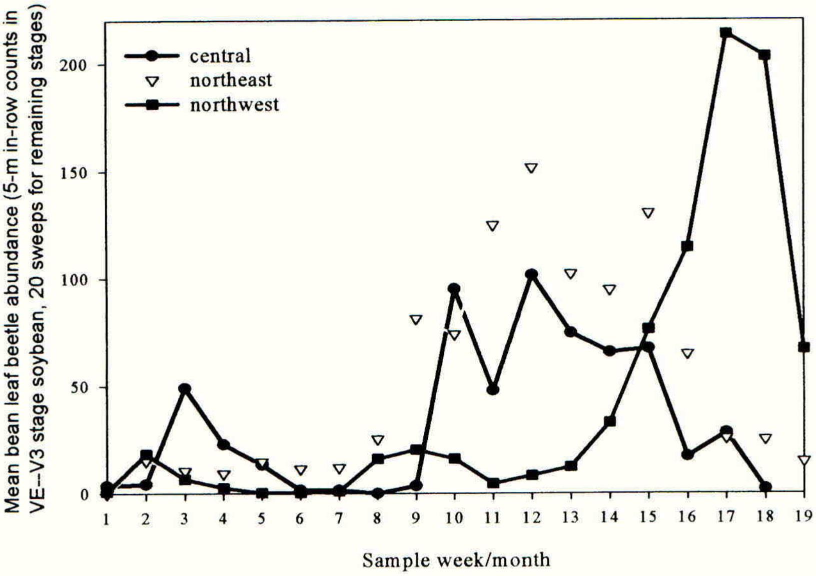
MATERIALS AND METHODS

Studies were conducted at three locations: Central, Northeast, and Northwest Iowa, US. To assess the efficacy of seed treatments, 6 and 7 treatments were chosen in 2002 and 2003, respectively: 1) thiamethoxam (seed applied), 2) lambda-cyhalothrin (foliar application) at soybean emergence, 3) lambda-cyhalothrin at emergence of first bean leaf beetle generation, 4) thiamethoxam (seed applied) plus lambda-cyhalothrin at approximately 10 days post-emergence (in 2003), 5) thiamethoxam (seed applied) plus lambda-cyhalothrin at emergence of first bean leaf beetle generation, 6) two lamba-cyhalothrin applications (one at soybean emergence and one at emergence of first bean leaf beetle generation), and 7) untreated control. Treatments were arranged in a randomized complete block design with 4 (2002) and 8 (2003) replications. Treatment plots were 30ft wide by 100ft long (12 rows per treatment), replications were separated by at least 30ft of soybean.

Bean leaf beetles were sampled weekly from the middle four rows of each treatment. Beetles were sampled by 5-meter in-row counts per treatment for emergent through three-trifoliolate leaf-stage soybeans (Ritchie et al. 1992). Twenty, sweep-net samples were taken for soybeans at the four-trifoliolate leaf-stage or older and counted in the laboratory. Soybean tissue samples were taken periodically throughout the summer (four in 2002, six in 2003) to correspond to bean leaf beetle population abundance. For a measure of virus incidence, twenty uppermost, fully-expanded, soybean leaves were collected randomly from each treatment. Tissue was tested for bean pod mottle virus using an enzyme-linked immunosorbant assay, similarly to *Soybean Mosaic Virus* (Steinlage 2002). Data were analyzed using analysis of variance (SAS 2003).

RESULTS

In our untreated plots, adult bean leaf beetle abundance tended to have a different pattern at each field site (Figure 1). However, beetle abundance was significantly suppressed by insecticide treatments, during three periods of increasing abundance, for northwest and northeast Iowa in 2002 (Figure 2) relative to an untreated control. This suppression in beetle abundance increased yield for some treatments, although not significantly (Figure 3). At each location the early-season lambda-cyhalothrin (treatment 2) treatment significantly reduced beetle abundance in sample weeks 1—4 (Figure 2). However, at northwest and northeast locations, thiamethoxam (treatment 1) provided better mid- and late-season suppression of bean leaf beetle abundance than the early-season lambda-cyhalothrin treatment. The addition of a lambda-cyhalothrin application (treatment 4 & 5) to an early-season thiamethoxam or lambda-cyhalothrin application gave season-long suppression of bean leaf beetle abundance at least at two locations (Figure 2A & B). The unexpectedly low abundance of second-generation beetles at the northeast- and central-Iowa locations probably had a negative impact on the treatment effects. These differing phenologies are probably due to a prolonged emergence from overwintering sites and abundant mid-season rains (unpublished data). Virus-assay data from sample week 13 at the northwest location indicate a trend for highest virus incidence in the untreated control (Figure 4). Additionally, virus incidence apparently is lower in treatment 5 (thiamethoxam plus lambda-cyhalothrin). Further analysis is needed to explain the seasonal epidemiology for all treatments at all locations for both years. Collection of data for 2003 is in progress.



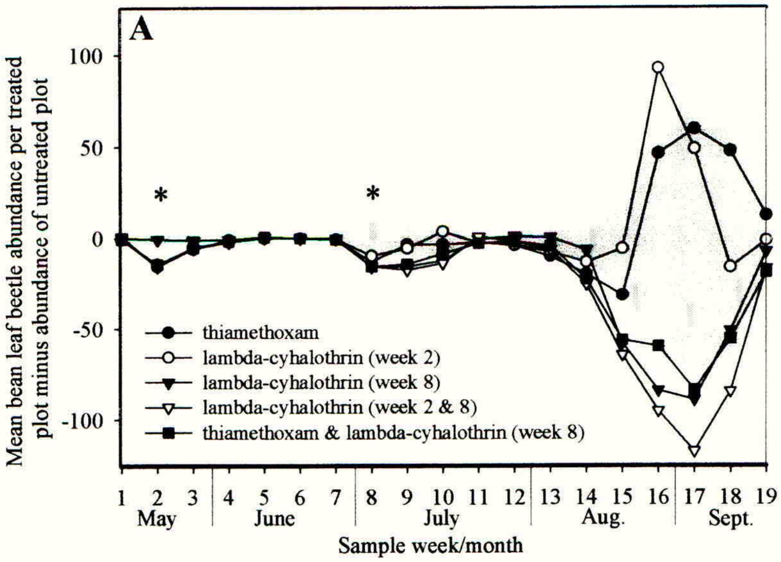
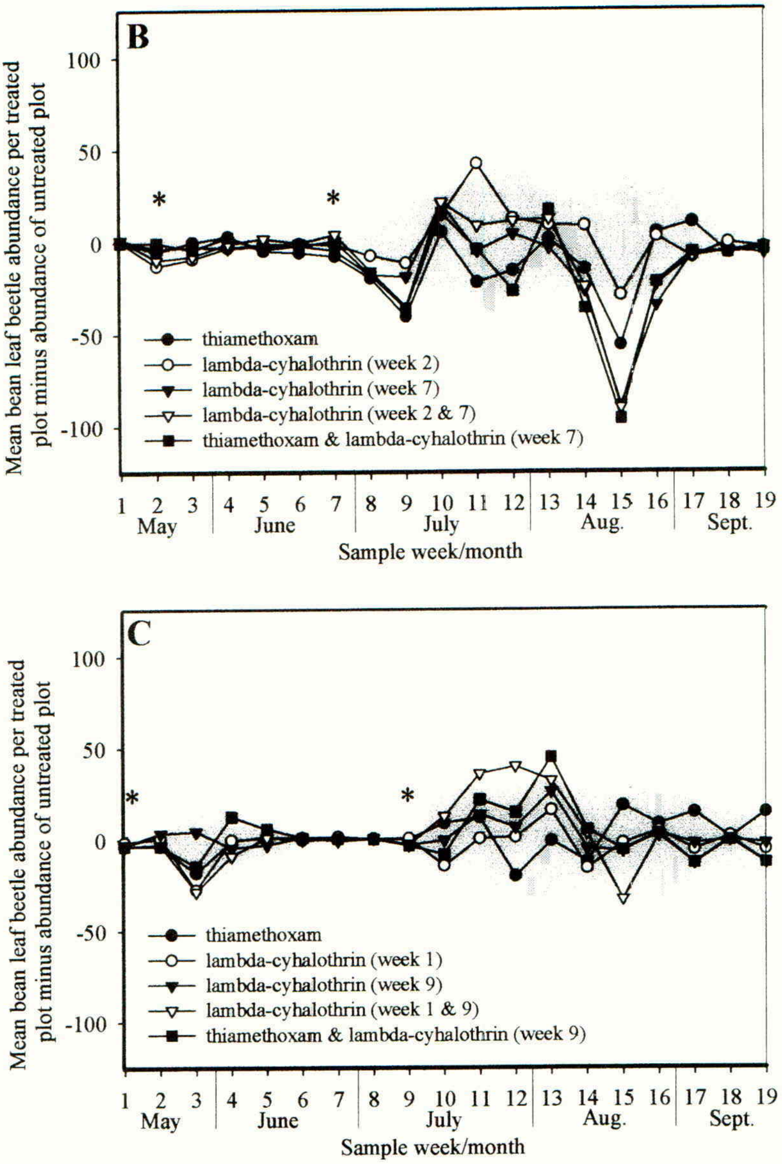


Figure 2. Mean difference of bean leaf beetle abundance in insecticide-treated from untreated soybean plots (origin=untreated control), A) northwest, B) northeast, and C) central Iowa, USA. Boundary of shaded area equals LSD (α=0.05). Approximate times of foliar applications of lambda-cyhalothrin shown with an asterisk, thiamethoxam applied to seed at planting.



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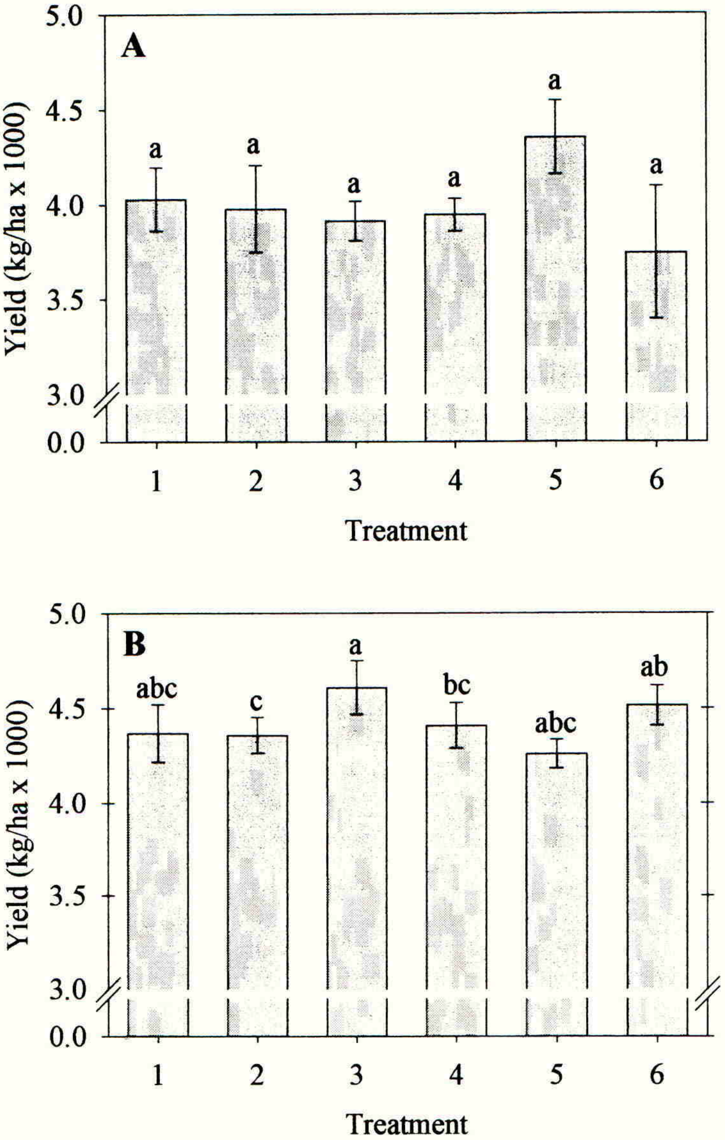
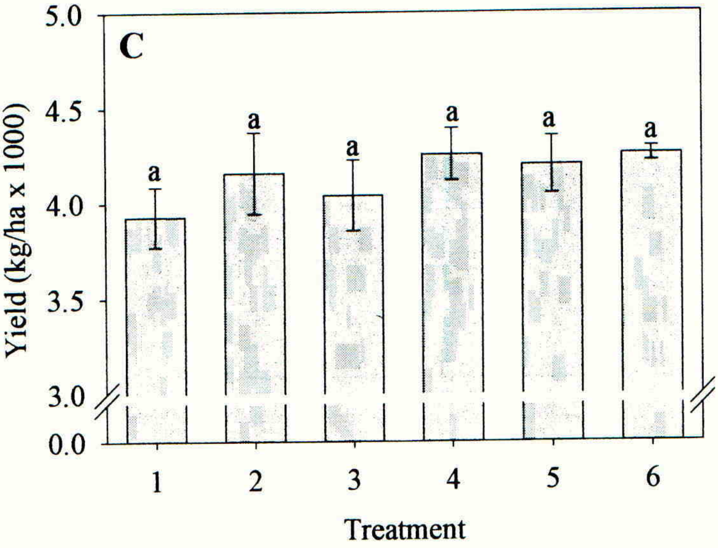


Figure 3. Mean yield of soybean in seed- and foliar-insecticide treated and untreated field plots at three locations: A) northwest, B) northeast, and C) central Iowa. Yield is statistically significant at northeast location (α=0.05) — treatments with the same letters are not significantly different. Treatments: 1. Thiamethoxam, 2. Lambda-cyhalothrin (May), 3. Lambda-cyhalothrin (early July), 4. Lambda-cyhalothrin (May & early July), 5. Thiamethoxam + Lambda-cyhalothrin (early July), 6. Untreated control.



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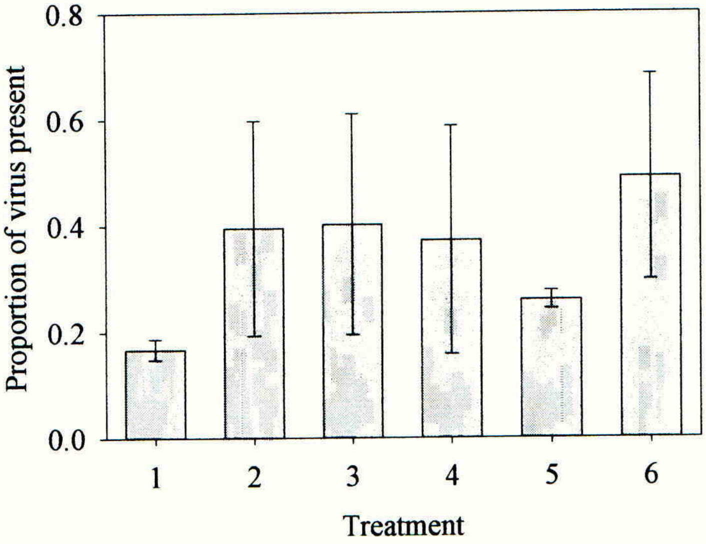

Figure 4. Proportion of *Bean pod mottle virus*-infected soybean in 20 samples per treatment from early August, in northwest Iowa. Treatments: 1. Thiamethoxam, 2. Lambda-cyhalothrin (May), 3. Lambda-cyhalothrin (early July), 4. Lambda-cyhalothrin (May & early July), 5. Thiamethoxam + Lambda-cyhalothrin (early July), 6. Untreated control.

CONCLUSIONS

The application of foliar insecticides to seedling soybean can be difficult in Iowa because of weather conditions in late April to Early May (cool, wet weather). However, Krell (2002) found that an early- and mid-season application of insecticide is needed to protect soybean yield and quality from *Bean pod mottle virus*. Seed-treated insecticides may be an alternative strategy to foliar-applied insecticides for the short-term management of this pest complex.

However, seed quality and virus titer within soybean may not be highly correlated for all varieties (Hill, unpublished data). Therefore, virus incidence alone from one sample date should be interpreted with caution. Additionally, our data indicate that the management of over-wintered and first-generation bean leaf beetles, for reducing virus incidence, can provide late-season benefits by suppression of second-generation beetles.

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Transgenic mycoherbicides for effective, economic weed control

J Gressel, Z Amsellem
Department of Plant Sciences, Weizmann Institute of Science, Rehovot, 76100, Israel
Email: Jonathan.Gressel@weizmann.ac.il

ABSTRACT

Agents proposed for biocontrol of major weeds in arable row-crop agriculture have not met expectations due to an evolutionary balance between microorganism and weed, even when a mycoherbicide is used at "inundative" levels (>10^4 spores/cm^2). Enhanced efficiency can be achieved by transferring virulence factors to the microorganism, tipping the evolutionary balance. Virulence was doubled when auxin overproduction was engineered into one mycoherbicide. Virulence was increased 9 fold, was more rapidly effected, and the requirement for a long dew period was decreased by introducing *Nep*1 encoding a phytotoxic protein to an *Abutilon theophrasti*–specific, weakly mycoherbicidal strain of *Colletotrichum coccodes*. The parent strain was at best infective on juvenile cotyledons of this intransigent weed yet the transgenic strain was lethal through the three-leaf stage, a sufficient time window to control this asynchronously germinating weed. Similar results were achieved when the *Nep*1 gene was transformed into a *Fusarium arthrosporioides* attacking *Orobanche* spp. (broomrapes). Strategies of coupling virulence genes with failsafe mechanisms to prevent spread (due to broadened host range) and to mitigate transgene introgression into crop pathogens could possibly open a new future to biocontrol of major weeds in row crops.

INTRODUCTION

There have been few successes in controlling weeds using phytopathogenic microorganisms. Unfortunately, most of the successes have not been where they are most needed – in row crop situations to augment chemical herbicides, especially where the latter are not cost-effective or even effective. The few successes in using microorganisms to control weeds have been in "classical" situations, where an axenic alien (imported) weed has proliferated, and its native pathogens were then imported to bring it in balance. In these "classical" cases there is no weed control in the agronomic sense, just the placing of a weed in its original ecological context of being a less competitive wild species.

Those imbued with chemical herbicides have decried biocontrol agents as useless because they will not kill a broad spectrum of weeds in the same manner as a chemical herbicide. The high specificity of many pathogens is their prime advantage, but this relegates them to niche and not-so-niche applications.

Niche application does not necessarily mean an unimportant application, because the application may be where chemicals have failed, or have never been cost effective or acceptable.

These niche applications include:

1. Weeds that have never been controlled cost-effectively by herbicides.

Pathogens can often distinguish between a crop and a closely related weed, where selective herbicides have be ineffectual. Biocontrol should be cost-effective for weeds invading pastures such as leafy spurge (*Euphorbia esula*) in the northern US plains and ferns in Europe, *Xanthium* spp in Australia. It also includes shrubby trees invading forests. Vegetatively-propagated *Cyperus rotundus* (purple nutsedge) is the World's Worst Weed (Holm *et al.*, 1977), and is becoming worse yet in conservation (minimum) tillage systems, yet is exceedingly hard to control by chemical herbicides.

2. Weeds requiring high rates of herbicides for control. One particular weed in an agro-ecosystem e.g. *Abutilon threophrasti* (velvetleaf), often requires a high rate yet most weeds are controlled by lower rates.

3. Weeds that have evolved resistance to herbicide, especially multiple and cross resistances. This includes *Echinochloa* spp. that have evolved resistance to most herbicides used to control them in rice, worldwide and *Lolium* spp., especially in Australia where there is no wheat-selective herbicide that can control some resistant biotypes (Gressel, 2002).

4. Urban allergenic weeds that need to be eliminated without affecting other species.

5. Narcotic weeds. The use of specific pathogens to cause specific epidemics of opium poppies or coca, especially when applied from the air, has been the subject of science as well as the first case where microbiocontrol starred in a science fiction novel (Cook, 2001). Clearly application of biocontrol agents to cause a species-specific epidemic is superior to the recent aerial application in Colombia of glyphosate, which kills most crops and vegetation.

Inundative biocontrol, where a specific pathogen of the weeds (not necessarily one from its center of origin), is used inundatively in the same manner as a chemical herbicide, has not been effective and thus not adopted by farmers. A few made it to market, where one sporadically remains – "Collego", a *Colletotrichum gleosporioides* for control of *Aeschynomene virginica* (northern jointvetch). Many possible reasons for the lack of success are elegantly discussed in a different vein by Auld and Morin (1995). No reason alone fully explains the problem, and single solutions have not brought cost-effective agents. The answers will come from synergistically combining solutions.

Besides the paltry sums invested in research and development of mycoherbicides, the lack of success stems from:

A placid acceptance of Van der Plank's thresholds

The erudite exposition of the need for threshold levels of inoculum to form a lethal infection by a compatible pathogen (Van der Plank, 1975) is accepted by most plant pathologists, as thresholds are typical in field epidemiology. Typically, threshold levels of 10^3- 10^5 propagules/cm^2 of leaf surface are used (Gressel, 2002). This is akin to ancient herbicides where tens of kg/ha were applied. Assuming that one propagule/cm^2 represents 100% efficiency, there are orders of magnitude to improve inoculum efficiency. There are now chemical herbicides that are active at tens of grams per hectare; and we must strive to lower the inoculum thresholds by similar magnitudes.

An over-interpretation of Koch's postulates

Koch was right in demanding the demonstration that a single organism was responsible for a disease by passing it through the plant, seeing that the symptomology was there, and re-isolating it. Nature rarely uses single pathogens, as we rarely find a single organism in a lesion. There are synergizing or facilitating organisms that typically assist the primary (Koch's) pathogen in infecting the plant. We now know of a few cases where pathogen mixtures are synergistic in killing weeds (Auld Morin, 1995) and should we be looking for more. Those searching for mycoherbicidal pathogens may have ignored that there might be pathogen-facilitating bacteria.

Formulation and dew period

Initially it was thought that aqueous spore suspensions would be sufficient for biocontrol. This is not sufficient for chemical herbicides, why for spores? Medium components, surfactants, and sticking agents were added to keep propagules alive during a storage period after production until use and to keep the biocontrol agent wet for the typical 12-24 h dew period required, which cannot be met in most field climes. This could only be met easily with *Collego,* used in rice paddies. Humectants were added to hold water, inverse emulsions to preclude evaporation by having an outer layer of oil around the water on the spore until it germinated, penetrated and became established. Conventional wisdom had it that anything that could not be sprayed with a conventional sprayer through standard nozzles could never be commercialized. They ignored that in many areas most pesticides are applied by custom applicators that constantly purchase new equipment. If bioherbicides were found that really met a unique need, yet required special application equipment (e.g. air assisted sprayers), the innovators in agriculture would have purchased it. The products seem not to exist that justify such equipment, none were sufficiently virulent to compete with chemicals.

SUCCESSES THROUGH TRANSGENICALLY ENHANCING VIRULENCE

Pathogens typically use toxic proteins or secondary metabolites to enhance virulence. These secondary metabolites are many enzymes removed from primary metabolism, and the enzymes are often encoded by clustered genes. The same clusters are found scattered among species in diverse orders of fungi, which has been used as a support for horizontal gene (cluster) transfer during evolutionary time (Walton, 2000). Similarly, a necrosis eliciting virulence protein (NEP) is found with similar sequences from Oomycetes to Ascomycete-like imperfecti, as well as bacteria (Fellbrich *et al.*, 2002).

Typically in a compatible infection, the plant has evolved some defenses to such virulence factors. Enhanced virulence can be achieved by transferring virulence factors from one pathogen species, to another where they have not previously been used. This approach has been successfully used to enhance the virulence of biocontrol agents attacking insects (St. Leger & Screen, 2002) and pathogenic fungi (Lorito *et al.*, 2001), as well as mammals (rabbits) (Kerr *et al.*, 2001). Such approaches have only recently been used for the biocontrol of weeds (Amsellem *et al.*, 2002).

The authors' group has tested transgenically enhancing virulence by using 'soft' genes (overexpressing phytohormones normally found in human food) and 'hard' genes (expressing a phytotoxic protein). Pathogens often excrete the plant hormone indole acetic acid (auxin), and mutants losing this ability lose virulence. The possibility that overexpression of auxin might enhance virulence had not been tested, even though a major group of herbicides mimics the effects of high auxin levels when killing weeds. When the two genes encoding auxin production (*iaa*H and *iaa*M) were overexpressed virulence was doubled (Cohen *et al.*, 2002). While this was statistically significant, it was not the order of magnitude needed to partially achieve the goal of weed control in row crops. Additionally, the organism was only effective when exogenous tryptophan, the substrate of the first enzyme, was added to the growth medium of the fungus before the fungus was applied to the soil as a drench. The application of such an organism with tryptophan could be a controlling failsafe mechanism with a foliar-applied pathogen; it has hypervirulence with tryptophan, but is similar to wild type without the amino acid. This was indeed the case when the *iaa*H and *iaa*M construct was engineered into a *Colletotrichum coccodes* specific to *Abutilon theophrasti*, the transgenic mycoherbicide was no different from the wild type when sprayed without tryptophan. The transgenic was lethal with 2,4-D-like (auxin) symptomology when tryptophan was added, but tryptophan had no effect with the wild type (unpublished results).

A 'hard' gene *nep1* was chosen to try to confer hypervirulence in two systems; a *Colletotrichum coccodes* (Amsellem *et al.*, 2002) that had been envisaged as a mycoherbicide for the pernicious weed *Abitulon threophrasti* (velvetleaf) (Watson *et al.*, 2000), but was never commercialized because it was only active on cotyledons. This weed has a continuous springtime germination, requiring control through the three leaf stage. Not only were the transformants effective (Figure 1), the length of the dew period was considerably shortened (Amsellem *et al.*, 2002), but possibly not sufficiently unless superior formulants are used.

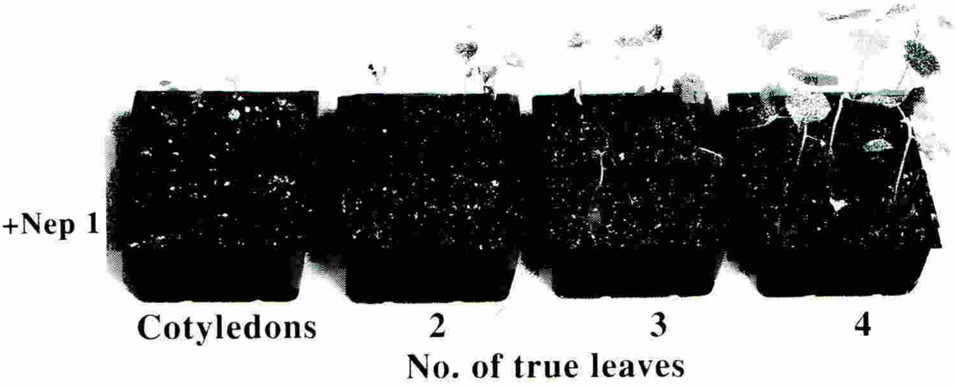

Figure 1. The superior control of *Abutilon* plants through the three true leaf stage by *nep*1 transformed *Colletotricum coccodes* (lower row) compared with using wild type (upper row). The plants were sprayed to runoff with 6 x 10^6 chopped mycelial propagules per ml of water, and kept 24 h at 100% humidity, and photographed 8 days after inoculation (see Amsellem et al., 2002).

The same *nep1* gene construct was tested with two *Fusarium* species attacking the crop-parasitic weed *Orobanche* (broomrape). It was inactive in transforming a *Fusarium oxysporum forma speciales* specific to this weed, even though the gene was integrated into many transformants (Amsellem and Gressel, unpublished).

It was possible to obtain hypervirulent transformants of a *Fusarium arthrosporioides* pathogenic on the same weed (unpublished). The NEP1 protein is phytotoxic to tomato, when applied as the protein (Jennings *et al.*, 2000) or when the gene is in the *Colletotrichum* pathogenic to *Abutilon* (Amsellem *et al.*, 2002) enlarging its host range. This was not the case with the *nep*1 transformed *Fusarium arthrosporioides* colonizing the tomato rhizosphere; it was only hypervirulent to *Orobanche*, and not to the tomato.

Other genes are appearing that might be useful: tomatinase from *Septoria* not only breaks down saponins that are part of plant defenses, the degradation products suppress induced defenses (Bouarab *et al.*, 2002). Genes enhancing oxalate production should also be effective.

BIOSAFETY MECHANISMS FOR TRANSGENIC BIOCONTROL AGENTS

Various failsafe and liability–limiting mechanisms are needed for use with transgenically or otherwise hypervirulent organisms. The dispersal of biocontrol agents beyond the target could be limited by rendering them asporogenic, using mycelial inocula (Gressel, 2001) as had been previously proposed for the broad host range *Sclerotinia* (Sands *et al.*, 1990). This would not prevent heterokaryotic or more mysterious horizontal gene transfer between the transgenic bioherbicide and crop-pathogenic organisms. Such transfers are known in *Colletotrichum* (Chacko *et al.*, 1994), although they have not been reported among the numerous, seemingly stable *forma specialis* of *Fusarium*.

Gene transfer can be mitigated by using constructs where the hypervirulence gene is flanked by genes that are 'neutral' to the biocontrol agent, but would be negative to the recipient of any such gene transfer, rendering it unfit to compete. Such TM (transgenic mitigating) genes include sporulation prevention genes, genes such as (anti) hydrophobin genes, which reduce spore dispersiveness and vitality, anti-melanin genes, which would decrease appressorial attachment and would increase UV susceptibility. The many possibilities for such genes have been discussed elsewhere at length (Gressel, 2001; Gressel, 2002)

The use of efficient biocontrol agents is bound to lead to litigation, as with other pest control systems. It will be necessary to demonstrate that it was not your organism or drift or gene introgression from your organism that causes off target damage. It was thus proposed that all such agents contain a "biobarcode" as part of the hypervirulence gene construct (Gressel & Ehrlich, 2002). Such a marker is easy to decode as an assigned and registered short (nonsense, non coding) DNA sequence will be included. Biobarcodes begin and end with a universal sequence (similar in concept with barcodes) that is recognized by a pair of universal PCR primers. If the universal PCR primer picks up something in the proverbially attacked tomatoes, it is sequenced to ascertain whether the unique coding sequence came from your organism or from someone else's.

SUCCESSES THROUGH OTHER APPROACHES

Better formulations

Some novel formulations are coming about; applying to crop seed to control the parasitic *Striga* (witchweed) species that attack crop roots (A. R. Watson in press). Similarly, aerial application of anti-narcotic species specific pathogens growing heterotrophically on seeds has been used as a method of propagating the pathogens after application, as well as assisting them to penetrate into the soil (Sands & Pilgeram, 2001).

It was possible to formulate chopped dehydrated mycelia from fermenter cultures of various fungi in silica, oil, modified starch, micro-pellets (Amsellem *et al.*, 1999), with a much higher yield of propagules per fermenter volume than spores. The mycelia grow almost instantaneously from the micro-pellets upon rehydration, shortening the dew period requirement. The chopped dehydrated mycelia remained viable for more than a year, albeit at 4°C. The ability to formulate mycelia allows the use of asporogenic mutants, preventing spread off-target (Gressel, 2001).

Overcoming host defenses – biochemically

Early attempts at biocontrol assumed that compatible agents should cause death. Too often the pathogen was isolated from a senescing adult stage of the weed, yet was expected to control the vital juvenile stages. Little attempt was made to "know thine enemy". Some attempts were made to add herbicides randomly to pathogens in the hope of achieving synergy (Christy *et al.*, 1992). There were equally random inklings of success. A more plodding yet sleuthful approach was taken to see how *Cassia obtusifolia* was defending itself from a specific compatible pathogen *Alternaria cassiae*. The weed produced massive amounts of a novel phytoalexin upon infection. After its phenylpropanoid structure was elucidated, it was possible to demonstrate that sub-lethal doses of the shikimate-pathway inhibiting herbicide, glyphosate, prevented the biosynthesis of the phytoalexin. This synergistically facilitated killing the weed without synergizing the pathogen on soybeans, a crop in the same family as the weed (Sharon *et al.*, 1992)

Mycoherbicidal pathogens were synergized by compounds interacting with calcium. One group noted that pathogens often secrete oxalate, so they added this calcium binding compound to a mycoherbicide and obtained synergy (Watson & Ahn, 2001). The other group noted that callose production is in the first line of defense against many pathogens. Callose synthase has an obligate requirement for large amounts of calcium (as a co-factor) and fungi require infinitesimally low levels of calcium to survive. They synthesized a series of lipophillic calcium chelators that would traverse the plant cuticle when applied with the pathogen. These chelators, as well as a calcium channel blocker and oxalate all inhibited callose biosynthesis and synergized the pathogen (Gressel *et al.*, 2002). Removing calcium may also block other calcium-dependent defenses.

Mechanical enhancement

Rapid penetration by bioherbicidal pathogens is facilitated by wounding and penetration via the lesion. Indeed incompatible pathogens can become pathogenic when there are many lesions (Amsellem *et al.*, 1991). The lesions can be made chemically, e.g. with a necrotic spot forming herbicide such as paraquat, or even by rancid oils that make holes through the cuticle. The lesions can also be biological; a compatible pathogen synergistically penetrated and killed a weed via pustules formed by a non-lethal rust fungus (Morin *et al.*, 1993). The effectiveness of a bacterial pathogen in controlling *Poa annua* on golf courses was enhanced by applying the bacteria to lawn mower blades; without affecting the lawn species. Similarly, mycoherbicides applied to cut stumps of brush species prevented regrowth, in forestry situations (Harper *et al.*, 1999). The recent development of highly efficient herbicide applications on a rotating wet cutting blade (Wahlers *et al.*, 1997) may be even more applicable to biocontrol agents than to herbicides.

Genetically enhancing virulence

The over-production of particular single amino acids by mycoherbicidal organisms has been pioneered by one group (Sands & Pilgeram, 2001). They selected for overproducing mutants using analogs of the amino acids in question. The logic behind their concept is that a single amino acid provided to a plant is often toxic because it shuts down whole pathways; e.g. high levels of valine feedback inhibit acetolactate synthase, turning off the production of all branch chain amino acids emanating from this key enzyme.

CONCLUSIONS

The biocontrol preparation of the future will probably be a combination of a weed specific pathogen bearing a number of hypervirulence genes, in a superior formulation that assists in keeping it on target, overcoming host defenses, and providing the sustenance to guarantee establishment. It will be engineered with failsafe mechanisms to prevent spread and gene flow. The various parts seem to be coming forth but there is a considerable distance to achieve superior systems that are competitive with chemical herbicides or that augment herbicides in row-crop conditions. Many of the solutions described increased virulence by nearly a factor of ten. This should shift biocontrol from being expensive and ineffectual to useful and economic.

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Genetically engineered Cry3Bb1 corn for controlling *Diabrotica* rootworms: Estimating the agronomic, economic and environmental benefits of transgenic biotechnology

M E Rice
Department of Entomology, Iowa State University, 110 Insectary, Ames, Iowa, USA
Email: merice@iastate.edu

ABSTRACT

In the US, corn rootworms, *Diabrotica* spp. (Chrysomelidae), cost farmers nearly \$1 billion annually in crop losses and control costs. Current corn rootworm control strategies require the use of insecticides or rotation of corn with another crop. Both of these methods are used widely, but each has its limitations and has occasionally failed to prevent yield loss. In 2003, transgenic rootworm corn (Cry3Bb1 protein) was approved for use by the US Environmental Protection Agency. The potential benefits of this biotechnology are expected to include: increased root protection; increased intangible benefits to farmers (safety of not being exposed to insecticides, ease of use and handling, time and labor savings, and better pest control); increased economic benefits to farmers (\$231 million from yield gains [\$25–\$75/acre relative to no insecticide control and \$4–\$12/acre relative to control with a soil insecticide] and \$58 million in reduced insecticide risks and time savings); reduced corn stalk rot; and increased yield protection (9–28% relative to no insecticide use and a 1.5–4.5% yield benefit relative to control with a soil insecticide). If transgenic rootworm corn is planted on 10 million acres, the annual impact will be a 5,340,000 lb a.i. (75.2%) reduction in insecticide use; increased resource conservation (3.07–5.23 million gallons of diesel fuel equivalents conserved that would have been consumed in the manufacture and delivery of insecticides); increased water conservation (5,657,734 gallons of water not used in insecticide application); aviation fuel conservation (68,845 gallons of aviation fuel not used); reduced farm waste (1,187,035 fewer insecticide containers used); increased planting efficiency; and possibly wildlife and non-target organism safety.

INTRODUCTION

Corn, *Zea mays*, was planted on almost 80 million acres in the US during 2002. It accounts for more than 90% of the total value and production of US feed grains. The western corn rootworm, *Diabrotica virgifera virgifera*, and the northern corn rootworm, *Diabrotica barberi*, are two pests that cost farmers nearly \$1 billion annually in crop losses and control costs (Agricultural Research Service, 2001). Current corn rootworm control strategies require the use of insecticides or rotation of corn with another crop. Both of these methods are used widely, but each has its limitations and has occasionally failed to prevent yield loss due to insecticide resistance or escaping crop rotation practices. Corn rootworm management will continue to include the use of insecticides, at least in the near future, and crop rotation, but it also will include the planting of transgenic (i.e. genetically engineered) corn that resists insect damage and protects grain yields.

Monsanto Company has genetically modified the *cry3Bb1* gene derived from *Bacillus thuringiensis kumamotoensis* (*B.t.k.*), to express a *B.t.k.* Cry3Bb1 protein in corn (Environmental Protection Agency 2001). This protein is selectively active against some species of Coleoptera. The intended technical effect of the genetic modification is to protect corn roots from corn rootworm feeding. In 2003, the US Environmental Protection Agency (EPA) approved transgenic rootworm corn with the Cry3Bb1 protein for commercial use (Environmental Protection Agency 2003). It has been stated that "transgenic insecticidal cultivars offer great potential to serve as the most exciting and effective tool for corn rootworm control in the pest management arsenal" (Gray 2001). In addition to insect control, the planting of transgenic rootworm corn is expected to generate numerous agronomic, environmental, and societal benefits. This article will discuss these expected benefits.

MATERIALS AND METHODS

Estimates of corn acres planted, and insecticide and water used during application are calculated based upon the available literature and independent reports. Potential benefits of transgenic rootworm corn are based upon these estimates.

RESULTS

Increased root protection

Transgenic rootworm corn will be as good as or better than soil insecticides in protecting corn roots from significant corn rootworm larval injury (Tollefson and Oleson, 2002). Two transgenic hybrids were compared against the soil insecticide terbufos and a nontransgenic hybrid with no insecticide. Both transgenic corn hybrids were 100% consistent in protecting roots from economically damaging root injury, whereas the insecticide was only 63% consistent and the untreated nontransgenic offered no protection from insect damage (Tollefson and Oleson, 2002). Transgenic rootworm corn does not offer total protection against larval feeding, but data suggest that the quantity of root injury is minor and should not translate into economic yield loss. An added benefit of transgenic rootworm corn is that root protection does not depend upon planting time, weather influences, calibration of application equipment, or soil conditions for optimum performance (Mitchell 2002). However, the narrow spectrum of activity against corn rootworm larvae could also be a limitation when secondary pests of corn roots are present in economically damaging densities.

Yield protection and economic increases

Over typical ranges for corn rootworm populations, transgenic rootworm corn would provide a yield benefit of 9–28% relative to no insecticide use and a 1.5–4.5% yield benefit relative to control with a soil insecticide (Mitchell 2002). For a reasonable range of prices and yields, the predicted value of the transgenic hybrid would be $25–$75/acre relative to no insecticide control and $4–$12/acre relative to control with a soil insecticide (Mitchell 2002).

It is estimated that if transgenic rootworm corn had been planted in 2000 on 100% of U.S. corn acres treated with a pesticide (14.2 million acres) for corn rootworm control, at a cost that was equal to per acre costs for corn rootworm insecticides, the total economic benefit would have been $460 million (Mitchell 2002). Of this benefit, $231 million from yield gains would be captured by farmers, $58 million would go to farmers in the form of reduced risks and time savings associated with reduced insecticide use, and $171 million to the technology developer and seed companies. The relative value of transgenic rootworm corn depends on the price of the transgenic seed, field performance, availability, and the price of close substitutes e. g. other corn rootworm-resistant varieties or insecticides (Alston, *et al.*, 2002). Higher pricing assumptions and a more realistic adoption on the number of acres would decrease the potential benefits.

Intangible farmer benefits

Farmers likely to adopt transgenic rootworm corn recognize several intangible benefits that bring additional value to this new technology (Alston, *et al.*, 2002). Surveyed corn growers noted that advantages of a transgenic technology combined with a corn seed treatment (for minor seed-feeding or root-feeding pests) would include 1) the safety of not handling an insecticide (30% of farmers); and 2) ease of use and handling (21%), all-in-one product insect control (21%), time and labor savings (14%), and better pest control (14%). The total value of perceived benefits among likely adopters of the technology is $16.08/acre (Alston *et al.*, 2002).

Reduced insecticide use

Replacing insecticides with transgenic plants will reduce pesticide use against the target pest. Estimates of the U.S. corn acreage treated with insecticides in 2000 for corn rootworms range widely from 13,305,233 acres (Doane Marketing Research, 2000) to 14,196,990 acres (Alston, *et al.*, 2002) to 20–25 million acres (Agricultural Research Service, 2001). Using the conservative estimate of 13,305,233 insecticide-treated acres in 2000, if 10 million acres are planted to transgenic rootworm corn, the result would be a 75.2% reduction in insecticide use. Calculating a number of assumptions based upon available data and a 10 million-acre replacement of insecticides with transgenic rootworm corn, the amount of insecticide (active ingredient) not placed into the environment can be reduced yearly by 5,340,000 lb.

The use of transgenic corn hybrids to control insect pests has a history of reducing insecticide use. Approximately half of surveyed farmers in five Midwestern US states that planted transgenic corn used an insecticide to manage European corn borers *Ostrinia nubialis*, and the percentage that decreased their insecticide use nearly doubled from 13.2% in 1996 to 26.0% in 1998 (Pilcher, *et al.*, 2002). Farmers who decreased insecticide use on their farms increased the percentage of transgenic corn acres they planted significantly from 19.7% in 1996 to 47.1% in 1998 (Pilcher, *et al.*, 2002). Transgenic Bt corn for control of European corn borer offers several advantages to the farmer, the most important being yield protection when the pest population is large, less insecticide in the environment, and less exposure of farm workers to insecticides (Pilcher and Rice, 1998). Similarly, a survey of 1,313 farmers from Illinois, Indiana, Iowa, Minnesota, and Nebraska predicted that the three primary benefits from transgenic rootworm corn would be less exposure of farmers to insecticides (69.9%), less insecticide used (68.5%), and better yields (53.2%) (Rice, unpublished data).

Reduced corn stalk rot

The stalk rot complex represents the most serious, widespread disease problem in corn (Munkvold and Hellmich, 1999). Fields affected by stalk rot are usually damaged by more than one fungal species. *Gibberella*, *Fusarium*, and *Colletotrichum* are the most frequently reported stalk rot pathogens. Yield losses due to the stalk rot complex occur as a result of premature plant death and lodging. Stalk rot development is greatly affected by plant stress and stalk rots often enter the plant through damaged roots (Munkvold and Hellmich, 1999). Transgenic corn that reduces the feeding of corn rootworm larvae on roots should significantly decrease the incidence of the stalk rot complex in corn.

Insect resistance management

The US EPA will require an insect resistance management (IRM) plan of any transgenic corn developed for commercial purposes. The IRM plan for corn rootworms requires a refuge of nontransgenic corn consisting of no less than 20% of a farmer's corn acreage and planted within or adjacent to the transgenic rootworm corn (Monsanto Company, 2003). This IRM strategy should extend the useful life of transgenic rootworm corn and help delay the development of insect resistance to the technology.

Increased resource conservation

The energy inputs for manufacturing, formulation, packaging, and transport of three types of insecticide formulations have been calculated as 311 MJ/kg for granules, and 257.3 MJ/kg for wettable powders and miscible oils (Pimentel 1992). Other estimates for the total indirect and direct energy inputs average 214 MJ/kg active ingredient for 11 insecticides (Green, 1987), 185 MJ/kg for 5 insecticides (Food and Drug Administration, 2001), or 315 MJ/kg for generic pesticides (Meir-Ploeger, *et al*., 1996). For comparison, 1 gallon of diesel fuel produces 146.3 MJ of energy (Green, 1987). Most of these energy estimates are based on the production of chlorinated hydrocarbon, organophosphate, or carbamate insecticides, of which the latter two are still used for corn rootworm control. However, the trend is towards production of pesticides that, although more energy intensive in manufacturing per unit, are applied at a very low rate per unit area (Helsel, 1992).

Using data from several sources, the energy saved by replacing insecticides with transgenic corn rootworm technology on 10 million acres can be estimated. The annual energy savings for the range (185-315 MJ/kg) of averaged energy requirements needed for insecticide manufacturing and transport are estimated to be 3.07–5.23 million gallons of diesel fuel equivalents. Additionally, fuel and water are conserved by not applying liquid insecticides for corn rootworm control on 2,764,974 of the 10 million acres. It is estimated that on an annual basis 68,845 gallons of aviation fuel will be saved, and 5,657,734 gallons of water conserved.

Reduced farm waste

Corn rootworm insecticides are packaged in several types of containers. The most common packaging is a plastic sack containing 50 lb of granule insecticide. More recent innovations deliver granules in a self-contained system that prevents the on-farm user from directly contacting insecticide granules during the planting operation but this container is not used for many products. Liquid formulations are mostly packaged in 1- or 2.5-gallon plastic jugs although large bulk containers are occasionally used. It is estimated that by not applying 5.34 million lb of insecticides that 1,187,035 fewer insecticide containers will be used.

Increased farm worker safety

Nearly all corn rootworm insecticides (both granule and liquid formulations) are labeled as restricted use products by the US EPA. Restricted use pesticides are products that, without additional regulatory restrictions, would have been found to cause unreasonable adverse effects on the environment, including injury to the applicator (EPA, 1996). Restricted use pesticides are moderately or highly hazardous to applicators by at least one mode of entry. However, the transgenic insect-control technologies pose no such safety risks to the farmer (Alston, *et al*., 2002). Use of transgenic rootworm corn can decrease farm worker exposure to chemical insecticides. By planting transgenic rootworm corn on 10 million acres, estimates are that farm workers would not be exposed to 5.34 million lb of insecticide per year.

Increased labor efficiency

Savings in planting costs also may occur because the insecticide application equipment attached to the corn planter will no longer be necessary, although some farmers may choose to use this equipment on their refuge acres. With the insecticide application equipment eliminated, larger seed boxes can be installed on the planter. The amortized purchase price of this equipment will mean a slight increase in per acre capital cost, but a larger hopper should cut seed refilling time in half, resulting in handling and labor time savings at a value of \$1.94/acre (Alston, *et al.*, 2002). Planting time saved could be 5.32 h for each 1000 acres of corn for equipment with no insecticide boxes. Also, there would be reduction in aerial application and 195 10-h work days of flying will be eliminated.

Non-target safety

Transgenic corn can conserve insect biodiversity in a cornfield. The preimaginal development, survival, and field abundance of several species of beneficial predators (*Coleomegilla maculata*, *Orius insidiosus*, and *Chrysoperla carnea*) are unaffected by Bt corn (Pilcher, 1999; Pilcher, *et al.*, 1997). However, there are concerns that the benefits of transgenic "insecticidal" corn are limited because the ecological effects on nontarget species are poorly documented and need additional study (Obrycki, *et al.*, 2001). Others (Ortman, *et al.*, 2001) counter that previous research may not necessarily predict all possible interactions, but acknowledged that regardless of whether a pest is controlled by a resistant plant, a biological control agent, an insecticide, a cultural technique, or any other method, that if the pest is reduced then there will be some impact on the biological community.

CONCLUSIONS

The corn rootworm species complex poses a serious and annual threat to the economic production of corn in the United States. Millions of pounds of insecticide are applied annually to control either the larval or adult stages of these pests, or cornfields are rotated with another crop to escape economic damage the following year. However, problems of incomplete crop protection with insecticides, the development of resistance to insecticides, and the biological adaptation of rootworms to crop rotation have diminished the effectiveness of these pest management tactics.

A review of the literature and interpretation of the available data suggest that, compared with conventional broad-spectrum corn rootworm insecticides, there are potentially numerous environmental, societal, and economic benefits associated with incorporating transgenic rootworm technology into a corn production system. Specifically, these benefits would potentially include increased crop protection, reduced insecticide use, reduced stalk rot, increased yield protection, increased farm worker safety, increased energy and resource conservation, increased producer efficiency, increased economic return, insect resistance management, and non-target safety. Transgenic rootworm corn has the potential to dramatically transform integrated pest management efforts in the US; however, this tool must be managed and used wisely if farmers expect to sustain the benefits of the technology into the future.

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Penoxsulam, a new broad spectrum rice herbicide for weed control in European Union paddies

D Larelle
Dow AgroSciences, 6 avenue Charles de Gaulle, BP 93, 78151 Le Chesnay Cedex, France
Email: dlarelle@dow.com

R Mann
Dow AgroSciences, 9330 Zionsville Rd, Indianapolis, Indiana 46268, USA

S Cavanna
Dow AgroSciences, 25 via d'Azeglio, Bologna, Italy

R Bernes
Dow AgroSciences, 72 Cerro del Castanar, 4A, Madrid, Spain

A Duriatti
Dow AgroSciences, 1095 chemin du Bachas, 30000 Nîmes, France

C Mavrotas
Dow AgroSciences, 85 Vouliagmenis Av., 16674 Glyfada, Athens, Greece

ABSTRACT

Penoxsulam (DE-638) is a new post-emergence rice herbicide for applications in drained and semi-flooded paddies. Dow AgroSciences will commercialize penoxsulam in the European Union under the trade name Viper as an Oil Dispersion containing 20 g a.i./litre, which does not require any additional adjuvant. Penoxsulam is a systemic herbicide that is absorbed mainly via leaves, and secondarily via roots. Penoxsulam is a member of the triazolopyrimidine sulfonamide chemical family with ALS (Acetolactate Synthase) inhibition as its mode of action. It has a favorable toxicological and environmental profile. Small plot replicated field trials (1998 - 2002) have demonstrated that penoxsulam at 40 g a.i./ha is a broad-spectrum herbicide that controls *Echinochloa* spp. (all tested biotypes) and major broad-leaf and sedge weeds including *Alisma plantago-aquatica*, *Ammania coccinea*, *Cyperus difformis*, *Cyperus serotinus*, *Scirpus maritimus* and *Scirpus mucronatus*. When applied between the two-leaf and mid-tillering stage of rice, penoxsulam at 40 g a.i./ha has demonstrated excellent safety to rice with no negative effect on yields or seed quality. Key attributes of penoxsulam include a wide window of application, good selectivity to all Indica and Japonica varieties of rice, excellent control of a broad-spectrum of *Echinochloa* spp., broad-leaf and sedge weeds, rainfastness in one hour after application, no rotational crop issues and water management flexibility.

INTRODUCTION

In the European Union, rice is cultivated in 5 countries and covers about 387 000 hectares. Ranking by importance the order is - Italy (215 000 ha), Spain (100 000 ha), Portugal (30 000 ha), Greece (26 000 ha) and France (16 000 ha). Water-seeded rice represents about 90% of the total area and dry-seeded rice in Italy covers the remaining 10%. Japonica varieties represent about 80% of the total area, the rest being Indica varieties.

The control of *Echinochloa* species is a major problem for Southern Europe rice growers, as this is the rice weed that is most frequent and difficult to control. Several existing products can provide satisfactory control, but either with multiple applications, high rates of active per hectare or when applied during narrow application timeframes. Thus, there is a need for a new post-emergence and flexible herbicide, controlling all *Echinochloa* biotypes in one shot.

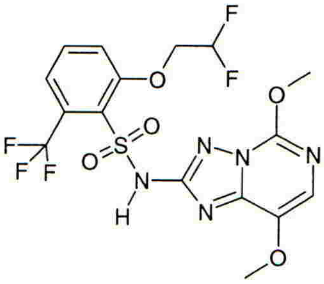
Penoxsulam, which was discovered and will be developed globally by Dow AgroSciences, also meets the European needs:

- Safety to all rice varieties (Indica & Japonica) cultivated in all countries and situations (water and dry seeded crops).
- Favorable toxicological and environmental profile, well adapted to rice growing areas.
- Control of a wide range of weeds, including all *Echinochloa* spp. biotypes, *Cyperus difformis*, *Scirpus mucronatus*, *Alisma plantago-aquatica*, *Ammania coccinea* and others.
- Commercial product pre-formulated with adjuvant, increasing convenience to growers.

This paper presents information on the active ingredient – physico-chemical properties, toxicological and environmental profiles, and biological performance - when applied in the European Union rice paddy conditions.

CHEMICAL AND PHYSICAL PROPERTIES

Common name (ISO)........... Penoxsulam (provisionally approved)
Structure:



Chemical name (CAS)	2-(2,2-difluoroethoxy)-*N*-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide
Chemical family	Triazolopyrimidine sulfonamide
Code names tested	DE-638, XDE-638, XR-638, DASH-001, DASH-1100
Empirical formula	$C_{16} H_{14} F_5 N_5 O_5 S$
Molecular weight	483.373

Vapor pressure	7.16×10^{-16} mm Hg or 9.55×10^{-14} Pa at 25 °C 1.87×10^{-16} mm Hg or 2.49×10^{-14} Pa at 20 °C
Dissociation Constant (*p*Ka)	5.1
Water solubility at 20 °C	5.7 mg/litre @ *p*H 5 408 mg/litre @ *p*H 7 1460 mg/litre @ *p*H 9

Octanol/water partition coefficient at 19 °C: $\log K_{OW} = -0.354$ (unbuffered water)

FORMULATION

Penoxsulam is formulated for the European Union as an Oil Dispersion (OD) containing 20 g a.i./litre. Penoxsulam is in suspension in vegetable oil optimizing the biological performance without the need for additional tankmix adjuvants.

TOXICOLOGY

Acute Oral LD_{50} (rat)	> 5000 mg/kg b.w.
Acute Dermal LD_{50} (rabbit)	> 5000 mg/kg b.w.
Eye Irritation (rabbit)	Mild transient ocular irritation
Skin Irritation (rabbit)	Very slight and transient skin irritation
Skin Sensitization (guinea pig)	Non-sensitizer
Mutagenicity Tests (Ames test, CHO-HGPRT, micronucleus assay, mouse lymphoma assay)	Non-mutagenic

ECOTOXICOLOGY

Avian Oral LD_{50}	Bobwhite Quail	> 2025 mg/kg b.w.
	Mallard Duck	> 2000 mg/kg b.w.
Avian Dietary LC_{50} (8 day)	Bobwhite Quail	> 4411 ppm
	Mallard Duck	> 4310 ppm
Fish 96-h LC_{50}	Rainbow trout	> 102 mg/litre
	Bluegill Sunfish	> 103 mg/litre
	Common Carp	> 101 mg/litre
Aquatic invertebrates	*Daphnia magna*	24- and 48-h EC_{50} > 98 mg/litre
Bees	48-h oral LD_{50} > 110 µg/bee ; 48-h contact LC_{50} > 100 µg/bee	

ENVIRONMENTAL FATE

In water, the major route of degradation is through a combination of photolysis and biological degradation. Laboratory studies showed a photolysis half-life in water of two days under conditions simulating summer sunlight at 40°N latitude (e.g. Valencia in Spain or Thessaloniki in Greece). Half-lives under laboratory aerobic aquatic conditions using European sediments and associated surface waters averaged 23 days (11-34 day range). Field studies in rice paddies in Italy and Spain using typical agronomic practices gave half-lives in water of 6.1 and 5.6 days, respectively.

In soil, penoxsulam degradation is mainly a microbiological process, influenced by temperature. Half-lives using European soils under laboratory aerobic conditions averaged 32 days at 20°C (22-58 day range) and 6.6 days under anaerobic conditions (20°C). Under European paddy conditions (Italy and Spain), no quantifiable residues of penoxsulam were found in soil beyond one day after application (residues moved into the water after flooding).

This rapid degradation of penoxsulam minimizes the risk of damage for cultivation species following rice harvest. Various field carry over studies were set up in Italy, France and Greece with different soil types (*p*H from 5.4 to 8.1, organic matter from 0.4 to 2.1 %, sand from 33 to 83 % and silt from 11 to 48%). Penoxsulam applied at 40 and 80 g a.i./ha under recommended conditions did not induce any negative effect on tested succeeding crops, which were sown either in autumn (durum wheat) or in spring (maize, soybean, sunflower and cotton).

RESIDUES IN RICE AND GRAIN QUALITY

Crop residue trials carried out to GLP standards in Italy, Spain and Greece have shown there are no penoxsulam residues in rice grain when applied at 40 g a.i./ha according to label recommendations (limit of determination = 0.002 mg/kg). In addition, four studies were conducted in Italy, France and Greece to evaluate the grain quality at harvest. It was shown that penoxsulam does not induce any negative effect on the following attributes: 1000 rice caryopsis weight, milling yield, immature and damaged kernels, protein and amylose content and gelation time.

TRANSLOCATION, MODE OF ACTION AND RESISTANCE MANAGEMENT

Penoxsulam is a systemic, phloem and xylem mobile herbicide that is absorbed primarily via leaves and shoots and secondarily via roots. The compound is translocated in plants to meristematic tissues and induces plant chlorosis and necrosis. Complete desiccation of susceptible plants may occur in 7-10 days under ideal growing conditions, but may take longer under less ideal conditions.

Penoxsulam is a triazolopyrimidine sulfonamide whose mode of action is the inhibition of the Acetolactate Synthase (ALS) enzyme in plants. Several sulfonylureas, with the same mode of action, are already used for weed control in rice. Thus, to minimize the risk of development of ALS-resistant species in Southern European paddies, it will be advised to apply this ALS-inhibition mode of action - including penoxsulam - only once per season.

INTENDED USES IN THE EUROPEAN UNION

Penoxsulam is a post-emergence herbicide to be used in dry and water-seeded rice (after the 2-leaf stage). Maximum single application rate is 40 g a.i./ha, which means 2 litres/ha of formulated product. There is no need to add any adjuvant as the formulation performance is optimized. Penoxsulam can be applied when paddy is drained (most common practice in the European Union) or partially flooded (with about 4-5 cm of water depth). It can be used also in dry-seeded rice before flooding the paddy.

CROP SELECTIVITY

Selectivity to rice is due to a differential metabolism of penoxsulam in crop plants compared to susceptible weeds. Rates of penoxsulam metabolism to inactive molecules contribute to the differential selectivity between species. Penoxsulam half-life in rice is 0.6 to 1.6 day, whereas in *Echinochloa crus-galli* its half-life is 4 to 5 days. Forty European varieties (33 Japonica and 7 Indica types) were tested in 4 years of field trials. Penoxsulam has shown the same – very good – selectivity level on all varieties. Penoxsulam applications can induce some slight transient symptoms on rice, mainly stunting or sometimes slight white spotting, but this does not affect further rice development (Table 1).

Table 1. Penoxsulam injury symptoms assessed in 31 field trials

Active ingredient	Rate per ha	1-2 weeks after spray	3-4 weeks after spray	5-6 weeks after spray	7-8 weeks after spray
Penoxsulam	40 g	1.9 (0-9)	0.3 (0-3)	0.3 (0-5)	0.0 (0-1)
Azimsulfuron*	20 g	9.1 (0-35)	1.6 (0-13)	1.0 (0-8)	0.1 (0-1)

* Tank mixed with an adjuvant = non-ionic surfactant at 0.1 % v/v
Mean percentage (0-100 scale) of visual injury (and minimum-maximum values)
Greece, Italy, Spain and Portugal - 2000 & 2001

This good penoxsulam selectivity was confirmed in harvested trials set up in weed-free conditions on 4 Japonica varieties (Drago, S.Andrea, Savio, Zeus) sprayed between the two-leaf and one-tiller stage. Yield in plots treated with penoxsulam was excellent (Table 2).

Table 2. Yield results with penoxsulam applied in 5 weed free selectivity trials

Active ingredient	Rate per ha	Yield : tons/ha
Penoxsulam	40 g	5.59 (4.03 – 6.60)
Penoxsulam	80 g	5.57 (4.44 – 6.72)
Azimsulfuron*	20 g	5.37 (4.20 – 6.45)
Azimsulfuron*	40 g	5.26 (3.94 – 6.67)

* Tank mixed with a non-ionic surfactant at 0.1 % v/v at single rate and 0.2 % v/v at double rate
Mean yield results (and minimum-maximum values)
Italy, France and Portugal - 2001 & 2002

EFFICACY TRIALS

Penoxsulam has a broad-spectrum of activity, with good control of all *Echinochloa* biotypes and many sedge and broad-leaf weeds. Paddies can be re-flooded from 1 to 5 days after application without affecting final performance. This water management flexibility is a great advantage to facilitate rice growers' activity. Results were consistent across all Southern Europe countries and therefore have been combined to present the penoxsulam efficacy profile (Table 3).

Table 3. Penoxsulam performance on key rice weeds in drained paddies and direct comparison to azimsulfuron standard (in same trials)

Weed species	Weed growth stage at treatment	Number of studies	Penoxsulam 40 g a.i./ha	Azimsulfuron* 20 g a.i./ha
Echinochloa spp (all stages)	BBCH 12-23	40	95 (62-100)	68 (18-98)
Echinochloa spp (early stage)	BBCH 12-21	32	96 (75-100)	74 (34-98)
Echinochloa spp (late stage)	BBCH 22-23	8	91 (62-100)	46 (18-94)
*Alisma plantago-aquatica***	BBCH 12-16	2	100 (100-100)	100 (99-100)
Ammania coccinea	BBCH 12-16	6	89 (69-100)	94 (91-98)
*Cyperus difformis***	BBCH 12-15	9	92 (68-100)	93 (67-100)
Cyperus serotinus	BBCH 13-16	4	81 (65-94)	96 (93-99)
Scirpus maritimus	BBCH 12-18	19	84 (56-97)	94 (86-99)
*Scirpus mucronatus***	BBCH 12-15	3	93 (90-98)	94 (88-98)

* Tank mixed with an adjuvant = non-ionic surfactant at 0.1 % v/v

** Biotypes that are resistant to azimsulfuron are not included in this table

Mean percentage (%) of control (and minimum-maximum values)

Italy, Greece, France, Spain and Portugal - 1998 to 2002

These data demonstrate that:

- Penoxsulam controls all *Echinochloa* biotypes outstandingly well. Best performance is achieved when plants are sprayed up to 1 tiller stage (96 %). Efficacy with applications on weeds at 2-3 tillers is still very good (91 %), showing great flexibility in application timing for the farmer.
- Penoxsulam controls a wide range of sedges and broad-leaf weeds very well (e.g. *Alisma plantago-aquatica, Ammania coccinea, Cyperus difformis* and *Scirpus mucronatus*). Control of *Scirpus maritimus* and *Cyperus serotinus* may not be sufficient in some conditions (severe weed pressure).

Penoxsulam also controls "minor" weed species such as *Bacopa rotundifolia, Bergia capensis, Bidens tripartita, Butomus umbellatus, Heteranthera limosa, Lindernia dubia, Nasturtium officinale, Polygonum persicaria.*

CONCLUSION

Penoxsulam is a new post-emergence herbicide for broad-spectrum weed control in rice. It fits European market needs very well showing a favorable toxicological and environmental profile, no grain residues, no anticipated crop rotation restriction, water management flexibility, excellent selectivity and very good control of the most important weeds (*Echinochloa* spp, sedges, broad-leaf weeds) in one unique application.

ACKNOWLEDGMENT

The authors wish to thank all Dow AgroSciences colleagues who contributed to the discovery and development of penoxsulam.

Metamifop: a new post-emergence grass killing herbicide for use in rice

T J Kim, H S Chang, J W Ryu, Y K Ko, D W Kim, K Y Cho
Korea Research Institute of Chemical Technology, P. O. Box 107, Daejeon 305-600, Korea
Email: tjkim@krict.re.kr

C H Park, O Y Kwon, B J Chung
Dongbu Hannong Chemical Co., Ltd, Hwasung, Gyeonggi 445-960, Korea

ABSTRACT

Metamifop [DBH-129, (*R*)-2-[4-(6-chloro-1,3-benzoxazol-2-yloxy)phenoxy]-2'-fluoro-*N*-methylpropionanilide] is a new aryloxyphenoxypropionate (AOPP) herbicide being developed by Dongbu Hannong Chemical Co Ltd, Korea. Like other AOPPs, metamifop provides excellent control on a wide range of annual grass weeds. However, unlike other AOPPs, it shows robust safety on rice. Applied post-emergence in paddy and direct-seeded rice cultivation, metamifop at the rates of 90-200 g a.i./ha gives excellent control of the major grass weeds including *Echinochloa* spp., *Leptochloa chinensis, Digitaria* spp. and *Eleusine indica.* Diverse field trials have been conducted globally to register metamifop both as 3.3-10% EC and as 0.67-1.6% GR formulation for rice cultivation in Asia regions, including Korea and Japan. Metamifop has a favorable toxicological, ecotoxicological, and environmental profile.

INTRODUCTION

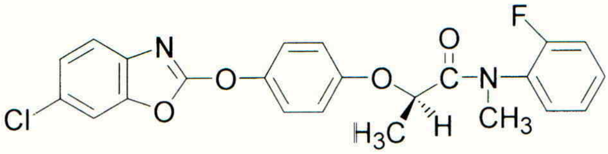
Since their introduction in the mid 1970s, the aryloxyphenoxypropionate herbicides (AOPPs), which exhibit absolute selectivity between grasses and the other species, have been widely used as selective post-emergence herbicides in broad-leaved crops. With this chemistry, creating selectivity in grass crops has been a long challenge, but a few successes have been made.

Metamifop [coded DBH-129, (*R*)-2-[4-(6-chloro-1,3-benzoxazol-2-yloxy)phenoxy]-2'-fluoro-*N*-methylpropionanilide] is a newly developed post-emergence herbicide, discovered initially by the Korea Research Institute of Chemical Technology (KRICT). Although a member of the AOPP class of herbicides, metamifop shows exclusive rice safety with high control efficacy on a wide range of annual grass weeds. Metamifop has an extended application window to control 1-6 leaf stages of important grass weeds in rice cultivation, such as *Echinochloa* spp., *Leptochloa chinensis*, *Digitaria* spp., and *Eleusine indica*. This allows the possibilities to mix metamifop with several other herbicides.

Dongbu Hannong Chemical Co Ltd has conducted many global field trials to develop metamifop as both an EC and a GR formulation for use in rice and all rotational broad-leaved crops.

CHEMICAL AND PHYSICAL PROPERTIES

Discovery code: K-12974
Common name (ISO): Metamifop
Code name: DBH-129
Chemical name (IUPAC): (*R*)-2-[4-(6-chloro-1,3-benzoxazol-2-yloxy)phenoxy]-2’-fluoro-*N*-methylpropionanilide
Empirical formula: $C_{23}H_{18}ClFN_2O_4$
Molecular weight: 440.87
CAS Reg No.: *[256412-89-2]*
Structural formula:



Appearance: Pale brown powder
Melting point: 77.0-78.5°C
Partition coefficient: (octanol/water) log P = 5.45 at 20°C (pH 7)
Vapor pressure: 1.51×10^{-4} Pa at 25°C
Henry’s constant: 6.35×10^{-2} Pa m^3/mole at 20°C
Solubility in water: 0.69 mg/litre at 20°C (pH 7)

TOXICOLOGICAL PROPERTIES

Acute toxicity

Rat, oral: $LD_{50} > 2{,}000$ mg/kg
Rat, dermal: $LD_{50} > 2{,}000$ mg/kg
Rat, inhalation: $LD_{50} > 2.61$ mg/litre
Non irritant to skin and eye
May cause sensitization by skin contact

Genotoxicity

Ames test: Negative
Chromosomal aberration: Negative
Cell mutation: Negative
Micronucleus test: Negative

Ecotoxicity:

Daphnia acute toxicity: EC_{50} (48 hr) 0.288 mg/litre
Algal growth inhibition: EC_{50} (72 hr) > 2.03 mg/litre
Honeybee: $LD_{50} > 100$ µg a.i./bee (contact, dietary)

ENVIRONMENTAL SAFETY

Metamifop shows moderate degradation in soil under standard conditions, with a half-life range of 40-60 days at 25°C. Soil degradation is through both chemical and microbial means. An aqueous photolysis study carried out according to OECD guideline showed seven detectable metabolites identified by LC-MS, and DT_{50} values ranging between 18 and 120 days in various water conditions.

FORMULATION

As a solo product, metamifop is formulated as a 3.3-10% EC for a foliar application to control annual grass weeds in both paddy and direct-seeded rice cultivation. To obtain a broad range of weed spectrum, GR formulated combination products containing 0.67-1.6% metamifop plus 0.07-0.21% pyrazosulfuron-ethyl have been under detailed investigation as a water injection application in paddy rice cultivation.

MODE OF ACTION

Metamifop is an inhibitor of ACCase, which catalyses the first committed step in fatty acid biosynthesis in plants. Similar to the other AOPPs, metamifop strongly inhibits ACCase, and the I_{50} value is approximately 0.6 μM in partially purified barnyardgrass ACCase (Figure 1).

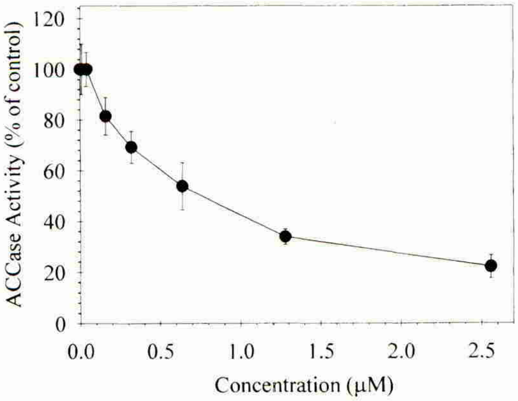


Figure 1. Dose-response to metamifop on the partially purified barnyardgrass ACCase.

BIOLOGICAL PROPERTIES

Although it is classified as an AOPP herbicide, the most important feature of metamifop is that it offers excellent whole plant rice safety. In susceptible species, the symptomology associated with metamifop is chlorosis in developing leaves accompanied by growth inhibition within a few days after application. Dependent upon the species, final death with severe desiccation occurrs approximately 2 weeks after application.

Weed spectrum

Table 1. List of annual grass weeds controlled to a level over 90% through a foliar application at 90-200 g a.i./ha of metamifop

Alopecurus aequalis
Anthraxon hipidus
Brachiaria platyphylla
Cynodon dactylon
Digitaria sanguinalis
Digitaria ischaemum
Echinochloa crus-galli
Echinochloa crus-galli var. *caudata*
Echinochloa crus-galli var. *formosensis*
Echinochloa crus-galli var. *oryzicola*
Echinochloa colonum
Eleusine indica
Leptochloa spp.
Panicum dichotomiflorum
Poa annua
Sorghum bicolor
Setaria spp.

When applied alone as a foliar spray, metamifop provides an excellent level of weed control on a broad range of annual grass species (Table 1). In 3 years of experiments, problematic grass weeds in rice cultivation, such as *Echinochloa* spp., *Leptochloa* spp., *Digitaria* spp., *Eleusine indica* and *Alopecurus aeqularis*, were excellently controlled (90-100%) with the dose rates of 90-200 g a.i./ha metamifop. Metamifop controlled *Echinochloa* spp. grown up to tiller stage effectively, offering a wide application window. The most favorable time for application was between the 2-leaf stage and end of tillering.

Registration trials in rice cultivation (2000-2003)

In two consecutive years of field experiments, barnyardgrass at the 2-3 tiller stage were completely controlled by a foliar application of 99 g a.i./ha metamifop (Table 2). At the double use rate of 198 g a.i./ha metamifop, no rice injury was observed after application.

Table 2. Late-stage barnyardgrass control following a foliar application with 3.3% EC formulation of metamifop in transplanted rice cultivation (2001-2002 trials in Korea)

		Transplanted rice			Barnyardgrass		
Treatment	Rate	Plant height	Tillers	Injury	Plants remaining	Dry weight	Efficacy
	(g a.i./ha)	(cm)	(no/plant)	(%)	(no/m^2)	(g/m^2)	(%)
Metamifop	99	53.5	22.3	0.0	1.3	4.0	96.3
	198	52.7	22.5	0.0	0.5	1.5	98.6
Cyhalofop-butyl	250	52.7	22.5	0.0	6.0	9.8	91.0
Hand weeding		53.8	22.2	0.0	0.0	0.0	100.0
Untreated		-	-	-	27.3	108.6	0.0

The herbicides applied at the 2-3 tiller stage of both plants; data collected 40 days after treatment.

Table 3. List of annual and perennial weeds controlled to a level over 90% through a water injection application with 250 g a.i./ha of metamifop plus 21 g a.i./ha pyrazosulfuron-ethyl (2000-2002 field trials in Korea)

Annuals	Perennials
Echinochloa spp.	*Scirpus juncoides*
Monochoria vaginalis	*Eleocharis kuroguwai*
Bidens tripartita	*Scirpus nipponicus*
Lindernia pyxidaria	

A mixture of metamifop plus pyrazosulfuron-ethyl significantly improves the weed control spectrum. With a dose rate of 250 + 21 g a.i./ha of this mixture, annuals including *Echinochloa* spp., *Monochoria vaginalis* and *Lindernia pyxidaria*, some perennials including *Scirpus juncoides*, *Eleocharis kuroguwai* and *Scirpus nipponicus* were effectively controlled (Table 3). A double use rate of this mixture at 500 + 42 g a.i./ha did not cause any rice injury 40 days after application (Table 4). Therefore, metamifop opens the possibility for using a 'one-shot' post-emergence application with appropriate partners. Dongbu Hannong Chemical Co Ltd is applying to register a GR formulation of 0.67-1.6% metamifop plus 0.07-0.21% pyrazosulfuron-ethyl as an effective product that maximizes the utility of both compounds.

Table 4. Rice safety through a flood water injection application with a GR formulated mixture of metamifop plus pyrazosulfuron-ethyl

Treatment	Rate (g a.i./ha)	Plant height (cm)	Tiller (no/plant)	Visual injury (%)
Metamifop + pyrazosulfuron	250 + 21	61.4	30.6	0
Metamifop + pyrazosulfuron	500 + 42	59.8	30.0	0
Hand weeding	-	60.2	30.6	0

CONCLUSIONS

Although classified as an ACCase inhibitor, metamifop shows excellent rice safety and controls annual grass weeds following post-emergence application. Metamifop applied alone at 90-250 g a.i./ha shows effective control of important annual grass weeds grown up to the tiller stage in rice cultivation. A mixture of metamifop plus pyrazosulfuron-ethyl with a use rate of 250 + 21 g a.i./ha clearly enhances the weed spectrum, controlling both annual grasses/broadleaves and some perennials. Favorable toxicological, ecotoxicological and environmental profiles do not indicate any substantial risk at practical level.

ACKNOWLEDGEMENTS

The authors would like to thank all colleagues in KRICT and Dongbu Hannong Chemical Co Ltd who were involved in achieving the present state of knowledge on metamifop.

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Flucetosulfuron: a new sulfonylurea herbicide

D S Kim, S J Koo, J N Lee, K H Hwang, T Y Kim, K G Kang, K S Hwang, G H Joe, J H Cho
LG Life Sciences Ltd, 104-1, Yuseong-gu, Daejeon 305-380, Korea
Email: dosoonkim@lgls.co.kr

D W Kim
Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea

ABSTRACT

Flucetosulfuron (LGC-42153) is a new post-emergence sulfonylurea herbicide for the control of broadleaf weeds, some grass weeds and sedges in rice and cereal crops. In rice, the herbicide provides excellent control of *Echinochloa crus-galli*, which is not controlled or only marginally controlled by commercial sulfonylurea products, and controls annual broadleaf weeds, sedges and perennial weeds of rice with similar efficacy to other sulfonylurea rice herbicides. Flucetosulfuron can be applied both to soil and foliage in rice, and its use rate is 15 to 30 g a.i./ha. In cereal crops, the herbicide also provides excellent control of broadleaf weeds including *Galium aparine*, *Matricaria* spp. and *Papaver rhoeas* with a good safety to the cereal crops, wheat and barley. It can be applied to foliage, and its use rate is 20 to 30 g a.i./ha.

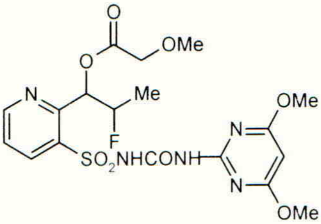
INTRODUCTION

Flucetosulfuron is a new post-emergence sulfonylurea herbicide discovered by LG Life Sciences Ltd for use in rice and cereal crops. Like other sulfonlyurea herbicides, its primary target site is the enzyme acetolactate synthase (ALS) (Hwang *et al.*, 2003). Sulfonylurea herbicides have contributed greatly in weed control in rice and cereal cultivations since their first introduction in the mid 1980s.

In rice cultivation systems particularly in Korea and Japan, sulfonylurea herbicides have provided good control of annual and perennial broadleaf weeds and sedges. However, as they have no or low activity against *Echinochloa crus-galli*, herbicide mixtures (so called "one-shot" herbicides) always come with grass killer partners to give complete weed control by a single application.

Unlike these herbicides, flucetosulfuron controls *E. crus-galli* very effectively as well as the other rice weeds by soil and foliar application, demonstrating a significant advancement in this chemistry. In cereal crops, flucetosulfuron provides good control of *Galium aparine* and other broadleaf weeds such as *Matricaria* spp. and *Papaver rhoeas*. In this report, we introduce the chemical, physical, toxicological and biological properties of flucetosulfuron studied up to the current status.

CHEMICAL AND PHYSICAL PROPERTIES

Code Name:	LGC-42153
Common Name:	Flucetosulfuron (ISO approved in 2003)
Chemical Name:	*N*-[[(4,6-dimethoxy-2-pyrimidinyl)amino] carbonyl]-2-[2-fluoro-1-(methoxymethyl carbonyloxy) propyl]-3-pyridine sulfonamide
Structural Formula:	
Molecular Formula:	$C_{18}H_{22}FN_5O_8S$
Molecular Weight:	487.3
CAS RN:	[*412928-75-7*]
Appearance:	Odorless, solid white powder at 25°C
Melting Point:	178~182°C
Solubility (water at 25°C):	114 mg/litre
Partition (octanol/water) Coefficient:	logP = 1.05
Vapor Pressure:	$<1.86\times10^{-5}$ Pa at 25°C
pKa value:	3.5

TOXICOLOGICAL AND ECOTOXICOLOGICAL PROPERTIES

Rat acute oral toxicity	LD_{50}: >5000 mg/kg
Mouse acute oral toxicity	LD_{50}: >5000 mg/kg (M/F)
Dog acute oral toxicity	LD_{50}: >2000 mg/kg (M/F)
Rat 13 weeks oral toxicity	NOAEL 200 ppm (dietary)
Ames test	Negative
Chromosome aberration test	Negative
Micronucleus test	Negative
Fish acute toxicity (carp)	LC_{50} >10 ppm
Algae acute toxicity	EC_{50} >10 ppm
Daphnia acute toxicity	LC_{50} >10 ppm

FORMULATION

For soil application, flucetosulfuron is formulated as 0.07 and 0.1% granule in Korea and Japan, respectively. For foliar application, it is formulated as a water dispersible granule of various contents from 10 to 50%. We have also developed a self-dispersible labor saving granule for rice.

MODE OF ACTION

Flucetosulfuron is a sulfonylurea and, thus, inhibits acetolactate synthase (ALS) (Hwang *et al.*, 2003), the first committed enzyme for the biosynthesis of the branched-chain amino acids, valine, leucine and isoleucine. This compound can be absorbed via roots, stem and leaf, and its translocation via leaf is faster than that of glyphosate and pyribenzoxim (Lee *et al.*, 2003). The symptoms of herbicidal action include growth cessation, chlorosis, death of apical meristems, and subsequently whole plant death in 2-3 weeks. The selectivity mechanism to this herbicide is assumed to be due to selective metabolism as more rapid recovery of ALS activity was observed in rice as compared with *E. crus-galli* although the initial inhibition of rice ALS was similar to that of *E. crus-galli* ALS (Hwang *et al.*, 2003).

BIOLOGICAL PROPERTIES

Rice

Flucetosulfuron applied to soil or foliage provides broad-spectrum weed control including annual broadleaf weeds, sedges, some grasses such as *Echinochloa* spp., and perennial weeds (Table 1). Particularly in rice, its weed control spectrum is very similar to conventional sulfonylurea rice herbicides such as pyrazosulfuron-ethyl, but the advanced characteristics of flucetosulfuron is control of *Echinochloa* spp. When applied to soil, flucetosulfuron controlled *E. crus-galli* completely even at 10 g a.i./ha (Figure 1A). Moreover, foliar applied flucetosulfuron also controlled *E. crus-galli* at 20 g a.i./ha, and its overall efficacy was better than that of pyribenzoxim (Figure 1B). Therefore, the efficacy of flucetosulfuron alone is similar to that of the conventional one-shot mixtures for soil application in Korea and Japan. Based on this advantage in biological efficacy, flucetosulfuron can be developed either as a solo product or mixtures for soil and foliar application.

Table 1. List of weeds controlled to a level of > 90% by flucetosulfuron applied to soil or foliage in direct-seeded or transplanted rice.

Very susceptible (at 10-20g a.i./ha)		Susceptible (at 20-30 g a.i./ha)	Moderately susceptible (at 30-40 g a.i./ha)
Alisma spp.		*Aeschynomene indica*	*Cyperus serotinus*
Ammannia coccinea	*Monochoria vaginalis*	*Butomus umbellatus*	
		Eleocharis kuroguwai	
Cyperus difformis	*Rorippa silvestri*	*Sagittaria pygmeae*	
Echinochloa spp.	*Rotala inidica*	*Sagittaria trifolia*	
Fimbristylis spp	*Scirpus juncoides*	*Sparganium erectum*	
Lindernia spp.	*Scirpus mucronatus*		
	Scirpus maritimus		

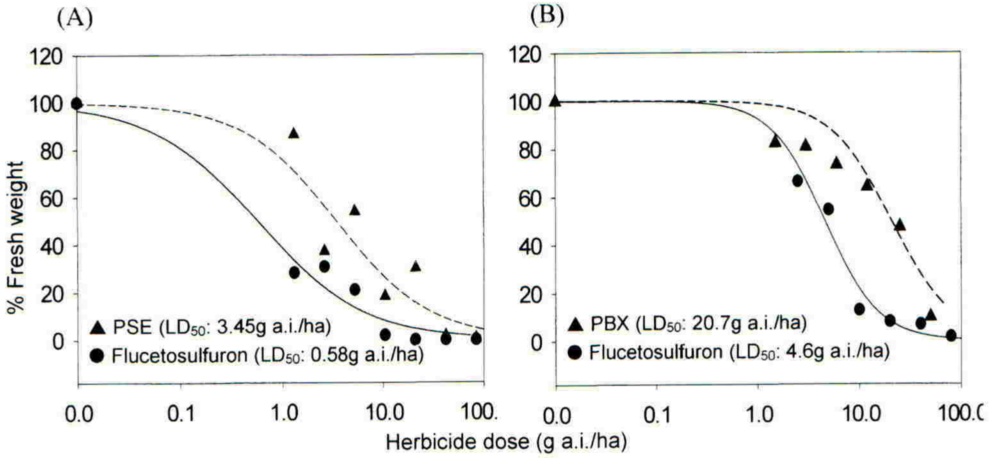


Figure 1. Dose responses of *E. crus-galli* to soil-applied flucetosulfuron and pyrazosulfuron-ethyl (PSE) (A), and to foliar-applied flucetosulfuron and pyribenzoxim (PBX) (B). The growth stage of *E. crus-galli* at the time of application was three leaf stage for both soil and foliar application.

Flucetosulfuron offers good selectivity to rice when applied to foliage and soil. The dose-response study of rice to flucetosulfuron revealed that the LD_{50} value of flucetosulfuron was 182.9 g a.i./ha, while that of pyrazosulfuron-ethyl was 323.1 g a.i./ha (Koo *et al.*, 2003). As recommended application dose range of flucetosulfuron is 20-30 g a.i./ha, it can be concluded that flucetosulfuron is very safe to rice.

Cereal Crops

Flucetosulfuron applied to foliage offers excellent levels of weed control, mainly broadleaf weeds including *Galium aparine*, *Matricaria* spp. and *Papaver rhoeas* at less than 30g a.i./ha (Table 2). Its weed control spectrum is similar to metsulfuron-methyl, but its novel characteristics include excellent activity against *G. aparine*. In comparison with some other herbicides developed for *Galium* control, flucetosulfuron provides better efficacy against *Matricaria* spp., *P. rhoeas* and *S. media*.

Table 2. List of weeds controlled to a level of > 90% by flucetosulfuron applied to foliage in winter cereal crops.

Susceptible (< 30 g a.i./ha)		Moderately susceptible (30-50 g a.i./ha)	Moderately tolerant (> 50 g a.i./ha)
Capsella bursa-pastoris	*Papaver rhoeas*	*Veronica hederifolia*	*Veronica persica*
Galeopsis tetrahit	*Raphanus raphanistrum*	*Viola arvensis*	
Galium aparine	*Senecio vulgaris*		
Lamium purpureum	*Sinapis arvensis*		
Matricaria spp.	*Stellaria media*		
Myosotis arvensis	*Thlaspi arvense*		

In particular, for the control of *G. aparine*, flucetosulfuron controlled the weed very effectively even at 12 g a.i./ha (Figure 2). Its efficacy was equivalent to or better than that of the commercial herbicides developed for control of *G. aparine* and broadleaf weeds. Field evaluations in the UK also demonstrated excellent performance of flucetosulfuron in controlling *G. aparine* treated at various timings from early March to April (Kim *et al.*, 2003). Another advancement of flucetosulfuron is its consistent performance at various temperature regimes, being particularly effective at low temperature as compared with fluroxypyr (Kim *et al.*, 2003). Our study also revealed that the addition of adjuvants improved the efficacy of flucetosulfuron to *G. aparine*, indicating that adjuvants can ensure stable or better performance of flucetosulfuron (Kim *et al.*, 2003).

Flucetosulfuron offers very good safety to wheat and barley. Even at 96 g a.i./ha, wheat and barley tolerated flucetosulfuron with little or no growth inhibition (Data not shown). Field evaluations conducted at more than 50 sites in 2002 and 2003 also showed that flucetosulfuron is very safe to wheat and barley.

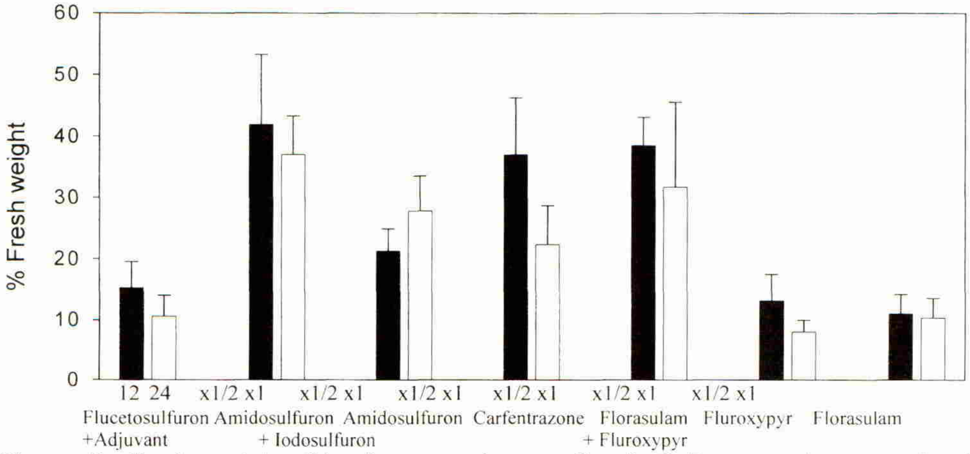


Figure 2. Fresh weight (% of untreated control) of *Galium aparine* treated with flucetosulfuron (g a.i./ha) and commercial standard herbicides (x1/2 and x1 of recommended doses) at its three-whirl stage.

CONCLUSIONS

Flucetosulfuron is a novel sulfonylurea herbicide with particularly high efficacy to *E. crus-galli* in rice and *G. aparine* in cereal crops. Due to its excellent activity to *E. crus-galli*, flucetosulfuron alone at 15-30 g a.i./ha can be an one-shot herbicide without a grass-killer partner, and offers various options for development of diversified products in rice. Flucetosulfuron also offers an alternative option for control of *G. aparine* and broadleaf weeds in cereal crops at 20-30 g a.i./ha.

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SYP-Z071: a new broad-spectrum fungicide candidate

L X Zhang, Z CH Li, B Li, K Sun, Z J Zhang, F K Zhan, J Wang
Shenyang Research Institute of Chemical Industry, Shenyang, 110021, P R China

S H Shaber
Dow AgroSciences, Indianapolis, Indiana 46268, USA

ABSTRACT

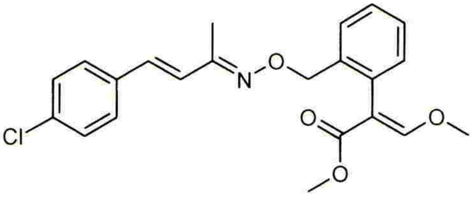
SYP-Z071 is a highly active fungicide providing broad spectrum disease control. It has demonstrated activity against major Ascomycete, Basidiomycete, Deuteromycete and Oomycete plant pathogens in *in vitro* and *in vivo* studies. SYP-Z071 controls fungal strains sensitive and resistant to C14-demethylase inhibitors, phenylamides, dicarboximides and benzimidazoles. The chemical, physical properties and bioactivity of SYP-Z071 are reported in this paper.

INTRODUCTION

Becker *et al.* (1981) first reported that the fungicidal activity of the natural products strobilurin A, strobilurin B, oudemansin A and myxothiazol, all derivatives of β-methoxyacrylic acid, stemmed from their ability to inhibit mitochondrial respiration by blocking electron transfer between cytochrome b and cytochrome c. Subsequent work has indicated that these natural products bind at a specific site on cytochrome b (Mansfield & Wiggins, 1990). Many companies have been involved in the study and development of strobilurin compounds. Several novel broad-spectrum systemic strobilurin fungicides, such as azoxystrobin, kresoxim-methyl, trifloxystrobin, picoxystrobin and fluoxastrobin have been developed. SYP-Z071 is a new strobilurin fungicide developed by Shenyang Research Institute of Chemical Industry.

CHEMICAL AND PHYSICAL PROPERTIES OF SYP-Z071

Chemical name:	methyl 2-{2-[3-(4-chloro-phenyl)-1-methyl-allylideneaminooxymethyl]-phenyl}-3-methoxy-acrylate
Code number:	SYP-Z071
Structural formula:	



Molecular formula:	$C_{22}H_{22}ClNO_4$
Molecular weight:	399.87

Appearance: pale yellow oil
Solubility: very soluble in acetone, ether, and chloroform
Formulation: 25% EC

MAMMALIAN TOXICITY

Acute oral LD_{50} (Rat): 926 mg/kg (M)
749 mg/kg (F)
Acute dermal LD_{50} (Rabbit): > 2000 mg/kg (M)
> 2000 mg/kg (F)
Eye irritation (Rabbit): slightly irritant
Skin irritation (Rabbit): non-irritant
Ames test (Rat): negative

MATERIALS AND METHODS

Laboratory and greenhouse experiments

(a) The bioassay method of cucumber downy mildew (CDM)

Cucumber plants were cultivated in a greenhouse and then the leaves were removed. Each leaf was placed in a petri dish containing a thin layer of water. For the protective activity test, the leaf was sprayed with the test chemical solution first, then inoculated with a suspension of *Pseudoperonospora cubensis* sporangia (3.2×10^5 sporangia/ml) 24 hr later. The petri dishes containing treated leaves were cultured in a chamber (24 °C, 78% RH, 12-hr light cycle). Efficacy was evaluated visually 7 days after inoculation with the classification system of 0, 50, 75, 80, 85, 90, 95, 99, and 100% disease control according to the diseased leaves of the standard sample and untreated checks. For residuality, the efficacy was evaluated 15 to 20 days later. For the curative activity test, the test chemical solution was applied 24 hr before inoculation.

(b) The bioassay method of cucumber gray mold (CGM)

The culture procedure and treatment method are as previously described, with the exception that the leaves were artificially inoculated with a mycelia disc of *Botrytis cinerea* that had been cultured on PDA medium. The development of disease was estimated visually 3 days later.

(c) The tests of wheat powdery mildew (WPM) and cucumber powdery mildew (CPM)

Wheat and cucumber plants were cultivated in the greenhouse and when the wheat plants reached the 2-leaf stage, and cucumber plants reached the 1-leaf stage, the test solution was applied. After 24 hr, the plants were artificially inoculated with *Blumeria graminis* or *Sphaerotheca fuliginea* spores from diseased plants. Disease control was evaluated 7 to 10 days later.

Field trials

All of the field trials in Dandong and Shenyang were carried out in randomised plots with 3 to 4 replications. The size of plots varied from 12 m^2 to 15 m^2. All trials were sprayed 2 times (for CDM) or 3 times (for TLB) at intervals of 7 to 8 days with test fungicides using a hand-held plot sprayer at an appropriate spray volume. Disease control was assessed 8 or 11 days after the final application.

RESULTS AND DISCUSSION

Laboratory Efficacy

(a) Activity of SYP-Z071 against cucumber downy mildew (CDM)

The results of *in vivo* petri dish test using isolated cucumber leaves showed that SYP-Z071 provided good protective activity against CDM (cucumber downy mildew). The activity of SYP-Z071 was very similar to dimethomorph (Table 1).

Table 1. Efficacy of SYP-Z071 against CDM (% Disease Control)

Compound	Application rate (mg/litre)				
	400	200	100	50	25
SYP-Z071	100	100	90	75	50
dimethomorph	100	100	95	75	50

The activity of SYP-Z071 was the same as azoxystrobin against CDM, but slightly higher than kresoxim-methyl (Table 2).

Table 2. Efficacy of SYP-Z071, kresoxim-methyl and azoxystrobin against CDM (% Disease Control)

Compound	Application rate (mg/litre)		
	100	25	6.25
SYP-Z071	100	100	99
kresoxim-methyl	95	85	85
azoxystrobin	100	100	99

In the greenhouse, the residual test of SYP-Z071 showed good protective results after 15 and 20 days after application at 100-200 g a.i. / ha (Table 3).

Table 3. Residual test of SYP-Z071 against CDM (% Disease Control)

Treatment	Rate	Percent of disease control (%)			
	(g a.i./ha)	7 days	10 days	15 days	20 days
SYP-Z071	200	100	100	100	97
	100	100	100	100	95
	50	100	100	88	68
metalaxyl	800	97	88	42	39
	500	77	73	39	32
dimethomorph	200	100	100	97	71
CK (disease index)		14	19	33	49

Table 4 shows that the curative activity of the two compounds, SYP-Z071 and azoxystrobin, was higher than that of kresoxim-methyl.

Table 4. Curative activity of SYP-Z071 against CDM (% Disease Control)

Compound	Application rate (mg/litre)		
	100	50	25
SYP-Z071	99	85	50
kresoxim-methyl	90	75	0
azoxystrobin	100	85	50

(b) Activity of SYP-Z071 against cucumber gray mold (CGM)

SYP-Z071 showed higher activity against CGM than that of the standard compound iprodione, which is widely used for CGM control in China (Table 5).

Table 5. Activity of SYP-Z071 against CGM (% Disease Control)

Compound	Application rate (mg/litre)			
	1000	500	250	125
SYP-Z071	100	90	80	75
iprodione	90	50	0	-

(c) Activity of SYP-Z071 against cucumber powdery mildew (CPM)

For cucumber powder mildew (CPM), the activity of SYP-Z071 was higher than that of triadimefon in the whole-plant test in the greenhouse (Table 6).

Table 6. Activity of SYP-Z071 against CPM (% Disease Control)

Compound	Application rate (mg/litre)				
	500	250	125	62.5	31.25
SYP-Z071	100	100	99	90	75
triadimefon	-	100	90	75	50

(d) Activity of SYP-Z071 against wheat powdery mildew (WPM) in the greenhouse

In the whole plant test in the greenhouse study, SYP-Z071 also showed very high activity against WPM (Table 7). The activity of SYP-Z071 was a little lower than that of myclobutanil, but higher than that of triadimefon.

Table 7. Activity of SYP-Z071 against WPM (% Disease Control)

Compound	Application rate (mg/litre)				
	500	250	125	62.5	31.25
SYP-Z071	100	100	99	85	80
triadimefon	-	100	95	75	50
myclobutanil	100	100	99	90	85

Field efficacy of SYP-Z071

(a) Field trials in Dandong, Liaoning province

Small plot research trials were carried out in a plastic sheet-covered cucumber field in Dandong city, Liaoning province. The results (Table 8) showed that SYP-Z071 as a 25% EC is a very effective fungicide for cucumber downy mildew control. Disease control of SYP-Z071 EC was higher than that of the standard compounds, dimethomorph and metalaxyl.

Table 8. Field efficacy of SYP-Z071 against Cucumber Downy Mildew* (Dandong)

Treatment	Rate (g a.i./ha)	Percent of disease control (%)				
		I	II	III	IV	Mean
SYP-Z071	500	100	100	100	100	100
	250	99	100	99	99	99
	125	96	94	96	96	96
dimethomorph	250	88	90	91	89	90
metalaxyl	500	46	49	50	51	49
CK (disease index)**		24	21	22	22	23

* Evaluations made 8 days after the final application.

** Leaf samples were divided into 6 levels of disease and the percent of disease control was calculated by the disease index.

(b) Field trials in Shenyang, Liaoning

The field trials were carried out in a plastic sheet-covered tomato field in Shenyang, Liaoning province. The results (Table 9) showed that SYP-Z071 25% EC is a very efective fungicide for tomato late blight control. Disease control with SYP-Z071 EC was similar to azoxystrobin, and much higher than that of the standard compound metalaxyl.

Table 9. Field efficacy of SYP-Z071 against Tomato Late Blight* (Shenyang)

Treatment	Rate (g a.i./ha)	Percent of disease control (%)			
		I	II	III	Mean
SYP-Z071	200	95	93	97	95
	100	94	92	95	94
	50	91	81	93	88
25% azoxystrobin	100	95	98	96	96
metalaxyl	500	51	25	50	42
CK (disease index)*		30	23	32	28

* Evaluations were made11 days after the final application.

Crop safety

In all of the laboratory, greenhouse and field experiments, SYP-Z071 produced no foliar injury on the tested crops.

CONCLUSIONS

SYP-Z071 is a broad-spectrum fungicide in the strobilurin class and it displayed good activity in laboratory and greenhouse experiments against important crop diseases, such as cucumber downy mildew, cucumber powdery mildew, cucumber gray mold and wheat powdery mildew. Field trial results indicated that SYP-Z071 is an excellent fungicide for cucumber downy mildew control in plastic sheet-covered cucumber fields. Primary toxicological research has shown that SYP-Z071 has a low toxicity to mammals. The spectrum of disease control and field performance against other important crop diseases is currently under investigation.

ACKNOWLEDGEMENTS

The authors are grateful to all of their colleagues who have participated in the SYP-Z071 project at the new pesticide discovery group of Shenyang Research Institute of Chemical Industry.

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MTF-753: a novel fungicide

K Tomiya, Y Yanase
Mitsui Chemicals, Inc., 1144, Togo, Mobara-shi, Chiba 294-0017, Japan

ABSTRACT

MTF-753, (*RS*)-*N*-[2-(1,3-dimethylbutyl)thiophen-3-yl]-1-methyl-3-trifluoromethyl -1*H*-pyrazole-4-carboxamide is a novel fungicide which belongs to carboxamide family. It is well known that early carboxamide fungicides have an activity against rust and *Rhizoctonia* diseases, but MTF-753 shows a remarkable activity against not only these diseases but also grey mold, powdery mildew and apple scab. Its mode of action is different from the other fungicides used to control these diseases. Here, we briefly describe how MTF-753 was discovered and also show its chemical and biological properties.

INTRODUCTION

It has been well known that carboxamide type fungicides such as carboxin have activity against rust and *Rhizoctonia* diseases, but BC-723, discovered by Mitsubishi Chemical Corporation and a type of benzamide derivatives, has an activity against grey mold (*Botrytis cinerea*). We had paid attention to the fact that *N*-phenyl benzamide compounds, which were a type of benzamide derivatives, had a weak, but broad-spectrum activity against pathogenic fungi. After long research, we found a novel carboxamide derivative which contained two heteroaromatic rings and had a high fungicidal activity, and found the fact that branched alkyl substitution on the heteroaromatic ring on the amino part of the carboxamide played an important role to expand its anti-fungal spectrum. After intensive research, we finally discovered MTF-753 that was classified as a carboxamide fungicide and it has a pyrazole unit and a thiophen ring.

Here we show the chemical and physical properties, the biological profile, and the toxicological and environmental behavior of MTF-753.

CHEMICAL AND PHYSICAL PROPERTIES

Code number:	MTF-753
Common name:	penthiopyrad (ISO proposed)

Structural formula:

Chemical name:	(*RS*)-*N*-[2-(1,3-dimethylbutyl)thiophen-3-yl]- 1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide
CAS RN:	*[183675-82-3]*
Empirical Formula:	$C_{16}H_{20}F_3N_3OS$
Molecular Weight:	359.42
Appearance:	white powder
Vapor pressure:	6.43×10^{-6} Pa at 25°C
Solubility in water:	7.53 mg/litre at 20°C

FORMULATIONS

MTF-753 is formulated as a 20% SC and a 15 % SC, both show good compatibility with conventional crop protection products

HUMAN SAFETY

Acute oral LD_{50}	Rat (male/female)	>2,000 mg/kg
Acute dermal LD_{50}	Rat (male/female)	>2,000 mg/kg
Acute inhalation LC_{50} (4 h)	Rat (male/female)	>5,669 mg/kg
Eye irritation	Rabbit	Slight-irritant
Skin irritation	Rabbit	Negative
Skin sensitisation	Rabbit	Negative
Mutagenicity	Ames test	Negative
	Chromosomal aberration	Negative

ENVIRONMENTAL SAFETY

Carp LC_{50} (96 h)	1.17 ppm
Daphnia LC_{50} (24 h)	40 ppm
Algae EC_{50} (growth rate 72 h)	2.72 ppm

BIOLOGICAL PROPERTIES

Laboratory evaluation

MTF-753 has fungicidal activity against various species of fungi. The activity of MTF-753 is shown in Table 1.

Table 1 Anti-fungal activity of MTF-753 on agar medium

Fungi	MIC (mg/litre)	Fungi	MIC (mg/litre)
Alternaria mali	15	*Monilinia mali*	3
Alternaria solani	3	*Monilinia fructicola*	5
Botrytis cinerea	4	*Mycosphaerella melonis*	2
Botrytis squamosa	9	*Mycovellosiella nattrassii*	10
Cercospora beticola	>50	*Phomopsis* sp	84
Colletotrichum acutatum	8	*Pyricularia oryzae*	7
Corticium rolfsii	7	*Rhizoctonia solani*	50
Diplocarpon rosae	105	*Sclerotinia sclerotiorum*	8
Elsinoe ampelina	40	*Ustilago maydis*	<0.4
Fulvia fulva	8	*Venturia inaequalis*	14
Glomerella cingulata	13	*Microdochium nivale*	35

Efficacy against resistant fungi

Table 2. Activity of MTF-753 against various resistant strains of *Botrytis cinerea*

Compound	S HR HR[1]	HR S S	HR MR HR	RAP[2]
Thiophanate-methyl	-	+++	+++	NT[3]
Procymidone	+++	-	+++	NT
Diethofencarb	+++	-	+++	NT
Mepanipyrim	NT	NT	NT	+++
MTF-753	-	-	-	-

Growth on Potato dextrose agar media where - indicates no growth of pathogen; +++ indicates good growth

[1] This strain is susceptible to benzimidazoles, highly resistant to dicarboximides and diethofencarb. MR designates moderate resistant.

[2] Resistant strain to anilinopyrimidine compounds

[3] NT = Not tested

Table 3. Activity of MTF-753 against resistant *Venturia inaequalis*

Compound	Susceptible strain	Resistant strain to DMIs	Resistant to strobilurins
Fenarimol	-	+	-
Azoxystrobin	-	-	+++
MTF-753	-	-	-

Growth on Potato dextrose agar media where - indicated no growth of pathogen, + indicates slight growth of pathogen and +++ indicates good growth of pathogen.

Inhibitory test against mitochondrial complex II

Mitochondria were isolated from mycelia of *Rhizoctonia solani*, *Botrytis cinerea*, and *Fusarium oxysporum*. Succinate dehydrogenase activity was assayed spectrophotometrically and the results are listed in Table 4 expressed as I_{50} (50% inhibition).

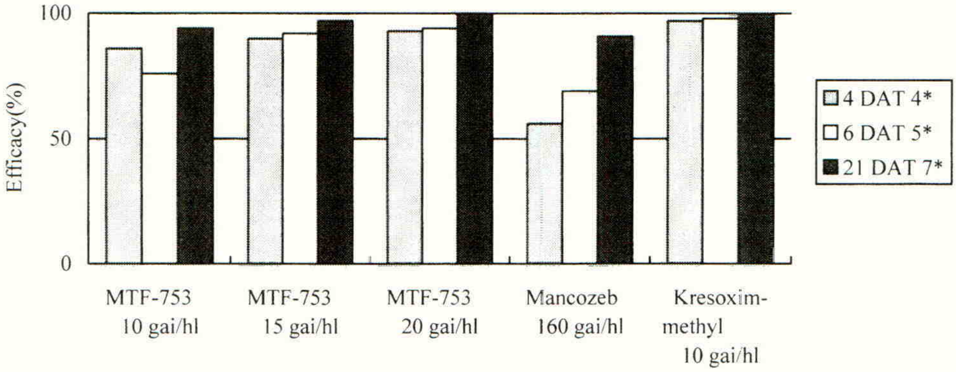
Table 4. Enzyme inhibition activity of MTF-753 as indicated by I_{50} (n mol)

Compound	*Rhizoctonia solani*	*Botrytis cinerea*	*Fusarium oxysporum*
Flutranil	372	<8000	800-2000
MTF-753	50	14	4-8

Field evaluation

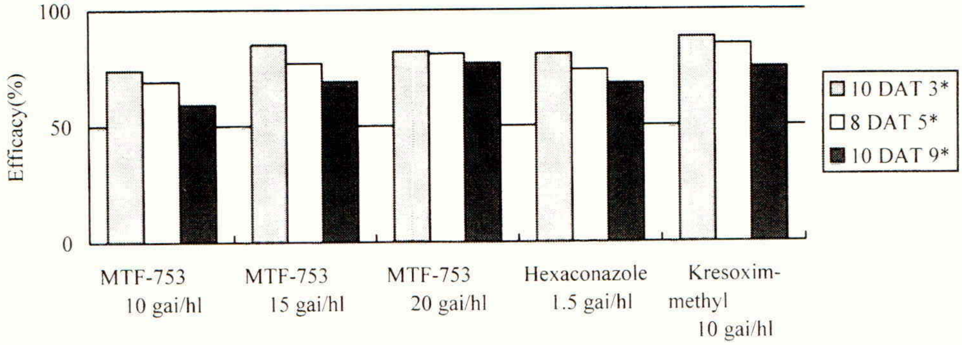
Many field trials were carried out in vegetable and fruit crops.

MTF-753 was proven to be highly active against apple scab (*Venturia inaequalis*, Figure 1) and powdery mildew (*Podosphaera leucotricha*, Figure 2) at 100-200 g ai/hl in comparison with other commercial standards. Also MTF-753 showed a good effect on grape grey mold at 100 ppm (Figure 3) and on powdery mildew in cucumber at 25 ppm (Figure 4).



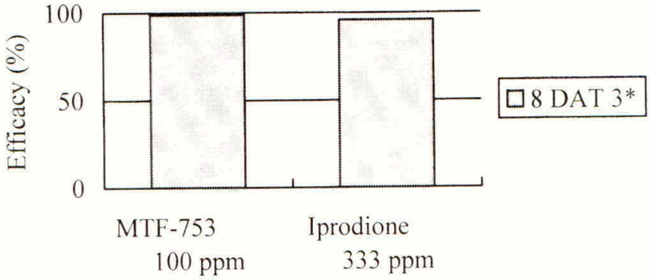
*4 DAT 4 = assessment date 4 days after the 4th treatment

Figure 1. Efficacy of MTF-753 15% SC against apple scab - foliar application (France, 2002)



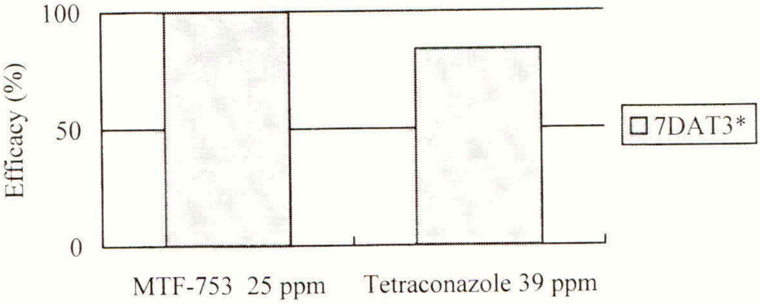
*10 DAT 3 = assessment date 10 days after the 3rd treatment

Figure 2. Foliar efficacy of MTF-753 against apple powdery mildew (France, 2002)



*8 DAT 3 = assessment date 8 days after the 3rd treatment

Figure 3. Efficacy of MTF-753 20% SC against grape grey mold - foliar application (Japan, 2002)



*7 DAT 3 = assessment date, 7 days after the 3rd treatment

Figure 4. Efficacy of MTF-753 20%SC against cucumber powdery mildew - foliar application (Japan, 2001)

CONCLUSION

From laboratory and field tests, MTF-753 showed good efficacy against a wide range of fungal pathogens. In field trials, MTF-753 gave an excellent activity against scab, grey mold and powdery mildew.

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Effects of a novel fungicide benthiavalicarb-isopropyl against Oomycete fungal diseases

Y Miyake, J Sakai, I Miura, K Nagayama
Life Science Research Institute, Kumiai Chemical Industry Co, Ltd, 3360, Kamo, Kikugawa, Ogasa, Shizuoka, Japan
E-mail:y-miyake@kumiai-chem.co.jp

M Shibata
K I Chemical Research Institute, Co, Ltd, Fukude, Iwata, Shizuoka, Japan

ABSTRACT

Benthiavalicarb-isopropyl, isopropyl [(*S*)-1-[(*R*)-1-(6-fluorobenzothiazol-2-yl)-ethylcarbamoyl]-2-methylpropyl] carbamate is a novel fungicide which is active against Oomycete fungal pathogens of various crops. Present studies show that it effectively controls potato and tomato late blight caused by *Phytophthora infestans* and downy mildews caused by *Plasmopara viticola*, *Pseudoperonospora cubensis* or *Peronospora parasitica*. Benthiavalicarb-isopropyl is strongly inhibitory to mycelial growth, sporulation and germination of sporangia and cystspore. It does not show cross-resistance to phenylamide and strobilurin fungicides, suggesting a different mode of action. This compound has not only strongly preventive activity, but also curative and penetrant activity, with excellent residual effects and rainfastness. Benthiavalicarb-isopropyl has a very favorable toxicological and environmental profile and does not cause phytotoxic symptoms on a number of crops, vegetables and fruits.

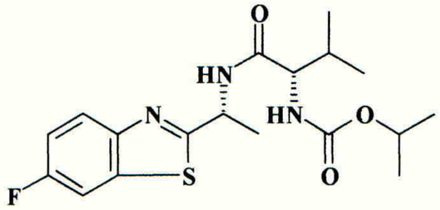
INTRODUCTION

Late blights and downy mildews are important and devastating diseases of crops world wide. Benthiavalicarb-isopropyl, a novel fungicide jointly invented by Kumiai Chemical Industry Co. Ltd and Ihara Chemical Industry Co. Ltd is active against Oomycete fungal plant pathogens. It is being developed in combination with contact fungicides such as mancozeb and folpet. This paper describes the chemical, physical and fungicidal properties of benthiavalicarb-isopropyl and its performance in greenhouse and field trials.

CHEMICAL AND PHYSICAL PROPERTIES

Chemical name :	Isopropyl [(*S*)-1-[(*R*)-1-(6-fluorobenzothiazol-2-yl)-ethylcarbamoyl]-2-methylpropyl] carbamate
Common name :	benthiavalicarb-isopropyl
CAS number :	[*177406-68-7*]

Structural formula :



Molecular formula :	$C_{18}H_{24}FN_3O_3S$
Molecular weight :	381.46
Appearance :	white, odorless, powder
Melting point :	169.2℃
Vapor pressure :	$<3.0\times10^{-4}$ Pa
Solubility :	13.14 mg/litre
Log P_{ow} :	2.52

TOXICOLOGY

Mammalian toxicity

Acute oral mouse (LD_{50}):	>5000 mg/kg (male and female)
Acute oral rat (LD_{50}):	>5000 mg/kg (male and female)
Acute dermal rat (LD_{50}):	>2000 mg/kg (male and female)
Inharation rat (LC_{50}):	>4.6 mg/kg (male and female)
Eye irritation:	not irritating to eye of rabbits
Skin irritation:	not irritating to skin of rabbits
Dermal sensitization:	no skin sensitization observed in guinea pigs
Mutagenicity:	Ames negative
Teratogenicity:	not a teratogen in rats and rabbits
Reproduction:	no adverse effects
Chronic toxicity:	no carcinogenic potential

Toxicity to wildlife

Avian (bobwhite & mallard):	
Acute oral (LD_{50}):	>2000 mg/kg
Dietary (LC_{50}):	>5000 mg/litre
Aquatic: Rainbow trout (LC_{50}):	>10 mg/litre
Bluegill (LC_{50}):	>10 mg/litre
Daphnia (LC_{50}):	>10 mg/litre
Beneficials: Honeybee (LD_{50}):	>100 µg/bee
Earthworm (LC_{50}):	>1000 mg/litre

MATERIALS AND METHODS

The antifungal activity against various plant pathogens was determined by measuring mycelial radial growth on agar culture medium confining benthiavalicarb-isopropyl. The efficacy of benthiavalicarb-isopropyl on various life-stages of *Phytophthora infestans* was investigated by measuring control *in vitro* as a percentage of that of the untreated. For pot tests, tomato, vine and cucumber seedlings were sprayed with the test compounds at predetermined concentrations before or after inoculation of a zoospore suspension of the pathogens. In the curative trials, the compounds were applied 8 hours after inoculation of *Phytophthora infestans* on tomato seedlings and 24 hours after inoculation of *Plasmopara viticola* on vine and *Pseudoperonospora cubensis* on cucumber seedlings. Control activity was assessed as a percentage of that on the untreated by observing the percentage of the leaf area infected. Since 1997, field trials have been conducted in various European and other countries. All field trials were laid out to a randomized block design and replicated 3 or 4 times. Each plot consisted of 8-10 plants for potato, 6 plants for cucumber and 1-3 plants for vine, respectively. Test compounds were applied at a volume of 250 litre/ha to potato and 1000 litre/ha to tomato, cucumber and vine. Spray timing followed the normal growers' practices of the area (7-10 day intervals). Disease evaluations consisted of assessments of frequency and intensity of infected leaves.

RESULTS AND DISCUSSION

In vitro fungitoxicity

Benthiavalicarb-isopropyl exhibited fungicidal activity only against Oomycetes (except for *Pythium* spp.) and not against Ascomycetes, Basidiomycetes and Deuteromycetes. It was considered that the minimum inhibitory concentration (MIC) against mycelial growth of *Phytophthora* spp. was in the range of 0.03-0.3 ppm (Table 1).

Table 1. Fungicidal activity against *Phytophthora* spp.

Pathogen	Inhibition of mycelial growth *MIC
Phytophthora infestans	0.1-0.3 ppm
Phytophthora capsici	0.03-0.1 ppm
Phytophthora palmivora	0.1-0.3 ppm
Phytophthora cactorum	0.03-0.1 ppm
Phytophthora nicotianae	0.1-0.3 ppm
Phytophthora porri	0.1-0.3 ppm
Phytophthora katsurae	0.03-0.1 ppm
Phytophthora megasperma	0.1-0.3 ppm

* Minimum inhibitory concentration

Mode of action

Against *Phytophthora infestans,* benthiavalicarb-isopropyl was strongly inhibitory to sporulation, germination of sporangia and cystspore at low concentrations, but zoospore release and motility were not affected (Table 2). Similar results were obtained in other trials with *Plasmopara viticola*, *Pseudoperonospora cubensis* and *Peronospora parasitica.* The precise biochemical mode of action of benthiavalicarb-isopropyl is currently under investigation. It was active against phenylamide resistant strains of *Phytophthora infestans* and strobilurin resistant strains of *Pseudoperonospora cubensis*, suggesting a different mode of action from the fungicides of these chemical groups. Benthiavalicarb-isopropyl did not affect respiration, synthesis of nucleic acid and protein, or the function of plasma-membrane of *P. infestans.*

Table 2. Biological activity against *Phytophthora infestans in vitro*

Treatments	LC_{90} (mg a.i./litre)				
	Spoluration	Zoospore release	Zoospore motility	Cystspore germination	Sporangium germination
Benthiavalicarb-isopropyl	0.6	>100	>100	0.07	0.03
Dimethomorph	2.9	22	>100	0.1	0.08
Mancozeb	>100	3.3	1-3	1-3	66

Laboratory characterization

Benthiavalicarb-isopropyl demonstrated excellent biological properties in greenhouse trials. Preventive treatments at the low rates of 1-3 mg a.i./litre and curative treatment at the rate of 10 AI mg/l highly showed excellent controls of tomato late blight, vine and cucumber downy mildews, respectively (Table 3 & 4). The residual activity and rainfastness of benthiavalicarb -isopropyl equalled or surpassed those of standard fungicides (Table 5). It was confirmed, by using cucumber seedlings with only the upper sides of the leaves treated, that benthiavalicarb -isopropyl had good penetrant activity against *Pseudoperonospora cubensis* (Table 6).

Table 3. Preventive activity against tomato late blight, vine and cucumber downy mildews in the greenhouse

Treatments	Rate (mg a.i./litre)	% disease control		
		Tomato late blight	Vine downy mildew	Cucumber downy mildew
Benthiavalicarb-isopropyl	10	100	100	100
Benthiavalicarb-isopropyl	3	100	98	100
Benthiavalicarb-isopropyl	1	97	84	94
Dimethomorph	10	89	78	100

Metalaxyl	10	79	100	100
Mancozeb	10	27	75	100
Untreated*	-	(100)	(84)	(100)

* Disease severity

Table 4. Curative activity against tomato late blight, vine and cucumber downy mildew in the greenhouse

Treatments	Rate (mg a.i./litre)	% disease control		
		Tomato late blight	Vine downy mildew	Cucumber downy mildew
Benthiavalicarb-isopropyl	30	100	100	100
Benthiavalicarb-isopropyl	10	100	98	100
Benthiavalicarb-isopropyl	3	39	59	86
Dimethomorph	30	0	58	38
Metalaxyl	10	0	100	100
Untreated*	-	(100)	(95)	(100)

* Disease severity

Table 5. Residual activity and rainfastness against tomato late blight in the greenhouse

Treatments	LC_{50} (mg a.i./litre) Ratio*	
	Residual activity	Rainfastness
Benthiavalicarb-isopropyl	1.5	2.3
Dimethomorph	2.2	25.2
Mancozeb	3.7	3.0

* Residual activity: LC_{50} of 7days/LC_{50} of 0 day

Rainfastness: LC_{50} of rainfall (30mm/hr for 1hr)/LC_{50} of no rain

Table 6. Penetrant activity* against cucumber downy mildew in the greenhouse

Treatments	Rate (mg a.i. /litre)	% disease control
Benthiavalicarb-isopropyl	100	100
Benthiavalicarb-isopropyl	30	82
Dimethomorph	100	46
Metalaxyl	30	97
Mancozeb	100	22
Untreated**	-	(100)

* Upper surface to under surface of a leaf

** Disease severity

Field trials

Benthiavalicarb-isopropyl alone at the rates of 25-75 g a.i./ha and in combination with reduced rates of protectant fungicides was tested for the control of *Phytophthora infeatans, Plasmopara viticola* and *Pseudoperonospora cubensis*. Benthiavalicarb alone at the rates of 35-75 g a.i./ha and in combination with mancozeb demonstrated excellent efficacy against potato late blight when sprayed at 7-day intervals (Table 7). The activity was equal to or surpassed that of the standard fungicides.

Table 7. Field efficacy of benthiavalicarb-isopropyl alone and in combination with mancozeb against *Phytophthora infestans* on potato (1997-2000)

Treatments	Rate (g a.i./ha)	% disease control			
		Netherlands	Germany	France	
		1998	1998	1997	2000
		3DAT11*	8DAT8	10DAT4	5DAT9
Benthiavalicarb-isopropyl	75	95	-	86	90
Benthiavalicarb-isopropyl	50	-	56	74	91
Benthiavalicarb-isopropyl	35	-	-	62	86
Benthiavalicarb-isopropyl	25	-	-	39	-
Benthiavalicarb-isopropyl	35 + 1400	99	-	-	93
+ mancozeb	(25 + 1400)	-	(68)	-	-
Dimethomorph + mancozeb	180 + 1200	-	59	-	-
Mancozeb	1600	-	-	58	-
Mancozeb	1400	-	-	-	88
Fluazinam	200	99	70	66	-
Untreated*	-	(95)	(60)	(96)	(83)

** 3DAT11 = 3 days after 11th treatment

* % infected

Against vine downy mildew, excellent efficacy was achieved alone at the rates of 35-75 g a.i./ha and in combination with folpet applied on both leaves and bunches by spraying at 10-day intervals (Table 8). The activity equalled that of the standard fungicides. Benthiavalicarb-isopropyl, both alone at the concentrations of 25-75 ppm and in combination, proved to be highly effective against cucumber downy mildew by spraying at 7-day intervals (Table 9). The activity surpassed that of the standard fungicides.

Table 8. Field efficacy of benthiavalicarb-isopropyl alone and in combination with folpet against *Plasmopara viticola* on vine (1997-1999)

Treatments	Rate g a.i./ha	% disease control			
		Italy	France		
		1997	1997	1999	
		8DAT8	4DAT6	2DAT5	
		Leaves	Leaves	Leaves	Bunches
Benthiavalicarb-isopropyl	75	94	97	-	-
Benthiavalicarb-isopropyl	50	95	96	-	-
Benthiavalicarb-isopropyl	35	-	-	73	76
Benthiavalicarb-isopropyl	35 + 1000	-	-	78	82
+ folpet	(25 + 1000)	-	-	(79)	(80)
Dimethomorph + folpet	226 + 1200	-	-	82	81
Fosetyl-Al + folpet	2000 + 1000	97	91	-	-
Untreated*	-	(61)	(36)	(32)	(28)

* Disease severity

Table 9. Efficacy of benthiavlicarb-isopropyl alone and in combination with chlorothalonil against *Pseudoperonospora cubensis* on cucumber in Japan

Treatments	ppm	% disease control	
		8DAT3	19DAT3
Benthiavalicarb-isopropyl	75	92	78
Benthiavalicarb-isopropyl	50	90	76
Benthiavalicarb-isopropyl	25	88	46
Benthiavalicarb-isopropyl	25 + 250	89	69
+ chlorothalonil	(50 + 500)	91	77
Chlorothalonil	500	46	9
Metalaxyl + mancozeb	100 + 550	58	29
Untreated*	-	(84)	(99)

* Disease severity

Crop Safety

Benthiavalicarb-isopropyl was safe to the leading commercial varieties of many crops at the rates required for effective disease control.

SUMMARY AND CONCLUSIONS

A novel fungicide, benthiavalicarb-isopropyl, has strong preventive, curative and penetrant activities with excellent residual activity and rainfastness. In field trials, it was effective for control of potato and tomato late blight, vine and other downy mildew at the low rates of 25-75 g a.i./ha. Rates of 25-35 g a.i./ha of benthiavalicarb-isopropyl combined with contact fungicides provided excellent control of these diseases. To provide broad spectrum activities and resistance risk management, it is recommended that benthiavalicarb-isopropyl should be used in combination with other contact fungicides. Benthiavalicarb-isopropyl has a favorable toxicological and environmental profile, causing no phytotoxicity at the recommended rates of application. Being free from existing resistance problems, benthiavalicarb-isopropyl is a promising product for integrated pest and resistance management programs.

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